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Julius-Kühn-Archiv

Edited by
C.S. Adler, G. Opit, B. Fürstenau, C. Müller-Blenkle, P. Kern,
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Proceedings of the12th International Working Conference on Stored Product Protection (IWCSPP)

in Berlin, Germany, October 7-11, 2018

Volume 1

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

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Bundesforschungsinstitut für Kulturpflanzen

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Preface

Ladies and Gentlemen,

It is my pleasure welcoming all of you to the 12th International Working Conference for Stored Product Protection on behalf of the Federal Ministry of Food and Agriculture and the Federal Government. I am glad that Germany has the opportunity to host this important event for the first time. Facing challenges as the ever increasing world population, climate change and unrest in many places of the world, securing sufficient food supply is a crucial task in order to ensure food security.

The United Nation's Food and Agriculture Organization (FAO) estimates that approximately one third of the harvest gets lost before consumption. This is by far too much, and improvements are direly needed. Concerted research efforts and international cooperation are needed to find solutions in all fields of stored product protection from better conditions for on-farm storage to save and degradable food packaging. Therefore the purpose of the conference is to exchange new findings and ideas in order to improve stored product protection.

Sustainable intensification of crop production and improved stored product protection are two key approaches to improve the productivity of our agriculture. Both lead to better food supply and better protection of natural resources like bio-diversity, water, air and fertile soils. By contrast food losses are wasting resources and they are unethical. The broad and comprehensive range of topics at the conference underlines the diversity of challenges we are facing. We urgently need the exchange of thoughts and ideas amongst researchers, administration, civil society and entrepreneurs. I am convinced that the German Capital Berlin offers an excellent platform for your deliberations.

More than 60 nationalities take part in this conference. Thus, you resemble a large portion of this world's population and certainly take home some incitements for your important work and contacts for future collaboration. This conference could be realized because of the support and sponsorship of many organizations. Thank you very much to all of them. My special gratitude addresses the FAO and the WFP as well as the BMZ and the GIZ, who were strongly committed to this event. Last but not least I'd like to thank the President of Julius-Kühn-Institute and his staff for organizing the 12th IWCSPP in an excellent manner.

Julia Klöckner, Federal Minister of Food and Agriculture, Germany

Preface

Dear colleagues,

You hold in your hands the Program and book of abstracts of the IWCSPP 2018. To get this far, we needed some luck convincing the Permanent Committee of IWCSPP in 2014, and we learnt that many little challenges come along with organizing such a meeting. Now we hope, everything falls into place and you will have a good time.

This conference intends to cover all aspects of stored product protection. And we are convinced it comes at just the right time. There are new findings available on pest biology, chemotaxis, mycotoxins, there are new results regarding storage engineering, trapping, plant extracts and contact insecticides, on anoxia and fumigation, of course there are new regulations, and new results on physical and biological control. But we are also glad that extension of our research into the field is a topic in this meeting. And the global challenges we face by climate change, increasing unrest and the highest number of displaced people in decades will be discussed. Stored product protection has implications when we need to secure food for refugees, for people struck by severe droughts or other disasters. Are we prepared to take on these challenges? Should'nt we have more international research, more coordination, more funding, more cooperation across continents?

We are happy that you are here and that you participate in this meeting. Thanks to the many of you who sent their abstracts and prepared their presentations, thus adding value to this event.

We hope you will enjoy the few days of this conference and the chance to exchange thoughts. A number of organizations represent their tasks, many companies show their products and services. All thoughts and ideas are important like little mosaic stones that add up to give a complete picture of how stored product protection looks today or may look like in the future.

We hope you take home some happy memories of Berlin and Germany, about colleagues and new acquaintances, may be some new ideas or perspectives on stored product protection.

Your local IWCSPP organizers and Cornel Adler

Berlin, October 2018

Dear colleagues:

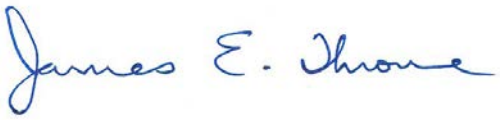
I welcome you to the 12th International Working Conference on Stored Product Protection (IWCSPP). This conference, which is held every four years, is the premier international conference for scientists and industry professionals working with stored agricultural commodities.

Since the first IWCSPP in 1974, the number of participants and the countries which they represent has increased along with the broad coverage of scientific topics related to storage of agricultural products including sessions on insect and pathogen biology, detection, and control, as well as engineering aspects of stored-product protection. The current conference also will have emphasis on protection of stored products in developing countries. The oral and poster presentations, along with specialized workshops, will inform participants on the latest advances in stored-product protection, while providing ample time to network with colleagues.

Berlin, the capital of Germany with a population of almost 4 million people, has much to offer historically (it was founded in the 13th century), culturally, and for outdoor activities. October is an ideal time to visit as the weather is very pleasant.

I welcome you to Berlin for what promises to be an exciting and productive conference for advancing the scientific fields involved in stored-product protection.

Sincerely,



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12th International Working Conference on Stored Product Protection

7 – 11 October 2018, Berlin, Germany

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IWCSPP, Past Conferences and Proceedings

1st International Working Conference on Stored-Product Entomology
Savannah, GA, USA, 1974 (articles available online)

2nd International Working Conference on Stored-Product Entomology
Ibadan, Nigeria, 1978 (articles available online)

3rd International Working Conference on Stored-Product Entomology
Manhattan, KS, USA, 1983 (articles available online)

4th International Working Conference on Stored-Product Protection
Tel Aviv, Israel, 1986 (articles available online)

5th International Working Conference on Stored-Product Protection
Bordeaux, France, 1990 (articles available online)

6th International Working Conference on Stored-Product Protection
Canberra, Australia, 1994 (articles available online)

7th International Working Conference on Stored-Product Protection
Beijing, China, 1998 (articles available online)

8th International Working Conference on Stored-Product Protection
York, UK, 2002

9th International Working Conference on Stored-Product Protection
Campinas, Brazil, 2006 (articles available online)

10th International Working Conference on Stored-Product Protection
Estoril, Portugal, 2010 (articles available online)

11th International Working Conference on Stored Product Protection
Chiang Mai, Thailand, 2014 (articles available online)

Link to former proceedings: http://bru.gmprc.ksu.edu/proj/iwcspp

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Session 1
Food Security and Challenges to Stored Product Protection

Food Safety and Global Challenges to Stored Product Protection – A WFP Perspective

Isabelle Mballa

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The United Nations World Food Programme (WFP) is the leading humanitarian organization fighting hunger worldwide, delivering food assistance in emergencies, assisting 80 million people in around 80 countries each year. In 2016, WFP delivered 3.5 million MT of food to 74 countries, of which 2.2 million MT travelled by sea. On any given day, WFP operates 5,000 trucks, 20 ships and 70 aircraft. Food is stored in a network of 650 warehouses worldwide and across thousands of retailers and distribution partners globally.

With my 20 years of experience with the WFP and particularly in my present capacity as the Chief of Food Safety and Quality Unit of the organization, I am made cognizant of the food safety challenges posed by a multi-modal international supply chain, particularly exacerbated by exposure to harsh variations in climates - from sub-zero temperatures in Canada and France to high 40's and 50's in Sahel within the span of a few months or sometimes even weeks.

WFP has moved in the last 15 years from providing more-stable "raw" grains products such as cereals and pulses like Maize, Sorghum, Wheat and Lentils to a more sensitive food basket that includes processed foods such as fortified flours, nutritious foods such as ready to eat nutritious pastes to treat malnutrition in infants and young children.

The integrity of these "evolved" products is subject to extremities of food storage in challenging conditions down to the last mile, without the protection of temperature or humidity control and sometimes lacking even the basic pest control programmes. In the best case scenario, this may lead to minor loss in nutrient profile of the product; in the worst, it may compromise the food safety of the product. In this lies a paradox on whether to spend public money to feed people or to provide temperature control in warehouses when the people we serve barely have a roof over their heads.

The next challenge in stored product protection lies in identifying the best possible packaging options to assist in maintaining the product's integrity. Losses incurred within the supply chain, though minimal, significantly comprise factors related to packaging failure; spillage due to improper sealing, product exposure due to inadequate packaging material, and so forth. Globally, food packaging has seen one of the fastest growth rates and innovations in the last decade and WFP is catching up with the best solutions to optimizing packaging while keeping costs within range.

One challenge pertinent to this forum is WFP's work in capacity building in developing contexts. The organization continues to work with governments, private sector food processors and small holder farmers to improve farm to fork food supply chains as well as public procurement platforms. Several food safety issues emanate from the harvest and post-harvest handling, including grass root storage of the produce. In this, the developing countries are also the source of various innovations and yet, still playing catch-up with many practices that are considered basic in developed countries. Pest control, protection against pesticides, appropriate crop drying methodologies all play a part in reducing food safety issues such as elevated mycotoxin levels.

Similarly, food processing industry in some contexts is marred by basic issues such as improper hygiene practices and lack of adequate sanitation facilities, which proliferate food safety issues, accentuated downstream in storage.

Lastly, the inability to detect these issues originates in lack of knowledge and lack of proper infrastructure to be able to identify and test the key food safety markers, which are context-dependent. For example, lack of reliable data on the presence of aflatoxins in maize in one country in East Africa along with the absence of a reference lab within the same country to test aflatoxins has hampered general awareness amongst policy makers and thereby the creation of policies, monitoring tools and mitigation measures – which, by some research estimates, has allowed aflatoxins in the crop to run rampant in the country and may be a primary cause of stunting amongst children.

In our line of work at WFP, food safety needs to be addressed throughout the supply chain, starting from the source. Storage of foods, whether raw or processed, falls under the bigger umbrella of food safety across the supply chain from harvest to consumption.

WFP has been and continues to liaise with the private sector to allow industry best practices to be channeled through its work at the grassroots and to bring about a transfer in knowledge to the people in need.

Yet, I personally believe that the solution to protection of stored foods across the supply chain lies in innovation. WFP strives to innovate in new ways of shortening the supply chain, such as by purchasing more locally and regionally; in packaging through research and development; in storage by using elemental energy to cool temperatures in the warehouses and so on.

WFP is a voluntary funded organization with the mandate of achieving Zero Hunger globally. It serves people in conflict contexts, on the move, malnourished children, pregnant and nursing mothers and some of the most vulnerable populations in the world. We deliver food through barges on Baro river in South Sudan and on the backs of donkeys in Nepal; it is stored under tents and in iron containers. While we strive to deliver the maximum food to these beneficiaries, the onus is also on the organization to provide safe food for consumption in an ever changing context.

Food waste and food losses - Importance of international partnerships and research

Friedrich Wacker

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More than 800 million people are still starving worldwide and around two billion humans are suffering from "hidden hunger". And the world population continues to grow, thus increasing the demand for food. Additionally, changed consumption patterns in emerging economies and an increased global demand for sustainable raw materials for the non-food area are leading to increased demand and competition for agricultural products. On top of this, global challenges such as climate change are putting considerable pressure on agriculture to adapt. At the same time, food waste and losses is one of the greatest challenges of our times. Around one third of all available food is spoiled or wasted before it is consumed. To improve the nutritional situation and to reduce food waste and losses worldwide in the long term, international cooperation of agricultural and nutritional research institutes, industries and the society is fundamentally important. The German Federal Ministry of Food and Agriculture (BMEL) supports long-term national and international partnerships with the objective to enhance the direct benefit of German research, innovation and technologies to develop high-performance, nutrition-sensitive and sustainable agri-food systems worldwide. The focus of BMEL is on an effective and efficient cross sector information and knowledge exchange to create a bridge between science and the practical application of research results by the society, industry and policy makers.

Stop the brain drain – Why we need stored-product protection research for food safety

Cornel Adler

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Abstract

In the history of human development, stored-product protection (SPP) is probably older than the invention of agriculture because even what was hunted and gathered needed to be stored to provide food for the bad days. One may think that the human race had enough time to find out everything that could be found out on SPP. But this is not the case. SPP problems often require a solution custom-made for the given product or storage situation, climate, socio-economic background, etc. Modern SPP research in the Americas, Asia, Europe, or Oceania was often started as a result of World War I or II, when hunger was an issue. But, with the absence of hunger, we witness another scary development: SPP research is dying out, institutions are closed down, e.g., CSL UK 2009, SGRL Australia 2009, DPIL Denmark 2010, INRA France 2015. Yes, research costs money. But, do we take into account that climate change may already have led to increased numbers of conflicts and increased mobility? That a lack of food safety can tear apart all advances of civilization and culture in the brink of a moment? Why are there no calls for SPP research under Horizon 2020? What happened to the Millenium Goal to cut down hunger by 50%? The FAO states that one third of our grains are lost between harvest and consumption. It is high time to improve food storages and SPP methods using all knowledge and technology available in order to reduce losses, it is high time to support international SPP research!

Keywords: Storage, research, food-safety, policy, starvation, risk-prevention, innovation, needs

Introduction

If we imagine human development before the onset of agriculture, hunters and gatherers needed to store and protect their food in order to survive bad weather conditions or seasons of scarcity. Thus, stored-product protection was needed even before the development of agriculture. One could expect that all of this time that man had to deal with difficulties such as moulds or two-, four, six-, and eight-legged competitors should have been enough to solve the problems, but this is not the case. According to the Food and Agriculture Organization of the United Nations (FAO 2011), one third of the harvest is not consumed but lost or wasted. While tropical and subtropical warm climates keep insect development and post-harvest losses high at all seasons, in temperate climates and industrialized countries a large portion of the harvest is wasted at the retail and consumer level (FAO 2011). If we try to grasp this gigantic loss, we need to think of one third of arable land, of plowing labour, of seeds, seeding, plant protection activities and products, irrigation, plus harvesting and storing in vain. If we take the grain harvest as an example, estimated by world-grain.com for 2016 at roughly 2.569 billion tons, one third, which roughly equals 850 million tons, will be lost. How is it possible that the human species allowing such a waste is called *Homo sapiens*? In less provocative terms, we could possibly agree upon the fact that there is a hidden treasure. Reducing such losses may help feed an ever growing population in the coming years, increase the productivity of agriculture, allow for more environmentally sustainable agricultural practices, and still leave some protected landscapes to maintain species diversity.

Climate, food security, and mobility

According to Diamond (1997), the availability of plant-based nutrition in early agriculture-driven societies allowed the development of different professions (farmers, hunters, warriors, doctors, chiefs). A society that cannot any longer supply sufficient food may easily fall apart. We should take into consideration that climate change and extreme climates with or without the influence of "El Niño" already cause drastic changes: It has been reported that in 2007 extreme climates caused unusually low harvests in Russia and the Ukraine which together with other factors (fires in Oceania, speculation) led to high grain-prices in the world market. This in turn caused hunger riots in

northern Africa and the so-called Arabic spring. The mass demonstrations of frustrated youths led to the demise of governments. Religious fundamentalism, war lords, and terrorist groups are more successful among young people that do not find a job nor see a future. We are facing times of increasing unrest and aggression in regions where adverse conditions drive desperate people to leave their homes and search for survival elsewhere. In Germany, we thought for a long time to be far away from such struggles, but in the past few years, we had to learn that considerable numbers of refugees made it all the way to our front door.

Thus, what does this have to do with stored-product protection research? Stored-product protection research could help reduce losses in both developing and industrial countries. Improved harvests and improved income from agriculture could help farming communities to be more productive and resilient. Europe claims the will to help improve living conditions in Sub-Saharan Africa and elsewhere. Reducing post-harvest losses would be among the most promising policies. Joint research could help to reduce not only losses but also improve quality of the harvested goods, e.g., by reducing infestation and mycotoxin levels. An example of this is hermetic storage in Purdue Improved Crop Storage (PICS) bags (Anon. 2008, Baoua et al. 2012). Stakeholders and researchers in many tropical countries also have innovative ideas, e.g., on how to improve solar drying, the use of plant parts or extracts, wood ash, zeolites, or other dusts. From Argentina came the innovation of hermetic grain storage in silobags (Bartosik 2012).

Less SPP research in industrialized countries

True stored-product protection research in the Americas, Asia, Europe, or Oceania was often started as a result of World War I or II, when hunger was an issue. But, with the absence of hunger, we witness another scary development: Over the past decades reducing numbers of researchers attended our SPP conferences. Especially in industrialized countries, less and less resources seem available for this kind of research. Research could help to improve storage, but from the Netherlands not one public stored-product protection scientist ever came to a meeting. There is no stored-product protection research done in Belgium, Sweden, or Norway. Denmark made its small Danish Pest Infestation Laboratory part of Aarhus University in 2007, where it was closed around 2010. The stored product protection group of the French Institute National du Recherche Agronomique (INRA), that hosted the IWCSPP in 1990, ceased to exist by the end of 2015, when the last colleague, Dr. Francis Feurat-Lessard, retired. The Stored Grain Research Laboratory in Canberra, Australia, that hosted the IWCSPP in 1994, was closed down in 2009, even though Australia is the 5th biggest grain exporter after Russia, the EU, the US, and Canada. The UK hosted the IWCSPP in 2002 and closed down its Central Science Laboratory after severe cuts in 2009. The few remaining scientists under the new roof of the Food and Environment Research Agency (FERA) cannot any more attend international conferences like this one. Obviously, many countries do not regard stored-product protection research as a priority and rely on other countries to develop the necessary innovation.

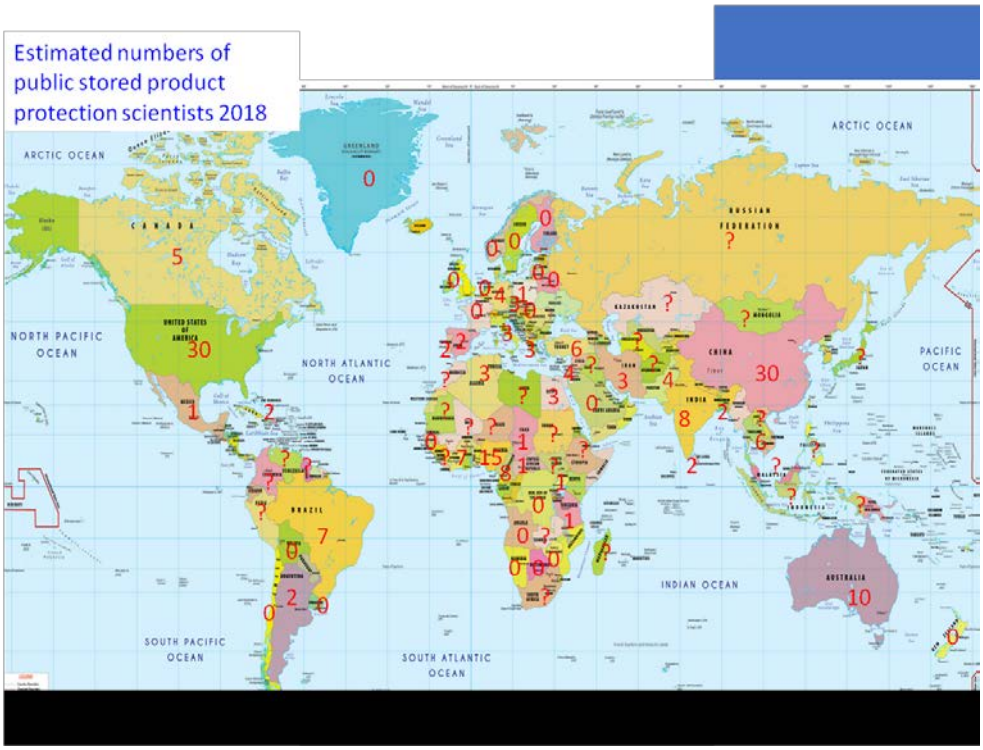

Fig. 1: Estimated numbers of public stored product scientists world-wide

We need stored-product protection research in industrialized countries because new ways of transportation, like bulk storage of cocoa in shipping containers and large horizontal storages, may be more cost effective on one hand but cause challenges like condensation, heating, and even damage by fire. We need research to develop and build better storage structures and to develop structures for the food processing industry that takes into consideration the latest information on insect behaviour. We need improved processing machines that give less opportunity for stored-product insect infestation. We need more research because with new and improved knowledge on pests, preventive methods, monitoring, and control can change. We need more research to learn which new species may find its way into our products or which known species is changing relative importance. Because biology never remains stagnant, we should be aware of changes. New materials can help us to improve packaging technology. Hermetic seals and vacuum could avoid or control pest infestation. Solar drying and aeration cooling could render storages unsuitable for arthropod survival at moderate costs. New camera equipment and computer chips can improve automatic pest detection, and new physical means like laser-technology can allow new methods of pest control (see IWCSPP 2018 publication Adler et al. "Starwars in food stores"). Improved lures with highly attractive volatiles could turn traps from monitoring tools into pest control equipment. A combination of acoustic detection with biological control could render the latter more effective and economically feasible (see IWCSPP 2018 publication Mueller-Blenkle et al. "A new approach to detect insects acoustically in grain storage").

How come we use computers, mobile phones, and other high-tech equipment in our every-day life. But our staple food is stored in storages that are often worse than those of our great-grandparents because the farmer is paid too little money per ton of grain. For decades, farmers were told to invest little into storage structures. Now it could make sense to implement IPM strategies, to propagate

preventive methods such as grain cleaning, drying, cooling, and pest-proof storage structures. Now we have fewer and fewer pest control options. But can we offer sufficient data to convince farmers?

What happened to the United Nations (UN) Millennium Development Goal to cut down hunger by 50%? How can stored-product protection research be helpful to reach this goal? And is there sufficient research done?

The European Union (EU) did not make stored-product protection a topic in its calls for Horizon 2020 even though early on a number of colleagues wrote to their respective national contact points. So far, just mycotoxin-research is funded, but that insects locally increase moisture and thus facilitate mycotoxin formation is not taken into consideration. Who decides research funding policy, and who has sufficient oversight? Is there a way to make research funding a more flexible tool?

At least within Germany, there were national funds available for research projects within the last six years. But international cooperation mainly depended on personal scholarships by sources such as DAAD or Humboldt Foundation.

What needs to change?

As stored-product protection researchers, we are usually analyzing a specific problem and searching for specific improvements or solutions. But if I would lift my head to look at the greater picture, I would like to utter the following wishes:

1. EU: Please make stored-product protection research part of the funding for FP9!

2. EU and member states: Please provide funding and facilitate research cooperation between European and non-European stored-product protection scientists (travel grants, smaller and larger projects), while keeping administrative hurdles at a minimum.

3. FAO and UN World Food Programme (WFP): Please help initiating and coordinating stored-product protection research according to your needs, in organizing exchange of ideas and concepts. Participate more regularly in scientific conferences.

4. UN: Please develop an improved method on how to reach consensus and a clearer perspective on how to tackle pressing challenges (e.g., overpopulation, malnutrition and starvation, scarcity of fresh water, pollution).

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Counting losses to cut losses: quantifying legume postharvest losses to help achieve food and nutrition security

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Abstract

Projections suggest that by 2050 global food production will need to have increased by 70% to meet food demands associated with the world's population growth. Such forecasts, alongside growing awareness of the socio-ecological costs of food loss, and political ramifications of food crises have seen postharvest loss (PHL) reduction reappearing as a development priority. Particularly so in sub-Saharan Africa, a region deemed highly vulnerable to the impacts of climate change, where 307 million people are already affected by severe food insecurity, and the population is projected to double by 2050. Targets for reduced PHL are emphasised in the African Union's Malabo Declaration and Sustainable Development Goal 12.3. However, crop postharvest systems are complex and losses occur in various ways at different activity stages and due to a host of diverse reasons. To better target and prioritise loss reduction investments and policies we need to understand how much food is being lost postharvest, where, and why. The African Postharvest Losses Information Systems (APHLIS), brought a rigorous knowledge management approach to cereal PHLs. We are now expanding this to include key legume and other crops and estimates of the nutritional and financial values of these losses. The scientific literature was screened to build profiles of the PHLs occurring along the value chains, and combined with contextual information, to provide science-based estimates of PHLs where direct measurements are not available. We discuss these legume PHL profiles and the related opportunities and knowledge gaps.

Keywords: Legume crops, postharvest losses, PHL metrics, loss estimates, African Postharvest Loss Information System (APHLIS)

1. Introduction

With the world's population expected to reach 9.8 billion people by 2050, two-thirds of whom will be living in cities (UNDESA, 2017), projections suggest food production will need to have increased by 60% if the growing and changing food demands are to be met (Alexandratos and Bruinsma, 2012). Such forecasts, alongside a developing awareness of the socio-ecological costs of food production, food loss, and political ramifications of food crises have seen postharvest loss (PHL) reduction reappearing as a development priority (World Bank et al., 2011; Gustavsson et al., 2011; Foresight Review, 2011; FAO, 2013; Hodges & Stathers, 2013; Affognon et al., 2015; Mvumi and Stathers, 2014; Sheahan and Barrett, 2017). This is particularly so in sub-Saharan Africa (SSA), a region deemed highly vulnerable to the impacts of climate change (Niang et al., 2014), where the population is projected to double by 2050 (UNDESA, 2017), and where 307 million people already suffer from severe food insecurity (FAO et al., 2017). Sustainable food security will not be achieved through focusing on reducing postharvest losses alone. Increased food production must be achieved with less impact on the environment, alongside actions to modify resource intensive consumption patterns and population growth, improve governance systems, and reduce food loss and waste (Godfray and Garnett, 2014).

Postharvest losses are not just a loss of valuable food, but also of all the resources invested in producing the food. As climate change impacts, population growth, environmental awareness, and competition for water for agriculture increase, so does the pressure to reduce losses. This recent recognition of the importance of and socio-ecological benefits of postharvest loss (PHL) reduction has led to significant investments in improved postharvest management, particularly storage technologies, by governments and donors across SSA. Major targets for reducing PHL have been set. African Union member states in the June 2014 Malabo Declaration for Africa Accelerated Agricultural Growth and Transformation for Shared Prosperity and Improved Livelihoods agreed to reduce current levels of PHL by 50% by the year 2025 (African Union, 2014). In 2015, all member states of the United Nations adopted a set of Sustainable Development Goals (SDGs). SDG 12 aims to ensure sustainable consumption and production patterns, and includes target 12.3 of 'by 2030, halving per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses' (UN General Assembly, 2015).

Crop postharvest systems cover a range of different activity stages and are typically spread spatially and temporally across different locations and actors, and are thus both complex and dynamic. They include the harvesting, transport from field, drying, threshing/shelling, cleaning and sorting, storage, packaging, further transport, marketing, processing, and consumption of the crop. Losses can occur in a multitude of ways at each activity stage and due to a host of diverse reasons (e.g.,

grain left in field at harvest, spilt during transport, or consumed by pests during storage, etc.). To decide how to reduce PHLs, and which investments and policies to implement, it is important to understand not just how much food is being lost postharvest, but at which activity stages these PHLs are occurring, how, and why.

The 2008 food crisis acted as the trigger for development agencies involved in improving food security across SSA to realise they needed a more detailed and accurate understanding of the level of postharvest loss of staple food crops occurring (World Bank et al., 2011; Hodges & Stathers, 2013). This led to the European Commission funding the development of an online African Postharvest Losses Information System (APHLIS) www.aphlis.net, which was launched in 2009, bringing a rigorous knowledge management approach to cereal PHL estimates (Rembold et al., 2011).

To create APHLIS, the scientific literature on cereal PHLs in SSA was screened and weight loss data extracted to build PHL profiles for nine key cereal crops. Seasonal data were then supplied by a network of experts for each province of 37 SSA countries on: the quantity of each of the cereal crops produced, whether rain had occurred at harvest, whether the devastating maize storage insect pest the larger grain borer (LGB), *Prostephanus truncatus* had been present, % of the crop marketed versus stored on-farm, typical storage durations, farm-scale proportions, and climate types. An algorithm was then used to adjust the PHL profile according to the seasonal factors supplied for each location to produce a contextualised science-based estimate of PHL occurring at each PH activity stage for each of the focal crops. This system provided an overview of PHLs by crop across countries and years. The PHL estimate was then presented as % weight loss and quantity of crop lost. The data were used by development agencies for refining their food security assessments. As such, APHLIS provides governments and international organisations and bodies with science-based estimates of cereal PHLs by crop, postharvest activity stage, province, and year, filling a valuable information gap for the majority of locations where direct measurements of PHLs have never been made. As transparency regarding how the loss estimates had been calculated was viewed as important, the APHLIS system enables the data and original studies behind the calculation of each PHL estimate and a rating of the reliability of each loss figure in a profile to be identified, and updated or improved where necessary (Hodges et al., 2014).

A sizeable body of literature exists that discusses and debates postharvest cereal loss assessment methods. Much of the work has focused on different methods for measuring weight losses occurring during cereal storage, which is viewed as a critical loss stage with crop storage in SSA typically occurring at farm-level and often for periods of up to 10 months. However, a focus on just the physical weight loss occurring at different PH stages underestimates the overall value and multi-dimensional nature of PHL, as the quality as well as the quantity of the crop can diminish postharvest. Qualitative losses include: the reduced financial value of damaged, contaminated, or aged produce; nutritional loss which may not always be directly proportional to the weight loss, as rodents and some insect species selectively feed on specific parts of the grains, such as the germ and thus may particularly remove fats or vitamins; reduced seed viability; commercial losses if control treatments have to be purchased, or legal costs are faced; and reputational losses (Boxall, 2001). Including qualitative as well as quantitative losses in PHL calculations would result in substantially higher figures and give a more accurate representation of their socio-economic impact. However, qualitative losses are more complex to measure and the perceived importance of loss in quality may be dependent on the: surrounding food availability situation, location, expectations and standards, intended use of the product (i.e., whether consumed as a whole grain, dehulled or milled product, or marketed), how easy they are to observe, knowledge about what caused them, etc. (Compton et al., 1998; Hoffman et al., 2013; Jones et al., 2014; Kadjo et al., 2016), and limited work has focused on them. They can also be complex to express, as many are not typically considered in monetary terms, i.e., well-being, farmer's time, wasted natural resource inputs, etc. As APHLIS further develops, elements of quality loss are being incorporated to help provide a more complete understanding of PHLs.

The original APHLIS focused on cereal grains. Whilst cereal grains are the main food staple crops in many areas of SSA, root and tuber and legume crops are also crucial staple foods; legumes are a major source of dietary protein in diets of the poor in SSA. In recognition of this, APHLIS is now expanding to include key legumes and other important staple food crops such as cassava. In the current paper, we present the legume PHL data and the process of developing legume PHL profiles and the related opportunities and knowledge gaps.

2. Materials and Methods

To create the PHL profile in APHLIS for each focal crop, the scientific published and 'grey' literature was screened, and reliable high quality data of the PHLs occurring in a specific context extracted and entered into a database along with details of where, when, at which PH stage, and how the loss figure was determined. This followed the method developed by Hodges et al. (2014), and ensures the PHL estimates are based on the best data available. Where limited SSA PHL data exists, the search was widened to include other countries with similar legume production and PH systems and climate types.

This complex multi-stage process involved a thorough search of the literature, followed by screening of the titles and abstracts of each potential PHL study identified during the search to determine whether quantitative data on PHLs was reported. The full versions of studies considered likely to contain quantitative PHL data were accessed and read. The loss assessment and sampling methodology, type of study, and presentation and interpretation of the results were critically examined to determine how reliable the measurements or estimates were likely to be, to determine whether the data should be included, and, if so, the quality rating of the study's data (high, medium, low, exclude). This screening process was based on that used by Hodges et al. (2014), and was similar to that followed by Affognon et al. (2015). If quantitative PHL figures had been collected during the study, they were extracted and entered into the appropriate crop group database (i.e., cereals, legumes, root, tuber, and banana).

For each PHL figure used, the accompanying data on the context in which that PHL occurred was recorded. This included the:

- crop type;
- PH activity stage that the loss figure occurred in (i.e., harvesting, field drying, stripping, transport to home, further drying, threshing, storage, transport to market, market);
- method used to obtain the loss figure (i.e., measured vs guestimate, and details of the loss assessment method, sampling technique, and accuracy of interpretation of results);
- type of study (i.e., field survey, field trial, or on-station trial);
- geographical location where the data were from;
- Koppen climate zone where the data were from;
- farm type and technology used (i.e., smallholder or larger-scale farmers and whether they were using an improved postharvest management method applicable to that PH stage);
- relevant details about the method and study (i.e., if storage stage, what storage container, treatment, duration, and sampling process that the loss was associated with);
- decision to include or exclude the study, and, if included, the quality rating of the study's data score (i.e., high, medium, or low)

Due to their importance as protein sources in the food systems of many African countries, the focal legume crops included are cowpeas (*Vigna unguiculata*), groundnuts (*Arachis hypogaea*), common beans (*Phaseolus vulgaris*), bambara nut (*Vigna subterranea*), pigeon pea (*Cajanus cajan*), and soy bean (*Glycine max*).

Many of the legume PH studies focus on the storage stage, but had recorded data on the % of insect damaged grains as opposed to the % weight loss, likely as a time-saving measure. Where these

studies were of 'high' or 'medium' quality rating, the percentage damaged grain data were converted to percentage weight loss using crop specific conversion formulae from published studies, e.g., for cowpeas ($y = -0.0025x^2 + 0.3551x - 3.31$, x = % damaged grain, y = % weight loss (Wright and Golob, 1999)). While the actual conversion rate between % damage and % weight loss is likely to be influenced by variety, storage insect pest species present, etc., it was judged to be beneficial to convert the % damaged grain data in order to increase and widen the geographical source of the number of PH loss figures being used to build the storage loss part of the PHL profile.

The dataset was then manipulated to provide an overview of what data of what quality exists for each legume crop, climate zone, and PH stage. Where major gaps in the available data exist, in terms of missing information on some of the postharvest stages for some legume crops, decisions are then made as to whether it is appropriate to use data from a similar legume crop for that stage or to include 'low' quality rated data as well as 'medium' and 'high', until higher quality studies for the PH stage of the specific crop are undertaken. This overview stage allows decisions to be made regarding which data will be used to create a profile of the PHLs occurring at each postharvest stage of the value chain for each crop. Details on key loss-causing factors at each stage are also collected and screened to determine what contextual data could be collected to indicate to what degree the main loss-causing factors occurred which will then be used in the algorithm to adjust the loss estimate for that particular context.

3. Results

3.1. Quantity, quality, focal PH activity stage and crop of legume PHL data

Although accessing and screening of the legume PHL literature is still ongoing, to date legume PHL figures from 63 studies have been identified, resulting in a dataset of 694 legume PHL figures.

Analysis of these figures reveals that 525 (76%) were categorised as of 'high' or 'medium' quality rating, and, of these, 75% were measured figures. When these 'high' and 'medium' quality legume PHL figures were grouped by PH activity stage, the majority were related to storage losses, with 57% giving data on losses during farm-level storage and a further 20% on losses during market storage stage (Table 1). Where storage data were provided as % damaged grains, it was converted to % weight loss; 58% of farm-level storage loss figures and 52% of market storage figures required conversion. Limited data on the losses occurring during the other PH stages exist, and data from cowpeas, groundnuts, and common beans dominate: 36, 28, and 19% of the legume PHL figures, respectively. Inclusion of lower quality data would increase the number of data points from the different PH activity stages but would reduce the reliability of the estimates produced using the profile.

Table 1 Number of legume postharvest loss figures obtained by crop and postharvest activity stage

Postharvest activity stages	Bambara	Common beans	Cowpea	Groundnuts	Pigeon pea	Soy bean	Total
Harvesting, field drying, pod stripping				24	2	4	30
Transport from field				6			6
Further drying				11	1	3	15
Threshing / shelling, winnowing			12	16	2	3	33
Storage on-farm	18	101	110	48	21	3	301
Packing, sorting, grading				8	1	1	10
Transport to market				13	1	1	15
Market	20		67	13	4	3	107
Processing				6	1	1	8
Total	38	101	189	145	33	19	525

3.2. Climatic and geographical nature of legume PHL data

The climate, in addition to the crop, activity timing, practices, and technologies, influences the level of PHL. When the 'high' and 'medium' quality PHL figures are viewed by the climate type of the

location where they occurred (using the Koppen climate classification), 46% are from tropical savannah (Aw) climate zones, 21% from warm semi-arid areas (BSh), 11% from humid subtropical climates (Cfa), and 8% from tropical monsoon areas (Am). These four climate types cover the majority of the crop producing areas of SSA.

Geographically, the legume PHL data came from a number of countries across sub-Saharan Africa, with 56 % of the studies coming from West African countries, particularly Nigeria, Ghana, and Niger (Figure 1). Relevant data from India, Brazil, and Thailand have also been included.

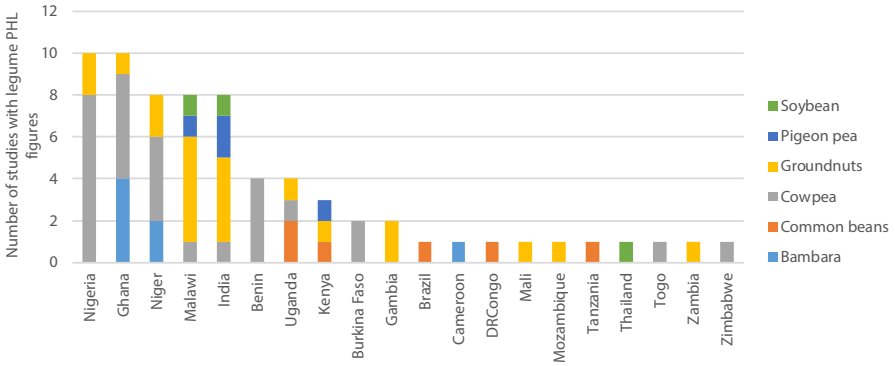

Figure 1 Number of studies with legume PHL figures by country

3.3. Age of legume PHL data

Analysis of the reporting year of the legume PHL data shows that 63% of the studies were published since 2000, reflecting the renewed interest in PHL reduction in SSA (Figure 2). Some high quality studies of legume PHL from before 1980 were also included.

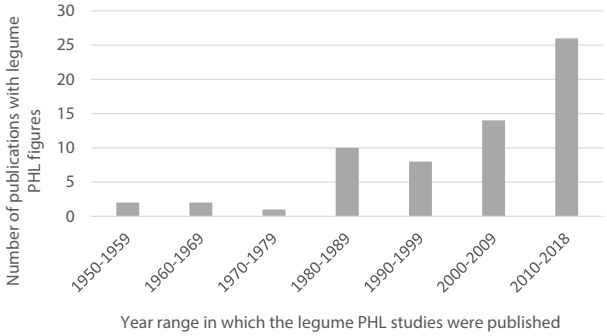

Figure 2 Age range of the legume PHL loss data

3.4. Storage losses from different treatments

The PHL data set includes 301 storage loss figures, and these are from a range of different storage treatments, with 36% from legumes stored untreated in sacks or outdoor granaries; 27% from legumes stored admixed with ash, sand, clay, botanicals, or above fire places; 17% from legumes stored in hermetic bags or other hermetic containers; and 3% from legumes stored admixed with synthetic pesticides (Figure 3). Where storage loss data were from surveys of a number of farmers who were using different treatments, including untreated, botanicals, and synthetic pesticides, it was recorded as 'range of treatments'. Most (70%) of the storage loss data came from legumes stored using the more traditional practices, e.g., untreated shelled or in pods, or admixed with ash,

sand, clay, botanicals, or kept above fire place. The majority of these storage loss data came from studies on cowpeas (39%), or common beans (36%). A preliminary comparison of the average loss levels occurring in the different treatments when loss figures were extrapolated to a standardised five month storage duration, revealed that the traditional practices resulted in average weight losses more than twice as high (>9% loss) as the improved treatments (<4% loss).

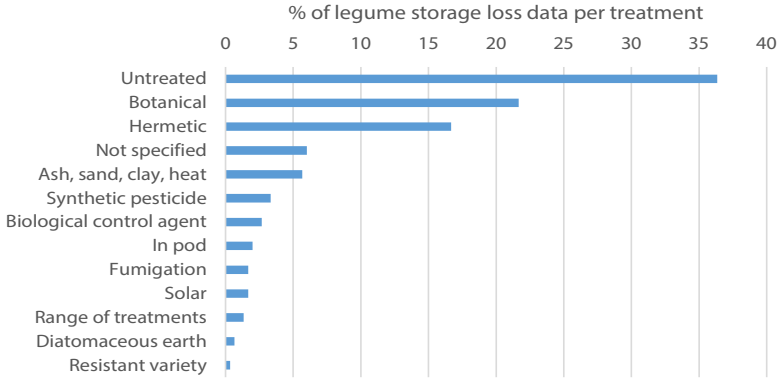

Figure 3 Overview of storage treatment methods from which the storage legume loss figures were obtained

While further calculations have been made using these data towards the calculation of PHL profiles for the different legume crops, it is premature to present this until further decisions are made by the legume technical expert panel regarding the sharing of data between crops and climate zones for cases where insufficient data exists on a particular crop at each PH stage in different climate zones.

During compilation of this legume PHL dataset, the loss causal factors have also been recorded, and these are being used to identify the seasonal or contextual factors that additional data is needed on to contextualise the losses to each location; these include: combining the PH activity timing with meteorological data (e.g., particularly for harvesting and drying), knowledge of the proportion of the focal population using different PH techniques, storage duration and number of harvests/ year, and the proportion of the crop marketed and the timing.

4. Discussion

The legume PHL scientific data are dominated by studies on cowpeas, groundnuts, and common beans, although other legume crops (e.g., pigeon pea) are widely grown and consumed in SSA. There is also more legume PHL data from West Africa than East, Southern, or Central Africa. Most research studies have focused on the storage losses which occur either on-farm or at the market place, and particularly those caused by insect pest damage. Very limited study has occurred of the losses which occur in legumes during harvesting, field drying, pod stripping, transport, further drying, threshing/ shelling, winnowing, sorting, or processing. A similar situation was observed with the cereal PHL data used to create APHLIS (Hodges et al., 2014), the durable crops included in the meta-analysis of PHL data from six SSA countries (Affognon et al., 2015), and a review of PHL in Organisation of Islamic Cooperation member states (Tomlins et al., 2016). However, whilst storage losses are clearly an important element of overall PHL and are the target of many PHL reduction investments, it is unlikely that a focus on reducing losses only during the storage stage will achieve the target of halving PHL by 2030. There is a need for losses at other PH activity stages to be reduced concurrently, and a more holistic and integrated view of pre- and postharvest crop systems.

To deepen understanding of legume PH systems and losses, studies of the non-storage stages are needed to provide greater insight into the proportional amounts being lost at each PH stage of the value chain and the reasons for these losses and opportunities for reducing them. This is important information to ensure available PHL reduction resources are being wisely targeted. Ideally, a PHL study should track the crop and its associated losses from field maturity stage onwards following it

through the different activity stages which will typically occur at and between different locations and times after harvest, and as it changes hands between actors along the value chain. However, such studies are rare, as the logistics of doing such a study at any scale, unless based close to the farms, are complex and costly. Such data would produce a more comparative understanding of where, why, and at what scale losses occur postharvest, and help by removing problems of lack of consistency between measurement methods, aims, geographies, data reporting styles, etc., which are well-known challenges. Many studies use 'guestimates', whereby farmers or other stakeholders are asked to provide verbal estimates of what percentage of their crop they lose postharvest, with the better ones of these studies asking for PHL estimates at each of the different stages and triangulating the responses with rankings, etc. However, these are perceived estimations, and highly subjective, and should not be confused with measured loss assessment. They are of course comparatively cheap and easy to obtain, but their accuracy is not well-understood and will vary by study, and the data obtained with them demonstrably prone to errors. If we think carefully about a simpler question of what % of the food in our home we lost or wasted during the last year or last 5 years, without measuring it and without having kept records, how accurate an estimate would we make? The growing research focus on food waste in developed countries has found quantitative estimates made from memory regarding the weight of food purchased and discarded are very prone to error (see Jorissen et al., 2015; FLW Protocol, 2017 for discussion). For more rapidly quantifying storage losses *in situ*, several visual scales have over the years been developed for different crops (e.g., maize cobs – Compton et al., 1991; cassava – Compton et al, 1992; millet – Hodges, 2013) (Compton and Sherrington, 1999; Hodges, 2013; Hodges et al., 2014).

Combining qualitative with quantitative losses provides a more realistic idea of the level and value of PHLs. However, the rejection criteria for produce varies by location, wealth group (Kadjo et al., 2016) and season (depending on food availability and typical quality) (Compton et al., 1998; Jones et al., 2014). Not all quality attributes are visible (i.e., aflatoxins, pesticide residues), and some studies suggest unobservable maize quality attributes affect farmers' food purchasing decisions and explain the large premium farmers place on maize they have grown themselves relative to that available for purchase (Hoffman and Gatobu, 2014; Kadjo et al., 2016).

There is a frequent misunderstanding that the weight loss occurring during storage is the same as the % of damaged grains, but this is not the case. The physical weight loss of grain is a fraction of the % of grains damaged, typical ratios for % weight loss : % damaged grains are: maize grain 1:8; sorghum 1:4; and paddy rice 1:2 (Adams and Schulten, 1978; Harris and Lindblad, 1978). Therefore a storage weight loss of 12% can mean the damage to the grain is so severe that unless there is extremely limited food availability, the grain would neither be eaten nor could it be sold, thus resulting in a total PHL of all the grain not just a 12% weight loss. There needs to be improved communication and understanding of this important topic, which could be helped with visual imagery. Currently APHLIS presents % weight loss data, and combines this with crop production figures for the different provinces of the focal countries to calculate what amount of each crop that province or country is losing. Preliminary work has begun in the APHLIS+ project to calculate the nutritional loss occurring as a result of these PHLs, and the financial value. Presenting PHLs in terms of dollars lost, or annual requirement of nutrient X for Y million people lost, or the number of extra acres of land farmed or cleared and associated quantities of seed, fertiliser, and water lost is likely to help increase public engagement with and concern about PHLs. However, the difficulty and complexity of including the more qualitative dimensions of PHL should not be underestimated. Attempts to estimate the economic impact of mycotoxins in SSA, for example, were thwarted by the lack of good data (Wu et al., 2011).

Looking beyond the use of APHLIS to calculate science-based estimates of PHL occurring at the different PH stages, there is interest in developing APHLIS to enable it to capture a more nuanced understanding of PHL and how these are or could change over time. Such development could enable it to become a useful support tool for PH investment scenario planning or a PHL M&E tool, for governments or Malabo Declaration or SDG 12.3 Global Food Loss Index M&E frameworks. For

example, the disaggregated storage loss data could be used to calculate changes in PHL as users adopt different improved crop storage practices. This could also be done for PHLs during non-storage stages if sufficient data were available. Governments wanting to better understand PH practices and technology use across their populations could ensure such questions were included in nationwide surveys such as the Living Standards Measurement Survey. However, it should also be noted that some 'improved' PH technologies or practices might be adopted to make a PH process less laborious or costly as opposed to reducing the quantity lost, and this may be of greater importance to the user.

Some improved pre or postharvest technologies or practices may actually increase PHLs, and these complex trade-offs need understanding; for example, some hybrid maize varieties had higher yields but were softer with poorer husk cover resulting in higher storage losses (Tyler, 1982, Boxall, 2001), mechanised harvesting and handling can result in higher levels of damaged grain which can render it more susceptible to attack by certain insect pests (Boxall, 2001), storage of milled rice is more susceptible to insect damage but takes up 25% less space than paddy (Boxall, 2001), double cropping may lead to increased annual production but may alter activity timings and disturb the traditional capability to conserve grain and lead to farmers putting wet season crop into store at higher moisture contents with markedly increased risks of spoilage (Wright, 1995; Boxall, 2001), and stricter food safety requirements and standards may result in increased removal of unsafe food from the food supply (Sheahan and Barrett, 2017). By contrast, a study in India reported rice showing signs of insect attack carried a price premium as it was taken as an indicator that the paddy was not freshly harvested and would taste better (Begum, 1991 cited by Wright, 1995). These examples highlight the importance of interaction and coordination between initiatives and a more holistic understanding of the whole interconnected agri-food system.

The rapid population growth and urbanisation occurring in SSA, the rise of the middle class (defined as those with purchasing power parity of 2 to 20 dollars a day (Ncube et al., 2011), and which is projected to reach 75% by 2040 (Tschirley et al., 2015)), and the growing consumption of food-away-from-home are also driving change in the agri-food systems. There are fears this will involve the consumption of more highly processed food, associated obesity, and unsustainable imports (USDA, 2013; Popkin, 2014), while hopes include demand for higher value and value-added agricultural products driving the creation of entrepreneurs and economic growth (Reardon et al., 2013; Badiane, 2014). Recent studies have found the share of dried legume and cereal grains in the diet reduces within the middle class, and the shares of fresh fruit, fresh fish, and eggs rise strongly, along with purchased maize meal replacing hand-pounded or custom-milled grain; and highly processed milk and vegetable oils and prepared food away from home rising sharply with income (Tschirley et al., 2015). This nutritional-transition will transform the agri-food system and very likely influence PHLs as diets diversify from staple roots, tubers, and grains to preferred cereals and increasing purchasing and consumption of more perishable dairy and meat products, vegetable oils, and fresh vegetables and fruits, which are known to have higher PHLs than cereal and pulses (Gustavsson et al., 2011). This will also come with environmental consequences, as many of these products are more land and water intensive to produce (Godfray and Garnett, 2014).

If PHLs are to be reduced by 50%, as per the Malabo and SDG 12.3 declarations, and make a serious and sustainable contribution to achieving food security in SSA, there is a need for: investment in deepening our understanding about and knowledge and awareness of the level, type, and reason for PHLs occurring along the value chain; institutionalised education of farmers and other stakeholders in postharvest management through practical hands-on learning opportunities (Hodges and Stathers, 2012) and ensuring postharvest management is woven into agricultural and agri-business curriculums; alongside supporting the promotion of appropriate and effective technologies and their distribution systems.

The APHLIS system has an important role to play in the postharvest system by providing science-based estimates of PHLs occurring at the different PH activity stages, for its focal crops by sub-national regions and years. These are useful to governments and development partners for

informing investment decisions and tracking progress. Other crops can be incorporated into APHLIS if sufficient PHL figures exist in the scientific literature, and APHLIS could be expanded to cover other geographical regions, e.g., Asia or the Middle East. The APHLIS team are always looking for new, carefully measured PHL figures to incorporate into APHLIS to keep increasing its accuracy and relevancy; please contact us if you have or plan to gather such PHL figures from SSA for any of the cereal, legume, or root and tuber focal crops.

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Food fights for life: Food diplomacy for food security

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Abstract

Stored food production is critical to food security. Food security refers to the physical availability of, the economic and physical access to, and the ability to utilize food (FAO, 2008, available at; http://www.fao.org/docrep/013/al936e/al936e00.pdf). Stored food production is a vital link in that chain: enabling the protection of (surplus) harvest to be made available when needed. Indeed, the means of stored food production constitutes an incentive for (surplus) harvest itself. However, food, food security, and alongside both, food *diplomacy* are not only practical concerns and challenges but also political. Furthermore, the politics of food are intrinsically related to health security, water security, and climate security, issues with increasing effects across the globe if at different orders of magnitude. Food insecurity may be measured higher in arid regions without adequate water and harvests and storage, but it also exists in 'urban deserts' without affordable access to (fresh) produce. In this presentation, I outline a cartography to depict the interconnections between local and global food securities using the characterization of diplomacy *of* food and *for* food, and food science *for* diplomacy. The aim is to enhance exchange of ideas and experiences to benefit food security – and reduced waste – in both food secure and food insecure settings.

Introduction

Food security is one of a litany of global challenges. Food security refers especially to the security *from* threats to food insecurity – including health threats, threats posed by climate change, and additional shocks such as economic upheavals (see Venezuela) and armed conflict. States alone, even if and when they want to act, can only do so much. That states themselves can constitute a threat to food security underscores a limit to this arrangement. "*The policy authority for tackling global problems still belongs to the states, while the sources of the problems and potential solutions are situated at transnational, regional or global level."* However, food (science) for diplomacy, by contrast, can promote health for security in both developing and developed states, especially when it emerges from developing country contexts and is communicated with developed states.

The politics of food go far beyond states though states arguably remain control of the in- and outflows of food. These include direct actions such as state-based agricultural subsidies; (in)direct mechanisms of state and private sectors, that influence the export of lifestyle, including food, paradigms; and also more diffuse influences such as preferential trade agreements and humanitarian aid programs which exert economic as well as social pressures at all levels of the food chain – from planting decisions to food choice. Food politics is also subject to external shocks, including health crises, such as the outbreak of Ebola in West Africa which led to decreased sowing as well as harvesting and subsequently to increased food insecurity in the region. Thus while any viable and sustainable response to food insecurity in view of such challenges requires state action, it also depends upon inter-national, as well as global and local action.

It is at the formal and informal diplomatic levels that knowledge exchange to recognize; design; maintain, cope; and adapt models and modes to rising threats to food insecurity, including innovative insight into improved stored food protection takes place. Indeed, continuously improved stored food production technology integrated with political decision-making can actively support food security. The alternative to continued knowledge exchange and innovation, especially as set against the continuing and intensifying challenges that increasingly impact food security worldwide, though in some instances appearing as inevitable, is resistance and withdrawal (Gaire, K.R., 2015). Yet 'doing nothing' is also a political and policy choice. It does not imply stagnation, however. It can lead to significant upleaval: for instance to population displacement, further contributing to instabilities and insecurities. In order to avoid uncharted change, planned – if not predictable – responses are necessary.

Three ways to chart possible diplomacies to interconnect local and global food securities are summed up as 'diplomacy of food, diplomacy for food, and food science for diplomacy'. First, diplomacy *of* food includes the elevation of food to an issue of international, notably security, concern. Second, diplomacy *for* food is broader, and includes diplomatic efforts on the parts of states to increase awareness not only of food crises but of solutions. These include diplomatic efforts by state and non-state actors to facilitate access to food according to the criterion of availability, access, and utilization. Third, food (science) for diplomacy in turn includes research and innovation enabling the development and production of, food – and food storage – interventions.

Food Rights and Responsibilities

The balance of food rights and responsibility is underscored by the discourse of human security, introduced in the 1994 Human Development Report, (UNDP, 1994), which emphasizes state and non-state responsibility to promote and protect the rights of its human beings, including to food. It proceeds on the assumption that the achievement of health, while vital, is but one of a litany of local / global challenges facing policy makers. Other prominent competitors for attention include state security writ large, as well as additional aspects of human security – economic, environmental, and food, to name a few.

This has led to the notion on the one hand that food rights are tied to state responsibility and to state security. Yet in practice, food security at the global level has mostly been left to World Food

Program and FAO, both dependent upon Member State financial contributions and votes to authorize distribution and support. On the other hand, the global human security narrative has advanced the claim that health rights are universal, their implementation the responsibility of the international and global communities. As globalization – in communication technology, travel, and climate change –accelerates, so too does the urgency of identifying and addressing rights, including those to food, and responsibilities globally and locally. This is especially critical as policy issues compete for priority: the crises of climate change, energy security, food production and the financial system "represent serious potential threats…in international politics, the prospects for global" "diplomacy, and the effectiveness of global health governance mechanisms."(Lee, et al, 2011, Filder, 2007).

Diplomacy <u>of</u> food

This section analyses the elevation of food to an issue of international, notably security, concern. It focuses on the role of diplomacy and diplomatic efforts to put food security on the international agenda.

Diplomacy <u>for</u> food

This second applies an analysis that is broader that that introduced above. It extends to diplomatic efforts on the parts of states to increase awareness not only of food crises but of solutions. These include efforts by state and non-state actors (NSAs) to facilitate access to food according to the criterion of availability, access, and utilization. As such, it represents a move from advocacy to action, notably on the part of states and NSAs. It traces the shift from food delivery to food production and trade (for example through the implementation of debit cards to enable local purchase and to spur local production).

Food (science) for diplomacy

This section looks briefly at tried and tested examples as well as new research and innovation enabling the development and production of, food – and food storage – interventions.

Discussion

These insights need to be shared: food security requires food diplomacy at all three levels, local, national and global, to recognize and respond to food insecurities across the board; and to critically exchange knowledge based on empirical evidence and (political and social) experience to surmount threats to such insecurities even at different orders of magnitude. This discussion also includes the anticipated impacts of climate change and migration on changing food needs and patterns.

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On farm grain storage – potential opportunity or risk- meeting the demands of food safety and quality, an Australian perspective

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Abstract

Traceability, product identity, food safety and quality assurance are increasingly required by end users and customers. The Australian on farm storage system has a unique opportunity to deliver grain to meet these requirements, provided the system is set up and managed to ensure the end product meets the market requirement.

Australian grain growers are becoming more aware of the changing nature of markets and their requirements, and the importance of managing storage to meet food safety requirements. With the increasing change in storage dynamics in Australia from a central receival system to a range of storage entities, of which on farm storage is becoming a major player, there is a growing need for the grains industry to ensure all who can affect grain quality and food safety are aware of and can meet their obligations.

There are many challenges for Australian growers to manage; including managing existing facilities, investing in new facilities, managing insects, managing grain quality and ensuring treatments are used in accordance with best practice. Despite these challenges, there are many opportunities and potential for the on-farm storage system to meet the demands required of them to deliver a quality and food safe product to the end-user.

This paper discusses the on-farm grain storage system, management of and the opportunity and risks for growers and end users to work together to ensure a quality and food safe product is delivered to the end-user.

Traceability, product identity, food safety and quality assurance are increasingly required by end users and customers. The on farm storage system has a unique opportunity to deliver grain to meet these requirements, provided the system is set up and managed to do this in collaboration with the end user and market.

Whilst grain growers are aware of the changing nature of markets and their requirements, it is fair to say food safety and how they might affect this is relatively new in their thinking. With the increasing change in storage dynamics from a central receival system to a range of storage entities, of which on farm storage is becoming a major player, there is a growing need for the grains industry to ensure all who can affect grain quality and food safety are aware of and can meet their obligations.

There are many challenges for growers to manage; including managing existing facilities, investing in new facilities, managing insects, managing grain quality and ensuring treatments are used in accordance with best practice.

Despite these challenges, there are many opportunities and potential for the on-farm storage system to meet the demands required of them to deliver a quality and food safe product to the enduser and customer.

This paper discusses the on-farm grain storage system, management of the system and the opportunity and risks for growers and end users to work together to ensure a quality and food safe product is delivered to the enduser and customer.

Introduction

With the increase in on-farm grain storage there has been a corresponding increase as to the impact it has on the supply chain. Traceability, food safety, product identity, biosecurity and quality assurance are required to differing extents by industry and the market place. Australia has long had a reputation for providing “clean and green” agricultural products, however there is an increasing demand from both domestic and international markets to prove that food products are safe, and the term food safety is increasingly common when describing market requirements.

Both the domestic and export grain markets continually change, however the need for suppliers to be customer focussed and respond to and manage changes in the market place, places the on-farm grain storage system in a unique position to meet and capitalise on these requirements. There is no question that grain growers are investing in their storage systems and have a unique opportunity

to put in place and improve their existing system to meet the current and future requirements of the supply chain and market.

The on-farm storage system can be developed into one which can manage product identity, quality, traceability, food safety and changing market demands, giving growers flexibility and choices in how they market and deliver their grain through the supply chain. Whilst some may view this as a negative, the growth in on-farm storage continues and can store and deliver grain precisely as the market needs.

The types of systems invested in give growers the ability to segment and manage grain in storage to provide a quality product to the market. Certainly the on-farm system needs to ensure it implements best practise and invest in technologies and training to support best practice, however there are many examples where growers are doing this and many more are seeing the examples of this and considering their own business opportunities.

The grains industry has developed a number of codes of practise as guidelines for Good Agricultural Practice (GAP), and has developed a QA program based on food safety principles using the internationally recognised HACCP (Hazard Analysis Critical Control Point) system. Graincare (the grains industry developed QA system) is well positioned as a HACCP based quality assurance program to deliver a food safe assured product to the market.

There are many potential opportunities and risks when sourcing grain from the on-farm system, however ensuring that the market and the supplier are clear about their needs and responsibilities and establish beneficial outcomes for both, the on-farm market is well placed to provide a quality, safe food product for the enduser.

The on-farm storage system

Traditionally the on-farm storage system was based around smaller silos up to 70 tonne capacity, sheds and in some areas ground storage such as pads and bunkers. In the past 10 years the size of silos has increased substantially, and there has been a trend to larger flat bottom style silos ranging in capacity from 500 – 5000tonnes capacity. The use of temporary systems such as silo bags has increased significantly, and are usually used as a short term option.

Grain in unsealed storage is typically treated with a contact grain pesticide treatment to protect the grain whilst in storage. Resistance to commonly used grain protectants has meant growers are looking for alternatives to control resistant insect pests.

In 2014 the only registered spray treatment to kill insects was taken off the market, currently the only way to kill an infestation is by fumigation. This is forcing growers to seriously consider their investment strategy in new systems because fumigation can only work properly in a sealed gas-tight structure.

With the deregulation of the export market growers storing grain became more aware of the PRF (pesticide residue free) requirement of the export market. Growers have also seen this transfer into domestic markets where they are increasingly being asked for grain to be stored without being treated with contact pesticide protectant treatments. This has meant growers have had to invest in gas-tight sealable storages to effectively fumigate grain to kill insects. There has been a significant increase in the investment in sealable storage, and in 2010 an Australian standard (AS 2628) for gas-tight sealable storage was gazetted providing growers with a benchmark for purchasing gas-tight sealable silos.

In Western Australia grain storage protectants are not registered for grain treatment in on farm storage. Growers are only permitted to fumigate grain, as such, sealed storage has been widely used in on-farm storage for over 30 years

Best practice when storing grain requires growers to implement an integrated approach, using physical and chemical interventions to manage quality and control insects. Over the past 20 years there have been significant changes in the storage types, design and ways growers have applied an

integrated approach. Implementing good grain and system hygiene ensures insect numbers are limited, understanding insect species and their ecology assists in managing pests, and using chemical treatments and fumigants correctly ensures insects can be controlled when needed. Cooling grain using ambient aeration systems has increased in the past 10 years and is gaining widespread acceptance as a way of managing insects and quality by reducing grain storage temperatures.

Growers are increasingly becoming aware of the need to understand the quality of their grain, particularly to ensure grain out turned from their system meets market specifications. One of the advantages of on-farm storage is the ability to segregate grain more readily by using a combination of small, medium and larger storages.

Provided growers are willing to invest in a system which meets market requirements, they are in a unique position to provide a package which delivers product identity, traceability, can meet the needs of food safety requirements and best practise. There is no question that the on-farm storage system can build on and become a larger component of the supply chain, providing confidence and integrity to the market

Food Safety – Can on-farm storage meet this requirement?

The on-farm storage system is well placed to demonstrate that the product stored is safe for consumption. The grains industry has produced a number of codes and guidelines for growers and industry to enable this. "Growing Australian Grain – Safely Managing Risks with Crop Inputs and Grain On-farm" is a guide for growers and advisors to help manage risks with inputs, grain handling and safety on farm.

Grain Trade Australia has produced in collaboration with industry the Australian Grain Industry Code of Practice for the post harvest/post farm sector. Both of these documents enable growers to begin the journey to manage the risks associated with grain production and storage. The grains industry has also developed GrainCare which is a HACCP based quality assurance system which directly enables the grower to demonstrate they meet food safety requirements and are independently audited and assessed.

With the development of a modern, fit for purpose on-farm storage system, which can manage quality, identity preservation, outturn and food safety risks, there is a growing opportunity for the supply chain and market to access grain post farm gate with the confidence that supply chain integrity is maintained.

Conclusion

There is no doubt that the on-farm grain storage system is an integral and growing part of the supply chain. Growers need to ensure they understand their role in the supply chain, and invest in technologies, systems and training which enable them to implement best practise in their grain storage system.

Ensuring that the integrity of the supply chain is maintained requires all parties to do their part and give feedback to all stakeholders. Growers can and will respond to the needs of their market, providing a product which can provide traceability, product identity and assure the product meets food safety requirements. Managed correctly, the on-farm storage system can be a growing opportunity for markets to access quality products direct from the grower, minimising the risk to the end user and supply chain.

Strengthening national food safety for improved food security in Nigeria

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Abstract

A review of literature concerning the quality and safety of eight key staple products in Nigeria, West Africa, was made. These products included stored rice, maize, cashew, yam, cassava, millet, sorghum, and beans. Food safety notifications, both national and international concerning mycotoxins, pesticides, and quality in these foods are highlighted. Across these commodities, a significant number of non-conformances were found, arising from a combination of factors including lack of technical knowledge, supply chain management, and public institutional and policy challenges. The paper discusses the subsequent impact on health, well-being, and the economy. Current strategies aimed at improving food quality and safety in the country was also examined. Recommendations in addressing some significant issues are given.

Keywords: Food security, Nigeria, cowpea, safety, HACCP

The accepted definition of food security is when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food to meet their dietary needs and food preferences for an active and healthy life. In Nigeria, only recently, is food safety seen as an integral part of food security. According to a report by the Nigerian Federal Ministry of Agriculture and Rural Development (FMARD), the country is beset with an inability to either meet domestic food requirements or export agricultural products of the desired quality or safety standards. The agricultural sector in Nigeria suffers from inadequate infrastructure and resources, inadequate financial investments, weak food control systems, obsolete food regulation systems as well as inability to enforce compliance to international standards. The country lacks effective functioning, comprehensive food inspection mechanisms. Laboratory support is also woefully inadequate. Most supply chains in the country are inefficient, with poor traceability systems (The Agriculture Promotion Policy, 2016-2020), and thus national food control is weak.

Cassava, is one of a number of targeted export crops for 2016-2018 by Nigeria's Federal Ministry of Agriculture and Rural Development FMARD. In addition, rice, cowpea (beans), and maize are three of the five targeted domestic crops prioritized for 2016-2018. In terms of nutritional losses and safety, numerous studies have shown many marketed samples of rice across the Nigeria to contain harmful mould causing mycotoxins which is a public health concern (Makun et al., 2011; Egbuta et al., 2015). Maize samples across the country have been found to contain harmful levels of mycotoxins, particularly aflatoxins (Egbuta et al., 2015). The extent to which cashew nuts pose a real food safety risk owing to contamination during storage and marketing is not clear as there are few reported studies. Yam and cassava, however, are commonly processed into dried products using traditional methods. Dried yam derivatives such a 'Elubo' is common amongst the Yoruba tribe as a weaning food for babies. There have been many instances where Elubo has been found to contain elevated levels of mycotoxins, lead, and iron. Gari, a popular cassava derivative has again been found to contain aflatoxins in particular. Millet and sorghum samples across the country have also been shown to contain harmful mycotoxins in a number of studies. The Standards Organisation of Nigeria (SON) has drafted Codes of Practice for cowpea. However, maintaining cowpea quality is posing a significant challenge for farmers and traders, who may store for up to a year. Cowpeas vary according to the size of the grain, color of the skin, texture of the skin, and amount of damage resulting from insects. Consumers prefer beans with few insects present. This has led to the use of unauthorised pesticides in some cases. Due to the detection of high quantities of the unauthorised pesticide dichlorvos, the European Commission Implementing Regulation (EU) 2015/943 temporarily suspended the import of dried beans from Nigeria to the EU in 2015. The ban is still in place.

The Rapid Alert System for Food and Feed (RASFF) of the European Union which highlighted the safety concerns of Nigerian cowpeas was put in place to provide food and feed control authorities with an effective tool to exchange information about measures taken responding to serious risks detected in relation to food or feed. The legal basis of the RASFF is Regulation EC/178/2002 which highlights the principles and requirements of food law, and procedures relating to food safety. Concerning agricultural exports, processed or unprocessed, Nigeria does not export many products in significant volumes, with the exception of raw cocoa. Nevertheless, there were around 200

(RASFF) food-related notifications between the period January 2013 and March 2018 originating from Nigeria, over 50% of which were classified as ' serious', ~40% 'not serious' and around ~10% 'undecided'. Cowpea (or beans) were responsible for over 40% of the serious notifications and resulted in border rejections. The non-conformances mainly concerned the presence and levels of unauthorised chemicals such as dicholorvos, cyhalothrin, chlorpyrifos, dimethate, proferiofos, and trichlorphos in cowpea though the National Agency for Food and Drug Administration and Control (NAFDAC) has produced guidelines and regulations for the import, manufacturing and distribution of pesticides and other chemicals, food additives and fats and oils, and port inspections (http://www.nafdac.gov.ng/). Certification and inspection of food and produce is carried out by the Standards Organisation of Nigeria (SON), Agricultural Quarantine Service (NAQS), NAFDAC, the Federal Produce Inspection Service (FPIS), or a combination of agencies. In addition, the Nigerian Food Safety and Applied Nutrition (FSAN) Directorate's mandate is to ensure that food manufactured, imported, exported, distributed, sold, and marketed in Nigeria meets the highest standard of Food Safety reasonably available and protect public health and consumer interests. It is evident that the environments in most rural areas which is where significant production, postharvest handling, and processing takes place, the monitoring and enforcement of safety standards, and marketing and storage conditions are not conducive to protecting the highlighted products from contamination.

A group of international food safety experts and regional representatives met in 2012 to determine the requirements for an African food safety authority, similar to the EU's European Food Standards Agency (EFSA), along with a communication system such as the RASFF. The effectiveness of RASFF is achieved by having a simple structure: it consists of clearly identified contact points in the Commission, EFSA, EFTA surveillance authority and at national level in member countries. One of the issues highlighted for the failure of the Nigerian government to address over fifty warnings on exported cowpeas before the ban was imposed, was the uncoordinated reporting structure to the various responsible agencies responsible for food safety (TAIEX Report, 2016). A representation of structure of the RASFF is depicted below (Figure 1).

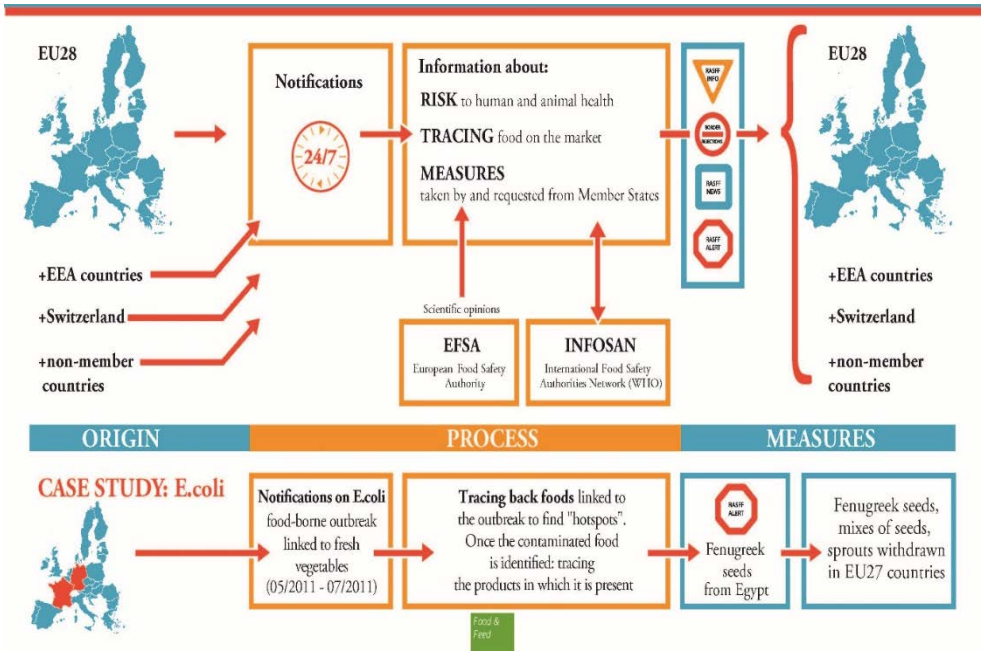

Figure 1: Workings of the European Rapid Alert System for Food and Feed

Source:https://ec.europa.eu/food/sites/food/files/safety/rasff/images/030614_how_does_it_work.jpg

Other serious RASFF alerts originating from Nigeria came in form of notifications relating to aflatoxin B1 (in nutmeg, groundnut, dried ugu leaves, dried bitter leaf, dried ginger, suya pepper, and sesame seeds in particular), various strains of Salmonella (in raw ginger, melon seeds, and sesame seeds in particular), *E. coli* (in ogbono, *Irvinia gabonensis*), and colouring Sudan Red in palm oil.

Unsafe food poses major economic risks. For example, the *E. coli* outbreak in Germany in 2011 was estimated to cost US$ 1.3 billion in losses for farmers. Food-borne disease outbreaks, such as cholera, typhoid, lassa fever, chemical contamination like lead and mercury as well as mycotoxin poisoning, is thought to be responsible for thousands of deaths in Nigeria. However, the exact numbers will not be known owing to poor surveillance and reporting mechanisms. However, Odeyemi (2016) estimated that well over 35 million people (~20%) in Nigeria are affected by foodborne illnesses annually. The true economic and health impact of these illnesses is yet to be properly quantified.

Many African countries including Nigeria are becoming increasingly interested in regional and international trade, with demands to strengthen their Sanitary and Phytosanitary (SPS) capacity, and are consequently trying to address their national food safety issues. As a result, a common framework for the countries is being developed. International cooperation and technical support for African countries in areas of agriculture, food security and food safety is centred around the Comprehensive Africa Agriculture Development Programme (CAADP). In spite of such efforts, it is unlikely significant changes in Nigeria will be made over the next 5 years. This is reflected in the absence of a realistic budget set aside by successive governments to transform the food security situation in the country, including a detailed timebound, auditable, and accountable implementation strategy.

Over 70% of the food in Nigeria is produced in rural areas where farmers and traders often have not gone beyond secondary school education. The Nigerian government must therefore develop a practical and workable strategy to sensitize and educate such stakeholders on good hygiene practices. Achieving food safety begins with ensuring good agricultural practices in production at the farm level. Further, open markets and vendors with basic facilities should be in place. Many foodborne illnesses are well known to be preventable when adopting proper handling, processing, and storage methods for foods guided by HACCP principles. Therefore, the provision or accessibility of appropriate infrastructure to facilitate this such as clean water, power supply, good processing facilities, and physical market design, alongside regular basic HACCP and food handling training of food handlers and vendors should be made in order to significantly reduce the number of incidents of foodborne illnesses and deaths and support the national economy.

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Insect Pests and Fungal Pathogens in Maize Stored in Ghana

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Abstract

Insect infestations and mycotoxin contamination contribute to postharvest degradation and crop loss in sub-Saharan Africa, including maize stored in Ghana. Surveys were conducted to assess the prevalence of insect pests and fungal pathogens in stored maize from the major and minor cropping seasons (September to December and January to April, respectively) that was stored on-farm and in retail markets in Ghana. Results show differences between the major and minor storage seasons for on-farm sites and retail markets. The presence of internal feeders such as *Sitophilus zeamais* (Motschulsky) was positivly correlated with insect-damaged kernels and percentage weight loss. Levels of aflatoxin were generally greater than the established threshold of 15 ppb early in the major crop storage season, while fumonisins were generally lower than threshold levels of 4.0 ppm in on-farm sites and in the retail markets.

Keywords: maize, storage, management, insects, mycotoxins

Introduction

Stored-product insects are a major threat to food security in sub-Saharan Africa, with loss estimates due to insects and associated mycotoxins ranging as high as 70%, depending on the specific commodity, storage site, and management strategies (Hell and Mutegi, 2011; Affognon et al., 2015; Kumar and Kalita, 2017). Major insect pests include the larger grain borer, *Prostephanus truncatus* (Horn), maize weevil, *Sitophilus zeamais* (Motschulsky), lesser grain borer, *Rhyzopertha dominica* (Fauvel), and Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Darfour and Resentrater, 2016). Interactions between insect infestations and subsequent prevalence of mycotoxins are known to occur (Lamboni and Hell, 2009). Many African countries have set tolerances for mycotoxins; for example, the allowable limits for aflatoxin and fumonisin in Ghana are 15 ppb and 4.0 ppm, respectively (Ghana Standards Authority, 2013). In 2015 to 2016, surveys were conducted by the Department of Crop and Soil Sciences of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, to assess insect pest populations and mycotoxin content of maize stored on-farm and in commercial markets. The United States Agency for International Development (USAID), through the U.S. Government's Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss (PHLIL) funded this work conducted in Ghana.

On-farm sites

The survey of on-farm sites was conducted in the Middle Belt crop production region of Ghana, which is the primary grain-producing region of the country. Complete details of this survey are described in Danso et al. (2017). In this study, a total of 51 farm sites were sampled from three separate geographic areas within the Middle Belt of Ghana: Ejura, Sekyedumase, and Amantin. Sampling was conducted on maize on stalks just before harvest, maize piled on the ground as unshelled cobs pending threshing (shelling), or maize shelled and then stored for distribution to grain markets. For the field sampling and sampling from ground piles, maize cobs were collected from different areas within the piles, then dehusked and mixed into 500-g replicate lots, and the kernels stored for subsequent analysis and processing. Sampling for the third category, dried maize, was done by collecting 2-kg samples, then sub-dividing and mixing into 500-g lots as described for the first two categories. For each sample, temperature, moisture content, and relative humidity (r.h.)

were assessed using a John Deere moisture meter (Armstrong et al., 2017). Samples were sieved to collect live insects. Separate subsamples were taken to analyze for aflatoxin and fumonisin, using a standard Romer Labs test kit (romerlabs.com). Complete data analyses are given in Danso et al. (2017) and Armstrong et al. (2017), and will be summarized here in general terms.

Data for each species were summed over the entire year and analyzed first by Chi-Square analysis (SAS Institute) to determine differences between the three sites at each geographic area, and then summed by the months associated with major season storage (September to December) and with minor season storage (January to April) to determine differences between storage season. The predominant species collected were *S. zeamais* and *S. cerealella*, with ground piles as the site where most were found (Table 1) in the respective geographic areas. The other species in order of abundance were *Carpophilus dimidiatus*, *Cathartus quadricollis*, *Cryptolestes ferrugineus*, and *Tribolium castaneum*, with ground piles again being the site where most *C. dimidiatus* and *C. quadricollis* were found (Table 1). *Cryptolestes ferrugineus* and *T. castaneum* were the least prevalent species (Table 1).

Table 1. Total numbers of *S. zeamais* (SZ), *S. cerealella* (SC), *C. dimidiatus* (CD). *C. quadricollis* (CQ), *C. ferrugineus* (CF), and *T. castaneum* (TC) collected from three types of on-farm areas where maize was stored after harvest (Field, Ground Piles, and Post-drying) in Ejura, Sekyedumase, and Amantin during September to April. Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, $P< 0.05$).

Location	Site	SZ	SC	CD	CQ	CF	TC
Ejura	Field	85b	71b	37b	48c	7a	0a
	Ground pile	200a	128a	76a	126a	9a	8a
	Post-Drying	181a	35c	26b	70b	15a	8a
Sekyedumase	Field	69b	48b	52a	40b	2a	0a
	Ground pile	149a	76a	70a	75a	6a	2a
	Post-Drying	141a	25c	63a	41b	10a	4a
Amantin	Field	112c	86b	17b	59a	6a	7a
	Ground pile	236a	125a	46a	76a	9a	13a
	Post-Drying	189b	58c	49a	65a	9a	6a

More *S. zeamais* were collected in the minor season compared to the major season in all three locations, but the only difference for *S. cerealella* occurred in Amantin (Table 2). More *C. dimidiatus* were collected from the major season compared to the minor season, while differences were mixed or not significant for the other four species (Table 2). Temperatures in all locations and sampling sites ranged from about 27.0 to 34.5°C during the period of the experiment. Moisture content was more variable, but in general ranged between 15 and 27% during the major season, and declined from September to December, with the low moisture content levels predominantly in the post-drying samples (as expected) (Danso et al., 2017). MC in the minor season ranged from about 9 to 17%, with less variation between sites, but MC was usually lowest in the post-dried samples. Neither temperature nor moisture content were correlated with the insect populations ($P < 0.05$). Numbers of *S. zeamais* were positively correlated with percentage of IDK and with kernel weight loss ($P < 0.05$). This was the primary species contributing to IDK and weight loss, as it is an internal feeder.

Average aflatoxin levels at all three locations were well above the tolerance level of 15 ppb during the major season, but ranged between 0.6 and 3.6 ppb during the minor season (Table 3). Fumonisin levels were below the tolerance level of 4 ppm.

Market Sites

The survey of market retail sites was also done in the Middle Belt of Ghana, in the geographic regions of Ejura, Techiman, and Amantin. The maize that was sampled was bagged mixed-variety white maize. Samples were taken monthly from September to April by randomly selecting 100-kg polypropylene or jute bags, inserting a 1.2-m grain probe into the bag (Seedboro, Chicago, IL, USA), and withdrawing a sample of approximately 350 g. Three samples were taken from the bag, mixed, and 500 g weighed out for sampling for insects. In selected months, a second 500-g sample was

collected for mycotoxin analysis, as described above. The maize was sampled from the same market location, but not from the same bags each time as this was an active retail market. Maize was also sampled for temperature, moisture content, and r. h. Collection procedures, sample preparation, and methodology for collecting insects, is the same as described above. More detailed descriptions of methodology are found in Danso et al. (2018), along with complete depictions of the results. Data from this study are re-analyzed and summarized here to present important findings from the market survey.

Table 2. Total numbers of *S. zeamais* (SZ), *S. cerealella* (SC), *C. dimidiatus* (CD). *C. quadricollis* (CQ), *C. ferrugineus* (CF), and *T. castaneum* (TC) collected in Ejura, Sekyedumase, and Amantin during the Major and Minor seasons (data for the three sites combined). Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, $P< 0.05$).

Location	Season	SZ	SC	CD	CQ	CF	TC
Ejura	Major	117b	111a	97a	95b	10a	1b
	Minor	349a	123a	42b	148a	21a	15a
Sekyedumase	Major	109b	73a	106a	72a	13a	2a
	Minor	250a	76a	79b	84a	5a	4a
Amantin	Major	121b	54b	94a	85b	7a	2b
	Minor	416a	215a	18b	115a	17a	24a

Table 3. Average aflatoxin values (ppb, means ± SE) during the major and minor seasons in Ejura, Techiman, and Amantin. Data from Danso et al. 2017. All comparisons by season were significant ($P < 0.05$, SAS, Tukey's Honestly Significant Difference Test).

	Major Season	Minor Season
Ejura	39.2 ± 9.1a	3.2 ± 0.1b
Sekyedumase	24.8 ± 0.8a	3.6 ± 3.6b
Amantin	23.4 ± 4.0a	3.6 ± 0.2b

There were six predominant stored-product insect species collected from the market samples: *S. zeamais*, *C. ferrugineus*, *C. quadricollis*, *S. cerealella*, *T. castaneum*, and *C. dimidiatus*. Data for each species were summed over the entire year and analyzed first by Chi-Square analysis (SAS Institute) to determine differences between markets, and then summed by the months associated with major season storage (September to December) and with minor season storage (January to April) to determine differences between storage season. The order of species abundance, in terms of total numbers, is arranged from left to right in Table 4, with *S. zeamais* as the predominant species. Varying levels of these six species were found in all markets, with no consistent differences between markets (Table 4).

Table 4. Total numbers of *S. zeamais* (SZ), *C. ferrugineus* (CF), *C. quadricollis* (CQ), *S. cerealella* (SC), *T. castaneum* (TC), and *C. dimidiatus* (CD) collected from three different maize markets in Ghana during September to April. Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, $P< 0.05$).

Market	SZ	CQ	SC	TC	CF	CD
Ejura	816b	192a	112c	121a	100b	80a
Techiman	960a	139b	180a	125a	67c	37b
Amantin	930a	116b	207a	85b	144a	62a

Data were then summarized to compare total numbers during the major versus the minor season. For 14 out of the 18 comparisons (6 species x 3 markets), there were more insects collected during the major versus the minor season, and only one instance where there were more collected during the minor versus major season (*C. quadricollis* in the Amantin market) (Table 5).

Table 5. Total numbers of *S. zeamais* (SZ), *C. ferrugineus* (CF), *C. quadricollis* (CQ), *S. cerealella* (SC), *T. castaneum* (TC), and *C. dimidiatus* (CD) collected from each market during the major season vs the minor season. Sum totals within columns for each market followed by different lower-case letters are significantly different (Chi Square, $P < 0.05$).

Market	Season	CF	SZ	CQ	SC	TC	CD
Ejura	Major	91a	702a	113a	90a	77a	77a
	Minor	8b	116b	73b	22b	43b	3b
Techiman	Major	60a	786a	82a	80a	58a	31a
	Minor	8b	174b	57b	100a	67a	6b
Amantin	Major	136a	811a	28b	140a	49a	58a
	Minor	7b	119b	88a	67b	36a	5b

Again, temperature combined for all markets during the storage months was about 27 to 32°C, well within favorable limits for insect population development. Moisture content combined for all markets ranged from a high of approximately 15% in September to a low of about 9% in December, then began to increase until April. However, most of the *S. zeamais* collected during the major season storage were collected in November and December (see Danso et al., 2018), the months with the lowest MC. There was no correlation between temperature or MC and insect pest populations ($P < 0.05$). Average aflatoxin levels at all three market sites were far above the tolerance level of 15 ppb during the major season, but less than 4 ppb during the minor season (Table 6). Fumonisin levels were below the tolerance level of 4 ppm.

Table 6. Average aflatoxin values (ppb, means ± SE) during the major and minor seasons in markets in Ejura, Techiman, and Amantin. Data from Danso et al. 2018. All comparisons by season were significant ($P < 0.05$, SAS, Tukey's Honestly Significant Difference Test).

	Major Season	Minor Season
Ejura	66.2 ± 14.6a	3.4 ± 0.4b
Techiman	58.9 ± 14.2a	2.9 ± 0.2b
Amantin	28.0 ± 8.7a	3.1 ± 0.2b

Conclusions

Temperature was generally within 27 to 32°C during these studies, which is within the optimum range for development of the collected species (Howe, 1965; Fields, 1992), and hence was not correlated with the insect populations. The predominant stored-product insect collected in the on-farm and market sites was *S. zeamais*, but it was surprising that no *P. truncatus* were collected given the extensive presence of this species in stored maize, particularly cob-stored maize, in western Africa. Few *R. dominica* were collected as well, and this species is also listed as one of the main storage pests in Africa. More *S. zeamais* were collected during the minor season compared to the major season in the on-farm sites, but the reverse was true for the market sites. During the minor season, the maize is left on stalks for long periods to dry, in contrast to the major season, which is a possible explanation for the greater incidence of S. zeamais during the minor season. The greater infestation in the market sites during the major season versus the minor season may be because the maize in the market sites could have already been infested when the maize was brought to those sites. In addition to the seemingly greater insect populations in maize with high MC, drying of newly-harvested maize is also essential to reduce fungal contamination. Results show improved storage management, which includes integrated pest management strategies for drying and storing maize, may be necessary to limit economic losses and ensure food security.

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Low-Cost Instrument to Measure Equilibrium Moisture Content of Bagged and Bulked Grain

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Introduction

Storage of grain in bags is common in Africa, Asia, and many other less developed countries. Because of this an *in situ* grain bag probing method is well-suited for moisture content (MC) measurement. A low- cost meter was developed under a USAID project to reduce post-harvest loss (PHL)(Fig 1). The meter, referred to as the PHL meter, measures the MC of maize and other grains based on relative humidity (RH) and temperature (T) measurements obtained by a small digital sensor located in the tip of a tubular probe that can be inserted into bags of grain or other grain bulks (Armstrong et al., 2017). Measurements are used in equilibrium moisture content (EMC) equations programmed into the meter to predict MC. A handheld reader connected to the probe provides a user interface.

Keywords. Equilibrium moisture content, Grain storage, Maize, Moisture content, Moisture meter, Post-harvest

The PHL moisture meter was evaluated based on laboratory studies in the U.S. and field studies in Ghana. Meter readings from field studies were compared to two commercial meters, a John Deere Chek-Plus-SW08120 grain moisture tester and a DICKEY-john GAC®2100 Agri meter. The John Deere portable moisture meter is a low cost meter by developed country standards (~US$250, 2016 price); the GAC 2100 benchtop moisture meter is an approved moisture tester by the U.S. Grain Inspection, Packers and Stockyards Administration (GIPSA) and has been a highly regarded and used electronic meter. Laboratory studies indicated that the PHL moisture meter may require up to six minutes to take a measurement due to the time required by the probe tip and sensor to equilibrate to grain conditions. Methods to reduce the measurement time by measuring temporal equilibration rates were developed. These can be programmed into the reader to shorten measurement time for many conditions. The accuracy of the PHL moisture meter was comparable to the GAC 2100 moisture meter for maize below 15% MC_{wb}. Average differences showed a positive offset of 0.45% for the PHL meter relative to the GAC 2100. The PHL meter provided an effective tool to probe bulk grain and bags.

A second generation (2G) PHL meter, Fig 2, has been developed with an emphasis on reducing manufacturing cost, improving the user interface, and increasing battery life. The 2G device also incorporates Bluetooth technology which can potentially reduce the cost further by eliminating the user screen.

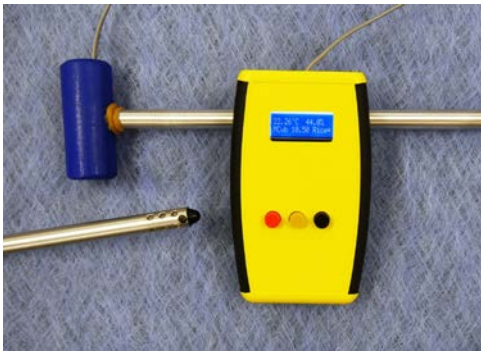

Fig. 1 PHL moisture meter based on measuring equilibrium moisture content.

Figure 2. Second generation PHL moisture meter

To expand the scope of the PHL meter, two crops, sesame and chickpea, were studied and included into the meter software by developing EMC equations specific to these crops. Both of these crops are important crops for Ethiopia as both are major exports providing small farmers and the country much revenue. There is a lack of information on fundamental equilibrium moisture content (EMC) relationships for these products which would help facilitate better monitoring and storage. For this reason EMC adsorption and desorption prediction models based on temperature (T) and relative humidity (RH) were developed for the modified Chung-Pfost and modified Henderson models for kabuli chickpea (KC), black sesame (BS), and white sesame (WS) seeds. Samples for adsorption and desorption tests were conditioned to various moisture content (MC) levels for EMC tests. Samples of approximately 500 g were placed in multiple sealed enclosures equipped with T and RH sensors, placed in an environmental chamber, and exposed to three temperatures (15, 25, and 35°C). For KC samples, the MC_{db}% ranges used for model development were 11.6-19.5% and 8.9-16.9% for adsorption and desorption, respectively; for BS, the range was 5.0-8.7% and 4.3-6.9%, respectively, and for WS, 4.2-8.7% and 3.5-7.6%, respectively. Nonlinear regression was used to determine model coefficients for the modified Henderson and modified Chung-Pfost equations. Prediction statistics for KC adsorption and desorption models yielded a SEE of 0.53 and 0.68% MC_{db}, respectively; for BS, SEE was 0.23 and 0.13%, respectively; and for WS, SEE was 0.28 and 0.25%, respectively. Model coefficients were incorporated into the PHL moisture meter.

EMC equation development for additional crops is needed which may include pulses, other grains and processed food products that span harvesting, drying, storage, conditioning, and processing operations.

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Stored Grain Protection: cases studies in Portugal

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Abstract

Considering the edibility of insects' species associated with storage ecosystem, chemical control methods can be easily replaced by environmental and economically sustainable alternatives.

Use of biogenerated atmospheres is an inexpensive method that tolerates insect presence. In Portugal, during one year, hermetic bags were used to store paddy under 65-75-85% relative humidity (RH) and 14-17-24ºC temperatures. Brown rice infested with *Sitophilus zeamais* adults was placed inside the hermetic bags.

Biogenerated atmosphere was naturally produced inside the hermetic bag, at 85% RH, with low O_2 and high CO_2 contents, showing that *S. zeamais* can survive but has no progeny at 14º-17ºC, or attained 100% mortality before producing progeny at 24ºC. The most abundant fungi isolated were *Alternaria alternata* and *Epicoccum nigrum*. The results showed the importance of the RH on changes in atmospheric gas content of paddy, due to biological agents' activity.

Analysing the edibility of insects species associated with stored grain, preliminary studies were carried out to evaluate the nutritional value of immatures stages of *Tribolium castaneum*. Larvae of *T. castaneum* had a content of 21.4% protein, 9.1% lipids, 8.8% fiber, and a relevant content of eight essential amino acids and also manganese and copper. The edibility of insects must be consider given their high nutritional value, low emissions of Green House Gases (GHGs), low requirements for land, and by reducing and mitigating the need for chemical control.

Keywords: insect edibility, paddy, hermetic storage, biogenerated atmosphere, *Tribolium castaneum*, *Sitophilus zeamais*

1. Introduction

Rice in Portugal and in Europe in general is a seasonal crop. Among pest species of stored rice, *Tribolium castaneum* (Herbst, 1797) is common but *Sitophilus zeamais* Motschulsky, 1855 is considered the key pest. The most common practice to control insects of stored grain is the use of fumigants to prevent and suppress insect development. The development of resistance of these two insect species to fumigants has decades of history (Champ and Dyte, 1977), and consumer concern over the use of pesticides in food oblige the research for alternative methods of insect control in order to get food product free of pesticide residues. Also, insect protein can be a good alternative to livestock production because it is more efficient feed/food conversion, lower greenhouse gas and ammonia production, less land area needed, and has potential to be grown on organic byproducts (Huis et al., 2013). Concerning producing benzoquinone-containing defensive secretions by *T. castaneum*, IARC (International Agency for Research on Cancer) officially states that no epidemiological data are available on the carcinogenicity of 1,4-benzoquinone, which consequently is not classifiable as to its carcinogenicity to humans (IARC and WHO, 1999).

On the other hand, fungal mycotoxin producers are the major cause of loss during long-term storage periods without efficient control of temperature and, above all, moisture content of stored grain (Christensen and Kaufmann, 1969; Wicklow, 1995; Fleurat-Lessard, 2017). One of the most important phenomena caused by fungi is the "hot spot" previously associated with insect infestations but currently identified as a consequence of fungal development in situations of poor storage conditions, such as high moisture content. The microclimate generated, i.e., temperature and moisture, attracts the insect populations (Fleurat-Lessard, 2017).

Based on these assumptions, preliminary trials were developed to analyze the edibility of immatures stages of *T. castaneum*. Also, trials were carried out using hermetic bags to store paddy rice, as a green and inexpensive alternative method to control insects and fungal development, under 65-75-85% RH and 14º-17º-24ºC. Moreover, rheological tests were performed on a MARS III controlled-stress rheometer to analyse the viscoelastic functions of the respective rice pastes.

The main objective is considering hermetic storage as a sustainable technology to reduce insect presence and fungal development, mitigating pesticide effect in food and feed.

2. Material and methods

2.1. Modified atmosphere

2.1.1. Sample preparation

Experiments were conducted in a warehouse located in Alcácer do Sal, Portugal. Three trials were carried out: T_1 the first trial, four months, temperature average 14ºC (December to April); T_2 the second trial, seven months, temperature average 17ºC (December to July); T_3 the third trial, four months, temperature average 24ºC (July to November).

For experiments, GrainPro® SuperGrainbag® Farm™ were used to store two rice varieties: Ronaldo, a japonica variety, and Sírio, an indica variety. Paddy rice, was stored in jute bags, as the control.

In all experiments, the two varieties were stored as paddy and submitted to three different relative humidities: 67, 75 and 85% RH, at three different average temperature (14, 17 and 24ºC). The RH and temperature were monitored by Hobo ® Data loggers, with probes placed inside the bags.

Both jute bags and SuperGrainbag® have a capacity of 50 kg, and for each treatment and variety three replications were carried out, for a total of 48 samples per trial.

At the end of all experiments, CheckpointII Portable O_2 and CO_2 Gas Analyzer was used to assess gas contents, at the bottom and top of each bag, totalizing six measures per treatment. The gas content is expressed in % by volume in air. After, the bags were opened and paddy samples were collected, samples were taken to be dehusked and milled to analyze water activity (a_w), with three replications per treatment, and rheology tests were performed. HygroPalm HP23 Rotronic was used to estimate a_w.

2.1.2. Bioassays

To evaluate insect response to each treatment, *Sitophilus zeamais,* the maize weevil, was chosen because it is the main pest of rice in Portugal (Carvalho et al. 2012). *Sitophilus zeamais* was originally collected from Portuguese rice mills and reared in climatic chambers, at 25±2ºC and 70% RH, at laboratory of Instituto Superior de Agronomia, University of Lisbon.

For experiments, 20 g of brown rice were infested with ~20 one-week-old *S. zeamais* adults and placed inside paper bags. One paper bag was set up inside of each paddy bag, totalling three replications per treatment.

2.1.3. Mycoflora analysis

From samples collected at the end of T_1 and T_2 trials, three samples were collected in sterilized containers and taken into the laboratory. In the laboratory, the rice samples were subdivided into samples with 100 grains. The surfaces of these grains were disinfected with 1% sodium hypochlorite for five minutes, as described by Pitt and Hocking (2009) and Magro et al. (2008). Ten disinfected grains were placed on Petri dishes with 20 mL of Potato Dextrose Agar (PDA) medium with chloramphenicol (1%). There were ten replicates for each sample.

2.1.4. Rheology measurements

Rheology tests were carried out for T1 trial and performed on a MARS III controlled-stress rheometer (Haake) coupled with a temperature controlling Peltier unit, using 35-mm-diameter serrated parallel plates and 0.5-mm gap. Aqueous flour suspensions (10% w/w) were held 5 min at 20ºC, between the plates, before testing. Stress sweeps were performed to ensure that all measurements were within the viscoelastic region. Then, the rheological study using SAOS (Small-Amplitude Oscillatory Shear) was performed according to previously optimized conditions (Torres et al., 2014).

2.2. Tribolium castaneum analysis

Moisture content was determined by placing approximately 2 g of each sample in a drying oven at 60ºC for at least 48 hours until constant weight. Protein was determined using the Dumas method as described by Saint-Denis & Goupy (2004), using a LECO FP-528 (LECO, St. Joseph, USA) calibrated with EDTA. The conversion factor of 6.25 was used to calculate total protein values. Total fat was determined by extraction with petroleum ether of around 0.5 g of sample using a Soxtec HT apparatus, and total fibre was determined according to the Weende method by extraction of non-fibre components from 0.5 g sample with sulphuric acid (0.2 M) and potassium hydroxide (0.2 M), followed by acetone washing in a FibreTec apparatus. Amino acids determination was performed by HPLC (Agilent 1100 HPLC, Agilent, Palo Alto, USA), using a Phenomenex Gemini ODS C18 110 Å column (4.6 × 150 mm, 5 µm, Phenomenex Inc., Torrence, USA) and a fluorescence detector according to the method described in Henderson et al. (2000). Mineral elements were determined by flame atomic absorption spectrophotometry (Unicam Solaar M, Thermo Electron GmbH, Dreieich, Germany) following acid digestion of 0.5 g of dried sample in 7.5 mL nitric acid and 2.5 mL hydrochloric acid using an SCP Science heat block (1.5 hours at 110 ºC).

2.3. Data analysis

All the computations and graphs were performed with software R (R Core Team, 2017). Function lm was used to fit and test for significance of the linear models.

3. Results and Discussion

3.1. Hermetic storage and *Sitophilus zeamais*

The average temperatures were similar for the same experiment and type of rice, but the individual days showed different values that are summarized in Table 1: T_1 had a temperature average of 14ºC, and was never higher than 20ºC; T_2 had an average temperature of 17ºC with 43 days above 20ºC and 13 days above 25ºC; and T_3 had a temperature average of 24ºC with 103 days above 20ºC of which 60 days were over 25ºC and 34 days above 27ºC.

Table 1- Number of days of each experiment, mean temperature, and number of "hot days".

Trial	**[T_1]**	**[T_2]**	**[T_3]**
*** days**	**139**	**215**	**121**

Temperature average (ºC)	14±1	17±4	24±3
* days with Temp>20ºC	0	43	103
* days with Temp>25ºC	0	13	60
* days with Temp>27ºC	0	0	34

**days*- the number of days with a determined mean temperature.

Tables 2 and 3 report the results of the population growth of *S. zeamais* in each type of rice, Indica and Japonica, under different conditions of temperature, RH, atmosphereic composition, and water activity. When we consider the progeny, the first 20 weevils adults used, per replication, were eliminated from results, to better understand if there were offspring produced during trials. Under hermetic conditions at low O_2 and high CO_2 contents in trials in which the RH was 85%, at all temperatures there were no progeny although the initial insects were still alive. In trials at RH of 75% under hermetic conditions at medium oxygen and CO_2 contents, progeny were found, and the number of F1 individuals was dependent on temperature. In trials at RH of 67% under hermetic conditions, there was no change of the atmospheric content, and at 14 and 17ºC there were fewer adults alive than the trials at 75% RH. The population showed a significant increase when the temperature average was 24ºC.

Table 2 – Indica rice. Average number of *Sitophilus zeamais* adults: alive, dead, and progeny (alive + dead-20) for each pair of temperature and relative humidity (RH) conditions. Values correspond to averages across the replications, with small bags containing rice initially contaminated with 20 insects. The amounts of oxygen (O_2, %), carbon dioxide (CO_2, %) and water activity (a_w, %) at the end of each experiment are also shown.

Trial	Temp (ºC)	RH (%)	CO_2	O_2	a_w	*S. zeamais* alive	*S. zeamais* dead	Progeny
[T_1]	14	67	1.2	20.4	0.5	4.3	44.5	28.8
	14	75	3.9	18.3	0.5	13.7	37.3	31.0
	14	85	20.6	4.4	0.5	10.3	7.7	0.0*
[T_2]	17	67	1.5	19.4	0.4	8.5	33.8	22.3
	17	75	9.2	10.5	0.4	24.5	46.0	50.5
	17	85	23	0.8	0.5	0.7	20.3	1.0
[T_3]	24	67	1.4	19.9	0.6	84.0	93.3	157.3
	24	75	6.5	14.4	**0.7**	86.3	64.0	130.3
	24	85	21.8	2.6	**0.7**	0.0	20.0	0.0

*at end less than 20 insects were found

Table 3 – Japonica rice. Average number of *Sitophilus zeamais* adults: alive, dead, and progeny (alive + dead-20) for each pair of temperature and relative humidity (RH) conditions. Values correspond to averages across the replications, with small bags containing rice initially contaminated with 20 insects. The amounts of oxygen (O_2, %), carbon dioxide (CO_2, %) and water activity (a_w, %) at the end of each experiment are also shown.

Trial	Temp (ºC)	RH (%)	CO_2	O_2	a_w	*S. zeamais* alive	*S. zeamais* death	Progeny
[T_1]	14	67	1.3	20.3	0.6	1.3	69.7	51.0
	14	75	3.5	18.7	0.5	12.5	41.0	33.5
	14	85	17.3	7.4	0.6	27.7	9.3	17.0
[T_2]		67	1.6	19.3	0.5	3.3	32.3	15.7
		75	4.0	16.9	0.5	36.5	20.0	36.5
	17	85	26.7	0.2	0.4	0.0	25.3	5.3
[T_3]	24	67	1.4	19.8	0.6	108.0	71.0	159.0
	24	75	3.8	17.8	0.6	16.0	43.0	39.0
	24	85	22.0	3.8	0.7	0.0	20.0	0.0

3.2. Hermetic storage and mycoflora analysis

Thirteen species of fungi were found on paddy hermetically stored during T_1 trial with average temperatures of 14ºC, and 20 species were found after seven months of storage with average temperatures of 17ºC [T_2]. Fungi identified in more than 20 grains were *Alternaria alternata*, *Aspergillus flavus, A. fumigatus, A. niger, Epicoccum nigrum, Nigrospora.* sp, and *Rhizopus* sp. After four months of storage at 14ºC (T_1), the majority of the fungi identified were associated with field species.

After seven months of storage and higher average temperature (17 ºC) (T_2), *Aspergillus spp*. became more abundant and dominant relative to field fungi species. From all samples collected, the a_w was lower than 0.6 and was considered secure for the non-development of mycotoxins, as the husk is most certainly a barrier to protect grain from water changes (Fig.1).

3.3. Hermetic storage and rheological measurements

Rheological tests were carried out at end of trial T_1. Figure 2 shows the gelling point (when G' overcomes G'') of 10% (w/w) pastes of japonica and indica rice flour heated from 20 to 90ºC, from paddy flours kept under the three different RHs. For the japonica rice paste, there is a slight decrease in the gelling point temperature with the increase of RH: hermetic storage at 67% RH and control started gelling at about 87ºC; hermetic storage under 75% and 85% RH, started gelling at 85 and 82ºC, respectively. For the indica rice pastes, the results were reversed: the control and hermetic storage at 85% RH started gelling at 85ºC, and both hermetic conditions of 67% and 75% RH paddy pastes started gelling at 82ºC.

Figure 3 shows the mechanical spectra of the paddy pastes by variety and RH conditions. The paste obtained from rice stored at lower RH values, i.e., 67%, is slightly more structured than rice stored at higher humidity and control, i.e., the values of the viscoelastic parameters G' (storage or elastic modulus) and G'' (loss or viscous modulus) are slightly higher for the pastes from rice stored under lower RH conditions, showing a better structured paste (Nunes et al., 2006).

3.4. T. castaneum chemical analysis

Singh and Sinha (1977) studied the changes in protein levels in the developmental stages of *Sitophilus oryzae* (L.) and *S. granarius* (L.) reared on whole wheat at 30°C and 70% RH. In S. *oryzae* life cycle, the average values of this nutrient (percentage of dry body weight) ranged between 50.0 (L4) and 78.3% (adult). In *S. granarius* life cycle, the comparable protein values ranged between 47.0% (prepupa) and 75.7% (adult). The protein contents in S. *oryzae* increased up to the prepupal stage, declined slightly in pupae, and increased again in adults; whereas in *S. granarius* it constantly increased through the life cycle stages up to adult emergence.

The larvae of *T. castaneum* also is particularly rich in several essential elements confirming that the enrichment of different flours with this insect will improve its global nutritional value.

The values for the nutritional analysis presented in this work (Table 4) are typical of other insects from the same order, with a relatively high protein content, although the total fat is lower than the usual values, taking into account that these values are highly dependent on species and on the feed type (Ghosh et al., 2017).

Regarding the amino acid analysis, the larvae of *T. castaneum* have a higher amino acid content compared to the regular flour confirming that the presence of this insect will enrich it in several amino acids. Leucine was the most abundant essential amino acid followed by valine and threonine. Among these three essential amino acids, threonine is strictly indispensable since it is not transaminated and its deamination is irreversible (Belluco et al., 2013).

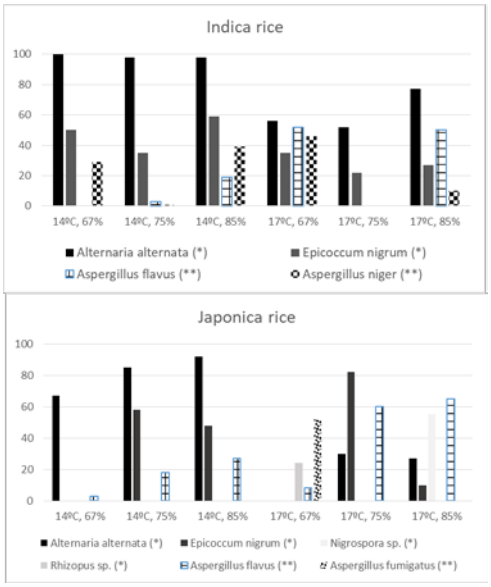

Figure 1 - Fungi incidence in 100 grains tested in indica and japonica rice stored for four months at 14ºC temperature average and stored for seven months at 17ºC temperature average. (*) field fungi (**); storage fungi.

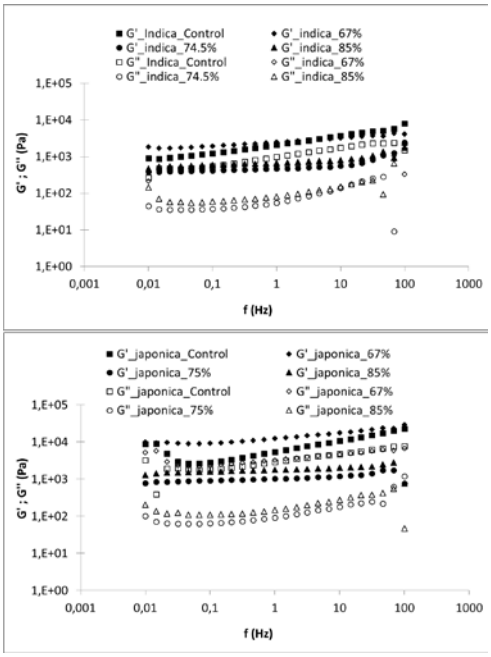

Figure 2- Indica and japonica rice: Mechanical spectra at 20ºC (G'- elastic modulus; G''- viscous modulus).

Table 4 – Chemical and nutritional composition of *Tribolium castaneum* larvae.

Water content (%)	42.23 ± 2.11	**Total fat (%)**	9.07 ± 4.09
Protein (%)	21.37 ± 0.45	**Fibre (%)**	8.76 ± 1.07
Amino acids (%)		**Mineral elements (mg/kg)**	
Histidine	0.64 ± 0.06	Fe	71.3 ± 8.1
Threonine	0.97 ± 0.06	Cu	6.1 ± 1.2
Valine	1.00 ± 0.07	Zn	48.2 ± 1.7
Lysine	0.84 ± 0.10	Mn	6.7 ± 0.5
Methionine	< LQ	Mg	41.5 ± 1.8
Leucine	1.23 ± 0.07	Na	199.5 ± 25.2
Isoleucine	0.71 ± 0.06	K	219.1 ± 8.6
Tryptophan	< LQ		
Phenylalanine	0.90 ± 0.07		

LQ: limit of quantification, 9 pmol/µL

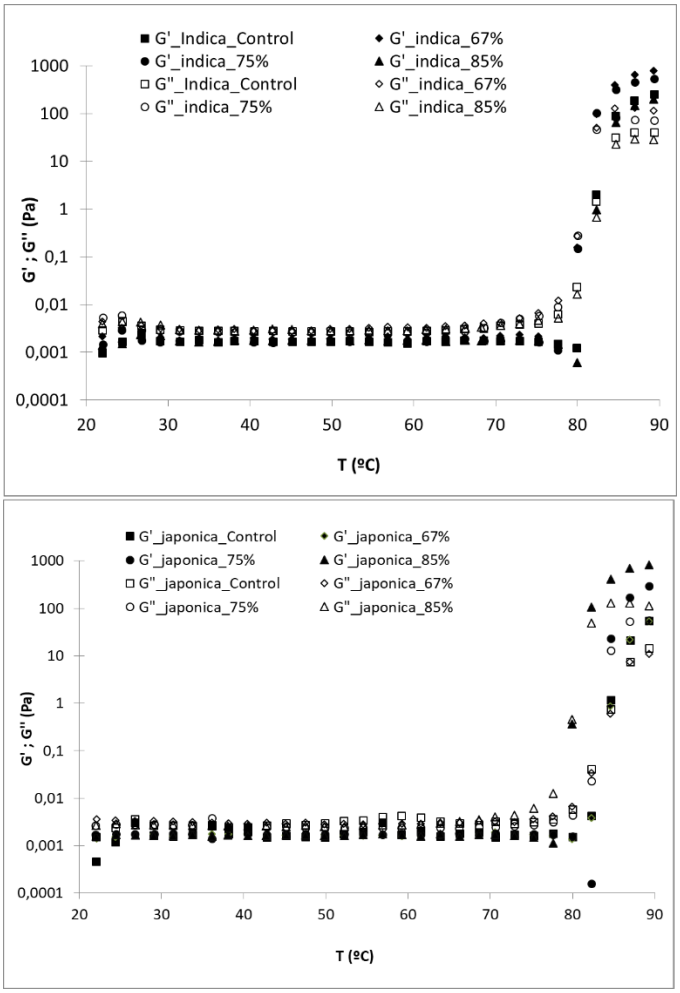

Figure 3 - Indica and japonica: **Starch gelatinization**. Heating from 20ºC to 90ºC at 2ºC/min (G′ elastic modulus; G′′ viscous modulus).

López-Vergé et al. (2013) evaluated the protein content of *Tenebrio molitor, Ephestia kuehniella*, and *Tribolium confusum* on a dry matter basis, and the results obtained in crude protein ranged from 42.47 to 58.77% and in ether extract ranged from 24.99 to 34.13%, supporting the idea that they can be incorporated into the feed diet. The same authors carried out a feeding trial in order to compare the effect of adding larvae of insect species *Sitophilus zeamais* to the diet on the performance parameters. Animals fed the insect-infested diet had higher final body weight (*P*=0.015) and higher average daily feed intake (ADFI, P=0.015) compared to animals fed the untreated (control) diet (López-Vergé et al., 2013).

Reuters announced in November 23, 2017, that a Finnish baker launched bread made from crushed crickets explaining that it «*offers consumers a good protein source and also gives them an easy way to familiarize themselves with insect-based food*».

4. Conclusion

Concerning the studies of hermetically stored paddy under different environmental conditions, the results showed that RH is the key factor for the modified atmosphere, attained only on trials under

75 and 85% RH at any average temperature, mainly due to respiration of paddy and fungi. As the time of storage increased, more fungal species developed, mainly associated with stored products. However, considering the determined a_w value was always below 0.6, the environment was considered safe from mycotoxin development. The modified atmosphere produced inside the hermetic bag with low O_2 and high CO_2 contents at 85% RH and at average temperatures of 14 and 17ºC demonstrated that *S. zeamais* can survive but produce no progeny. At the same conditions but higher average temperature of 24 ºC, ~100% mortality of *S. zeamais* occurred but progeny still were produced.

The increase in respiration rate at higher RH values, reduced the viscoelastic functions and changed the starch gelatinization point of indica and japonica rice.

The enrichment of different flours with larvae of *T. castaneum* can improve its global nutritional value.

It is our intention to break the misconception that insects are always harmful and to contribute positively to the concept of edible insects in the sector of stored products. It can be used for the incorporation of their protein as food/feed ingredients, and also to allow some tolerance to their presence during the storage period in order to suppress the use of chemical control methods, contributing to the sustainability of the environment and human health and animal welfare.

Following this concept, hermetic storage can be one of the sustainable and green methodologies to be used on grain storage. Storing paddy hermetically at low RH, did not change atmospheric content but maintained the viscoelastic functions of the rice pastes, low fungal incidence, and reduced insect population growth.

Acknowledgments

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Survey of dermestids of the genus *Trogoderma* in grain storages in Spain

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Several *Trogoderma* species of the family Dermestidae are important pests of stored products. Among them, *Trogoderma granarium* Everts, is one of the most harmful pests of cereal grains for all countries that are major exporters of agricultural commodities and for their trading partners (Athanassiou et al., 2016). Therefore, in most countries a very strict quarantine legislation exists to prevent the introduction of this pest (Myers and Hagstrum, 2012).

Trogoderma granarium is considered an endemic species in the southern Mediterranean region, and it has been intercepted or eradicated in many European countries. Nevertheless, global warming and the increase in international trade of raw materials are favoring its expansion. The establishment of *T. granarium* can likely occur in countries with more than 4 months per year with an average temperature higher than 20ºC (EPPO, 2011). However, temperatures in storage facilities can be higher than in open field, so there is also a risk of establishment in colder climatic areas.

According to the EPPO, *T. granarium* is present in Spain with a restricted distribution. But, while it has been detected in the country, there is no evidence of its establishment. It was found in 1952, but, after that record, there have been no new records of its presence (Banks 1977, Rebolledo and Arroyo 1993). Therefore, it is important to know whether *T. granarium* is present or not in Spain to take the necessary measures for its eradication or management. In the present study, a survey of the species of the genus *Trogoderma* has been conducted to determine the species present in grain storage facilities in Spain and their phytosanitary importance.

In 2016 and 2017, we sampled with traps baited with the pheromone of *Trogoderma* spp. in fifteen warehouses and grain silos along the Spanish Mediterranean coast. Monthly samples were collected in each sampling location using five PC floor traps placed in the storage facilities and three probe traps inserted in the grain piles. Taxonomic keys were used for the identification of the specimens found, as well as *T. granarium*-specific molecular markers by conventional PCR analysis.

A total of 3,276 *Trogoderma* specimens were captured in almost all locations sampled. However, no *T. granarium* were found. The majority of them were *T. inclusum* Leconte, and some were *T. variabile*

(Ballion), which can be distinguished by the male genitalia (Green, 1979) (Fig. 1). Captures were particularly abundant from June to September. These species are considered secondary pests affecting stored grain, processed dry foods, and animal feeds. Some other pest species were captured with both type of traps, including the Coleopteran *Anthrenus* sp., *Sitophilus* sp., *Rhyzopertha dominica* (F.), *Oryzaephilus* sp., and *Tribolium*. sp. According to our results, the presence of *T. granarium* in the sampled areas is not confirmed. Therefore, this species does not seem to be established in Spain.

Fig 1. Male genitalia of *T. inclusum* (left) *T. variabile* (center) and *T. granarium* (right):

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Performance Assessment off a Commercial Scale Solar Biomass Hybrid Dryer for Quality Seed Maize Production

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Abstract

Though several maize varieties have been developed and introduced over the years in Ghana, farmers still face challenges of access to quality seed maize. Among the major constraints is lack of proper drying systems to quarantee quality of seed produced. Peculiar to most parts of Africa, drying of maize in the open, on bare ground along shoulders of roads is still a common practice in Ghana. In this study, a 5-tonne capacity solar biomass hybrid dryer was developed for drying maize for seed and food/feed in Ghana. Effect of drying air temperature in the dryer on the physiological quality and germination of maize kernels was investigated. Maize grains were dried in the open sun simulating farmers practice and using the dryer at 4 varying levels (L1, L2, L3 and L4) with corresponding heights (0.6m, 1.2m, 1.8m and 2.4m) respectively. Harvested maize at 22.8% moisture content

was dried at the varying levels until reaching overall mean moisture content of 12.8 ± 0.2% (wb). Results showed that, drying air temperatures in the dryer increased in accordance with height with lowest mean temperature of 44.4 ± 4.6˚C recorded at L1 and mean maximum of 52.8 ± 5.4 ˚C at L4. The increase in drying temperature at L4 increased kernel stress crack index by an average of 14% and reduced germination by 33%. However, drying temperatures at L1-L3 and in the open sun had no significant effect ($p > 0.05$) on the germination potential of maize grains. This satisfies the dryer's potential to be used for drying maize grains for high quality seed production on commercial scale.

Keywords: Solar biomass hybrid dryer; drying; maize grain; germination.

1. Introduction

Maize (*Zea mays*) is an important cereal food crop extensively cultivated worldwide for food and as livestock fodder. In Ghana and sub-Sahara Africa, maize is the most important cereal crop produced and is also the most widely consumed staple food. The production of maize in Ghana is dominated predominantly by small-holder resource poor farmers mainly under traditional production practices and rain-fed conditions resulting in yields well below attainable levels (Amanor-Boadu, 2012). Maize yields in Ghana average approximately 1.9 metric tonnes per hectare, however, achievable yields as high as 6 metric tonnes per hectare are possible, if farmers use improved seeds, fertilizer, mechanization and irrigation (MoFA, 2013). However, availability of quality improved seeds is one of the main challenges faced by farmers in Ghana resulting in their over reliance on their own seed stock for production. After harvest, it is common for maize grains to have moisture contents considered inadequate for safe storage for seed. Under such situation, there is clearly a need for reduction of this characteristic to preserve the physiological quality of seeds for at least eight months, impeding possible chemical and physical changes that may come about during storage up to sale of the seeds (Carvalho et al., 2016). Drying maize after harvest from high moisture content (20-30%) to low safe storage moisture content (12-14%) is therefore necessary to ensure storability and preservation of maize grains as seed lots in warm and humid countries like Ghana. Post-harvest activities such as drying and storage are among the key areas along the maize value chain that is of critical importance to small-holder farmers/traders in Africa (Akowuah et al., 2015). However, traditional drying methods where farmers rely on leaving their crops to dry in the field or in the open sun next to farmers' homes or along shoulders of roads either on bare ground or on tarpaulins are unhygenienic and can be detrimental to the quality of the dried seed grain. During unfavorable weather condtions, drying can take up to 10 to 14 days before a safe storage moisture content of 12-14% is reached. Inadequate drying especially among peasant farmers in rural communities poses a serious threat to food safety and security in Ghana, since it creates favourable conditions for fungal growth and insect damage during storage (Folaranmi, 2008) leading to substantial losses grains for seed or food. A significant percentage of the current post-harvest losses and aflatoxin contamination can be attributed to improper and/or inefficient drying of foodstuffs such as maize and groundnuts (Togrul and Pehlivan, 2004). However, the introduction of heated air mechanical dryers is not desirable by most small-holder farmers in Ghana due to high drying charges. Also, drying air temperatures as high as 70 -100 ˚C may be reached with these dryers. These temperatures are considered excessive by most farmers for seed drying. The most severe constraints are on beans (35 ˚C), rice (45 ˚C), and all grains if they are to be used for seed (45 ˚C) (Weiss and Buchinger, 2015). The viability of grain is therefore directly linked to the temperature attained during drying. Seed embryos are killed by temperatures and therefore for seed grains, low temperature drying schemes must be used. Seed grain may be dried in any type of dryer provided it is operated at a low temperature and preferably with high flow rates than generally used (Weiss and Buchinger, 2015). Hassan (2010), reported maximum average germination percentage and viability of dried paddy seed of 86% and 98% respectively after drying paddy seeds at 44 ˚C in a hybrid solar drier. Tonui (2014), attested to the effect of rapid drying under high temperatures in mechanical drying which often results in stress cracks, reduction of the milling quality of grains, discolouration and reduced germination potential. Fast drying due to high temperature are also likely to induce seed cracking including internal fissures due to trapped moisture. Conventional solar dryer's may be a solution to

these challenges but due to its high weather dependency, its usage is limited during rainy periods, cloudy weather conditions and at night. Due to this, the commercialization of solar dryers has generally not been successful leading to limited or non adoption of such systems by farmers in Ghana (Sekyere et. al., 2016). However, Kaaya and Kyamukangire, (2010) reported that, maize grain dried using biomass-heated natural convection dryer did not significantly reduce the kernel viability.

In this study, the performance of a 5-tonne capacity solar biomass hybrid dryer (SBHD) that integrates both solar and biomass energy for seed maize drying on commercial quantities is investigated. Specifically, effect of drying temperature on germination rate and stress crack on grains were determined.

2. Materials and Methods

The experiment was conducted in January, 2017 using a 5-T capacity SBH dryer purposely constructed for a commercial seed maize distributor at Wenchi in the Brong Ahafo Region of Ghana.

2.1 Dryer Description

Fig. 1 shows the chematic and constructed views of the 5-tonne capacity SBHD at the site. The SBHD is based on a greenhouse structure design with an overall dimension of 10.7 × 6.5 × 3.3 m. It is partitioned inside into three drying sections i.e., the Right Section (RS), Left Section (LS) and a Middle Section (MS). Each of these stations have four levels of drying shelves/racks. The drying shelves are arranged from top to bottom in order of Level 4 (L4), L3, L2 and L1 respectively. The dryer is coupled with a biomass burner enclosed with a crossflow type heat exchanger to raise the temperature of ambient air that is blown into the SBHD with a 0.374-kW axial-flow fan that draws in drying air through the heat exchangers and passes it through the drying beds. The SBHD was test-run during the minor maize production season under load conditions during the month of January 2017 which is one of the driest month in the year.

Fig. 1*: Solar Biomass Hybrid Dryer; schematic view (left) and constructed view (right)*

2.2 Experimental Procedure and Setup

To assess the performance of the SBHD, white shelled maize at 22.8 % moisture content wet basis was obtained from a local farmer and dried in the dryer and in the open sun. Drying occurred using experimental cages (0.3m × 0 .3m × 0.05m). Each cage was filled with maize seed samples weighing 1kg and were replicated three times at each level within the dryer. A control set-up of equal maize seeds (1kg) in triplicate was also dried in the open sun as practiced by most rural farmers in the area. The experimental trays containing the maize samples were taken out every hour and weighed to monitor the moisture reduction of the maize samples. The moisture content at the i^{th} time during the drying period was calculated using Euqtaion 1 until the required final moisture content was attained.

Moisture content (%); $MC_i = (1 - W_o \frac{(1-Mo)}{Wi})$ …….. Equation 1

MC_i = moisture content at i^{th} time (%)

M_o = initial moisture content in decimal

W_o = initial weight of samples in grammes (g)

W_i = weight of samples at ith time in grammes (g)

The drying process started at 09:50 am to 16:50 pm, over a period of seven (7) hours. During this period, Tinytag TGP-4017 data loggers (Gemini Data Loggers, Chichester, U.K.) were installed near the experimental cages to monitor and register variations in heat and humidity in the drying environment. Five data loggers were positioned, as shown in Fig. 2, at each of the four levels and the average used to represent the drying conditions at each level. Drying conditions in the dryer were compared with the ambient conditions.

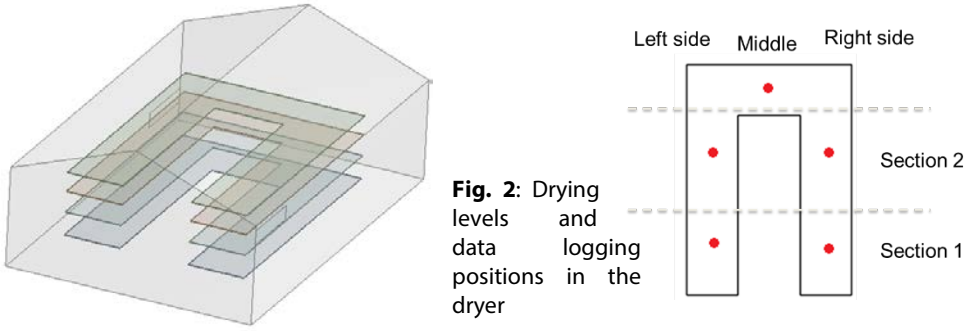

Fig. 2: Drying levels and data logging positions in the dryer

2.3 Germination Test

The germination test was carried out in accordance with the criteria established in the Rules for testing seed (ISTA1985). Four 100-seed sub-samples for each drying level and the open sun were randomly selected and planted in germination trays fill with soil fetched from a river bank. The setup was kept under room temperature conditions. Emergence counts started from the fifth day after planting and continued on the sixth and seventh days. The percentage of normal seedlings was calculated eight days after the test was set up. The percentage germinated seeds were calculated using Equation 1 as stated by Azadi and Younesi (2013).

$$\% \text{ Germination} = \frac{\text{number of seeds germinated}}{\text{number of seeds set for germination}} \times 100\% \ldots\ldots\ldots \text{ Equation 1}$$

2.4 Stress Crack Test

Maize grains were selected by random sampling from each station per layer of dried grains. For each sample, 100 seeds free from insect attack were counted and analysed for stress cracks. The samples were placed on a light box and checked for single, double, multiple or no cracks. The stress crack index (SCI) for each of the analysed sample was determined using Equation 2 as proposed by Kirleis and Stroshine (1990).

$$\text{SCI} = (1 \times \text{single crack}) + (3 \times \text{double crack}) + (5 \times \text{multiple crack}) \ldots\ldots\ldots \text{Equation 2}$$

2.4 Data Analysis

The experiment was conducted in a complete randomized design and data obtained were subjected to analysis of variance (ANOVA) using GenStat statistical software version 12 at a significance level of 5% ($p \leq 0.05$). Results were presented in tables and graphs using Microsoft Excel using the mean values obtained.

3. Results

3.1 Temperature variations during drying

The temperature profile recorded during the drying of the maize grains for seed in the SBHD is presented in Fig. 3. Drying air temperature at level 4, increased during the first four hours, from 41.1 ˚C to a maximum of 58.9 ˚C by noon and reduced to 43.2 ˚C at the end of the drying process. Over the drying period of 7 hours, a mean temperature of 52.8 ± 5.4 ˚C was recorded at L4. Similar variations in temperature trend were oberserved for L3, L2 and L1 in the dryer with mean temperatures of 49.8 ± 5.6˚C, 45.4 ± 5˚C and 44.4 ± 4.6˚C respectively. Comperably, the overall mean temperature inside the dryer (L1-L4) of 48.1 ˚C was higher than the ambient temperature by 12 ˚C. This could be attributed to the solar insolation during the time of the experiment (Tibebu et.al, 2016). Also, the hot air from the biomass furnace rises due to the force convection from the blower with the heat accumulated at the upmost part of the dryer leading to the recorded higher temperatures at L4.

3.2 Changes in maize grain moisture content during drying

As the drying temperatures increased, the moisture content of the maize grains reduced. Maize grains at 22.8% moistute content was dried to an overall final average moisture content of 12.8± 0.2% (wb) within 7hrs. However, samples at L4 and L3 reached average moisture content of 13% within 5 and 6hrs of drying respectively while samples at L1 and L2 reached final moisture content of 12.7% by the 7th hour. Comparably, samples dried in the sun reduced from 22.8% to 12.9% in 7hrs. The result showed that overall drying rates of 1.3%/hr were acheievd in the dryer compared to 1.2%/hr for samples dried in the open sun. The high moisture reduction rate achieved in the dryer could be attributed to the additional heat from the biomass furnace attached to the dryer which facilitated the faster drying of grains to the required moisture content. Fig. 4 shows the variation of moisture content of maize samples dried at different levels in the SBHD as compared to the samples dried in the open sun.

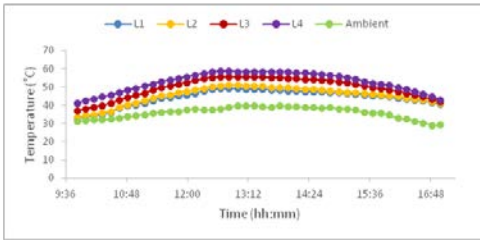

Fig. 3: Temperature trend at different levels in the SBHD vs ambient

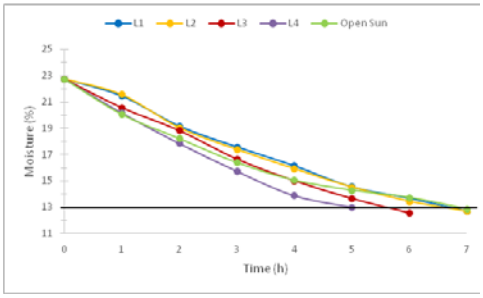

Fig. 4: Moisture content across levels in the SBHD vs open sun

3.3 Effect of drying temperature in SBHD on maize kernel viability

Drying maize using the SBHD did not significantly reduce the viability of the kernels. However, there was a 9% reduction in overall germination potential of grains dried in the SHBD compared to grains dried in the open sun. From the temperature effect, it was observed that, due to the increase in temperature of drying air at the upper level (L4) of the SBHD, the percentage of normal seedlings decreased compared to the lower levels (Table 1). Germination rate of grains dried at various levels with mean temperaures of 52.8 ± 5.4 ˚C, 49.8 ± 5.6˚C, 45.4 ± 5˚C and 44.4 ± 4.6˚C were 44, 61, 64, and 78% respectively (Table 1).

Tab. 1: Percentage germination of maize dried using SBHD*

Layer	Mean temperature (°C)	Germination (%)
Level 4	52.8 ± 5.4	44 c
Level 3	49.8 ± 5.6	61 b
Level 2	45.4 ± 50	64 b
Level 1	44.4 ± 4.6	77 a
Open Sun	35.99 ± 3.2	71 ab
LSD (p≤0.05)		11.95

*Within a column, means followed by the same letter are not significantly different (P>0.05)

3.4 Stress Crack Analysis

Percent stress-cracked kernels in different categories for maize grains dried at the various levels in the SBHD are presented in Table 2. It was observed that, there was much variation among the different stress-crack categories for grains dried at the different levels in the dryer. In all categories, grains dried at level 4 (L4), had higher stress crack values compared to grains dried at L3, L2, L1 and the open sun. Among the stress-crack categories, the percentage multiple stress cracks were the lowest at all the levls followed by the doubles and the singles in that order. To establish variations in drying temperature effect on the quality of the grains, the SCI was determined for grains dried at the various level. As shown in Table 2, the highest SCI of 160 was recorded for Level 4 followed by L3, L2, L1 and the open sun with SCI of 70, 24, 22 and 14 respectively

Tab. 2 shows the stress crack analysis for the dried samples at each level comapared to the samples dried in the open sun. It is revealed that temperature has an effect on the final quality of the dried maize in terms of the grains susceptibility to cracks. Samples at L4 had the highest stress crack index (SCI) of 160 due to relatively high average temperature experienced at that level during the trial. This high SCI value indicates a high susceptibility of grains dried at L4 to cracking. With relatively low temperatures experienced in the ambient, low SCI value of 14 was observed.

Tab. 2: Stress crack analysis of sampled maize at different levels

Level	Stress Crack Categories				SCI
	No crack (%)	Single (%)	Double (%)	Multiple (%)	
Level 1	90	5	4	1	22
Level 2	90	4	5	1	24
Level 3	68	18	9	5	70
Level 4	38	26	23	13	160
Open Sun	94	2	4	0	14

4. Discussions

The maximum temperature recorded during drying in the SBHD was 58.9°C which was recorded after 4 hours at the top shelve (Level 4). Similar temperature recordings of 55.8, 51.1 and 49.4 °C were recorded for the lower drying shelves (L3, L2, and L1) respectively. Comparably, ambient temperature of 39°C was recorded at the same time. The high temperatures recorded in the dryer compared to the ambient temperature could be attributed to the transparent cover material used in construction of the SBHD which has the ability to retard the heat from escaping by acting as a heat trap for infrared (thermal) radiation thereby forming a confinement for the heated air. Similar results were obtained by Achint et al., (2017) during corn drying in a solar cabinet dryer. The additional heat from the biomass furnace also contributed to the high temperature trend in the dryer compared to the ambient temperature agreeing with the findings of Kaaya and Kyamuhangire (2010).

In the solar biomass hybrid dryer, as the drying temperature increased the drying time and grain moisture content decreased irrespective of the grain position (L1 –L4) in the dryer. Mean moisture content decrease steadily from 22.8% to 12.2% (wb) during the 7 hour drying period. This agrees with the findings of Agona et al., (1998), who reported that drying of maize cobs in a biomass natural

convection dryer takes between five and six hours to reduce the moisture content to 14%. Similar results were also obtained for the drying of eggplant (Ertekin and Yaldiz, 2001), and coroba slices (Corzo et al., 2010).

The effect of temperature across levels on the stress crack of the dried maize grains was distinct. Increase in drying air temperature across the drying trays/shelves in the SBHD did not significantly affect the quality of grains dried at the lower shelves with only 10% of grains samples dried at L1 and L2 showing visible signs of stress cracks. However, grains dried at the top tray (L4) had over 60% of dried grains samples showing visible signs of stress cracks with about 50% recorded under the single or double stress crack category and little over 10% found in the multiple range category. The maximum grain temperatures reached in the dryer at the top trays (L4) may have contributed to this occurrence. Similar works (Lewicki and Pawlak, 2003; Sadjad and Saeid, 2014) have showed similar results where increase in drying temperature increased moisture gradient, creating internal tensions, cracks, breakages, and fractures in dried corn. Chakraverty (1988), also reported that these changes create internal stresses, resulting in cracks, which lead to damage and fractures in the structure of agricultural products.

Evaluation of seedling performance also manifested the negative effects of increase in drying temperature on the physiological quality of maize seeds. It was observed that, maize grains dried at the upper level (L4) of the SBHD where the highest mean temperature (52.8 ± 5.4 ˚C) and highest SCI (160) where recorded had the lowest maize seed percentage germination of 44%. Similar observation was made by Ullmann et al., (2015) in evaluation of sweet sorghum seeds in which a reductionin in germination was recorded , especially at temperatures above 40 °C. As suggested by Afrakhteh et al. (2013); Menezes et al. (2012), and also observed in this study, this could be attributed to the high drying temperature at the upper level (L4) resulting in the formation of stress cracks in the seed coat and microfissures in the cotyledons thereby affecting the quality of the seeds.

However, maize dried at the lower levels (L1-L3) of the SBHD did not significantly reduce the germination potential of the grains as the recorded percentage germination of 61, 64 and 77% (L3, L2 and L1 respetively) were close or above the acceptance percentage germination of 70% recommended by the Plant Protection Act (1976). It was observed that, a 10 ˚C drop in drying temperature across the levels from L4 to L1, led to about 33% increase in germination rate of maize seeds. Similar observations were made by Kaaya and Kyamukangire, (2010), who reported that, drying maize using a biomass-heated convection dryer did not significantly reduce germination potential. Agona et al., (1998), also reported that grain dried using the natural convection dryer technology is good for seed production.

The suitability of the solar biomass hybrid dryer for the production of seed maize was investigated. The dryer has the potential to be utilised for drying maize grains for seed on commercial basis but users are encouraged to use the lower drying shelves; Level 1 through to Level 3 since maize grains dried at these levels met the standard commercial limit recommended for sale of maize seeds in Ghana. Moreover, the upper level (level 4) could be potentially utilized for drying maize grains for food or feed.

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Evaluation of AgroZ Hermetic Storage Bag against insect pests on stored maize

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Abstract

A study on AgroZ airtight bag was conducted against two major storage insects under simulated farmers' storage practice. Two (2) lots of 50kg white maize of Pioneer variety were put into AgroZ bag and polypropylene woven bag to serve as a control. Four replications of each bag type were used. In each bag, 50 adults of unsexed larger grain borer, *Prostephanus truncatus*, and maize weevil, *Sitophilus zeamais*, each were introduced. AgroZ bag had one liner placed inside polypropylene bag to provide support and handling convenience. Each liner had been tested for air tightness before use. The AgroZ bags were securely tied to ensure airtightness thus leading to a hermetic environment. The bags were then randomly placed in a barn on pallets in a randomised complete design (RCD). Sampling was done every 4 weeks up to 24 weeks. A 500g sample was initially taken using a compartmented long spear probe from each bag for baseline data, and subsequent ones at 4, 8, 12, 20 and 24 weeks. Repeated sampling from the same storage device reflected farmer practices of opening the device at regular intervals to draw grain for use as household food. Gas analysis in AgroZ bags showed oxygen level dropping rapidly to 7% within 4 weeks and later increased gradually to 10% at 12 weeks. Conversely, carbon dioxide level increased sharply to 10% and declined gradually to 9% over the same period. The number of insects and percentage damaged grains between AgroZ bag and polypropylene bag significantly differed from 12th week to 24th week. AgroZ bag outperformed the polypropylene bag commonly used by farmers and conveniently protected maize from insect infestation within the 6-month storage period.

Introduction

Maize (*Zea mays* L.) is a major grain staple in Sub-Saharan Africa that provides calories and income for many households (Zia-Ur-Rehman, 2006). Whereas the crop is harvested every season, substantial amount of the grain is lost to insect pests during storage because to control these pests remains a challenge to resource-poor smallholder farmers. This is aggravated by lack of effective, appropriate and affordable storage devices (Baributsa *et al*., 2014). As a result of insect feeding, damage, and contamination, the volume of stored grain and quality, grain value, and marketability is reduced (Affognon *et al*., 2015). To avoid the risk of losing the harvested crop to insect pests, some farmers sell their maize early at low price while others treat it with dilute insecticidal dusts, but satisfactory protection is never achieved (Obeng-Ofori, 2011). The major insect pests of stored maize are the larger grain borer, *Prostephanus truncatus* (Horn), and the maize weevil, *Sitophilus zeamais* Motschulsky (DeGroote *et al*., 2013). The former is the most damaging pest, and in endemic areas causes weight loss estimated at 30% while the maize weevil causes 10-20% weight loss when untreated maize is stored in traditional structures (Boxall, 2002; Mutambuki & Ngatia, 2012; Likhayo *et al*., 2016).

Hermetic storage technology offers farmers' effective alternative for protection of stored maize against insect pests. The technology functions by creating modified atmospheres around the grains through physical and biological means to retard the activity and survival of insects in the stored grain (Anankware *et al*., 2012). In recent years, the technology has received much attention by the private sector as the means of safeguarding stored grain because it is cheaper compared to metal silo and safe (Murdock et al., 2003). Currently, there are two hermetic bags available commercially, namely, Purdue Improved Crop Storage (PICS) and GrainPro bags. Whereas these bags effectively control storage insect pests of crops such as maize (DeGroote *et al*., 2013), cowpeas (Moussa *et al*., 2014) and beans (Mutungi *et al*., 2015), the plastic film (liner) has been found perforated (Garcia-Lara *et al*., 2013; Martin *et al*., 2015; Likhayo *et al*., 2016) thus compromising the integrity of the bags. With changing behaviour of insect pests, it is imperative to continually evaluate novel and newer hermetic bags to address this concern. Hermetic grain storage bags of 100kg or less capacity offer smallholder farmers the desired flexibility and control of their produce. AgroZ bag is a low permeability plastic bag developed by A to Z Textile Mills Ltd (Tanzanian manufacturer) for the storage of maize and pulses against postharvest insect pests.

In an effort to contribute to the Public-Private Partnership, the manufacturer submitted samples of AgroZ bags to KALRO-Kabete for local validation through a structured evaluation. The aim of this study was therefore to validate the efficacy of the bag in protecting stored maize against the larger grain borer and other important storage insect pests under field conditions.

Materials and methods

The field trial was conducted at Kiboko sub-centre, about 200 km from KALRO-Kabete, along Nairobi Mombasa highway. The choice of the site was due to prevalence of the larger grain borer and a barn where simulation of farmers' storage condition was adopted. Two (2) lots of 50kg white maize of Pioneer variety (H614D) that had been fumigated was put into AgroZ bag and polypropylene (farmer) bag to serve as a control. Four replications of each bag type were used. In each bag, 50 adults of unsexed *P. truncatus* and *S. zeamais* each (based on 1 adult insect per kg) were introduced. AgroZ bag had one liner placed inside polypropylene bag to provide support and handling convenience. Prior to placing the liners in polypropylene bags, they were tested for air tightness or leakage by filling the air to form a pouch before compressing them with both hands. The AgroZ bags were securely tied to ensure airtightness thus leading to a hermetic environment. Any bag that leaked was discarded. The bags were then randomly placed in a barn on pallets (dunnage) in a randomised complete design (RCD). Sampling was done every 4 weeks up to 24 weeks. A 500g sample was initially taken using a compartmented long spear probe from each bag for baseline data, and subsequent ones at 4, 8, 12, 20 and 24 weeks. Repeated sampling from the same storage device reflected farmer practices of opening the device at regular intervals to draw grain for use as household food.

Each grain sample was sieved to separate dust from insects and grain. Grain moisture was determined using Dickey-John Multi-Grain® moisture tester (Dickey-John Corporation, Aurburn, IL, USA) (wet basis). Moisture content was measured at the beginning of experiment and at every sampling time. Three readings of each sample were taken, and the average recorded. The sample was then divided using a riffle divider until four sub-samples of approximately 65g were obtained. Grains in three of the sub-samples were sorted into undamaged, damaged, discoloured, and broken grain categories which were counted and weighed. The damaged grain was expressed as a percentage of the total grain in the sub-sample. The fourth sub-sample was reserved for reference. Percentage grain moisture content, number of live adult insects, and percentage grain damage were parameters used to judge the efficacy of each treatment. Upon termination of the trial, the hermetic bags were inspected for perforation (holes) made by adult *P. truncatus* and the number of holes recorded.

Statistical analysis

The number of insects was $\log_{10}$ (count +1) transformed, while percentage moisture content and damaged and discoloured grain data were square-root transformed in order to stabilize the variances. The transformed data were analysed using General Linear Model procedure of GenStat Release 12.1 (VSN International Ltd 2009), with bag type and storage period as main effects. Insect counts, grain moisture content, and percentage damaged grains at each time-point post-treatment were the response variables. Significant differences between the means were separated by Tukey test at $P<0.05$. However, for ease of understanding untransformed means are presented. The means of gas composition levels at each sampling time-point were computed.

Results

Effect of bag type on grain moisture content

There were significant differences ($F_{1, 33} = 16.43$; $P<0.001$) in grain moisture content between bag type and storage period ($F_{5, 33} = 21.82$; $P<0.001$) but the interaction was not significant ($P>0.05$). Although significant differences were observed, the moisture content did not change markedly between bag types throughout the entire storage period and, remained below the recommended limit of 13.5% (Table 1). The grains stored in polypropylene bag (12.1%) and AgroZ bag (12.3%) recorded almost same moisture contents after 24 weeks of storage, respectively.

Table 1: Mean percentage (±SE) grain moisture content changes during storage

Bag Type	Storage period (weeks)					
	0	4	8	12	20	24
AgroZ	12.3 ± 0.2cd	11.9 ± 0.0a	12.4 ± 0.0cd	12.6 ± 0.0d	12.3 ± 0.0cd	12.3 ± 0.0bcd
Polypropylene	12.2 ± 0.0bcd	11.6 ± 0.0ab	12.1 ± 0.1bc	12.5 ± 0.0cd	12.1 ± 0.1bc	12.1 ± 0.0bc

Means within the same column or row followed by the same letter are not significantly different at P = 0.05 level (Tukey test)

Effect of bag type on adult insect counts (both live and dead)

In this study *P. truncatus*, *S. zeamais*, and *Tribolium castaneum* (Herbst) were detected. At the start of the trial, the maize did not have emergent infestation. Interaction effect between treatment and storage duration was significant ($F_{5, 33}$ =73.6; P<0.001). AgroZ bags supressed increase in insect population compared to control (polypropylene) bags. On all sampling occasions starting at 12 weeks, least number of adult insects was recorded in the grains stored in AgroZ bags (Table 2). Significant numbers of insects became evident starting from 12th week of storage in the polypropylene (4) bags (Table 2). At the end of the trial, holes perforated by *P. truncatus* were detected in AgroZ bags. Two replicates had 3 and 4 holes each while the other 2 had no holes hence only half of the plastic liner bags used were perforated.

Table 2: Mean number (±SE) of adult insects (both live and dead) per grain sample

Bag Type	Storage period (weeks)					
	0	4	8	12	20	24
AgroZ	0 ± 0f	2 ± 0de	1 ± 0ef	2 ± 1d	2 ± 1de	1 ± 0def
Polypropylene	0 ± 0f	1 ± 0ef	2 ± 2de	4 ± 0c	10 ± 0b	16 ± 1a

Means within the same column or row followed by the same letter are not significantly different at P = 0.05 level (Tukey test)

Effect of bag type on grain damage

There were significant interaction differences ($F_{5, 33}$=185.3; P<0.001) between treatments and storage duration. Grain damage for the treatments is presented in Table 3. At the start of the trial, the maize showed little damage. From 12 weeks' storage duration, no further grain damage was detected in AgroZ bags (Table 3). In contrast, grain damage in the control bags increased steadily from 8th week of storage and reached 11.7% at the end of the trial. If by the 24th week the damage was adjusted by subtracting the baseline damage, actual damage for AgroZ bag was only 1.1% compared to 10.4% for polypropylene bags.

Table 3: Mean percentage ((± SE) grain damage due to insect infestation during storage

Bag Type	Storage period (weeks)					
	0	4	8	12	20	24
AgroZ	1.6 ± 0.2hi	2.0 ± 0.2gh	2.2 ± 0.1efg	2.5 ± 0.2ef	2.8 ± 0.2de	2.7 ± 0.2de
Polypropylene	1.3 ± 0.2i	1.8 ± 0.1ghi	3.1 ± 0.2d	5.7 ± 0.1c	8.4 ± 0.2b	11.7 ± 0.2a

Means within the same column or row followed by the same letter are not significantly different at P = 0.05 level (Tukey test)

Changes in gas composition in AgroZ bag

Although the storage period was 24 weeks, gas composition levels in AgroZ bags was only measured for 12- week storage period (Figure 1). Gas composition levels determined after closing the bags at the onset of the storage were 20.7 ± 0.0% oxygen and 0.9 ± 0.0% carbon dioxide. Oxygen level dropped rapidly to 6.7 ± 0.1% within four weeks and thereafter increased gradually to 10.7 ± 0.1% at 12 weeks. Carbon dioxide level on the other hand increased sharply to10.3 ± 0.1% then declined gradually to 8.9 ± 0.1% within the same period.

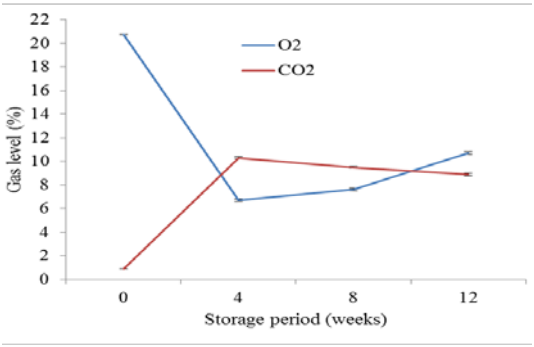

Figure 1: Oxygen and carbon dioxide levels in AgroZ bag over 12 – week of maize storage. Data shown are means ± standard error for four replications.

Discussion

Smallholder farmers store their maize grain to assure supply between the harvests. However, factors such as use of improved susceptible varieties and the spread of the exotic storage insect pest like the larger grain borer (*P. truncatus*) could negatively impact effective storage practices. Despite farmers applying insecticides and traditional protective measures, fewer achieved satisfactory control of the insect pests. Grain damage due to insect infestation is a serious concern that threatens food security and livelihood of rural farmers.

The modified environment created by respiration of th maize grains and insects effectively suppressed insect survival and as a consequence stopped grain damage. Low oxygen levels and enhanced carbon dioxide of inter-granular atmosphere is the basis of insect infestation suppression in hermetic storage. Evidently, extreme oxygen depletion and carbon dioxide build-up levels were not achieved in the AgroZ bags probably due to opening of the bags during sampling. The depletion of oxygen and build-up of carbon dioxide is a function of, among other elements, storage containers; insect population; grain moisture; and gas-tightness. Development of a low oxygen environment is very slow in the absence of insects and predominance of dry grains (<13% moisture content), even in containers where high standard of gas-tightness is achieved. This is attributed to low aggregate oxygen demand in the containers (Moreno-Martinez *et al*., 2000). Other studies, however, reported gradual decrease of oxygen to 8.4% within 30 days in clean maize stored without insect infestation under hermetic conditions (Moreno-Martinez *et al*., 2000). Further, Ng'ang'a *et al*. (2016) reported that oxygen level dropped to 4.9% and carbon dioxide increased to 10.5% within the first seven weeks of on-farm storage of maize in PICS bags. The gas composition levels reported in this study did not differ markedly from those documented by these researchers suggesting hermeticity of AgroZ bags is comparable to that of PICS bags.

This study has demonstrated significant grain damage in maize stored in polypropylene bags compared to that which was stored in AgroZ Plus bags. A study by Njoroge *et al*. (2014) reported 3.4% grain damage when maize (variety H614D) was stored in PICS bags in the presence of *P. truncatus* at ambient conditions for six months. The same maize variety was used in the current study, and a difference in grain damage reported (0.9%) is very small to be important. Therefore, the grain damage recorded by AgroZ bags compares well with that of PICS bags. Although insect pest multiplication was not very high in the control bags as expected, the grain damage levels observed were mainly a result of insect infestation attributed to favourable ambient conditions. Conversely, multiplication of insect pests was drastically reduced in AgroZ bags because of the modified environment (low oxygen and high carbon dioxide levels) within the bags.

Upon termination of the trial, inspection showed physical damage (perforation) of AgroZ bags. These bags are made of tougher polyethylene (PE), 90 μm thick, with good gas and water barrier properties. Therefore, grain volatiles would not be released to the outside to elicit movement of

insects into the bags looking for food while the insects inside the bags died due to depleted oxygen levels (hypoxia). Although the holes were evident to the naked eye, their examination by use of hand-held magnifying glass showed that the scratch and tear were less marked around the holes on the side from which the insects perforated the liner, an indication of exit holes (Riudavets *et al*., 2007). The holes might have been made by *P. truncatus* attempting to escape from the bags when exposed to oxygen-depleted environment. *P. truncatus* has the ability to bore through hard materials such as a 35mm thick plastic (Li, 1988). The holes were made near the bottom of the bags. The holed bags therefore failed to attain air-tight conditions resulting in ineffective control of the storage pests. The observation is in agreement with that made by Ognakossan *et al*. (2013 – not in References) when maize was stored in PICS bags for 150 days and 180 days in SuperGrain™ bags (De Groote *et al*., 2013). Cowpea bruchid *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) was found to bore PICS bags during storage in Niger (Baoua *et al*., 2012) but the hermetic condition was not completely lost because of the imperforated second liner.

Conclusion and Recommendation

AgroZ plastic bag effectively prevented insect multiplication, changes in moisture content, and grain damage as demonstrated in the field trial. Without perforations or a few as observed in the trial, the bag maintained air-tight condition leading to death of insects and hence translating into very minimal grain damage. Owing to this good performance, AgroZ bag is recommended as a storage grain protectant against storage insect pests.

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Impact of Rodent Infestation on Availability, Safety and Nutritional value of Maize Stored On-farm in Lowland Tropical Zone of Kenya

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Rodents are the second most important storage problem after insects during on-farm maize storage in Kenya, and the greatest storage problem in the lowland tropical agro-ecological zone. However, there is limited information on the actual magnitudes of food lost, and food safety issues associated with rodent grain damage. Such information would help to improve maize postharvest management. Farmer stores were monitored over 3 months under natural infestation conditions to quantify actual weight losses due to rodents. Rodent trapping was also carried out to determine rodent species associated with the losses and their population. Additionally, samples of rodent-damaged and non-damaged grain were analysed for total mould count (CFU/g), mould incidence, total aflatoxin contamination, proximate content, and amino-acid and fatty acid profiles. Cumulative weight losses ranged from 2.2 to 6.9% in shelled maize grain, and from 5.2 to 18.3% in dehusked cobs during 3 months of storage. Rattus rattus was the only rodent species captured over the whole trapping period with a trap success rate of 0.62 -10%. Total mould count and Fusarium spp. incidence were significantly higher in rodent-damaged grains than in the non-damaged ones (P= 0.001; P= 0.011, respectively), whereas no significant difference was observed for Aspergillus spp incidence (P=0.239) and total aflatoxin contamination (P = 0.077). Contents of methionine, valine, proline and all fatty acids were significantly lower in the rodent-damaged grains.

Postharvest losses of agricultural commodities in Trincomalee, Sri Lanka

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Abstract

In Sri Lanka, postharvest losses vary with the geographical area; higher losses are reported in warmer areas. A survey was conducted in Trincomalee district, one of the hottest areas in Sri Lanka, to ascertain the status of crop cultivation and postharvest losses of cultivated crops. Farming is the main livelihood of the people in the area. The main crops cultivated are paddy, red onion, chili, brinjal, tobacco and manioc; the average land extent possessed by a farmer family and the yield varies with the crop. Paddy, onion, and tobacco are stored for 6, 3, and 12 months, respectively. Paddy is stored indoor in bags, onion as racks (indoor), and tobacco as piles (indoor and outdoor) under shade conditions. During harvest, drying and storage losses occur in paddy and onion. *Sitophilus oryzae, Rhyzopertha dominica*, *Sitotroga cerealella*, and rats are the major problems during paddy storage. Pesticides are not used regularly by the farmers. Instead they practice traditional pest management methods.

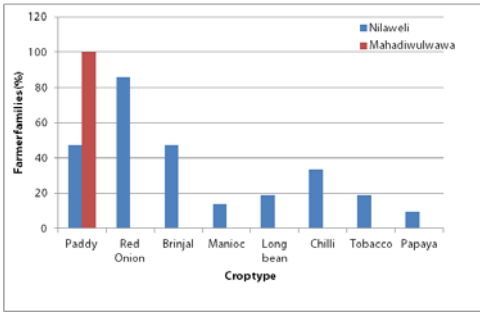
1. Introduction

Postharvest losses of agricultural commodities are much higher in the tropics (Wijayaratne et al., 2018). Insects are major cause for these losses (Hill, 1990). Trincomalee is located in the northeast area of Sri Lanka, and belongs to the dry zone. Due to the limited availability of water, crop cultivation in this region is mainly seasonal. The harvested yield is stored for consumption during the off seasons. Despite the high postharvest losses of agricultural commodities in Sri Lanka, a detailed study has not been conducted recently in Trincomalee area. Therefore, this survey was conducted to determine the status of crop cultivation and postharvest losses of cultivated crops in two cropping areas in Trincomalee, Sri Lanka.

2. Materials and methods

Two geographical areas in Trincomalee district having storage practices, Nilaweli and Mahadiwulwawa, were selected for the study. From each location, 21 families were selected. Using a questionnaire, information on crop growth and postharvest practices were gathered.

3. Results



. Fig. 1. Crop cultivation (as a percentage of all families) in Nilaweli and Mahadiwulwawa areas.

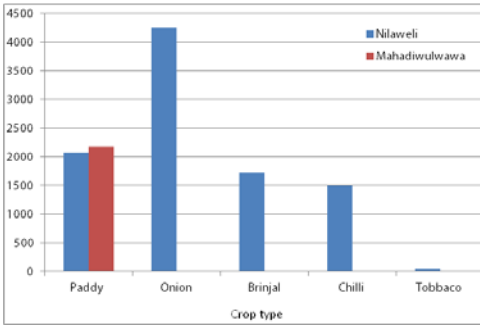


Fig. 2. Annual crop yield (kg) in Nilaweli and Mahadiwulwawa areas.

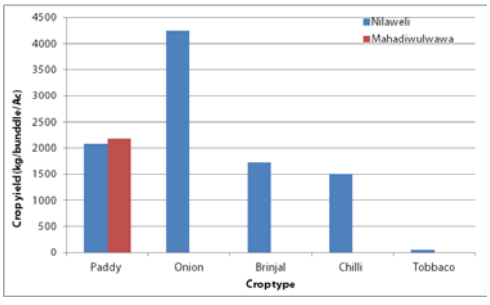


Fig. 3. Post-harvest losses in Nilaweli and Mahadiwulwawa areas.

Several economically-valuable crops are grown in Nilaweli area whereas paddy is the only economically valuable crop in Mahadiwulwewa area. Most of the families in Nilaweli area tend to grow red onion. As a crop grown in the two areas surveyed, the highest annual yield is obtained from onion cultivation. Yield losses during storage happen due to stored-product insects, rodents, and unfavorable conditions of food stores.

Acknowledgements

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Abundance of insects in rice mills in Polonnaruwa, Sri Lanka

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Abstract

Monitoring of insect population is a prerequisite for integrated pest management attempts. The complex structures/machines in rice milling facilities, however, limit surveying attempts aggravating the ignorance of insect fauna associated with such facilities. Furthermore, insect surveys conducted in Sri Lanka are very rare. The objective of the current study was to determine the presence, diversity, and abundance of insects in rice mills of varying capacity as found in a major rice processing area in Sri Lanka. A group of large-, medium-, and small-scale mills were used for the survey. Samples were collected from different locations in the mills, and the density of insects at each location was determined. Insect species and their abundance varied with the type of mill as well as with the location in the mill. This information is useful to design and implement pest management for the mills.

Keywords: small scale, medium scale, large scale, abundance, insect

1. Introduction

Rice is the staple food of Sri Lankans. In Sri Lanka, the annual paddy production in 2015 in one season was 1.9 million MT. Of this production 14% is from Polonnaruwa district (Department of Census and Statistics, 2015). Furthermore, rice milling is popular in Polonnaruwa area. Insects in rice mills are a challenge as they cause quantitative and qualitative losses in the milling products (Hagstrum and Subramanyam, 2006; Wijayaratne et al., 2018). However, lack of information on insect pest populations and their diversity in rice milling facilities restrict the development of integrated pest management programs. No proper survey has been conducted in rice mills in Sri Lanka on the presence of insects. Therefore, this survey was carried out to determine the presence, diversity, and abundance of insects in rice mills in Polonnaruwa district, Sri Lanka.

2. Materials and Methods

Using a questionnaire, information was collected from large-scale, medium-scale and small-scale rice mills on the abundance of insects and their diversity. Samples from different parts of the mill were collected: store house, rice polishers, destoner, dehusker, separator, silky machine, grader,

elevators, paddy cleaner, flour machine, and polisher. The insects were identified using morphological characters. The abundance of insects in each sample was determined.

3. Results

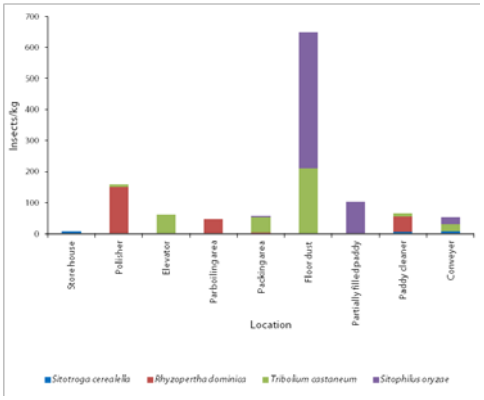

Figure 1. Abundance of insects at different places in the mill-large scale.

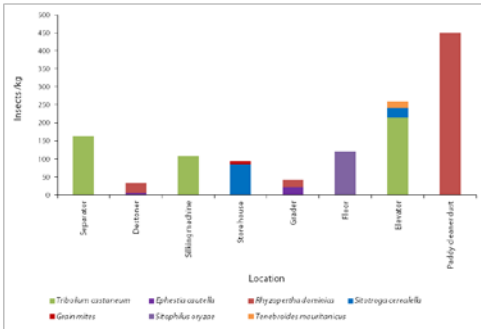

Figure 2. Abundance of insects at different locations in the mill-medium scale.

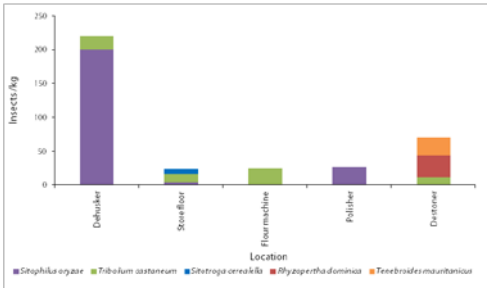

Figure 3. Abundance of insects at different locations in the mill-small scale

Insect diversity and their abundance varied with the type of the mill surveyed. As pest management methods, fumigation and vacuum cleaning are practiced in the large- and medium-scale mills whereas the small-scale millers rely on botanicals.

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Loss of animal feed due to infestation by Rhyzopertha dominica

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Abstract

Despite the use of natural food for livestock production, different animal feeds are currently available at the market. Long-term storage of these animal feeds lead to deterioration and contamination by insects. Therefore, it is important that the loss of these animal feeds be determined and methods to control the damage be sought. This study was conducted to determine the loss of eleven types of animal feed commonly used in Sri Lanka due to infestation by *Rhyzopertha dominica*, a major granivorous insect species.

Twenty newly emerged adults of *R. dominica* were introduced separately to each animal feed: fish feed, rabbit feed, dog feed, cat feed, chick mash, grower mash, layer mash, broiler starter, broiler finisher, bird feed (Bajiri), and rice polish. Each animal feed was maintained either aerated or air tight. These parent adults were maintained for 21 days in the media under ambient environmental conditions (30°C, 65% relative humidity), and then removed. The progeny adults emerged in each feed sample were removed and the weight of the samples was determined at monthly intervals. In general, weight loss of animal feed varied with the feed type, duration of exposure, and aeration condition. Attention needs to be paid to protect those animal feeds that recorded higher losses due to *R. dominica* during storage.

Keywords: Animal feed, *Rhyzopertha dominica*, Weight loss, Duration, Aeration

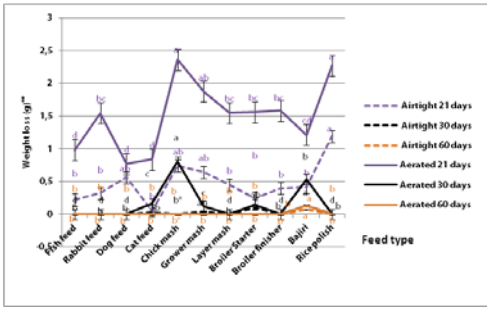
1. Introduction

Traditional practice to feed farm animals is by natural vegetation, but different animal feeds are now available at the market as an alternative. During storage, these animal feeds can be infested by insects. The lesser grain borer, *Rhyzopertha dominica*, is a major pest of stored cereals (Chittenden, 1911), pharmaceuticals, leather stuffing, and packing materials (Riley, 1882; Chittenden, 1911; Winterbottom, 1922; Hoffman, 1933; Potter, 1935). There is a potential that animal feeds can be infested by *R. dominica*. The objective of this research was to examine the potential of *R. dominica* to damage eleven types of animal feed commonly used in Sri Lanka.

2. Materials and methods

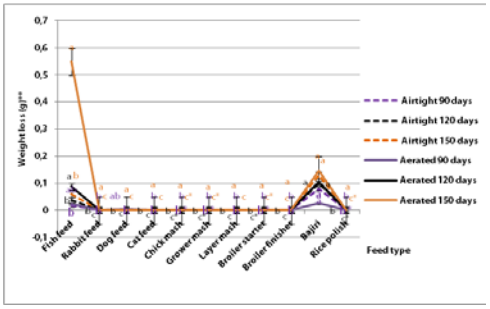
Eleven types of animal feed were used in the study: fish feed, rabbit feed, dog feed, cat feed, chick mash, grower mash, layer mash, broiler starter, broiler finisher, bird feed (Bajiri), and rice polish. Twenty adults of newly emerged *R. dominica* were introduced to 20 g of each animal feed in separate vials and maintained either aerated or air tight under the ambient environmental conditions (30°C, 65% relative humidity). The experiment was conducted using four replicates. After 21 days, the parent adults were removed. The progeny adults were counted and the weight of each animal feed was determined at monthly intervals for five months.

3. Results



*Fungal contamination
**For a combination of a 'given aeration (aerated or air tight) and duration', weight loss followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA.

Fig. 1. Weight loss in animal feed at 21, 30 and 60 days following infestation under aerated/air tight condition.



* Fungal contamination
**For a combination of a 'given aeration (aerated or air tight) and duration', weight loss followed by the same letter are not significantly different at P=0.05 according to Tukey's test following ANOVA.

Fig. 2. Weight loss of animal feed at 90, 120 and 150 days following infestation under aerated/air tight condition.

4. Discussion

Aerated samples of a given animal feed demonstrated higher weight loss than air-tight samples. The maximum weight loss occurred in chick mass and Bajiri. The minimum weight loss was recorded in dog feed and cat feed. Discard of certain animal feed samples due to fungal contamination seemingly interrupted the smooth increase of weight loss when the duration increased.

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Quality and Safety Conditions of Flocked Oats (*Avena Sativa* L.) Stored in Bags

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Abstract

Oats (*Avena sativa* L.) have reached the healthy food market worldwide due to its special nutrients composition and fiber high quality. Therefore, quality & safety control is a must, both during the storage and commercialization stages. The current study evaluated the physicochemical characteristics (flakes size/variation %, pH, moisture content-mc, water activity-aw), living organisms (insects & mites / mycoflora - fungi load& genera identification), mycotoxins(ochratoxin A – OTA / zearalenone – ZON / aflatoxins – AFLs / esterigmatocistin – EST)andthe storage conditions of flocked oats stored inbags.Regarding the oats physicochemical characteristics, flakes particle size varied, however most of the samples present size uniformityand only one sample had high percentage of residue. That indicates high insects and other living organisms activity (consumption / proliferation) of oats starch and other nutrients. The analysis through

stereomicroscope showed intense presence of insects and mites. Samples were seen also sheltering those living organisms (27%), which are not allowed by regulation (no soils, parasites and larvae presence). As expected, mc (10.8-13.2%) and/or aw (0.61-0.90) varied, however they kept on the safer levels (< 13% / 0.90) insects/mites and fungi growth wise. With respect to pH, it varied from4.1to 5.85, indicating some rancidity/fermentation reactions taking place, thus changes in organoleptic parameters. The total fungi load ranged from $3x10^2$ to $1.29x10^5$ CFU/g, with*Aspergillus* and *Rhizopus*the genera more identified. Only one sample was toxin contaminated (OTA - 80 µg/kg). Insects are known vectors of fungal spores and can spread their hyphae on their dead/live skeleton, apart from mites that can trigger allergies in humans and animals. Therefore, current data demonstrate that despite the storage conditions control application, living organisms can occur in flocked oats (stored in bags) and it is necessary to apply decontamination methods to control/prevent their proliferation.

Key words: oats, storage, bags, insects, fungi, toxins.

Introduction

As part of the demand for a healthy diet, oats (*Avena sativa* L.) have gained more and more popularitydue to its functional claims (mainly due to its composition). Oats have large amounts of beta-glucans and soluble fibers, which are able toreduceglucose absorption and increase intestinal transit. Several studies have shown its effectiveness in preventing diabetes and cardiovascular diseases, and in reducingglucose levels and blood pressure. It is important to emphasize that processing steps do not alter the concentration of oats nutrients (De Sá et al, 1998).

During grain storage, moisture and temperature reduction and control is required. Despite that, grains need to be harvested in the most efficient way so that there is no mechanical damageallowing insect infestation and fungiproliferation. Storage locations vary fromwarehouses, bags or silos, and also in more modern ways, such as hermetic silos (Marini et al., 2007).

Oats, like others cereals, are susceptible to a number of fungi, including those of field and storage, such as *Fusarium*, *Aspergillus*, and *Penicillium*. Fungi, under favorable conditions, can cause deterioration in grains and produce mycotoxins. These toxins canaffect animal and human health, being more severe in some animals, such as swines and equines.We are exposed to fungal spores at all times as they are easily transported through the air. These fungalspores can be toxigenic - those that are able toproduce toxins harmful to health.As these spores are not easily perceived due to their microdimensions, we only identify their presence in foods when they are well developed, spoiling their tissues and producing mycotoxins. More than 300 mycotoxins have been isolated in food, however there are five main ones, among them aflatoxins (AFLs), ochratoxin A (OTA), T-2 toxin, deoxynivalenol, and fumonisins (Scussel, 2002; Agais, 2005).

Some foods may contain mycotoxins and are apparently healthy, which leads us to consume these foods without the full certainty of safety. It is necessary to monitor the quality of the grains stored and marketed, so that the population is aware of what they are consuming.

Therefore, this work evaluated the quality and safety conditions of flocked oats stored in bags.

Materials and Methods

Material

Samples: flocked oats stored in bags.

*Culture media and reagents:*potato dextrose agar (PDA) and peptone bacteriology media were purchased from Himedia (Curitiba, Parana, Brazil) and chloramphenicol were from Vetec (Duque de Caxias, RJ, Brazil), phenolphthalein and sodium hydroxide from Merck (Darmstadt, Germany).

*Equipment:*autoclave, Phoenix (Araraquara, SP, Brazil); microwave oven, Philco (Sao Paulo, SP, Brazil); tweezers, Prolab (São Paulo, SP, Brazil);caliper, Digimatic (Mitutoyo, Tokyo, Japan); drying oven, Olidef-cz (Ribeirao Preto, SP, Brazil); a_w meter, Aqua- Lab4TE, Decagon (Sao Jose dos Campos, SP, Brazil); Peagameter, Model Schott-gerate CG 818 (Schott,, Mainz, Germany); laminar flow cabinet, Veco (Campinas, SP, Brazil); fume cabinet, Quimis (Diadema, SP, Brazil); rotary shaker, Marconi (Piracicaba, SP, Brazil); microbiological incubator, Quimis (Diadema, SP, Brazil); colonies counter,

Phoenix (Araraquara, SP, Brazil); sieve system, mesh (2-1mm) Beffer (Caieiras,SP, Brazil).Microscopes - light (LM), CH-Bl45-2, Olympus (Shinjuku, Tokyo, Japan); stereo microscope (SM), Opzt coupled to a color image-capture camera, model OPT14 MP, Opticam Microscopy Technology (Doral, Fl., USA).

Methods

Sample collection and preparation: samples (300 g) were collected from stored bags, then sealed, labeled, and transported to the Laboratory of Mycotoxicology and Food Contaminants for analysis; *(b) preparation* - each oat sample was homogenized and then divided into two main portions: (b.1) integral i.e., its original flakes characteristics (analysis: pH, mycology, and aw) and (b.2) ground, for mc and mycotoxins.

Granulometry of oat flakes: sample portions (100 g) were subjected to separation by a Screen System (sieves) with different apertures (Mesh: 9; 16; 200, corresponding to 2.0; 1.0 and 0.75 mm) (Lorini et al., 2015) then %/mesh calculated.

Physicochemical analysis: pH, acidity, and moisture content (mc) were determined by the international official AOAC methods (Peisino et al., 2015; AOAC 2005). The water activity (a_w)was determinedusingthe Aqualab apparatus at 25ºC (n=3) (Decagon, 2001).

Total fungi load and genera identification: the enumeration technique and genera identification of Da Silva et al. (2007) and Pitt (1979) were used.

Storage conditions: the environmentalconditions of the storage- ventilation / refrigeration, application of pest control system, cleaning of the premises - were evaluated (Souza et al., 2013).

Multi-toxin analysis: the method of Soares&Rodrigues-Amaya (1989) was applied for the determination of multi-toxins [AFLs (AFB1, AFB2, AFG1, AFG2), ZON, EST and OTA].

Results

From the data obtained, it was possible to observe that part of the flocked oats stored in bags showed that the flocculation process applied and the bags storage condition in which they were submitted were efficient. Despite that, some oat flakes presented different physicochemical conditions ideal for the development of insects, mites, andfungi.Table 1 shows the total fungal load, genera, and humidity of flocked oats samples (*Avena sativa* L.).

Insects and mites: they were detected in all oats samples (at different percentages), enphasizing the concern on the storage conditions and safety. Part of the samples (32%) presented insects and mites when analyzing under stereoscopic microscope. Thereboth living and dead insects present. Figure 1shows by sterescopy (a, b) *insects*and (b) *mites* isolated from oat samples.

Humidity: the mc of the samples analyzed varied from 10.8 to 13.2%, indicating a small variation of the products stored and process. With respect to a_w, the samples varied from 0.4782 to 0.5906 and the pH ranged from 4.1 to 5.85 indicating some rancidity and fermentation process, thus flavor alterations.

*Fungi:*as expected, the total load was high ranging from $3x10^2$ to $1.29x10^5$ CFU / g.The genera isolated were *Aspergillus* and*Rhizopus*, the first with possible toxin formation and the second only deterioration. Figure 2presents the light microscopy of fungi (a) *Aspergillus* and (b) *Rhyzopus*isolated from oat samples.

Mycotoxins: a single sample showed contamination by OTA (80 µg / kg) well above the regulations of several countries (OTA daily intake: 3; maximum tolerant level: 50 µg / kg – FAO/WHO, 2017).

Table.1 Physico-chemical characteristicsand fungi of flocked oats (*Avena sativa* L.) stored in bags

Flakes oats		Physico-chemical			Fungi		Mycotoxins* (ug/kg)			
Number	Code	Humidity		pH	CFU/g	Genera	AFLs	EST	ZON	OTA
		mc (%)	a_w							
1	A	11.48	0.5429	5.75	ND	ND	ND	ND	ND	ND
2	B	11.97	0.5712	5.535	ND	ND	ND	ND	ND	ND
3	C	11.92	0.5547	5.853	$1.05x10^4$	*Aspergillus/ Rhizopus*	ND	ND	ND	ND
4	D	12.94	0.5514	5.6	$3x10^2$	*Rhizopus*	ND	ND	ND	ND
5	E	12.42	0.5159	5.39	$7.0x10^4$	ND	ND	ND	ND	ND
6	F	12.88	0.5904	4.095	$6x10^{-2}$	*Aspergillus/Rhizopus*	ND	ND	ND	80
7	G	12.11	0.5239	5.53	$1.47x10^4$	*Aspergillus*	ND	ND	ND	ND
8	H	10.81	0.4781	5.25	ND	ND	ND	ND	ND	ND
9	I	13.08	0.5728	4.61	$1.29x10^5$	*Aspergillus*	ND	ND	ND	ND
10	J	12.83	0.5416	4.595	$8.85x10^4$	*Aspergillus*	ND	ND	ND	ND
11	K	13.24	0.5061	5.665	ND	ND	ND	ND	ND	ND

Mc: moisture content a_w: water activity CFU/g: colony-forming units AFLs: aflatoxins OTA: ochratoxin A EST: sterigmatocistin ZON: zearalenoneND: not detected *LOQ: 2 ug/kg each

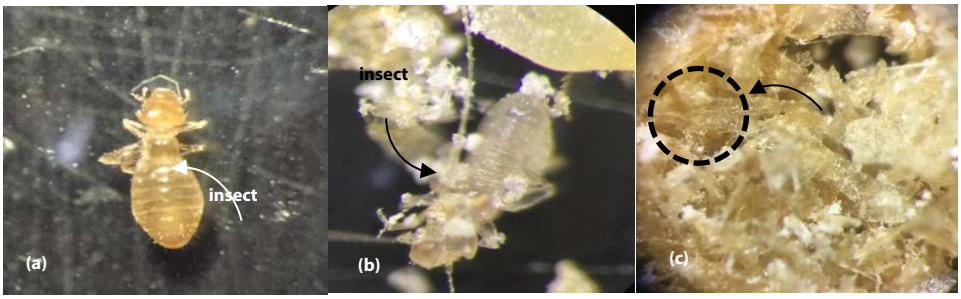

Fig.1 Insects and mites detected in flocked oats (*Avena sativa* L.) samples stored in bags understereomicroscopy [x60].

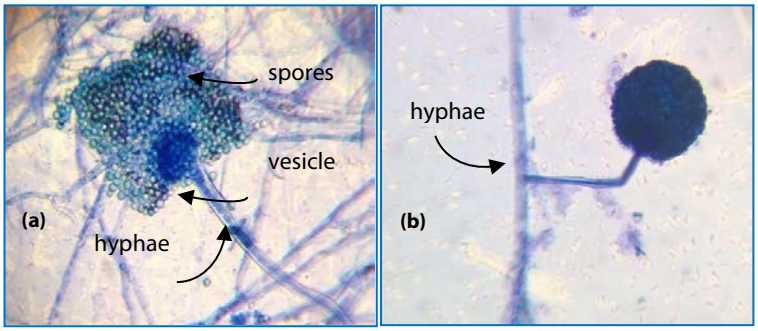

Fig.2 Reproductive structures of fungiisolated from flocked oats (*Avena sativa* L.)by light microscopy (a)*Aspergillus* and (b) *Rhyzopus*genera [x400].

Discussion

Insects and mites are vectors of fungal contamination, as spores and hyphae may develop and be carried / transported in their exoskeleton.In addition, mites can trigger allergies in humans. Franzolin (1998) has identified that when spores of *Aspergillus flavus* adhere to the body of mites, they do so as a means of spreading, causing viable spores to proliferate in grains stored incorrectly.

Soares et al. (2018) isolated fungi of the genus *Aspergillus* and *Penicillium* adhered to the exoskeleton of beetles *Alphitobiusdiaperinus*, considered a secondary pest in storage units.

The mc values detected in the samples were higher than those found by Sandrin (2013) in oat flakes where authors got 10.04%. Gutkoski &El Dash(1999) suggest that after hydrothermal processing followed by flocking, the oats flakes should reach 10% mc.When the mc is higher than 13% over time, the acidity index increases very fast, indicating grain deterioration (Gutkoski and Pedó, 2007; Rupollo et al, 2004). All samples analyzed showed high pH, which leads us to conclude that the deterioration and rancidity process started, generating a characteristic odor, however, only in one sample studied. The oil extracted from oats presents a great amount of unsaturated fatty acids, with linoleic being the main one. Unsaturated fatty acids have double bonds in their structure, making themmore unstable to the rancidity process. Hydrolytic and oxidative rancidity are factors that adversely affect quality (Gutkoski and El-dash, 1999). They can be caused by enzymes (active at acidic pH) present naturally in the grain or by contaminating microorganisms.The acidity of sample F (pH: 4.1), followed by samples J (pH: 4.60) and I (pH: 4.61), can be explained by that reason (enzyme catalyte activities). Samples I and J both presented microorganisms contamination, one of the causes of rancidity byoxidative enzymes.

Only 2.8% the samples were out of the standards required for total fungal load. Unsatisfactory conditions at the storage time, such as high temperature and humidity favor the development of fungi (Scussel, 2002).With the data obtained from the total fungal load it is concluded that there was a high contamination in storage due to the presence of *Aspergillus* (storage) and *Rhyzopus* (deterioration) genera. The presence of fungi in the product can cause spoilage, alter organoleptic properties, and present health risks (DallaVecchia&Castilhos-Fortes, 2007).

Influenced by extrinsic factors such as a_w, fungi can develop, causing serious problems for the grain. According to Iamanaka et al. (2013) the values that favor fungal development and toxin formation vary between 0.60 and 0.90. However, these values obtained from samples C (aw: 0.5547), F (aw: 0.5904), and I (aw: 0.5728) were favorable to the development of some genus of filamentous fungi. The mycotoxin production isdirectly related to the quality of storage. According to Gerez et al. (2014), the optimal conditions for *Aspergillus niger* to produce OTA was a_w of 0.995. Despite that, the toxin can be produced from a_w as low as 0.60, depending on other factors such as temperature and substrate composition.Other authors such as Esteban et al. (2006) also reported lower values of $a_w$for OTA production.

In a single sample (F) the highest $a_w$detected was 0.590.Consequently it was contaminated by OTA. Kuzdralinski et al. (2013), when analyzing oat grains, reported that 42 of58 samples were contaminated with OTA. In another study by Sacchi et al. (2009) authors did not find AFLs and ZON in their samples, corroborating what was found in the present study.

Conclusion

The samples showed uniformity in the flakes size. However, the presence of insects and mites, which exposes the grain to other types of contaminants such as fungi, was registered along with high colony forming units of *Aspergillus* and *Rhyzopus*. The high pH detected in the samples leads us to conclude that deterioration and rancidity were in the process of initiation. Other characteristics such as the levels of mc and a_w also favor the development of fungi and their metabolites.

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The impact of two drying methods on the quality of high-moisture rice

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Abstract

In this experiment, freshly harvested rice was dried by natural and mechanical methods. For natural drying, paddy rice was spread on a cement floor under a shelter at a thickness of 4cm, and it was turned twice a day. At a temperature of 19.3°C and a relative humidity of 58.8%, a total of 28 days was needed to reduce the water content from 23.11 to 14.38%. For mechanical drying, the Guwang 5HXG-15B circulating dryer was used, drying temperature was set to 42°C, and it took a total of 5 hours to reduce the water content from 23.1 to 11.8%. The changes in spore count, fatty acid value, germination rate, waist burst rate, whole polished rice rate, and taste value of rice mold after drying were studied. The results showed that compared with mechanical drying, the

drying rate of air-dried rice was slower, and the number of mold spores increased from 0.65×10^5/g to 3.05×10^5/g, a 3.7 times increase. The number of mold spores in dried rice was not significant. Dried rice fatty acid value of 25.1mg/100g for natural drying was higher than the value of 19.9mg/100g for mechanical drying. High temperature affected rice seed vigor: mechanically dried rice germination rate was 58.0%, far lower than the 87.5% for natural drying. The blasting rate, polished rice rate, and taste value of mechanically dried rice were 5.33%, 57.9%, and 83.7, respectively, which was 2.33%, 58.9%, and 89.3 for naturally-dried rice. The processing quality and taste quality were even worse. Therefore, the drying process of the optimized circulation dryer should be further adjusted to reduce its impact on rice processing quality and taste quality.

Key words: rice; natural drying; circulating dryer; processing quality; taste quality

1. Introduction

Rice is one of the world's major grains, and 50% of the world's population is rice-based. In China, as one of the three major grain consuming countries, the yield of rice ranks second only after corn. In 2017, China's rice cultivation area was 30 million hectares, and the output was about 210 million tons. The planting area and output all ranked first in the world. The moisture content of rice after harvest is usually high. At this time, the rice itself has a strong respiration. At the same time, the biological activity of grain reserves is intense. If it is not dealt with in time, it can easily cause adverse effects such as high temperature, germination, and mildew. According to statistics, China's high-moisture paddy after harvest was too late to reach the safe storage moisture content, resulting in losses of up to 5% in storage, transportation, and processing. Rice drying is a necessary processing step after harvest. Rice is a heat-sensitive grain, and unreasonable drying methods can cause changes in the physical and chemical characteristics of the main components of rice. Therefore, it is important to choose an effective and appropriate drying method. The natural drying method is a technique for ventilating and drying food by means of solar energy and natural wind in a natural environment. The natural drying method does not require special equipment. Although it is largely limited by weather conditions, it is still the main method of grain drying in China. With the rapid development of agricultural machinery, grain dryers have become more and more widely used. They can be roughly divided into continuous and circulating types. Compared to corn and wheat, rice has higher requirements for drying process due to its heat sensitivity, and circulating dryer with small batches and low temperature is more suitable.

In this experiment, removal of water was performed on high-water rice by using two drying modes: natural rice drying and circulating dryer. The changes of water and fungal spores in rice during the natural drying process were studied. The quality of the dried rice was compared between the two methods, and the differences in the changes were studied to provide reference for the effective and reasonable method of rice drying in the post-harvest treatment of rice.

2. Materials and Methods

2.1 Test materials

Test material was new rice that was harvested on September 13, 2017. The variety was Shenliangyou 5814, and the place of production was Xinjin County, Chengdu. The initial quality indicators of rice are shown in Tab.1.

Tab.1 rice intial quality indicators

Moisture （%）	Impurity rate （%）	Brown rice rate （%）	Thousand kernel weight （g）
23.11	1.83	76.36%	31.16

2.2 Test equipment

FA22048 Electronic Balance: Shanghai Jingke Tianmei Scientific Instrument Co., Ltd.; 101-1A Electric Heating Blast Drying Box: Beijing Zhongxing Weiye Co., Ltd.; JFSD-100 Crushing Machine: Shanghai Jiading Grain and Oil Instrument Company; JJSD Type Filter: Shanghai Jiading Grain and Oil Instrument Company; JLG-1 Huskers: Chengdu Grain Storage Research Institute, China Grain Storage; JNM-III Milling Machine: Chengdu Grain Storage Research Institute, Middle Grain Storage;

SMART Biological Microscope: Beijing Zhongxian Hengye Instrument and Meter Co., Ltd.; HPS-250 Biochemical incubator: Harbin Donglian Electronic Technology Development Co., Ltd.; JSWL rice taste detector: Beijing Dongfu Jiuheng Instrument Technology Co., Ltd. and Japan Satake Company; TH802A mechanical temperature and humidity meter: Meideshi Instrument Co., Ltd.; DTS418 type three-phase four Line Electronic Energy Meter: Changan Group Co., Ltd.

1.3 Test methods

1.3.1 Rice natural drying

Fig.1 Rice natural drying

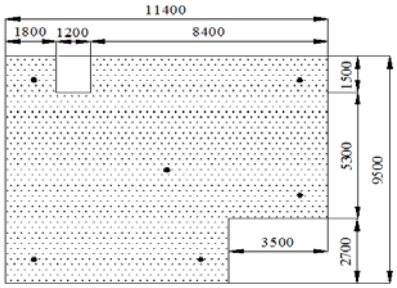


Fig.2 Sampling point layout

In accordance with the common farmer's pattern of grain drying, we chose a well-ventilated area to lay the wet rice on the cement floor under the shelter (Fig.1). The total weight of rice was 1.60t, and the area of the site was 11.4×9.5-1.2×1.5-3.5×2.7=97.05m^2, and the thickness was about 4cm. Samples of rice moisture content and fungal spores were routinely taken at 8:30 daily. The location of the sampling points was set as shown in Fig.2. Six sampling points were located at five corners and at the center. The sampling point on the corner was 1.00m away from both adjacent sides. The six sampling points can more fully reflect the change of moisture in the rice during the drying season. In accordance with the method of foodstuffs for farmers, daily sun drying was carried out. [Taking into account the relationship between autumn temperatures, the use of wooden clogs is repeated every day at 9:00 and 14:00.What does this mean?] We placed a thermometer and hygrometer on the drying area and recorded the temperature and humidity changes from 9:00 to 17:00 every hour.

2.3. 2 Rice mechanical drying

We used a mechanical dryer to dry 10.00t of the same batch of wet rice. Dryer model GuWang5HXG-15B, a cross-flow circulation dryer.

2.3.3 Determination of indicators

2.3.3.1 Determination of moisture content was according to GB/T 5497-1985. In the early drying period, the moisture content of rice exceeds 18%. Therefore, it was necessary to use two drying methods. The specific steps were: Weigh out 20g of rice (accurate to 0.001g), and put it in a baking box with a diameter of 10cm and a height of 2cm. Bake at 105±2°C for 30-40min. Take out and cool to constant weight. (The difference between two weighings does not exceed 0.005g. This is the weight of the sample after the first baking). After smashing the first baked rice sample, use an aluminum box that is baked to a constant weight, weigh about 3g of the sample (accurate to 0.0001g), put the aluminum box cover on the bottom of the box, and put it into the drying oven . Take it out after 105±2°C for 3 hours, remove it, cap it, place it in a desiccator and cool it to room temperature. Take it out and weigh it, then re-bake it according to the above method, take it out and cool and weigh it once every 30min, baking it before and after. The difference between the two weights should not exceed 0.005g. If the latter weight is higher than the previous weight, the previous weight is calculated. The moisture content of rice was calculated according to the following formula:

$$\text{Moisture}\ (\%) = \frac{W \cdot W_2 - W_1 \cdot W_3}{W \cdot W_2} \times 100$$

Where: W - the weight of the sample before the first baking, g;

W_1 - weight of the sample after the first baking, g;

W_2 - weight of the sample before the second baking, g;

W_3 - Sample weight after the second baking, g.

2.3.3.2 The number of fungal spores was determined according to the fungal spore count method proposed by Cheng et al. (2009). The specific operation procedure was: Take 10.0 g of rice sample, add 30 mL of deionized water in a 50 mL test tube, add stopper, shake vigorously for 1 min, and filter with 300 mesh. The cloth was filtered and the filtrate was counted under a microscope for fungal spores.

2.3.3.3 Determination of waist burst rate According to GB/T 5496-1985.

2.3.3.4 Determination of germination rate According to GB/T 5520-2011.

2.3.3.5 Determination of roughness According to GB 5495-2008.

2.3.3.6 Determination of the rate of polished rice According to GB/T 21719-2008.

2.3.3.7 Determination of fatty acid value According to GB/T 5510-2011

2.3.4 Test site

Chengdu Qingbaijiang National Grain Reserve of Sichuan Province

3. Results

3.1 Natural drying rice moisture changes

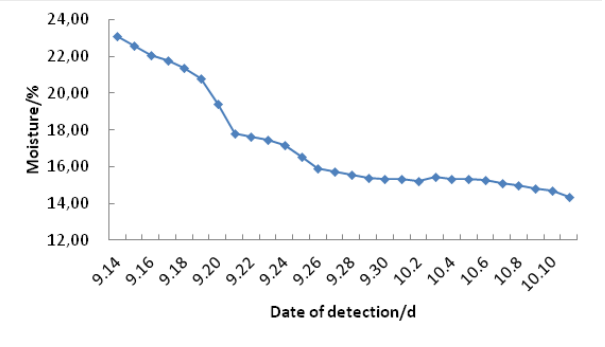


Fig.3 The curve of rice moisture

Tab.2 Daily precipitation and temperature and humidity of rice correlation analysis

		Temperature	Relative humidity	Daily water loss?
Temperature	**correlation coefficient**	1.000	-0.502**	0.529**
	Sig.	-	0.009	0.005
Relative humidity	**correlation coefficient**	-0.502**	1.000	-0.220
	Sig.	0.009	-	0.281
Daily water loss?	**correlation coefficient**	0.529**	-0.220	1.000
	Sig.	0.005	0.281	-

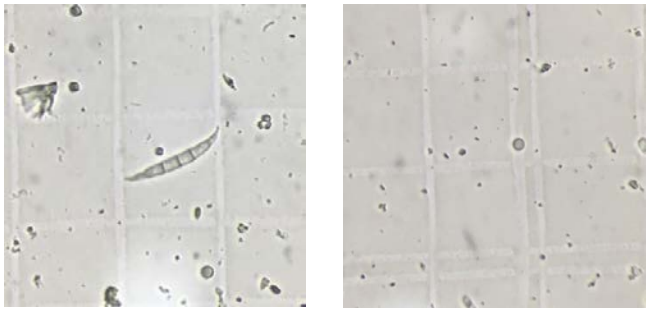
Note: ** indicates a significant correlation at the α=0.01 level, and * indicates a significant correlation at the α=0.05 level.

As shown in Table 3, there is a positive correlation between daily water loss and temperature, and the correlation is extremely significant. The daily water loss is negatively correlated with the average relative humidity, and the correlation is not significant. Therefore, under the conditions of natural

drying of rice in this experiment, the effect of temperature on the drying rate is greater than the humidity.

3.2 Rice fungi spores change during natural drying

During the natural drying, due to the slower rate of water loss, rice is in a state of unsafe moisture for a long time. Because the ambient temperature is not high, the external conditions are extremely suitable for the growth of fungi.



(a) Fusarium spore (b) Aspergillus niger spore

Fig.4 Microscopic view of fungal spores

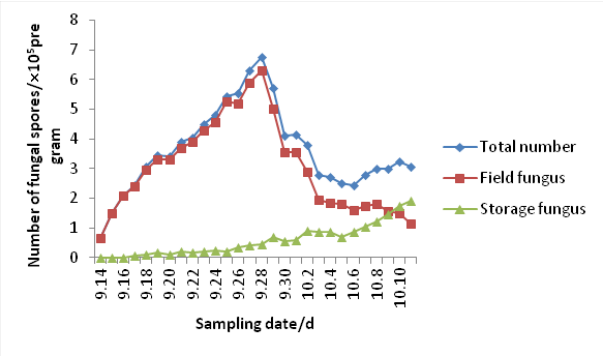


Fig. 5 The number curves of the of spores of rice fungal

Tab.3 The evaluation criteria of spoilage fungi spores in stored grains

Safty level	Fungal spores number	Safty
Level I	$<1.0\times10^5$	Security
Level II	$(1.0\sim9.9)\times10^5$	Critical control criticality
Level III	$(1.0\sim9.9)\times10^6$	Harm
Level	$\geq1.0\times10^7$	Serious harm

According to the ecological group, microorganisms can generally be divided into field fungi (infested by field growth, mainly parasitic and parasitism) and stored fungi (all kinds of saprophytic bacteria). Field fungi include *Alternaria*, *Fusarium*, etc., and storage fungi include *Aspergillus niger* and *Penicillium*[3]. Shown in Fig. 4 are the spores of the *Fusarium* spp. and *Aspergillus nigrum* under the microscope. During the experiment, samples were taken daily to detect field and stored fungal spores. A graph showing the change in the number of spores in rice cultivars is shown in Fig. 5. It can be seen from the figure that during the natural drying of rice, the total number of fungal spores first increased and then decreased. On September 28, the total number of fungal spores reached the maximum value of 6.75×10^5/g. According to Table 3, the number of fungal spores that are hazardous to rice is at the critical control threshold, although this did not reach the level of harm. However, there is a great threat to the safe storage of rice. During the whole drying period, the

stored spores showed a gradual upward trend, and the number of spores in the field increased first and then decreased. Referring to Figure 3 and Figure 5, when the total number of fungal spores in the field reached the peak value, the rice water content was 15.55%. After that, the rice water reached a stable period with less than 1% change. Therefore, it is possible that the decrease in total moisture is the main reason for the decrease in the number of spores in the field.

3.2 Two methods of drying results analysis

Tab.4 Drying results in two ways

Dring method	Initial moisture (%)	Final moisture (%)	Drying amount (t)	Drying time (h)	Drying speed (1% moisture/h)
Natural drying	23.1	14.4	1.6	648	0.0134
Mechanical drying	23.1	11.8	10.0	5	2.26

Comparing Tab.4 with the drying results of two kinds of drying methods, it can be seen that natural drying has the characteristics of small treatment volume and slow drying speed, while mechanical drying has a large amount of processing and a high speed relative to rice batches.

3.3 Analysis of the impacts of two kinds of drying on rice quality

Tab.5 Rice crack ratio with two drying methods

Drying method	Natural drying	Mechanical drying
Crack ratio(%)	58.90±0.15a	57.90±0.36a

Note: Different letters represent significant differences, the same below.

The waist burst rate is an important indicator for assessing the drying process of rice. During the drying process, due to the different water loss rates of the inner and outer layers of the rice grains, a water gradient is generated, causing internal stress, and cracks appear when the stress exceeds the tensile strength of rice[4]. In the case of rice with a high waist rate, especially when the cracks are large and deep, it is not appropriate to process high-precision rice, otherwise it will increase broken rice and reduce the rice rate. Since the natural drying process does not assist the heating of rice, the waist rate is lower than that of the dryer. It can be clearly seen from Table 5 that the popping rate of natural dry rice is significantly lower than that of mechanical drying. [Table 5 shows no significant difference in crack rate between the two drying methods.] This is due to the high temperature environment in the mechanical dryer causing excessive water loss in the outer layer of rice grains.

Tab.6 Head rice yield with two drying methods

Drying method	Natural drying	Mechanical drying
Head rice ratio(%)	58.90±0.15a	57.90±0.36a

The head rice rate is the most important trait in the quality of rice milling, which affects the taste quality of rice to a large extent. Studies have shown that the higher the rate of whole polished rice, the greater the volume of rice swell and the better the quality of rice consumption[5]. According to Tab.6, it can be seen that the roughness of natural dry rice is significantly less than mechanical drying, and the percentage of whole rice is greater than that of mechanical dry rice, but the difference is not significant.

Tab.7 The germination rate of rice with two drying methods

Drying method	Natural drying	Mechanical drying
Germination rate (%)	87.5±5.5a	58.0±2.0b

The germination rate of rice is an important indicator for comprehensively measuring the new graininess of rice. The freshness of the rice and the quality of the food can be reflected by changes in the germination rate[6]. From Tab.7, it can be seen that compared with naturally-dried rice, the germination rate of mechanically dry rice is low, and the difference is significant. The results indicated that the vitality of natural air-dried rice embryos was well preserved, while the low-

temperature circulation dryer had a lower hot air temperature than the continuous dryer, but it still had a strong destructive effect on the internal physiological structure of rice, which seriously affected the growth of rice.

Tab.8 Fatty acid value of rice with two drying methods

Drying method	Natural drying	Mechanical drying
Fatty acid value (mgKOH· (100g)$^{-1}$)	25.11±0.57a	19.8±0.90b

Rice lipids are oxidized to produce free fatty acids, which are represented by fatty acid values. Fatty acid values are an important indicator of rice freshness, and the extent of rice quality changes can be judged based on changes in fatty acid values [7]. It can be seen from Tab.8 that the fatty acid values of rice obtained by natural drying are significantly greater than those of mechanical drying, and the differences are significant. The growth rate of free fatty acid content is significantly positively correlated with the relative activity of lipase[8]. Since natural drying has a lower ambient temperature, lipase maintains a higher relative activity during the drying process. In addition, the natural drying time is lower. Longer, higher accumulation of free fatty acids may result in relatively higher values of natural dry rice fatty acids.

Tab.9 The taste value of rice with two drying methods

Drying method	Natural drying	Mechanical drying
Taste value(%)	89.3±0.41a	83.7±0.41b

The main principle of the taste meter is to accurately determine the components of amylose, protein, water, and fatty acids that determine the taste of rice by the difference in absorbance generated by the near-infrared light at a specific wavelength, and then to compare different ingredients data with experimental rice taste. The data is combined and scored by simple numerical values to objectively reflect the purpose of eating rice[9]. As shown in Table 9, the taste value of natural dry rice was significantly greater than that of mechanical drying, indicating that rotary bins can better protect the food quality of rice, while mechanical dried rice has the poorest food quality, and is significantly different from the former two.

4. Discussions

In this study, the water loss characteristics of naturally dried rice field were studied. At a temperature of 19.3°C and a relative humidity of 58.8%, a total of 28 days of water reduction from 23.11 to 14.38% was observed. The water loss of rice showed a trend of rapid change and slow change. The analysis of the correlation between daily water loss and ambient temperature and humidity shows that the daily water loss is positively correlated with the ambient temperature, and the correlation is significant. It is negatively correlated with the relative humidity of the environment and the correlation is not significant. During the drying period, the number of fungal spores in the rice field gradually increased in the early stage, began to decline after reaching the highest value on September 28, and the number of stored fungal spores gradually increased, and exceeded the number of field spores in the late drying period. Mechanical drying was performed using a Guwang 5HXG-15B circulating dryer. The drying temperature was set at 42°C, and the water content was reduced from 23.1 to 11.8% after a total of 5 hours. The changes of spore count, fatty acid value, germination rate, waist burst rate, whole polished rice rate, and taste value of rice mold after drying were studied. The results showed that compared with the mechanical drying, the natural rice has a lower popping rate, a higher rate of whole milling, and a better processing quality. The germination rate of the dried rice is much lower than that of natural dry rice. In addition, the fatty acid value of natural dry rice is greater than mechanical drying, which may be caused by the natural drying time is too long. Using the taste meter to comprehensively score the rice obtained by the two drying methods, the natural dry rice score was 89.3, which was greater than the mechanical drying of 83.7, and it was found that the nature of natural drying and taste was better.

Comparing the drying results of the two methods of drying rice, it can be seen that natural dry rice has a small amount of processing and slow precipitation, but it has a good protection effect on rice processing, germination, and taste quality. While mechanical drying is relatively large and rapid with respect to rice batches, it has a great influence on rice quality. With the increasing living standards of the people, more and more importance is attached to the quality of rice. Therefore, in the future research process, it is necessary to further improve the drying machinery technology and improve the quality of rice.

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Germination rates of frozen grain legume seeds in Cameroon

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Abstract

A project on collection and conservation of genetic resources was carried out in Cameroon in 2014 in villages around Yaounde, Mbalmayo, and Ebolowa. Samples of all grain legume species cultivated by the farmers were collected from the 15th of March till early May 2015. Farmers in these zones cultivate mostly ground nuts, followed by soybean and cowpea. A total of 39, 13, and 45 samples were collected from Yaounde, Mbalmayo, and Ebolowa, respectively. After collection, samples were sun-dried, treated, labeled, plasticized, and stored in the freezer at -20°C in the Institute of Agricultural Research for Development (IRAD) store room at Nkolbisson, Yaounde. A trial was carried out at IRAD Kumba experimental farms in 2016 to purify and maintain 14 cowpea and 12 groundnut samples from the freezer, under the C2D project. There were highly significant differences ($P< 0.05$) amongst samples (treatments) for the germination rate. Cowpea samples had a germination rate ranging from 0.33 to 47.67%, while germination rates for groundnuts were between16.67 to 68.33%. Out of the 26 samples, only 5 (19%) had germination rates above 50%. Due to irregular power supply, freezing turned out to be an ineffective storage method for grain legume seeds. Seeds are now being maintained in vivo in small quantities and on seasonal basis which renders the job of plants breeders very difficult and ineffective. Alternatives storage methods and facilities for grains and seeds in developing countries like Cameroon remain an urgent need to boost research and ensure food security.

Keywords: conservation, grain legume, germination, seeds, developing countries.

Introduction

Biodiversity is the third component of biological diversity (the other two being the specific diversity (the individuals) and ecosystem diversity (populations and their habitats)). Biodiversity may refer to wild biodiversity and agricultural biodiversity. Since the Conference in Rio de Janeiro in 1992 organized by the Convention on Biological Diversity (CBD), to which Cameroon is party, other international legal instruments have been put in place in order to ensure the implementation of the relevant provisions of the CBD. Among these instruments signed and/or ratified by Cameroon, we may cite the International Treaty on Plant Genetic Resources for Food and Agriculture on the

facilitated access and benefit-sharing in the multilateral system of the Treaty, and the Protocol of Nagoya on access to genetic resources and the fair and equitable sharing of benefits arising from their exploitation.

Genetic resources are, in effect, the basis of variation. The development of plant varieties and animal breeds constitutes the main axis of research in order to increase agricultural production to ensure the food security of populations. This can be done either by conventional selection methods or by biotechnology which lead to the creation of genetically modified organisms (GMOs). Therefore, genetic resources, both exotic and landraces, constitute the essential raw material for researchers in selection and improvement of cultivated plants and animals.

The diversity of cultivated plants, animals, fisheries, and forest products are the 4 main components of agricultural biodiversity, and also are essential to the satisfaction of tastes and preferences of consumers that change constantly following culture and economic fluctuations.

The diversity of landraces cultivated by farmers is not sufficiently known to researchers, but nevertheless represents a reservoir for the search for genes important in the creation of varieties adapted to the unpredictable changes in the environment. These landraces, having been cultivated for many years, have acquired a stability of performance (Marchenay and Lagarde, 1986).

In the context of Cameroon, Nya Ngatchou Fondoun published a report of 385 pages in 1987 entitled "Inventories of plant genetic resources in the structures of the Institute of Agronomic Research (IRA)". This document, devoted to plant genetic resources, contained collections in the fields and in the cold rooms with a total of 9500 accessions. Of these, 5240 accessions were unimproved genetic material and 4260 accessions were improved. At that time, the Institute of Agronomic Research (IRA) still enjoyed a relative comfort in terms of preservation infrastructures and financial and human resources. The economic crisis of the mid-1980s led to financial hardship, and the samples were abandoned in the cold rooms. These lasted for 20 years, during which significant losses of genetic material were recorded in both *in situ* and *ex situ* collections. Recently, few studies have been carried out on the collection and characterization of grain legumes in Northern Cameroon (Gonne et al., 2013) and nation wide (Atemkeng and Yousseu, 2017). The results indicated that most of the landraces collected in the 1980s have become extinct. There was therefore the need to recollect and conserve local plant genetic resources for the development of new improved crop varieties. The objectives of this project was to collect and ensure the conservation of the major grain legume genetic resources cultivated by farmers in Southern Cameroon.

Materials and Methods

With funding from the public investment budget (PIB 2014), a project was carried out entitled: collection and conservation of genetic resources in Cameroon. A team made up of researchers, technicians, agricultural extension workers, and village facilitators worked as three groups in villages around Yaounde (Nkolfoulou, Minkoameyos, Elig Essomballa, Elumdem, Nkometou III, Mendong, Simbock), Mbalmayo (Nkolnguet, Melombo, Ekombitie) and Ebolowa (Nkoemvone, Biba, Assoo'seng, Ndengue). Samples of all grain legume species cultivated by the farmers were collected from the 15th of March till early May 2015. Farmers were contacted on the farm, market, and at times at home in the evening. In the process, the objective of the project was explained to farmers and some incentives in form of in-kind donations given to them to supply the samples. After collection, samples were sun-dried, treated, labeled, plasticized, and stored in the freezer at -20^{o}C in the Institute of Agricultural Research for Development (IRAD) store room at Nkolbisson, Yaounde (Figure 1). A trial was carried out at IRAD Kumba experimental farms from September 2016 to December 2016 to purify and maintain 14 cowpea and 12 groundnut samples from the freezer, under the C2D project. The data collected were germination rate, days to first flowering, days to 50% flowering, number of seeds per pod, number of pods per plant, pest and disease scores, and grain yield per hectare. Here only data on germination rates is reported.

Data analysis

Analysis of variance was done using the R – package version 2016 and multiple mean separations were done using the Tukey test.

Fig. 1 Grain Legume Samples frozen at -20°C.

Results

Farmers in these zones cultivate mostly ground nuts, followed by soybean and cowpea. Very few farmers cultivate common bean. The samples ranged from 20 to 50 grams per sample (Figure 2). A total of 39, 13, and 45 samples were collected from Yaounde, Mbalmayo, and Ebolowa, respectively. There were highly significant differences (P< 0.05) amongst samples for the germination rate in cowpea, while no significant differences existed among groundnut samples. Cowpea samples had a germination rate ranging from 0.33 to 47.67% (Table 1), while germination rates for groundnuts were between16.67 to 68.33% (Table 2). Out of the 26 samples, only 5 samples (19%) had germination rates above 50% (Tables 1 and 2).

Discussion

The results indicate that only 19% of the frozen samples had germination rates above 50%. This might have been caused by irregular power supply and low voltage. Consequently, freezing, which is very effective for storing grains for years, turned out to be an ineffective storage method for grain legume seeds in Cameroon. The efforts of the team went in vain, and the budget allocated for the project was sort of wasted as phase two of the project could not be implemented due to low germination rates. Seeds are now being maintained *in vivo* in small quantities and on a seasonal basis which renders the job of plant breeders very difficult and ineffective. Alternative storage methods and facilities for grains and seeds in developing countries like Cameroon remain an urgent need to boost research and ensure food security.

Fig. 2: Grain legume samples from farmers ranged between 20 to 50 grams per sample.

Tab. 1: Germination rates of cowpea seeds frozen for two years at -20°C.

Cowpea gerplasm	Germination rate
Accession 1	0.33a
Accession 2	16.67ab
Accession 3	30.00ab
Accession 4	47.67bc
Accession 5	20.00ab
Accession 6	15.00ab
Accession 7	36.67ab
Accession 8	13.67ab
Accession 9	17.00ab
Accession 10	21.67ab
Accession 11	14.67ab
Accession 12	9.67ab
Accession 13	16.67ab
Accession 14	20.33ab
Accession 15	**76.67c**
DF	14
F	1.715
P	<0.001

Means followed by the same letters are not significantly different ($P< 0.05$). Highlighted samples had germination rates above 50%.

Tab. 2 Germination rates of Groundnut seeds frozen for two years at -20°C

Variety	Germination rate (%)
Aboul Niveau	**68.33**
Afoumou	36.33
ICGV 86003	**59.67**
JL 24	50.67
Manipinta	**58.00**
Mfoumou	29.33
Minkonga	**54.00**
Ngomomou	20.00
Ngomomou Congo	25.67
Ngoxomou	34.00
Ossa owondo	45.33
Zebedee	16.67
DF	11
F	0.959
P	0.506

Means are not significantly different ($P< 0.05$). Highlighted samples had germination rates above 50%.

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Bioefficacy of Cameroonian Hemizygia welwitschii Rolfe-Ashby (Lamiaceae) leaf powder against Callosobruchus maculatus Fabricius in stored cowpeas seeds

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This work aims to evaluate the efficacy of Cameroonian Hemizygia welwitschii leaf powder against C. maculatus. The H. welwitschii leaf powder was applied at four different dosages 0.25, 0.5, 1 and 2 g/50g (corresponding to 5, 10, 20 and 40 g/kg) and SilicoSec (positive control) at 0.025, 0.05, 0.075 and 0.1 g/50g of cowpea (corresponding to 0.5, 1, 1.5 and 2 g/kg) and the untreated control (0 g/50g). 20 unsexed adults were introduced into the test jars to evaluate adult mortality and F1 progeny. To assess damage and seed viability, 30 unsexed insects were added to jars treated at the same concentration. Adult?s mortality was recorded at 1, 3, 5 and 7 days after treatment (DAT), damage and seed viability were evaluated after three months of storage. All the experiments were arranged in a completely randomized design with four replications. From the results obtained, the highest mortality rate (82.50%) was recorded in jar treated with H. welwitschii at 40 g/kg compared to 100% for SilicoSec (2 g/kg) at 7 DAT. Like SilicoSec, H. welwitschii significantly ($P < 0.001$) reduced the number of F1 progeny compared to the untreated control. Seed damage was found to decrease with increase in concentration of insecticide within the three months of storage. Germination rate of cowpea seeds treated with the highest dosage (40 g/kg) of H. welwitschii powder were 72.50% and for SilicoSec was 87.50% (1.5 g/kg). Our findings show that the leaf powder of H. welwitschii is very effective in protecting stored cowpea seeds against C. maculatus infestation and could be exploited by farmers.

Session 2
Biology, Ecology and Behavior

Insect infestation sources in stored maize grain; what is more important resident versus incoming infestation?

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Abstract

Most studies targeted pest control inside stores; incognisant of the population dynamics in the store vicinity; leading to product re-infestation. Distinction between storage insect pest source and sink grain patches is important for effective pest management strategies. We examined the role of resident versus incoming insect infestation in phosphine-fumigated closed or open and unfumigated closed or open maize farm stores. Grain quality measurements were recorded over 32 weeks for two storage seasons. Whether open or closed, fumigated grain had significantly lower ($p < 0.001$) grain damage and lower grain weight loss ($p < 0.05$) than unfumigated grain. Fumigated open stores had significantly higher ($p= 0.004$) grain damage and weight loss than closed ones. Grain damage was higher in unfumigated-closed than fumigated-open, evidence that resident infestation inflicted higher food loss than incoming infestation. *Prostephanus truncatus*, *Cryptolestes ferrugineus* and *Tribolium castaneum* had significantly higher populations ($p < 0.001$, $p = 0.018$ and $p = 0.001$; respectively) at bottom levels of unfumigated and fumigated grain (*T. castaneum*). *Sitotroga cerealella* and *Sitophilus zeamais* were significantly higher ($p < 0.001$) at the top of closed than open unfumigated compartments. Grain suffers less infestation and quality loss when it is a sink patch than when it is a source patch. Population build-up and 'settling' to inflict significant food loss takes longer for incoming compared to resident infestation. These results have ecological implications on postharvest IPM.

Key words: Grain sink-source patches, closed and open grain stores, fumigated and unfumigated grain, grain insect damage, grain weight loss, storage insect pests

1. Introduction

Stored product insect pests ecology has not been accorded the systematic scientific investigation it deserves, but effective stored product IPM from any perspective requires the understanding of insect pest behaviour and bionomics. Stored grain, compared to any other insect pest habitats resembles a unique and largely homogeneous habitat in which food availability for many storage pests is unlimited, making a perfect ecological system from which we can better understand the population dynamics, relationships and associations between storage pests (Athanassiou et al., 2005; Nansen et al., 2009). Studies on the ecology of most storage pests of maize have been done in laboratories giving results that are thus limited in scope of application to the farm situation. Farmer-managed stores have very diverse spectra of species, complex levels of inter-, and intra-specific competition, environmental conditions and the presence or absence of natural enemies that influence field ecological studies (Mvumi et al., 2003; Nansen et al., 2009). Therefore a study of the ecology of the maize pest complex on-station is meant to determine *in situ* activities of insect pests and associated trends in grain damage and weight loss. Many stored product pests are highly mobile and can freely move in and out of storage facilities (Campbell and Arbogast, 2004), however, it is often thought that grain insect pest infestation is largely facilitated by human activities during grain exchange, transportation and way of storage. Since insect pest status is often partly derived from their mobility to colonise unexploited grain patches, we set out to determine whether storage losses are higher when the grain patch acted as a sink (colonised only by incoming new infestation)

or as a source (colonised only by resident infestation). In the process, we determine insect succession in grain infestation, the abundance and length of storage period. We relate this to the damage and weight loss to get an indepth understanding of the stored grain ecosystem to enable development of postharvest IPM (Athanassiou et al., 2005; Carvalho et al., 2013). It has been reported, that the key to controlling stored product pests is to explore the potential connection between resident infestation (inside stored grain) and outdoor populations (Campbell and Toews, 2005). This is important to reduce the cost and risk associated with chemical pesticides (Campbell and Arbogast, 2004) in stored grain. The main objectives of the current study were to (1) determine which source of insect pest infestation between the resident (field infestation) and incoming (re-infestation) caused more grain damage and weight lossthan the other, (2) determine the trends in populations of different insect pest species over a storage season both on pest-free (fumigated) and on field-infested (unfumigated) grain; and (3)to investigate population dynamics, grain damage and weight loss and associated pest species in bulk grain in relation to granary depth as in (Athanassiou et al., 2005).

2. Materials and Methods

2.1 Granary preparation

The experiment was carried out at the Institute of Agricultural Engineering (IAE, Harare, Zimbabwe) in the granaries. Three granaries were selected, repaired, thoroughly cleaned and re-plastered using clay and small amounts of cow-dung (to prevent the clay from cracking), as per typical farmer practice.

2.2. Treatments

One tonne of shelled maize (SC 637 hybrid variety) was fumigated using phosphine tablets (Phostoxin®, Detia-Degesch GmbH, Aluminium phosphide 56% w/w + inert ingredients 44% w/w) at the recommended rate. Fumigation was done in a metal silo of volume 2.395 m3. The metal silo was placed on a strong iron bench and loaded with the grain. Ten tablets were applied to the grain at different levels (3 at the bottom, 4 at the middle and 3 at the top). This was achieved by driving a metal pipe to the desired level and then dropping the tablet through the metal pipe. The spouts of the metal silo were then immediately closed using custom-made tight fitting lids followed by extensively wrapped with packaging tape to make the silo air tight.

About 900 kg of the fumigated grain were weighed and separated into six portions of 150 kg each. These portions were loaded into granary compartments in three granaries (blocks). Each granary had two compartments loaded with the fumigated grain, immediately after loading one compartment was closed and sealed completely while the other was left open. The closed compartments (Fumigated Closed and Unfumigated Closed) were fitted with tightly closing doors whose surfaces were then plastered using clayey soil to make a continuous seal with the wall plastering. The same was repeated with un-fumigated grain. The grain treatments are shown in Table 1.

Tab. 1 Grain treatments.

Grain treatment	Entrance status	Treatment code
Fumigated	Open	FO
Fumigated	Closed	FC
Not fumigated (unfumigated)	Closed	UFC
Not fumigated (unfumigated)	Open	UFO

2.3 Grain sampling frequency

After every four weeks, grain samples were withdrawn collected using a multi-slotted double tube brass sampling spear (about 1.2 m long). The spear was dipped vertically inside the grain whilst it

was closed, it was then opened when its tip touched the bottom, before being shaken to enable grain to enter, then it was closed. The sampling pattern in each granary compartment was as shown in Figure 6. The depth of the grain (60 cm) in each compartment enabled sampling to be conducted from the top (50-60 cm), middle (20-30 cm) and bottom (0-10 cm) positions in the granary. Grain sampled from each level was packed and labelled separately. This was meant to enable observation of the differences in grain damage and pest densities and distribution between the top, middle and bottom layers of stored grain. Samples from each point per level were bulked to make a composite sample of size approximately 1 kg.

2.4. Data collection and analysis

For each 1kg grain sample, all insect pest species, were identified, counted and recorded. Insect-damaged and undamaged grains were separated, counted and weighed. This was achieved by dividing each sample into four equal sub-samples using a riffle sample divider. A sample was first poured out from the sample bag into the riffle divider to produce two equal sub-samples. These were each further divided in the same manner to produce a total of four equal sub-samples. Grain from three sub-samples were each poured out into white plastic trays and examined for insect damage. The fourth sub-sample was not considered. Data from the three sub-samples were averaged to give a sample average for damage and weight loss. Trash weight and insect counts were done for the entire sample. Data on grain damage (%) was arcsine square root–transformed before being analysed. Data on grain weight loss (%) were analysed without any transformation. Data on insect numbers for each species were $\sqrt{(x+1)}$ -transformed (Fowler et al., 1998). All the data were then subjected to one way analysis of variance (ANOVA) in STATISTICA 13.3.Where the F-ratio was significant ($p < 0.05$), means were separated by Tukey-Kramer's HSD test.

3. Results

In season 1, grain damage started increase notably in the unfumigated grain (UFC and UFO) from week 12 - 32 (Fig 1A) and from week 8 – 32 in season 2 (Fig. 1B). Generally, unfumigated grain showed consistently significantly higher ($F_{(24, 288)} = 2.810$, $p = 0.0002$) grain damage than the fumigated grain regardless of being closed or open from week 12-28. In both seasons, at week 32, only the unfumigated open (UFO) had significantly higher grain damage ($p < 0.001$) than fumigated closed (FC). However, lack of significant differences between fumigated open (FO) (no resident infestation) and unfumigated closed (UFC) (with resident infestation) in both seasons signified that both sources of infestation were equally important over time (Fig 1 A and B). In both seasons, there was a significant interaction ($F_{(24, 288)} = 2.810$, $p = 0.0002$) (Season 1) and ($F_{(24, 288)} = 1.7711$, $p = 0.0161$) (Season 2), signifying that grain damage was significantly affected by the treatments over time (Fig 1A &B).

Grain weight loss was more pronounced in season 2 than in season 1 (Fig 2A & B). As observed in grain damage, significant increase was observed from week 12. Generally, unfumigated grain (UFC and UFO) specifically showed persistently significantly higher grain weight loss ($F_{(24, 288)} = 2.7946$, $p = 0.0003$ than fumigated grain (FC and FO) between 12-28 weeks in season 1. Again, in season 1, UFC showed consistently high grain weigh loss ($p < 0.001$) than FC between 12-28 weeks but was not significantly different from UFO. At week 32, although UFO had significantly higher grain weight loss than both FO and FC ($p < 0.001$), there were no significant differences between FO and UFC, again signifying that the visiting infestation (FO) and resident infestation (UFC) had equal similar impact on grain weight loss (Fig 2A). In season 2 however, there were no notable increases in grain weight loss from 0 – 24 weeks. Nevertheless, from week 28 – 32, unfumigated (UFC and UFO) grain started showing higher grain weight loss ($F_{(8, 297)} = 16.556$, $p = 0.0001$ than the fumigated grain (FC and FO). This showed that resident infestation had more negative impact than incoming infestation.

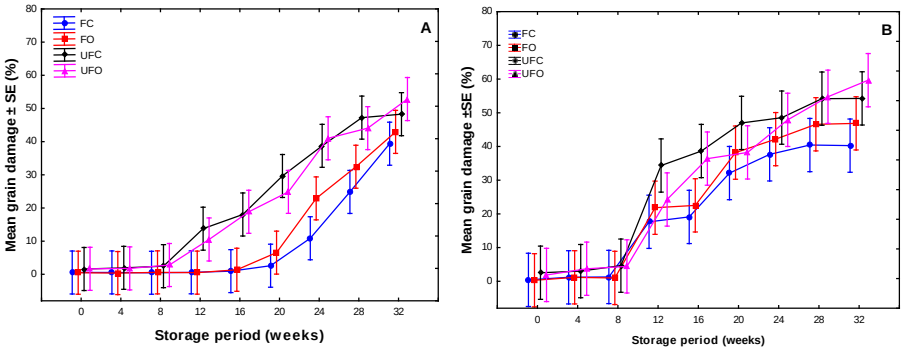


Fig. 1 Grain damage in (A) season 1 and (B) season 2 for different treatments: FC = Fumigated closed; FO = Fumigated open, UFC = Unfumigated closed and UFO = Unfumigated open

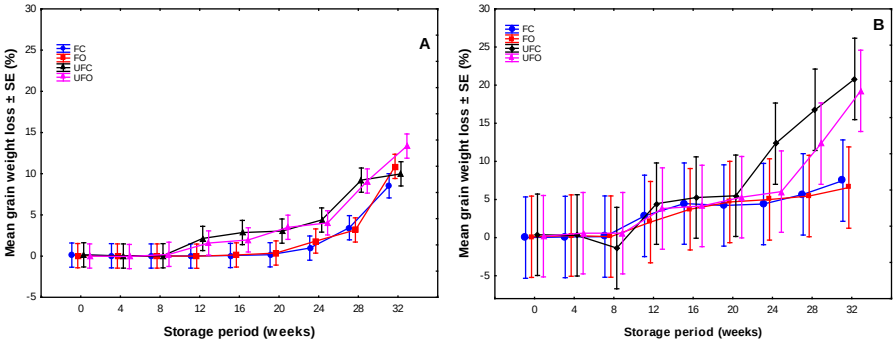


Fig. 2 Grain weight loss in (A) season 1 and (B) season 2 for different in different treatments: FC = Fumigated closed; FO = Fumigated open, UFC = Unfumigated closed and UFO = Unfumigated open.

In both seasons the opening or closing of the granary entrance did not show significant effect on grain weight loss compared to fumigation and non-fumigation. There was a significant interaction ($F_{24, 288} = 2.7946$, $p = 0.0003$) between the length of storage period and treatments on grain weight loss for season 1, showing that the length of storage period affected grain weight loss for each treatment. However, this was not the case for some treatments in season 2 ($F_{(16, 297)} = 1.2473$, $p = 0.231$).

We assessed the evolution of grain damage along the depth of the grain. In the granary in both seasons, grain damage was consistently low and constant for the first 8 weeks; significant increase changes at was observed from 12 weeks (Fig. 3A and B). Generally, the TOP layers of the grain had consistently higher grain damage in both season 1 ($F_{(16, 297)} = 2.3306$, $p = 0.00295$) and season 2 ($F_{(16, 297)} = 2.8282$, $p = 0.00027$) than the middle (MID) and the bottom (BOT) levels. The latter were not significantly different from each other in both seasons (Fig. 3A and B).

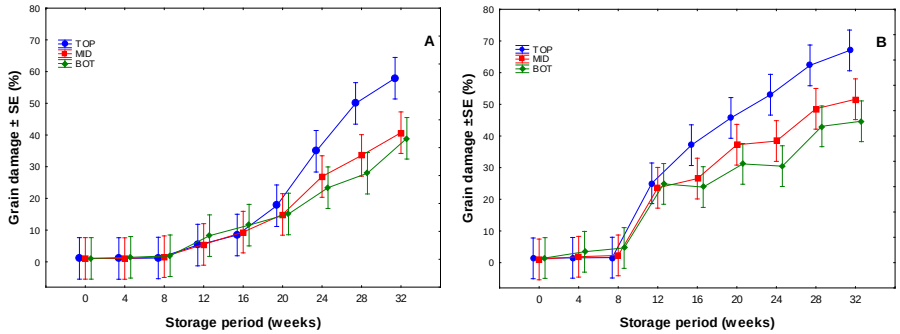


Fig. 3 Grain damage along the depth of grain at top (TOP), middle (MID) and bottom (BOT) in (A) season 1 and (B) season 2.

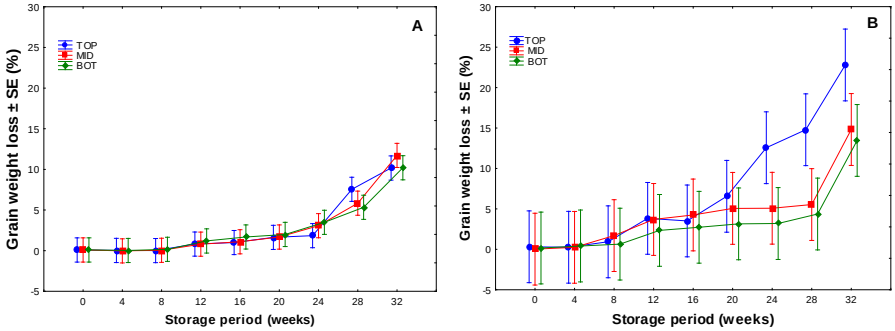
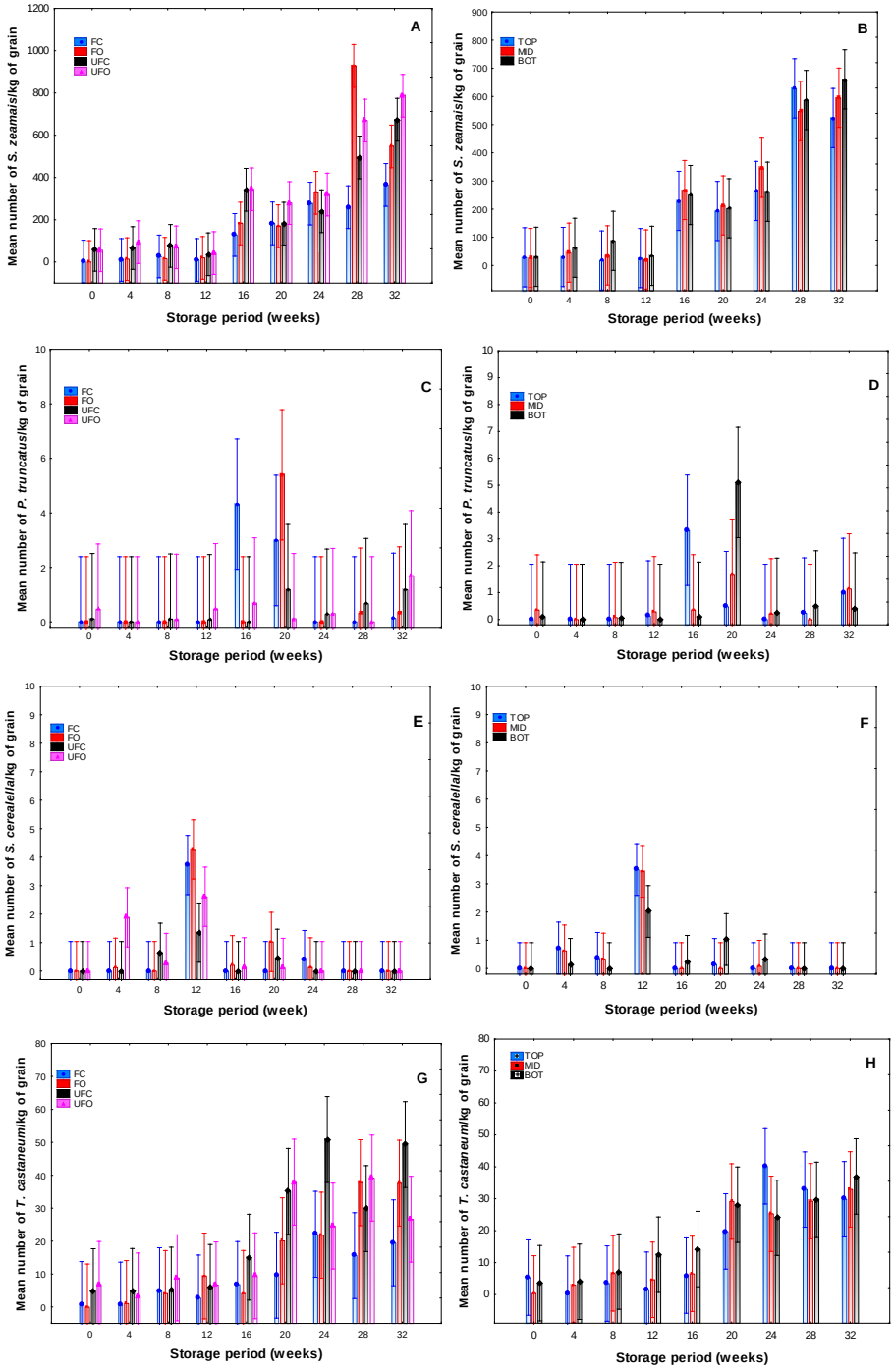


Fig. 4 Grain weight loss along the depth of grain at top (TOP), middle (MID) and bottom (BOT) in (A) season 1 and (B) season 2.

For grain weight loss however, notable increase was observed in week 28 through to week 32; with MID and BOT showing significant differences ($F_{(8, 297)} = 68.086$, $p < 0.0001$) between week 28 and 32. Nevertheless, the three levels did not show any significant differences ($F_{(16, 297)} = 0.66814$, $p = 0.82477$) among each other in season 1 (Fig 4A). This was inconsistent with season 2 which showed significantly higher ($F_{(2, 297)} = 16.555$, $p < 0.0001$) grain weight loss at the TOP level than the MID and BOT (28 weeks) and on the BOT only in week 32 (Fig 4B).

There was no significant interaction ($p = 0.82477$) (season 1) and ($p = 0.23100$) (season 2) between the level of grain and the length of the storage period on grain weight loss. This implies that in our results, length of storage period did not significantly influence grain weight loss for each grain level sampled.

For the sake of brevity, results reported for insect pest populations are for the second storage season only (2013/14). General increase in *S. zeamais* populations in grain was observed from 16 weeks of storage through to 32. Significantly higher ($p < 0.001$) was recorded in FO (922.3 insects/kg of grain) at 28 weeks.


A
Mean number of S. zeamais/kg of grain
FC
FO
UFC
UFO
Storage period (weeks)
B
Mean number of S. zeamais/kg of grain
TOP
MID
BOT
Storage period (weeks)
C
Mean number of P. truncatus/kg of grain
FC
FO
UFC
UFO
Storage period (weeks)
D
Mean number of P. truncatus/kg of grain
TOP
MID
BOT
Storage period (weeks)
E
Mean number of S. cerealella/kg of grain
FC
FO
UFC
UFO
Storage period (week)
F
Mean number of S. cerealella/kg of grain
TOP
MID
BOT
Storage period (weeks)
G
Mean number of T. castaneum/kg of grain
FC
FO
UFC
UFO
Storage period (weeks)
H
Mean number of T. castaneum/kg of grain
TOP
MID
BOT
Storage period (weeks)

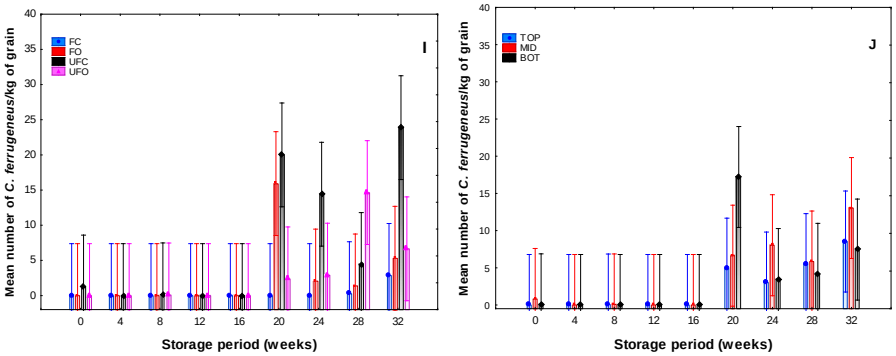


Fig. 5 Number of adult insects recorded over a 32 week storage period for each treatment and at different levels of grain depth (A &B) *S. zeamais*, (C & D) *P. truncatus*, (E & F) *S. cerealella*, (G & H) *T. castaneum*, and (I & J) *C. ferrugeneus*. (FC = fumigated closed, FO = fumigated open, UFC = unfumigated closed, UFO = unfumigated open; TOP, MID and BOT represent the top, middle and bottom level of grain depth in granary).

Unfumigated grain showed consistently high populations of *S. zeamais* up to 783.04 and 676.7 insects/kg of grain respectively at 32 weeks which did not significantly differ from each other. At termination, UFO (774.9 insects/kg) had significantly higher ($F_{(24, 288)} = 4.4915$, $p < 0.001$) *S. zeamais* populations than FC (357.9 insects/kg) (Fig 5A). Along the depth of the grain, although there was a general increase in populations from week 16-32, *S. zeamais* did not show significant ($F_{(16, 297)} = 0.40373$, $p = 0.9814$) preference for any specific level (Fig 5B). On the other hand, *P. truncatus* (Fig 5C and D) was detected in much lower (<10 insects/kg of grain) compared to *S. zeamais* and did not generally show significant differences between treatments ($F_{(24, 288)} = 0.84815$, $p = 0.67324$) and grain depth levels ($F_{(16, 297)} = 1.0419$, $p = 0.41205$ (Fig 5D). At peak populations (20 weeks) however, the bottom (BOT) level had significantly higher ($p < 0.001$) *P. truncatus* than the top (TOP) (Fig 5D), signifying *P. truncatus* tendency to concentrate at the bottom. *Sitotroga cerealella* increased quite earlier in storage (12 weeks)(Fig 5E) compared to other insect species. FO and FC had significantly higher ($F_{(8, 288)} = 13.175$, $p < 0.001$) populations than UFC, signifying that resident infestation had less impact in population build up compared to incoming infestation for this species. At peak populations, *S. cerealella* was significantly ($p < 0.001$) concentrated at the TOP and MID levels than the BOT (Fig 5F).

Tribolium castaneum and *Cryptolestes ferrugenius* were the major secondary pests recorded in this study. Significant increases in *T. castaneum* were observed from week 20 – 32, where it fluctuated in abundance between different treatments (Fig 5G and H). At week 20, *T castaneum* was more dominant in unfumigated grain (UFC and UFO), whereas at week 28, it was more dominant in both fumigated (FO) and unfumigated (UFO) open granaries (Fig 5G). This indicated that incoming infestation played a major role in population built up. On the contrary, at week 28 and 32, high *T. castaneum* populations were recorded in unfumigated closed (UFC) (Fig 5G) grain signifying the important role of resident infestation in population buildup. Although each of TOP, MID and BOT showed a significant increase in *T. castaneum* population over the storage period ($F_{(8, 297)} = 14.578$, $p < 0.001$), there were no significant differences between the different grain depths ($F_{(2, 297)} = 0.49571$, $p = 0.60964$) (Fig 5H). *Cryptolestes ferrugenius* was dominant in unfumigated closed (UFC) grain at weeks 20, 24 and 32 where it was significantly higher ($F_{(24, 288)}=1.8132$, $p = 0.001276$) than FC and UFO signifying the dominance of resident infestation(Fig 5I) There were no significant differences ($F_{(2, 297)}=.41696$, $p =.65943$) in the number of *C. ferrugenius* between different grain depths (Fig 5J).

4. Discussion

Regardless of being closed or open, the unfumigated grain recorded more damage and weight loss in both seasons, suggesting that resident infestation is very critical in food loss. However, open granaries generally recorded higher damage than closed ones especially at the top surfaces

signifying the importance of visiting infestation (re-infestation). Nevertheless, the significant differences observed in grain damage between the fumigated and unfumigated treatments attests to the fact that resident infestations play the major role in both grain damage and grain weight loss. This coupled with the low insect numbers in all fumigated closed and open compartments meant that incoming insects, although it should be carefully considered, does not play a key role in building up enough populations to ellicit significant grain damage and weight loss in initially pest-free grain especially in the short term.

The trend of *S. zeamais* populations remained fairly stable for the first 8 weeks, and began to show rapid increases from week 12, where higher numbers were observed at the middle and the bottom than the top layers of the granaries. In the unfumigated open environments, there were consistently higher *S. zeamais* populations at the top from around week 16. This agrees with reports by Mvumi et al. (2003) that *S. oryzae* (closely related to *S. zeamais*) in sorghum is consistently concentrated at the top levels. It is also interesting to note that there were very low populations in fumigated grain whether it was kept closed or open especially in the first 16 weeks. This shows that incoming infestations take time to build up as compared to resident ones. In open granaries, *S. zeamais* populations started to increase significantly at 16 weeks and were mainly concentrated at the top grain layers. In the closed granaries, the populations were higher at the bottom and middle layers. Campbell et al. (2006) explained that inside and outside grain storage structures, *S. zeamais* has patchy spatial and temporal distributions around the food source without a specifically apparent pattern (see also Throne and Cline, 1989). This is because of their high mobility on stored grain. Another possible explanation is that when the granary is open, insects are attracted to light and concentrate at the top layers, in addition, this within-store spatial distribution is also affected by temperature (seasons) (Athanassiou et al., 2005). Open granaries enabled insects to communicate with the outside environment by voluntary in and out movements.

Prostephanus truncatus did not occur in large numbers, but where it occurred, it was mainly found at the bottom layers, confirming reports by Vowotor et al. (2005) that the bostrychid favours bottom layers. It is postulated that bottom levels provides pressure from the grain above and *P. truncatus* manipulates this pressure to anchor its hind legs and bore into compacted maize kernels in straight lines (Vowotor et al., 2005). *Prostephanus truncatus* population trends showed that it began to appear at 16-20 weeks in fumigated open granaries at the bottom layers albeit at relatively lower populations compared to other species. This suggested that the population developed from incoming rather than resident infestation, from this standpoint, the low *P. truncatus* populations can also be explained by the fact that maize grain may not have as strong volatiles that attract *P. truncatus* compared to other comodities, e.g Cassava (Pike et al., 1994). Like *P. truncatus*, although there were fluctuations in the numbers, *S. cerealella*, was mainly detected in UFO and FO mainly at top and middle levels. This again resonates with Mvumi et al. (2002) who reported the same vertical gradient of *S. cerealella* along the depth of the grain. The invasion of fumigated grain by *S. cerealella* shows its ability to invade new territories as a primary moth and almost always appearing as the first pest on clean undamaged grain. The low numbers of *S. cerealella* observed in this study are attributable to the rapid movement and invasive nature of the moths as also reported for a similar moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Campbell and Arbogast, 2004) which could not be captured in significant populations due to the limitations of the sampling methods employed.

The source of infestation for all the fumigated closed compartments is not clear. Possibilities are that grain was infested in transit from the fumigation site to the granaries, or the re-plastering in granaries was not thorough enough to block resident insects in cracks and crevices inside the granary compartments. It is also possible that grain was infested during the short periods when these compartments were opened for sampling. Resistance of these species to the fumigant aluminium phosphide cannot also be ruled out (Daglish et al., 2004). Benhalima et al. (2004) reported detecting phosphine resistance by *S. oryzae* in Morocco and acknowledged receiving similar reports from many other countries due to the overuse of the fumigant.

We conclude that grain suffers more damage when it acts as the source patch than the sink patch. Resident infestation elicits more grain damage and weight loss than visiting infestation in the short term; but both elicit equal losses in the long term. *P. truncatus* and *C. ferrugenius* prefer the bottom levels of grain, whereas *S. cerealella* prefers top levels. *T castaneum* and *S. zeamais* did not show any specific grain depth preferences.

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Climate change and its implications on stored food grains

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Abstract

Safe food grain storages are considered as a measure to adapt to the changing global climates and as a channel to food security, particularly in periods when agriculture fails. However, grain storage themselves can be heavily affected by changing global climates. One main aspect of the 'climate change' is the rise of global temperature that may lead to an increase in atmospheric humidity. This climate change, warm and humid, are not suitable for grain storage. At such a scenario, stored grain is at a risk due to the favorable conditions developed for the growth of insect pests. Predicting the future ecological impact of climate change drivers requires understanding how these same drivers have acted in the past on the dynamics of insect's population. In the past ten years there has been a detailed documentation on the biotic and abiotic conditions of two storage sites in Israel. This historical ecological data can reveal long-term consequences of multiple drivers of climate change. The changes can be evident at the level of the species and at the level of the societies of insect-pest in the grain storage. The differences between two storages located at different climate regions in Israel further predict the direction current IPM practice may lead to. Following this understanding, we hope to develop feasible mitigation strategies that might overcome the changes ahead of us.

Keywords: Climate change, Historical ecology, grain storage insects

Introduction

The annual consumption of grain for human and animal consumption in Israel is about 5.1 million tons worth about 1.5 billion dollars. The insects living in grain storage can cause a great deal of economic damage: reduction in quantity and quality of the grains. In developing countries the damage can reach 30-50% and in developed countries the weight loss of grain after a prolonged storage period reaches up to 5-10%. In recent years, there is an increase in the population of grain storage insects [1]. There is a struggle to control the increase via raising the quantities and frequency of the provision of pesticides. This study examines the effect of climate change on the increase of insect populations in storage.

Much work has been done to examine the appropriate climatic and geographical position for grain storage. However, there is limited information to allow careful examination of the effect of climate change on the biotic factors, their interactions and their impact on the vulnerability of the grain storage. Insects infesting grain storages, among them: *Sitophilus oryzae* (Linnaeus), *Tribolium castaneum* (Herbst), *Rhizopertha dominica* (Fabricius), and *Oryzaephilus surinamensis* (Linnaeus), have a major effect on the quality and quantity of stored grains. They constitute a real threat that amounts to large economic losses [1]. The climatic changes can lead to changes in the geographical distribution of the pests, but also within the storage itself, such as changes in the rate of population development, an increase in the number of generations, an increase in the season of activity and changes in synchronization between the time of gathering the seeds from the field and the time of insect activity [2, 3, 4 ,5].
One of the most prominent effects on insects' success in grain storages is temperature [6]. The temperatures at which the insects can survive in a grain storage range from 8-41 ° C [1] with the optimum temperature ranging between 25 °C and 35 °C [1]. Environmental changes outside of this temperature range can directly and indirectly affect the storage insects. Climate changes can affect interactions and lead to the strengthening and / or extinction of species from the warehouse [7]. This work examines the possible significant interactions in the storage and their character (negative or positives) in light of future climate changes.

Materials and Methods

Storage facility

The study was conducted at two sites; northern site (NS) located in the Mediterranean climate zone in Israel (Jazreel Valley) and southern site (SS) located in a semi-arid zone, in the Negev desert. Each site has several storerooms (NS-10, SS-7). The storerooms are made from concrete and have solid roof. Grain introduction and fumigation are conducted yearly.

Sampling

In each storeroom temperature in-between flour grains, captured moisture of seeds and species presence were estimated monthly ranging between 1-34 locations on the grain mount, depending on the amount stored. Temperature in-between grains was estimated via a thermometer probe pushed 1 and 2 meters inside the grain mount. Grain moisture content was estimated in the lab. The presence of live insects was directly estimated via 1 kilo of seeds collected.

Results

In total, 147,328 insects were found during the entire sampling period. These can be characterized in 8 Coleoptera species and 2 Lepidoptera species (Table 1). The most abundant Coleoptera were *S. oryzae* and *O. surinamensis*. These two species correspond to >>50% of the total number of the individual collected. For Lepidoptera the most numerous Lepidoptera species were *P. interpunctata*, corresponding to 1% of the number of individuals found. Grain moisture content was in the range of 5-25%, temperature at the depth of 1 meter form the surface of the grain mount was 15-58 °C and 2 meter below surface 15-46 °C. Whereas the range of temperature in Israel during the sampling period 6-43 °C.

Site characteristics

Abiotic factors outside the storage- NS and SS annual temperature and humidity similarly deviate from the mean temperature and humidity 2008-2017. Whereas southern site is characterized in significantly higher temperature ($t_{(106)}$=5.276, P<0.0001, Fig. 1.1a,b) and lower humidity ($t_{(106)}$=3.142, P<0.0001).

Abiotic factors inside the storage- grain moisture content, temperature at 1 meter below grain surface and temperature 2 meters below surface were significantly higher at the SS (Table 2).

Insects' dynamics

SS had in total more insects than the NS (Table 2). At both sites there is a significant linear regression between the years since the commodity started to function and the level of infestation (NS; R^2=0.870, P<0.001), SS; R^2=0.737, P<0.003). In 2010 *S. oryzae* is the dominant pest both NS and SS. After 2010, in the north the dominancy alternates between *S. oryzae* and *O. surinamensis* and in the southern storage *O. surinamensis* uniquely dominates the storage. There is a significant negative correlation between *S. oryzae* and *O. surinamensis* in both sites (NS; Pearson -0.956, P<0.0001 and SS; Pearson -0.983, P<0.0001, Fig. 1). There is no evident correlation between these species and the other species in both sites (Pearson ranges between -0.204 to -0.083, P value between 0.848 to 0.628).

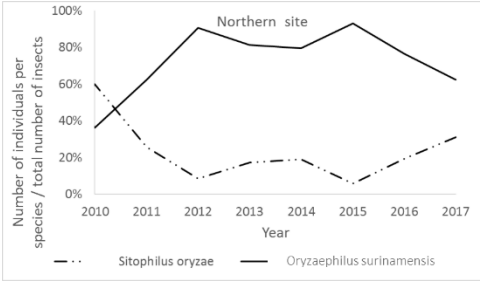

Fig. 1a Proportion of two main species found in the grain storage at the Northern Site.

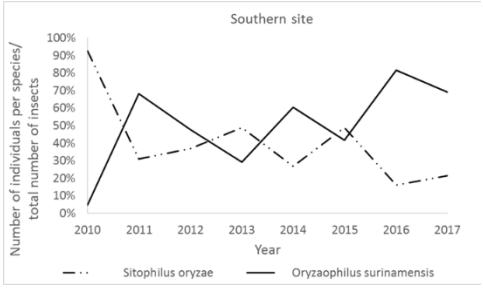

Fig. 1b Proportion of two main species found in the grain storage at the southern site.

Table 1. Insects found during the entire sampling period.

Species/taxa	*%Southern Site*	*%Northern Site*
Coleoptera		
Silvanidae		
Oryzaephilus surinamensis	55.542	66.941
Curcullonidae		
Sitophilus oryzae	24.133	14.987
Tenebrionidae		
Tribolium castaneum	12.859	11.072
Tenebrio molitor	0.000	0.014
Tenebrio mauritanicus	0.078	0.011
Bostrichidae		
Rhyzopertha dominica	0.311	0.909
Mycetophagidae		
Typhae stercorea	0.041	0.032
Cucujidae		
Cryptolestes ferrugineus	6.549	1.539
Lepidoptera		
Gelechiidae		
Sitotroga cerealella	0.063	1.384
Phycitidae		
Plodia interpunctella	0.424	3.111

Table 2. In-between grain temperature at 1 and 2 meters below grain mount surface, grain moisture content and total number of insects (both latter collected from the surface of the grain mount).

	Site	**n**	**Mean**	**± std**	**F**	**P**
Temperature (°C)-1 meter	NS	449	28.07	5.193	8.622	0.003
	SS	1300	28.23	4.547		
Temperature (°C)-2 meter	NS	796	30.67	4.273	5.390	0.020
	SS	1681	31.08	4.032		
Grain moisture content (R.h)	NS	1946	11.70	0.969	33.178	<0.0001
	SS	3734	12.09	2.897		
Total number of insects	NS	1974	18.46	66.02	16.589	<0.0001
	SS	3782	25.25	46.69		

Discussion

Our understanding of climate change and its implications on stored food grains, is still limited. The historical ecological data collected in the north and south of Israel can unravel long-term consequences of multiple drivers of climate change. The most prominent result of this study is that infestation levels are higher at the southern site of Israel. Such an observation stands in accordance with the faster developmental rate at higher temperature and humidity (so long it does not exceed the maximum temperature of survival). The main insect species that dominant the northern and southern grain storage are *S. oryzae* and *O. surinamensis*. The results of this study reveal significant negative correlation between the species. There are two non-mutually exclusive explanations; 1- dynamics between primary, *S. oryzae*, and secondary pests *O. surinamensis*. Grain damage caused by primary pest are known to facilitate colonization by secondary pests and reduce the infestation level of the primary pests [8]. 2- Each species has a unique range of optimal temperature, humidity and time of activity. This explanation stands in accordance with previous studies indicating that they have different spatial distribution [9] and temporal distribution (personal observation, Gottlieb Daphna). Although both explanation can explain the phenomenon the first is less likely as it assumes that there is a limited amount of grains in the storages. We are currently conducting a detailed analysis of monthly data to reveal the possible interaction between these species.

In both sites there is a significant linear regression between the years since the commodity started to function and the level of infestation. This can suggest that the facility itself, during the course of the years, accumulates increased amount of insects and treatment in-between storage is not sufficient or the insects developed resistance to pesticides (e.g. previous studies [10]). We are currently studying populations' dynamics within the year to reveal if a new harvest of grains initiates with high infection or if the infection level is equal in all years at the beginning of the storages but reacts differently to insecticides.

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Innovative stored plant products in Germany and the potential threat by native and invasive pest insects

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Abstract

Climate change, economic-political developments as well as new trends in diet and in bio-economy considerably influence the assortment of cultivated plants in Germany and thereby, determine the plant products which have to be stored after harvest. In the light of the International Year of Pulses 2016 and also, as a result of the European Soya Declaration, the acreage cultivated with new plants such as pulses, stress tolerant wheat varieties and also oil seed rape expanded worldwide. Due to increasing stocks of novel commodities, the emergence of economically important insects infesting stored products and the possible risk caused by native and invasive pest species have to be generally considered during storage. In this overall context, we studied the capacity of various stored-product pest insects to infest two important pulses. In laboratory tests different varieties of soy and lupine have been offered as whole seeds, grist and flour to selected moth and beetle species common in Germany. Over 14 weeks we examined the developmental time from egg to eclosion as well as the number of adults in the F1 generation compared to control insects reared on their standard feeding substrate. First findings under laboratory conditions (20-25 °C, 65-70 % RH) indicate that these innovative stored products, and in particular its simply processed plant products are highly susceptible to moths (i.a. *Ephestia elutella, Plodia interpunctella*) and to a much lesser extent also to some beetle species (i.a. *Callosobruchus chinensis, Tribolium confusum*), but the usally recommended optimal storage conditions (T ≤ 16 °C, RH ≤ 65%) can prevent a loss of volume and quality.

Keywords: pulses, soy, lupine, stored-product pests, risk of infestation

Introduction

Following the goals of the United Nations Agenda 2030 adopted in 2015 (United Nations, 2016) and the European Union's Sustainable Development Strategy adopted in 2001 (Commission of the European Communities, 2001), the German Federal Ministry of Food and Agriculture (BMEL) has started to prioritise food security and improve nutrition. In addition, the BMEL has stepped up his support for more sustainable and resilient agricultural systems, responding to advancing climate change, social-political developments and new consumer trends in diet and bio-economy.

As part of the German engagement, promoting the production of legume crops destinated for animal and human consumption, a so-called "protein crop strategy" has been initiated to improve cultivation of field beans and peas as well as that of more alternative protein consisting crops such as soy bean and various lupine species ("Eiweißpflanzenstrategie", BLE announcement, No. 20/17/31, 2017). Moreover, in July 2017 fourteen European ministers have signed the European Soy Declaration (Council of the European Union, 2017), an initiative launched by the German and the Hungarian Minister of Agriculture. Both political strategies underline that legume crops are vital to the agricultural system in Europe. As only 3% of the arable land in Europe is used for legume crops, the political guidelines aim to encourage the cultivation of pulses on the one hand and the scientific research related to the primary production of protein plants on the other. In this way, the participating countries will gain greater independence from non-European food and feed imports and also, support a diversification of crop rotation.

The domestic lupine (*Lupinus* spp.) and soybean (*Glycine max*), which have been introduced to Europa 150 years ago, represent excellent alternative protein sources and a protein-rich feedstuff. Both pulses have a protein content of up to 45% and thus, provide valuable plant-based protein for the production of food, feed and nutritional supplements (Bader et al., 2009; Hartman et al., 2011).

The yields of these legume crops per ha in Europe are comparatively high (e.g. to date in Germany the yield of soy is 34.4 dt/ha and the yield of lupine 18.2 dt/ha; Federal Statistical Office, 2017) and for soybean similar to those recorded in the USA and Brazil (www.usda.com; www.soystats.com). Nevertheless, Germany has a protein gap of approx. 2.5 million tons, which represents 65% of the 3.66 million tons of protein consumed in 2015 and therefore, still needs to import ca. 3.5 million tons of soy per year (http://www.fao.org/faostat/en/#home; www.ovid-verband.de). For this reason, it is a first success of the European and national politics that the area of soybean, cultivated mainly in southern parts of Germany, has been increased from estimated 5,000 ha in 2011 to 19,000 ha in 2017 (Donausoja, 2017). The harvested amounts for their part have increased from 13,000 to 66,000 t which represents a fourfold increase of the amount of inland harvested soybeans (Federal Statistical Office, 2017).

Currently, three agricultural lupine species are cultivated in Germany, the blue lupine (*Lupinus angustifolius*), the yellow lupine (*L. luteus*) and the white lupine (*L. albus*) (Bader et al., 2009; Bremer, 1999). The blue sweet lupine species, for example, are cultivated on about 30,000 hectares in the North German region (Ruge-Wehling et al., 2016) and about 53,000 t were harvested in 2017 (Federal Statistical Office, 2017).

In total, the arable acreage of pulses in Germany reached nearly 197,000 ha in 2017 (Federal Statistical Office, 2017). With increasing yields the stored amounts of these pulses will grow as well and aspects of stored-product protection have to take into consideration. Nevertheless, in case of infestation some main pesticides could be used for chemical protection of pulses, oilseeds and expeller but no pesticide especially approved for the use in stored soy and lupine is available in Germany.

Moreover, very little is known about stored-product pests associated with soy and/or lupine and their potential to develop on these plant products and consequently the damage they could provoke in middle latitudes. More than 60 insect species are described to occur in soy storages

(Hagstrum and Subramanyam, 2009) but central European storekeepers state that up to date pest insects do not represent an economic risk during storage of soy. Only the Mediterranean flour moth has been observed in greater numbers during storage in big bags (Ghosh and Jayas, 2010).

To get recommendations for good storage practice, the present study should clarify the following questions:

1) Can local stored-product pests infest stored soy and lupine and develop successfully?
2) How do they develop compared to those reared on standard (control) feeding substrate?
3) Does the processing level/degree of plant products have any influence on the development of pest insects?
4) How is the respective damage pattern provoked by the tested pest species?

Materials and Methods

To study the potential of common (native and invasive) stored-product pests to develop on soy and lupine adults or eggs of the respective beetle and moth species were placed together with 200 g of whole beans as well as of the simply processed plant products (grist and flour) of these pulses in rearing glass jars (3 L) covered with cotton cloth (Tab. 1). For experiments with soy the differently processed substrates were infested by adding 10 adult moths or 30 adult beetles. Whole beans of lupine were infested by adding 100 moth eggs or 50 adult beetles. Test beetles were all of the same age without taking into account sexes. Additionally, grist and flour of a mix of 4 blue sweet varieties (*Boregine, Boruta, Mitrabor, Probor*), one white sweet variety (*Energy*) and one blue bitter variety (*Karo ZS*) were infested by adding 100 moth eggs of *Plodia interpunctella* and *Ephestia elutella* at the beginning of the experiment. As control the indicated amounts of the respective standard laboratory feeding substrates (equivalent to the test substrates) for each pest species were used (Tab. 1). Each treatment was replicated 6 times.

After one week the beforehand added adults were removed and glass jars were maintained at 22 or 25 ± 1 °C and 65-70 % RH in climate chambers (Tab. 1). The glass jars were checked every two days and newly hatched adults were removed. We measured the time until first hatchings of new (F1) adults and counted the number of fully developed individuals. The mean development time from egg to adult and the number of hatched adults on soy and lupine were compared with those reared on control feeding substrate (Tab. 1). Experiments were stopped when in control jars no further hatching was observed during 5 days.

Results & Discussion

In the present study we showed that the stored-product moth species *E. elutella* and *P. interpunctell*a have the potential to multiply on stored pulses, especially on its simply processed forms, grist and flour (Tab. 2 - 4). Progeny of both moth species tested here developed well on soy beans and the corresponding processed substrates (Tab. 2). Mean number of hatched adult *P. interpunctella* on soy grist and flour was comparable to those on the control standard feeding substrate. But the development time was significantly longer on soy and hatching start of F1 adults was shifted for 3 weeks in *E. elutella* and for 2 weeks in *P. interpunctella* (Tab. 2).

Moreover, *P. interpunctella* showed the potential to develop on lupine beans and new F1 adults hatched on all varieties tested but also, significantly later and to a much lesser extent than on the control feeding substrate (Tab. 3). Most adults were counted on the white sweet lupine variety *Energy* followed by the blue bitter one *Karo ZS* and the blue sweet variety *Boruta*. On the processed plant material of lupine, *P. interpunctella* and *E. elutella*, developed better than on whole beans and in comparable numbers to the standard feeding substrate but hatching start was shifted for 2-3 weeks as well (Tab. 4). Highest numbers of hatched larvae of *P. interpunctella* were found on grist and flour of the sweet lupine mix (100% compared to control). In the two experiments, live larvae and adult individuals of moths were found and damage was displayed by feeding traces, feces and webs (Tab. 4). However, under laboratory conditions the tested stored-product pest beetles did not

develop or not at all on the different substrates and thus, demonstrated a very low risk of infestation. This is possibly due to the biology of these species which are most probably specialized on other feeding substrates than soy and lupine. Even bruchids that might start to colonize these seeds in the field directly on plant could not develop properly. In this context, no live adults of *Sitophilus granarius* were found and the substrate was totally undamaged. Some *Callosobruchus chinensis* individuals developed at 22°C on soy beans and at 25°C on soy grist as well but significantly fewer adults were found compared to the control substrate. *Tribolium confusum* developed better on the processed grist and flour than on whole soy beans but hatching start of F1 adults was shifted for more than 5 weeks (Tab. 2). Observed damage patterns were live individuals and the typical smell associated with *Tribolium*-infestation. On the six varieties of sweet and bitter lupine beans none of the three tested beetle species developed and only some *Rhyzoperta dominica* adults were found (Tab. 3). The significant longer development time until hatching of new *P. interpunctella* and *T. confusum* adults on soy and flour at 25°C and 65% RH compared to the standard feeding substrate (Tab. 2) is comparable to data from literature (Cox and Simms 1978).

Tab. 1 Experimental design to test the development time from egg to adult (F1) of different stored-product pest species (moth and beetle) on 200 g of whole beans, grist or flour of one soy variety (*Sultana*) and six lupine varieties (blue sweet (*bs*): *Boregine, Boruta, Mitrabor, Probor;* white sweet *(ws): Energy;* blue bitter *(bb): Karo ZS*), as well as on the corresponding standard fedding substrates as control (N=6).

PESTS \ PULSES			SOY beans, grist, flour	LUPINE beans (***I-VII***), grist+flour (***V-VII***)						
	CONTROL	Variety	***Sultana***	***I) Boregine*** (*bs*)	***II) Boruta*** (*bs*)	***III) Mirabor*** (*bs*)	***IV) Probor*** (*bs*)	***V) Sweet mix†*** (*bs*)	***VI) Energy*** (*ws*)	***VII) Karo ZS*** (*bb*)
Moths	**Standard feeding substrate**		**Infested with**							
Ephestia elutella	*Wheat bran (200g)*		10 adults	--	--	--	--	100 eggs*	100 eggs*	100 eggs*
Plodia interpunctella	*Wheat bran/almond grist (185/15g)*		10 adults	100 eggs	100 eggs	100 eggs	100 eggs	100 eggs*	100 eggs*	100 eggs*
Beetles										
Acanthocelides obtecus	*Black-eyed beans (100g)*		--	50 adults	50 adults	50 adults	50 adults	--	50 adults	50 adults
Callosobruchus chinensis	*Peas (200g)*		30 adults	--	--	--	--	--	--	--
Callosobruchus maculatus	*Mung beans (100g)*		--	50 adults	50 adults	50 adults	50 adults	--	50 adults	50 adults
Rhyzopertha dominica	*Wheat grain (200g)*		--	50 adults	50 adults	50 adults	50 adults	--	50 adults	50 adults
Sitophilus granarius	*Wheat grain (200g)*		30 adults	--	--	--	--	--	--	--
Tribolium confusum	*Wheat grist/yeast (191/9g)*		30 adults	--	--	--	--	--	--	--

† Mix of 4 blue sweet lupine varieties: *Boregine, Boruta, Mirabor, Probor.*

* Additional experiment by infesting grist and flour of lupine sweet mix (***V***) and varieties ***VI*** and ***VII*** with 100 moth eggs each.

Since development not only depends on the feeding substrate but also on factors such as temperature and humidity (Dettner and Peters, 2011), the experiments presented here (under specified temperature, product moisture and relative humidity) only give a first indication of whether the tested species represent a real risk to stored soy and lupine. In fact, higher temperatures seem to favor the potential of beetles and pests of tropical origins to develop on the tested feeding substrates. Therefore, during cold, dry and well-ventilated storage these pest insects probably do not represent a high risk for the stored pulses. Here, the temperature effect has been observed on *C. chinensis* (Tab. 2) which may be an indication of the potential for increased reproduction on soy at higher temperatures. This in turn implies that the potential to develop on innovative stored products (pulses) in Germany may rise with increasing global warming.

In any case, a thorough cleaning before storage of soy and lupine is to be recommended, in order to prevent the spreading of harmful insects, even after a nonmonitored infestation. Consequently, the most important preventive measures against pest insects and future infestations in practice are

well-cleaned storage facilities, cool storage temperatures (10-16 ° C) and for long-term storage kernels with no more than 11% residual moisture (Landwirtschaftliches Zentrum für Sojaanbau und Entwicklung, 2015).

Tab. 2 *The potential risk of stored soy beans, grist and flour to get infested by common stored-product pests.* Summary of experiments analyzing the capability of different moth and beetle species to develop on whole beans, grist and flour of the soy variety (*Sultana*) and measuring the developmental time from egg to adult (F1) compared to standard control substrates.

PESTS ON SOY *(Sultana)*	**Development time compared to control** (weeks)			**Mean n° of hatched adults compared to control** (%)			**Damage pattern**	**Risk of infestation**
	beans	*grist*	*flour*	*beans*	*grist*	*flour*		green levels: low risk red levels: potential risk
Moths								
P. interpunctella (at 25°C)	>	>	>	**22.1**	**75.7**	**80.1**	Feces Webbing Larvae	High potential to infest soy, especially the processed forms, grist and flour. Loss of quality due to moth webs and larvae. Moth develop well.
E. elutella (at 25°C)	>>	>>	>>	**20.2**	**57.1**	**54.8**	Living individuals Feces Webbing Larvae	High potential to infest soy, especially the processed forms, grist and flour. Moth develop well. Loss of quality due to moth webs and larvae.
Beetles								
T. confusum (at 24°C)	>>>	>>>	>>>	**0.3**	**8.2**	**6.3**	Living individuals Typical smell	Higher risk of infestation on soy grist and flour at warmer temperatures.
C. chinensis (at 25°C)	>>	>>	*X*	**3.1**	**5.0**	*X*	Living individuals Laid eggs No drill holes	Risk of infestation on soy beans and grist increases with increasing temperatres .
C. chinensis (at 22°C)	>>	*X*	*X*	**0.5**	*X*	*X*	Living individuals Laid eggs No drill holes	Very little risk of infestation and only on soy beans.
S. granarius (at 20°C)	*X*	*X*	*X*	*X*	*X*	*X*	None	No expected infestation since beans are too big and without the necessary endosperm.

>: Development time slightly longer than on control substrate (shift ca. 2 weeks)
>>: Development time longer than on control substrate (shift ca. 3 weeks)
>>>: Development time much longer than on control substrate (shift > 5 weeks)
***X*:** No development (no adult individuals hatched)

Tab.3 *The potential risk of stored lupine beans to get infested by common stored-product pests.* Summary of experiments analyzing the capability of different moth and beetle species to develop on whole beans of six lupine varieties (*Boregine, Boruta, Energy, Mitrabor, Probor, Karo ZS*) and measuring the developmental time from egg to adult (F1) compared to standard control substrates.

PESTS ON LUPINE whole beans	**Development time compared to control** (weeks) / **Mean n° of hatched adults compared to control** (%)						***Damage pattern***	***Risk of infestation***
	Boregine (*bs*)	***Boruta*** (*bs*)	***Mirabor*** (*bs*)	***Probor*** (*bs*)	***Energy*** (*ws*)	***Karo ZS*** (*bb*)		green levels: low risk red levels: potential risk
Moths								
P. interpunctella (at 25°C)	>> / **3.0**	>> / **5.1**	>> / **2.5**	>> / **3.8**	>> / **8.5**	>> / **5.0**	Living individuals Feces Webbing Larvae	Some potential to infest all varieties of sweet and bitter lupine whole beans. Loss of quality due to moth webs and larvae.
Beetles								
R. dominica (at 25°C)	>>> / **0.2**	>>> / **0.4**	>>> / **0.2**	>>> / **0.3**	>>> / **0.4**	>>> / **0.1**	Very low	Almost no risk of infestation on all varieties of lupine whole beans.
C. maculatus (at 25°C)	*X*	*X*	*X*	*X*	*X*	*X*	None	No expected infestation.
A. obtecus (at 25°C)	*X*	*X*	*X*	*X*	*X*	*X*	None	No expected infestation.

>>: Development time longer than on control substrate (shift ca. 3 weeks)
>>>: Development time much longer than on control substrate (shift > 5 weeks)
***X*:** No development (no adult individuals hatched)

Tab.4 *The potential risk of lupine grist and flour to get infested by common stored-product pests.* Summary of experiments analyzing the capability of *P. interpunctella* and *E. elutella* (100 eggs initially) to develop on grist and flour of a mix of 4 blue sweet varieties (*Boregine, Boruta, Mitrabor, Probor*), one white sweet variety (*Energy*) and one blue bitter variety (*Karo ZS*) and measuring the developmental time from egg to adult (F1) compared to standard control substrates.

PESTS ON LUPINE grist and flour	**Development time compared to control** (weeks) / **Mean n° of hatched adults compared to control** (%)						***Damage pattern***	***Risk of infestation*** green levels: low risk red levels: potential risk
	Sweet mix (*bs*)		***Energy*** (*ws*)		***Karo ZS*** (*bb*)			
	grist	*flour*	*grist*	*flour*	*grist*	*flour*		
P. interpunctella (at 25°C)	> / **100**	> / **100**	> / **89**	> / **76**	> / **98**	> / **97**	Living individuals Feces Webbing Larvae	High potential to infest processed lupine (grist and flour). Moth develop well. Loss of quality due to moth webs and larvae.
E. elutella (at 25°C)	> / **83**	> / **92**	> / **90**	> / **81**	> / **98**	> / **97**	Living individuals Feces Webbing Larvae	High potential to infest processed lupine (grist and flour). Moth develop well. Loss of quality due to moth webs and larvae.

>: Development time slightly longer than on control substrate (shift ca. 2 weeks)

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Biological abilities of storage pests required for the successful penetration of food packages or seeds

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Abstract

Storage pests cause enormous damage to stored seed commodities and packaged food. Most of the work published on pest risk assessment concentrates mainly on the effects of "pest –package" or "pest-seed" interactions: i.e. if some species is able (or not able) to penetrate in a sound kernel or package. Based on such "YES-NO outcomes", the particular stored product pest species is then categorized to either as a "primary" or "secondary" seed feeder; or "penetrator" or "invader" of packages. However, less research attention is paid to the functional explanations of the observed interaction-outcomes. This work therefore deals with comparison of morphological adaptation in various species storage insects with regards to their penetration abilities. For this analysis our original data as well as data from literature were used. As the most important morphological (pre-) adaptations, modulating penetrative/invasive success of storage insect pests, have been recognized: (i) shape and hardness of mandibles, (ii) size and strength of mandibular muscles, (iii) morphology of tarsi enabling climbing and/or firm stance on smooth surfaces. In addition to the morphological adaptations the specific genetically pre-programmed behavioural patterns and abilities may also play a significant role. It will be demonstrated that the above morphological abilities must be taken into account while establishing standard methods of testing of various packages in terms of their sensitivity to penetration/invasion by various species s of storage pests.

Keywords: food packages, morphology, madibulae, tarsi, claws, *Sitophilus granarius* and *Rhyzoperta dominica*

Introduction

Storage pests cause profound injury and damage to stored seed commodities (Stejskal et al., 2014) and packaged food products (Essig et al.1943; Hubert et al., 2011; Stejskal e al., 2015). In order to reach protected food resources, pests must be able to overcome physical and chemical defences present on the surface of seeds and food packages. As a natural defence, many types of plant parts (seeds, fruits, and leaves) have very smooth and/ or waxy surfaces (Al Bitar et al., 2009). In addition, seeds are equipped with hard and smooth protective layers (e.g. Fig. 1) that are impenetrable for many morphologically maladapted stored product pests. Unlike undamaged seeds, the processed food (i.e. cereal products, energy fruit bars, and cornflakes) is usually served without any protective hard surfaces. In order to protect food from pest infestation and/or contamination, early civilisations came up with an idea of "artificial- peel" centuries ago that is nowadays known as protective food packaging. During the course of human history, many types of packaging materials have been developed (Athanassiou et al., 2011). However, their protective properties still differ profoundly: chemical composition and number of layers of the film were recognized among the most important factors affecting film resistance against pest penetration (e.g. Lee et al., 2017; Trematerra and Savoldelli, 2014, Stejskal et al., 2017). It has been also shown that various pest species differ in their ability to penetrate or invade protective food-packaging films (Cline, 1978). Riudavets, et al., (2017), based on SEM microscopy, described various types of physical injuries and damages caused by particular species of stored product pests.

Most of the work published on pest risk assessment concentrates mainly on the effects of "pest – package" or "pest-seed" interactions: i.e. if some species is able (or not able) to penetrate in a sound kernel or package. Based on such "YES-NO outcomes", the particular stored product pest species is then categorized to either as a "primary" or "secondary" seed feeder; or "penetrator" or "invader" of packages. However, less research attention is paid to the functional explanations of the observed interaction-outcomes. This work therefore deals with comparison of morphological adaptation in various species storage insects with regards to their penetration abilities. For this analysis our original data as well as data from literature were used. As the most important morphological (pre-) adaptations, modulating penetrative/invasive success of storage insect pests, have been recognized: (i) shape and hardness of mandibles, (ii) size and strength of mandibular muscles, (iii) morphology of tarsi enabling climbing and/or firm stance on smooth surfaces.

Shape and hardness of mandibles

Protective surface of various seeds (such as seeds of bean; pea, barley; wheat; corn and pearl millet - Fig.1) and packages are usually hard. Storage pests have differential morphological ability and hardiness of mandibles to penetrate seed surface. Based on biological abilities, the particular stored

product pest species is then categorized to either as a "primary" or "secondary" seed feeder. The relationship between mandible morphology and diet has been studied on different insect taxa, e.g. on grasshoppers (Patterson, 1984; Smith and Capinera, 2005), carabid beetles (Acorn and Ball, 1990) or ladybirds (Samways et al., 1997). Generally, there coud be differences in relative molar and incisor length, in mandible apex (multidentate/unidentate), or in general mandible shape (width/length ratio) according to type of food (i.e. herbivorous *vs* carnivorous, graminivorous *vs* forbivorous etc.). Nevertheless, there is no research on relationship between morphological characters and ability to penetrate food packages in stored pests. Besides the mandible shape, hardness (which is caused manily by presence of metals in cutting edge) of mandibles can also play a significant role in ability of infest packed food. For example, high contrentations of zinc and manganese were detected in mandibles of stored pest larvae that bore into the seed, whilst in species that feed on already damaged seed there was no metal in the mandibles (Morgan et al., 2003).

Size and strength of mandibular muscles

Even very hard and sharp mandibular tools cannot efficiently serve their purpose without being equipped an adequate muscle system. However, the size and strength of mandibular muscles has not been studied in stored pests so far. In reality, there exists little information about biting forces in insects at all. In carabid beetles, it seems that mandibular force is not dependent on size of the species (Wheater and Evans, 1989), so the species size is probably not a good predictor of the species penetration ability. On the other hand, there are indices that size of mandibular (adductor) muscle is related to the mandibular and head size (Li et al., 2011). Weihmann et al. (2015) found that there is relationship between mandibular adductor size and diet in different insect taxa.

Morphology of tarsi enabling climbing and/or firm stance on smooth surfaces

Various seeds (Fig.1) or food packages show diverse structure of their surfaces: from rough, to smooth. To be evolutionary successful, phytophagous pests have developed differential climbing and surface attachment morphological devices and adaptations. Tarsal claws are adapted for movement on rough surfaces, while various adhesive tarsal devices (i.e. pads, arolium, pulvilli, etc.) enable to attach to smooth surfaces. Although there are studies on movement and adhesive abilities of insects (mainly in context of plant vs plant pest/pest predator; e.g. Al Bitar, et al., 2009, Gorb and Gorb, 2002; Eigenbrode, 2004) and other organisms (spiders, geckos, etc.; e.g. Bhushan, 2012; Wolff and Gorb, 2012), studies dealing with tarsal morphology and its relation to the climbing performance in stored product pests are surprisingly lacking. One of the very few work on this topic showed high variability in climbing abilities of stored product pests on several packaging materials (Cline and Highland, 1996). For example, whilst some species (e.g. Sitophilus oryzae, Lasioderma serricorne, Oryzaephilus surinamensis) had no problem to climb in angle 90°, several species (Rhyzopertha dominica, Attagenus megatoma) were almost unable to move on the materials. This work thus raises a question which morphological features stand behind the variability in the ability of climbing on artificial smooth surfaces.

Previous studies showed morphological adapatations on attachment ability on smooth (e.g. arolium in Blattodea, Lepidoptera and Hymenoptera, pulvilli in Diptera or setal tarsal pads in Coleoptera) and rough (claws – Fig.2, different types of setae in adhesive pads) surfaces. Hence, thanks to their variability in attachment ability, stored product pests may serve as an additional organism group for study of morphological (pre-) adaptations of climbing abilities.

Conclusions

The article summarized the selected morphological abilities that must be taken into account while establishing standard methods of testing of various packages/ seeds in terms of their sensitivity to penetration by various species of storage pests. In addition to the morphological adaptations the specific genetically pre-programmed behavioural patterns and abilities of phytophagous stored product insects may also play a significant role.

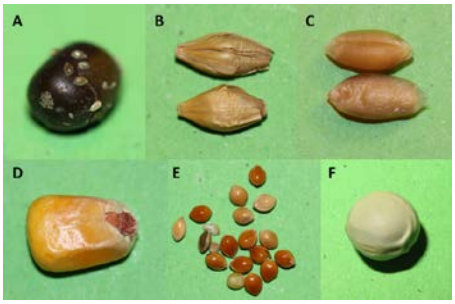

Fig. 1 Protective surface of various seeds are usually hard and smooth: A- beans; B- barley; C – wheat; D- corn; E- pearl millet; F- pea. Storage pests have differential climbing and attachment morphological ability (shape of tarsal claws or adhesive pads) to smooth surface of seeds as well as different ("primary" or "secondary" seed feeder) morphology and hardiness of mandibles to penetrate seed surface.

Fig. 2 Comparison of tarsal claws of two primary pests *Sitophilus granarius* and *Rhyzoperta dominica.* The relative length of claws is considerably larger in *R. dominica* (cca 25% of tarsal length) than in S. granarius (cca 12% of tarsal legth).

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Constraints in Grain quality management: A warehouse journey

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Abstract

India produces about 150 million tons of food grains per year. The major components of production are 47 million tonnes of wheat, 64 million tonnes of rice, and 13 million tonnes of pulses. Seasonal fluctuations in harvesting of grains impose efficient design for long term storage. Quality of grains will be retained by proper storage. Post harvest processing and storage conditions such as temperature, humidity, aeration, insect infestation, rodents, fungus, etc., at a particular geographical location influence the qualitative and quantitative losses of grains. Approximately about 10% of produce wasted during post production such as harvesting, threshing, and storage which means that about 15 million tons of grains are being washed out per year. Main intention of any government in warehousing is to offer a safe buffer stock during off-season. Knowledge about existing storage criteria creates a vision to develop new strategies. Based on this concept, a compartment in a godown of dimension 37.2m x 24.2m x 8m made of concrete and asbestos roof, with six doors and thirty-four windows was selected for the research. The stacks of dimension 6.5m x 3.9m x 6.1m with two hundred and sixty-four numbers of gunny bags filled with grains arranged above the wooden dunnage were selected for insect and chemical analysis. Temperature, humidity and aeration rate were recorded at four corners and at center of the stack and also at 26 different spots in whole godown. The influence of various factors on insect infestation in grains during storage was studied. The results will help to design an advanced scientific grain storage godown for safe storage of grains in gunny bags for longer duration.

Keywords: Godown, Dunnage, Insect infestation, Temperature, Humidity.

Introduction

Agricultural products such as grains, cereals are stored for facing shortage of commodities during off-season, droughts and natural calamities. They are usually stored for 3–12 months by farmers, traders and by the public sector agencies like Food Corporation of India, the Central Warehousing Corporation, State Warehousing Corporations and State Civil Supplies Corporations which handle

about 30% of the production (TIFAC, 1996). In many developing world, post harvest losses of cereals accounts to 10-15% (Lucia and Assennato, 1994). Post harvest losses are mainly due to insect infestation which found their food and shelter and also contaminate the grains by their by-products and making them unfit for consumption resulting in qualitative as well as quantitative losses.

Tropical and humid areas are mostly prone to pest infestation on stored foods. The tropical climate of India is highly favourable for continuous survival of storage insect pests throughout the year. Insects gain access to storage area at various stages of processing of grains; during the development seeds/grains, processing in threshing yards, during transport or during storage. Major sources of infestations are old bags, storage structure, old containers, and cross over infestation (Pruthi and Singh, 1950).

Infestation of whole storage area is facilitated by movement of grains from one area to another or by active flight of insect pests as some of the adult insects are strong fliers. Monitoring the stored grain pest, by finding the insect population or infestation level in a period of time helps to understand the behaviour of insects with respect to environmental conditions. These will further help to determine the time for pesticide application and effectiveness of pest management actions. The emphasis of tropical storage pest management is thus on constraining the increase and spread of such infestations. Thus the following study was undertaken to determine the population pattern of insects with respect to tempertature and humidity in a godown.

Materials and Methods

A compartment in a godown of dimension 37.2m x 24.2m x 8m made of concrete and asbestos roof, located in Thanjavur, Tamil Nadu, India was selected for the research. The compartment was ventilated with six doors and thirty four windows. Paddy was stored in the gunny bags. About with two hundred and sixty four numbers of gunny bags filled with grains were arranged above the wooden dunnage. Individual stack has the dimension of 6.5m x 3.9m x 6.1m. Among them one stack was selected for studying the population behavior of insects. The temperature and humidity were recorded using HOBO data logger at points near the ventilation and far from ventilation from 10.00 am to 4.00 pm at an interval of 2 hours. Simultaneously, insect population at the top, middle and bottom of the stack was also counted. The study was conducted during the post monsson season (December) with an average outdoor temperature of 25° C. Based on the readings, the population pattern of insects with respect to temperature, humidity and time was investigated.

Results

The results of the study showed that temperature was found maximum in the interior part of compartment while ventilated areas near door and windows recoreded minimum temperature. The humidity was found to be higher in ventilated areas than interior part. It was observed that five fold increase in insect population at the top of stack during day time and about seven fold increase in insect population after 4.00 pm.

During storage, the paddy was attacked by many insects including the *Sitotroga cerealella,, Rhyzopertha dominica, Tribolium castaneum, Sitophilus oryzae,* etc., But the major pest was identified as *Tribolium* sp., which feeds on broken grains resulted in dust formation. Similarly Rajan *et al*. (2018) explained that the most abundant species caught was *T. castaneum* across all of the localities sampled. Infested grains emitted sour and pungent smell, which was due to some secretions of beetles.

Discussion

The observation on the insect population or infestation level in a period of time along with temperature and humidity helps to understand the behaviour of insects with respect to environmental conditions. In general, the minimum temperature threshold for *T. castaneum* flight initiation in the laboratory being 25° C (Cox *et al.,* 2007). The results of the present study showed that temperature was found maximum in the interior part of compartment while ventilated areas

near door and windows recoreded minimum temperature. The humidity was found to be higher in ventilated areas than interior part.

Tab. 1 Population strength of insects with respect to temperature, humidity and time

Time	Temperature (ºC)		Relative Humidity (%)		Insect Count at T_1			Insect count at T_{avg}		
	T_1	T_{avg}	T_1	T_{avg}	B	M	U	B	M	U
10.00 am	25.8±0.5	26.8±0.7	73.4±3.3	73.2±2.2	1±0	2±1	2±1	1±0	1±1	4±1
12.00 noon	26.7±0.2	27.8±2.0	72.5±3.3	69.9±2.3	1±1	0±1	4±2	1±1	0±1	6±3
2.00 pm	26.7±0.3	28.9±1.0	70.9±3.8	68.0±5.0	2±1	2±2	9±3	1±2	3±3	13±7
4.00 pm	27.0±0.5	28.5±0.9	70.0±3.9	68.5±5.0	5±3	6±3	14±4	2±2	4±1	17±11

T_1 – Near the ventilation area; T_{avg} – Far from ventilation; B – Bottom of stack; M – Middle of stack; U – Upper portion of stock

It was observed that five fold increase in insect population at the top of stack during day time and about seven fold increase in insect population after 4.00 pm. The results are in line with the report of Rajan *et al.* (2018) who reported that vast numbers of *T. castaneum* take flight inside godowns in the late afternoon. The resutls of the present study will help to design an advanced scientific grain storage godown for safe storage of grains in gunny bags for longer duration. It will also help develop effective management tactics to reduce the severity of infestations caused by stored product insects.

5. Future Progress

Effect of temperature, humidity and ventilation on insect population was studied for short duration. To establish the efficient pest management practices, the influence of all the above said factors on microbial growth and chemical analysis has to be studied. Further pest management by integrating the different methods based on the results of the study in large scale godown has to be studied.

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Modelling of population dynamics of insects in any ecosystem with several distributions of insect development: A Review

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Abstract

Predicting the occurrence of insects with a high accuracy requires the estimation of insect development time and the variation among individuals for each life stage and species under different environmental conditions such as fluctuating temperature, variation of relative humidity, different body sizes and stages of the insects, levels of crowding, and food supply. This review summarized the modeling methods of population dynamics of

insects with several distributions of insect development, assumption and prediction accuracy of these developed models, and disadvantages and advantages of these modelling methods. These modeling methods include degree day model, nonlinear model, and distribution delay models. The structure of most common models are cohort, Leslie matrix, simulation, and individual based. The relationships among the modeling assumptions, effects of temperature, and other environmental factors, and structures of the developed models were examined. A new modelling approach such as physiological-biological time scale and chaos theory was suggested.

Key words: Degree day, nonlinear model, distribution delay, Leslie model, chaos.

Introduction

Predicting the occurrence of insects in an ecosystem with a high accuracy is essential for conducting integrated pest management. These predictions require the estimation of insect development time and the variation among individuals for each life stage and species under different environmental conditions such as fluctuating temperature, variation of relative humidity, different body sizes and stages of the insects, food sources, and levels of crowding. This adds another level of complexity to models already complicated by accounting for the variable time of development (Stinner et al. 1974, Wagner et al. 1984 a and b, Briere et al. 1999, Gramig et al. 2015), variations under different temperatures (Anderson et al. 1982, Worner 1992, Regniere et al. 2012, Damos and Savopoulou-Soultani 2012, Moore and Remais 2014), variations among different growth stages and ages, and discrepancies under constant and fluctuating temperatures (Hagstrum and Milliken 1991, Nachman and Gotoh 2015). However, complexity does not assure more accuracy in all cases. Therefore, the right mathematical approach and theoretical assumptions should be developed to model population dynamics of insects with several distributions of development time. This review outlines the basic modelling methods, the disadvantages and advantages of the methods, reasons for the low accuracy of these developed models from their applications, and suggestions for future model development.

Temperature effect and modelling

Temperature is the only considered factor in almost all, if not all, of these published models in the literature (Damos and Savopoulou-Soultani 2012) because temperature is the most critical factor affecting insect development. Damos and Savopoulou-Soultani (2012) reviewed the temperature-driven models for insect development. Empirical and semi-theoretical mathematical models have been developed. Even though none of these models is based on accepted biophysical laws such as Eyring's absolute reaction rate theory, temperature effect on enzymes has been recognized by the empirical equations of Van't Hoff's law and Arrhenius (Schoolfield et al. 1981). The basic assumption of the enzymes was used for most model development such as degree day and nonlinear models. To calculate the temperature effect, three general types of models are developed: degree-day summation, non-linear temperature inhibition, and distribution delay. These empirical or semi-theoretical equations predicting average development rates are in exponential or logistic format and explain the thermodynamics of complex biological processes by the laws of chemical reactions. This provides biologists with a greater understanding of the temperature-dependent and developmental responses for a given insect species. With this approach, developed models could be extended beyond the range of temperatures specified by model theory or beyond the range of temperatures measured. However, these empirical and semi-theoretical models are not valid for most practical cases because exponential or logistic increase is observable on a limited range and not throughout all temperature regimes. Therefore, different researchers modified these empirical equations (Sharp and DeMichele 1977). However, these modified equations have limited application because of their limited fit to laboratory data, and usually these developed models have not been validated with field data because it is difficult to collect field data; there are inherent variations in the field data; and predictions from models are uncertain.

There are three issues with the modelling temperature response of insects: development rates, development times at temperatures near thresholds (extremes) where excessive mortality or

developmental abnormalities can occur, and individual variation from the average developmental rates and reproductive responses. These three issues are handled in the models of degree day, nonlinear, and distribution delay differently.

Degree day models

To model the effect of temperature, the development of an organism is viewed as a biological clock that measures time thermal units, and it is often referred to as thermal time. Although physiological time accelerates or slows under different temperature fluctuations, the thermal time unit to complete a particular developmental event under both field and laboratory conditions is assumed to be the same by modellers because thermal requirement is the basis for insect development. This is the basic assumption of the degree day model. Degree-day models were initially developed for agricultural applications and have been widely used since the 1730s in many research areas related to farming (Moore and Remais 2014). The degree-day model, without considering the individual variation from the average developmental times or rates and reproductive responses, is a simplistic representation of a potentially complex developmental process (Moore et al. 2012).

Degree day model is the most widely used mathematical format to estimate insect development time because it requires minimal data for formulation, calculations are easy and applications are simple, and often yields approximately correct values. Most of degree day models only estimate the average development time, and do not consider the individual variation from the average developmental times or rates and reproductive responses. This simple format of degree day model is widely used in many agricultural areas. Recently, Nachman and Gotoh (2015) developed a biological age model with the consideration of the individual variation from the average developmental rates and reproductive responses. This newly developed model used probability distribution to estimate the individual variation and this probability distribution was related to temperature effect and development time mathematically. This mathematical relationship among development probability distribution and temperature effect and development time is the modification of the degree day model. Therefore, this new developed model combined the degree day model with distribution delay model. This modeling framework was successfully used to model several insect species (Skovgard and Nachman 2017).

Even though different researchers used different mathematical equations to calculate the degree days (Moore and Remais 2014), and used different assumptions to quantify the relationship between the sum of degree days and the insect development, the developed degree day models have a low prediction accuracy. A degree day model developed under constant temperature cannot be used to predict insect population under fluctuating temperature (Hagstrum and Milliken 1991, Jian et al. 2017). Developmental times of many species under constant temperature differ from these under fluctuating temperatures with the same mean (Hagstrum and Milliken 1991) because short periods of colder or warmer temperatures under fluctuating temperatures may have an overriding influence on development rate when compared to the mean temperature over a longer period of time (Hagstrum and Milliken 1991). Low prediction accuracy of the degree day model under fluctuating temperature will have a large drawback because most life tables studied are conducted at constant temperature under laboratory conditions. The development differences between constant and fluctuating temperatures could not only increase the complexity of the degree day model, but also make the degree day model unsuitable. Researchers have shown that these differences could be resolved by integration of temperature developmental times over the fluctuating temperature cycle to predict development times at fluctuating temperatures (Dallwitz 1984, Hagstrum and Milliken 1991). Therefore, combination of the degree day model with other mathematical tools might be one of the choices to improve prediction accuracy. However, deciding the integration interval is part of arts and science, and different researchers used different assumptions

The main reason causing this low prediction accuracy of degree day models might be the basic assumption of the degree day model. The basic assumption of the degree day model is that: the

completion of a given stage in development requires an accumulation of a definite amount of heat energy, thus, degree day models apply the accumulated temperatures as the heat energy to establish the relationship between the development and the environmental conditions without considering the additive effect of the accumulated energy and morphological change of the organisms. Insects might have a mixture of liner and non-linear development. It is difficult to find the right equation because both linear and non-linear models generally cannot represent the complete effects of temperature on an organism. Nonlinear indicates that the whole becomes something greater than the mere sum of its individual parts or linear parts due to the interaction effects between factors. Moore and Remais (2014) found the difference between linear and non-linear predictions of *Nephus bisignatus* (Bohrman) (Coleoptera: Coccinellidae) emergence can be up to a week, which is not trivial and have important implications for the use of degree-day models in ecological applications. It is common that temperature not only influences the rate of chemical reactions, but also induces conformational changes in biological systems. For any degree day model, the most challenging task is to find the base temperature. Insects under fluctuating temperature may not have a distinct low temperature development threshold, and development rates become asymptotically smaller as temperature decreases (Eubank et al. 1973). The organism's response to high and low temperatures, as well as to the specific methods used to estimate accumulated degree-days, can lead to markedly divergent model predictions. Therefore, prediction of the degree day model is very sensitive to the tailored system, region, and time scale. This requires development of models that are tailored to the specific system, region, and time scale under a good fit. Therefore, a new modeling framework to cater these effects on insect development should be developed.

Nonlinear models

The initial objective for most of the nonlinear regression models is to describe developmental rate of insects over the full range of temperatures. This modeling procedure can be easily generated using several different software if the developed models are only used to predict the average developmental time or rate (reverse of the average development time, lifespan, or LT50). Most of these developed models considered the maximum and minimum development temperatures. These maximum and minimum development temperatures correspond to the assumption that there is no growth below the minimum temperature threshold, while developmental rate increases to reach a maximum at optimal temperature, and then declines rapidly approaching zero at the maximum temperature threshold that is often considered as the lethal temperature. To include the prediction of the distribution of the development time delay, this developed nonlinear model becomes complex because probability and/or likelihood estimation must be used.

One type of nonlinear temperature inhibition models is the biophysical model. Biophysical models are developed based on Van't Hoff's law which states that the rate of chemical reactions increases between two- and three-fold for each 10°C rise. The Arrhenius equation relates the chemical reaction rate to temperature and the activation energy of the reaction in an exponential equation. However, these models usually have a large prediction error because exponential increase is observable in a limited temperature range and not throughout all temperature regimes, and temperature affects not only the rate of chemical reactions, but also induces morphological changes in biological systems (Schoolfield et al. 1981, Sharpe and DeMichele 1977, Briere et al. 1999). Nonlinear models predicting average development rate with considering minimum and maximum development temperatures are usually complex (most have more than four thermodynamic parameters) and can only be used for the insects for which the model was initially developed (Schoolfield et al. 1981, Wagner et al. 1984 a and b, Wang and Engel 1998, Briere et al. 1999, Hansen et al. 2011, Regniere et al. 2012). These thermodynamic parameters were found to be highly correlated (Schoolfield et al. 1981, Briere et al. 1999). To eliminate this correlation, different researchers (SchoolÞeld et al. 1981, Briere et al. 1999) re-parameterized these models, and some researchers just used different mathematical equations to best fit the data which cover the entire

insect development temperature (Jian et al. 2007). This parameterization in turn results in a nonlinear model with no or few biophysical assumptions.

Distribution delay models

Life history studies usually require to determine recruitment (actual number), duration, and survivorship for each life stage. The most used methods to model this life history are Leslie matrix format, distribution delay model (referred to as distributed maturation models or variable development rate models), and combination of both. In a Leslie matrix format, the organism's life cycle is divided into sub-stages with a length equal to the length of the shortest stage. At each time step, all individuals in the population are advanced to the next sub-stage and the time step is usually set as the sub-stage length. All individuals in a cohort can advance in age at the same rate (development index model) or change from one stage to the next at the same age (sojourn time models). Mathematically, the development index model is a special case of the sojourn time model (Schaalje and Vaart 1989). The Leslie matrix model has been of limited use in ecology because it models exponential population growth. One format of the modified Leslie matrix is the distribution delay. In the distribution delay format of the Leslie matrix model, advance of an individual from one stage to the next is not only based on the mean length of development time, but also the variability among individuals. The advance of an individual can be calculated from a probability distribution based on the mean and standard deviation or is assigned a predetermined probability (Schneider and Ferris 1986). Survivorship of an individual can also use the same method as that used for the advance of an individual or use mathematical equations describing the survivorship pattern. Weibull function is mostly used to describe this probability distribution (Schneider and Ferris 1986). Erlang probability distribution can be used to describe the asymmetric and positively skewed development rates within the population (Wegner et al. 1984 a and b, Schneider and Ferris 1986). These asymmetric and positively skewed development rates are assumed as the effect of temperature and enzyme concentration (Curry et al. 1978, Sharpe and DeMichele 1977). The stochastic treatment of the Leslie matrix model can include the insect density effect and stochastic process on the survivorship and development rate by making the elements of the projection matrix vary with the age distribution or density (Leslie 1959, Vansickle 1977, Desharnais and Cohen 1986, Desharnais and Liu 1987, Liu and Cohen 1987). More complex models could be formulated to allow for time delays, but the above are commonly used. During the model development of a Leslie matrix, the temperature effect is usually implicit because it is difficult to combine the effect of fluctuating temperatures in each time step when the Leslie matrix is advanced to the next time step. For example, if temperature is changed every hour and this fluctuating temperature effect will influence the Leslie matrix, then the Leslie matrix should be calculated every hour. This will increase the difficulty of parameter estimation for the Leslie model calculation. There is no model developed in this way because the use of the Leslie matrix formulation allows the overall model to be stated concisely and this small time step will downgrade this advantage. Because the fecundity and death rate may change abruptly as an individual matures from one stage to the next, the Leslie, Von Foerster (Longstaff 1988) and related models are implicitly formulated in terms of growth stages. Cuff and Hardman (1980) calculated the fecundity and survival rate by considering the effect of temperature, moisture content, weight of free water, weevil density, and oxygen concentration. These calculated fecundity and survival rates were the basic components of the Leslie matrix in each time step. Other environmental factors such as respiration of insects, feeding, and egestion activities were also considered by modifying the Leslie matrix in each time step. Because these environmental factors influence the insect development rate, Cuff and Hardman (1980) used both physiological and chronological time scale to track insect age in each sub-stage and advance the sub-stage, respectively. This increases the complexity of the Leslie matrix model. This might be one of the reason few Leslie matrix models with distribution delay were developed after the 1990s.

Models that predict this stochastic development distribution usually involve application of probability distributions and likelihood estimation. These developed models are similar except they use different 1) variables, such as mean or median development time (Wagner et al. 1984 a and b,

Gramig et al. 2015) or development rate; 2) forms of frequency distribution, such as probability or cumulative density function; 3) types of probability distribution such as normal quadratic and beta; and 4) equations, such as Erlang probability distribution function, Weibull function (Wagner et al. 1984b), nonlinear functions with different assumptions (Gramin et al. 2015, Nachman and Gotoh 2015). The most commonly used model strategies are distributed delays (Nisbet and Gurney 1982, Wagner et al. 1984 a and b, Schneider and Ferris 1986), cohort-based (Sharpe and DeMichele 1977, Nachman and Gotoh 2015, Skovgard and Nachman 2017), Leslie model based (Longstaff and Cuff 1984, Henson 1999), simulation based (Longstaff 1988, Maggi et al. 2013), and individual-based (Regniere et al. 2012). The parameter values estimated from these developed models are usually not comparable even for the same stage of an insect species under different environmental conditions because the chronological time is used as the time scale and these factors are changing with time. The distribution of development time and the variation in the chronological time scale is different under different temperatures.

Other environmental factors

The major constraint of most developed models is directly related to temperature and do not take into account other climatic variables such as photoperiod, humidity, nutrition, as well as crowding and competition at different density levels and in different patch sizes. Incorporating more factors in the equations, temperature-driven models have the potential to describe the general ecological behaviour, abundance, distribution, and outbreaks of insects on a regional or even global scale, with important practical applications. Nachman and Gotoh (2015) claimed their developed model framework could simulate the growth of an insect population in a variable environment by modifying the response variable y in the equation (y was a product of limiting factors) with the assumption of a multiplicative relationship between the environmental factors. Nisbet and Gurney (1982) considered the quantity of food. Cuff and Hardman (1980) considered other environmental factors. However, this modelling approach has not been verified. This modelling approach increases the complexity and the y in Nachman and Gotoh's model has no basic biological meaning. Therefore, a new modeling approach should be developed to effectively predict the effect of these environmental factors with sound biological meaning.

Future model development

Physiological or biological time is intuitively obvious to some extent, but has been explicated in various ways in the literature. It is referred to as heat units and is measured in degree-days and development accumulation as the basis of physiological time scale in model development. Physiological age as a life-history event are sometime related to cyclic event such as biological rhythmicity. Nachman and Gotoh (2015) used biological age as a measure of the cumulated day-degrees an individual has achieved while in a given stage. From the view of an insect body, ageing might be the result of physiological and biological advance in the chronological time scale. Therefore, a physiological-biological time scale which can normalize the distribution of the development time and the variation should be developed.

The time delays, cyclical patterns of insect populations (periodic forcing), and nonlinearities in population models are the typical characteristics that lead to chaos in the natural ecological world (Logan and Allen 1992, Boeing 2016). Even though whether the insect populations have the chaos is still in debate, this debate has largely been carried out on theoretical grounds, and chaos occurring in the time series of forest insect pests have been proven (Turchin 1990, Turchin and Tylor 1992). Analysis of insect-population data collected inside lab or controlled field conditions for the signature of chaos presents significant limitations because: 1) chaos analysis is unrealistically data intensive; 2) data collected under lab and/or controlled field conditions usually reduce the dimensionality such as that interactions between species or among species and food sources are usually simplified and completely sampling of the entire population is usually difficult or impossible, and this simplification will rarely occur in nature; 3) chaos characteristics usually show after a few

generations of insect populations (Turchin and Tylor 1992), and collation of the lab or field data are usually interrupted before chaos shows; and 4) analysis of a complex ecological system in reduced dimensionality will tend to obscure complex dynamics and ignore the chaos (Logan and Allen 1992). Therefore, study of chaos in a real system by using mathematical modelling is critical. To analyze the chaos of population dynamics, this developed model should be a reasonable representation of the natural system and parameter values should be in a realistic range. This requires complex models which represent the reality in nature and the developed model should also be validated.

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High Quality Genomic Resources for Stored Product Insects

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Abstract

The expansion of genomic resources for stored product insects has largely been hampered by cost, time required for inbreeding, and technical issues that can arise during genome assembly from pooling multiple individuals together for DNA isolation and library preparation. However, newer library methods, such as 10X Chromium libraries, largely overcome these issues in that sufficient DNA can be recovered from a single individual for library prep and allelic variants are assembled as separate phase blocks, eliminating the need for inbreeding. Using 10X Chromium libraries coupled with 150 x 150 bp HiSeqX sequencing to a depth of at least 60X coverage, we are developing high quality draft genome assemblies for eight different stored product insect species, including Dermestidae (*Trogoderma variabile*, *Trogoderma granarium*, and *Dermestes maculatus*), Tenebrionidae (*Tribolium confusum*), Anobiidae (*Lasioderma serricorne* and *Stegobium paniceum*), Bostrichidae (*Prostephanus truncatus*), and Pyralidae (*Plodia interpunctella*). Overall, BUSCO (Benchmarking Using Single Copy Orthologs) scores exceeded 95% in all assemblies with few fragmented or duplicated genes, suggesting a high quality assembly of the gene space. Further, scaffold N50s exceeded 1 Mb in many cases and further

improvements to these scaffolding metrics will be made using linkage maps and Hi-C libraries. Overall, this approach will yield high quality assemblies for eight different insects and could be used to quickly and efficiently generate draft assemblies of invasive or emerging stored product pests.

Keywords: khapra beetle, Bostrichidae, Dermestidae, Anobiidae, Pyralidae.

Introduction

Genome sequences have provided tremendous insight into the physiological and metabolic capabilities of various insect species, led to the identification of causative mutations associated with pesticide and fumigant resistance (Schlipalius et al., 2012), facilitated the identification of taxonomically informative loci for DNA barcoding (Chesters et al., 2015), and identified copy number expansions that may allow insects to exploit new ecological niches (McKenna et al., 2016). Despite these utilities, only one stored product insect genome is publicly available (*Tribolium castaneum*) (Tribolium sequencing consortium, 2008) while a small, but growing number of transcriptome assemblies, are available for other stored product species. Since its initial assembly, the *T. castaneum* reference assembly has been used to identify mutations in a gene coding for dihydrolipoamide dehydrogenase (DLD) associated with phosphine resistance (Schlipalius et al., 2012), biorational gene targets for pest control via RNAi (Dönitz et al., 2014), and causative mutations associated with sensory system defects (Angelini et al., 2009). This assembly has even facilitated the discovery of a mutation associated with phosphine resistance in lesser grain borer (*Rhyzopertha dominica*), which happens to occur in a DLD ortholog (Schlipalius et al., 2012). However, the biologies of stored product insects vary tremendously across various taxonomic groups. Further, different taxonomic groups may evolve different strageies for overcoming biotic and abiotic stresses. In this case, having genome references available would greatly facilitate genome wide association analyses to identifiy causative mutations associated with tolerance to stress. In other cases, some species are more inherently tolerant to certain biotic and abiotic stresses and genome sequences could lead to the identification of genetic factors associated with tolerance.

Historically, genome sequencing for insects has been cost prohibitive; however, new library approaches coupled with the reduced cost of sequencing is making genome assembly more affordable and accessible than ever. One major challenge faced by those working with insects is obtaining sufficient quantities of DNA for library preparation and assembly. For Illumina mate-pair and PacBio long-read libraries, over 10 ug of DNA must be provided. This requires pooling multiple individuals for sequencing. This practice leads to multiple allelic variants derived from the same locus in the DNA pool, which can introduce bubbles and breaks into the assembly graph, reducing overall contiguity. Although sequence variations among individuals can be reduced through inbreeding for several generations, this often involves a significant time investment and the number of backcrosses required to obtain homogeneity varies by species and their recombination rates. One new library approach (10X Chromium) largely overcomes these limitations in that sufficient quantities of DNA can be recovered from a single insect for library construction and haplotypes are assembled as separate phase blocks, reducing the number of bubbles in the assembly graph and improving contiguity. In addition, during library construction, HMW DNA is separated into microfluidic chambers designed to hold exactly one molecule of DNA per chamber. Within each chamber, DNA is fragmented and DNA derived from the same molecule is tagged with the same barcode. In this manner, sequencing reads with the same barcode can be linked together during the assembly stage to form long scaffolds and contigs. This approach greatly improves assembly contiguity compared to other short read assembly methods.

In order to improve genomic resources for stored product insects, we sequenced 10X Chromium libraries derived from eight different species of stored product insects from the families Dermestidae (*Trogoderma variabile*, *Trogoderma granarium*, and *Dermestes maculatus*), Tenebrionidae (*Tribolium confusum*), Anobiidae (*Lasioderma serricorne* and *Stegobium paniceum*), Bostrichidae (*Prostephanus truncatus*), and Pyralidae (*Plodia interpunctella*). Genomes were assembled using the program Supernova and assemblies will be superscaffolded to chromosome

scale using linkage maps and/or chromatin contact maps. Overall, these assemblies exceeded the quality of many publicly beetle genome assemblies and thus, represent a viable strategy for generating genome sequences for underreprented groups of insects. Not only will these assemblies be useful for mapping traits, conducting population genetics studies, and understanding genetic similarities and differences between various stored product species, but they will also allow for broader evolutionary analyses regarding gene order, gene duplications, and the evolution of different gene families across different taxonomic groups. Such broad scale analyses can lead to the identification of convergent strategies for overcoming stress, such as mutations in orthologous genes associated with stress response shared across species, and can also shed light on family-, genus-, or species-specific adaptations.

Materials and Methods

High molecular weight (HMW) DNA was isolated from single individuals using several different approaches. For *S. paniceum*, *T. confusum*, *T. granarium*, *D. maculatus*, and *P. interpunctella*, DNA was isolated using the Qiagen MagAttract HMW DNA Kit (Gaithersburg, MD) following the manufacturer's directions. Unfortunately, insufficient quantites of HMW DNA were recovered from *L. serricorne*, *P. truncatus*, and *T. variabile* for 10X Chromium library preparation using this approach, so other approaches were attempted. For *L. serricorne* and *T. variabile*, an agarose isolation method was used. In brief, single insects were macerated with a pestle in a nuclei isolation buffer containing 2% Triton X-100 (w/v), 10 mM EDTA, 100 mM KCl, 4mM spermidine, 1 mM spermine, and 17.1% sucrose. 50 µL of the supernatant was transferred to 75 µL molten InCert agarose (Lonza, Basel, Switzerland). The solution was placed in a gel mold and allowed to set for 10 mins at 4°C. The sample was lysed overnight at 50°C in a solution containing proteinase K and 1% sarkosyl. The gel plugs were rinsed in TE buffer and proteinase K was deactivated for 1 hour using phenylmethane sulfonyl fluoride (PMSF). Four washes in TE buffer were used to remove PMSF and DNA was recovered from the gel plug using an agarase treatment (Wieslander, 1979). For *P. truncatus*, a salting out approach was employed. In brief, insects were macerated in a lysis buffer containing 10 mM Tris-HCl, 400 mM NaCl, 2 mM EDTA, and 0.5% SDS, and incubated overnight at 37°C with mixing. 1.2 mL of 5 mM NaCl solution was added to 'salt out' the DNA and the sample was centrifuged for 15 mins at 4°C under low speed (1,000 x g). The supernatant containing the HMW DNA was washed with ethanol and centrifuged at medium speed (6250 x g) for 5 mins. Ethanol was removed and the pellet was resuspended in TE buffer. The full protocol can be found at https://support.10xgenomics.com/genome-exome/sample-prep/doc/demonstrated-protocol-salting-out-method-for-dna-extraction-from-cells.

In all cases, the final concentration of the DNA was validated using the dsDNA High Sensitvity Assay on the Qubit Fluorimeter (Thermo Fisher Scientific, Waltham, MA) and, when sufficient DNA quantities were available, the quality of the DNA was validated using Pulsed-Field Gel Electrophoresis (PFGE). 1-5 ng of HMW DNA were used to make 10X Chromium libraries at Hudson Alpha Biotechnology Institute (Huntsville, AL) and libraries were sequencing using 150 x 150 bp reads on the Illumina HiSeq X-ten instrument to a depth of at least 60X. All genomes were assembled using the Supernova assembler with barcode subsampling in order to normalize coverage across the barcodes and improve the contiguity of the assemblies. Subsampling was performed from 30 to 70% to determine how much subsampling was needed to produce the most contiguous assembly, which was gauged using the programs QUAST (Gurevich et al., 2013) and BBTools (https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/). Criteria that were used to select the most optimal assembly included scaffold N50, maximum scaffold length, recovery of conserved single copy orthologs (BUSCOs) (Simao et al., 2015), and scaffold length accumulation curves.

Once the Supernova assemblies were finalized, either linkage maps or HiC chromatin contact maps were prepared in order to obtain chromosome scale assemblies. Linkage maps were pursued for non-quarantine and non-invasive species with short generation times that were relatively easy to

rear, including *L. serricorne*, *T. variable*, *D. maculatus*, and *T. confusum*. In brief, crosses were set up between males and females collected from different and isolated populations of each insect species. F_1 individuals were collected and sibling crosses were performed from the F_1 through the F_3 generations to facilitate recombination. At the F_4 generation, four sets of F_3 parents and 40-50 offspring derived from each set of parents were collected for genotyping using ddRAD-Seq. Samples were barcoded and sequenced on a single lane on the Illumina HiSeq 2500 platform using 100 x 100 bp paired end libraries. Prior to mapping, the genome was masked for repeats using RepeatModeler for *de novo* repeat analysis and RepeatMasker. The *T. castaneum* repeat library was used for masking in addition to the predicted repeats from RepeatModeler. The Stacks pipeline is currently being used for genotyping and variant calling and LepMap-3 will be used to identify and order linkage groups (Rastas et al., 2015). For HiC contact maps of *T. granarium*, *P. interpunctella*, *P. truncatus*, and *S. paniceum*, pools of insects were macerated and treated with formaldehyde to cross-link DNA and chromatin complexes (Belton et al., 2012). Endonucleases and restriction enzymes were used to digest the uncrosslinked regions, cross linking was reversed, and DNA was sequenced to a depth of at least 50 million 100 x 100 paired end reads on a HiSeq 2500 platform. Reads will be eventually mapped to the 10X assemblies using BWA (Li and Durbin, 2009) and LACHESIS (Burton et al., 2013) will be used to identify regions of DNA that shared chromatin contacts.

Results

HMW DNA was successfully acquired from single insects in sufficient quantities to generate 10X Chromium libraries for all eight stored product species. No major differences in DNA quality were noted across species. It was more difficult to obtain HMW DNA from *P. truncatus* using either the gel plug extraction method or the Qiagen MagAttract kit; however, HMW DNA was obtained using a salting out approach that had been previously used for prepare HMW DNA for 10X Chromium libraries for other insect species. Additionally, HiSeqX yields across all eight species were relatively consistent and ranged from 700-875 million reads. All reads were initially used for Supernova assemblies; however, barcode subsampling was employed to normalize read coverage across barcodes, which can vary significantly for genomes smaller than 1 Gb. The amount of barcode subsampling required to produce the most contiguous assembly varied by predicted genome size, with larger genomes requiring less subsampling compared to smaller genomes. Genome size estimates ranged from 150 Mb (*L. serricorne*) to 500 Mb (*D. maculatus*). Examples of assembly improvements with subsampling are shown for *T. variabile* and *L. serricorne* in Tables 1 and 2. For *T. variabile*, subsampling 160 million reads generated the best assembly metrics, including longest contig and scaffold N50s, longest maximum contig and scaffold lengths and the highest percentage of the assembly in scaffolds > 50 Kb (Table 1). Additionally, a significantly higher percentage of the genome was present in long scaffolds when 160 million reads were subsampled (Figure 1a). Similarly, subsampling 350 million reads led to the best assembly metrics for *L. serricorne* (Table 2) while subsampling either 300 or 350 million reads led to the highest percentages of the assembly in long scaffolds (Figure 1b).

Tab. 1 Assembly improvement with barcode subsampling for *Trogoderma variabile*. Assemblies were performed using Supernova with various levels of subsampling to generate the most contiguous assembly. Subsampling 160 million reads of a total of 812 million reads led to the assembly with the highest contig and scaffold N50s, the highest maximum contig and scaffolds lengths, and the highest percentage of the genome in scaffolds > 50 kb. Thus, this assembly was selected as the most optimal.

	140M	**150M**	**160M**	**170M**	**All**
Number contigs	12,688	12,643	12,903	13,622	32,292
Contig N50	250 Kb	277 Kb	294 Kb	274 Kb	33 Kb
Max Contig Length	748 Kb	605 Kb	650 Kb	619 Kb	185 Kb
Number of Scaffolds	7,003	6,894	7,122	7,687	21,917
Scaffold N50	3.8 Mb	4.9 Mb	7.0 Mb	6.3 Mb	1.0 Mb
Max Scaffold Length	17 Mb	14 Mb	22 Mb	17 Mb	8.7 Mb

Number of Scaffolds > 10 Kb	640	679	586	722	1,727
% of Genome in Scaffolds > 50 Kb	89.3%	89.2%	89.7%	88.1%	71.4%
Total Contig Assembly Length	264 Mb	265 Mb	265 Mb	269 Mb	272 Mb
Total Scaffold Assembly Length	271 Mb	274 Mb	273 Mb	278 Mb	310 Mb
% Gap	2.6%	2.8%	2.8%	3.1%	12.4%

Tab. 2 Assembly improvement with barcode subsampling for *Lasioderma serricorne*. Assemblies were performed using Supernova with various levels of subsampling to generate the most contiguous assembly. Subsampling 350 million reads of a total of 870 million reads led to the assembly with the highest contig and scaffold N50s, highest max contig and scaffold lengths, and the largest percentage of the assembly present in scaffolds > 10 Kb. Thus, this assembly was selected as the most optimal.

	160M	**270M**	**300M**	**350M**	**400M**	**All**
Number contigs	9,705	10,270	10,288	10,057	10,405	17,892
Contig N50	52 Kb	61 Kb	69 Kb	79 Kb	72 Kb	27 Kb
Max Contig Length	776 Kb	3.8 Mb	2.1 Mb	3.8 Mb	2.0 Mb	1.6 Mb
Number of Scaffolds	9,266	9.653	9,594	9.315	9,540	16,588
Scaffold N50	55 kb	75 Kb	87 Kb	119 Kb	118 Kb	38 Kb
Max Scaffold Length	851 Kb	3.8 Mb	3.8 Mb	3.8 Mb	2.9 Mb	2.8 Mb
Number of Scaffolds > 10 Kb	2,562	2,313	2,105	1,963	2,125	3,061
% of Genome in Scaffolds > 50 Kb	56.7%	61.1%	64.4%	66.4%	64.2%	42.0%
Total Contig Assembly Length	143 Mb	154 Mb	154 Mb	156 Mb	161 Mb	160 Mb
Total Scaffold Assembly Length	144 Mb	155 Mb	155 Mb	154 Mb	159 Mb	164 Mb
% Gap	0.1%	0.2%	0.2%	0.2%	0.3%	0.4%

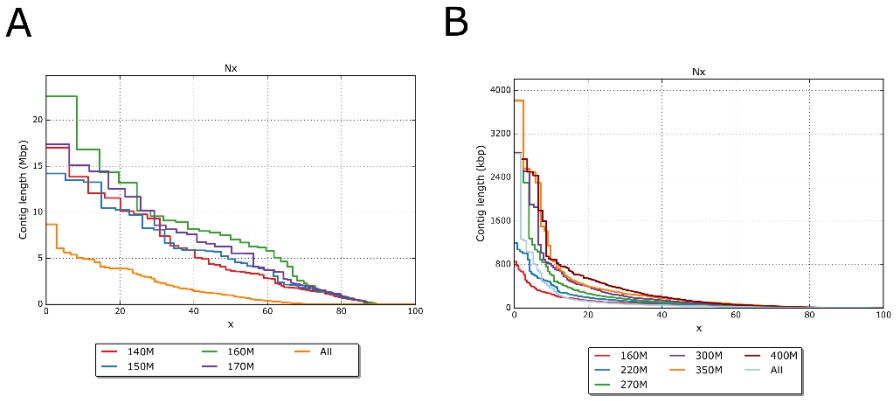

Fig. 1 Scaffold size distribution for a) *Trogoderma variabile* and b) *Lasioderma serricorne*. Y-axis represents scaffold length and X-axis represents number of scaffolds at each length. For *T. variabile*, subsampling 160 million reads led to the highest representation of long scaffolds in the assembly and for *L. serricorne,* subsampling either 300 or 350 million reads led to the highest representation of long scaffolds.

Assemblies for *T. granarium T. confusum*, and *D. maculatus* were similarly optimized. Although the assembly contiguity varied among these three insects, with contig N50s ranging from X to Y, scaffold N50s ranging from Z to Q, maximum contig lengths ranging from Y to Z, and maximum scaffold lengths ranging from blah to blah, all three assemblies had over 80% of their total assembly lengths in scaffolds > 50 kb and over 94% of conserved single copy orthologs were detected (Tables 3 and 4). While we are still awaiting sequencing data for *P. interpunctella, P. truncatus, and S. paniceum*, libraries have been prepared. We are also in the process of improving our scaffolding metrics using linkage maps and HiC contact maps.

Tab. 3 Assembly metrics for Trogoderma granarium, Tribolium confusum, and Dermestes maculatus optimized with barcode subsampling.

	T. granarium	***T. confusum***	***D. maculatus***

Number contigs	25,392	16,079	32,623
Contig N50	50 Kb	129 Kb	102 Kb
Max Contig Length	862 Kb	734 Kb	930 Kb
Number of Scaffolds	18,835	10,607	23,540
Scaffold N50	570 Kb	1.2 Mb	876 Kb
Max Scaffold Length	3.6 Mb	11 Mb	6.4 Mb
Number of Scaffolds > 10 Kb	1,596	702	1,747
% of Genome in Scaffolds > 50 Kb	80.1%	90.1%	83.1%
Total Contig Assembly Length	297 Mb	294 Mb	456 Mb
Total Scaffold Assembly Length	329 Mb	306 Mb	460 Mb
% Gap	9.5%	3.9%	3.8%

Tab. 4 BUSCO (Benchmarking Using Single Copy Orthologs) metrics for stored product assemblies completed to date.

	L. serricorne	***T. variabile***	***T.*** *granarium*	***T. confusum***	***D. maculatus***
Complete/Single Copy	94.3%	98.3%	94.9%	96.9%	96.6%
Duplicate/Single Copy	0.8%	0.7%	0.9%	0.8%	0.5%
Missing	2.8%	0.5%	0.5%	0.5%	2.0%
Fragmented	2.9%	0.5%	1.9%	1.8%	2.7%

Discussion

Overall, the 10X Chromium libraries alone produced high quality assemblies that recovered significant percentages of the predicted gene space as the recovery of BUSCOs ranged from 92 to 98%. Assembly qualities were also relatively consistent regardless of genome size or taxonomic group and in all cases, over 80% of the assembly was present in less than 1,000 scaffolds, suggesting that these libraries can be a good first approach for assembling high quality draft genomes from insect species from many underrepresented taxonomic groups. In addition to the assemblies presented here, 10X Chromium libraries have been also used to produce high quality assemblies of aphid, butterfly, and dipteran genomes, providing further support for the use of this technique (Talla et al., 2017). Using linkage maps or HiC analyses, higher order assemblies will be obtained and will be almost to chromosome scale. Assemblies of this caliber can lead to the identification of syntenic orthologs across species. The identification of syntenic orthologs is important because orthologous genes present in the same chromosomal locations across species often have conserved functions, which can greatly facilitate functional annotation for non-model species (Zheng et al., 2004). In addition, the identification of syntenic orthologs may also expedite the identification of genetic targets for pest control (Futahashi, et al., 2011). For example, if knocking down a syntenic ortholog in one species reduces fitness or causes lethality, knocking down the same gene in other species that share synteny will likely also cause similar phenotypes. Although long-read sequencing approaches, such as PacBio, can generate assemblies of similar contiguity, the cost of the 10X libraries and the accompanying seuqencing is substantially less, potentially facilitating larger-scale comparative genomics studies that can be used to address broader evolutionary questions. In addition, because inbreeding is not necessary, 10X libraries can be generated much more rapidly relative to long-read sequencing approaches, which may greatly expedite genome assemblies for emerging or invasive pests.

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DNA barcode of stored-product Pests based on Mitochondrial Cytochrome Oxidase I Gene

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Abstract

The stored-product pests are economically important that can be spread through grain trade. Most stored-product pests, including eggs, nymphs, and adults, are very small and difficult to identify morphologically. Also the classification and identification of them have always been hindered by the overwhelming number of species, widely distribution. Here, we collected 43 stored-product pests from 46 geographical locations in China and other countries. The mtDNA COI gene sequences were sequenced. Software MEGA 5 was used to analyze the sequence comploition and genetic distances. Three molecular phylogenetic trees of Platypodidae were recomstructed using PAUP4.0 according to distance/ the neighbour-joining (NJ) and maximum parsimony (MP). The molecular results were compared with the morphological taxonomy. The interspecific genetic distance of the stored-product pests was significantly higher than the intraspecific genetic distance according to the barcoding gap analysis. This work provides a practical approach for the precise and rapid diagnosis of stored-product pests.

Keywords: DNA Barconding, stored-product pests, mtDNA COI gene, phylogeny

Introduction

Stored-grain insects and mites are great economic significance for grains and other products during storage. They are closely related with human life. The rapid identification is the precondition and basis for the comprehensive prevention and control of the pests. The modern identification of stored-grain insects by molecular biology techniques is able to get rid of the influence of the growth situation of individual specimen and the environment, and get accurate and reliable result from their DNA.

Traditionally, the species have been identified based on morphological characteristics of the adult. However, the identification of the species based on immature stages (i.e., egg, larva or pupa) or adult body parts, which lack distinct diagnostic characteristics, is very difficult and somethimes not reliable (Li et al., 2011). Traditional morphological identification is also time-consuming, requires specialized taxonomic knowledge and microscopy techniques (Yang et al., 2013; Jiang et al., 2014)

Recently, molecular identification based on nucleotide sequence analysis has become an effective method used to complement traditional taxonomic identification. For some important insect pests of stored products, AFLP has been used to diagnostic *Liposcelis* (Qin et al., 2008; Li et al., 2011), *Sitophilus oryzae* and *Sitophilus zeamais* (Hidayat et al., 1996), DNA barcode technology for *Liposcelis entomophila* (Yang et al., 2012). Some recent studies have implemented by PCR with species-specific primer pairs (Zhao et al., 2016). Species-specific primer identification is a PCR-based procedure that yields a unique band of known size and allows a species to be identified directly after gel electrophoresis (Wu et al., 2016). In animals, mitochondrial cytochrome c oxidase I (COI) gene, has been shown to be a reliable, quick and cost-effective tool for the identification of organisms of various taxa in all life stages. More and more important insect pests have been identified by this way (Namikoshi et al., 2011; Zhang et al., 2012; Jiang et al., 2014). It is a great advantage especially for the identification of small size pests.

DNA barcoding is a DNA-based species identification system which offers a promising supplemental technique with standardized portions of the genome (Hebert et al., 2003). The most commonly used barcode gene, mitochondrial (mt) DNA cytochrome c oxidase I (COI), has been shown to be a reliable, quick and cost-effective tool for the identification of organisms of various taxa in all life stages (Augot et al., 2010; Cywinska et al., 2010). A threshold of 2-3% mtDNA COI sequence divergence was recommended to define separate species for insects and mammals (Hebert et al., 2003). In studies of butterflies and ants, DNA barcoding has been successful in defining species boundaries by genetic distance thresholds (Hebert et al., 2004; Smith et al., 2005); however, there is no established universal distance threshold value to distinguish between taxonomic groups.

In the present study, we describe a reliable and efficient method based on conventional PCR with mtDNA COI gene, and we set up a DNA barcode data bace for stored-product Pests, which we hope will prove useful for the rapid diagnosis.

Materials and Methods

Specimens used in this study are collected from different provinces in China, including 46 geographical locations as fig. 1, some mites form Czech Republic, and except *Liposcelis bostrychophila*, *L. entomophila*, *L. decolor*, *and L.paeta*, the samples of *Liposcelis* from the Plant quarantine laboratory of China Agricultural University (CAUPQL). There are 43 species of stored-grain insects/ mites in total, 415 individuals, every species at least 5 specimens.

Total genomic DNA was extracted from the entire body of individual adults using the TIANamp Genomic DNA kit (DP304, TIANGEN, China) following the manufacturer's protocol for animal tissue. Five individuals from each species were used. PCR was performed with a pair of universal primers, LCO1490 (fw) 50 GGT CAA CAA ATC ATA AAG ATA TTG G 30 and HCO2198 (rev) 50 TAA ACT TCA GGG TGA CCA AAA AAT CA 30, amplifying an approximately 710 bp fragment of the standard mtDNA COI-5 barcode (Folmer et al., 1994). PCR products were separated on a 1.0% (w/v) agarose gel (1 × TAE), stained with ethidium bromide, and visualised under UV light. The agarose gel slice

containing the PCR amplicon of interest was excised and placed in a centrifuge tube. The agarose gel slice containing the PCR amplicon of interest was excised and the DNA was gel extracted. Bidirectional sequencing reactions were carried out from a single individual of each geographical isolate (Beijing Aoke Biotechnology Co., Ltd.).

DNAMAN software (Lynnon Biosoft, Vaudreuil, Quebec, Canada) was used for DNA multiple sequence alignment using an optimal alignment method. Genetic diversity was estimated for haplotype diversity (Hd) and nucleotide diversity inDnaSP version 4.10.1 (Librado & Rozas, 2009). Pairwise genetic distances for COI were calculated using the Kimura-2-Parameter (K2P) distance model implemented in the software Molecular Evolutionary Genetics Analysis 5 (MEGA 5; Tamura et al., 2011). All phylogenetic analyseswere carried out using the program PAUP 4.0 (Swofford, 2002). Two different types of phylogenetic trees, neighbour-joining (NJ) andmaximumparsimony (MP), were graphically displayed and compared. A heuristic search was employed using tree bisection and reconnection (TBR) branch swapping and randomaddition for 100 replicates, and bootstrapping was performed using 1000 replications.

Results

In this study, we tested and evaluated the general genes of COI, which is an appropriate gene for identifying the DNA barcode of stored-grain insects/mites – a section of 650bp COI gene in mitochondria (primer pair LCO490/HCO2198). The mtDNA COI sequences of 415 obtained in this study. The sequences were all trimmed to a 650 bp core region that could be unambiguously aligned to one another. No sequences contained indels or nonsense codons, allowing for easy alignment and supporting their origin in the mitochondrial gene.

Fig. 1 Distribution of sampling sites for stored grain insects and mites in China

A rapid identification DNA barcode sequence database of stored-grain insect/mite is established, including 43 species of stored-grain insects/ mites. Every species has at least 5 specimens, we get the COI barcode sequence of 415 specimens. 98 haplotypes, among which only 1 haplotype is found in 20 species of insects/mites, showing the diversity of stored-grain insect is relatively low. Also most showed low intra-species divergence. Based on DNA barcode sequence and distance method, the neighbour-joining (NJ) and maximum parsimony (MP), the phylogenetic tree of stored-grain insect/mite is built. Both the NJ and MP phylogenetic analysis of the COI gene generated the same tree topology. The resulting trees showed a clear clade and every species has individual branch.

DNA barcode in the mitochondria COI sequence can be used to identify the species of stored-grain insects/mites rapidly and accurately.

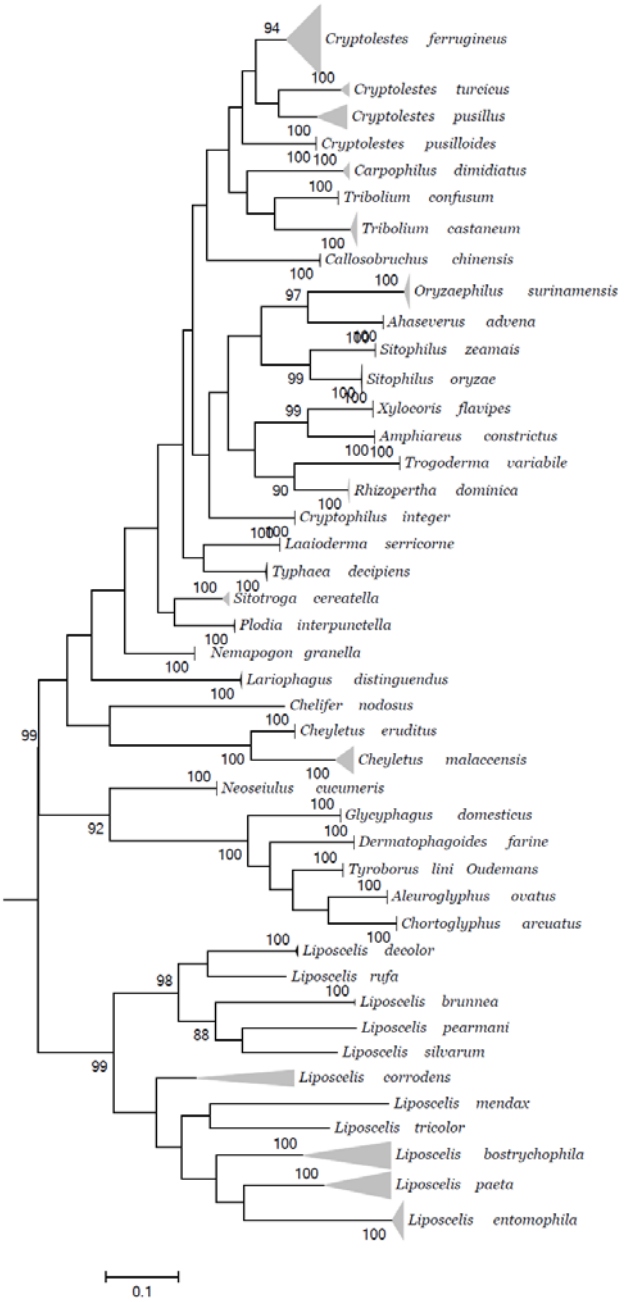

Fig. 2 Neighbour-joining tree of stored product insects and mites

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Effect of delayed mating on reproductive performance of *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae)

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Abstract

With the ban of methyl bromide and the many problems associated with the use of other synthetic chemicals, current research have focused on non-chemical alternatives and integrated pest management approach for the control of stored product insect pests. Mating disruption is one technique being investigated for its effect on stored product insects. In this study, we determined the effect of age at mating on the reproductive rate and longevity of the cigarette beetle, *Lasioderma serricorne* (Coleoptera: Anobiidae). We disrupted the mating approach by delaying the insects from mating for different time periods in days. Same age virgin male and female cigarette beetles were paired to mate soon after emergence (0 d old), or delayed from mating for 1–14 d. In another experiment, we maintained the age of the male at 0 d old and varied the age of the female from 0–14 d old and vice versa. Insects were observed daily for longevity and F_1 progeny was recorded 7–10 weeks after mating pairs were put together. Progeny production generally decreased with age of adults at mating. The number of F_1 progeny produced by same age adults varied from 10 per female to 59 per female. Similarly, the number of progeny decreased the longer one sex was delayed from mating. Findings from this study may provide information for the development of mating disruption techniques that can delay mating and may be effective in keeping populations of *L. serricorne* below levels that would warrant a control action.

Keywords: cigarette beetle, stored products, mating disruption, progeny production, methyl bromide alternatives

1. Introduction

Lasioderma serricorne (F.) (Coleoptera: Anobiidae), commonly known as the cigarette beetle or the tropical warehouse beetle, is a common stored product insect pest of feed mills and retail stores. *L. serricorne* causes significant damage to grain-based products, tobacco products, and other commodities of animal or vegetable origin (Arbogast, 1991; Dimetry et al., 2004; Mahroof and Phillips, 2008). The damage caused by this pest can account for millions of dollars in the Food and Feed Industries (Arbogast, 1991).

The ban of methyl bromide and the development of resistance to phosphine by the cigarette beetle (e.g. Savvidou et al., 2003; Sağlam et al., 2015; Fukazawa and Takahashi, 2017), has resulted in the search for potential non-chemical alternatives for the control of this pest (including Adler, 2003; Roesli et al., 2003; Conyers and Collins, 2006; Yu, 2008; Mahroof and Phillips, 2014).

Delayed mating techniques have been widely studied and used successfully in the control of many insect pests (including Ellis and Steele, 1982; Lingren et al., 1988; Fadamiro and Baker, 1999). Mating disruption involves the use of synthetic pheromones that mimic the natural sex pheromone normally released by the female. The release of high concentrations of the synthetic chemical 'confuses' the male which expends energy in finding the source of the pheromone and ends up delaying mating or not mating all together. To our knowledge, however, limited studies have been carried out on stored product beetles on mating disruption and mating delays. Few studies have been carried out on lepidopteran insects including the Indian meal moth, *Plodia interpunctella* (Hübner) (including Mbata, 1985; Huang and Subramanyam, 2003), with only one published studies on *L. serricorne* (Mahroof and Phillips, 2014). Mbata (1985) reported that a delay in mating resulted in a significant reduction of the number of eggs laid by *P. interpunctella* female as mature eggs were retained in the ovaries. Huang and Subramanyam reported that fecundity in female *P. interpunctella* significantly decreased by about 25 eggs for each day mating was delayed. The authors also reported that delaying mating in both sexes for 5 d resulted in the production of non-viable eggs by the female. Mahroof and Phillips (2014) studied the effect of the synthetic form of the predominant sex pheromone, serricornin, on the mating disruption of *L. serricorne*. The inhibition of proper orientation behavior of the males to females disrupted mating, resulted in delay in mating, and reduced the mating success. As a result, a significant reduction in the population size of subsequent generations was reported. From this study it was not clear why males fail to locate females in an environment purged with high concentration of synthetic pheromone. False trail following, masking of natural female pheromone or habituation of olfactory receptors may delay the age of mating (Mahroof and Phillips, 2014). The objective of this study was therefore to

investigate the effect of adult age at mating on the fecundity of females and the longevity of adult *L. serricorne*.

2. Materials and Methods

2.1. Insects

L. serricorne used for this study were from colonies which had been maintained at the Stored Products Entomology Research Laboratory at South Carolina State University since 2010. Prior to the bioassays, new colonies were established by transferring newly emerged adults to 473 ml rearing jars (Ball Corporation, Broomfield, CO, USA) with food made of 95% whole wheat flour and 5% yeast. The adults were allowed to lay eggs for 48 h and the rearing jars were then incubated for 31–35 days to attain the pupal stage of the insect. Cigarette beetle pupae were sexed using differences in the genital papillae (Halstead, 1963) and kept separately in jars containing some food. The jars were checked daily for adult development. Adults that developed in each jar were collected daily and kept in separate jars to be used when required. Adults of 0–14 d old were used in this study.

2.2. Mating of insects

One male and one female adult cigarette beetles of the same age were paired in a 5 cm high, 2 cm diameter plastic vial that contained 2 g of the diet mix. Same age insects (0-14 d) were paired up in a 5 cm high, 2 cm diameter plastic vial that contained 2 g of the diet mix. For each of the 15 ages, ten vials were set up. The vials were kept in an incubator at approximately 27.6 ± 0.1°C and 60.8 ± 0.8% RH. We determined the longevity of mated adult insects. The vials were checked daily until all adults died.

In another set of experiments, newly emerged (0-d old) virgin males were paired up with newly emerged virgin females or with 1–14 d old virgin females. Also, newly emerged virgin females were paired up with newly emerged virgin males or with 1–14 d old virgin males. Each mating treatment was done in a 10 cm high, 2 cm diameter plastic vial that contained 5 g of the diet mix. Each mating treatment was replicated 10 times.

2.3. Data analyses

The number of adults that developed in each vial was recorded weekly beginning 7 weeks after set up until 10 weeks. Data on the number of F_1 progeny produced in each mating treatment were subjected to one-way ANOVA and means were separated using Tukey's Honest Significant Difference (HSD) test when the ANOVA was significant at $P \leq 0.05$ (PROC GLM, SAS Institute, 2013).

We also determined the relationships between the longevity of the adults and their age at mating using regression analysis in TableCurve 2D software (Systat Software Inc., 2002).

3. Results

3.1. Effect of delayed mating on progeny production

The number of F_1 progeny produced by same age adults was significantly different as delay in mating increased ($F = 33.36$; $df = 14, 135$; $P < 0.0001$). Fewer progeny were produced by newly emerged adults (0-d old), with the highest progeny production by adults delayed from mating for 1- or 2-d. Delaying mating for two days significantly reduced the number of progeny produced (Fig. 1). The number of progeny produced by 6–11 d old adults did not differ significantly among each other.

The number of F_1 progeny produced when newly emerged males (0-d old) were paired with newly emerged females or with 1–14 d old females was significantly different ($F = 39.47$; $df = 14, 135$; $P < 0.0001$). The highest number of progeny were produced when both parents were 0 d old but it was

not significantly different from when the female was 1 d old. The number of offspring produced by 1–4 d old females were similar. The longer mating was delayed, the fewer the offspring produced (Fig. 2).

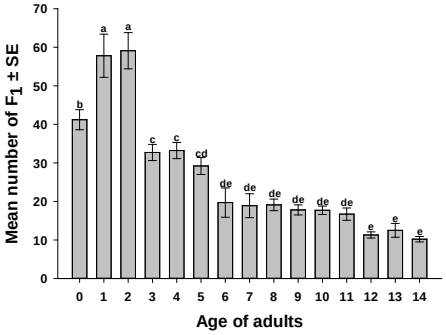

Fig. 1 Mean progeny production ± SE in same-age adult *Lasioderma sericorne* delayed from mating for different days. Bars with different letters represent means that are significantly different (Tukey's Honest Significant Difference test, $P < 0.05$).

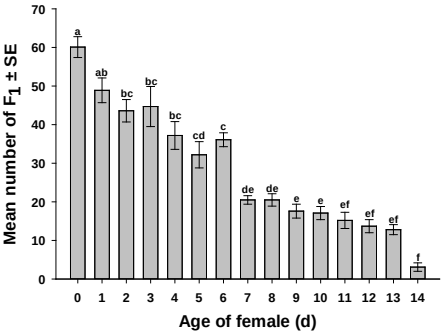

Fig. 2 Mean progeny production ± SE in adult *Lasioderma sericorne* delayed from mating for different days. Newly emerged (0-d old) males were mated with 0–14 d old females. Bars with different letters represent means that are significantly different (Tukey's Honest Significant Difference test, $P < 0.05$).

When 0-d old females were mated with 0–14 day old males, the number of F_1 progeny produced varied significantly ($F = 10.31$; df = 14, 135; $P < 0.001$) (Fig. 3). The trend was similar to that of the mating treatments where females were delayed from mating with newly emerged males. Similarly, newly emerged adults produced the highest number of progeny, however, not significantly different from progeny produced as a result of mating 0 d females with 1 d old males. Generally, the older the male, the fewer the number of progeny produced.

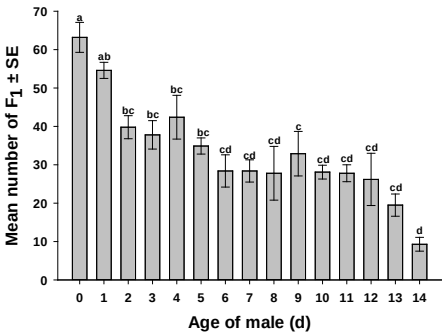

Fig. 3 Progeny production in adult *Lasioderma sericorne* delayed from mating for different days. Newly emerged (0 d old) females were mated with 0–14 d old males. Means followed by different letters are significantly different (Tukey's Honest Significant Difference test, $P < 0.05$).

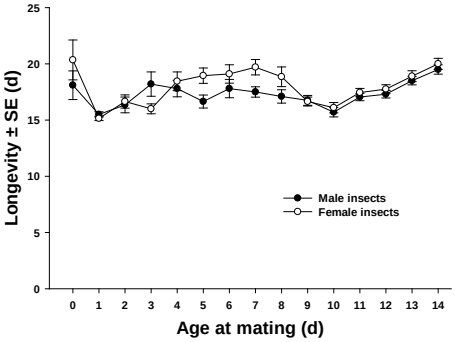

Fig. 4 Longevity of same-age adult *Lasioderma sericorne* mated at different ages. Longevity data accounts for their age at mating and the number of days they lived post-mating.

3.2. Effect of delayed mating on adult longevity

Average longevity ranged from 15.5 ± 0.2 to 19.5 ± 0.4 d in mated males and 15.2 ± 0.2 to 20.4 ± 1.8 d in mated females. Longevity was reported as the total lifespan of the insects, accounting for their

age at mating and the number of days they lived post-mating. There was a weak relationship between female longevity and age at mating ($y = 17.77 + 0.0000019^{x}$; $n = 20$; $r^2 = 0.14$) but a moderate relationship between male longevity and age at mating ($y = 17.05 + 0.0000022^{x}$; $n = 20$; $r^2 = 0.42$) (Fig. 4).

4. Discussion

The effect of delayed mating on progeny production in *L. serricorne* was investigated in this study. Progeny production was highest when there was no mating delay. Mating without delay may encourage multiple mating, probably because the adults are able to start mating early, subsequently resulting in an increase in the number of progeny produced (Huang and Subramanyam, 2003). As either sexes (Fig. 1) or one of the sexes (Figs. 2 and 3) age, fewer progeny was produced. Our findings were similar to those of other authors that reported the significance of multiple mating in progeny production in insect pests (including Huang and Subramanyam, 2003; Jiao et al. 2006; Yu, 2008). Huang and Subramanyam (2003) reported a significant reduction in fecundity of *P. interpunctella* for each day mating was delayed. The authors also reported that the majority of eggs laid by the female were laid within 4 d of mating. Jiao et al. (2006) reported a significant decrease in fecundity with increasing age at mating in the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae). Yu (2008) reported a significant decline in daily egg production in *L. serricorne* females 7 d after being paired with males. In our study, for each day that mating was delayed in any of the two sexes, 8–57 less progeny were produced. Delaying male or female mating for 2 d or more may have a significant impact on fecundity of *L. serricorne* and this could lead to a significant suppression in the population size of subsequent generations.

Studies have shown that increased fecundity in some multiple-mated females, and therefore increase in progeny production, may be due to the repeated transfer of some compounds including nutrient secretions and other hormones from the male to the female during copulation (Benz, 1969; Henneberry and Clayton, 1984; Park et al., 1998).

Many factors including mating status and diet have been reported to affect the longevity of stored product insect pests. Huang and Subramanyam (2003) reported that mated *P. interpunctella* moths lived for approximately 4–7 d. Yu (2008) reported that mated *L. serricorne* adults lived for 17–23 d, while unmated adults lived for 29–35 d. Findings in our study are similar to those of Yu (2008). In our study, we reported longevity of approximately 15–20 d in mated males and 15–22 d in mated females. Although not presented here, we observed that unmated males lived for approximately 28 d while unmated females lived approximately 3 d longer. The mating status of *L. serricorne* therefore seems to have an effect on the longevity of the insect. Adult longevity in *L. serricorne* has also been reported to be influenced by the diet on which the insect is raised (Mahroof and Phillips, 2008). The authors reported that adult longevity varied from 10–20 d depending on the food source.

Although the age of insects at mating has been established to be important in determining the fecundity (Mbata, 1985; Makee and Saour, 2001; Huang and Subramanyam, 2003), other factors such as diet, temperature, light have been shown to be equally important as well (Mbata, 1985; Shinoda and Fujisaki, 2001; Mahroof and Phillips, 2008; Vukajlović and Pešić, 2012). These factors may also be investigated to help develop pest management techniques. Findings in this study may be useful in the development of mating disruption techniques as an alternative control method that may be essential in managing *L. serricorne*.

Acknowledgement

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Larvae of Trogoderma respond behaviorally to whole body extracts

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Abstract

Behavioral responses to semiochemicals by *Trogoderma* (Coleoptera: Dermestidae) stored product pests were assayed in a small arena. Hexane extracts were obtained from Khapra beetle, *Trogoderma granarium*, warehouse beetle, *Trogoderma variable*, and the larger cabinet beetle *Trogoderma inclusum* that were killed by being frozen for 48 hours at -20° C. These extracts were analyzed using gas chromatography coupled with mass spectrometry (GC/MS), and it was confirmed that they contain several cuticular hydrocarbons, fatty acids and sterols. Two choice experiments were performed inside Petri dish arenas, with filter paper fully covering the bottom surfaces. Two smaller 3cm filter papers were placed on opposite ends within each arena. Each of the smaller papers were folded three times in parallel to present a corrugated surface that the insects could move underneath if they chose. In each case, one paper had a 100µl aliquot of one of the extracts, and the other 100µl of hexane as a control. 10 late instar larvae of the same species as the treatment extract were placed in the arena and allowed to acclimate overnight in a dark room. For all three species, it was found that larvae were more likely to be found on the side of the Petri dish with the hexane control rather than the conspecific larval extract. They were also more likely to be on or near the smaller corrugated filter paper treated with the control as opposed to the filter paper treated with the larval extract. Thus repellency of the conspecific extract was demonstrated at that particular dose. Further assays using different doses of the raw extracts and their individual chemical components are planned. The use of these semiochemicals in novel management strategies will be considered.

Keywords: Aggregation, behavior, khapra beetle, management, pheromone

Introduction

The khapra beetle (KB), *Trogoderma granarium* is a serious pest of stored products and is the only stored products pest that is currently quarantined in the United States. KB larvae feed on a wide range of dry food products of plant and animal origin including cereal grains, dried fish and museum specimens (Hagstrum et al. 2013). It currently has an extensive distribution throughout warm and arid regions of Eurasia and Africa. It is a quarantine pest in the United States, which has a history of interception at ports and successful eradications of various scales, at specific locations (Armitage, 1958, Myers and Hagstrum 2012). Preventing establishment of the khapra beetle in the US is crucial to maintaining access of products to export markets. Various kairomone attractants have been developed for monitoring the species. Additionally a pheromone produced by adult females is used in current APHIS-PPQ monitoring efforts (Barak, 1989).

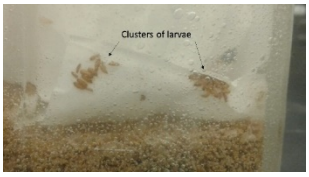

Fig. 1. KB larvae assembling in clusters on paper placed in a laboratory colony jar.

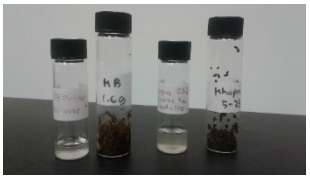

Fig. 2. Hexane extracted from KB larvae. The extract is contained in the hexane in vial on the left, which was removed from the one on the right containing the dead larvae.

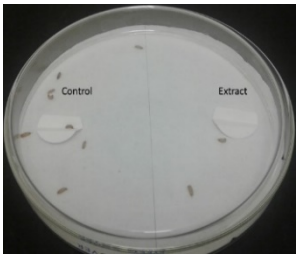

Fig. 3. Bioassay showing that in this instance, KB larvae preferred to remain on the left side of the arena, but without substantial clustering on the smaller treatment paper.

The possibility that pheromones affect the movement of larvae has not been investigated. Any such chemicals potentially could be incorporated into novel management strategies. For larvae, it was shown some time ago that carbon dioxide and certain food odors can be attractive, while many short chain (3-7 carbon) alcohols and organic acids can be repellant (Spangler 1965). Currently the adult pheromone and host associated kairomones are used in traps. However, it is readily observed that KB larvae, as well as those of other Trogoderma species will assemble in clusters, even on non-food sources such as papers placed in laboratory colony jars (Figure 1). The question of whether there may be semiochemicals that mediate this particular behavior has not been investigated. In this study, this possibility was researched in KB, as well as two related species, the warehouse beetle (WB), *Trogoderma variable*, and larger cabinet beetle (LCB), *Trogoderma inclusum*. We included the additional species because it is of interest to what degree such behaviors are common in the genus, particularly since several species share the same adult pheromone.

Materials and Methods

Hexane extracts were obtained from late instar larvae and adult that were killed by being frozen for 48 hours at -20° C (Figure 2). Extracts were made at a ratio of 4ml of hexane/ 1 g of larvae of each species. These extracts have been analyzed using gas chromatography coupled with mass spectrometry (GC/MS), indicating the presence of a number of compounds with fragmentation patterns that are consistent with those of cuticular hydrocarbons. Previously published research confirms that similarly prepared extracts of khapra beetle larvae using dichloromethane rather than hexane contained a number of cuticular hydrocarbons (Maliński et al., 1986). Furthermore adults also have a similar hydrocarbon profile (Dubis et al., 1987). In our newly prepared extracts, there were no noticeable differences among the traces for any of the different species. Furthermore each contained a region of several compounds indicative of hydrocarbons, all at similar retention times. The identities of such compounds in our extracts have not been confirmed, but there is little reason to believe they are different from the chemicals described in the literature. There were also a number of other compounds in the extracts, including particular fatty acids and sterols. These compounds also may potentially affect behavior.

For assessing whether the extracts can influence the behavior of the larvae, two choice experiments were performed in static air enclosures. Inside of 15cm glass petri dish arenas, a filter paper was placed that fully covered the bottom surface (Figure 3). Within each of these arenas, two smaller 3cm filter papers were placed, which were used for presenting chemical treatments and providing a possible clustering surface. To encourage clustering, each of the papers were folded three times in parallel to present a corrugated surface. For each experimental replicate that was performed in an arena, one of the smaller corrugated papers received a 100µl aliquot of one of the extracts, and the other smaller paper 100 µl of hexane as a control. This was a dose of roughly five larval equivalents. Ten late instar larvae were placed in the arena and allowed to acclimate overnight in a dark room.

Long-chain hydrocarbons have very low volatility, and if behaviorally significant, generally will function as close range pheromone attractants. Thus the static air environment of the Petri dishes is not different from the context within which the larvae are likely to respond to such signals. The dose of five larval equivalents was selected because it would represent the chemical equivalent of an existing cluster of larvae.

Results

For all three species, the larvae were more likely to be found on the side of the Petri dish with the hexane control, while less likely to be on the side with the conspecific larval extract. A total of 36 replicates were performed for each species and the percentages of larvae on the control side of the arenas were 63% for KB, 72% for WB, and 71% for LCB. Most of these larvae were not found on or under the folded control or treated filter papers. Thus 66% of KB, 65% of WB, and 69% of LCB, were found in other parts of the arena. When not clustered on the folded papers, several were often

clustered in other locations. Among those that were on the clustered papers, 80% of KB, 76% of WB, and 75% of LCB, were on the control paper, versus the treated one. Thus the conspecific extract was repelling the assembly of the larvae of all three species. The attraction that was expected given the observations of clustering behavior did not occur.

Discussion

In considering these results, it should be noted that the dose applied was greater than the equivalent of a single larvae, and thus the concentration of the chemicals may indicate a biologically unrealistic situation. Furthermore, it is not clear yet whether the cuticular hydrocarbons are producing this reaction or if some of the other compounds in the extracts, such as the fatty acids and sterols may be causing repulsion. It is possible that production of these same compounds, or others were elicited by the stress of the insects being frozen to kill them before the extraction. Any such a compound would thus function as an alarm pheromone that repels other insects from joining with and aggregating near other larvae that are distressed.

Whatever the causal factor of the repellency may be, understanding the mechanism may provide a product useful for the management of KB. For example it may be possible to incorporate such a compound into treatments of products or their packaging in a way that repels the larvae to protect the products. There is also the possibility that a repellent compound could perhaps be used in push-pull trapping strategies.

Additionally, it may also be worth revisiting the idea of whether clustering can occur if perhaps only the cuticular hydrocarbon portions of the extracts are used. If there is another compound causing repellency, it could be masking the effects of attractive compounds. There are also potential dosage issues with any behaviorally active compound. It could be that repellent compounds are attractive at other doses. Thus much additional work will be needed to fully utilize the capabilities of the behaviorally active components of such extracts. However, it is promising that at this stage in our investigations, behavioral activity has been clearly demonstrated.

Another final consideration may be whether adults have similar responses to such compounds. We did attempt to assess the response of adults to such extracts in the assay described above. However, it became clear very quickly that the adult insects were highly mobile and did not settle in clusters like the larva. Many were actively crossing between the sides of the Petri dish as they were being evaluated at the end of the overnight period. Thus, the evaluation of the adult behavior with respect to these extracts may require a different assay. For example, a small scale wind tunnel, or two-choice olfactometer with moving air, may be more applicable.

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Necrobia rufipes (De Geer): an emerging pest associated with pet store chain in Europe

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Abstract

Necrobia rufipes is a cosmopolitan pest, causing considerable damage to stored commodities such as copra (dried coconut), cheese, dried fish, ham. The present study was undertaken to investigate the occurrence of these insects on pet store chain in Europe. In the last year *N. rufipes* was found associated with pet food, especially in Mediterranean countries, causing considerable economic damage and loss of product. The causes of such sudden diffusion are not known but some considerations are reported. Future studies will be needed to collect data on development on pet food and on the possibility to monitor *N. rufipes* in wharehouses and pet stores.?

Keywords: pet food, pest infestation, red-legged ham beetle, Cleridae.

Necrobia rufipes (De Geer) is a beetle, belonging to family Cleridae. Riley (1874) gave it the common name of red-legged ham beetle, while in the Pacific Island is known as copra beetle (Froggatt, 1911). Riley made the first economic investigation, citing cases of extensive injury to hams in St. Louis and Boston (USA). It is a cosmopolitan pest, associated to copra (dried coconut), cheese, dried fish, cured ham and bacon. However it is reported to feed on other pests that infest products or decaying animal matter (Simmons and Ellington, 1925; Ashman, 1963; Peck and Thomas, 1998). *N. rufipes* was also found on mummies (Panagiotakopulu, 2001), and it is considered in forensic entomology since it is usually found on carrion after most of the flesh has been remove, presumably feeding on other insects rather than on the carrion itself (Kulshresthaa and Satpathy, 2001).

In 2003 the red-legged ham beetle was found in retail pet store and in 2007 it was reported infesting pet food in Brazil, but the origin of infestation was unknown (Roesli et al., 2003; Gredilha and Lima, 2007).

In the present note we report the *N. rufipes* presence in Mediterranean countries, especially associated with pet food warehouses and retail pet stores. The first report dates back 2015 in Israel, in 2016 it was found in Southern Italy (Puglia region), in 2017 in Greece, Turkey, Montenegro, Germany and Czech Republic, Southern France, Spain, Northern Italy, all Southern regions and island.

The causes of such a wide spread of this pest are, for now, unknown. Pet food products are rich in animal protein content, particularly suitable for *N. rufipes* development, and pet food packaging are often not resistant to insect infestation. Different Authors suggest that the presence of *N. rufipes* is mainly linked to the predation of other pests (Kulshresthaa and Satpathy, 2001; Roesli et al., 2003). The red-legged ham beetle is reported as a facultative predator on larvae of *Lasioderma serricorne* (F.), *Oryzaephilus mercator* (Fauvel), *Carpophilus dimidiatus* (F.) (Simmons and Ellington, 1925; Ashman, 1963). In effect, in three cases of pet food infestation we verified the simultaneous presence of *N. rufipes* and the sawthoothed grain beetle, *Oryzaephilus surinamensis* (L.).

An important way of diffusion of the pests is represented by pallets and packaging materials. Several *N. rufipes* larvae were found on crevices of pallet wood and packaging like carton, protected in a white pupal chamber produced by themselves. Furthermore adults are able to fly and are attracted by food odors from long distance. We observed in several retail pet stores an incorrect management of waste material; broken pet food bags, pallets, cartons were stored near the entrance doors. We also verified that pallets and packaging are preferred by *N. rufipes* larvae for pupation and this is the way to spread infestation.

A critical point to manage *N. rufipes* infestation is that the monitoring is difficult, as there are no

commercial lures specifically available for baiting traps. A sexual attractant was identified and described in a Chinese patent, but not commercially available (Lei and Guangwei, 2016). *N. rufipes* larvae and adults were captured with commercial pitfall traps baited with food oil and pheromone lures (not specific for *N. rufipes*) from Trécé, in pet food retail stores (Roesli et al., 2003), but currently there is not a trap specifically set up for *N. rufipes*. This is a weak point for the management of infestations as it is not possible to constantly monitor the presence of the insect in industries, warehouses, pet stores.

Another aspect to take in account is the lack of specific information on control strategies. Only Roesli and Subramanyam (2002) reported that precision targeting using sanitation alone did not have an impact on *N. rufipes* adults, but reduced larval presence. Precision targeting with sanitation followed by cyfluthrin spray greatly reduced both larvae and adults.

We can conclude that the diffusion of *N. rufipes* is actually favored by a lack of specific monitoring traps, by incorrect application of pest prevention tecniques, combined with the incorrect management of storage warehouses and retail stores.

Further researches are needed to better understand food preference and behavior of this emerging pest associated with pet store chain.

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The orientation of *Tribolium castaneum* adults in the presence of aggregation pheromone 4,8-Dimethyldecanal and food oils

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Abstract

Monitoring of *Tribolium castaneum*, the red flour beetle, involves the use of aggregation pheromone 4,8-dimethyldecanal (4,8 DMD) and kairomones such as cereal oils. Despite their present use, certain information which maximizes the efficacy of these compounds is still lacking. These experiments tested the effects of distance from the pheromone and edible oils on the orientation of *T. castaneum* adults. The movement of adults toward the aggregation pheromone was determined by changing the distance from the pheromone or the air flow. The adults released inside a glass apparatus tested their orientation either toward the food oils or the empty vial. The maximum trap catch was recorded at distances up to 60 cm from the pheromone and with the presence of air flow. The oils having botanical origin successfully attracted adults than those of animal origin. It is concluded that the orientation of *T. castaneum* adults varies with the distance from pheromone, air flow and the nature of food oil.

Keywords: Aggregation pheromone, distance, Kairomone, air flow, Tribolium castaneum

1. Introduction

Tribolium castaneum (Herbst), the red flour beetle, is a serious pest of stored agricultural products (Rees, 2004; Trematerra and Sciarretta, 2004). The most popular control measures for stored-product insects include the use of contact insecticides (Ghimire *et al*., 2016) and fumigants (Hill, 1990). Due to the negative impact of the residual effect of insecticides on human and the environment, control methods using compounds other than neurotoxic chemicals are emphasized for stored-product protection.

Male adults of *T. castaneum* release the aggregation pheromone 4, 8-dimethyldecanal (4,8 DMD) that attracts both sexes (Suzuki *et al*., 1984). Commercial pheromone lures use a combination of pheromone and kairomone (Campbell, 2012). However, certain information that maximizes the efficiency of this aggregation pheromone is still lacking. Also limited research has been conducted on the response of *T. castaneum* adults to food volatiles (Campbell, 2012). Therefore, the objectives of this research were to determine the effect of distance from the aggregation pheromone, air movement and food oils on the orientation of *T. castaneum* adults.

2. Materials and methods

One-month-old *T. castaneum* adults (50) were released at distances 30-120 cm from the trap having pheromone 4,8 DMD. From each distance, three replicates were tested. The control experiments were done using only the plastic trap (without pheromone or kairomone). The beetles trapped following releasing was counted. The effect of airflow was tested by using an exhaust fan.

A glass chamber having two holes on the bottom plate and vials underneath was used to test the effect of edible oils on *T. castaneum* movement. The food oils, egg albumin, the commercial kairomone solution (Trece Inc., USA) or two pheromone septa (Trece Inc., USA) was placed inside one vial. Fifty *T. castaneum* adults were released at the center of the chamber, and the number of beetles in each vial was counted.

3. Results and discussion

The attraction of *T. castaneum* adults to pheromone decreased when the distance at which the beetles released was increased (Tables 1 and 2).

Tab.1 *Tribolium castaneum* adults trapped when released at different distances from the trap- presence of air flow.

Distance (cm)	Trapping in control (%)*	Trapping in treatment (%)*
30	0d	21.33a
60	0d	18.66a
120	0d	2.66c

* Percentage trapped followed by the same letter in a column are not significantly different at p=0.05 according to Tukey's test.

Tab. 2 *Tribolium castaneum* adults trapped when released at different distances from the trap- absence of air flow.

Distance (cm)	Trapping in control (%)*	Trapping in treatment (%)*
30	0c	13.33a
60	0c	10a
120	0c	1.33cb

* Percentage trapped followed by the same letter in a column are not significantly different at p=0.05 according to Tukey's test.

Olive oil, kairomone solution (Trece), pheromone solution and Mee oil attracted significantly higher number of adults than their controls. The adults attracted to rice bran oil, corn oil, cod-liver oil, ghee, coconut oil or sunflower oil were not significantly different from their controls. Lowest attraction was shown by the egg albumin and the mustard oil.

Tab. 3 *Tribolium castaneum adults* attracted by different food oils, pheromone or kairomone.

Source	Trapping in Treatment (%)*	Trapping in Control (%)
Olive oil	29edc**	15**
Rice bran oil	21ed	25
Coconut oil	52ab**	8**
Kairomone	44abc**	5**
Pheromone	53ab**	17**
Sunflower oil	36bdc	17
Mee oil	59a**	14**
Mustard oil	29ecd**	26**
Gingelly oil	22ed	21

* Percentage trapped followed by the same letter in a column are not significantly different at p=0.05 according to Tukey's test.

**denotes significant difference from the control.

The aggregation pheromone 4, 8 DMD released from *T. castaneum* adults is dispersed effectively up to 60 cm from the trap. Air flow increases the beetle orientation towards the source. Coconut oil and Mee oil equally attract adult beetles as the synthetic pheromone and kairomone.

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The responses of *Tribolium castaneum* to wheat germ oil and fungal produced volatiles

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Abstract

The red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is a significant pest affecting a wide variety of different stored products around the Globe. Despite its economic impact, there is evidence that the lures currently used in traps to monitor for this species are largely ineffective. Based on the evolutionary history of *T. castaneum*, and the ecological niche it occupies, the volatiles of wheat germ oil and volatiles produced by grain-associated fungi have the potential to act as attractants for this species. We used electroantennography (EAG) to measure the electrophysiological response elicited by sixty-eight volatile compounds found in wheat germ oil and/or grain-associated fungi in two *T. castaneum* strains; an established lab population (CTC12 strain) and a recently caught wild population. Many volatile compounds from both sources elicited strong antennal depolarisations, and the responses of both strains were highly correlated. We then tested whether the compounds that triggered the strongest antennal depolarisations also elicited behavioural responses by using Y-tube olfactometer bioassays and identified several compounds attractive to both strains. The discovery of novel compounds that elicit strong EAG signals and behavioural responses could prove useful in the design of

improved lures for *T. castaneum* and other stored product pests. Our future research will identify how effective these attractive volatiles might be when used in combination, and when used under conditions that more closely replicate a stored product environment.

Key words: *Tribolium castaneum*, electroantennography, Y-tube olfactometer, fungal volatiles, wheat germ oil

Introduction

Tribolium castaneum and its sister species *T. confusum* are both economically significant pests of the stored product industry. They are particularly damaging owing to their global distribution and the wide variety of food products that they can infest, including nuts, milled grains and dried cereal products (Bell, 2014). Severe *Tribolium* infestations can also produce a "conditioning" effect in the medium they infest, which is characterised by depletion of the nutrient value of the medium, the accumulation of toxic benzoquinones secreted by the beetles and a build-up of debris such as larval casts and dead adults (Ghent, 1963). *Tribolium* infestations are a particular problem in stored product warehouses where damage by insects, mites and other microorganisms accounts for an estimated global annual post-harvest loss of 10-15% (Neethirajan *et al*., 2007). This problem is especially severe in developing countries, where post-harvest losses can be as high as 20% (Phillips and Throne, 2010).

One of the main ways that infestations of *Tribolium* species and other stored product pests can be detected and monitored is using lure baited traps. These lures typically contain insect pheromones in a slow-release formula and are commercially available for over 20 species of stored product insect, including *T. castaneum* and *T. confusum* (Phillips and Throne, 2010). Multispecies lures, containing the pheromones of different insects, are also available and are a common feature of integrated pest management strategies as they overcome the need to have multiple different lures for the different pest species encountered (Cox and Collins, 2002). Many of these lures combine insect pheromones with a food based kairomone such as wheat germ oil (Campbell, 2012), and this can make up 90% of the concentration by weight of these products. Wheat germ oil appears to be attractive to *T. castaneum* (Phillips *et al*., 1993), but the specific compounds in this mixture that elicit this attraction have not yet been identified. Despite the widespread use of these lures, there are reports from users in the stored product industry that they are not very effective (Semeao *et al*., 2011). This is supported by experimental data showing that the responses of *T. castaneum* to these pheromone baited traps is limited in ideal conditions and can be minimal in an environment with no air-flow (Campbell, 2012). In a simulated warehouse experiment, less than 2% of *T. confusum* released within a 60 cm distance from a pheromone trap were caught (Hawkin *et al*., 2011). As a result of this there has been a focused effort towards improving the efficacy of lures for *T. castaneum* and other stored product pests (Cox and Collins, 2002).

One major barrier in improving the ability of these lures to attract *T. castaneum* and other stored product insects is the lack of knowledge about the specific odours that attract these insects to stored product environments. This is particularly true for *T. castaneum* where little is known about its attraction to specific food related volatiles (Campbell, 2012). As the common name of *T. castaneum,* the red flour beetle, implies, flour and other milled grains are a major food source for this species. However, experimental data show that the odours of flour are only marginally attractive to this species (Campbell, 2013). *Tribolium castaneum* appears to be more attracted to cereal grains that exhibit signs of damage from decay or pest infestation (Trematerra *et al*., 2000). They are also more attracted to wheat kernels with other insects present, and to those that have been damaged by other pest species (Trematerra *et al*., 2000). *Tribolium castaneum* also exhibits attraction to fungal odours, specifically the volatiles of fungi associated with cotton seed lint (Ahmad *et al*., 2012). Indeed, they were shown to be more attracted to these odours than to the odours of conventional food sources such as wheat (Ahmad *et al.,* 2012). These preferences could be explained by the fact that *T. castaneum* is a 'secondary pest' which primarily feeds on grains that are rotten or have been damaged by the infestation of other insects, or mechanically processed by humans, i.e. milled

(Trematerra and Sciarretta, 2004). The presence of wheat germ and fungal volatiles would therefore be an indicator that the grains are in a suitable condition for *T. castaneum* to feed on.

Despite the knowledge that *Tribolium* species are generally attracted to the odours of both wheat germ oil and fungi the specific volatiles underpinning this attraction have not been clearly identified. As many current lures contain wheat germ oil as a food based attractant there is the potential to improve the efficiency of these traps by only including the specific volatiles in wheat germ oil that attract *T. castaneum*. The incorporation of attractive fungal volatile compounds also has the potential to improve the attractiveness of lures. To identify specific volatiles of wheat germ oil and grain-associated fungi that are attractive to *T. castaneum* we have used a combination of electroantennography (EAG) and behavioural bioassays. We have used EAG as an efficient method of identifying which compounds can be detected by the antennae of *T. castaneum* and have determined whether *T. castaneum* are attracted to the volatiles that they can detect using Y-tube bioassays. A variety of different compounds have already been tested for *T. castaneum* using EAG (Balakrishnan *et al.*, 2017; Verheggen *et al.*, 2007), but this is the first screen focusing specifically on the volatiles found in wheat germ oil and produced by grain associated fungal species.

Methods

Tribolium husbandry

Two *T. castaneum* strains were used in the experiments; the CTC12 strain and a wild captured Zimbabwean population. The CTC12 strain originates from an organophosphate resistant strain from Australia (Champ and Campbell, 1970) that has since been cultured in the laboratory. This strain was used to represent an established laboratory population. The wild captured population were cultured from a population found inside a shipment of infested grain from Zimbabwe in 2017. Cultures for both strains were maintained at 30°C in containers of 200 g of whole grain flour with the addition of 10 g yeast powder (as an additional protein source) and 1 g of the antimicrobial agent Fumagillin (to inhibit fungal growth in the cultures). All beetles used in the experiments were aged between 4 and 8 weeks post-emergence.

Volatile compounds

Sixty-eight volatile organic compounds that are either present in wheat germ oil or produced by grain-associated fungi were used (Table 1). The 34 wheat germ oil volatiles used in these experiments had been previously identified through headspace-solid phase microextraction of a sample of wheat germ oil (Niu *et al.*, 2013). Fungal compounds (28) were identified from a review listing the volatiles produced by common fungi grown on cereal and grain substrates (Magan and Evans, 2000). Six compounds were identified as being found in both wheat germ oil and produced by grain-associated fungi. Synthetic DMD (4,8-Dimethyldecanal), the *Tribolium* spp. aggregation pheromone, was used as a positive control as it is known to be behaviourally attractive and elicit strong antennal depolarizations in *T. castaneum* (Levinson and Mori, 1983). All odorants were diluted to working concentration using hexane, an established solvent for use in insect olfactory behavioural experiments that has previously been shown to not elicit significant EAG depolarisations or behavioural attraction in *T. castaneum* (Verheggen *et al.,* 2007). All compounds were obtained from commercial suppliers.

Table 1. The environmental volatiles used in our experiments and whether they were identified as being found in wheat germ oil, produced by grain-associated fungi or as both.

Compound	Source	Compound	Source	Compound	Source
5-methyl-3-heptanone	Wheat germ oil	3-octanone	Fungal	3-methyl-1-butanol	Both
trans-2-heptenal	Wheat germ oil	butyl acetate	Fungal	hexanal	Both
ethyl hexanoate	Wheat germ oil	benzaldehyde	Fungal	1-octen-3-ol	Both
Limonene	Wheat germ oil	3-methylanisol	Fungal	1-hexanol	Both
trans-trans-2,4,-heptandinal	Wheat germ oil	2-methylacetophone	Fungal	nonanal	Both

2-heptanone	Wheat germ oil	1-pentanol	Fungal	ethanol	Both
trans-2-pentenal	Wheat germ oil	trans-2-hexen-1-al	Fungal		
iovaleraldehyde	Wheat germ oil	2-methyl-2-butanol	Fungal		
Octanal	Wheat germ oil	damascenone	Fungal		
amyl acetate	Wheat germ oil	3-octanol	Fungal		
trans-2-octene	Wheat germ oil	dimethyl benzene	Fungal		
1-penten-3-one	Wheat germ oil	styrene	Fungal		
ethyl benzene	Wheat germ oil	2-butanol	Fungal		
trans-2-pentanal	Wheat germ oil	naphthalene	Fungal		
1-octene	Wheat germ oil	1-butanol	Fungal		
trans-cinnamaldehyde	Wheat germ oil	2-methyl-1-propanol	Fungal		
ethyl octanoate	Wheat germ oil	2,2,4-trimethylhexane	Fungal		
Pentane	Wheat germ oil	2-nonanone	Fungal		
2-pentylfuran	Wheat germ oil	acetone	Fungal		
trans-2-octenal	Wheat germ oil	butyl acetate	Fungal		
Undecane	Wheat germ oil	2-methylfuran	Fungal		
1-heptene	Wheat germ oil	octyl acetate	Fungal		
Nonane	Wheat germ oil	2-pentanone	Fungal		
trans-5-decene	Wheat germ oil	1-phenylethanol	Fungal		
Toluene	Wheat germ oil				
trans-3-octene	Wheat germ oil				
2-methyl-2-butene	Wheat germ oil				
p-anisaldehyde	Wheat germ oil				
trans,trans-2,4-decadienal	Wheat germ oil				
trans-2-decenal	Wheat germ oil				
4-allylanisol	Wheat germ oil				
octanoic acid	Wheat germ oil				
Tridecane	Wheat germ oil				
Hexane	Wheat germ oil				

Electroantennography

The electroantennography protocol was adapted from the Syntech Electroantennography manual (Syntech, 2004). Only female beetles were used, as in preliminary experiments (not reported) we found there were no significant differences between the responses of male and female beetles. The same finding has recently been reported by Balakrishnan *et al.* (2017). For each strain, a live female beetle was carefully positioned on a glass slide with adhesive tape to restrict movement and allow EAG recordings to be taken (N=8). A thin strip of double-sided adhesive tape was placed under the head of the beetle. This was sufficient to prevent movement of the antenna with the addition a small drop of cyanoacrylate glue to stick down the head of the beetle. Care was taken to not get any glue on the antenna of the beetles. Small holes were pierced into the tip of the antenna and through the eye of the beetle with an electrolytically sharpened tungsten wire to allow glass capillary electrodes filled with Ringer solution, in contact with silver wire, to be inserted. Filtered air continuously flowed over the restrained beetle and the test odorants were delivered by an air-puff from a Syntech stimulus controller. When triggered the stimulus controller delivered a one second puff of air to the end of a Pasteur pipette pointed at the head of the restrained beetle. Strips of Whatman filter paper with 5 µl of 20% vol/vol dilution in hexane of each volatile compound were inserted into this pipette to present the beetles with the different volatiles used in the experiments. Every 10 volatiles the responses of the beetles were tested against DMD (positive control) and hexane (negative control) and the responses of the preceding 10 volatiles were normalised against the DMD response. The EAG potential was recorded on a computer using a signal amplifier, IDAC convertor and EAG 2000 software.

Y-tube bioassay

The Y-tube olfactometer apparatus consisted of a 20 cm long, 6 cm in diameter, glass cylinder that branches in the middle to form a two-armed (Y-shaped) glass tube. The Y-tube was connected by PTFE tubing to a vacuum pump, which drew an air-flow through each of the two Y-arms at a rate of 0.2 L/min. Each arm was in turn connected by PTFE tubing to three sealed vials, the first containing Whatman paper disks to which 5 µl of a 200 ng/µl dilution of the test volatile was added, the second

containing activated charcoal and the third containing water. The Y-tube olfactometer had a sealable hole on the main stem that allowed for insects to be inserted while the vacuum pump was running. A single beetle was inserted into the Y-tube through this hole for each trial and its movements were observed for five minutes. Once a beetle had walked 2 cm down one of the two branches of the Y-tube it was recorded as having chosen that arm of the olfactometer. If no choice was made within five minutes the beetle was deemed to be non-responsive and was discarded. The odorants connected to each arm of the olfactometer were switched over every 10 trials to prevent the direction of the arms from biasing the choices of the beetles. Only females were used as other researchers have suggested that aggregation pheromone could be produced by males within the olfactometer, which could influence the behaviour of beetles used in subsequent trails (Ahmad et al. 2012). All trials were conducted in a 20°C controlled temperature room.

Statistical analysis

Differences between the EAG responses of the two strains to the different volatiles tested were analysed with a two-way mixed ANOVA. Where significant differences were found they were followed up with pairwise paired t-tests (for within-strain differences) or unpaired t-tests (for between-strain differences). To correct for the error associated with number of statistical tests the significances were adjusted using a false discovery rate method. All statistics were performed using IBM SPSS Statistics 24.

Results

Electroantennography

The average antennal depolarisations elicited by the volatiles presented to female *T. castaneum* after being normalised against the response to the DMD positive control are shown in Figure 1. It was noted that the absolute depolarisations of the CTC12 strain were much larger than that of the wild strain for all volatiles tested with the compounds on average eliciting depolarizations of twice the response from the CTC12 strain as from the wild strain. However, after normalisation of the data, the overall trend in the responses of both strains to the different volatiles was remarkably similar. A two-way mixed ANOVA revealed a highly significant effect of the volatiles ($F_{1,66}$= 10.59, p< 0.001), and a significant effect of the interaction between strain and volatile ($F_{1,66}$= 1.362, p= 0.032). This indicates that the size of the antennal response changed depending on the volatile tested and that there were some differences between responses of the two strains to the same volatile. However, no significant effect of strain alone was found ($F_{1,66}$= 1.384, p= 0.259Post hoc paired-t tests with a false discovery rate applied were performed to identify volatiles that were significantly different from the hexane control. The relationship between the responses of the two strains is also shown by a bivariate plot of the normalised responses of the two strains to the different volatile compounds (Fig. 2), which revealed a strong correlation between the responses of both strains to the different volatiles ($r = .809$, $n = 68$, $p < .001$).

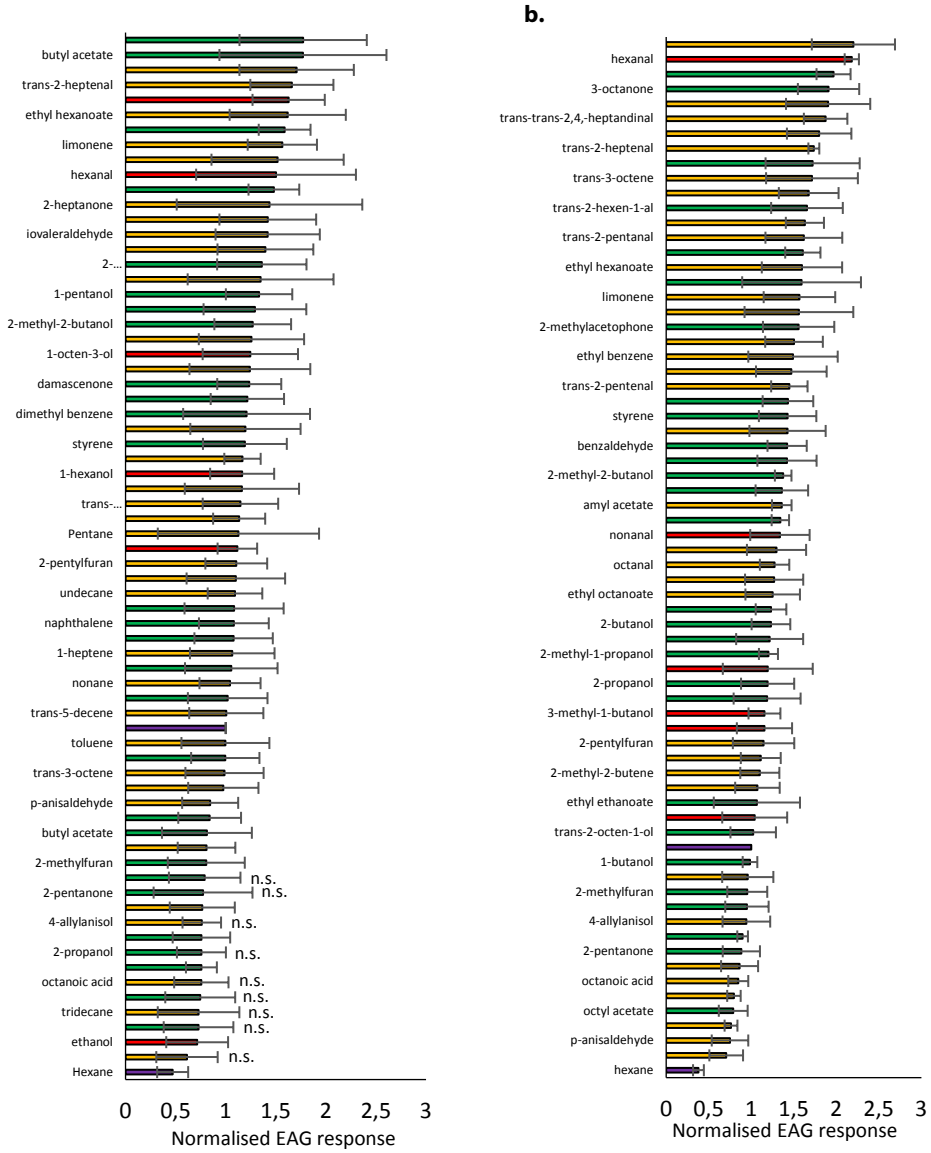

Figure 1 Average EAG responses of 8 CTC-12 strain (a) and 8 wild captured *Tribolium castaneum* (b), normalised against a DMD control, to 68 volatile organic compounds found in wheat germ oil or produced by grain associated fungi, plus the *Tribolium* aggregation pheromone, DMD as a positive control. Columns, arranged by descending EAG response, represent the average depolarization across eight individuals, and the error bars represent the standard deviations of the mean. Yellow bars represent volatile compounds found in wheat germ oil, green bars represent compounds identified as being products of grain associated fungi, red bars represent compounds we identified as being produced by both sources, and purple bars represent the two control compounds, DMD (positive control) and hexane (negative control). Compounds that did not elicit a significantly different EAG response compared to the hexane control are indicated with "n.s"; no compounds were found to be significantly different in the wild population beetles.

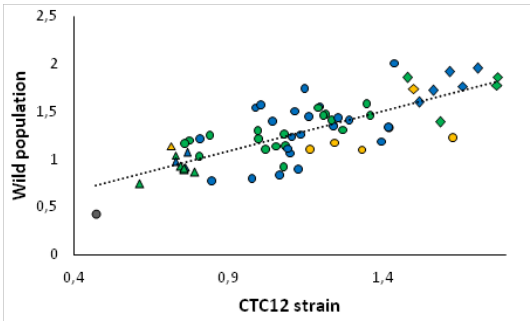

Figure 2 A bivariate plot showing the correlation between females of the CTC12 strain and females of the wild population strain in their average (n=8) normalised EAG responses to 68 different volatile organic compounds found in wheat germ oil or produced by grain associated fungi. Blue points represent volatile compounds found in wheat germ oil, green points represent compounds identified as being produced by grain associated fungi, yellow points represent compounds we identified as being produced by both sources, the grey point represents the control compound hexane. Pearson product-moment correlation indicated a strong positive correlation between the responses of the two strains to the different volatiles ($r = .809$, $n = 67$, $p < .001$). The ten compounds that elicited the largest average responses across both strains are indicated with diamond shaded points, and the ten compounds that ellicited the smallest average EAG responses are indicated with triangle shaped points. The responses to these indicated volatiles was also tested behaviourally using a y-tube olfactometer.

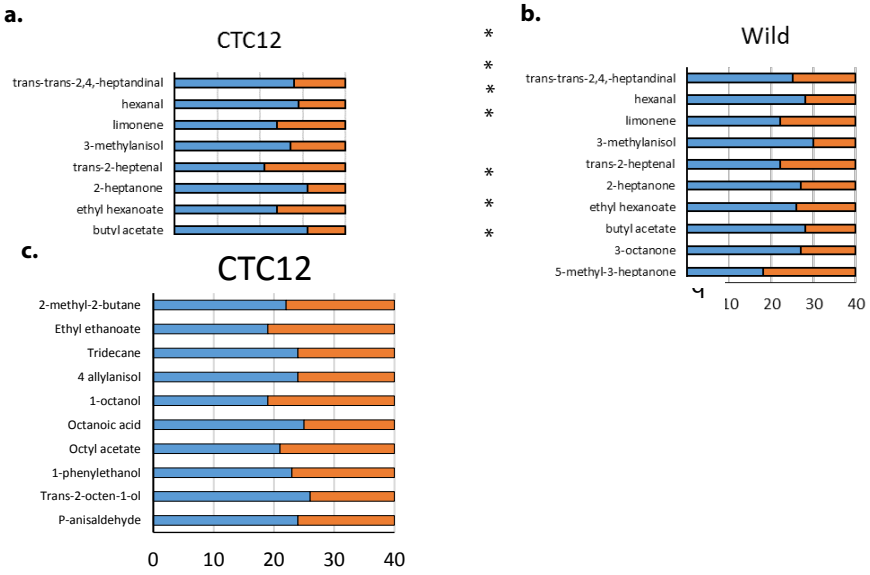

Figure 3 The attraction of female CTC-12 (a.) and wild population (b.) *T. castaneum* beetles to the ten volatile compounds that elicited the largest average EAG responses across both strains (see Fig. 2). The blue bars indicate the number of beetles (out of 40 individuals tested) that chose the Y-tube arm containing the test volatile, the orange bars indicate the number that chose the arm containing the hexane control solvent. Asterisks indicate a statistically significant bias for one arm of the Y-tube over the other (chi-square of goodness-of-fit <.05)

Y-tube olfactometer

Figure 3 shows the behavioural responses of female CTC12 (a) and wild (b) strain beetles to the ten compounds that elicited the highest EAG responses across both strains (indicated with diamond shaped points in Figure 2) and the responses of CTC12 strain females to the 10 compounds that elicited the smallest average EAG response across both strains (c) (indicated with diamond shaped points in Figure 2). The results show that, of the ten most attractive compounds, seven of the compounds elicited a statistically significant attractive response in the CTC12 strain (chi-square of goodness-of-fit $p < .05$), whereas five were shown to be significantly attractive to the wild population with all the compounds that were behaviourally attractive to the CTC12 strain also attractive to the wild population. None of the 10 compounds that elicited the smallest average EAG responses were found to be significantly attractive to the CTC12 strain.

Discussion

The results of our EAG and Y-tube olfactometer experiments give new insights into the physiological and behavioural responses of *T. castaneum* to common environmental volatile compounds that could have relevance to its future pest management. The results of the EAG experiments reveal that many of the compounds tested elicited large EAG responses relative to the DMD positive control, with around two-thirds of them eliciting larger responses than to DMD when used at the same concentration. This is encouraging given that insect aggregation pheromones (i.e. DMD) form the basis of many current lures. Strong antennal and behavioural responses were observed following exposure to a subset of volatiles found in wheat germ oil. This is perhaps unsurprising, given what was already known from the literature, and the current composition of insect lures. Interestingly, we also observed strong antennal and behavioural responses to volatiles from grain-associated fungi. *Tribolium castaneum* is known to have been associated with humans for at least 4,500 years, having been found sealed within Pharaonic urns in Egypt (Dawson, 1977). However, prior to the existence of anthropogenic food stores, *Tribolium* species must have fed on a different source of food. As many species in the same *Tenebrionidae* family as *T. castaneum* primarily feed on rotting tree bark, and other decaying plant matter, it is possible that this was the original food source of this species, before it switched to feeding on anthropogenic stored products. It has also been theorised that *T. castaneum* may have first adapted to feed on rotting grains stored in the burrows of rodents, and other sources of rotten grains, before switching to feed on mechanically processed grains stored by humans (Dawson, 1977). Therefore, *T. castaneum* may have co-opted an ancient ancestral attraction to fungal volatiles, derived from rotting plant matter, to find human stored grain products. Our findings lend some tentative support to this idea.

Although *T. castaneum* was attracted to volatiles from both wheat germ oil and grain associated fungi, there was no clear pattern between the responses to these two sources, with both groups of volatiles containing within them individual compounds that elicited very strong antennal responses, as well as volatiles that did not elicit strong responses. There was also no clear relationship between the type of compound and the size of the antennal depolarizations recorded. Some alcohols, ketones and compounds with methyl groups were found to elicit EAG depolarisations, while other compounds of the same chemical group did not. This agrees with a previous large-scale EAG screen which found no clear pattern between the size of depolarisations and the chemical class of the compounds tested in *T. castaneum* (Balakrishnan *et al*., 2017). This suggests that this species is responding to very specific compounds associated with stored products, rather than to a broad range of chemically related compounds.

Before normalisation of the EAG responses, a striking difference was observed between the two *Tribolium* populations tested, with the depolarisations of the wild caught population typically being half the amplitude of the laboratory strain. However, after normalising the responses against a DMD positive control to correct for variation in antennal resistance over the course of taking recordings, the responses of both strains were found to be highly correlated. This demonstrates that the overall trend across the volatiles was the same in both strains, and could indicate that the composition of

odorant receptors is similar between both strains. However, to ensure we could record strong EAG responses, the concentration at which we tested these volatiles were much higher than would be encountered typically in a stored product environment, so it is possible that the responses to the two strains could still differ when they encounter these volatiles at more natural concentrations. It should also be noted that the results of the EAG on their own do not reveal how attractive these compounds are, only the degree to which they are detected at the insects' antenna. The amount of antennal depolarisation and the attractiveness of compounds are often not strongly correlated, and insects can have different responses when encountering blends of volatiles at different ratios (Bruce *et al.,* 2005). A previous study that examined behavioural differences between freshly caught and established laboratory populations of *T. castaneum* found little difference in their responses to traps baited with food and pheromone lures (Campbell, 2012). These results could suggest that fungal and wheat germ oil volatiles might elicit similar responses in *Tribolium* populations from diverse ecological backgrounds, which is important if these volatiles are to be used in a general-purpose lure and suggest that the results of previous behavioural studies conducted in lab strains should be applicable to wild populations, and vice versa. However behavioural experiments testing the responses to these compounds under more natural conditions would be needed to confirm this idea.

Owing to the very small response elicited to the hexane control volatile, all the volatiles tested in the wild population, and almost all the volatiles tested in the CTC12 strain, were found to elicit significantly different electrophysiological responses compared to this control. This is similar to the results of previous EAG experiments in *T. castaneum*, which found almost all of the compounds tested gave "a measurable EAG response" (Balakrishnan *et al.*, 2017). However, it was not clear from the study by Balakrishnan *et al*. (2017) what level of antennal depolarisation would predict a significant behavioural response from the beetles. When the ten volatiles that elicited the highest average EAG responses in the current study were tested for behavioural responses in the CTC12 strain, seven of them were found to be significantly attractive. In contrast, when the ten volatiles that elicited the smallest average EAG responses were tested, none of them were found to be significantly attractive. This would suggest that a certain threshold depolarisation must be reached before compounds become behaviourally attractive or that these compounds elicit a response that the Y-tube olfactometer does not measure, e.g. they are repellent or arrest the beetles by stimulation oviposition. However the results also show that even compounds that elicit relatively large EAG responses will not necessarily be attractive owing to complex relationship between odour perception and behavioural response in insects (Bruce *et al*., 2005; Bruce and Pickett, 2011). It is possible that the same volatiles will be attractive when tested at different concentrations, or when tested together as a blend. Although attractiveness to most of the volatiles used in this study has not previously been demonstrated for *T. castaneum,* some of the volatiles have previously been used in research involving *Tribolium* species. For example, 3-octanone has been identified as a volatile that can be found in *Tribolium* infested flour, but is absent from clean flour (AbuelInnor *et al*., 2010), and this could explain the advantage of strong attraction of both strains to this volatile. In addition, hexanal has also been shown to be attractive to *T. confusum* when used in a blend with other plant volatiles (Wenda-piesik *et al*., 2016).

Taken together, the results of our EAG and behavioural experiments have revealed previously unidentified attractive compounds for *T. castaneum*, which have the potential to be used to improve the effectiveness of commercial *Tribolium* lures. There are also several wheat germ oil and fungal derived volatiles that elicited strong antennal depolarisations that have not yet been tested behaviourally, compounds that also have the potential to be highly attractive to *T. castaneum*. We are now exploring whether there are any synergistic effects of the attractive volatiles we have identified when they are encountered together. If fungal volatiles are indicators that grains are in a condition that *T. castaneum* can feed upon, it is likely that a stronger attractive response will be elicited when wheat germ oil and fungal volatiles are encountered together. This could be an important factor in adapting these volatiles for use as a *T. castaneum* lure. *Tribolium castaneum* has

been shown to be less attracted to lures when tested in an environment without a strong airflow (Campbell, 2012). We are therefore also doing behavioural experiments in environments closer to those encountered in a warehouse, which should provide better information about how attractive these compounds are to *T. castaneum* under real world conditions.

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The potential of host-specific volatiles from *Tribolium confusum* larval faeces for luring the ectoparasitoid *Holepyris sylvanidis*

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Abstract

The ectoparasitoid *Holepyris sylvanidis* (Bethylidae) attacks larvae of different stored product pest beetles. Previous studies on the olfactory host search of *H. sylvanidis* revealed that female parasitoids are strongly attracted to volatiles released from *Tribolium confusum* larval faeces, in particular to (*E*)-2-nonenal and 1-pentadecene. We suggested that these host-specific key compounds may serve the parasitoid as long-range attractants for host location. In this context, we propose that the attractive volatile blend could be used to establish a new approach within the biological control of stored product pests by guiding the parasitoid to its host and thus, increasing the host finding success. We investigated the potential of the identified host-indicating volatile cues to attract *H. sylvanidis* from a distance by offering the two key compounds to female parasitoids. Their walking behaviour and the covered distance were analysed on a Kramer sphere. Moreover, in semi-field trials both attractive volatiles were loaded onto rubber septa which were placed next to 4th instars of *T. confusum* at 1.5 m distance from the parasitoids. We studied the host finding success of *H. sylvanidis* by (i) measuring the mean time to locate and parasitise *T. confusum* larvae and (ii) counting the number of parasitised and unparasitised host larvae as well as the number of newly hatched parasitoids compared to the control without additional olfactory cues. First results showed that *H. sylvanids* females can locate the provided host larvae from a distance. Parasitistion of host larvae started four days after the release of parasitoids. No effect of the additionally offered host-specific key volatiles ((*E*)-2-nonenal and 1-pentadecene) on the parasitoid´s host finding success was observed at the given conditions and used amounts of compounds. Further studies are required to determine the right odour blend and concentrations for attraction of parasitoids over a distance and finally to show that the addition of host-derived kairomones may support the host finding success of *H. sylvanidis*.

Keywords: biological control, stored-product pests, semi-field trial, long-range attractants, Bethylidae.

Introduction

Over the last years, social concerns about the usage of synthetic pesticides for protection of food commodities or stored products against insect infestation have been increased considerably, mainly due to possible side-effects for humans (e.g. food contaminations, health risks for users) and environment (e.g. persistence of chemical residues) as well as well as the risk of developing resistance within pest populations (Field, 1992; Arias-Estevez et al., 2008). That in turn has led to an increased demand for alternative non-chemical pest management strategies (Phillips and Throne, 2010). Within the field of integrated stored product protection, the use of natural enemies (e.g. parasitoids) of stored-product pests as biological control method represents a promising and environmentally-friendly approach, but more research on the biology and behaviour of parasitoids is needed (Flinn and Schöller, 2012; Trematerra, 2012).

For instance, Adler et al. (2012) showed that the release of the larval ectoparasitoid *Holepyris sylvanidis* at a two-week interval was sufficient to control the local population of the confused-flour beetle *Tribolium confusum* in a grain mill. As a result, additional heat treatment, that had to be adopted in the past, was not necessary during the further experiment. Therefore, and

basend on previous studies and assumptions, *H. sylvanidis* is a potential candidate for the biological control of beetle larvae infesting stored products, in particular *T. confusum* and other *Tribolium* species (Evans, 1977; Fürstenau et al., 2016; Amante et al., 2017; Fürstenau and Hilker, 2017).

However, one important weak point in previous applications of parasitiods in general was the lack of specific traps and attractants which could help to monitor natural occurring beneficials as well as those additionally released. The development of suitable lures and traps for monitoring present populations of natural enemies requires profound and further research on the olfactory host finding process of the respective parasitoids with the aim to identify behavioural active, host-associated compounds for their application (Philipp and Thrones, 2010; Trematerra, 2012).

Previous studies on the odour-mediated host foraging behaviour of *H. sylvanidis* revealed that parasitoid females use volatiles released from larval faeces of *T. confusum* to locate its hosts. Two compounds of the faecal odour, (*E*)-2-nonenal and 1-pentadecene, were highly attractive to the parasitoid, particularly, in the presence of odour from host's feeding substrate, (i.a. wheat grist) (Fürstenau et al., 2016). Since the corresponding bioassays of this study were performed in a static 4-field olfactometer it still needs to be confirmed whether these two host-specific compounds ((*E*)-2-nonenal and 1-pentadecene) may act as long-range attractants for host location from a distance and whether these volatiles are possible candidates to lure and monitor *H. sylvanidis* individuals in the field. Therefore, the present study aimed to investigate (long-range) attraction effects of a two-component mix, consting of the specifically-host associated key compounds, on the host finding success and the efficiency of the parasitoid to locate *T. confusum* larvae in a semi-field trial from a distance.

Materials and Methods

Insects

Test insects (female *H. sylvanidis* and 4th instar host larvae of *T. confusum*) were taken from a permanent rearing at the JKI (Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany) as described previously (Fürstenau et al., 2016; Fürstenau and Hilker, 2017). According to Brindley (1930) and Sokoloff (1974), we defined 25-to-30-days-old *T. confusum* larvae (3-4 mm long) as 4th instars under our rearing conditions (25±1°C and 65±5 % relative humidity).

Preparation of the host-specific two-component mix (2CM)

The two-component mix, hereafter abbreviated to 2CM, was prepared by adding 2 mg (*E*)-2-nonenal (97%, purchased by Sigma Aldrich) and 1 mg 1-pentadecene (95%, purchased by TCI Europe) to 10 µl *n*-hexane (98%, purchased by Merck). The resulting solution was stored at -20°C until its use in subsequent bioassays.

Semi-field trial to evaluate the host finding success of H. sylvanidis from a distance

To investigate possible effects of two specifically host-associated compounds identified from *T. confusum* larval faeces on the foraging behaviour of *H. sylvanidis* females a semi-field trial was conducted. We evaluated the potential of 2CM to attract *H. sylvanidis* females over a 1.5 m-long distance and to improve the host finding success by guiding the parasitoid to its host. Experiments were performed in specifically manufactured boxes (0.75 x 2.0 x 1.0 m), consisting of a metal frame. Head and foot end as well as three side panels were made from gauze or cotton fabrics; four doors were embedded in the front side of the box. Each box was installed on a wooden panel. At the head end of the box we put a Petri-dish (Ø 5.5 cm) with fifty 4th instar host larvae of *T. confusum* on a plastic tray (23.5 x 30 cm) filled with wheat grist. Above the Petri-dish we put one odour dispenser which had been loaded with either 10 µl 2CM (treatment) or 10 µl of *n*-hexane (control) and evaporated for 24 h. As *H. sylvanidis* females generally transferres host larvae to hiding places (Ahmed et al., 1997) loose pipette tips were randomely put in each corner of one tray. Two boxes

for test and control trials each were placed in two separate rooms to avoid biased results due to interferences between test and control treatments. During the semi-field trial, room temperature and relative humidity depended on the outside conditions and were recorded by a datalogger; the average room temperature and relative humidity were 22 ± 1°C and 33 ± 7%, respectively.

At the beginning of the two-weeks-lasting experiment, twenty 1-to-8-days-old, mated parasitoid females were released at the opposite side of the box, 1.5 m away from the Petri-dish with host larvae. On day 1, 4 and 6 after the release of parasitoids we checked whether we could find paralysed and/or parasitised *T. confusum* larvae in the pipette tips and outside the tray. Pipette tips having paralysed and/or parasitised host larvae were replaced by new ones; all parasitised larvae were transferred to the climate chamber. After seven days the tray filled with wheat grist, the Petri-dish with *T. confusum* larvae and the pipette tips were renewed and the number of remaining *T. confusum* larvae found inside the Petri-dish and the wheat grist were counted. Twenty new parasitoid females were released in each box. As described above for 1st week, the number of *T. confusum* larvae outside the tray and the proportion of parasitised and unparasitised host larvae were measured. The semi-field trial was stopped after 13 days; each trial was repeated twice.

Since *H. sylvanidis* females drag the paraslysed host larvae to hiding places for parasitation we defined as a successful host finding event when *T. confusum* larvae were found in the pipette tips or outside the tray. In addition to the mean number of successful host finding events, we also calculated the parasitation rate and the ratio of hatched parasitoid (females and males) compared to the control. Data were statistically analysed by a Welch Two Sample *t*-test for the rat of successful host finding events and a Wilcoxon rank sum test with continuity correction for the parasitation rate. All analyses were done using the statistical programm "R" version 3.4.1 (R Core Team, 2017) with packages "car" (Fox and Weisberg, 2011) and "pastecs" (Grosjean and Ibanes, 2014).

Results and Discussion

In the present semi-field experiments a mix of two highly attractive, host-associated odours, (*E*)-2-nonenal and 1-pentadecene, identified from the volatile blend collected from *T. confusum* larval faeces was offered to *H. sylvanidis* test females in combination with host larvae to test the influence of these additional odours on the parasitoid's host finding behaviour. We measured the rate of successful host finding events by counting the number of *T. confusum* larvae found in hiding places (pipette tips) and outside the tray 1, 4 and 6 days after the release of parasitoids. In both treatments the number of *T. confusum* larvae which had been displaced by *H. sylvanidis* increased with the duration of experiment (number of experimental days; Tab. 1). On day 1 after start of the experiment, all host larvae were still in their respective Petri-dishes in test and control treatment. Six days after releasing the parasitoids, 4.88 (±1.63) and 1.25 (±0.75) displaced host larvae were counted in the control and the 2CM-treatment, respectively. Overall, *H. sylvanidis* could locate and displace ca. 25% of fifty host larvae offered to parasitoids in the control-treatment; the rate of successful host finding events was twofold higher than in the 2CM-treatment (ca. 13%) but did not differ significant (Tab. 1). In both treatments the number of parasitised host larvae was lower compared to the number of displaced host larvae. In the control treatment ca. 8% of the fifty offered host larvae were parasitised whereas the parasitation rate was fourfold lower in the 2CM-treatment (ca. 2%). When calculating the parasitation rate we excluded all larvae which were not found at the end of the experiment. Regarding the high number of not recovered host larvae (4.25±2.04 larvae in the control and 4.38±1.97 larvae in test treatment, Tab.1) one could assume that the actual parasitation rate might be higher in both treatments.

The host finding ability under storage-like conditions has been examined previously for a few parasitoid species (Steidle and Schöller, 2002; Adler et al. 2012; Niedermayer et al. 2016). For example, Niedermayer et al. (2016) showed that the two larval parasitoids *Lariophagus distinguendus* and *Anisopteromalus calandrae* could locate approximately 20% of wheat kernels infested by the granary weevil *Sitophilus granarius* when they were released at 1 m distance. In comparison, the rate of successful host finding events of *H. sylvanidis* was higher in the

control treatment (ca. 25%). Regarding environmental conditions, the field trial described before in comparison to our semi-field experiments differed considerably. The test parasitoids of *L. distenguendus* and *A. calandrae* were released into an open environment (two buildings of 150 m^2 and 45 m^2) where the host location process might be influenced by several local predominat conditions such as air flow, light incidenc, variable temperature and humidity (Niedermayer et al., 2016). In contrast, we conducted our experiments in a constant dark and closed environment as we used 2 m long boxes placed in two separated rooms. The host location and ability of *H. sylvanidis* under storage-like conditions is not known yet and needs to be tested in further studies. However, Adler et al. (2012) already demonstrated that a mass release of laboratory reared *H. sylvanidis* is sufficient to temporally suppress the growth of a natural occurring *T. confusum* population in a grain mill. This result indicates that the rate of successful host finding events might be the same or even higher than what we measured in the here presented semi-field trial.

Tab. 1: Effect of additionally deployed host-specific volatiles on the host finding success of Holepyris sylvanidis in semi-field experiments (N=2).

	N° of displaced *T. confusum* larvae (mean ± SE [a])			N° of not found *T. confusum* larvae [b] (mean ± SE [a])	Rate (%) of host finding events (mean ± SE [a])	*p*-value [c]	Parasitation rate (%) (mean ± SE [a])	*p*-value [c]
	Days after parasitoid release							
Treatment	**1**	**4**	**6**					
Control (hexane)	-	3.38 ±1.53	4.88 ±1.63	4.25 ±2.04	25.00 ±5.96	*0.126*	8.50 ±2.35	*0.054*
2CM [d]	-	0.88 ±0.40	1.25 ±0.75	4.38 ±1.97	13.00 ±4.26		4.00 ±1.00	

[a] SE = Standard error of the mean.
[b] Number of *T. confusum* larvae which were not found in Petri-dishes, wheat grist, pipette tips or outside the tray during and at the end of the experiment
[c] To compare the different treatments a Welch Two Sample *t*-test for the host finding success events and a Wilcoxon rank sum test with continuity correction for parasitation rate were applied.
[d] 2-component mix of specifically host-associated compounds ((*E*)-2-nonenal: 2 mg, 1-pentadecene: 1 mg)

Initially our suggestion was that the addition of host specific volatiles (2CM) loaded onto dispensers may facilitate the host search of *H. sylvanidis* by attracting and guiding the parastioid to its host and thus, increasing the host finding success. Surprisingly, the rate of successful host finding events (13%) and the parasitation rate (2%) were both lower in the test treatment, offering 2CM, compared to the control (rate of successful host finding events = 25%; parasitation rate = 8%; Tab. 1). A possible explanation for the different performance when using 2CM might be that concentrations of both compounds ((*E*)-2-nonenal = 2 mg and 1-pentadecene = 1 mg) used here were (much) too high and therefore possibly reppelled the parasitoids instead of attracting them. Regarding this, in preliminary studies on a Kramer sphere, however, we observed noticeable differences in the walking behaviour of *H. sylvanidis* individuals when 2CM was tested compared to the control without offering volatiles. Usually, two characteristics of the walking behaviour of insects on the Kramer sphere is a higher walking speed and therefore, a longer track length when the test individuals are strongly attracted to an odour source as Thiery and Visser (1986) have shown for the Colorado potato beetle *Leptinotarsa decemlineata* and its preferred host plant, *Solanum tuberosum*. In contrast, *H. sylvanidis* females walked slower and covered a smaller distance in presence of 2CM compared to the control treatment. Additionally, *H. sylvanidis* frequently turned back while walking on the Kramer sphere or rested more time in the test treatment (personal observations). The reverse movements of *H. sylvanidis* in presence of 2CM probably indicate that parasitoid female re-examined the area for a potential host. A similar behaviour was observed when females of the larval parasitoid *Tiphia vernalis* could not find immediately a host at the end of a trail of their preferred host, the Japanese beetle *Popillia japonica*. Parasitoid females stayed nearby the trail's end and searched the area to locate potential hosts in the soil (Rogers and Potter, 2002). Therefore, we can not exclude that the application of two highly attractive, host-indicating compounds (2CM) may influence the host finding behaviour of *H. sylvanidis* by attracting or repelling the parasitoid. Further

studies on dispenser emission of 2CM are required to identify the correct blend (concentration, ratio etc.) and finally to show that these host-specific compounds can support the host finding success of *H. sylvanidis*.

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(Z, E)-9, 12-Tetradecadienyl Acetate (ZETA) disrupts mating of *Ephestia cautella*

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Abstract

The tropical warehouse moth *Ephestia cautella* is a major pest of stored products in Sri Lanka, and difficult to control using currently-available insecticides. The sex pheromone (*Z, E*)-9, 12- tetradecadienyl acetate (ZETA) emitted by the females attracts males of this species. Hence it can potentially be used in the management programs but the limited information on pheromone concentration and air movement impede the potential use of this pheromone in pest management programs. This experiment was conducted to determine the effects of ZETA concentration and air movement on the mating disruption of *E. cautella.* The male and female moths of *E. cautella* were introduced into a cubicle in which ZETA was placed at different concentrations. Later, the female moths were dissected to determine the presence/absence of spermatophore. All the pheromone concentrations tested recorded lower mating percentages than the hexane control. Mating disruption varied with the pheromone concentration and the availability of air flow. This study reveals that ZETA can be used to disrupt mating in *E. cautella*.

Keywords: *Ephestia cautella*, Mating disruption, Spermatophore, Concentration, ZETA

1. Introduction

The Tropical warehouse moth *Ephestia cautella* (Lepidoptera: Pyralidae) is a major pest of stored products (Hill, 1990) and reduce the quality of food commodities (Boshra, 2007). The current control measures by synthetic chemicals or extreme temperature exposure accompany disadvantages/limitations. Therefore, the grain-handling personnel seek for alternatives. The female moth releases the sex pheromone (Z, E)-9, 12-tetradecadienyl acetate (ZETA) to attract the males for mating (Kuwahara *et al.*, 1971). This is a promising pest management tool through mating disruption (MD) (Trematerra *et al.,* 2011) but certain information on the effective concentration and air movement on MD of *E. cautella* is not yet available. The objectives of this study were to evaluate the effect of pheromone (ZETA) concentration and the presence/absence of air movement on MD of *E. cautella*.

2. Materials and methods

Ephestia cautella adults were reared under ambient environmental conditions (30±2°C and 60±5% relative humidity), sexed at the pupal stage, and the adults emerged were used in the experiments. A cubicle (2.5 m×2.5 m× 2.5 m) having two opposite sides and the top covered by polythene, and the remaining two opposite sides covered by insect proof net was used to test the mating disruption. Each of the four pheromone concentrations prepared using commercially-available ZETA, diluted in hexane, was placed at the middle of the cubicle separately. Equal number of male and female adults of *E. cautella* was released into the cubicle at each concentration. The insects were recaptured after 24 hours and the female moths dissected to determine the mating status (Ryne *et al.*, 2001). The control experiments were conducted by using hexane solution. The highest mating disruption with respect to the pheromone concentration or the air flow was determined.

3. Results

Tab. 1 Mating disruption of *Ephestia cautella* at different ZETA concentrations (absence of air flow).

ZETA concentration (mg)	Mating disruption (%)*

0.05	25b
0.1	25b
1.0	37.5b
4.5	50a

*mating disruption (%) followed by the same letter are not significantly different at p=0.05 according to contrast option in binary logistic regression.

Tab. 2 Mating disruption of *Ephestia cautella* at different ZETA concentrations (presence of air flow).

Pheromone concentration (mg)	Mating disruption (%)*
0.05	37.5c
0.1	37.5c
1.0	62.5b
4.5	75a

*mating disruption (%) followed by the same letter are not significantly different at p=0.05 according to contrast option in binary logistic regression.

4. Discussion

This study reveals that MD of *E. cautella* increases with the increase in ZETA concentration and the presence of air flow. The higher MD with the presence of air flow compared to that without the air flow may be due to the increase in the dispersion of ZETA through the air.

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Suitability of Poaceae seeds for *Plodia interpunctella* development

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Abstract

One of the most important pests of stored grains is *Plodia interpunctella* (Hübner), whose larvae feed primarily on germinal part of the kernels, causing a reduction of seed germination and seed viability. This is detrimental for seeds of high category. However, seeds of different species within the same taxonomic family have different morphology (thickness of seed-coat, presence or absence of palea, palea loose or firmly attached to the seed etc.), which affects the susceptibility of seeds to *P. interpunctella* attack. The hypothesis was that seed hardness and the absence of palea could also significantly influence the life history of this pest. We assessed the suitability of different seeds from family Poacae (maize, wheat, barley, oats, ray, forage sorghum (variety), forage sorghum (hybrid), Sudan grass and millet) for *P. interpunctella* development and seeds susceptibility to pest attack (expressed in Susceptibility index –SI). The following parameters were monitored: larval mortality, adult emergence, mean developmental duration (from egg to adult) and female fecundity. Observations were carried out weekly, for 49 days. Data were statistically analyzed using Duncan's multiple range Test. The highest larval mortality, the lowest number of emerged moths and the lowest fecundity were recorded on millet, Sudan grass

and forage sorghum (variety and hybrid). However, the shortest larval development (27.8 days) and the highest fecundity (109.5-115.6 eggs) were on standard laboratory diet, maize and wheat. Morphometric measures of moths indicate that on unsuitable mediums like millet, Sudan grass, and different sorghum varieties the body lengths were statistically significantly shorter (0.5-0.6 cm) compared to other treatments (0.8-0.9 cm). According to the SI, the most susceptible were maize, wheat, barley, oats and ray, while moderately resistant were Sudan grass and millet. Testing kernel hardness and continuous improving of kernel resistance to storage insect pests could provide lower losses in stored grain quality and quantity.

Key words: *Plodia interpunctella*, Poaceae seeds, development, life history parameters

1. Introduction

Post-harvest losses and reduction of seed quality is one of the main restraints in achieving food security in developing and under developed countries (Rounet, 1992). During storage, the presence of insects is one of the major causes of deterioration of grain quality, reduction of grain weight, nutritional and market value. Indian meal moth, *Plodia interpunctella* (Hübner), is one of the most important polyphagous pests of grains, processed cereals and their products, oilseeds, nuts and manufactured products (Perez-Mendoza, 2003; Rees, 2004; Ozyardimci et al., 2006 Mohandasset al., 2007). It can be found on whole and/or damaged grains in storages, but since larvae feed mostly on germinal part of the seed and a bran layer (Almaši, 1984; Rees, 2004; Silhacek and Murphy, 2005), they lead to the reduction in seed germination and viability, which is detrimental for seed of high category.

Seeds of all cultivated Poaceae species (grains) are vulnerable to insect attack in warehouses because of usually prolonged period of storage. The growing importance of cereal (grain) production, primarily wheat, maize, barley and oats, lies in the fact that grains are the major carbon hydrate source in human and animal nutrition (FAO, 2011). Recently, there is also a growing interest in sorghum production because it has the potential to be used as bioenergy crop (Berti et al., 2013) and it is an attractive forage crop for many tropical and subtropical areas (Naeini et al., 2014). In 2013, sorghum was cultivated on over 300 thousand acres in Europe, while the world production of sorghum took place on the surface of over 42 mill acres (FaoStat, 2014) which also indicates at the growing importance of this crop.

The susceptibility of different grains (Poaceae seeds) to *P. interpunctella* attack and suitability for pest development depend on different characteristics of grains. In the first place it depends on the type of grain (hulless seeds like wheat, maize and rye, or seeds with palea like oats, barley, millet, Sudan grass), the type of palea (firmly attached to the seed – millet and Sudan grass, or loose palea -oats and barley) and the grain hardness (depends on grain density, structure of the grain, and the level of moisture). As a rule, grains without palea have higher density (Anonymous, 2017). Also, there can be a difference in seed characteristics between hybrids and varieties of the same species. For example, a variety of forage sorghum has palea firmly attached to the seed, while a hybrid of forage sorghum (crossing of male line of Sudan grass and female line of grain sorghum) does not have palea, since the female line is the grain sorghum.

This work aimed to assess susceptibility of different Poaceae seeds (wheat, maize, barley, oats, ray, millet, three genotypes of sorghum) for *P. interpunctella* attack and suitability for insect development.

2. Material and methods

2.1. Seed commodities

The experiment was carried out with seeds of nine different cultivated species from family Poaceae. Maize (*Zea mays* L.), wheat (*Triticum vulgare* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), ray (*Secale cereale* L.) and millet (*Pennisetum glaucum* L.) were used as nutrient medium for *P. interpunctella* development. Three varieties of sorghum (*Sorghum bicolor* Moench) were also used: a hybrid of forage sorghum, a variety of forage sorghum and Sudan grass. According to the agronomic classification based on different methods of sorghum cultivation and use, *S. bicolor*

species is divided into agronomic forms: grain sorghum, forage (sweet) sorghum, broomcorn and Sudan grass (Sikora and Berenji, 2011). Forage sorghum hybrids are obtained by crossing grain sorghum as the female parent and Sudan grass as the male parent (Pataki et al., 2010). This process of breeding provides seeds of sorghum without palea, which is the reason why it is more susceptible to insect attack. All seeds were obtained from the Institute of Field and Vegetable Crops, Novi Sad, Republic of Serbia, vegetation season 2016.

The grains were not treated with insecticides and prior to the experiment and were exposed to deep freezing (-80 °C) in order to eliminate the presence of other pests and/or superficial harmful organisms.

2.2. Experimental design

P. intepunctella parental population originates from a laboratory population reared in plastic containers, at 28 ±1 °C, R.H. 60 ± 10% and 14:10 (L:D) photoperiod, on a standard laboratory diet (SLD) for *P. intepunctella* (Silhacek and Miller, 1972). 50 one-day-old eggs were placed on 100 g of grains into 0.25 L glass jars. Jars were sealed with a cotton swab for proper aeration. The experiment was set in 4 replicates and carried out at the same conditions as the rearing of parental population.

The following life history parameters of *P. intepunctella* were monitored: larval mortality, mean developmental duration (from egg to adult), adult emergence, adult lifespan and female fecundity. The observations were carried out weekly, until the last larva pupated. Once the emergence of adults began, assays were checked each 24 h and the number of the emerged moths was recorded. Newly emerged unmated moths from the same treatment were paired and each pair was isolated in a separate test tube until the oviposition. The fecundity was defined as the total number of eggs laid after the mating.

The susceptibility of different Poaceae seeds was assessed based on Susceptibility index (SI) described by Dobie (1974):

$$SI = \frac{(\ln(F_1))}{D} \quad 100$$

ln – The natural logarithm of the mathematical constant *e*

F1 – average number of emerged moths per treatment

D –average developmental duration (egg to adult) in days

Seeds were rated as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) according to Mensah (1986) as follows:

$0 \leq SI \leq 2.5 (R)$

$2.6 \leq SI \leq 5 (MR)$

$5.1\ SI \leq 7.5 (MS)$

$7.6 \leq SI \leq 10 (S)$

2.2. Statistical analysis

Data were statistically processed using Duncan's multiple range Test to analyse the differences between life history parameters on different grains, in software package SPSS 21 (P>0.05).

3. Results and discussion

3.1. Larval mortality

The highest mortality of *P. intepunctella* larvae was recorded on millet (34.9%) and Sudan grass (21.5%) and was significantly higher compared to other grains (0.9-15.3%). The lowest larval mortality was on SLD (0.7%), during the entire experiment (Tab. 1). The difference between treatments was statistically highly significant (F=300.66**, P<0.01). According to Subramanyam

(1995), the mortality of *P. intepunctella* larvae can reach 28% on yellow maize, while in this work it was significantly lower on maize (12.8%).

3.2. Mean development duration

The results on *P. intepunctella* developmental duration on nine different Poaceae seeds and SLD are presented in Tab. 1. The fastest development was on SLD (27.8 days), while the slowest was on millet (49.8 days) followed by forage sorghum hybrid, forage sorghum variety and Sudan grass (41.8, 42.0 and 42.0 days, respectively). The differences between developmental duration were statistically highly significant ($F=96.14^{**}$, $P<0.01$). As reported by Williams (1964), duration of *P. intepunctella* development could range from 36 to 327 days. Developmental rate depends on maize variety (Abdel-Rahman et al., 1968) and also kernel damage. Mbata (1990) reported that the shortest development from egg to adult on the whole maize kernels was 31.2 days (tested on 13 varieties) and the slowest was 37.0 days, which was similar to the results of this work. Also, developmental dynamics of *P. intepunctella* depends on nutritive quality and mechanical state of food (Locatelli and Limonta, 1998; Vukajlović and Pešić, 2012; Kočović, 2014). Many researchers reported that life-cycle of *P. interpunctella* ranges from 27 to 52 days depending on factors such as temperature, food odor, presence of oil in diet, type of food, size and physiological state of females, availability of drinking water, food source and temperature (Allotey and Goswami 1990; Johnson et al., 1992; Nansen and Phillips, 2003; Mohandass, 2006). However, diet is the most important factor for determining the developmental period of the insects (Mbata 1985; Johnson *et al.,* 1992; Nansen and Phillips, 2003; Silhacek, et al., 2003; Silhacek and Murphy, 2005) and according to Vukajlović et al. (2017), when reared on whole wheat and rye kernels, this moth successfully completes life-cycle.

3.3. Adult emergence

The highest number of emerged adults was recorded on SLD (36.0), wheat (30.0) and maize (28.0), with statistically significant difference between treatments ($F=172.12$, $p<0.01$). The lowest number of emerged adults was on millet and Sudan grass (6.2, and 7.5, respectively). Other seeds were also less suitable for larval development, based on the number of emerged moths (10.5-18.5 moths). Essien (2006) reported that emergence of adult insect can be enhanced by the diet chemical composition, which was proven in this work, based on other life history parameters.

3.4. Female fecundity

The highest female fecundity (115.6 eggs) was recorded for females reared on SLD (Tab. 1). Between different grains, females reared on maize and wheat laid significantly higher number of eggs (110.2, and 109.5 eggs, respectively) which was at the same level of significance with the number on SLD. Females reared on millet laid the lowest number of eggs (16.2 eggs). The difference in fecundity among females reared on different grains and SLD was statistically highly significant ($F= 432.43^{**}$, $P<0.01$). The food source is an important factor for determining fecundity of moths which can be influenced by different diets (Mohandass et al., 2007; Fathipour and Naseri, 2011; Madboni and Pour Abad, 2012), thus the low fecundity indicates at relatively poor nutrient medium (Arbogast, 2007). Values of *P. interpunctella* fecundity reported in the literature vary widely. Allotey and Goswami (1990) reported fecundity of 96.8 eggs per female on wheat and 174.2 eggs for moths reared on split maize kernels. According to Onaolapo (2017), the fecundity on formulated diet can reach 161 eggs, while Almaši (1984) reports only 26 laid eggs on whole wheat grains. In the research of Mbata (1990), among 13 tested Nigerian maize hybrids, some were more some less attractive for oviposition, regardless on the type of maize. This indicated at the presence of certain ques, i.e. ovipositional attractants that were not related to the type of maize. Babić et al. (2013) emphasized that dent type of maize kernels are the softest kernel type since it contains higher percentage of floury endosperm. Thus, we can speculate that the consumption of dent kernels is easier and the lower energy is needed for breaking the kernel pericarp, which might lead to higher mean fecundity.

3.5. Moth lifespan

The lifespan of moths differed depending on the grains, i.e. Poaceae species. The longest lifespan was recorded for moths reared on forage sorghum hybrid (9.5), millet (9.0), forage sorghum variety (8.5), barley (8.5) and Sudan grass (8.0), which was significantly longer compared to SLD and wheat - 6.0 days (F=6.11*, P>0.01). Subramanyam (1995) reported that the longevity of adult stage depends primarily on the environmental factors (temperature and humidity), occurrence of mating, opportunity for oviposition and the presence or absence of water for consumption.

3.6. Moth body lengths

Moths reared on SLD had the longest body sizes, 0.9 cm on average (Tab. 1). The smallest average body lengths were measured for moths reared on millet, Sudan grass, forage sorghum variety and hybrid (0.5-0.6 cm). The difference between body lengths of moths reared on different Poaceae seeds were statistically highly significant (F=66.32**, P<0.01). Akinneye (2009) reports that adult moths reared on the formulated diet produce the highest body lengths, which is in accordance with the results of this work since the longest body sizes were on SLD.

Tab. 1 *Plodia intepunctella* life history parameters on different Poaceae seeds and SLD

Commodity	Mortality of larvae (%)	MDD (days)	Adult emergence	Fecundity	Moth lifespan	Body lengths
Maize	12.8 ±1.25cd	28.3 ±0.58 c	28.0 ±2.08 b	110.2 ±2.64 a	6.5 ±0.56 c	0.8 ±0.08 ab
Wheat	2.9 ±0.85 e	32.0 ±0.50 c	30.0 ±1.82 b	109.5 ±0.96 a	6.0 ±0.81 c	0.8 ±0.02 ab
Barley	10.3 ±0.96 d	34.7 ±0.81 c	18.5 ±1.29 c	58.2 ±3.55 b	8.5 ±1.29 ab	0.7 ±0.09 ab
Oats	11.3 ±1.71 d	32.8 ±0.52 c	16.3 ±0.50 c	44.2 ±1.71 d	7.5 ±0.58 b	0.6 ±0.05 b
Ray	0.9 ±0.30 f	32.0 ±1.71 c	10.5 ±1.29 d	51.8 ±2.62 c	7.0 ±0.96 bc	0.6 ±0.05 b
Forage sorghum (hybrid)	13.0 ±1.29 c	41.8 ±1.25 b	14.0 ±1.71 cd	22.8 ±1.63 e	9.5 ±0.58 a	0.6 ±0.05 c
Forage sorghum (variety)	15.3 ±1.50 c	42.0 ±0.81 b	11.8 ±0.95 d	24.5 ±1.71 e	8.0 ±0.00 b	0.5 ±0.13 c
Sudan grass	21.5 ±1.29 b	42.0 ±1.00 b	7.5 ±0.96 e	27.5 ±1.71 e	8.0 ±0.81 b	0.5 ±0.09 c
Millet	34.9 ±1.03 a	49.8 ±1.29 a	6.2 ±1.26 e	16.2 ±1.55 f	9.0 ±0.00 a	0.5 ±0.21 c
SLD	0.7 ±0.37 f	27.8 ±1.29 d	36.0 ±1.00 a	115.6 ±3.40 a	6.0 ±0.58 c	0.9 ±0.08 a
F value	**300.66****	**96.14****	**172.12****	**432.43****	**6.11****	**66.32****

Mean values ±SD, Values with the same letter in the column are on the same level of significance, ** - P<0.01, * - P<0.05, ns - P>0.05

3.7. Susceptibility of Poaceae seeds to *P. intepunctella* development

The calculated SI (Tab. 2) indicates that maize and wheat kernels (SI=11.90, and 10.62, respectively) were the most susceptible to *P. intepunctella* attack (S), while the least suitable were millet and Sudan grass (SI=3.65, and 4.95, respectively), that were rated as moderately resistant (MR).

Grain resistance to environmental factors is influenced by the characteristics of the seed coat that covers its entire surface. The seed coat consists of extinct woody cells with thickening walls, made of cellulose, hemicellulose, lignin and other materials that provide high strength, and also additional resistance towards insect pests (Anonymous, 2017).

According to Weipert (1996) wheat kernels are much preferable to insect pests than rye, primarily because rye kernels are much harder and this is in accordance with the result of this work, where rye was less susceptible to *P. interpunctella* attack. Although, *P. intepunctella* larvae have strong mandibles, they do not easily break the pericarp of wheat and especially of rye kernels, so whole kernels are not the most suitable food for this moth (Locatelli and Limonta, 1998; Kočović, 2014), which is why *P. intepunctella* larvae are easily developed on crushed grain, rather than on whole ones (Lecato, 1976; Kočović, 2014). Barley seeds differ from the wheat and rye because palea is firmly attached to the seed coat and its share in the grain is 7-15% (Anonymous, 2017). This part of seed

may provide additional protection insect attack, so we can speculate that it is a reason for higher mortally of larvae. The outer layer of maize seed coat is more developed than in other cereals, but without palea. The total thickness of the maize seed coat (6 - 10% of the total grain weight) can be 0.5 mm, thus is easier to be damaged by insects, while for sorghum seeds, the seed coat thickness and the presence of palea depends on agronomic form.

Tab. 2 Susceptibility of different Poaceae seeds and SLD for development of IMM

Commodity	Susceptibility Index	Rating
Maize	11.90	S
Wheat	10.62	S
Barley	9.03	S
Oats	8.66	S
Ray	7.19	S
Forage sorghum (hybrid)	5.71	MS
Forage sorghum (variety)	6.28	MS
Sudan grass	4.95	MR
Millet	3.65	MR
SLD	12.80	S

R- resistant; MR – moderately resistant; MS – moderately susceptible, S - susceptible

Considering the above mentioned, it is obvious that aside from standard measures for control of storage insects, especially *P. interpunctella*, host plant resistance is one of the promising practices and more sustainable in integrated pest management, but also cheaper and ecologically safer (Abebe et al., 2009; Tefera et al., 2011).

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Population growth and development of *Liposcelis obscurus* Broadhead (Psocodea: Liposcelididae) at constant temperatures and relative humidities

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Abstract

The effects of nine temperatures (22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, and 42.5°C) and four RHs (43, 55, 63, and 75%) on the population growth and development of the parthenogenetic *Liposcelis obscurus* Broadhead (Psocodea: Liposcelididae) were investigated in laboratory studies. Results showed that *L. obscurus* did not survive at 43% RH at all temperatures tested. At 55% RH, *L. obscurus* survived at 22.5, 25, and 27.5°C; none survived at 42.5°C and ≤63% RH. *Liposcelis obscurus* survived and the population increased 56–fold from an initial population of five adult females at 42.5°C and ≤75% RH. Population growth was highest at 40°C and 75% RH, where population increase was 215-fold. *Liposcelis obscurus* has three-to-five nymphal instars, and the percentages of third, fourth, and fifth instars were 52, 41, and 7%, respectively. Temperature-dependent developmental equations were developed for *L. obscurus* eggs, individual nymphal, combined nymphal, and combined immature stages. *Liposcelis obscurus* populations grew much faster at 30–42.5°C and 75% RH. These

data provide a better understanding of *L. obscurus* population dynamics, and can be used to develop effective management strategies for this psocid.

Keywords: psocid, stored-product, population growth, development rate, booklouse

1. Introduction

Psocids of the genus *Liposcelis* (Psocodea: Liposcelididae) have emerged as important pests of stored products worldwide over the last two-to-three decades (Nayak et al., 2014). Psocids are mostly found in grain food stores, food processing facilities, and they thrive on a variety of food products (Opit and Throne, 2008a; Athanassiou et al., 2010). Psocid infestations do not only cause grain weight loss but also result in significant germination failure by feeding on the germ and endosperm of seeds (Kučerová, 2002; Gautam et al., 2013). Psocids have a short generation time at elevated temperatures which allows them to rapidly colonize new habitats (Nayak et al., 2014). The economic importance of psocids in a commodity is not just limited to direct feeding and contamination but they can also lead to rejection of infested commodities from domestic and international markets (Nayak, 2006). Psocids are difficult to control using standard practices of protection and disinfestation (Wang et al., 1999; Beckett and Morton, 2003; Athanassiou et al., 2009; Huang et al., 2009).

In the US, *Liposcelis* and *Lepinotus* are two genera of psocids that are found in large numbers in grain storages and are of economic importance (Gautam, 2010; Opit et al., 2011). Four *Liposcelis* species of notable economic importance worldwide are *L. bostrychophila* Badonnel, *L. entomophila* (Enderlein), *L. decolor* (Pearman), and *L. paeta* Pearman, but examples of other species that are economically important include *L. corrodens* Heymons, *L. brunnea* Motschulsky, *L. obscurus* Broadhead, and *L. rufa* Broadhead (Lienhard and Smithers, 2002; Gautam et al., 2010).

The psocid species *L. obscurus* Broadhead has been found infesting storage structures in the US. *Liposcelis obscurus* is an obligate parthenogen (Mockford, 1993). The only ecological study conducted on *L. obscurus* published in scientific literature investigated the effects of temperature and food on the reproductive parameters of this species (Khalafalla, 1990). In the present study, objectives were to determine the effects of constant temperatures and relative humidities on the population growth of *L. obscurus* and to quantify the effects of temperature on the development of this species.

2. Materials and Methods

2.1. Insects

Cultures of *L. obscurus* used in this study were started using insects collected from peanut (*Arachis hypogaea*) warehouses in Oklahoma (USA). Voucher specimens of 100 *L. obscurus* preserved in 95% ethyl alcohol that were used in this study were deposited at the K.C. Emerson Entomology Museum at Oklahoma State University under lot numbers 119 (females). Psocids were reared on a mixture of 93% cracked wheat (*Triticum aestivum* L.) (Duster variety), 5% Rice Krispies (Kellogg North America Company, Battle Creek, MI), and 2% wheat germ (The Quaker Oats Company, Chicago, IL) (wt/wt) in 360-ml glass canning jars with mite-proof lids (Opit and Throne, 2008b). The top one-third of the inner surface of each jar was coated with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent psocids from accessing and gathering on the inside of the lid. Cultures were placed inside a growth chamber maintained at 30 ± 1°C in plastic boxes (42 x 29 x 24 cm high) painted black, which had saturated NaCl solution beneath perforated false floors to maintain a RH of 75 ± 5% RH. The boxes were painted black to mimic dark conditions in which psocids are typically found.

2.2. Effects of temperature and relative humidity on population growth

The effects of nine temperatures (22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, and 42.5°C) and four RHs (43, 55, 63, and 75%) on the population growth of *L. obscurus* over a 30-d period were determined. The

inner sides of 108 Petri dishes (100 x 25-mm high) were coated with Fluon to prevent psocids from escaping. Into each Petri dish, 5 g of red colored diet, 1 g of cracked duster wheat, and 0.5 g wheat germ (hereafter referred to as diet) were placed. The mixture of red colored diet, cracked duster wheat, and wheat germ was used as diet because *L. obscurus* did not survive well on only cracked wheat diet. The plastic Petri dish lids were replaced. Red colored diet was made by mixing 100 g of Rice Krispies with a solution of 5 ml of red food dye (Global Chem Sources Inc., Cedar Grove, NJ) in 300 ml of water, drying the mixture in a mechanical convection oven (model HTM 85, Precision Scientific, Inc., Chicago, IL) for 6 h, and then grinding the dried mixture in a Wiley Mill. A U.S. Standard #20 sieve (0.85-mm openings) (Scientific Apparatus, Philadelphia, PA) was used to sieve the diet. Petri dishes with diet were randomly put in four plastic boxes (42 x 29 x 24 cm high) containing each of the saturated solutions of K_2CO_3 (43%), NaBr (55%), $NaNO_2$ (63%), and NaCl (75%) (Greenspan 1977) beneath perforated false floors to maintain the required RH. Petri dishes were kept at the four RHs to equilibrate the diet in them at room temperature for 4 wk. Each box had 27 Petri dishes.

To obtain 1- to 2-wk-old *L. obscurus* adult females required for the experiment, 300 female late-instar nymphs of *L. obscurus* were picked from culture jars and placed in six 9-cm Petri dishes with Fluon-coated sides. Each Petri dish had 5 g of colored psocid diet, 1 g of cracked duster wheat, and 0.5 g of wheat germ in it. The Petri dishes were placed on perforated false floors of one black Rubbermaid plastic box (32 x 18 x 13 cm). The late instar nymphs were maintained at 75 ± 5% RH for 2 wk.

After 4 wk of diet equilibration, five 1- to 2-wk-old adult *L. obscurus* were placed in each of the 108 Petri dishes containing equilibrated diet. Nine incubators (Thermo Fisher Scientific; Waltham, MA) were set at temperatures of 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, and 42.5°C, where four plastic boxes (17 x 17 x 12 cm high) containing saturated solutions of K_2CO_3, NaBr, $NaNO_2$, and NaCl were placed. Three Petri dishes containing diet, equilibrated at room temperature and each RH, were randomly assigned to the corresponding RH box in all incubators. Methods by Gautam et al. (2010) and Aminatou et al. (2011) were then followed.

The experiment had three temporal replications, and the experimental design was a randomized complete block design (RCBD) with subsampling. Statistical procedures were done by using Statistical Analysis System software version 9.4 (SAS Institute, 2014). PROC MIXED was used for analysis of variance (ANOVA) to determine the effects of temperature and RH on the number of psocids in the Petri dishes. Data on psocid numbers were transformed using the square root transformation to stabilize variances before analysis. Untransformed means and standard errors are reported for straightforward interpretation. We used the least significant difference (LSD) test to determine differences among mean numbers of psocids produced at the various temperatures and RHs despite the quantitative independent variables, because we were not able to quantify the relationship using a biologically meaningful equation (TableCurve 3D) (Systat Software, Inc., 2002a).

2.3. Effects of temperature on development

Eggs were obtained by placing 1 g of red colored diet, 5 particles of wheat germ, and 30 adult female psocids of unknown age from our psocid cultures in each of eighty 35-mm-diameter Petri dishes (Greiner Bio-One, Kaysville, UT), which had a coat of Fluon on the sides. Procedures used to obtain the red colored diet were similar to those in Opit and Throne (2008). The Petri dishes were placed in two black Rubbermaid plastic boxes (30 x 23 x 9 cm high) that contained saturated NaCl solution (75% RH) beneath a perforated false floor. Boxes were placed in an incubator maintained at 40 ± 1°C. After 2 d, adult females were taken off, and the diet in each Petri dish was examined for eggs by using a dissecting microscope at 25x magnification. Procedures used for setting up the experiment to monitor development of eggs were analogous to those used by Opit and Throne (2018). Thirty centrifuge caps (associated with vial caps and Petri dishes) were randomly placed in each of nine Rubbermaid plastic boxes (37 x 22 x 13-cm high; 270 centrifuge caps total) that were painted black and contained saturated NaCl solution to maintain 75% RH. One box was placed in each of the nine incubators set to maintain treatment temperatures of 22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5, 40.0, and 42.5°C. Temperatures above 42.5°C were not tested because preliminary

experiments had shown that *L. obscurus* eggs do not hatch at temperatures above 42.5°C. The experiment had two temporal replications. To estimate the incubation period of eggs and to mark insects after egg hatch to determine when one developmental stage ended and the next began, procedures analogous to those used by Opit and Throne (2018) were used.

2.4. Data analysis

In the determination of the effects of temperature on the duration of development of *L. obscurus,* PROC MIXED was used for analysis of variance (ANOVA). The experimental design for the analysis of the proportions of viable eggs and nymphs that developed to the adult stage was an RCBD. Regression (TableCurve 2D; Systat Software, 2002b) was used to describe the relationship between temperature and development time for the egg, individual nymphal, combined nymphal, and combined immature stages. Fitting curves with nonlinear regression showing the relationship between temperature and development time for the individual developmental stages were constructed using SigmaPlot version 10.0 (Systat Software, 2006). The selection of an equation used to describe the data was based on the magnitude and pattern of residuals, lack-of-fit tests, and whether the curve had a reasonable shape to describe the data. In the analysis of the proportions of viable eggs and nymphs that developed to the adult stage, the design for analysis was a RCBD. To analyze the proportions of viable eggs and nymphs, PROC MIXED was used for ANOVA after arcsine square-root transformation to stabilize variances.

The lower developmental threshold for *L. obscurus* was determined by fitting a linear equation to development rate (reciprocal of development time) and temperature data using TableCurve 2D (Systat Software Inc., 2002b). The upper developmental thresholds for *L. obscurus* developmental stages were found by determining the temperature at which the rate of development begins to decrease (Zilahi-Balogh and Pfeiffer, 1998). The upper developmental thresholds were obtained by fitting the appropriate equation to all the development rate and temperature data and by using the "EVALUATION" procedure in TableCurve 2D (Systat Software Inc., 2002b) to determine the upper developmental thresholds.

3. Results

3.1. Effects of temperature and relative humidity on population growth.

The nine temperatures and four RHs tested affected *L. obscurus* population growth (Fig. 1). No live *L. obscurus* were found at 43% RH for all temperatures; at 55% RH and 30–42.5C; and 63% RH at 42.5°C. Numbers of *L. obscurus* at 35 and 37.5°C and 75% RH were very similar—approximately a 143-fold increase in population, in 30 d, for each temperature. Population growth was highest at 40.°C and 75% RH, where population increase was 215-fold (Figure 1). At 42.5°C and 75% RH, *L. obscurus* populations increased 56–fold from an initial population of five adult females.

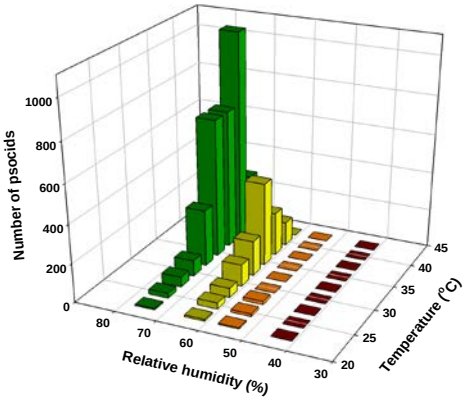

Fig. 1. Effects of temperature and relative humidity on *Liposcelis obscurus* population growth.

3.2. Effects of temperature on *L. obscurus* development.

3.2.1. Eggs

Incubation varied with temperature and the relationship between temperature and incubation time was well described by a quadratic equation (Fig. 2A). The optimal incubation temperature is 40.0°C, and development is completed in 4.1 d.

3.2.2. Nymphal and Combined Nymphal Stages

Duration of the nymphal and combined nymphal stages varied with temperature (Fig. 2B–E). Quadratic equations described the relationship between temperature and development time well for individual nymphal and combined nymphal stages. Temperature had a significant effect on development time for N1 (first instar), N2 (second instar), and N3 (third instar) (Fig. 2B–D); where development time decreased with increasing temperature. Based on analysis of data for all nymphs that developed to adults, combined nymphal development time averaged 28.6 d at 25ºC and declined to 11.6 d at 40ºC. However, developmental time increased slightly at 42.5ºC and development is completed in 11.8 d, respectively. Based on the quadratic equation for the combined nymphal stages, the predicted optimal developmental temperature is 41.1ºC and development is completed in 11.7 d.

3.2.3. Combined Immature Stages

The analysis of data for all individuals that developed to adults showed that temperature had a significant effect on total developmental time from egg to adult, and a quadratic equation fit the data well (Fig. 2F). Total developmental time from egg to adults averaged 42.7 d at 25°C and declined to 15.8 d at 40°C. However, developmental time increased slightly at 42.5°C and development is completed in 16 d. The upper developmental threshold was estimated as 43.9°C. The lower developmental threshold was estimated as 13.2ºC using a linear equation that best described the development rate and temperature relationship. Based on this study, *L. obscurus* has three to five nymphal instars, and the percentages of third, fourth, and fifth instars were 52, 41, and 7%, respectively.

3.3. Effects of Temperature on Egg Viability and Nymphal Survivorship

Temperature affected egg viability (F = 3.8; df = 7, 7; P = 0.049), which ranged from 83% to 100% and averaged 91.5% for all temperatures. Temperature had no effect on nymphal survivorship (F =

1.0; df = 1, 1; $P = 0.50$). Proportions of nymphs surviving to adults at the nine different temperatures ranged from 65–73%.

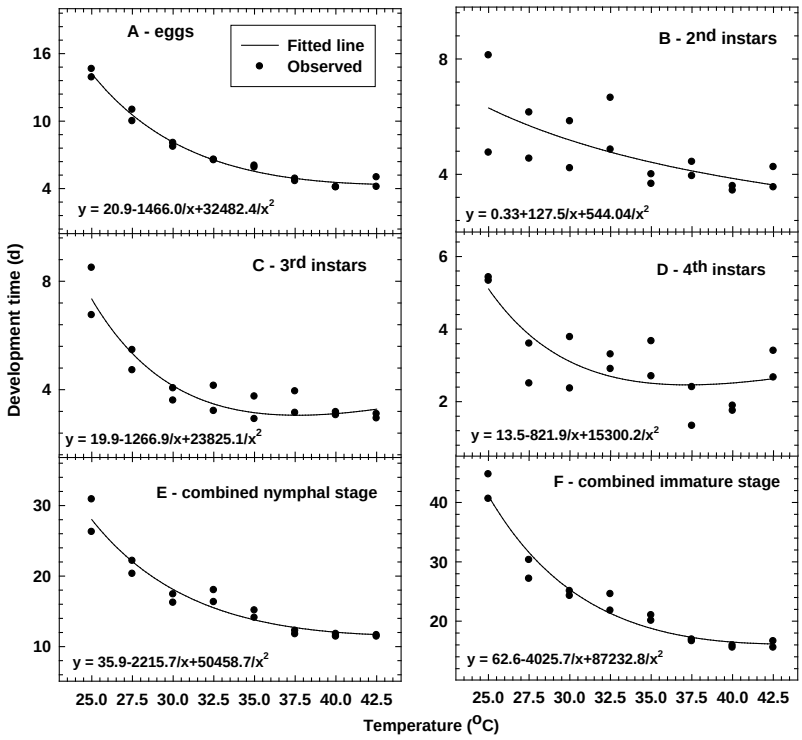

Fig. 2. Development of female *Liposcelis obscurus* at constant temperatures and 75% RH: (A) eggs, (B) second, (C) third, and (D) fourth instars, and (E) combined nymphal and (F) combined immature stages.

Discussion

Results from this study show that *L. obscurus* did not survive at 43% RH at any of the temperatures tested; at 55% RH and 30–42.5°C; and at 63% RH and 42.5°C. The optimal temperature and RH for population growth of is *L. obscurus* are 40°C and 75% RH. *Lepinotus reticulatus*, *L. brunnea*, *L. rufa*, *L. pearmani*, and *L. fusciceps* have also been reported not to survive at 43% RH (Opit and Throne, 2008b; Opit and Throne, 2009; Gautam et al., 2010; Aminatou et al., 2011; Gautam et al., 2015). Although *L. obscurus* survived and barely multiplied at 55% RH and 22.5–27.5°C over 30 d, data indicate it will not thrive at this low RH. At 63% RH, a low temperature of 22.5°C results in limited increase in population, and a higher temperature of 42.5°C kills all psocids. At 75% RH, a low temperature of 22.5°C results in limited increase in *L. obscurus* population. Rees and Walker (1990) observed that *L. bostrychophila, L. entomophila,* and *L. paeta* did not survive at low RHs (<60%). Knulle and Spadora (1969) stated that below the equilibrium RHs of psocids, death occurs. According to Devine (1982), high atmospheric water vapor of ≥60% RH is necessary for psocids to maintain body water levels by absorption; however, below this level, more moisture is lost than gained, which results in dehydration and death. At 30.0°C and 55% RH, *L. obscurus* did not survive, but *L. brunnea*, *L. rufa*, and *L. fusciceps* populations grew, although growth was slow (Opit and Throne, 2009; Gautam et al., 2010). *L. brunnea*, *L. rufa*, and *L. fusciceps* are probably well adapted in a manner that enables them to absorb atmospheric water vapor even when RH is as low as 55%.

The highest population growth for *L. obscurus* occurred at 40ºC and 75% RH. RH of 75% has also been found to be optimal for the population growth of *L. reticulatus*, *L. rufa*, *L. pearmani*, and *L. fusciceps* but 63% RH was optimal for *L. brunnea*. Optimum temperatures for these species were 30C° for *L. fusciceps;* 32.5°C for *L. reticulatus*, *L. pearmani, and L. brunnea;* and 35°C for *L. rufa* (Opit and Throne, 2008b; Opit and Throne, 2009; Gautam et al., 2010; Aminatou et al., 2011; Gautam et al. 2015). Optimal RH for *L. brunnea* (63%) explains why it mainly occurs in the relatively drier parts of US compared with other species (Gautam et al., 2010). However, its distribution may be limited by high temperatures of 35.0°C or higher (Opit and Throne 2009). Rees and Walker (1990) showed that the optimum conditions for *L. bostrychophila*, *L. entomophila,* and *L. paeta* are 30.0°C and 80% RH, 30°C and 70% RH, and 33°C and 70% RH, respectively. *L. rufa* barely survives at 40.0°C (Gautam et al., 2010). Therefore, higher temperatures may limit *L. rufa* distribution although it reproduces relatively well at lower RHs and temperatures (55% RH and 22.5–30.0ºC) compared to *L. obscurus*. The optimum conditions for *L. obscurus* (40.0ºC and 75% RH) imply that it is expected to have a broader distribution than *L. rufa,* and be more abundant in hot and humid areas. Based on this study, *L. obscurus* is capable of surviving and multiplying at moderately high rates at 42.5°C.

L. obscurus has three to five nymphal instars, and the percentages of third, fourth, and fifth instars were 52, 41, and 7%, respectively. *L. brunnea* females were also found to have three to five nymphal instars with a higher percentage having four nymphal instars (78%) compared with *L. obscurus* which has a higher percentage of insects with three nymphal instars. However, Khalafalla (1990), reports that the *L. obscurus* strains found in Egypt have exactly four instars. Opit and Throne (2008b), report that *L. reticulatus* (a parthenogenetic species) also has four nymphal instars. Males and females of bisexual *Liposcelis* species are found to have two to four and two to five nymphal instars, respectively (Gautam et al., 2010; Aminatou et al., 2011; Gautam et al., 2015). Due to the additional number of instars female psocids have, the developmental period of females is longer than that of males. The evolution of a variable number of *Liposcelis* instars may be to prolong their survival in adverse conditions (Aminatou et al., 2011). According to Mockford (1993), psocids usually have four to six nymphal stages.

The optimal temperature for *L. obscurus* development from egg to adult was 40ºC and development was completed in 15.8 d. The optimal temperature for development of female *L. badia*, *L. bostrychophila*, *L. reticulatus*, *L. pearmani*, and *L. tricolor* was 32.5ºC and development were completed between 17 and 31 d (Wang et al., 2000; Dong et al., 2007; Jiang et al., 2008; Opit and Throne, 2008; Aminatou et al., 2011). For *L. brunnea*, *L. entomophila*, *L. decolor,* and *L. fusciceps*, the optimal temperature for development was 35.0°C and development was completed in 23.6, 21.7, 16.1, and 19.0 d, respectively; also, *L. paeta* and *L. rufa*'s development were completed in 11.5 and 21.6 d, respectively, at 37.5°C (Tang et al., 2008; Wang et al., 2008; Opit and Throne, 2009; Gautam et al., 2010; Aminatou et al., 2011; Gautam et al., 2015). At the optimal temperature of 40.0°C, development of *L. obscurus* from eggs to adult takes a slightly shorter time compared to other psocids that have been studied.

This study demonstrates how temperature and RH affect *L. obscurus* population growth and development. *L. obscurus* is not expected to be a serious pest in grain storages where temperatures are 27.5°C or less. Given that *L. obscurus* had a relatively higher population growth over a 30-d period compared to other *Liposcelis* species at higher temperatures of 35–42.5°C and 75% RH, we expect it to be (or become) a predominant pest in hot and humid areas. Finally, the temperature dependent equations developed for this species could be used to understand *L. obscurus* population dynamics and to develop effective management strategies.

4. Conclusions

Based on this study, *L. obscurus* is predicted to be more abundant and a pest in hot and humid areas of the world. That being said, to the best of our knowledge *L. obscurus* has only been reported twice — it was found infesting a peanut warehouse in Oklahoma, USA and in stored rice in Egypt. Possible

reasons for why *L. obscurus* has not been frequently reported may be due to lack of research or misidentification of this species.

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Circadian Rhythm of *Liposcelis entomophila* and *Liposcelis paeta* in Paddy Warehouse

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Abstract

Booklice is a small but serious stored grain pest, and understanding the circadian rhythm of booklice help to control. In this study, circadian activity of booklice were monitored with sticky traps in the grain bulk surfaces of two warehouses stored paddy rice in two different provinces in China. The results showed that the species of booklice were different and were *Liposcelis entomophila*, and *Liposcelisp paeta* for Nanning's and Zhanjiang's warehouses respectively. In term of *L.entomophila*, its activity intensity gradually decreased from 0 am to 12 pm and reached the lowest level of daily activity at 12pm. After this, there was a steady and straight upward trend, and the peak of its activity intensity is reached at 8 pm. Its circadian activity trend can be represented as: $y = -0.971x^3 + 21.88x^2 - 139.5x + 353.4$(x: time; y: quantity of booklice). Over the same period, the activity intensity of *L.paeta* varied greatly. It gradually increased, reached a peak at 8 am, dropped dramatically at 12 pm and then climbed the second peak at 6 pm.

Keyword: sticky trap, monitor, *L.entomophila*, *L.paeta*, circadian rhythm

1. Introduction

In control of stored grain pests, insect population dynamics monitoring and density inspection are important. The species, density, distribution, and damage status data of grain stored pests in the grain bulk can be timely detected, predicting the development trend of insects, avoiding unnecessary prevention cost and the economic losses, and providing scientific strategy for insect control (Bai Xuguang, 2002).

At present, the pest detection technology is traditional screening method. There are many disadvantages, such as, labor intensive, low efficiency, and imprecise. Base on these, trapping detections are developed. They are convenient, fast, environmentally friendly, and highly automated（LI Zhishen, 2014）. Sticky board trapping is one of these technologies and has been widely used in the monitoring and control of agricultural and forestry pests. However, there are few reports in stored grain insect pests. In this study, the sticky board trapping technology was applied to monitor the population dynamics of the booklice in two paddy warehouses in Guangdong and Guangxi, southern China. The aim in work reported in this publication was to assess sticky board trapping as an alternative to detection.

2. Materials and Methods

2.1 Test Warehouse

No. 32 large warehouses in Nanning Shajing Grain Warehouse examined was a length of 26.69 m and a width of 19.73 m, storage ability 1250t. The stored grain is indica rice and has been stored since September 2013. The moisture content and impurity ratio of this grain is 12.8%, and 1%, respectively.

No.4 large warehouses （50 m ×20 m）in Guangdong Zhanjiang North Station Grain Warehouse was selected for trails. The indica rice in this warehouse had 10.5% grain moisture content and been stored since August 2013. .

Both warehouses had been fumigated with phosphine.

2.2 Application of sticky board trap

Sticky board traps (20 cm × 25 cm, Beijing Ikoman Bio-Technology Co., Ltd.) were applied different positions of the surface of indica rice in each of two warehouses, including corner, under the fan, window, and check door. The numbers applied were four. The position of the trap location in the real warehouse is shown in Fig. 1.

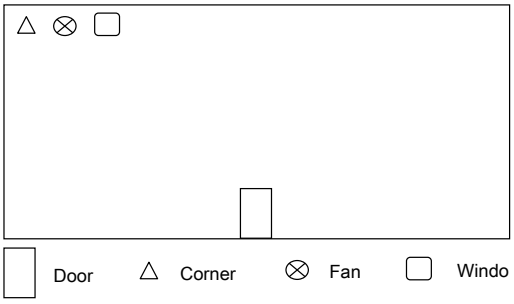

Fig. 1 sampling points in the warehouse

2.3 Observing the circadian rhythm of the booklice in warehouse

General procedures: the methods of assessment were based on procedures developed to measure booklice population on grain. The methods were:

- Counting of booklice in marked areas.
- Leaving sticky board traps for defined periods (2 hs), shaking out the booklices into trays and counting.
- Sampling grain with a bottom-opening probe, sieving (Φ 1.5 × 2.5 mm) and count insects.
- The whole test lasted for 24 hours.

The temperature and relative humidity of the pf the warehouses were monitored by Vaisala VAISALAHM34 High-precision Temperature and Humidity Table (VAISALA, Finland).

3. Results

3.1 Circadian rhythm of L.entomophila in paddy warehouse in Nanning Shajing Grain Warehouse

The species of booklice in Nanning Shajing Grain Warehouse was *L.entomophila*. Within 24 hours, the temperature ranged over time from 27.48 °C to 29.28°C and the humidity ranged from 68.95% to 73.05% (Fig. 2). The warehouse temperature showed an overall downward trend during the period from 12 am to 10 pm and a rise after 10 pm. During this period, the humidity showed an overall downward trend, except that it was an abnormally high point at 10 am. The trend continued until 2 pm. The quantity of booklice trapped generally decreased during the period from 12 am to 10 am, and reached a minimum at 12 pm. After 12 pm, the temperature in the warehouse experienced an increase, while this phenomenon appeared 4 hours later and 2 hours later in the humidity and the quantity of booklice trapped respectively. Therefore, the Circadian Rhythm can be inferred from the quantity of booklice attracted by the sticky trap at different time periods. The activity frequency of *L.entomophila* in the warehouse is correlated with the temperature and humidity in the warehouse. From early morning, the frequency of booklice gradually decreased, and booklice activities entered a relatively quiet period at 12 pm. After this, with the overall recovery of temperature and humidity, pest activity gradually became active again, and reached relative activity peak period from 6 pm to 2 am. The daily activity trend of *L.entomophila* can be expressed as $y = -0.971x^3 + 21.88x^2 - 139.5x + 353.4$ (x is the 24-hour time, and y is the quantity of booklice)

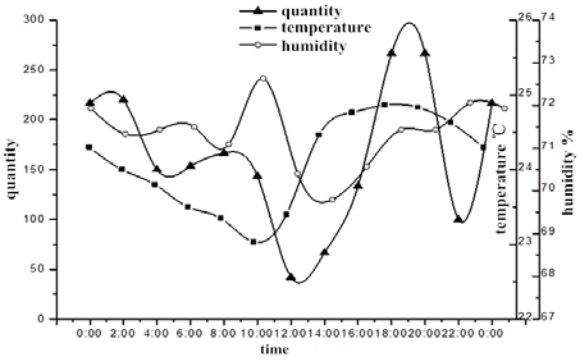

Fig. 2 Changes in Temperature, Humidity, and quantity of *L.entomophila* trapped with time in the warehouse of No. 32 Shajing Grain Depot

3.2 Circadian Rhythm of L.paetain Zhanjiang Warehouse

The species of booklice in Zhanjiang Warehouse is *L.paeta*. In Zhanjiang North Station State Grain Storage No. 4 warehouse, the temperature varied significantly with time, ranging from 27.30°C to 29.27°C. However, the humidity vary slightly with time, ranging from 74.40% to 79.27% and reached an unusually high point around 2 am. There was a certain correlation between the quantities of booklice and humidity after 10 am. The trend in Fig. 3 showed that the quantity of trapped *L.paeta* was relate to temperature. Interestingly, 12 pm was not the lowest temperature of the day, but the quantity of trapped *L.paeta* reached a minimum value as same as that of *L.entomophila* in Nanning Shajing. This might be caused by other factors expect temperature and humidity, such as insect daily activity rhythm. The peak activity of the *L.paeta* appeared at 2 pm.

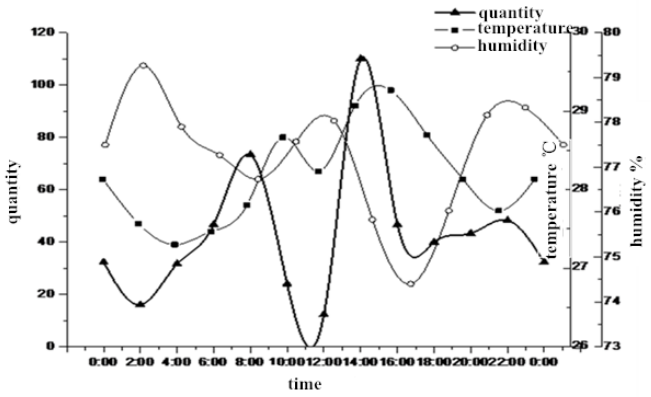

Fig. 3 Changes in temperature, humidity, and quantity of *L.paeta* trapped with time in Zhanjiang No. 4 warehouse

4. Discussion

In recent years, booklice has also became a new threat to global grain security (Zhang Shengfang, 1998), which is the problem that needs to be solved urgently (Muhammad Shoaibet al, 2010). The sticky trap in the study can be considered as a physical control method. It provides a new green and effective means for the prevention and treatment of booklice.

Since both the *L.entomophila* and *L. paeta* have obvious light-shielding properties (Yan Xiaoping et al, 2008), the principle of sticky trap remains to be further studied. It may be related to the fact that the *L.entomophila* prefers high humidity environment because of the stickiness. The glue on the glue sheet causes an increase in humidity.

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Development of a suitable rearing media for *Tribolium castaneum*

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Abstract

Tribolium castaneum is a serious pest of cereal flour and flour-based products, and thus a test insect in stored-product research. The composition of the rearing medium affects the progeny production, their performance and handling efficacy. The objective of this research was to develop a suitable rearing media for *T. castaneum*. The research tested wheat flour, crushed broiler feed, crushed dog feed and corn flour alone and in different combinations. Twenty adults of *T. castaneum* were introduced to each medium separately, and removed after 2 weeks. The progeny adults emerged in each rearing medium was determined. The progeny produced differed with the food medium. In general, the rearing media having a combination of ingredients produced more

progeny than a particular component alone. Different ratios of these food ingredients need to be tested to further increase the progeny production in *T. castaneum* and to determine the efficacy of these media on the progeny production in other species.

Keywords: *Tribolium castaneum*, rearing media, adult emergence, progeny

1. Introduction

Food security is a major issue throughout the world, and storage of food always has been a challenge due to infestation by insects (Wijayaratne et al., 2009; Wijayaratne et al., 2018). *Tribolium castaneum* larvae and adults cause quantitative and qualitative losses in stored products (Wijayaratne and Rajapakse, 2015), and hence appropriate control methods need to be developed. Thus *T. castaneum* is a test insect in many research and need to be handled in large numbers. A rearing media which facilitates healthy development of *T. castaneum* cultures and convenient handling of them is therefore important. Therefore this research was conducted to develop an effective rearing media for *T. castaneum* using food ingredients available at the local market.

2. Materials and methods

Tribolium castaneum reared in wheat flour at 30°C, 65% R.H. were used in the experiment. Wheat flour, crushed broiler feed, corn flour and crushed dog feed were used alone and in different combinations as given in Table 1. From each medium prepared, 100 g was weighed into a plastic vial and 20 adults (without sexing) were introduced into each vial. The adults were removed after two weeks. The progeny emerged in each container was counted at one and three months.

3. Results and Discussion

Progeny produced by *T. castaneum* varied with the medium (Figures 1-3). The medium that contained wheat flour, crushed dog feed and crushed broiler feed at 2:1:1 ratio produced the maximum adult progeny.

Tab. 1 Rearing media tested for progeny production by *Tribolium castaneum*.

Treatment	Wheat Flour(g)	Crushed Dog feed(g)	Crushed Broiler feed(g)	Corn flour(g)
A	100	-	-.	-
B	-	100	-	-
C	-	-	100	-
D	-	-	-	100
E	50	50	-	-
F	50	-	50	-
G	50	-	-	50
H	50	25	25	-
I	50	-	25	25
J	50	25	-	25
K	25	25	25	25

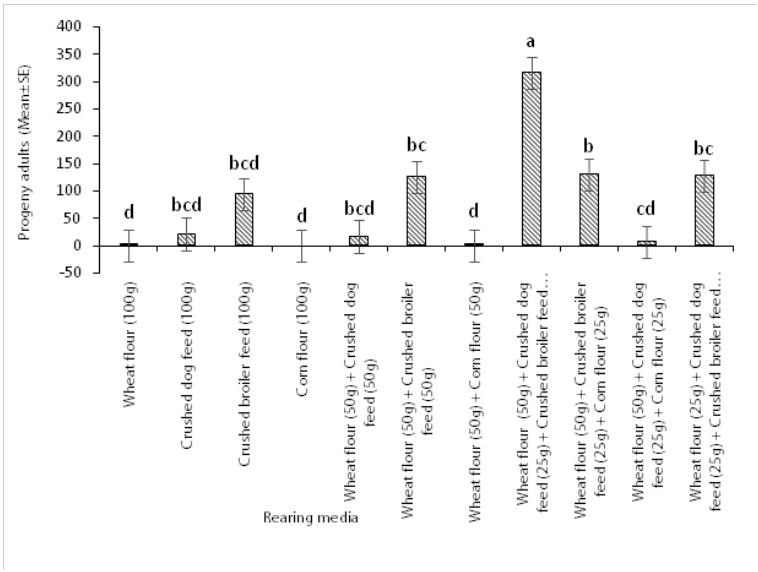

Fig. 1 Progeny *Tribolium castaneum* adults (mean±SE) emerged on different rearing media one month following infestation (n=4).

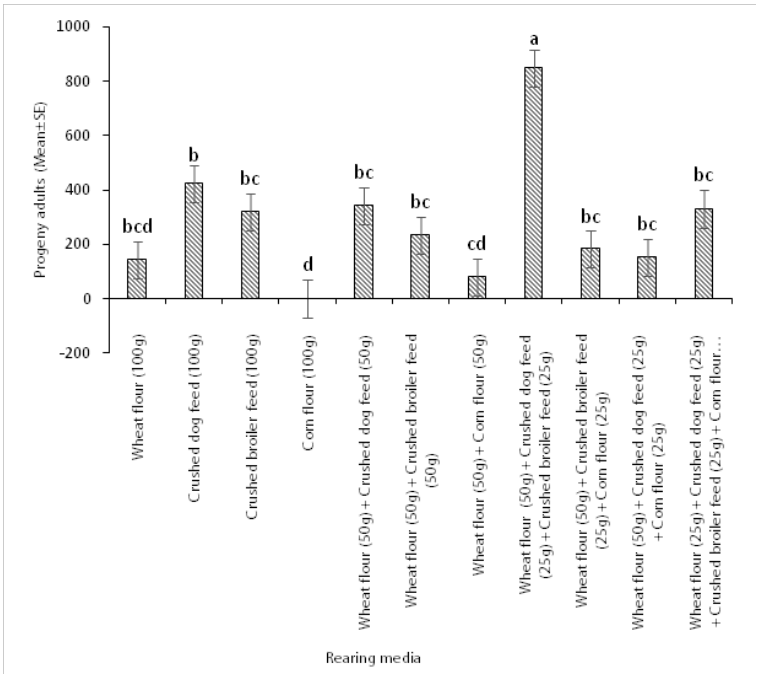

Fig. 2 Progeny *Tribolium castaneum* adults (mean±SE) emerged on different rearing media two months following infestation (n=4).

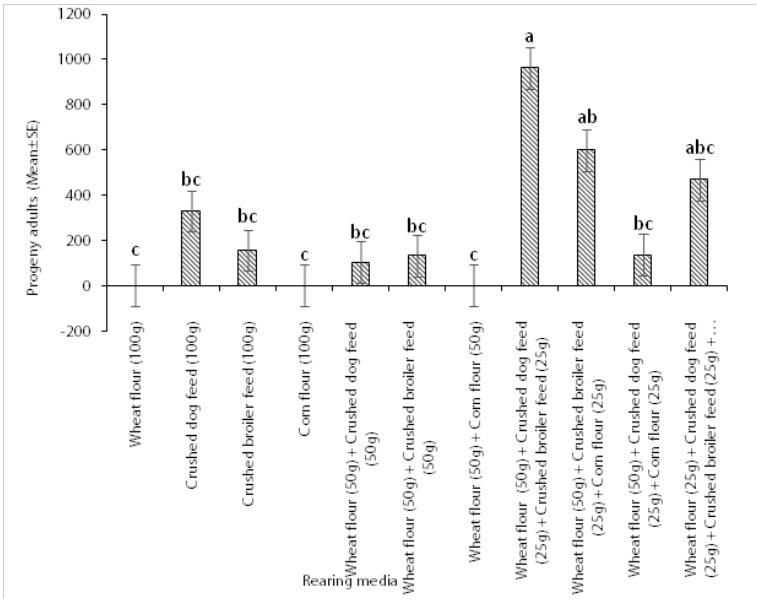

Fig. 3 Progeny *Tribolium castaneum* adults (mean±SE) emerged on different rearing media three months following infestation (n=4).

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Sitotroga cerealella (Olivier) resilience to extreme temperature and desiccation may explain its increasing pest status in changing climates

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Abstract

The mechanisms underlying *Sitotroga cerealella* survival under variable and increasing mean thermal and desiccation environments typical under global change is currently unknown. To understand how *S. cerealella* survives extreme abiotic stressors typical of stored-grain environments, we measured *S. cerealella* tolerance temperature and desiccation. The results showed that to survive desiccating grain storage environments, *S. cerealella* relied more on high body water content (BWC) (70.2 ± 3.72%) compared to lipid reserves (9.8± 0.81%). In desiccating environment, *S. cerealella* showed a reduced water loss rate (0.056mg/h) (equivalent of 1.81% of body water/hour) which would require 19.31 h to reduce the insect body water to its critical minimum (35.23% body water content at death), which is 50.20% of normal initial body water. Similarly *S. cerealella* exhibited high basal heat tolerance with critical thermal maximum of 46.09 ± 1.042°C and a heat knockdown time of 7.97 ± 1.64 minutes. Basal cold tolerance was relatively compromised (critical thermal minima of 4.52 ± 1.06°C and chill

coma recovery time of 5.80 ±1.17 minutes), following 1h at 0°C. We found no significant correlation ($P > 0.001$) between BWC and the measured thermal tolerance traits. Low water loss rates reported here may be an evolutionary resistance mechanism for desiccation tolerance. Observed abiotic stress tolerance may explain the ubiquitous distribution of *S. cerealella* in Africa which is likely to enhance its survival and increase its pest status under global change.

Keywords: storage insect pests, abiotic stress, thermal tolerance, desiccation tolerance, stored cereal grain, stress tolerance mechanisms.

1. Introduction

The Angoumois grain moth, *Sitotroga cerealella* (Olivier) is a cosmopolitan primary coloniser of cereal grains in warm regions of the world (Hansen et al., 2004; Bushra and Aslam, 2014, Demissie et al., 2014). It is a dominant component of the cereal grain pest complex typical in small-scale farmers' stores along with *Sitophilus* species, *Prostephanus truncatus* (Horn.), *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) (Hansen et al., 2004). It is one of the most problematic pests of sorghum (Mvumi et al., 2003; Mubayiwa et al., 2018) which is the second most important cereal in sub-Saharan Africa (SSA) (Mubayiwa et al., 2018). The high pest status of *S. cerealella* stems from its larval internal kernel feeding habit which minimises contact with insecticides (Bushra and Aslam, 2014). Moreover, this internal feeding has been reported to contribute to insecticide resistance (Bushra and Aslam, 2014). Tunnels made by the larvae result in grain quantitative and qualitative losses, exposure to secondary pests and microbial attack (Akter et al., 2013). In addition, the feeding frass and scales from dead moths reduce the aesthetic value and hence economic value of the grain. In consequence, it is imperative to understand its ecological characteristics specifically abiotic stress responses to enable the development of alternative non-chemical control methods. The current cereal grain storage methods by small-scale farmers in developing countries (Nukenine, 2010; Nyagwaya et al., 2010) promote the unabated proliferation of *S. cerealella*, making it one of the most abundant problem pests especially in small grains (Mvumi et al., 2003; Hansen et al., 2004). However, to-date no study has focussed on the abiotic stress tolerance of *S. cerealella*, especially temperature and desiccation, two environmental stressors mainly used as proxy to determining insects' survival (Kelley 2014) and potential pest status.

Most terrestrial arthropods including insects are vulnerable to extreme temperature stress (Chown and Nicholson, 2004) and water loss due to their high surface area to volume ratios (Gibbs, 1997; Weldon et al., 2013, 2016). Different species have developed different mechanisms to enhance dehydration tolerance to survive low relative humidity environments (Weldon et al., 2013) such as stored grain habitats. We hypothesize that *S. cerealella*'s ability to withstand desiccation and extreme temperatures typical in tropical climates where it is dominant, likely contributes to its enhanced survival and hence, continued increase in pest populations and grain damage. The objectives of the current study were therefore to determine (1) *S. cerealella* heat and cold tolerance in comparison to like species and in relation to prevailing tropical temperatures; (2) determine whether *S. cerealella* desiccation survival is due to high lipid or body water storage or both and how water loss control may contribute to the moth's desiccation survival.

2. Materials and Methods

2.1 Insect rearing and handling

Insects were reared in the Eco-physiological Entomology Laboratory at Botswana International University of Science and Technology. Field-collected moths were placed in sterilized maize grain (-15°C for 14 days followed 7 days of preconditioning at room temperature of 25 ± 1°C and 60± 5% RH) in 35 × 35cm^2 bugdorm insect cages (BugDorm®, MegaView Science Co., Ltd. Taiwan). The bugdorms were placed in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) maintained at 25± 1°C , 10:14 day and night photoperiod, and 65 ± 10% RH. The moths were fed on 10% sugar solution through the dental-wick method (Shelton et al., 2012) and supplied with randomly folded black-dyed filter paper for egg laying and resting. After 7 days, the moths were

removed from the grain leaving grain and filter papers with eggs. F_1 hatched larvae were allowed to feed on the grain until adult stage. Unsexed 2-3 day old F_1 adult moths were used for the experiments. To reduce mortality, moths damage and excessive loss of scales, resting moths were carefully handled by individual trapping into 0.6 ml polypropylene eppendorf tubes with minimum direct contact.

2.2 Body water and lipid content (BWC and BLC)

To determine Body water content (BWC), 50 moths were collected from the bugdorms and weighed (RADWAG®, Wagi Elektroniczine, Model As220.R2, Radom, Poland) to 0.0001 g in 0.6 ml numbered and uniformly perforated polypropylene eppendorf tubes. These were placed on eppendorf-tube-holders and transferred to a laboratory oven (UF160, Memmert GmbH + Co.KG, Germany) set at 60°C for 48 h. Moths were re-weighed after 48 h and the BWC was calculated as the difference between the initial and the final body mass. (Lease and Wolf, 2010)

For Body lipid content (BLC), 50 moths were individually placed in uniformly perforated, pre-weighed and numbered 0.6 ml polypropylene eppendorf tubes. These were dried in an oven (UF160, Memmert GmbH + Co.KG, Germany) at 60°C for 48 h. The moths were immediately weighed after drying to determine dry weight and transferred to unperforated 2ml eppendorf tubes containing 1.5ml diethyl ether. The tubes were gently agitated at 37°C for 24 h before the insects were removed and re-dried in the oven (60°C) for 24 h (Lease and Wolf, 2010). After drying, the moths were weighed and the BLC was calculated as the difference between the initial insect dry mass and the final (lipid-free) dry mass.

2.3. Water loss rates (WLR)

Desiccation tolerance experiments were conducted following standard protocols (Gibbs, 1997; Weldon et al., 2013). After initial mass of the moths were recorded, 50 moths were individually placed in numbered 0.6 ml uniformly perforated polypropylene eppendorf tubes. The tubes were placed in small loose granules of desiccant (Drierite, W.A. Hammond Drierite Co. Ltd, Xenia, USA) with < 10% RH at 25°C in a large airtight laboratory glass bowel. Vials were inspected every 3 h and mortality was recorded.

2.4. Critical thermal limits (CTLs)

Critical thermal limits (CTmin and CTmax) were measured using a protocol developed by Nyamukondiwa and Terblanche (2010). Ten individual moths were placed in a series of test tubes floating in insulated double-jacketed chambers or "organ pipes" connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol and subjected to a constant cooling or heating rate. Moths were first given 10 minutes to equilibrate at 25°C before the temperature was ramped up or down for CTmax or CTmin respectively at a three different rates; 0.12, 0.25 and 0.5°C/min. This was repeated three times to yield sample sizes of n = 30 individuals per treatment. A thermocouple (type T 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into the middle (control) test tube to monitor temperature. In the current study, CTmax and CTmin were defined as the maximum or minimum temperature, respectively; at which each individual moth lost coordinated muscle function, following mild prodding with a thermally inert object (see Nyamukondiwa and Terblanche, 2010).

2.5. Chill coma recovery (CCRT) and heat knockdown time (HKDT)

CCRT and HKDTs were conducted following a method developed by Weldon et al. (2011). For CCRT, moths were individually placed in 0.6 ml eppendorf tubes and then loaded into a large zip-lock bag which was subsequently submerged into a water bath (Systronix, Scientific, South Africa) filled with 1:1 water: propylene glycol mixture, was set at 0°C for 1 hour. After 1 hour of exposure, the tubes

were removed from the water bath and transferred to a Memmert climate chamber set at 25°C, 65% RH for moth recovery. The chamber was connected to a camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) that was linked to a computer where observations were recorded. In this study, CCRT was defined as the time (in minutes) taken by an individual moth to recover and stand upright on its legs. For HKDT, ten moths were individually placed in 0.6 ml eppendorf tubes and placed in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) set at 48 ±1°C and 65±5% RH. Observations were recorded as explained for CCRT. In this study, HKDT was defined as the time (in minutes) at which moths lost activity following exposure to high (48°C) temperature. Both CCRT and HKDT were repeated three times (n = 30 individuals moths).

2.6. Data analysis

Data analyses were carried out in STATISTICA, version 13.2 (Statsoft Inc., Tulsa, Oklahoma). For CTLs, ramping rate was used as a factor in one–way Analysis of Variance (ANOVA) and statistically significant means were separated using Tukey-Kramer's post-hoc test. However, for BWC, WLR, LC, HKDT and CCRT mean values were presented and compared to like species, e.g *Plutella xylostella* (L.) and *Tuta absoluta* (Meyrick).

3. Results

3.1. Body water content, water loss rates, water loss at death and body lipid content

Sitotroga cerealella had a mean body water content (BWC) of 1.80 ±0.517 mg with an average body mass (BM) of 2.61 ±0.691 mg resulting in 70.2% BWC. The water loss rates of 0.056 mg/h (1.81% BW/h) were low, showed that *S. cerealella* would need 19.31 h of exposure to a desiccating environment ($\leq$ 10% RH) to reach its critical body water at death, 35.23% of initial body water. There were no significant correlation ($P > 0.001$) between BWC and thermal traits (CTLs, HKDT and CCRT). The BLC of (9.8± 0.81%), was relatively low compared to BMC indicating that lipids were unlikely used as a source of metabolic water.

3.2 Thermal tolerance (CTmax, CTmin, HKDT and CCRT)

At a benign ramping rate of 0.25°C/min, *S. cerealella* had a CTmax of 46.1±1.04°C which was significantly higher ($F_{(2, 87)} = 83.921$, $P < 0.0001$) than the CTmax at lower ramping rate (0.12°C/min) , 42.8±1.19°C (Fig 1A). However, higher ramping rate (0.5°C/min) did not have a significant effect on CTmax (Fig. 1A).

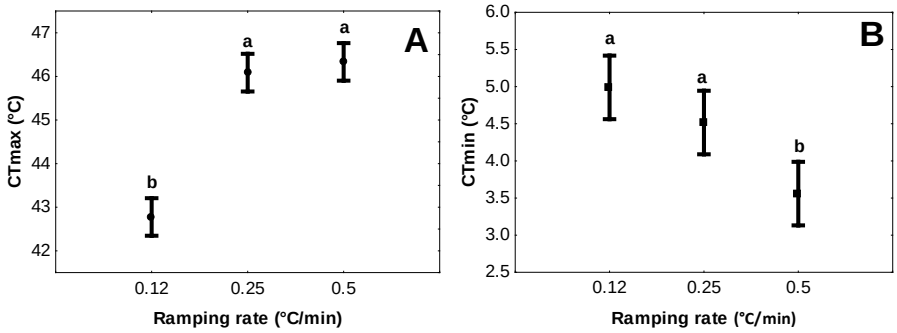

Fig. 1 The critical thermal limits (CTLs) for *S. cerealella* at different ramping rates: (A) critical thermal maxima (CTmax); and (B) critical thermal minima (CTmin).

Similar to CTmax, the CTmin for *S. cerealella* was significantly affected by the ramping rate. At the benign ramping rate of 0.25°C/min, *S. cerealella* showed a CTmin of 4.5±1.06°C, which was significantly higher ($F_{(2, 87)} = 11.443$, $P = 0.0004$) than the CTmin at a higher (0.5°C/min) ramping rate (3.6±0.98°C) (Fig. 1B). However, lower ramping rate (0.12°C/min) did not have a significant effect on CTmin (Fig. 1B).

Tab. 1 Thermal traits of *S. cerealella* compared to like species.

Insect species	CTmax (°C)	CTmin (°C)	HKDT (min)	CCRT (min)	Reference
S. cerealella	46.1±1.04	4.5±1.06	7.9±1.64	5.8±1.17	Current study
Plutella xylostella	46.6±0.52	-3.2±0.41	3.8±0.65	2.48±0.40	Machekano et al., 2018a
Tuta absoluta	44.1±0.43	-5.2±0.23	*	*	Machekano et al., 2018b

*Denotes that data on that thermal trait were not available.

Table 1 shows the comparison of thermal traits between *S. cerealella, P. xylostella and T. absoluta.* The CTmax is comparable to *P. xylostella* and *T. absoluta* (Machekano et al., 2018a & b, upcoming). Compared to other economic Lepidopterans *S. cerealella* shows almost double the time (7.9±1.64 minutes) needed to be knocked down by heat stress (HKDT) compared to *Plutella xylostella* (3.8±0.65 minutes). Its CTmin (4.5±1.06°C) however, was higher (4.5±1.06) than *P. xylostella* (-3.2±0.41°C) and *T. absoluta* (-5.2±0.23°C) (Table 1). In addition, the chill-coma recovery time (CCRT), was almost two-fold that of *P. xylostella* (Table 1). To understand whether *S. cerealella* was likely to cope with prevailing field temperatures, we compared its critical thermal limits to field meteorological data for the 2015-2016 seasons (Fig. 2).

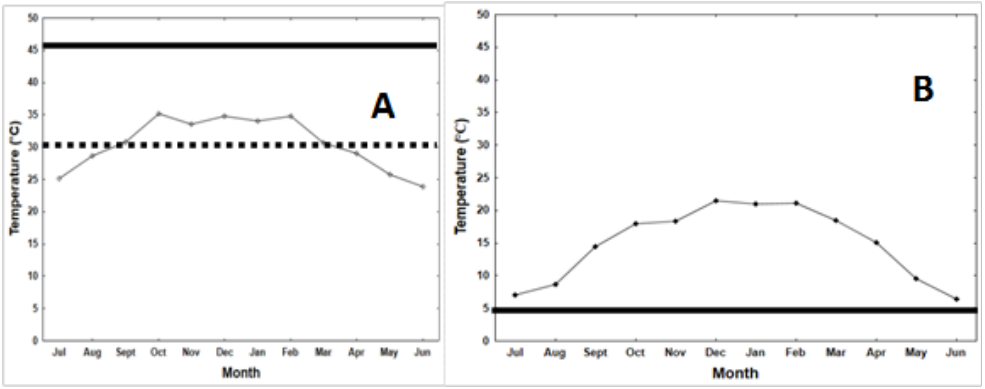

Fig. 2 (A) The mean maximum field temperature data in Botswana (2015-16) (black curved line) compared to *S. crealella* CTmax (black solid line) and optimum temperatures (black dotted line), (B) the mean minimum field temperatures (black curved line) compared to *S. cerealella* CTmin (black solid line).

The difference between the highest recorded temperature (35.2°C) (October, 2015) and *S. cerealella* CTmax was~10°C (Fig. 2A), evidence of a very high thermal safety margin (Deutsch et al., 2008) for *S. cerealella* considering a 30.0°C optimum temperature (Hansen et al., 2004; Demissie et al., 2014). Similarly, the mean field minimum temperatures were well above *S. cerealella*'s CTmin (Fig. 2B), indicating that it is not strained by low temperatures in nature under typical tropical conditions. Like other economic Lepidopterans (Table 1), *S. cerealella* is likely not under thermal physiological stress under current and projected global change. Such adaptations to increasing temperatures may contribute to high pest activity, short generation time and high population growth with potential deleterious effects on stored cereal grains.

4. Discussion

Sitotroga cerealella's high BMC (70.2%) of total body mass, was comparable to desiccation-resistant *Drosophila* species observed by Gibbs and Matzkin (2001). This entailed that *S. cerealella* likely used high body water storage to tolerate low relative humidity, typical of grain environments to survive desiccation. This is so because, high levels of internal body water content extends the time required for dehydration to critical levels that would induce mortality. The observed low water loss rates showed that *S. cerealella* would need 19.31 h of exposure to a desiccating environment to reach its critical body water at death. This suggests that, *S. cerealella* likely uses two mechanisms; high body water and low water loss rates to tolerate desiccation in dry stored-grain habitats. The exact physiological mechanisms used by *S. cerealella* to reduce water loss need further investigation. Lack of significant correlation ($P > 0.001$) between BWC and thermal traits (CTLs, HKDT and CCRT) further suggests that high body water content solely played a significant role in desiccation tolerance. This is supported by the relatively low body lipids (9.8± 0.81%), which explains that lipids were unlikely to be a source of metabolic water but probably energy for this species (Arrese and Soulages, 2010). Demissie et al. (2014) reported that low relative humidity did not have a significant effect on the growth and development of *S. cerealella* except egg hatching, suggesting that apart from the egg stage, all stages of *S. cerealella* are capable of tolerating desiccation.

Our data and previous reports suggest that *S. cerealella* is heat-tolerant. Its CTmax is comparable to the thermally resilient *P. xylostella* (Machekano et al., 2018a) and the invasive *Tuta absoluta* (Machekano et al., 2018b, upcoming). However, on the low temperature scale, *S cerealella* showed compromised CTmin (4.5±1.06°C) compared to like species. The time taken by the moths to recover from chill-coma was almost two-fold that of *P. xylostella*. Both CTmin and CCRT responses suggest limited low temperature tolerance for *S. cerealella*. High temperature tolerance likely explains why it is a major pest in the warm tropical climates especially SSA (Hansen et al., 2014; Bushra and Aslam, 2014) as similarly reported for fruitflies (Nyamukondiwa et al., 2010) and stemborer species (Mutamiswa et al., 2017).

Only low ramping rate had an effect on CTmax, but higher ramping rate (0.5°C/min) did not significantly shift the CTmax. This result suggests the inability of *S. cerealella* to adjust its CTmax in the short-term or rapid heat hardening but only in the long term. Similar to CTmax, the CTmin for *S. cerealella* was significantly affected by the ramping rate. On the low side of the temperature scale, only the higher ramping rate (0.5°C/min) significantly dipressed the CTmin. This result suggests faster ramping rates enhanced low temperature tolerance, measured as CTmin. This plastic effect has been observed in similar Lepidopterans (Machekano et al., 2018a and b) and likely facilitates adaptation to novel but stressful environments (Nyamukondiwa et al., 2010). Compared to other economic Lepidopterans, *S. cerealella* showed two-fold time needed to be knocked down by heat stress compared to *Plutella xylostella* further attesting its thermal resilience on the higher side of the temperature scale.

The difference of ~10°C between the highest recorded field temperature and *S. cerealella* CTmax, is evidence of a very high thermal safety margin (Deutsch et al., 2008) from its 30.0°C optimum temperature (Hansen et al., 2004; Demissie et al., 2014). Similarly, the mean field minimum temperatures were well above *S. cerealella's* CTmin (Fig. 2B), indicating that it is not strained by low temperatures in nature under typical tropical conditions. Like other economic Lepidopterans (Table 1), *S. cerealella* is likely not under thermal physiological stress under current and projected global change. Such adaptations to increasing temperatures may contribute to high pest activity, short generation time and high population growth with potential deleterious effects on stored cereal grains.

We conclude that *S. cerealella* uses high body water storage and low water loss rates to tolerate desiccation in low humidity stored grain habitats. *Sitotroga cerealella* shows high basal heat but not cold tolerance, and coupled with potential plasticity and behavioural regulation, this may aid its survival under abiotic stressful environments. Grain cold treatment may be used as an effective pest control method against *S. cerealella*.

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Suitability of hemp seed for reproduction of stored-product insects

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Extended Abstract

1. Introduction

Hemp, or industrial hemp, is a high value alternative crop that has seen major increases in acreage in Canada since commercial production was legalized in 1998. The term industrial hemp applies to non-psychoactive varieties of *Cannabis sativa* L. There have been reports of insect infestations on stored hemp seed in Manitoba. The moths *Plodia interpunctella* (Hübner) Indianmeal moth, and *Ephestia küehniella* (Zeller) Mediterranean flour moth feed on hemp seed (Hagstrum and Subramanyam, 2009). Our objectives were to determine which stored-product beetles can reproduce on hemp and the effect of dockage and seed moisture content.

2. Materials and Methods

Twenty adult insects were placed on 15 g of hemp seed at two different moisture contents (~8% or ~15%) and two different dockage levels (~0% or~15%) and held at 30^{O}C and 60-70% relative humidity. The number of live and dead insects were counted at 3, 5, 7 and 9 weeks. Only live adults were returned to vials.

3. Results and Discussion

These insect populations increased over the 9 weeks; red flour beetle [*Tribolium castaneum* (Herbst)], drugstore beetle [*Lasioderma serricorne* (F.)] saw-toothed grain beetle [*Oryzaephilus surinamensis* (L.)], warehouse beetle (*Trogoderma variabile* Ballion). These insect populations did not increase: rusty grain beetle [*Cryptolestes ferrugineus* (Stephens)], lesser grain borer [*Rhyzopertha dominica* (F.)], rice weevil [*Sitophilus oryzae* (L.)], flour mill beetle (*Cryptolestes turcicus* (Grouvelle), confused flour beetle (*Tribolium confusum* Jacquelin du Val), cigarette beetle [*Stegobium paniceum* (L.)]. In general, higher dockage led to higher populations. The effect of moisture content was variable.

Keywords: *Cannabis sativa,* reproduction, dockage, moisture

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The use of long-lasting insecticide netting to prevent dispersal of stored product insects

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Abstract

The lesser grain borer, *Rhyzopertha dominica*, and red flour beetle, *Tribolium castaneum*, are two notorious primary and secondary pests of stored products. Extensive research has been done to prevent the establishment and subsequent infestation of the insects in stored product facilities. Long-lasting insecticide netting (LLIN) on mosquitoes has proved effective in controlling the spread of malaria, but little research has been conducted on the LLIN's behavioral effects of stored product insects. In this study, a movement and dispersal assay were performed. In the movement assay, the video-tracking software, Ethovision, recorded the movement of *R. dominica* and *T. castaneum* after 1-10 min exposures to LLIN or control netting and a waiting period of 1 min, 24 hr, 72 hr, or 7 days after netting exposure. In the dispersal assay, *R. dominica* and *T. castaneum* were observed after 5 minutes of exposure to LLIN or control netting to measure the insects' ability to reach new food patches at three different distances. The results from the movement assay showed a significant reduction in horizontal movement and significant increase in angular velocity for beetles exposed to LLINs, indicating that movements were more erratic and less directed. The dispersal assay revealed that exposure to LLIN had a significant effect on the dispersal ability of both *R. dominica* and *T. castaneum* with averages of 0-3 from a group of 20 beetles reaching the new food patch. These results indicate that LLINs can be an effective tool for the prevention of stored product insect establishment and colonization.

Keywords: polyethylene netting, integrated pest management, behavior, sublethal effects, bed nets

1.Introduction

Together, the major three stored grains in the US (corn, soybean, and wheat) alone represent a value of $85.9 billion (NASS, 2018), much of it exported to help feed the world's growing population. The world's population is estimated to reach 9 billion people by 2050 (Godfray et al. 2010), and agricultural output will have to more than double by that point (Ray et al. 2013). Insects are our main competitors for food on the planet, resulting in 10-50% yield loss of products after they have been harvested from the field. The key to many integrated pest management programs (IPM) for stored products is sanitation to prevent infestation by insects (Phillips and Throne, 2010). However, this is often difficult because of the success with which stored product insects can immigrate to new facilities (McKay et al. 2017; Campbell and Arbogast 2004).

In particular, the lesser grain borer, *Rhyzopertha dominica*, and red flour beetle, *Tribolium castaneum*, are two notorious primary and secondary pests of stored products. These represent radically different life histories among stored product insects. *Tribolium castaneum* is a secondary feeder (Hagstrum and Subramanyam 2006), feeding on already broken grain, is a relatively weaker flier, and is mostly confined to facilities and local areas around which grain is processed (Drury et al. 2009; Ridley et al. 2011). By contrast, *R. dominica* is a primary feeder, boring into whole kernels, depositing eggs, and developing inside the grain (Hagstrum and Subramanyam 2006), while also being a strong flier (Edde et al. 2006) and long-distance disperser (Mahroof et al. 2010).

Extensive research has been done to prevent the establishment and subsequent infestation of the insects in stored product facilities. One potential alternative management tactic that has not been evaluated for control of stored product insects is long-lasting insecticide netting (LLIN). Since the 1990s, LLINs have proved effective in reducing mosquito populations to control the spread of malaria (Lengeler 2004; Kitchen et al. 2009; Alonso et al. 1991) and to kill vectors of other arthropod-borne diseases (Dutta et al. 2011). LLINs are constructed such that insecticide moves to the surface of the netting material over time, producing multi-year residual efficacy (Martin et al. 2007). In the past few years, LLINs have been evaluated for their utility in protecting crops before harvest in agriculture. This has included as a kill mechanism in traps for the brown marmorated stink bug (Kuhar et al. 2017; Morrison et al. 2017; Rice et al. 2018). Most recently, LLINs are now being considered for their ability to control post-harvest insects (Scheff et al. 2018; Rumbos et al. 2018). However, one challenge with currently available LLINs is that stored product insects are small enough to pass through the netting material, and it takes extended durations of exposure to elicit outright mortality. As a result, a natural question is whether the netting will have sufficiently pronounced effects on the behavior of stored product insects to prevent their dispersal after contact.

For LLINs to be an effective control measure, they must be compatible with the biology and behavior of stored product insects. Pyrethroids, which are the active ingredient in many LLINs, may have deleterious behavioral side effects in some arthropods, such as repellency (Katz et al. 2008). This would prevent the use of LLINs from effectively intercepting pests as they immigrate to stored product facilities. However, Scheff et al. (2018) importantly found no evidence of long-distance or contact repellency from LLINs against *T. castaneum* and *R. dominica*. Nonetheless, there are several other considerations that must be met for LLINs to be behaviorally compatible with the behavior of stored product pests and be potentially effective as a control tactic. Specifically, LLINs must 1) swiftly decrease the locomotion and result in the loss of coordinated movement by stored product insects, and 2) prevent dispersal to new food patches after brief contact with the material. In this study, a movement and dispersal assay were performed. We employed either a deltamethrin-incorporated polyethylene netting at 0.6% a.i. (ZeroFly, Vestergaard-Frandsen, Inc., Switzerland; LLIN hereafter) or netting with identical physical characteristics but not insecticide (control netting).

2.Materials and Methods

In the movement assay, the video-tracking software, Ethovision, recorded the movement of *R. dominica* and *T. castaneum* for 2 h after 1, 5, or 10 min exposures to LLIN or control netting and a waiting period of 1 min, 24 hr, 72 hr, or 7 days post-exposure (Fig. 1). The movement variables characterized were the total distance moved (cm) by adults and their mean angular velocity (deg/s). In the dispersal assay, *R. dominica* and *T. castaneum* were observed after 5 minutes of exposure to LLIN or control netting to measure the insects' ability to reach new food patches at three different distances (25, 75, or 175 cm) at the conclusion of a 48-h period. These were performed in laboratory spaces and environmental chambers under standardized abiotic conditions (30˚C, 65% RH, 14:10 L:D). The data did not conform to the assumptions of normality, and thus were log-transformed prior to running the final model. Data were analyzed with an ANOVA, and upon a significant result from the model, Tukey's HSD were performed for post-hoc pairwise comparisons. All data was analyzed using the R Software (R Core Team, 2017).

3.Results

In the movement assay (Fig. 1), brief bouts of 1-min exposure to LLINs resulted in the same numbers of affected and dead as longer 10-min exposure to the same nets for both species (ANOVA: *T. castaneum*, $F = 0.073$; df = 2, 404; $P < 0.93$); *R. dominica*, $F = 1.38$; df = 2, 407; $P = 0.25$). Importantly, movement was descreased by 3-fold for both species after exposure to LLINs (*T. castaneum*: $F = 102$; df = 1, 404; $P < 0.0001$; *R. dominica*: $F = 28.2$; df = 1, 407; $P < 0.0001$). There was some recovery of *T. castaneum* at 72 h and 7 d, but not for *R. dominica*. Movement was immediately reduced by half after exposure, and by 24-72 h later, movement was reduced by 4 to 9-fold compared with adults exposed to control netting. The behavioral effects of exposure extended out to 7 d later for both species where movement was still reduced by half or more compared to control netting-exposed adults. Angular velocity was elevated for LLIN-exposed adults compared to those exposed to control netting (*T. castaneum*: $F = 289$; df = 1, 404; $P < 0.0001$; *R. dominica*: $F = 38.1$; df = 1, 407; $P < 0.0001$), though this effect attenuated by 7 d after exposure for *R. dominica*, but not *T. castaneum*.

The dispersal assay (Fig. 2) revealed that exposure to LLIN had a significant effect on the dispersal ability of *T. castaneum* ($F = 2151$; df = 1, 89; $P < 0.0001$) with averages of ~1 from a group of 20 beetles reaching the new food patch after exposure to LLINs, whereas almost the full set of 20 control netting-exposed adults reached the novel food patch. Out of over 1,400 *R. dominica* tested, not a single LLIN-exposed adult reached the novel food resource ($F = 701$; df = 1, 54; $P < 0.0001$). The distance that adults had to disperse did not impact their ability to disperse; the primary determinant was whether they were exposed to netting with insecticide. The dispersal distance did not affect the dispersal capacity of either species for the range of distances tested (*T. castaneum*: $F = 1.59$; df = 2, 89; $P = 0.21$; *R. dominica*: $F = 2.31$; df = 2, 54; $P = 0.11$).

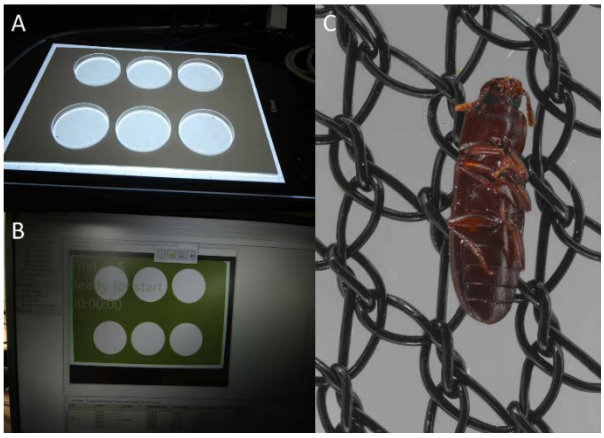

Fig. 1 The movement assay used with A) individual adult *Tribolium castaneum* or *Rhyzopertha dominica* placed in 100 × 15 mm petri dishes, 2) their movement tracked with a video camera and sent to software on a computer, and C) the effect of netting on the mobility of *T. castaneum*. Please note that figures (photographs or graphs) shall be provided in the best possible resolution without frames.

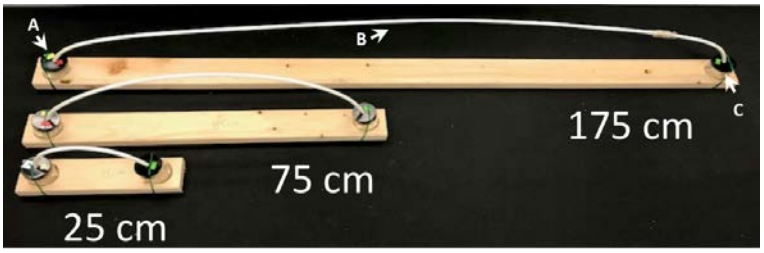

Fig. 2 The dispersal assay that tested the ability of *Tribolium castaneum* and *Rhyzopertha dominica* to move to a novel food resource after a 48 h period, with A) 20 adults introduced in the introduction chamber, B) a single line of twine threaded through the system from the bottom of the introduction chamber to halfway down the jar with the novel food resource so movement was only unidirectional, and C) a dispersal chamber with 15 g of organic, unbleached flour.

4.Discussion

This is the first study to examine, in-depth, the sublethal effects of exposure to LLINs on any stored product insects. We have shown here that even brief exposure times of 1-min are sufficient to induce the same dramatic decreases in movement and increase in disorientation as longer 10-min exposures compared to controls. Exposure to LLINs reduced adult movement of both species by 3 to 4-fold. In addition, a moderate exposure time of 5-min was sufficient to substantially reduce or effectively prevent the dispersal of adult stored product insects, with *R. dominica* the more susceptible of the two species studied. Radically diminished dispersal capacity held steady even after a 2-3 d period during which adult *T. castaneum* or *R. dominica* could have recovered, but did not. As a result, this suggests that while mortality may be initially incomplete after exposure, brief bouts of contact with LLIN are adequate in preventing adults from reaching novel food patches. Overall, these results indicate that LLINs are a promising tool for the prevention of stored product insect establishment and colonization.

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Evaluation of the attractiveness of an organic litter compared to breeding substrate

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Abstract

In a pet shop warehouse, stored food pest insects can develop in various preserved animal feeds (dog's pasta, puffed rice, kibble). However, there is another commodity that is rarely considered, such as the organic litter which is composed of bran, flours and other residues of the screening of corn that may result attractive to the same pest insects. The purpose of this laboratory test was to evaluate the attractiveness of organic litters on *Plodia interpunctella*, *Tribolium confusum*, *Oryzaephilus surinamensis* in comparison with breeding substrate. The results confirmed that the test insects were attracted by the breeding substrate rather than by the organic litter.

Keywords: stored food pest insects, pet food, organic litter, *Plodia interpunctella*, *Tribolium confusum*, *Oryzaephilus surinamensis*.

1. Introduction

The stored food insects can attack several food products, the importance in stored food losses are estimated about 16% (World Bank et al., 2011). Food industries pest are also involved in losses in pet food industries. Roesli et al. (2003) reported that it is possible to record up to thirty insect species belonging to 20 families in four pet stores chain during February to August 2001. The most common and abundant species were *Plodia interpunctella* (Hübner), *Oryzaephilus mercator* (Fauvel), *Tribolium castaneum* (Herbst) and *Sitophilus* spp.

In the pest food stores this attack by stored pest food can cause damage to various preserved animal feeds (dog's pasta, puffed rice, kibble). However, there is another commodity that is rarely considered, such as the organic litter which is composed of bran, flours and other residues of the screening of corn that may result attractive to the same pest insects. In July 2017, a package of dog's pasta found infested by *O. surinamensis* in a pet food store was delivered to LEAA (Laboratory of applied entomology Agroblu). The origin of infestation was investigated, and it was discovered it had developed from a pallet of ecological litter for pets. Thanks to these events, the aims of the present work is to investigate the attractiveness of organic litter applied to *O. surinamensis*, *P. interpunctella* and *T. confusum*. in comparison with a balanced diet, typical of breeding. This test was combined to a development evaluation test of the same species on such substate.

2. Materials and Methods

2.1 Insects

The insects used in the test was provided by the Agroblu Laboratory of Applied Entomology (LEAA: via Isonzo 20, Rozzano- Milano – Italy) where are reared at 26 ± 2 °C 70% RH and photoperiod light darkness 16L:8D. The test organisms used were typical insects infesting food industries and also collected in pet stores. For the test were employed larvae II instar of *Plodia interpunctella* (Hübner), adult stage of *Tribolium confusum* (Jaqcquelin du Val) and *Oryzaephilus surinamensis* (Linnaeus). Table 1 reported the species and stage used for the test.

Tab. 1 Species, stages and substrates used for the test.

Insect	Stage	Quantity	Substrate TNT	Substrate T
P. interpunctella	II instar larvae	20	Honey, glycerin, white flour, semolina, yellow flour, oatmeal, sesame, bran	Organic litter
T. confusum	Adult	20	Semolino, brewer's yeast, bran	Organic litter
O. surinamensis	Adult	20	Honey, glycerin, white flour, semolina, yellow flour, oatmeal, sesame, bran	Organic litter

2.2 Substrate

The test was conducted to compare the attractiveness of a commercial organic litter and a balanced diet normally used for breeding. *P. interpunctella* and *O. surinamensis* were reared on a diet composed as follow honey 15%, glycerin 5%, white flour 20%, semolina 20%, yellow flour 15%, oatmeal 5%, sesame 15%, bran 5%.

T. confusum diet was composed 70% semolina, 29% bran and 1% brewer's yeast.

The composition of the organic litter, vegetable granules, obtained by extracting and drying the fibrous part of the corn's ear, was reported in table 2.

Tab. 2 Composition of organic litter.

Component	Range
Raw ashes	1 – 2%
Raw protein	0,5 – 1,5%
Raw lipidis	0,1 – 1%
Raw fiber	33 – 40%
Extraction inazotati	50 – 60%
Humidity	4 – 10%.

2.3 Y-olfactometer

To test the preference between the two substrates, a plexyglas Y-olfactometer was used. The arms of the structure were 10 cm long and 3 cm wide. At the end of the arms a plastic panel (3x3 cm) was fixed with a 0,5 cm wide hole to allow the air flow created thanks to an extractor fan. To allow the assessment, the Y-olfactometer was fixeded on a rectangular plexiglass panel. On the top of the structure, another rectangular plexiglass panel was placed, featuring a rubber gasket to avoid insects escape.

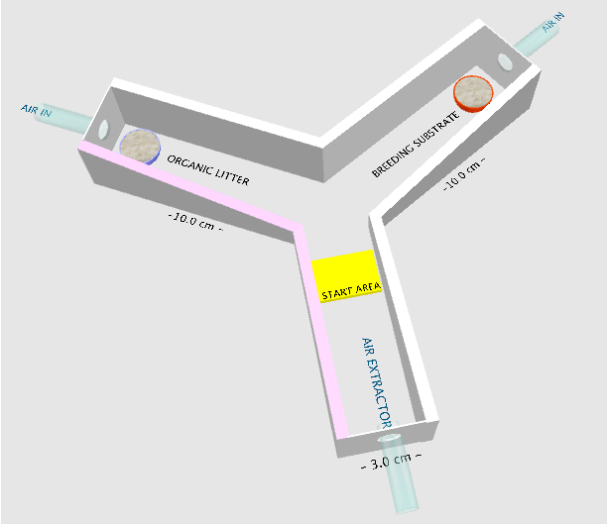

Fig. 1 Scheme of the plexyglass Y-olfactometer setup for the test

2.4 Test System

The Y-olfactometer was set as follow:

- Principal arm: starter point of insect;
- Arm 1: TNT (breeding substrate);
- Arm 2: T (organic litter);

The position of the two alternative substrate was randomized in the replicates. (figure 1).

2.5 Replicates

The test was replicated 20 times.

2.6 Test site

The test was conducted in the Peet Grady Room of LEAA at 26 ± 2 °C 70% RH and photoperiod light darkness 16L:8D.

2.7 Application method

2.7.1 Coleoptera

For *O. surinamensis* and *T. confusum* 50 adult insects were sampled from the breeding and placed in a Petri dish for 72 hours. After this period a single insect was placed in the principal arm of the olfactometer to choose between two alternative substrates.

2.7.2 Lepidoptera

For *P. interpunctella*, 50 larvae at the II instar, high trophic activity development stage, were collected and placed in a petri dish for one hour. The larvae were placed in the principal arms individually to choose between two alternative substrates.

2.8 Evaluation method

2.8.1 Coleoptera

To ensure accurate assessments, the insects were observed up to their choice for 5 minutes, as choices adopted after 3 minutes do not statistically differ from choices taken within the first 3 minute (Wakefield et al. 2004).

2.8.2 Lepidoptera

For *Lepidoptera* the assessment lasted longer because the choice took place in max 25 minutes. The choice of substrate and the time of choice was recorder and compared with t-test.

3. Results

The results showed that all the species taken into consideration, preferred to head towards the substrate typically used for breeding (table 3).

Tab. 3 Percentage of choise between T and TNT.

Test insect	T-Organic litter	TNT-Breeding substrate
Plodia interpunctella	15%	85%
Tribolium confusum	30%	70%
Oryzaephilus surinamensis	20%	80%

The observed mean values were significantly different for $p<0.05$ (t-test) for all the species and stages considered (table 4).

Tab. 4 Mean of insects that have chosen the test substrate (organic litter). Means followed by "*" are significantly different ($p<0,05$) than the response to the control (t-test).

Test insect	T-Organic litter	TNT-Breeding substrate
Plodia interpunctella	0,15*	0,85
Tribolium confusum	0,30*	0,70
Oryzaephilus surinamensis	0,20*	0,80

The mean time of choice of the two alternatives was recorded and compared with t-test. *P. interpunctella* larvae took more time than Coleoptera to choose, the mean time of choise of the preferred substrate for Coleoptera was respectly 3.21 minutes for *T. confusum* and 1.63 minutes for *O. surinamensis*, only the mean choice time of *T. confusum* between organic litter and breeding substrates was statistically significant for $p<0.05$ (t-test) (table 5).

Tab. 5 Mean time used by insects in chosen the test substrate (organic litter). Means followed by "*" are significantly different ($p<0,05$) than the response to the control (t-test).

Test insect	Choice time (min)	
	T-Organic litter	TNT-Breeding substrate
Plodia interpunctella	11,67	12,76
Tribolium confusum	5,00*	3,21
Oryzaephilus surinamensis	1,75	1,63

4. Discussion

The results obtained show that the test insects in front of a choice between a balanced diet substrate and a commercial litter, prefer the first substrate. This result complete and integrate the information available in literature (Phillips *et al*., 1994, TsuJi, 2000, Mowery *et al*., 2002). These data are preliminary and require further investigations on the possible attractiveness of organic litter compared to other commodities stored in pet food shops by other stored food pest or its attractiveness in interaction with other volatile components.

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Evaluation of the difference in the development of stored insect pests on organic litter

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Abstract

On July 2017 in a warehouse of pet food shop in Italy an infestation of *Oryzaephilus surinamensis* was found on a pallet of organic litter, near an infested pallet of dog's pasta. In order to investigate the origin of the infestation, and to support the risk assessment by the pest control operator, one test was conducted at Agroblu Laboratory of Applied Entomology (LEAA) to observe the feasibility of development of *O. surinamensis*, *Plodia interpunctella* and *Tribolium confusum*, in a substrate of 2,5 g of organic litter and to compare it to a balanced diet substrate.

The results showed that only *T. confusum* was able to develop with no statistical difference both on the breeding diet and the organic litter.

Keywords: stored food pest insects, organic litter, *Plodia interpunctella*, *Tribolium confusum*, *Oryzaephilus surinamensis*

1. Introduction

The stored food insects have a high economical importance because of they contribute to the post harvest losses around 16% (World Bank et al., 2011). Food industries pest are also involved in losses in pet food industries. A limited number of surveys were conducted to determine insect species associated with retail grocery, and pet stores, however there are some experience that record the presence in warehouses and in the pet food stores of the common stored pest insects (Loschiavo and Okumura, 1979; Platt et al., 1998, Roeslli et al., 2003). This autor reported that the common species recorder in a pet store were *Plodia interpunctella* (Hübner), *Oryzaephilus mercator* (Fauvel), *Tribolium castaneum* (Herbst) and *Sitophilus* spp.

In a pet store, stored pest food can cause damage to various preserved animal feeds and to another commodity that is rarely considered, such as the increasingly popular organic litter for cats, hamsters, reptiles and amphibia. Organic litter is composed of bran, flours and other residues of the screening of corn that may result attractive to the same pest insects. On July 2017, an infestation by *O. surinamensis* of a package of dog's pasta has been reported in a pet food store. The origin of infestation was investigated and was discovered it had developed from a pallet of ecological litter for pets. Thanks to these events, the aims of the present work is to investigate the development faeseability for *O. surinamensis*, *P. interpunctella* and *T. confusum* on organic litter in comparison with a balanced diet.

2. Materials and Methods

2.1 Insects

The insects used in the test was provided by the Agroblu Laboratory of Applied Entomology (LEAA) placed in Via Isonzo 20, Rozzano Milan, where are rearing at 26 ± 2 °C 70% RH and photoperiod light darkness 16L:8D.

The test organisms used were typical insects infesting food industries and also collected in pet stores.

For the test were employed eggs 72 h laid of *Plodia interpunctella* (Hübner), adult stage of *Tribolium confusum* (Jaqcquelin du Val) and *Oryzaephilus surinamensis* (Linnaeus). Table 1 reported the species and stage used for the test.

Tab. 1 Species, stages and substrates used for the test.

Insect	Stage	Quantity	Substrate TNT	Substrate T
P. interpunctella	Eggs 72 h laid	50	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran	Organic litter
T. confusum	Adult	10 adults	Semolino, brewer's yeast, bran	Organic litter
O. surinamensis	Adult	10 adults	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran	Organic litter

2.1 Substrates

The test was conducted to compare the level of development on organic litter with a substrate normally used for breeding. *P. interpunctella* and *O. surinamensis* were reared on a diet composed as follow honey 15%, glycerin 5%, white flour 20%, semolina 20%, yellow flour 15%, oatmeal 5%, sesame 15%, bran 5%.

T. confusum diet was composed 70% semolina, 29% bran and 1% brewer's yeast.

The composition of the organic litter, vegetable granules, obtained by extracting and drying the fibrous part of the corn's ear, was reported in table 2

Tab. 2 Composition of organic litter.

Component	Range
Raw ashes	1 – 2%
Raw protein	0,5 – 1,5%
Raw lipidis	0,1 – 1%
Raw fiber	33 – 40%
Extraction inazotati	50 – 60%
Moisture	4 – 10%.

2.3 Test unit

The substrates whit the test species were put into small plastic container 6 cm diameter and 6,5 cm high. The container was covered with a plastic twist cap provided with a small hole 1cm diameter, covered with a special filter that avoid the escape of insects and allow the air exchange.

2.4 Test site

The test was conduced in the Peet Grady room of LEAA at 26° ± 2 °C 70% RH and photoperiod light darkness 16L:8D in 4 replicates.

2.5 Application method

2.5.1 Lepidoptera

For the test with *P. interpunctella* the eggs were collected from breeding and examineted at the stereo-microscope to verify their integrity and to exclude the presence of mites. After the check, 8 groups of 50 eggs were sorted each in one plastic container four of which were filled with the balanced diet (TNT) and the other four have been filled with organic litter (T) to give the possibility to the newborn larve to feed.

2.5.2 Coleoptera

For *O. surinamensis,* 8 groups with 10 adults were sorted in 8 plastic containers 4 with balanced diet (TNT) and 4 with test substrate. For *T. confusum* 10 new born larvae per group were arranged as above.

2.6 Evaluation method

After the start of the test, the experimental units were checked every seven days and the development stage of the test species at the time of the assessment was noted. In according to the scale shown in table 3 the qualitative data was converted in number for statistical analysis.

Tab. 3 Table of conversion for data analysis.

Index	Stage achieved (at least one individual)
0	No development
1	Newborn larvae
2	Mature larvae
3	Pupae
4	Adults

The obtained data was statistically elaborated with t-test. For all test species the test was stopped at the appearance of adults.

2. Results

The results showed a difference in development in all the species. Only *T. confusum* showed the ability to complete the life cycle to organic litter successfully. The table 4 below showed the results observed.

Tab. 4 Table of results (mean of four replicates). Means followed by "*" are significantly different (p<0,05) than the response to the control (t-test).

P. interpunctella	***T. confusum***	***O. surinamensis***

	T	TNT	T	TNT	T	TNT
T 1	0	0,25	2	2,5	0	0
T 2	0,25*	1	2,75	2,5	0*	3,25
T 3	0,25*	1,5	3,5	3,75	0*	2,75
T 4	0,5*	2	4	4	0*	2,5
T5	0,5*	3,25	4	4	0,25*	4

P. interpunctella was not able to develop on organic litter, indeed young larvae failed to develop in any replicates. *O. surinamensis* too showed difficulties in develop on organic litter and only one adult emerged by one replicates in all the test.

T. confusum showed a significative difference in development only in the first assessment. The following assessments showed no significant difference to the control.

3. Discussion

These data confirmed and integrated the avaible information in literature about the influence of diet on development of stored pest, with reference to *P. interpunctella* and *O. surinamensis* (Fields *et al.*, 1992; Johnson *et al.,* 1995, Hagstrum and Milliken, 1988; Waldbauer and Bhattacharya, 1973).

With reference to *T. confusum*, this study showed that it was able to complete successfully his development on organic litter. These data are preliminary and require further investigations on the possible development on organic litter by other stored food pest in addition to adjustments to the experimental protocol.

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Unusual cases of product contamination by 'wandering' larvae of the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae)

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ABSTRACT

Upon hatching, the larvae of the Indian meal moth (IMM), *Plodia interpunctella*, disperse vigorously. Within a few hours, they establish themselves on the crevices of food or enter packaged product through small openings and cracks. When on food the larvae intensively feed in or near a tunnel-like case made of frass and silk they web together. The number of larval instars varies from five to seven, depending on temperature, humidity and available food quality. Most mature larvae leave the food medium and search for a suitable place to spin a cocoon in which they pupate or hibernating (diapause). At the end of larval development, the larvae usually chews a hole in a packaging foil, and leave the medium to pupate outside in corners and cracks and also behind

items on walls. Fully grown larvae of the IMM may travel a considerable distance before pupating in a location that is frequently away from the larval food source. It will be proven and illustrated that during this time larvae the IMM may penetrate the packaging material of some household items that were not their food source. Unusual cases of product contamination by 'wandering' larvae will be described. Client claims are thus frequent as only a few larvae in a package with their webbing and frass are very repulsive to homeowners. Impact of product contamination by 'wandering' larvae of the IMM to the firm marketing the products will be discussed.

Key words: Indian meal moth, *Plodia interpunctella*, larva, food products, contamination, client claims

1. Introduction

Females of the Indian meal moth (IMM), *Plodia interpunctella* (Hübner), lay 200-400 eggs singly or in a small batches on food products or near them (Mullen and Arbogast, 1977), sometimes spatially aggregated in some fashion (Arbogast and Mullen, 1978). These eggs are rounded or elongated (0.3 x 0.6 mm) and white, turning orange over time. The larvae (L1) that emerge from these eggs are very small and barely visible to the human eye. They usually disperse vigorously in a search for food, and after detection of the food odor they move into its direction and find food source finally (Sedlacek et al., 1996). Within a few hours, they establish themselves on the crevices of food or enter packaging through cracks and crevices. The larvae eat the stored products in or near a tunnel-like case of frass and silk they web together (Mueller, 2010). Larvae feed greedily on various food products, grow quickly and molt. Fully grown larvae called also the 'wandering larvae' are 9-19 mm in length, with an average of about 1,25 cm. Their color is usually dirty white, but may range from pink to brown to a greenish tinge. The number of larval instars varies from five (Allotey and Goswami, 1990) to seven, depending on temperature and available food (Tzanakakis, 1959).

Thus, the IMM larvae contaminate food products with their presence and webbing containing larval excreta (frass) and exuvia (cast skins). Customers usually find the food product to be infested when larvae grow up and produced a vast amount of webbing.

What is more, the fully grown larvae of the IMM (the wandering larvae) leave the food medium, and they may travel a considerable distance before pupating in a location that is frequently away from the larval food source. Before and during this migration the larvae may penetrate the packaging material of many household foods (Robinson, 1996). Simply, some products (food and non-food) kept in storage are indirectly contaminated by wandering larvae that usually search not for food, but for pupation sites. The presence of larvae within these products causes consumer complaints and rejection of these products.

This paper will prove and illustrate the cases that during search for pupation sites larvae of the IMM may penetrate the packaging material of some household items that were not their food source. Unusual cases of product contamination by 'wandering' larvae will be described. Client claims are thus frequent as only a few larvae with their webbing and frass in a package are very repulsive to homeowners. Therefore, the impact of product contamination by 'wandering' larvae of the IMM to the firm marketing the products will be also discussed.

2. Materials and Methods

Within last 3 years, a company "Trojszyk" Entomological Consulting, Warsaw, Poland, received various products contaminated by pests or sometimes pictures illustrating the pest contamination as customer complaints. These were provided with the orders of manufacturers to determine an insect pest to the species, and to explain each case of product contamination. Among requests there were some cases of unusual contamination of different food and non-food products, and they were accompanied by the repulsive customer claim. These non-typical cases were selected, analyzed and presented in this paper. Cases were illustrated with the pictures, a part of them done by the customers, therefore some of them are of low quality.

3. Results

The typical and unusual cases of collateral contamination product by IMM larvae from another food sources are illustrated and described below.

Case No. 1:

A consumer provided a chocolate bar that was contaminated only with large fecal pellets (frass) and delicate webbing that were produced by the fully grown IMM larvae. No larvae and no other signs of pest activity were noticed.

This chocolate bar was traditionally packed, and the aluminium foil and paper were used as packaging materials. Wrapping materials did not constituted a barrier that prevented the larval invasion. Thus, fully grown larvae visited the product for a short time, and they laid down only fecal pellets and a few threads of silk webbing on the surface of chocolate bar. Probably, a nearby product was infested and it formed a source of wandering larvae.

Fig. 1 Excreta (frass) of the IMM larvae on the surface of the chocolate bar with no other signs of the larval activity.

Case No. 2

Chocolate and nut candies were provided for the evaluation. The thorough investigation of these candies under a microscope revealed that these candies were contaminated only on the external surface of candy wrappers (Fig. 2 & 3). No signs of pest activity were found on the candy surfaces.

A live pupa of the IMM (Fig. 2) and remains of pupal skin (Fig. 3) left by an emerging moth were found in spaces of the wrapper that were suitable for pupation. Silk cocoon was produced during warm months as it was made of delicate silk webbing. The product was contaminated by the fully grown larvae that were interested only to find no food but a proper space for pupation. Some other products (may be other candies of the same lot) were contaminated by the pest, and these products were a source of larvae.

Case No. 3

IMM larva was found under a cap of the bottle with mineral water (Fig. 4). It was fully grown larva (wandering stage) within the cocoon made of delicate silk webbing. Thus, the cocoon was produced during the summer months. Mineral water as a content of the plastic bottle was not contaminated by larva or larval excretes. Possible entries to the space confided by a cap are indicated in Fig. 5 with arrows. This is essentially collateral damage from another food product.

Fig. 2 A live pupa of the IMM within the candy wrappers.

Fig. 3 Remains of pupal skin left by an emerged IMM moth

Case No. 4:

A customer consumed one third of content of the ketchup jar, and on the following day found larva under the jar lid (Fig. 6). The consumer documented this case of product contamination by the picture, and reported a complaint to the manufacturer.

The larva found under the jar lid build already a delicate cocoon, indicating the summer contamination. This larva seems to be freshly pupating, thus it contaminated ketchup jar 1-2 days ago, and it happened in the customer house, not at a premise of the manufacturer nor in the retail food store.

Fig. 4 Larva of the Indian meal moth under the cap of bottle with mineral water.

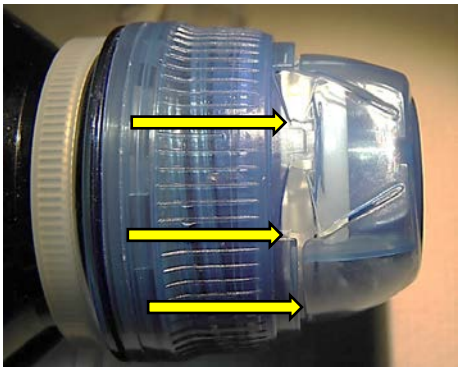

Fig. 5 Possible entries for the larva of the Indian meal moth

Fig. 6 IMM larva in a pupal cocoon found under jar lid.

Fig. 7 Pupa of the IMM in a hibernation cocoon found under lid of jam jar

Case No. 5

The Case No. 5 is similar to the Case No. 4. A pupa of the IMM was found under a lid of the jam jar (Fig. 7) that was freshly bought at the local retail food shop. The pupa was alive, and its body color was deep brown. Pupa was confined within a dense silk cocoon. Pupal stage of the IMM lasts 15-20 days under prevailing room temperatures about 20oC (Sedlacek et al., 1996). Thus, the cocoon was spun during a cold month (made of a dense webbing), and development of pupal stage was advanced (deep brown coloration of pupa). The customer provided the receipt indicating that the jam jar was purchased a few days ago. All these facts prove that the pest contaminated the jam jar at the retail food shop.

Again, the final product was contaminated by wandering larva of the IMM. That larva left the product in which it was feeding and developing, and after searching around for the hiding place larva finally found a proper place under the lid of glass jar, constructed a silk cocoon there and pupated.

Case No. 6

An unusual food contamination by live larvae of the IMM was reported on November 2016 by a customer which opened the originally closed glass jar, and found two moving larvae on the surface of jam (Fig. 8). Label data indicate that jam was manufactured on March 2016. Thus, the product was not infested at the manufacturer site, as it was not possible for IMM larvae to survive more than a half of year on the surface of jam kept in a tightly closed glass jar.

Simply, larvae of the IMM fall down on the surface of jam when a customer removed a lid off the jar, devastating a silk construction of the cocoon that was formed outside of jar, just under its lid.

Fig. 7 Pupa of the IMM in a hibernation cocoon found under lid of jam jar

Fig. 8 Two live larvae of the Indian meal moth on the surface of the final product

Case No. 7

The most unusual case of product contamination is illustrated by Fig. 9. It presents several fully grown larvae of the IMM within the diapers (baby nappies). Packaging of diapers was not insect-proof, and wandering larvae readily penetrated into the bag with diapers. They were not searching for food, but only for a good hiding place for pupation. A nearby product was heavily infested by larvae of the IMM, and it should be removed from a premise as soon as possible to prevent the further spreading of wandering larvae.

Discussion

Indian meal moth (IMM) is a world-wide insect pest of stored products and processed food commodities. Cox and Bell (1991) noted that this moth 'has the widest distribution of all moths generally infesting stored foods and is truly a global pest'. Also, it is one of the most troublesome pests in retail shops or private households. Infestation of the stored food products by IMM can cause a direct product loss and indirect economic costs through pest control costs, quality losses, and considerable amount of consumer complaints.

The cause of consumer complaints are the IMM larvae or pupae in cocoons found within the product. Only one larva or a few larvae in a package with their webbing and frass are very repulsive to homeowners, and very costly to the companies that market the products. Consumer complaints about the presence of insects on or in packaged products can affect the reputation of the brand or manufacturer.

Fig. 8 Two live larvae of the Indian meal moth on the surface of the final product

Fig. 9 The wandering larvae of the IMM found a good hiding place for pupation within the diapers (nappies).

When full grown, IMM larvae usually leave by the hole chewed in packaging material of the product and pupate in a suitable place outside the package (Robinson, 1996). They then emerge from their food source and can travel some distance before spinning their cocoons in various crevices or at wall/ceiling junctions. Pupation usually occurs not only in the vicinity of their food (Mueller, 2010), but also away from their food source. Some larvae spin their cocoons in the food medium just below the surface, but cracks, crevices or other protected places, typically in dark locations, are preferred by the others. One infested package of product in a store can be a source of larvae that search out other products usually to pupate on the surface or interior spaces of the other packages. Thus, a collateral contamination with pest from another food products should be considered (Fig. 1-9). Therefore, control treatment with insecticides should be followed by the advanced inspection including a search for pupal cocoons in corners and cracks and even … behind items on walls. All food and non-food product must to be checked, even those perfectly sealed packages that contain non-food for the IMM larvae (Fig. 6 & 7) as well as the diapers (baby nappies) (Fig. 9).

Cocoons spun by the pupating larvae can be differentiated from those made by the hibernating or diapausing larvae. The hibernaculum (i.e., hibernation cocoon) is dense and completely closed (Fig. 7), whereas the pupal cocoon is flimsy, loose fitting, tapering, and opens anteriorly to permit exit of the adult (Fig. 6). Following hibernation, the larva opens a hole in the hibernaculum and either spins its pupal cocoon inside, or comes out and constructs the pupal cocoon nearby. It appears that larvae spin pupal cocoons outside the hibernaculum only when the hibernaculum is not large enough to include the pupal cocoon (Sedlacek et al., 1996). The differentiation between the pupal cocoons and dense hibernation cocoons should be always conducted when we want to explain the case of product contamination by IMM. For example, when a product was produced in February and some

pupal cocoons were found in August, then one may conclude that the product was not contaminated at the manufacturer site.

Diapause provides a means for the species to overwinter or survive periods of adverse environmental conditions at higher latitudes in unheated situations. The extent to which different strains diapause varies greatly, and those from the tropics or long reared in laboratories showing a reduced capacity. Diapause induced in response to short photoperiods (Bell, 1976), low temperature, or high population pressure (Tsuji, 1963) may greatly extend the developmental periods. At the limits of its range, IMM may have only one to two generations per year, but as many as eight generations per year may occur in warmer climates (Tzanakakis, 1959; Stratil & Reichmuth, 1984). Therefore, the use of the larval developmental time under the prevailing room temperatures is cumbersome to determine the moment of the product contamination by the IMM larvae. Only during warm months it is possible to indicate the time (and place) of product contamination when live larvae of the IMM or live pupae in the pupal cocoons are found within the infested product.

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Susceptibility of dried berries to infestation by *Plodia interpunctella* (Lepidoptera: Pyralidae) in correlation with total sugar content

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Abstract

By assessing the degree of resistance of stored products to infestation by insect pests and correlating it with physical, chemical and nutritional characteristics of products, we could gain a real insight in these pests feeding preferences, and consequently in their biology and ecology. The aim of this study was to assess the degree of resistance of five dried berry species (strawberry, raspberry, blackberry, black chokeberry and cranberry) to infestation caused by the major pest of dried berries, *Plodia interpunctella*. Susceptibility was rated based on the Index of susceptibility (IS) for insect development and the Susceptibility rating. Dried cranberries were

absolutely resistant to infestation by *P. interpunctella* (IS = 0) - no larvae reached the adult stage. Four other dried berry species were also resistant (IS ranged 2.01 – 2.44). In other words, dried cranberries are very unsuitable food for *P. interpunctella*, while other four tested species were slightly more suitable. The content of total sugars in dried berries varied from 24.2% (black chokeberry) to 72.8% (strawberry), but important correlation between IS and total sugar content was not found. By analysing feeding preferences of *P. interpunctella*, we can undertake different pest-management strategies for protection of stored dried fruits.

Keywords: Indian meal moth, dried fruits, infestation, index of susceptibility, susceptibility rating.

Introduction

During all stages of storage process and in all types of storages, dried fruits could be infested by different stored product insect pests like *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Tribolium castaneum* (Herbst) and *Oryzaephilus* spp. Periodically, some polyphagous moths, beetles and mites could also be found (Simmons and Nelson, 1975; Hagstrum and Subramanyam, 2009; Johnson et al., 2009, Almaši and Poslončec, 2010). The most important pest of dried fruits is Indian meal moth, *P. interpunctella* (Johnson et al., 2009), which larvae eat the inside and out of the fruit and cover it with excrements and silk, making it unusable for human diet (Burks and Johnson, 2012). In other words, the biggest losses are in the quality of goods. This moth is of the greatest concern for dried fruits processors (Burks and Johnson, 2012), although studies report that it develops poorly on dried fruits in the laboratory (Arbogast et al., 2005).

In Serbia, the most commonly used dried fruits are prunes, raisins, dried figs and apricots. Recently, different types of dried berries are becoming more popular in human diet and production of dried berries is increasing (Statistical office of the Republic of Serbia, 2018). There are no published data about losses and damages in dried berries caused by *P. interpunctella*. But still, in personal communication with a lot of small producers in Central Serbia, it is emphasized that *P. interpunctella* makes a lot of damages in storages of dried fruits and berries. Besides fumigant control with sulfuryl fluoride and phosphine, the most important methods in control of this pest are sanitation, pest exclusion, sanitary facility design and environmental conditions in storages, especially temperature control (Heaps, 2012).

Dried fruits and berries also have their own susceptibility to infestation by *P. interpunctella*. It depends on the type of fruit, particularly mesocarp density and structure, and also on its nutritional quality and level of moisture. Dried fruits contain > 10% of moisture, which makes them very suitable substrate for the development of this pest. If dried fruits contained < 10% of moisture, it would be more resistant to infestation, but would also be unattractive to human consumers (Sood, 2011). Besides water, dried fruits contain a lot of sugars, commonly > 30% (Cvetković et al., 2009). Proteins and fats, which are very important for insect development, are found in very small amount in dried fruits. Therefore, we hypothesize that the total sugar content could be an important factor that influences the suitability of dried fruits and berries to development of *P. interpunctella*. Based on this hypothesis, the aim of this study was to assess the degree of resistance of five dried berry species (strawberry, raspberry, blackberry, black chokeberry and cranberry) to infestation caused by *P. interpunctella* in correlation with total sugar content.

Materials and Methods

Parental *P. interpunctella* population used in this study was reared for ~50 generations in the Laboratory of General and Applied Entomology, Faculty of Science, University of Kragujevac, Serbia. The population was reared in climate chamber (27 ± 1°C, R.H. 60 ± 10% and photoperiod 14:10 (L:D)), in transparent plastic containers (1.2L in volume) and fed on standard laboratory diet (Silhacek and Miller, 1972). About 100 one-day-old moths *in copuli* were transferred from rearing containers to oviposition jars and one-day-old eggs were used in assays.

Suitability of five dried berry species commonly used in Serbia were tested as a nutrient medium for *P. interpunctella* larvae: strawberry (*Fragaria* × *ananassa* Duchesne), raspberry (*Rubus idaeus* L.), blackberry (*Rubus fruticosus* L.), black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) and cranberry

(*Vaccinium macrocarpon* Aiton), all bought in a local market. 100 mL of each dried berry species were measured and placed into separate glass jars (250 mL in volume). Assays were repeated 12 times for each dried berry species, with a total of 60 replications (jars). In each jar, 50 *P. interpunctella* eggs were added. Jars were then sealed with cotton swab, coated with cotton cloth, for proper aeration. The experiment was carried out in the same environment conditions as described for the rearing of the parental moth population.

Once the emergence of adults began, jars were checked every 24 h and the number of emerged adults and mean developmental duration (MDD) for each adult were recorded. The mean developmental duration was calculated as the average time (in days) from the start of the experiment to the emergence of each adult.

The degree of resistance of five dried berry species to infestation caused by *P. interpunctella* was calculated based on the Index of susceptibility (IS) for insect development (Dobie, 1974)

$$IS = \frac{(\ln(F_1))}{D} \quad 100$$

where F1 represents the mean number of *P. interpunctella* adults that emerged in twelve replications during the experimental period, while D represents MDD. Susceptibility rating (SR) was based on the calculated Indices of susceptibility as suggested by Mensah (1986):

- IS value 0.0 – 2.5 = resistant;
- IS value 2.6 – 5.0 = moderately resistant;
- IS value 5.1 – 7.5 = moderately susceptible;
- IS value 7.6 – 10.0 = susceptible;
- IS > 10 = highly susceptible.

The analyses of total sugar content in dried berries were conducted in Accredited Laboratory (ISO/IEC 17025:2005) of the Center of Hygiene and Human Ecology, Institute of Public Health Kragujevac, Serbia. Total sugar content was determined according to the Luff-Schoorl method for determination of total sugars after inversion, as described in Anonymous (1983). The results are expressed as mass percentage of the sample. Each value was measured three times and averaged with standard error.

Data were statistically analysed using IBM SPSS Statistics 21 software package. Means of Indices of susceptibility and the total sugar content were compared using the Bonferroni test ($p < 0.05$). Correlation between the Indices of susceptibility and the total sugars content was calculated using Pearson coefficient of correlation.

Results

Indices of susceptibility and susceptibility ratings of five analysed dried berry species are presented in Tab. 1. Cranberries were absolutely resistant to infestation by *P. interpunctella* (IS = 0.00). In this assay, one week after the beginning of the experiment, all larvae died. Four other dried berry species were also resistant to infestation by *P. interpunctella*, with higher IS values, ranging from 2.01 (black chokeberry) to 2.44 (blackberry). Index of susceptibility of cranberry was significantly lower than those of four other tested dried berry species ($p < 0.0005$). There were no significant differences established among any of four other dried berry species ($p = 1.0$).

The results of the total sugar content in five dried berry species are presented in Tab. 1. Dried strawberry had the largest content of total sugar (72.8%), while dried black chokeberry had the lowest (24.2%). Correlation between IS and the total sugar content was negative and weak, statistically insignificant ($r = -0.230$; $p = 0.709$).

Tab. 1 Mean values of Index of susceptibility (± SE), susceptibility rating and the total sugar content (%) of five dried berry species to infestation by *Plodia interpunctella*

Dried berry	Index of susceptibility (IS)	Susceptibility rating (SR)	Total sugar content (%)
Strawberry	2.26 ± 0.22 [a]	Resistant	72.80 ± 1.56 [a]
Raspberry	2.37 ± 0.41 [a]	Resistant	51.43 ± 0.80 [d]
Blackberry	2.44 ± 0.36 [a]	Resistant	60.40 ± 0.36 [c]
Black chokeberry	2.01 ± 0.18 [a]	Resistant	24.20 ± 0.52 [e]
Cranberry	0.00 ± 0.00 [b]	Resistant	66.74 ± 0.26 [b]

Vertical mean values of Index of susceptibility and total sugar content having different letters in superscript are statistically different by one-way ANOVA test and Bonferroni test at $p < 0.05$.

Discussion

Due to our knowledge, there are no data about life history of *P. interpunctella* on dried berries, although this moth is one of the most important pests of dried fruits in the world (Hagstrum and Subramanyam, 2009; Sarwar, 2015).

Values of indices of susceptibility and resistance of five tested dried berry species to infestation by *P. interpunctella* could be attributed to their nutritional and moisture content. A few studies showed that nutritional content is of primary importance for successful development of *P. interpunctella*, while moisture content is of secondary importance (LeCato, 1976; Sambaraju and Phillips, 2008; Burks and Johnson, 2012; Predojević et al., 2017). In this experiment, we hypothesized that total sugar content, as major nutrient in dried fruits, could be an important factor that influences the susceptibility of dried berries to infestation by *P. interpunctella*. Our result showed that total sugar content, if used alone as parameter and tested only at five dried berry species, did not show its influence. Sugars are very important for insect development, not alone, but in combination with other nutrients. For example, Arbogast et al. (2005) reported that *P. interpunctella* fails to develop on raisins in the laboratory, while in storage it completes development, thanks to the fungal presence, because the conidia of fungi supports neonate larval development.

In this experiment, five tested dried berry species were resistant to infestation by *P. interpunctella*. Dried cranberry was the most resistant. Value of IS for cranberry was 0.00, because seven days after the beginning of the experiment all larvae were dead. Values of IS of four other tested dried berry species were higher, especially for blackberry (2.44). These results indicate that damages of *P. interpunctella* to tested dried berries were small, but important for the quality of goods, because it makes them much less desirable for human consumption.

Numerous studies showed that some dried fruits are not as suitable for development of *P. interpunctella* as some other types of food (like nuts, maize etc.), especially in laboratory conditions, but still, it is the most important pest of dried fruits. Johnson et al. (1995) reported that prunes are unsuitable food for *P. interpunctella*, because only 0.7% of individuals emerged as adults and MDD lasted between 80 and 160 days. Studies of Johnson (2004), Sambaraju and Phillips (2008), Almaši and Poslončec (2010) and Vukajlović et al. (2017) also indicated that development of *P. interpunctella* on prunes lasts very long, while the number of survived individuals is low. Similar results were published for dried apricots (Almaši and Poslončec, 2010; Vukajlović et al., 2017) and dried cherries (Vukajlović et al., 2017). Recent study showed that dried apricots, prunes and cherries were resistant to infestation by *P. interpunctella*, with IS valued 0.00, 0.78, 1.01, respectively (Vukajlović et al., 2017).

By assessing the degree of resistance of different dried fruits to infestation by *P. interpunctella* and correlating it with physical, chemical and nutritional characteristics of dried fruits, we could gain a real insight in this pest feeding preferences, and consequently in its biology and ecology. On the other hand, by knowing these facts, we can undertake different pest management strategies for protection of stored dried fruits.

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Behaviour of the Angoumois grain moth (*Sitotroga cerealella* Oliv.) in different grain substrates and assessment of losses

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Abstract

The Angoumois grain moth, *Sitotroga cerealella*, is a primary stored grain pest, which development occurs within a single grain. The respond of the pest to various offered grain substrates was studied in no choice laboratory experiment (temperature 27±1ºC; relative humidity 60-80%), by rearing moth populations on entire grains (corn, wheat, barley, sorghum, millet, tall fescue and Kentucky bluegrass) and mechanically damaged grains (corn in fractions with/without embryo, polished rice). The pest behaviour was determined by observation of the entrance and exit hole position on different grains. The food consumption was estimated after adult emergence, by measuring of mass losses of infested grains. Mass losses were correlated with quantitative and qualitative grain parameters. The development was successfully accomplished in all grain substrates, except in Kentucky bluegrass. Strategies of larval penetration and exit hole position depended on morphological properties of grains. As a rule, the development of an individual was completed in a single grain, but in polished rice the transfer from one to another grain was observed. The highest loss of infested grain was recorded in corn grains (55.48 mg), the lowest in tall fescue grains (2.40 mg). Positive correlations were detected between the mass losses and protein, lipid and sugar content, negative in relation to cellulose and ash content.

Keywords: grain, Poaceae, *Sitotroga cerealella*, behaviour, losses.

Introduction

The Angoumois grain moth (AGM), *Sitotroga cerealella* (Olivier), is worldwide distributed primary stored grain pest. In tropical, subtropical and temperate regions with warmer climate it can also affect cereals in the field (Vukasović, 1940). The complete development of an individual generally occurs inside the kernel and therefore the survival directly depends on quantitative and qualitative properties of the available food resources, provided by a single infested kernel. The females of *S. cerealella* lay the eggs by attaching them to the grain surface, being stimulated by mechanical contact of the abdomen with the tight interspaces between the kernels (thigmotactism). After hatching, the larva penetrates the kernel and once inside it continues to feed and develop. Before the pupation, the mature larva extends its feeding chamber to the outside of the kernel, leaving intact only the epidermis of the bran, a symptom of infestation that is visible from outside as a circular transparent "window". After emerging, the adult pushes the "window" and leaves a small characteristic round exit hole. The development and survival of an individual is strongly directly depending on the available food resources, which are determined and limited (quantitatively and qualitatively) by a single inhabited kernel, by itself.

The objective of the present study was to evaluate the convenience of different cereal species/types of grains (whole grains or mechanically modified grain kernels) as feed for the AGM by comparing penetration modes of neonate larvae in different offered grain types, positions of adult exit holes, as well as by evaluation of the final results of infestation: number of emerged adults, mass losses of overall infested grain substrates and single infested kernels, as well as to determine on which extent the survival of AGM and consecutive losses depend on quantitative and qualitatative properties of grains.

Materials and Methods

The respond of the pest to various offered grain substrates was studied in no choice laboratory experiment in controlled conditions of temperature (27±1ºC), relative humidity (60-80%) and photoperiod (16h/8h light/dark), by rearing AGM populations on grains of different Poaceae plant species, including whole and mechanically damaged kernels of the following plant species:

- Corn, *Zea mays* L. (NS SC 444), whole grains
- Corn, *Zea mays* L. (NS SC 444), fraction with embryo
- Corn, *Zea mays* L. (NS SC 444), fraction without embryo
- Wheat, *Triticum vulgare* Host (Balkan), whole grains
- Barley, *Hordeum sativum* J. (NS 27), whole grains
- Rice, *Oryza sativa* L. (population), polished (white) grains

- Sorghum, *Sorghum vulgare* Pers. (NS-šećerac), whole grains
- Millet, *Panicum mileaceum* L. (population), whole grains
- Tall fescue, *Festuca arundinacea* Schreb. (NS- visoki vijuk), whole grains
- Kentucky bluegrass, *Poa pratensis* L. (population), whole grains.

The two fractions of corn grains were obtained by cutting each grain transversally at the line approximately corresponding to the middle grain length.

Eggs of the same age, with ongoing embryogenesis confirmed by the detection of the formed larva under the transparent chorion, were transferred to plastic jars (500 ml), filled with a grain substrate (10 different substrates, 4 replications, 100 eggs per unit). The successful hatching was confirmed by observations of empty transparent chorions.

Each of the 40 experimental units contained 37.5 g of a grain substrate, equivalent to the average weight of 100 undamaged corn grains, which as being the largest grain species could hypothetically assure the development of at least 1 larva/grain.

Quantitative and qualitative parameters of each grain species/type regarded: the measurement of the mass of uninfested kernels (with precision of 0.01 g), determination of sugar content (mg/g of dry matter), protein, lipid, cellulose and ash content (% of dry matter) and energy value (J/g) of each grain substrate before exposition to AGM. Standard methodology was applied for determination of protein content (Kjeldhal method), lipid content (Soxlet method) and cellulose content (Sharner-Kurschuer method), while the ash content was determined by heating at 550°C. Sugar content was determined by the method of liquid chromatography (VAN RIEL AND OLIEMAN, 1989) and the energy value by the method of calorimetry described in KRAJCOVIC AND REGAL (1976).

Emerged adults were extracted from the jars on daily basis and the total number of emerged adults was recorded in each experimental unit. The termination of the eclosion was assured by absence of newly hatched adults in the period of 14 days after the last recorded adult emergence.

The pest behaviour in relation to different offered grain species/type was determined by visual observation of the mode of penetration of neonate larva into akernel (i.e. position of entrance holes), as well as by the position of the exit holes of the emerged adults. Determination of infested kernels was conducted under magnification of binocular microscope Wild M400.

After the termination of adult eclosion, the measurements of the mass of infested substrates (expressed in grams) and single infested kernels (in milligrams; up to 50 kernels with a single exit hole/unit) were conducted in each experimental unit and compared with the related values obtained before the infestation in order to evaluate the mass losses. Average mass loss of an infested kernel (expressed in mg and %) represented directly the total feed consumption (i.e damage) of a single individual in larval stage that successfully developed and survived until adult emergence. Furthermore, the obtained data on mass losses of infested grain substrates and number of emerged adults served for calculation of average mass losses per survived individual (adult). Comparison of the results for each obtained quantitative parameter (number of adults, mass losses of grain substrate, mass losses of grain substrate/adult and mass losses of infested kernel) was conducted by Duncan Multiple Range Test for significance level of $p = 0.05$.

Finally, the impact of qualitative grain parameters to the expression of the number of emerged AGM adults and to the mass loss parameters (mass losses of grain substrate, substrate/adult and infested kernels) were identified by determination of correlation coefficients and the level of their significance. The statistical analyses were conducted in Statistica 13.2 software (Dell Inc. Dell Statistica (data analysis software system) version 13. 2016. (http://software.dell.com).

Results

The development of AGM was successfully accomplished in all tested grain substrates (with whole and mechanicaly damaged kernels), except in Kentucky bluegrass. Neonate larvae were not able to bore the entrance hole in Kentucky bluegrass, probably because of the structure of the husk. Indeed, during the carefully observation of the behaviour of neonates on Kentucky bluegrass grains under

the microscope magnification, it was remarked that the trichomes on the husk represent the mechanical barreier limiting the larval movement and causing letal injuries to larval body. Thus, Kentucky bluegrass may not be considered by us as a host plant species.

Observations of exit holes on infested kernels of other grain species/types demonstrated that usually only one AGM individual can complete the development in a single kernel. An exception was recorded in the substrate with whole corn kernels, where appart the most frequent combination of one individual per single kernel, also two AGM individuals could inhabit the same kernel and successfuly accomplish the development.

Entrance and exit holes

The position of the entrance hole of newly hatched larvae (neonate) on the kernel surface of the offered grain species/type indicated that larvae perform different strategies of penetration depending on grain the properties. In whole, undamaged grains (corn, barley, wheat, sorgum, millet, tall fescue), as a rule, the entrance holes were detected in the zone of the germ (embryo). In such cases, the exit holes were always located at the opposite end (latero-terminally), indicating that during the feeding a larva is following the direction of the longitudinal axis of the grain. Moreover, in some of the infested wheat kernels, the entrance hole was also detected on the dorsal side, in the zone of endosperm and in such cases the exit hole was located at the oposite, ventral side of the kernel. In millet grains, covered by tightly adhering husk (*palea* and *lemma*), which represents a hard, insuperable mechanical barrier, the precise location of the entrance hole coincided with the micropyle, as a naturaly present opening. In tall fescue, the larvae were able to bore the entrance hole through the husk covering at the ventral side of the kernel, whether in the zone of the germ (down below or through the *rachila*) or in the zone of endosperm, and the exit holes were detected on the terminal side of the kernel, exclusively. The hull present on barley kernels did not represent any kind of barrier for larval penetration in the germ zone.

The position of the entrance hole in the zone of the germ was also detected in mechanicaly alterated corn kernels containing the germ, and in this case the the exit hole was also located on the oposite side laterally, but never directly on in the transfersal cut.
In mechanicaly damaged corn kernels without the germ (fraction without embryo), the penetration of a larva occured in the zone of the transfersal cut, through the space left after the removal of the germ. The exit holes in this grain type were always located on the latero-terminal, intact part of the grain, as in the case of the entire corn grains.
Finally, polished white rice (free of husk and bran), the entrance hole of AGM was found on different parts of the kernel, with no particular rule, but in this substrate type the transfer of the larva from one to another kernel was remarked, suggesting that the food resource obtained by a single grain is not satisfying the food requirements of a larva to complete the development. After consuming the most of the single kernel, a larva was able to pass to the next one in order to continue the feeding. The transfer from one kernel to another occurred within the silky tunnel produced by the larva, which served as protective „bridge“ between the kernels. In some cases few (up to 6) kernels were connected with silky threads and some of them were damaged only superficially. The pupation occured whether inside or outside the kernel in the silky cocoon.

Losses

The suitability of different offered grain species/types for AGM was estimated by the number of emerged adults, mass losses of the grain substrates, including the mass losses calculated per emerged AGM individual (Tab.1) and mass losses of infested kernels (Tab. 2).

As demonstrated in Tab. 1 highly significant differences ($p<0.01$) in mass losses of grain substrates, number of emerged adults and estimated consumed mass of substrate per emerged (i.e. survived) individual were recorded among different grain species/type.

Tab. 1 Mass losses of different grain substrates caused by *Sitotroga cerealella* infestation and estimation of the individual consumption based on the number on emerged adults

Grain substrate	Mass losses of grain substrate*			Number of emerged adults**		Consumed mass of substrate per individual	
	Mean (g)	Sd	Mean (%)	Mean	Sd	Mean (mg)	Sd
Corn	4.86 a	0.59	12.97 a	79.75 b	2.75	60.99 a	7.24
Corn fraction without embryo	4.16 b	0.09	11.09 b	68.75 c	6.75	60.82 a	4.80
Corn fraction with embryo	4.24 b	0.13	11.30 b	88.25 a	4.57	48.11 b	2.76
Barley	2.71 c	0.19	7.22 c	86.75 ab	9.78	31.33 c	1.49
Wheat	2.05 d	0.08	5.46 d	84.00 ab	5.72	24.42 d	1.10
Rice-polished	0.94 e	0.08	2.49 e	19,50 d	1.91	48.08 b	2.96
Sorghum	0.97 e	0.04	2.59 e	70.00 c	4.24	13.93 e	0.97
Millet	0.16 f	0.03	0.42 f	19.25 d	2.87	8.17 f	0.27
Tall fescue	0.09 f	0.01	0.25 g	21.00 d	2.45	4.41 f	0.27
Kentucky bluegrass	0.00 f	0.00	0.00 h	0.00 e	0.00	.	.
Analysis of variance F	337.40		863.61	199.31		181.44	
Analysis of variance p	<0,01		<0,01	<0,01		<0,01	

*Each experimental unit initially contained 37.5g of grain substrate

**Each experimental unit was initially infested with 100 individuals in egg stage

Mean values labeled with the same letters are not significantly different according Duncan Multiple Range Test for significance level of p = 0.05. In the process of statistical analyses the % values were transformed in arcsin √%

The calculation of mass losses of the whole substrates took in account the losses caused by entire population of hatched larvae which consumed the feed, including also those that eventually died during the development. The highest mass loss was recorded in substrate with entire corn kernels (4.86 g; 12,97%), followed by corn in fractions without and with embryo, barley, wheat, sorghum and polished rice, millet and finally tall fescue with the lowest registered mass loss (0.09 g; 0.25%). In the substrate with Kentucky bluegrass, neither emerged adults nor mass losses were recorded. The highest average number of emerged adults was recorded in substrates with corn fraction with embryo, barley and wheat (88.25, 86.75 and 84.00, respectively), followed by the substrate with entire corn grains (79.75), which was not significantly different from the number of adults recorded in barley and wheat. Significantly lower number of adults were recorded in sorghum and corn fraction without embryo (70 and 68.75), the lowest in tall fescue and millet (21 and 19.25, respectively).

Another general picture of the infestation consequences in each substrate is demonstrated by estimation of the consumed mass of the substrate per emerged AGM individual (Tab.1). Here, the ordination of the substrates following the decreasing values is similar as in the case of comparison of mass losses of the substrate, with the highest consumption/AGM individual recorded in the case of corn entire grain and fraction without embryo (60.99 mg and 60.82 mg, respectively), the lowest in the cases of millet and tall fescue (8.17 mg and 4.41 mg, respectively). Surprisingly high consumption of grain supstrate per AGM individual was recorded in polished rice (48.08 mg) where low number of adults emerged. The estimated individual consumption in rice was not significantly different from the value recorded in the population reared in corn fraction with embryo (48.11 mg), where high number of adults emerged. In order to survive larvae of AGM reared in polished rice, had to consume as high quantity of feed as in corn fraction with embryo and therefore the consumption of more rice grains was required and obtained by the transfer from one rice kernel to another.

The most precise quantification of the larval damage was provided by calculation of the mass losses of single infested kernels (Tab.2), that were determined before the measurement by the presence of both entrance and exit holes. The average mass of a kernel before and after the infestation, as well as the resulting average mass loss of single infested kernel were significantly different depending on the grain species/type. All offered grain species/types had significantly different mass before infestation, with the highest value recorded in corn with entire kernel (375.03 mg), the lowest in Kentucky bluegrass (0.40 mg). As previosly mentioned, in Kentucky bluegrass the infestation of

the kernel was not observed and no losses in substrate mass were recorded. Therefore, within the serial of tested species/types recognized to host AGM, the lowest kernel mass that provides sufficient food resources for the successful development of AGM was recorded in tall fescue (2.80mg).

Tab. 2 Mass losses of single infested kernels of different grain species/type caused by *Sitotroga cerealella*

Grain species/type		Mass of a single kernel				Loss of a single kernel mass		
		Before infestation		After adult emergence		Mean (mg)	Sd	Mean (%)
		Mean (mg)	Sd	Mean (mg)	Sd			
Corn		375.03 a	0,29	319.55 a	7,67	55.48 a	7.61	14.75 a
Corn fraction without embryo		227.30 b	2,12	175.00 b	1,40	52.30 a	1.67	23.01 b
Corn fraction with embryo		143.28 c	0,79	95.60 c	2,51	47.68 b	2.67	33.26 c
Barley		51.43 d	0,17	21.90 e	0,68	29.53 c	0.66	57.41 f
Wheat		50.10 e	0,08	26.60 d	0.33	23.50 d	0.37	46.90 d
Rice-polished		19.63 g	0,30	5.73 g	0.17	13.90 e	0.20	70.83 g
Sorghum		20.75 f	0,06	9.75 f	0,10	11.00 e	0.14	53.01 e
Millet		7.43 h	0,05	1.95 gh	0.06	5.48 f	0.10	73.74 h
Tall fescue		2.80 i	0,00	0.40 h	0.00	2.40 f	0.00	85.71 i
Kentucky bluegrass		0.40 j	0.00	.	.	.	.	.
Analysis of variance	F	114831.83		6328.5		223.15		1472.8
	p	<0,01		<0,01		<0,01		<0,01

NOTES: Mean values labeled with the same letters are not significantly different according Duncan Multiple Range Test for significance level of p = 0.05. In the process of statistical analyses the % values were transformed in arcsin √%

In accordance with the availability of the mass of food resources determined by a single kernel (Tab.2), the highest average mass loss of an infested kernel (i.e. larval consumption) was recorded in grain species/types having the highest kernel mass (whole corn grain and in corn-endosperm fraction, 55.48 mg and 52.30 mg, respectively), the lowest in millet and tall fescue (5.48 mg and 2.40 mg, respectively). Despite the low number of emerged adults in kernels of low mass (e.g. millet and tall fescue), it was demonstrated that AGM is able to survive with remarkably limited amount of feed, consuming about 10-23 times lower mass of kernel than in optimal conditions provided by wheat, barley or corn. The lowest average mass loss of an infested kernel expressed in percentages was recorded in whole corn kernels (14,75%), the highest in grains of tall fescue where 85.71% of grain mass was consumed. Obviously, the utilization of food resources in grains of low kernel mass is significantly higher.

Statistically highly significant correlations were detected between the parameters of chemical composition of the grain substrates and number of emerged adults (Tab.3). Sugar, protein and lipid content had positive influence to the development of AGM, while cellulose and ash content had negative influence to the number of emerged adults. Highly significant positive correlation was also detected between the energy value of a substrate and number of emerged adults. Similarly, higly significant influences were also recorded when chemical composition parameters, as well as the energy value of grain substrates were correlated with mass losses of grain substrate, and with mass losses of single infested kernels. The impact of each of the tested chemical composition parameter to the mass loss of the supstrate per emerged adult (i.e. consumption of a survived individual), was also higly significant: positive when it regarded the influence of sugar, protein and lipid content and negative regarding the influence of cellullose and ash content. The only not significant correlation coefficient was established between the energy value of the substrate and mass losses of the grain substrate per emerged adult.

Tab. 3 Qualitative parameters of different grain species/types and correlations with the number of emerged adults of *Sitotroga cerealella* and mass losses parameters

Grain species/type	Chemical content					Energy value (kJ/g)
	Sugar (mg/g)	Protein (% d.m.)	Lipid (%d.m.)	Cellulose (% d.m.)	Ash (% d.m.)	
Corn	15.45	10.10	3.88	2.02	2.54	17.437
Corn - fraction without embryo	16.60	14.01	2.56	1.75	3.36	18.566

Corn - fraction with embryo		16.03	16.52	3.22	1.50	3.08	18.911
Barley		15.00	11.66	2.39	3.52	4.09	17.334
Wheat		14.56	12.53	2.10	2.33	2.81	16.196
Rice - polished		11.53	9.11	1.95	2.36	3.36	14.866
Sorghum		13.56	10.51	2.11	2.21	2.16	17.258
Millet		10.02	11.49	2.76	3.28	5.21	16.487
Tall fescue		9.23	5.06	1.47	3.71	6.83	16.911
Kentucky bluegrass		9.85	4.21	1.64	3.55	5.91	16.722
Correlation coefficients	Number of emerged adults	0.91**	0.76**	0.59**	-0.60**	-0.73**	0.56**
	Mass losses of grain substrate (g)	0.91**	0.68**	0.79**	-0.73**	-0.61**	0.65**
	Mass losses of substrate/adult (mg)	0.72**	0.47**	0.60**	-0.71**	-0.53**	0.32
	Mass losses of infested kernel (mg)	0.88**	0.61**	0.75**	-0.70**	-0.51**	0.66**

d.m.- dry matter ** highly significant ($p<0.01$)

Discussion

AGM is worldwide distributed oligophagous pest species that usually attack cereal grains in extensive storage conditions. In temperate regions, as demonstrated by TREMATERRA (2015) in Southern Italy, the infestations with AGM occurre both during preharvest plantation and postharvest storage, and therefore the author highlighted that warehouses, field-plots and wild hosts distributed on the teritory can each serve as sources of both reproduction and aggregation, depending on the time of the year. Stored grains of plant species that are frequently reported as hosts of AGM are corn, wheat, barley, sorghum, rice, ray, oatand millet, but apart these most usually cultivated cereals it can also develop in grains of some spontaneous Poaceae species of few genera, such as *Lolium* L., *Eleusine* Gaertn., *Phalaris* L. and *Echinochloa* Palisot de Beauvois (BALACHOWSKY, 1966; DAKSHINAMURTHY AND REGUPATHY, 1988). So far, the only available report on successful development of AGM in grains of tall fescue (*Festuca arundinacea*) is given by IGNJATOVIĆ ĆUPINA (2001).

Apart the cereal species commonly known as hosts of AGM (corn, wheat, barley, sorghum), this study confirmed the adaptability of the pest to survive in small grain species, such as millet and tall fescue, as well as in mechanically damaged kernels of common host species (corn grain fractions with and without embryo, polished rice), but not in Kentucky bluegrass. Despite the less favorable quantitative and qualitative conditions in host species of small grains, the survival of AGM was still evident at different extent, reflected by lower number of emerged adults and lower mass losses of the infested substrate.

Usually a single grain kernel is infested by only one, single AGM larva. The best conditions for the development of AGM are provided by corn grains, which offer enough food resources for the development of even more than one AGM individuals (up to 3) per single kernel, and such behavior was rarely recorded also in wheat grains (GRANDI, 1951; BALACHOWSKY, 1966; VUKASOVIĆ *et al*., 1972; MANOJLOVIĆ, 1987). According PRAKASH *et al*. (1982), the grain resistence to pest infestation depend on physical and biochemical grain properties, as well as on the pest feeding and/or oviposition preference.

Different modes of penetration of AGM neonate larvae into the kernel are described depending on the plant host species. In corn grains the penetration takes palce in the germ zone (VUKASOVIĆ *et al*., 1972), where the bran is thinnest and additionally such strategy provide the most nutritious matters contained in the germ during the initial feeding of the young larva. The same strategy of penetration in the germ zone in sorghum kernels was reported by WONGO (1990) and WONGO AND PEDERSEN (1990).

In the present research the boring of larvae in the germ zone was observed in grains free of hull (corn, corn-fraction with embryo, sorghum, wheat), but also in husked grains (barley, millet and tall fescue). According to VUKASOVIĆ *et al*. (1972) penetration of AGM larvae in the germ zone of wheat

kernels seems quite unusual. Penetration in the zone of endosperm in wheat kernels was detected in the present study, but not exclusively (some larvae preferred the germ zone for penetration).

Few authors observed that the hull of rice kernels represents an important protective structure that prevent the penetration of pest insects into the kernel and hard, thick and intact hull represents a resistance factor that affect the penetration of AGM larve (RUSSELL AND COGBURN, 1977; COGBURN AND BOLLICH, 1986; RAGUMOORTHY AND GUNATHILAGARAJ, 1988; SAUPHANOR, 1988; COGBURN *et al*., 1989; TAKESHITA AND IMURA, 1990).

In grains completely covered by intact hull, which reperesents a mechanical barrier, the penetration may occure through the abscission scar of the central vascular bundle, as described in rough rice kernels by COGBURN *et al*., (1983). In this research, such beheviour was observed in tall fescue kernels. Similar strategy of penetration was observed in millet kernels thightly enclosed by *palea* and *lemma*, where the larva also chose the natural opening (micropyle) to bore into the kernel. However, the hairy hull structure of Kentucky bluegrass represented the insuperable mechanical barrier for larval penetration into the kernel. The larval mortality of 100% in this grain substrate was obviously attributed to the husk structure, not to the grain size (i.e. food resources).

The imperfect hulls of barley kernels did not represent a barrier for penetration of larvae in the germ zone. Similar observation was reported by COGBURN *et al*. (1983) who stated that rice varieties with imperfect hull favored the infestation by AGM.

Furthermore, the hardness and thickness of the bran also contribute the resistance to penetration of insects into the kernel, as demonstrated in sorghum kernels where the lowest infestation occurred in varieties with the hardest bran layer (SHAZALI, 1985). In the present research, the penetration hole in corn fraction without embryo was always detected on the side of the transfersal cut (free of the bran), where the larvae penetrated through the crevice left after removal of the germ. The possibility of larvae to penetrate mechanically damaged corn kernels suggests that such substrates are equally susceptible to infestation by AGM as whole grains. ALLOTEY AND MOLOKO (2015) recorded higher emergance rate of AGM reared in substrates with whole grains of maize varieties than in mechanically altered grains (cut and ground grains). However, in the present study where the fractions with and without embryo were separately considered, the obtained results demonstrated the highest adult emergence rate in corn fraction with embryo, followed by whole corn grains and fraction without embryo. In contrast, in sorghum and millet varieties ALLOTEY AND MOLOKO (2015) recorded the highest number of adults in substrates with broken grains and such results were explained by the earlier exposure of endosperm for larval feeding and easier exit path between the broken grains. In the present study, small grain species, were offered to AGM only as whole grains. Nevertheless, the emergence rate of 70% of AGM reared in sorghum whole grains was similar to the values recorded by ALLOTEY AND MOLOKO (2015) in both whole or broken sorghum grains (66,3% and 68,8%, respectively), depending on the tested variety.

The most interesting behavior of AGM was observed in polished rice, where no particular rule of larval penetration was observed. Lacking the external mechanical barrier in this type of mechanically processed substrate the larvae were able to pass from one kernel to another by producing the silky tunel between the kernels, and additionally during the transfer some of the surrounding kernels were damaged superficially. Superficial damages on adjacent kernels were reported in rare cases when the food resource provided by a single kernel is scarce (BALACHOWSKY, 1966). Lacking the epidermis layer in polished rice kernels, the exit holes of AGM aduls did not have a typical "window" appearance. Whether the transfer to another kernel was conducted with ultimate pupation inside the kernel, or the pupation occurred outside in the interspace between the kernels in the cocoon, the silky threads connecting the kernels and frass were evident. At a first appearance, such infestation symptom might be incorrectly linked to some other stored product pests that are feeding externaly, such as *Nemapogon granella* (Linnaeus) or *Plodia interpunctella* (Hübner).

Appart the positive association of the integrity of the rice husk with the resistence to pest infestation, COGBURN AND BOLLICH (1986) emphasized that hardness and texture of the kernel surface are of a crucial importance for oviposition and further development, and suggested that nutritive compounds also play a role in grain resistance. However, several aspects are involved in grain resistence to infestation with store products pests, such as the absence of preferences (for oviposition and/or feeding), physical and chemical grain properties and changes during the grain processing (PRAKASH *et al*., 1982). Furthermore, the grain resistence to infestation depends on the grain size, as observed among different cultivars of pearl millet (SEIFELNASR AND MILLS, 1985). Numerous studies were conducted in order to estimate the survival rate and mortality of AGM in grains of different plant species, as well as in different varieties of the same plant species. According MANOJLOVIĆ (1987), the mortality rate during the post-embryonic developlent of AGM reared under the same conditions in corn hybrids of larger grain size ranged between 36.2% and 40.05%, while in smaller size hybrid it was 41.46%. In the same study, the mortality rate in wheat variety of smaller grain size was also higher (46.02%) than in the varieties of larger kernels (36.2-40.05%). The research conducted by COGBURN (1989) demonstrated that the survival rate of AGM reared in different species of rice with deliberately broken hulls ranged between 0.0% and 40.2% and significantly depended on the size and mass of the kernel. The resistence of different stored rice varieties, expressed through the number of emerged AGM adults was positively and highly significantly correlated with the weight loss (RIZWANA *et al*., 2011). In different tested varieties of sorghum, SRIVASTAVA (1996) determined significantly different mortality rates during the postembryonic development ranging between 7.47% and 41.64%.

In the present study a series of different grain species/types were tested to AGM infestation. Appart the external physical characteristics of the kernel that influenced the larval penetration, quantitative and qualitative grain properties had significant impact to the survival of AGM (i.e. number of emerged adults) and consecutive mass losses, as expected. Substrates with higher mass of kernels favored the development of AGM resulting in higher number of adults, higher mass losses of the infested kernels and higher mass losses of grain substrates as a whole. In kernels of lower mass, the number of emerged adults was significantly lower, but in such kernels the available food resources were efficiently utilized by the survived individuals (e.g. consumed kernel mass of 73.74% and 85.71% in mellet and tall fescue, respectively). The mass of consumed feed by an individual in such small kernels (5.48 mg and 2.40 mg in mellet and tall fescue, respectively) was significantly lower than in optimal conditions, such as provided by whole corn kernels where the consumption was 55.48 mg. Despite the low mass of consumed feed in sorghum kernels (11 mg/larva; 53,01% of kernel mass), the survival rate (i.e. number of emerged adults) in this small grain substrate was surprisingly high (70%), not significantly different from the survival rate recorded in corn fraction without embryo, but still significantly lower than in corn fraction with embryo, whole corn grains, barley and wheat. Similarly to our results, BORZOUI *et. al* (2017) also recorded significantly lower food consumption per larval individual in sorghum grains (about 27 mg), than in wheat and maize grains (about 52 mg and 65 mg, respectively), as well as significantly lower survival rate of immature stages reared in sorghum (about 46%) than in maize (65%), barley (68%) and wheat (90%). Our results demonstrated the high adaptability of AGM populations to survive in limited conditions of available feed, determined by the mass of kernels. Nevertheless, the food resource available in polished rice kernel was not sufficient to an individual, and therefore, appart the consumation of 13.90 mg of internaly infested kernel (70.83 % of the kernel mass), the larva was able to move to the next kernel and continue the feeding (supeficially or internaly). Therefore, the mass losses of polished rice substrate calculated per number of emerged individual was as high as recorded in corn fraction without embryo. However, the ability of AGM to transfer and infest more polished rice kernels did not result in high number of survived individuals.

Obvuiously, the chemical composition and the energy value of the feed were also significantly involved in the survival of AGM and consequently had the impact on mass losses parameters (mass losses of whole grain substrate, mass losses of grain substrate per adult and mass losses of infested

kernels). Reserches conducted by some authors on different varieties of the same plant species were not able to identify significant correlations between the chemical composition parameters (sugar, lipid, protein, starch, ash content) on one side and mass losses or pest survival rate (or index of population growth) on the other side. Such results were obtained in the studies conducted by PANDEY AND PANDEY (1983) in varieties of corn, as well as in the research of RIZWANA *et al.* (2011) in rice varieaties. However, DEMISSIE *et. al* (2015) also tested different varieties of corn for susceptibility to AGM and found significant positive correlation between the ash content and the number of emerged adults, while the impact of the moisture and phenolic content to the number of total progeny emerged was significantly negative. In addition, the same authors also have not found statistically significant correlations between other studied parameters of biochemical composition of the feed (i.e. content of crude oil, crude carbohydrate, crude proteins, crude fibre, amylose and amylase) and the total emerged progeny, as well as no significant correlations between biochemical composition paramethers and the percentage of weight loss, with the exception of crude proteins and amylose content that had significantly positive impact to the grain weight loss. Recent research of SAFIAN MURAD AND BATOOL (2017) demonstrated that varieties of wheat with higher protein and carbohydrate content, higher grain weight and lower grain hardness were more susceptible to AGM, since in such varieties significantly higher number of AGM adults emerged, and higher values of percent damage and percent weight loss were recorded. In our present research, where different plant species/types of grains were compared in relation to AGM development and consecutive losses, the impact of biochemical composition of the feed became clearly evident. Sugar, protein and lipid content were positively and highly significantly correlated with the number of emerged adults and mass losses parameters, while negative, also highly significant correlation was detected with the cellulose and ash content. The energy value of the feed had also statisticaly significant positive impact to the number of emerged adults, to the mass losses of the whole substrate and mass losses of infested kernels.

Based on the presented results, it can be concluded that kernels of small grain species with intact hulls, higher cellulose and ash content, lower sugar, lipid and protein content (e.g. millet and tall fescue), as well as mechanically/morphologically dammaged kernels (e.g. polished rice) had negative impact to the development of AGM populations, which resulted in lower number of survived individuals and lower mass losses of grain substrates. However, in such substrates AGM individuals could survive by consuming surprisingly low mass, but high percentage of available feed limited by a single kernel, as demonstrated in millet and tall fescue, or by successful transfer and infestation of more than one kernel in order to compensate the insufficient food resources, as observed in the substrate with polished rice.

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Progeny production by *Stegobium paniceum* in different spices

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Abstract

Spices have long been an important component in the preparation of food, and some have medicinal properties as well. *Stegobium paniceum*, the drugstore beetle, has been detected in spices but no detailed information is available on its infestation in certain locally-available spices. Objective of this study was to find out the degree of infestation by *S. paniceum* in ten different spices. Twenty adults of *S. paniceum* were introduced into a vial containing a particular spice, maintained for two weeks and shifted out. These were maintained under ambient environmental conditions and the progeny adults emerged in each medium was counted at two week intervals for three months. The progeny produced varied with the food medium; the highest progeny was recorded in coriander whereas the lowest progeny was recorded in cinnamon, clove, dill seeds, cardamom, chilli, pepper corn and turmeric powder. This study reveals that *S. paniceum* infests a wide array of spices at different levels. This information is important for taking necessary steps to protect the spices from the infestation of *S. paniceum*.

Keywords: *Stegobium paniceum*, Progeny, Spices, Infestation

1. Introduction

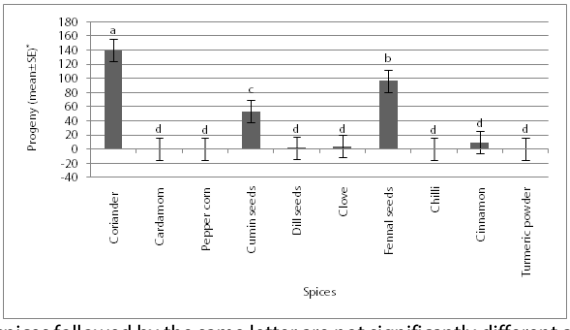
Stored-product losses are more in tropical countries than in temperate regions (Wijayaratne et al., 2018). Sri Lanka has the reputation for producing good quality spices. Drugstore beetle, *Stegobium paniceum* is a pest of stored spices (Cabrera, 2014). Infestation of spices kept in storage by *S. paniceum* is reported but a proper investigation has not yet been performed. Therefore, the objective of this study was to find out the infestation level of *S. paniceum* in ten spices locally available and frequently used as indigenous medicine.

2. Materials and Methods

Ten spices were used in this study: coriander, cardamom, pepper corn, cumin seeds, dill seeds, clove, fennel seeds, chilli pieces, cinnamon and turmeric powder. Drugstore beetles were reared in coriander medium inside the incubator at 30^{0}C and 60% RH. The progeny adults aged one month were used in the experiments. Twenty adults of *S. paniceum* were introduced into a vial containing 12 g of a particular spice, maintained for two weeks and sifted out. Four replicates from each treatment were maintained. Progeny adults emerged in each medium was counted at one month intervals for three months.

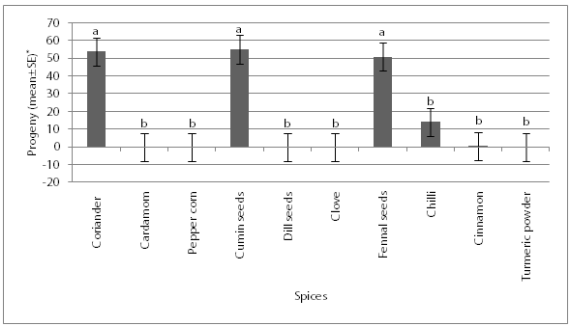
3. Results and Discussion

The progeny production differed with the spice and the duration. Highest infestation recorded in coriander. No progeny was produced in cardamom, pepper corn and turmeric powder.



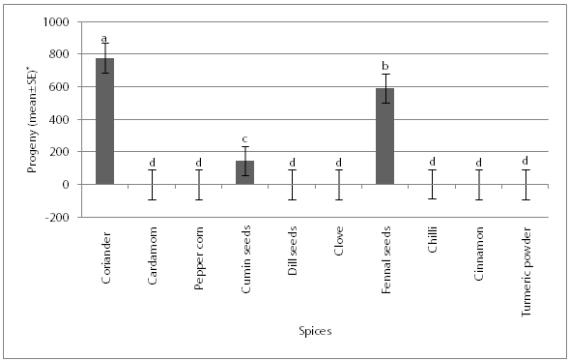
*Progeny produced in spices followed by the same letter are not significantly different according to Tukey's test.

Fig.1 Progeny adults emerged (mean±SE) in different spices one month following initial infestation.



*Progeny produced in spices followed by the same letter are not significantly different according to Tukey's test.

Fig. 2 Progeny adults emerged (mean±SE) in different spices two months following initial infestation.



*Progeny produced in spices followed by the same letter are not significantly different according to Tukey's test.

Fig. 3 Progeny adults emerged (mean±SE) in different spices three months following initial infestation.

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The developmental parameters of the minute brown scavenger beetle *Dienerella argus* (Coleoptera: Latridiidae)

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Abstract

Adults and larvae of *Dienerella argus* (Reitter) (Coleoptera: Latridiidae) feed on fungi and are frequently found in indoor, moldy areas. The basic biology of this species, other than its feeding habits, has not been determined. In this study, the developmental parameters of the beetle were investigated using dried hyphae and conidia from three fungi that are common in living areas. The developmental periods of the beetle on *Cladosporium cladosporioides*, *Penicillium citrinum*, and *P. decumbens* were examined at 16, 20, 24, 28, 32 °C / 70–75 % RH under dark conditions. The low developmental threshold temperatures and thermal constants calculated from egg to adult emergence were 10.5 °C and 526 DD (degree day), 9.0 °C and 500 DD, and 10.9 °C and 370 DD on *C.*

cladosporioides, *P. citrinum*, and *P. decumbens*, respectively. These developmental parameters indicate that these beetles can breed year-round in indoor areas that are in air-conditioned facilities.

Keywords: *Dienerella argus*, developmental period, low developmental threshold temperature, thermal constant, *Cladosporium*, *Penicillium*

1. Introduction

Latridiidae is a family of small (1–3 mm) mycophagous beetles that includes 761 species according to Rücker (2015). These beetles are named minute brown scavenger beetles, or plaster beetles for some indoor species, and are found in moldy areas, on debris and occasionally on flowers. At least 30 species have been listed as stored product pests, although they do not directly affect stored foods but feed entirely on the fungi that grow on foods (Hinton 1941). Most species associated with stored foods are dispersed worldwide, perhaps due to the international transportation of food commodities. *Dienerella argus* (Reitter) is one of these wide-ranging species that was introduced into Japan (Mito and Uesugi 2004). Stored-product pest species usually adapt to indoor environments and are frequently found in moldy areas such as plaster walls in a damp-dried state; under floors, garrets, and internal wall structures; and in air-conditioning and refrigeration systems, where dew condensation occurs, as well as hospitals or sterile drug processing areas (Carlton 1988; Robinson 2005; Tanaka 1986; Tani and Ito 2006). In manufacturing industries, the populations of these beetles sometimes increase explosively inside factories or warehouses, causing insect contamination in products. These beetles may possibly cause sanitary problems by spreading fungus spores (Robinson 2005). For example, Tani and Ito (2006) isolated the fungi *Cladosporium* spp. and *Penicillium* spp. from the body surface of *Dienerella costulata* (Reitter) and three fungus genera including *Aspergillus* from another latridiid beetle. Currently, little is known about the basic biology of these species, except for feeding habits. Since a successful artificial rearing method has been established for the beetles using dried hyphae and conidia of fungi, the low developmental threshold temperature and thermal constant of the beetle on three fungi, *Cladosporium cladosporioides*, *Penicillium citrinum*, and *P. decumbens*, were examined.

2. Materials and Methods

2-1. Collection and rearing method of *Dienerella argus*

Adult beetles were collected from the floor using a vacuum cleaner at a laboratory in the Manufacturing Technology Center, Japan Tobacco Inc. (Tokyo), where an outbreak of this insect had occurred. The insects were preserved on the dried hyphae and conidia of *Cladosporium cladosporioides* (NBRC6348) on potato dextrose agar (PDA). Approximately 30 adults that were 1–4 weeks old were placed on dried fungi in a petri dish and kept under 27 °C, 75 % RH and dark conditions. The adults were removed after two weeks. The next generation adults emerged after one month under these conditions.

2-2. Fungi

C. cladosporioides (NBRC6348) and *P. citrinum* (NBRC 6352) were obtained from the Biological Resource Center, National Institute of Technology and Evaluation, Chiba, Japan. *P. decumbens* was collected from above the ceiling of the laboratory where *D. argus* was collected. An open plate method was used in which the airborne particles were passively collected and preserved on PDA in a petri dish that was uncovered for 30 min. Then, the Petri dishes were kept under 25 °C, and fungal colonies were isolated. The primary culture and subculture of these fungi were prepared on PDA in 90-mm plastic Petri dishes for 3–6 weeks at 25 °C. Spore-formed cultures were dried under room conditions (23–28 °C and 40–70 % RH) and provided to the insects.

2-3. Developmental parameters

The developmental periods from egg to adult were examined on the three fungi *C. cladosporioides, P. citrinum*, and *P. decumbens*. Ten mating pairs were placed on the dried fungi in the Petri dishes. The Petri dishes were enclosed in 11.5 cm × 19.5 cm × 7.0 cm plastic containers with saturated NaCl solution in a 2.5 cmϕ × 5 cm cup to maintain humidity at 70–75 % RH and kept in a 28 ºC chamber. After 16–20 h, the adults were removed and the containers were transferred to chambers set at 16, 20, 24, 28, 32 °C under dark conditions. Two replications were carried out for each fungus and temperature. Actual temperatures in the containers were recorded at one hour intervals by the thermo recorder. Adult emergence was checked at 1–3 d intervals. The timing of eclosion was assumed to occur during the observation intervals that were in the middle of the study period. The low developmental threshold temperature (T_0) and thermal constant (K) were obtained from the regression lines of $1/D$ against T, where D is the developmental period in days from egg to adult, and T is temperature in °C (Kiritani 2012).

3. Results

Egg to adult development occurred for all fungi tested, and the adults that emerged were both viable and fertile. Table 1 shows the developmental periods under different temperature conditions, and the values of T_0 and K calculated for the three fungi. Development periods were slightly different among the fungi. The period was delayed by one week to ten days for complete growth on *C. cladosporioides* compared to the periods for *Penicillium* spp.

Tab. 1 Developmental periods at five temperatures and developmental parameters for *Dienerella argus* on three fungi.

Temperature, ºC	Developmental period in days, mean ± SD (Number of adults emerged)		
	Cladosporium cladosporioides	*Penicillium citrinum*	*Penicillium decumbens*
15.4	95.5 ± 4.3	85.0 ± 5.1	88.4 ± 3.0
19.4	54.2 ± 2.5	46.0 ± 3.7	45.8 ± 3.1
23.3	40.3 ± 2.6	31.3 ± 0.8	29.3 ± 0.8
27.3	31.5 ± 8.5	26.8 ± 2.9	21.1 ± 2.3
31.7	25.4 ± 3.9	22.2 ± 3.1	18.9 ± 0.4
Low developmental threshold	10.5 °C	9.0 °C	10.9 °C
Thermal constant	526 DD	500 DD	370 DD

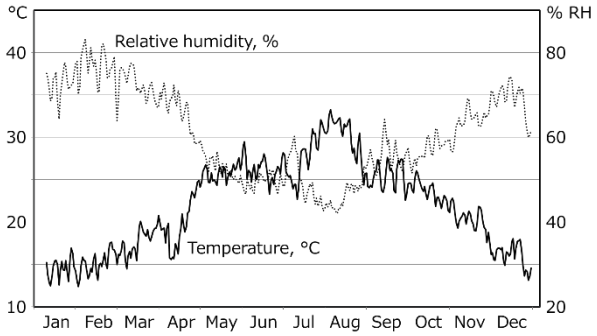

Fig. 1 Temperature and humidity above the ceiling panel of the laboratory where the outbreak of *Dienerella argus* occurred. Solid and dotted lines represent daily mean temperature and relative humidity, respectively.

Discussion

Although the developmental parameters for Latridiid beetles have not been elucidated, Hinton (1941) described the life histories of several species on cultures of *Penicillium glaucum* and *Mucor mucedo* on bread and cheese. The durations of the time the egg was laid to adult emergence were 27–32 d (egg to the second instar larval stage was at 15.6 °C and the third instar to pupal stage was at 20 °C) for *Cartodere nodifer* (Westwood), 36 d at 23.9 °C for *Dienerella filum* (Aubé), 51–52 d (egg at 17.2 °C and larva to pupa at 19.4 °C) for *D. filiformis* (Gyllenhal), and 40 d at 18.3 °C for *Corticaria fulva* (Comolli) (Hinton 1941). The period from egg to adult observed for *D. argus* in the present study was longer than the periods observed for *Cartodere nodifer* and *Corticaria fulva*, and was the same as the periods observed for the two *Dienerella* species at comparable temperatures. Based on the systematic review by Kiritani (2012), the average T_0 and K values from egg to adult for 31 coleopteran species are 10.9 ± 2.5 °C and 415 ± 239 DD (mean ± SD), respectively. The T_0 and K values for *D. argus* were 10.5 °C and 526 DD on *C. cladosporioides*, 9.0 °C and 500 DD on *P. citrinum*, and 10.9 °C and 370 DD on *P. decumbens*, all of which are average values for coleopterans.

The daily mean temperatures of the garret of the laboratory, the original location of the test insects, fluctuated within the range of 12–17 °C in the winter, constantly surpassing T_0 (= 9.0–10. 9 °C) (Fig. 1). This result strongly suggests that *D. argus* had bred there year-round. In fact, the adult beetles were caught irrespective of the season. Because the female adults require at least one week before starting oviposition at 25–30 °C, the thermal constant K for one generation on *P. decumbens* was assumed to be 470–500 DD (370 DD for egg to adult emergence + 100–130 DD for the pre-oviposition period). The accumulated daily mean temperature above 10.9 °C was calculated at 3974 DD in the garret of laboratory, and the number of generations per year was estimated at eight.

Acknowledgement

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Comparison of mandible morphology of two stored product bostrichid beetles, *Rhyzopertha dominica* and *Prostephanus truncatus*

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Abstract

Insect mandibles are most frequently encountered fragments in processed foods. Thanks to their sclerotised and darkly pigmented nature, they usually remain intact in foods and are relatively easily detectable. Moreover, because of their complexity and variety of shapes, stored product beetle mandibles may be useful in species determination. The present work deals with a comparative morphology of two stored product bostrichid beetles, *Rhyzopertha dominica* and *Prostephanus truncatus*. The mandibles were studied using by light and scanning electron microscopy and their morphological details, overall appearance and size are provided.

Keywords: mandibles, stored product pests, Bostrichidae, *Rhyzopertha dominica*, *Prostephanus truncatus*

1. Introduction

Beetle mandibles serve as effective tools for both intake and processing of food. From the stored product perspective, they are used for overcoming barriers imposed by a manufacturer thus enabling to infest a commodity (Stejskal et al., 2017). For this reason, external morphology of mandibles can provide information about mechanism of feeding and infesting potential of the particular insect species. Lesser grain borer *Rhyzopertha dominica* and larger grain borer *Prostephanus truncatus* are serious pests of stored grain in many regions worldwide (Stejskal et al., 2015). As both species are internal grain feeders with relatively inconspicuous adult way of life (Edde, 2012), early detection of the infestation is problematic. Nevertheless, thanks to their microscopic size and highly sclerotised nature, mandibles are most numerous fragments in the processed foods (Trematerra et al., 2011) and may thus serve as an indicator of level of a product contamination and for species identification.

2. Materials and Methods

Both species were reared at 27 °C and 75% relative humidity on wheat (*R. dominica*) or maize grains (*P. truncatus*). Only newly emerged, 1 – 7 days old individuals were used. The mandible measurements were taken using stereomicroscope Olympus SZX10 equipped with a Canon 1300D digital camera and analysed by QuickPHOTO INDUSTRIAL 3.1 software. Before examining with the JEOL 6380 scanning electron microscope, the mandibles were cleaned in 20% lactic acid for 24 hours, dried with critical point drying and mounted on aluminium plates.

3. Results

For both species, interspecific differences in size, shape, as well as morphological details were indentified. The mandibles of *P. truncatus* were described for the first time. The morphological characteristics and the most important differences are summarized below:

3.1 Rhyzopertha dominica (Fig. 1A)

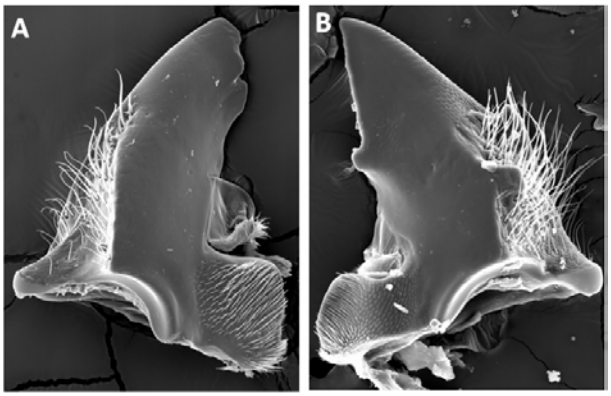
Mandible length 270 - 310 µm. Shape of incisive part: incisor lobe relatively short and blunt. A setal tuft relatively small, containing only 10 – 20 long branched setae. Mola quadrate from the dorsal aspect, smooth, without any apparent structure, only with shallow groove approximately in the middle. Setae along the molar area in dorsal part uniform. Lateral margin without any lateral protuberance.

3.2 Prostephanus truncatus (Fig. 1B)

Mandible length 390 - 440 µm. Incisor long, with blunt mesal protuberance in proximal part, primary incisor pointed. Wide setal brush containing > 100 short setae. Mola rounded, chewing area concave with coarse surface. Setae along the molar area of two types. Lateral margin with two large protuberances on lateroventral and laterodorsal aspect.

Fig.1 SEM photographs of mandibles of (A) *Rhyzopertha dominica* and (B) *Prostephanus truncatus*.

4. Discussion



Despite great variability of insect mandibles, there exist generalities in their morphology according to feeding habits of the species (e.g. Samways et al., 1997; Smith and Capinera, 2005). For example, pointed bifid or unidentate apex of mandibles serves as a piercer and is mainly present in predatory species. Similarly, highly developed mola is used for trituration of a dense material and is thus present in species feeding on hard material. The relative size and shape of mola and molar surface are different in both studied species and probably reflect their slightly different food source. Nevertheless, the well developed mola in both species is probably linked with presence of a dust in the infested commodities by these species (Kumar, 2002). Also, the incisors are adapted for scraping and play a role in removal of a food as well as in penetration of a hard material (e.g. wood, seed surface, or, secondary, food packages). Thus, the length and robustness of the incisive part may be a reason of a great penetration ability of the studied species (Stejskal et al., 2017).

In this work, we described mandible morphology in two stored product bostrichid beetles, *R. dominica* and *P. truncatus*. The mandibles of *P. truncatus* are described for the first time. We identified differences between the two species in size, shape as well as morphological details. We conclude that the two species can be easily identified based on their mandibles and that the species determination is possible at low magnifications by light microscopy.

Acknowledgement

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Behavioural responses of Callosobruchus maculatus to volatiles organic compounds found in the headspace of dried green pea seeds

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There is growing evidence that insects rely on chemical cues to locate food, hosts, predators, and potential mates. The pulse beetle Callosobruchus maculatus has been recognised for decades as the major post-harvest insect pest of legume seeds. In a previous study, we identified five volatile compounds in the headspace of dried green pea seeds as electroantennographically active in C. maculatus antennae: 1-pentanol, 1-octen-3-ol, (*E*)-2-octenal, nonanal and 3-carene. Volatile compounds are generally perceived by insects as blends, we hypothesized that *C. maculatus* might particularly show attraction to different mixtures of the aforementioned compounds. To test this we examined the behavioural response of *C. maculatus* towards volatile mixtures in a dual choice Y-tube olfactometer. The results showed that females were attracted to five mixtures while males were attracted only to two binary mixtures consisting exclusively of aldehydes. The other mixtures caused *C. maculatus* to move away. Further investigations with the attractive mixtures should be done in real storage conditions with the aim of developing a trap for the pulse beetle, *C. maculatus.*

Investigation on the Species and Distribution of Stored Grain Insects in Northwest China

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Abstract

To understand the diversity of stored grain insects in northwest China, we have fulfilled insect collection in 56 grain storage enterprises, 60 grain, oil and feed processing plants and 65 farmers situated in 26 cities of five provinces (Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang) in northwest China from 2016 to 2017. After systematical identification, totally 83 species of stored grain insects have been found in this investigation, belonging to five orders, namely Class Insecta Order Zygentoma, Order Coleoptera, Order Lepidoptera and Order Hymenoptera, as well as Class Arachnida Order Chelonethida, in which Order Coleoptera owns 74 species in 22 families, Order Lepidoptera owns six species in four families, Order Zygentoma and Order Hymenoptera own one species in one family respectively, and Class Arachnida Order Chelonethida has one species in one family. After the statistics of four insect investigations in northwest China during 1955-2017, this paper has analyzed the results of four insect investigations and the representative stored grain insects in northwest China.

Key Words: northwest China, stored grain insects, species, distribution, investigation

1. Introduction

Located in the hinterland of the Eurasian continent, the northwest region covers the first (high-cold and dry stored grain region), second (low temperature and dry stored grain region) and fourth

(medium-temperature and dry stored grain region) of China's seven stored grain ecological regions, and consists of Shaanxi, Gansu, Qinghai, Ningxia Hui Autonomous Region, and Xinjiang Uygur Autonomous Region. Wide area with scarce rainfall, the northwest region, as a traditional grain deficit area, has the most serious soil erosion, land drought and desertification problems in China. Main grains planted and stored in northwest region include wheat, corn, rice, highland barley, soybean, buckwheat, pea, naked oat, proso millet, flax and other small grains.

Since the founding of the people's Republic of China, there have been seven national insect investigations related to the grain system, in which Gansu, Ningxia, Xinjiang and other northwest provinces were not included in the first and second investigations (Qizong Chen, 1994; Xiaoping Yan, et al., 2008). Besides the national insect investigations, some provinces have carried out their own investigation independently. The coleoptera pest investigation in Shaanxi commercial warehouses carried out by He Jinyan, et al. in 1983-1984 discovered 30 species of insects in eight families, Coleoptera Order (Jinyan He, et al. , 1993), Gao Duping reported the main stored grain pests in Pingliang, Gansu, composed of 19 species in 10 families, three orders (Jinyan He, et al. , 1993), the stored grain pest investigation in Ningxia Hui Autonomous Region carried out by Zhu Desheng, et al. in 1983-1984 found 47 species of pests in 19 families, two orders (Desheng Zhu,1987); the stored grain pest investigation in Changji Hui Autonomous Prefecture, Xinjiang carried out by Li Mingshan, et al. in 1981 found 39 species of pests in 22 families, seven orders (Mingshan Li, et al.,1994); and the stored grain insect investigation in Tibet Autonomous Region carried out by Chen Qizong, et al. in 1987 found 73 species of stored product insects (Qizong Chen,1990) Over the past 20 years, the farming mode and storage environment in northwest region have changed greatly. Therefore, regular investigation and research on pest species, location, distribution area and object should be carried out for effective stored grain pest control. Considering 2015 Grain Public Welfare Industry Research Project and *Notice of State Administration of Grain on the Seventh National Stored Grain Insect and Mite Investigation* [GLBC (2016) No.95], under the great support of grain administrations and local grain bureaus of Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang as well as relevant enterprises, an investigation on the species and distribution of stored grain insects has been carried out in grain storage enterprises, grain, oil and feed processing plants, farmers and other relevant places within three stored grain ecological regions, namely high-cold and dry stored grain region (the first region), low-temperature and dry stored grain region (the second region), and medium-temperature and dry stored grain region (the fourth region), for a better understanding of the stored grain insect diversity in the northwest region and pest control.

2. 2. Investigation Method and Scope

2.1 Sampling Site

Within the scope of five northwest provinces (Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang), besides some representative cities selected in the light of each stored grain ecological region, some relevant enterprises in the east, west, south, north and middle of each province were also selected as sampling sites. Totally, 56 grain storage enterprises, 68 grain, oil and feed processing plants, and 65 farmers from 26 cities in the first, second and fourth stored grain ecological regions, participated in this investigation. (See Table 1)

Table 1 Number of Cities, Relevant Enterprises and Farmers in Five Provinces.

Stored Grain Ecological Region	Province	Number			
		City	Grain Storage Ent.	Processing Plant	Farmer
First: High-cold and Dry	Qinghai	4	7	7	4
Second: Low-temp. and Dry	Xinjiang	6	10	14	1
	Gansu (partial)	4	14	6	19
	Ningxia	3	3	8	1
Fourth: Med.-temp. and Dry	Gansu (partial)	3	12	5	15
	Shaanxi	6	10	20	25

2.2 Sampling Method

Field sampling and screening were adopted by this investigation, and the durations from July to September, both 2016 and 2017, were selected as its sampling time. Collected samples were preliminarily classified and processed on site with original information registration, such as host of pest, sampling time, sampling site, etc. After then, a series of follow-up processes were carried out at the laboratory, including further separation, processing, preliminary species naming, classification and preservation, as well as specimen preparation.

2.3 Insect Identification Method

In terms of traditional morphological characteristics of insects, the species of each sample was identified, named and then reviewed by an expert team composed of researcher Zhang Shengfang from China Academy of Inspection and Quarantine, Professor Bai Xuguang and Professor Zhou Yuxiang from Henan University of Technology, in case of any preliminary naming error.

3. 3. Investigation on Insect Species and Distribution

3.1 Catalogue of Stored Grain Insects of Five Provinces in Northwest China

Through this investigation on stored grain insects in the northwest region, 83 species of stored grain insects were identified, respectively belonging to 29 families in five orders, two classes (Class Insecta: 74 species in 22 families of Order Coleoptera, six species in four families of Order Lepidoptera, and one species in one family of Order Zygentoma and Order Hymenoptera respectively; and Class Arachnida: one species in one family of Order Chelonethida). There were 16 species of undetermined species, in which seven were natural enemies of stored grain pests.

3.2 Insect Distribution Difference in Different Grain Storage Environments

Main pests found in this insect investigation in northwest China totaled eight species, including *Sitophilus zeamais* (Motschulsky), *Sitophilus oryzae* (Linnaeus), *Rhyzopertha dominica* (Fabricius), *Tenebroides mauritanicus* (Linnaeus), *Bruchus rufimanus* (Boheman), *Araecerus fasciculatus* (Degeer), *Sitotroga cerealella* (Olivier) and *Plodia interpunctella* (Hübner). It is reported that *Sitophilus zeamais* (Motschulsky), *Sitophilus oryzae* (Linnaeus) and *Rhyzopertha dominica* (Fabricius) are the main wheat, corn and rice pests in many temperate and tropical countries [8, 9], while *Rhyzopertha dominica* (Fabricius) is more common in warm and dry wheat producing areas of China, Australia, India and Pakistan[10].

There exist obvious differences in species numbers collected in different storage environments. Grain, oil and feed processing plants usually have suitable temperature and humidity and difficulty in complete cleaning [11], especially the small flour and rice mills in rural area without any pest control measures, where more (72 in total) species of stored grain insects were found. As for grain storage enterprises, 50 species of stored grain insects were found, belonging to 24 families, four orders, in which 25 species were found in Sinograin depots, and 43 species were found in local grain depots. Relying on less stored grain types, standard management, regular fumigation and better storage conditions, the species number of pests founded in grain depots is less than that of processing plants. In recent years, few farmers store grains by themselves, and their grain storage environment is improved after the wide application of small steel barns, hence the occurrence of stored grain pests is reduced. As a result, only 26 species of stored grain insects were found in farmers' barns in northwest China. (See Table 2).

Table 2 Information of Stored Grain Insect Distribution in Different Environments.

Classification	Sinograin Depot	Local Grain Depot	Grain, Oil and Feed Processing Plant	Farmer
Order	3	4	5	4
Family	15	23	26	17

Species	25	43	72	26

3.3 Insect Distribution in Stored Grain Ecological Regions

The investigation on stored grain insects in northwest China involves three stored grain ecological regions, the first, second and fourth ones, covering five provinces. The species number of stored grain insects in each ecological area remains in a range between 46 and 60 (Tab. 3). Wang Dianxuan, et al. [12] discovered 16, 59, 34, 23 and 59 species of stored grain pests respectively in the flour mills of the third, fourth, fifth, sixth and seventh stored grain ecological regions. According to the overall data, the species numbers of stored grain pests in low-temperature and high-humidity stored grain region (the third region), medium-temperature and high-humidity stored grain region (the fifth region) and medium-temperature and low-humidity stored grain region (the sixth region) is less, which may be related to the limited numbers of sampling site, sampling point and sampling time.

Table 3 Species Numbers of Stored Grain Insects Collected in Different Stored Grain Ecological Regions (SGER).

Classification	High-cold and Dry Stored Grain Region (First)	Low-temp. and Dry Stored Grain Region (Second)	Medium-temp. and Dry Stored Grain Region (Fourth)
Order	4	5	4
Family	21	23	23
Species	53	60	46

4. Discussion

4.1 Analysis on Investigations on Stored Grain Insects in Northwest China

Totally, seven national investigations on the species and distribution of stored grain insects and stored product insects have been carried out within Chinese grain system, and because of partial overlapping, investigations in 1955, 1957 and 1956-1958 were recognized by this paper as an investigation, in which date related to investigation on stored grain insects in northwest China were sorted out in this paper. According to statistics, 199 species of stored grain insects were found in total, belonging to 40 families, eight orders, Class Insecta and Class Arachnida. Among then, Class Insecta: Order Coleoptera owns 174 species in 26 families, Order Lepidoptera owns 15 species in six families, Order Blattidae owns three species in two families, Order Hymenoptera owns two species in two families, Order Hemiptera, Thysanura, Diptera own one species in one family respectively; and Class Arachnida Order Chelonethida has two species in one family, comprehensively summarizing the stored grain insects in northwest China.

Zhao Yangchang, et al. carried out comprehensive investigation on pests in stored grain, oilseeds, livestock products, aquatic products, medicinal materials, archives, timber and other stored products in northwest China with an emphasis on stored grain pests in 1955-1960, discovering 113 species of store product insects in 33 families, six orders [13]. According to the data of the fourth national stored grain pest investigation organized by the Ministry of Commerce in 1974-1975 [14], the insect species distribution investigation group found 94 species of stored grain insects in 29 families, four orders in northwest China. The sixth national stored grain insect investigation organized by the State Grain Administration in 2004-2005 found 133 species of stored grain insects in 40 families, eight orders in the northwest region. Following the task stipulated in the 2015 Grain Public Welfare Industry Research Project – "Stored Grain Insect and Mite Region System Investigation and Pest Monitoring and Forecasting Technology Research" and the arrangement of the State Grain Administration's "Seventh National Stored Grain and Mite Investigation", a project team carried out investigation on stored grain insects in the northwest region from 2015 to 2017, indentifying and recording 83 species of stored grain insects in 29 families, five orders. According to previous investigation results, the species number of stored grain insects remained stable basically. However, due to the limitation of time, site and scope of these investigations, the species of stored

grain insects may not be comprehensive.

4.2 Representative Store Grain Insects in Northwest China

Among these four investigations in 1955-1960, 1974-1975, 2004-2005, and 2015-2017, 29 species of stored grain insects occurred in the northwest region in every investigation, basically covering the main stored grain pest species in China, namely *Cryptolestes ferrugineus* (Stephens), *Cryptolestes turcicus* (Grouvelle), *Tribolium castaneum* (Herbst), *Tenebroides mauritanicus* (Linnaeus), *Oryzaephilus surinamensis* (Linnaeus), *Ahasverus advena* (Waltl), *Stegobium paniceum* (Linnaeus), *Sitophilus granarius* (Linnaeus), *Bruchus pisorum* (Linnaeus), *Rhyzopertha dominica* (Fabricius), *Lyctus sinensis* (Lesne), *Ptinus japonicus* (Reitter), *Trogoderma variabile* (Ballion), *Cryptophilus integer* (Heer), *Carcinops pumilio* (Erichson), *Palorus ratzeburgi* (Wissmann), *Alphitophagus bifasciatus* (Say), *Alphitobius laevigatus* (Fabricius), *Tenebrio obscurus* (Fabricius), *Typhaea stercorea* (Linnaeus), *Migneauxia orientalis* (Reitter), *Thes bergrothi* (Reitter), *Holoparamecus ellipticus* (Wollaston), *Sitotroga cerealella* (Olivier), *Pyralis farinalis* (Linnaeus), *Ephestia cautella* (Walker), *Plodia interpunctella* (Hübner), *Tinea tugurialis* (Meyrick), and *Ctenolepisma villosa* Fabricius. Due to lack of data, poor test conditions, and other factors, difficulty in differentiating *Sitophilus zeamais* (Motschulsky) from *Sitophilus oryzae* (Linnaeus) may lead to an error in the 1955-1960 investigation. After combination of external genitalia anatomy and morphology was introduced into identification in 1975, differentiation between *Sitophilus zeamais* (Motschulsky) and *Sitophilus oryzae* (Linnaeus) and other allied species was finally achieved [1]. Therefore, all of the following three investigations in 1974-1975, 2004-2005 and 2015-2017 found *Sitophilus oryzae* (Linnaeus) in the northwest region, while *Sitophilus oryzae* (Linnaeus) found in the 1955-1960 investigation may be mistakenly identified as *Sitophilus zeamais* (Motschulsky).

The representative stored grain insects in the northwest region (incl. the first, second and fourth stored grain ecological regions) listed in the *Technical Specification for Grain and Oil Storage* (GB/T 29890-2013) total 13 species, namely *Sitophilus zeamais* (Motschulsky), *Sitotroga cerealella* (Olivier), *Plodia interpunctella* (Hübner), *Oryzaephilus surinamensis* (Linnaeus), *Tenebroides mauritanicus* (Linnaeus), *Tribolium castaneum* (Herbst), *Tribolium madens* (Charpentier), *Attagenus augustatus gobicola* (Frivaldszky), *Trogoderma variabile* (Ballion), *Niptus hololeucus* (Faldermann), *Gibbium psylloides* (Czenpinski), *Ptinus japonicus* (Reitter), and *Sitophilus granarius* (Linnaeus) (Xinjiang) [15]. In this investigation, *Sitophilus zeamais* (Motschulsky), *Sitotroga cerealella* (Olivier), *Plodia interpunctella* (Hübner), *Oryzaephilus surinamensis* (Linnaeus), *Tenebroides mauritanicus* (Linnaeus), *Tribolium castaneum* (Herbst), *Attagenus augustatus gobicola* (Frivaldszky), *Trogoderma variabile* (Ballion), *Niptus hololeucus* (Faldermann) and *Sitophilus granarius* (Linnaeus) were found, but *Tribolium madens* (Charpentier) *Gibbium psylloides* (Czenpinski), and *Ptinus japonicus* (Reitter) were not found. According to the newest *Stored Product Beetle*, *Tribolium madens* (Charpentier) *Gibbium psylloides* (Czenpinski), and *Ptinus japonicus* (Reitter) never occurs in China [16]. Therefore, the previous records may be naming errors. *Gibbium aequinoctiale* (Boieldieu) has been mistakenly recognized as the allied species of *Ptinus japonicus* (Reitter) in some domestic references, which coincides with the fact of discovery of *Gibbium aequinoctiale* (Boieldieu) rather than *Ptinus japonicus* (Reitter) in this investigation.

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Session 3
Detection and Monitoring

Stored Product Insects at a Rice Mill: Temporal and Spatial Patterns and Implications for Pest Management

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Abstract

Monitoring is fundamental to integrated pest management programs since it provides feedback on effectiveness of prevention programs and helps with targeting interventions as needed and evaluating their effectiveness. Rice mills are spatially complex facilities that have a combination of rough rice storage bins, buildings where rice is milled and processed, and warehouses and bulk storage bins where finished product is held before shipment. Each of these structures can have its own suite of insect species, different levels of risk, as well as different suites of management tools available. At a large rice mill in Brazil, stored product insect activity was monitored using food bait traps placed around rough rice receiving areas and storage bins; inside building containing white rice mill, rice flour mill, and packaging; and inside building for processing parboiled rice. The facility was monitored from 2010 to 2018 with 100 traps. Major pest species recovered at the facility included *Sitophilus oryzae, Sitophilus zeamais, Rhyzopertha dominica, Tribolium castaneum, Cryptolestes ferrugineus, Ahasverus advena*, *Oryzaephilus surinamensis*, *Typhaea stercorea, Anthicus floralis*, and Nitidulidae species. Temporal and spatial patterns in abundance were evaluated for each of the major species and for major functional groups (primary feeders, secondary feeders, and fungal feeders). Monitoring data generated was used to guide pest management programs and also provided the information needed to develop management thresholds.

Keywords: rice, monitoring, *Sitophilus* spp., *Rhyzopertha dominica*, *Tribolium castaneum*, spatial distribution.

Introduction

Rice is one of the three major food crops of the world, along with corn and wheat. After harvest, rice is vulnerable to infestation by a suite of stored product insect species as it is stored and processed. Rice mills consist of a complex of structures, including structures such as metal bins or concrete elevators where rough rice is stored in bulk, the mill building where the hull and outer bran layer is removed, milled rice storage structures which are typically warehouses for packaged rice, and storage areas for waste material such as rice hulls. Some facilities also have other structures or areas where additional processing occurs such as parboiling or milling into rice flour. These different areas of a rice mill complex are all vulnerable to stored product insect infestation, although the distribution of species and their inherent risk varies with area. Integrated Pest Management (IPM) for rice mills relies on a range of tactics to deal with insect infestation issues, including fumigation of rough rice and packaged rice with phosphine, treatment of mill building and warehouses with structural treatments such as fumigants or heat or aerosol insecticides, spot and surface insecticide treatments. However, the major focus of IPM for food facilities needs to be on prevention of insects entering storage and processing areas, and ultimately into the finished product. Stored product insects can be captured in large numbers outside of rice mills (McKay et al. 2017), so understanding patterns of activity outside of rice mills and the impacts of management tactics to target these outside populations is critical.

Brazil is a top 10 worldwide rice producer and the state of Rio Grande do Sul is the largest rice producing region in Brazil. The objective of this study was to monitor insect activity in and around a large rice facility in this region of Brazil. A high density of traps in place across multiple years was

used to determine the community of insect species that are active at the mill, seasonal patterns of activity, and the spatial patterns of distribution. This information is useful in determining times and areas at greatest risk and also for providing information to guide pest management programs and evaluate their success.

Materials and Methods

Stored-product insect abundance was monitored at a large rice facility located in southern Brazil. The rice facility included rice receiving areas, drying facilities, metal storage bins for holding rough rice, a structure for white rice and rice flour milling, and packaging/warehouse, and a structure for parboil rice manufacturing. Insects were monitored using 100 food-baited cage traps [based on Throne & Cline (1991) and adapted by Pereira (1999)] placed outside around the bins and inside the white rice and parboil rice plants. The bait used in the traps consisted of 150 g of whole corn kernels, broken corn kernels, whole rice, broken rice, whole wheat, and wheat germ that had been previously sifted and frozen for 7 days at -18ºC to kill any insect infesting the raw material. Personnel at the rice facility placed traps out for 15-day periods once a month and returned the traps so that the captured insects could be identified and counted. However, given the range of factors that arise from working with commercial operations, not all traps were returned for each monitoring period, there were gaps in the data collected, and for some of the early monitoring periods traps were out a couple times a month but only one of the 15-day intervals is presented here. Data are presented as the number of insects captured per 15-day period. Monitoring started in Jan 2010 and continued until January 2018.

Results

Across the total duration of the monitoring program, *Rhyzopertha dominica* was the most abundant species recovered, accounting for 47% of the stored-product species captured. Other pest species captured included *T. stercorea* (11%), *Sitophilus* spp. (8%), *Cryptolestes ferrugineus* (7%), *A. advena* (3%), *T. castaneum* (1%), and low numbers of *O. surinamensis* and *L. serricorne* (<1%, respectively). Sap beetles in the family Nitidulidae were the second most abundant group of insects in the samples, accounting for 22% of the species captured. Although was considerable variation in captures among years, for *R. dominica* there was a temporal pattern of greater captures during the summer months, between November and February (Fig. 1). This seasonal pattern also appeared to apply to *C. ferrugineus*, but captures of *T. stercorea* and *Sitophilus* spp. did not exhibit as strong a seasonal trend (Fig. 1). There was a significant relationship between average monthly temperature and total insect captures, with captures low and stable at average temperatures below 22°C and with peak captures around 26°C (Fig. 2).

Insect captures were greatest in the traps near the rough rice storage bins, followed by captures in traps in the receiving/drying area, with the least captures inside the rice mill and the parboil facility (Fig. 3). In the rough rice area, *R. dominica* (640±150 total adults/trap), Nitidulidae (262±106 total adults/trap), *Sitophilus* spp. (102±22 total adults/trap), *T. stercorea* (99±14 total adults/trap) and *C. ferrugineus* (75±22 total adults/trap) were the five most commonly captured species. In the receiving/drying area, *R. dominica* (273±103 total adults/trap), Nitidulidae (206±56 total adults/trap), *T. stercorea* (113±25 total adults/trap), *C. ferrugineus* (65±14 total adults/trap), and *Sitophilus* spp. (43±8 total adults/trap) were the five most commonly captured species. Inside the rice mill, *R. dominica* (34±15 total adults/trap), *T. stercorea* (29±14 total adults/trap), Nitidulidae (18±8 total adults/trap), *Sitophilus* spp. (15±2 total adults/trap, and *T. castaneum* (9±3 total adults/trap) were the five most commonly captured species. And inside the parboil facility, *R. dominica* (62±6 total adults/trap), Nitidulidae (61±24 total adults/trap), *Sitophilus* spp. (21±6 total adults/trap, *T. stercorea* (9±3 total adults/trap), and *T. castaneum* (5±2 total adults/trap) were the five most commonly captured species.

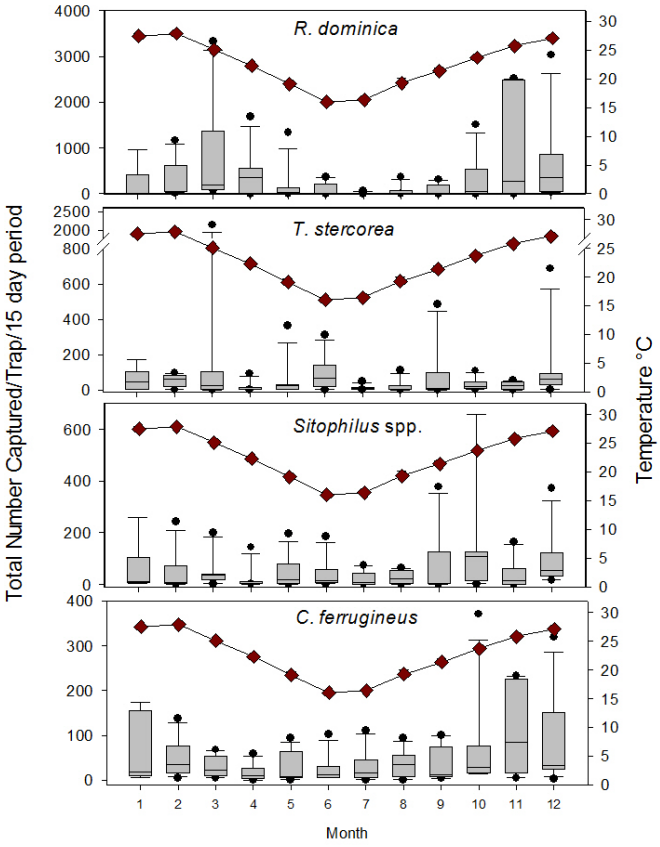

Fig. 1 The total number of each species captured per month across the eight years study shown as box plots, with 50% of data in the box, 95% in the whiskers, and outliers shown black circles. Average monthly temperatures obtained from a nearby weather station are shown as diamonds.

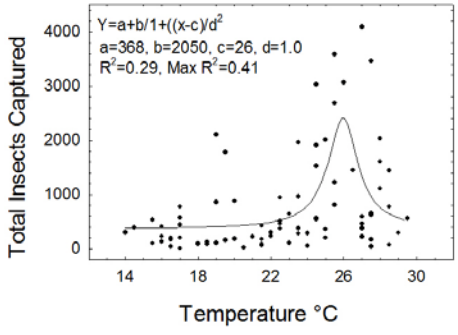

Fig. 2 Relationship between total number of insects captured and average monthly temperatures obtained from a nearby weather station.

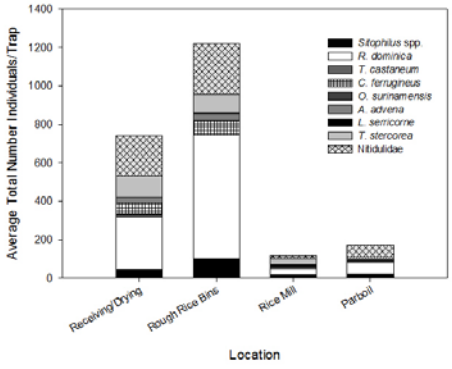

Fig. 3 Total number of individuals of each species captured over the course of the study in four areas of the rice mill facility.

Discussion

There were high levels of stored product insect activity throughout the rice mill facility, especially outside in the areas that handled rough rice – both the receiving and drying areas and the storage bins. Some of the major primary pest species were recovered in these areas, including *R. dominica* and *Sitophilus* spp. (*S. oryzae* or *S. zeamais*). These species were monitored outside of the rough rice bins, so it is not known how these activity levels relate to levels of infestation in the rice within the bin. Insects captured outside could originate from within the bins, from grain spillage accumulations onsite, or immigrate from offsite locations. However, these activity levels indicate the potential for insect movement into and out of the bins and potential for movement into the rice processing structures and ultimately into finished products (Campbell and Arbogast 2004; Campbell and Mullen 2004; Toews et al. 2006)

Stored product insect monitoring at other rice mill locations have indicated difference in relative species abundance, but these differences might be due to a combination of geographic location and monitoring method. In this study, food baited traps were used, but in other studies pheromone traps were used for monitoring. In Portugal, Carvalho et al. (2013), using pitfall traps with food and pheromone attractants inside a rice mill, found that *Sitophilus* spp. and *T. castaneum* were the most abundant species captured. In the USA, McKay et al. (2017) used pheromone-baited flight traps outside a rice mill and found that *Trogoderma variabile* was the most abundant species, although this species was not recovered at this rice mill in Brazil. High numbers of *Plodia interpunctella* were also captured in the McKay et al. study, and they were also present at this Brazil rice mill location, but were primarly captured in light traps and not in the bait traps. At the USA rice mill, *R. dominica* was captured in high numbers, but few *Sitophilus* spp. were captured, probably due to the monitoring method. Interestingly, at this Brazil rice mill the insect community inside the rice mill and parboil structures was similar to that in the rough rice areas, although overall numbers were much lower. In other studies, *Tribolium castaneum* is one of the most abundant and economically important pest species inside mills (Buckman et al. 2013).

Activity of stored product insects in this current study and in others has tended to show seasonal patterns. Temperatures inside rice mills tend to track those outside the mills and to be associated with levels of insect activity inside mill (Buckman et al. 2013). Captures of insects at this Brazil rice mill was associated with temperature, but rather than a linear or increasing relationship there appeared to be a threshold below which there was lower insect captures and above which there tended to be a peak in captures around 26°C. It is often difficult to determine consistent relationships with temperature (e.g., Carvalho et al. 2013; McKay et al. 2017) most likely due to other variables such as movement of grain and treatment activity having strong influences on abundance. Outside temperatures and captures of stored product insects in flight traps were positively related at a USA rice mill, but only at temperatures above 15°C and the nature of the relationship varied with species and year (McKay et al. 2017). Given the multi-year duration of this current monitoring study, it provides the opportunity to detect patterns that might otherwise be missed.

Understanding the stored product insect community at a location and its temporal and spatial patterns of distribution provides the foundation for IPM programs. Given the variation among locations this information is important in developing site-specific programs and for the continual evaluation of program success. The data from this study can be further evaluated to relate activity to specific locations and with management tactics implemented during the study. Understanding outside and inside insect activity can provide important insights into the sources of insect infestation and help more effectively target pest management.

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endorsement by the U.S. Department of Agriculture. The US Department of Agriculture is an equal opportunity provider and employer.

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From stored-product psocids to the other pests: the developments, problems and prospects on research and application of molecular identification

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Abstract

Psocids, beetles, moths and mites are regarded as the common kinds of stored-product pests in the world. The rapid and correct identification of stored-product pests is significant for quarantine, monitoring and control purposes. Molecular methods and techniques have been studied and applied for stored-product pest identification. Based on collection and analysis of literature in the last decade, this paper reviews the developments, questions and prospects for molecular identification of stored-product pests. As a representative model, the molecular methods and techniques for species identification of stored-product psocid pests were developed and applied systematically based on international collaboration involving China, Czech Republic, the United States and other countries. More than 10 studies on stored-product psocids related to RFLP, DNA barcoding, PCR, real-time PCR and gene chip have been published during this decade. Subsequently, DNA barcoding, PCR and real-time PCR techniques for the identification of common species of *Tribolium* and *Cryptolestes* pests of stored products have been reported by the same international team. Recently, a web system called Grain Pests DNA Barcode Identification System (GPDBIS) has been established in China using SOL SERVER and C#. Like a marathon that requires persistence, we should do our best to continue to promote research and application of molecular identification of stored-product pests with more international collaboration.

Keywords: stored-product pests, molecular identification, review, research, application

Globally, stored-product arthropod pests include a large number of species. The rapid and correct identification of stored-product pests is significant for quarantine, monitoring and control purposes. In recent decades, molecular methods and techniques have been studied and applied for stored-product pest identification. There is quite a substantial amount of literature related to stored-product pests and their molecular identification. In this work, literature from 1900 to 2017 was collected and analyzed using Web of Science (http://apps.webofknowledge.com/). The total count of articles on stored-product pests was found to be 32,123 whereas the total count of articles on molecular identification of stored-product pests was 179. The years with the highest counts for these two categories were 2015 and 2012, respectively. In decreasing order, countries with the most contributions to literature on stored-product insect pests were USA, China, UK and India (Figure 1).

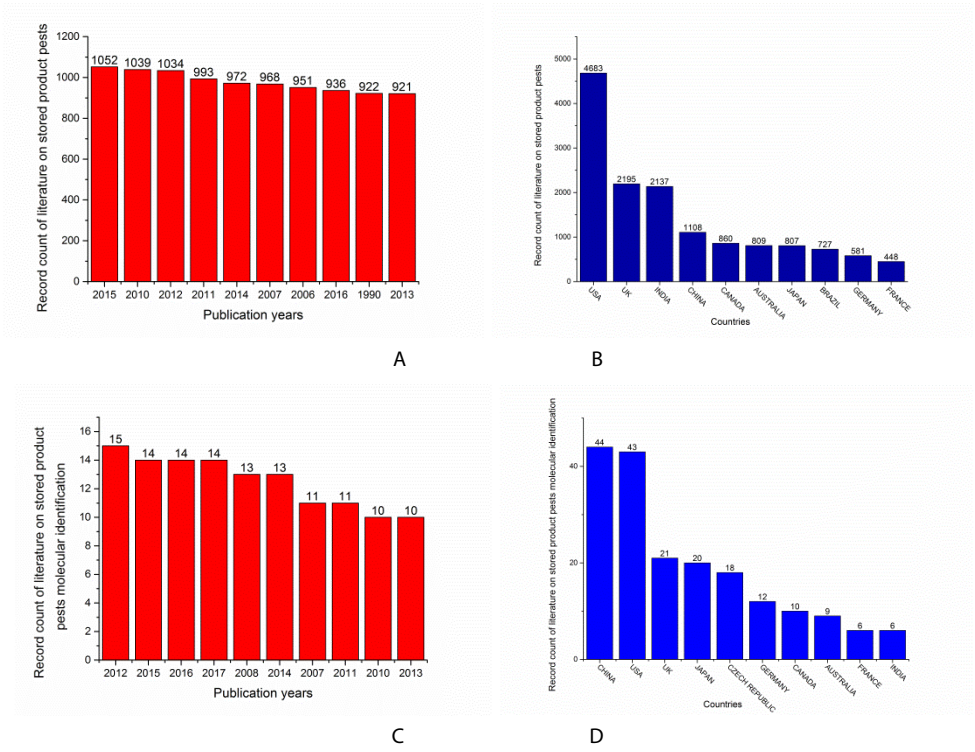

Fig. 1 The top 10 contributors to literature on stored-product pests and molecular identification. A: top 10 years of stored-product pest literature, B: top 10 countries contributing to stored-product pest literature, C: top 10 years of literature on molecular identification of stored-product pests, D: top 10 countries contributing to literature on molecular identification of stored- product pests.

Psocids, beetles, moths and mites are the common kinds of stored-product pests. From the number of articles on molecular identification of stored-product pests, most of the research and application have obviously been in the last 10 years (Table 1). As a representative model, the molecular methods and techniques for species identification of stored-product psocid pests were developed and applied systematically based on international collaboration involving China, Czech Republic, the United States and other countries. More than 10 articles, theses and dissertations on molecular identification of stored-product psocids have been published between 2008 and 2017; methods used including RFLP (Qin et al. 2008; Qin 2009), DNA barcoding (Li et al. 2011; Yang et al. 2012; Cui 2013; Yang et al. 2013b; Yang 2014), PCR (Arif et al. 2012; Yang et al. 2013a; Zhao et al. 2016), real-time PCR (Pang 2017), and gene chip (Liu et al. 2017). Recently, this team discovered the highly divergent mitochondrial genomes and indicated that *Liposcelis bostrychophila* was a cryptic species, which provided a taxonomic basis for species identification of stored-product psocids (Feng et al., 2018). Subsequently, the techniques such as DNA barcoding, PCR and real-time PCR have been reported for the identification of common species of *Tribolium* (Wang 2015; Zhang et al. 2016; Zhang 2017), *Cryptolestes* (Wang et al. 2014; Varadínová et al. 2015; Chen, 2018) and predatory mites (Wu et al. 2016) by the same international team. For more application of DNA barcoding, a web system which was entitled as Grain Pests DNA Barcode Identification System (GPDBIS) has been established in China using SOL SERVER and C# (Figure 2) (Li 2016; Wu et al. 2017).

Tab. 1 Number of articles on molecular identification of common stored-product pests during the period 2008–2017

Arthropods	2017	2016	2015	2014	2013	2012	2011	2010	2009	2008	Total
Beetles	9	7	10	8	7	8	3	4	2	8	66
Moths	3	2	1	1	2	2	0	2	0	0	13
Psocids	1	1	1	1	2	3	4	3	0	2	18
Mites	2	3	0	1	0	0	1	1	0	0	8
Total	15	13	12	11	11	13	8	10	2	10	105

A B C

Fig. 2 The main pages of molecular identification in GPDBIS. A: page of sequence input, B: page of sequence similarity, C: page of phylogenetic tree

Globalization accelerates the spread of stored-product pests among different countries and regions. What are the related questions and prospects for research and application on molecular identification of stored-product pests? Apparently, there is more need for molecular identification and common action for the prevention and control of stored-product pests. There is still a gap between the research and application. Like a marathon that requires persistence, we should do our best to continue to promote research and application of molecular identification of stored-product pests with more international collaborations that involve the sharing of more representative samples, development of more practical techniques, and establishment of a more common platform through further research, training and application.

Acknowledgement

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Enhancing surveillance for exotic stored pests in the Australian grains industry using a partnership approach with industry and government.

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Abstract

Verifying freedom from exotic pests such as Khapra beetle (*Trogoderma granarium*) & Karnal Bunt (*Tilletia indica*) is critical to supporting & maintaining access for Australian grain producers to international markets. Despite Australia's geographical isolation & strong quarantine systems, increasing levels of travel & trade continues to place pressure on our biosecurity systems, emphasising the need for improving our regional efforts in prevention, preparedness & surveillance to mitigate risks. The Australian Grains Farm Biosecurity Program (GFBP) is a national initiative to assist in the development & implementation of improved biosecurity practice, playing a vital role in the education of exotic pests & the role of surveillance by industry. The GFBP has undertaken a targeted surveillance program for stored product pests, with Khapra beetle as the main focus. A range of sites based on potential risk groups & pathways (e.g. farming enterprises, seed distributors & agricultural stores) were targeted, with different approaches used across the three grain growing regions of Australia depending on State

activities & pre-existing collaborators. All regions used a combination of pheromone traps & other sampling methods appropriate for host materials & environment. The surveillance is aimed at strengthening evidence of absence, building industry knowledge & participation in grain storage surveillance & promoting improved management practices around storage. These regionally specific engagement methods & surveillance efforts are discussed. Australia remains free of Khapra beetle.

Keywords: grains biosecurity, exotic pest surveillance, on-farm storage and hygiene, risk mitigation practices

Introduction

Exotic plant pests threaten production, market access and sustainability of Australian plant production systems. For the Australian grains industry, over 600 exotic pests have been identified of which 54 are considered high priority pests (HPPs), posing a significant threat. Despite Australia's geographical isolation and strong quarantine systems, increasing levels of travel and trade continues to place pressure on our biosecurity systems, emphasising the need for improving our regional efforts in prevention, preparedness and surveillance to mitigate risks. Verifying freedom from HPPs such as Khapra beetle (*Trogoderma granarium*) and Karnal Bunt (*Tilletia indica*) is critical to supporting and maintaining continued access for Australian grain producers to domestic and international markets (including biosecurity, food safety and quality assurance aspects).

Currently many surveillance activities are done through crop monitoring in the field and sample assessment through the bulk handling system, but little useful data is captured at the national level with regards to exotic stored grain product pests. This type of data is limited, and gaps exist particularly on-farm, where on-farm storage of grain is increasing and becoming common practice, particularly in eastern Australia. Thus, expanding surveillance efforts regionally on-farm to capture more evidence of absence is required.

The national Grains Farm Biosecurity Program

Within Australia, the Grains Farm Biosecurity Program (GFBP), a national initiative to assist in the development and implementation of improved biosecurity practice, plays an instrumental role in awareness and education about exotic pests (Taylor-Hukins et al, 2015). As Australia's flagship biosecurity extension program, the GFBP contributes to the Australian grains industry's risk mitigation activities (under the formal signing of government / industry agreements around biosecurity and emergency response (PHA, 2016) and has now been running for 10 years. The GFBP was acknowledged with a national biosecurity award in 2018 for its contribution to biosecurity and promoting a partnership approach involving government, industry and community (http://www.agriculture.gov.au/biosecurity/australia/public-awareness/aba#australian-biosecurity-award--government).

The GFBP emphasises the importance of surveillance and reporting by industry stakeholders to support and maintain market access and to detect an incursion early, increasing the likelihood of early detection and facilitating the eradication or containment thus reducing its impact on industry and community. A strength of the program is the ability to build collaborative networks for a wide range of activities at national, state, regional and local levels (Bellati et al 2010). This strength has been used to encourage general surveillance and the collection of data for key exotic grain pests through a variety of industry reporting avenues: e.g., National variety trails, state diagnostic laboratories, bulk handlers, researchers, industry consultants and grower groups. Data has been captured from over 90 surveillance programs from a range of broad acre crop types.

Whilst surveillance has been one of the GFBP key activities, it has also been one of the most challenging to execute and maintain, as its voluntary and relies on the 'good will' of those contributors.

The Project (Objectives)

The GFBP recently piloted a targeted surveillance monitoring program for stored product pests with the main target being Khapra beetle (*Trogoderma granarium*), one of the highest ranked exotic pests

for grains which is also listed as a prohibited or invasive species for many of Australia's export trading partners.

The key aims of the project were to strengthen evidence of absence data for Khapra beetle and to build industry awareness, knowledge and participation in grain storage surveillance regionally within the grain-growing regions of Australia. The program also aimed to identify and promote industry advocates and to assist industry in promoting and improving management practices around grain storage, especially in hygiene, and improving the use and efficacy of phosphine application in on-farm storage systems.

The Approach (Methods)

For the pilot surveillance program to be successful, it was imperative to use a partnership approach with reputable programs, networks and alliances for effective industry engagement and uptake. Benefits to using a partnership approach also allowed for a wider coverage of locations, took advantage of cost-sharing for required resources and ensured we were value-adding to contributors.

Different approaches for implementation were used across the three grain regions of Australia (southern, western and northern zones), depending on types of linkages and pre-existing collaborators, state government surveillance activities and industry networks and alliances that could assist and were willing to participate.

A range of sites based on potential risk groups and pathways were targeted. These included privately owned farming enterprises (grain, mixed production and intensive animal production systems), milling, processing and bulk handler establishments, importers of high risk materials, seed distributors, grain/ stock and/or feed producers/ wholesalers and regional agricultural re-sellers.

Host materials and target environments included: older silo systems; products with slow turnover/ minimal fumigation routines; longer term storage, containers and bulker bags (feed /seed, fertiliser/ baits, by-products, other); stockfeed and other dry food stuffs; packing/ bagging materials; cracks/construction joints in cement walling near product storage; areas of low hygiene and inadequate sanitation (within sheds, barns and around machinery and product storage areas) and; dark, dry and low movement corners spaces in processing and production areas.

A range of complimentary sampling techniques appropriate for host materials and favourable environments, for Dermestidae species, were used. These included vacuuming and visual inspection of grain and other host materials, and pheromone specific traps which improved participation and industry engagement due to its novelty.

Access to expert diagnostic support for identification of Dermestidae species was a critical component to the program and states had access to a service (paid or provided in-kind).

Regional specific (State) focus and development of surveillance efforts in 2016-2017 included:

- *Queensland (Qld):* Growers (grower groups) targeted - surveillance and monitoring is occurring, but not formally recorded; potential to develop a storage best management practice / accreditation based around storage and monitoring in conjunction with Qld grains storage research and extension team.
- *New South Wales (NSW):* Targets included privately owned farms, warehouses importing high risk products (e.g. rice, pulses, seed and spices), feedlots and stock-feed manufacturers and wholesalers. Partnered with regionally based NSW Local Land Services (NSW Govt.) that provided staffing to service the traps.
- *Victoria (Vic):* Intensive farming enterprises (e.g. poultry, feed lots, dairy) were targeted due to their tendency of having poorer hygiene practices around grain storages. Also, targeted grain mix and stock-feed manufacturers. The CropSafe program is being used for diagnostics support (http://agriculture.vic.gov.au/agriculture/grains-and-other-crops/cropsafe-program).

- *South Australia (SA):* Significant State government support provided an extensive extension of the program that allowed for a wide coverage of locations and a wider range of target groups surveyed compared to other states which included producers, milling/ processing, stock feeders, bulk handler/ seed distributors, agri-suppliers, regional high school, and a regional research centre. Program was also promoted across the supply chain through a State campaign (http://www.pir.sa.gov.au/primary_industry/crops_and_pastures/clean_grain).
- *Western Australia (WA):* Commercial agronomists targeted, and value added to the existing sentinel merchants and agronomist activities under the Biosecurity eSurveillance projects in WA, which was modelled on the successful Pantry Blitz campaign (an externally funded 'citizen science' project that demonstrated absence of Khapra beetle in WA with 2,252 reports (pers comm. L. Fagan, Department of Primary Industries and Regional Development, WA).

Outcomes (Results)

Over 100 target sites were surveyed and over 1000 'zeros' scored against Khapra beetle in 2016-17. The surveillance data captured is compliant with the Australian national minimum dataset specification for plant health surveillance and was entered into AUSPestCheck, a national database for plant pest surveillance (http://www.planthealthaustralia.com.au/resources/auspestcheck/).

As the program is currently on-going the large sample size being generated allows for comparisons and evaluation, in terms of target group risk profiles, suitability and effectiveness of trap types and where closely related Dermestidae species are found on-farm and within the farming environment regions.

The challenges and considerations identified to date included:

- trap positioning and suitability on farm; every place is different; trial and error due to other factors (e.g. abiotic and biotic factors)
- time length the traps and lures stayed out in the environment (dependent on remoteness of location and who could assist to service traps)
- surveillance program rigor, uniformity and geographical coverage across the regions
- finding voluntary participants and concerns of confidentiality
- reliability of contributors and their ability (skills and training requirements)
- processing and pre-sorting of multiple samples (stored grain insect identification training required)
- value of by-catches (non-targets) to producers and others (e.g., researchers)
- new technologies to assist / trial and to improve automation for data collection
- use of postal services (for sending lures to participants) to help reduce travel costs associated with servicing traps

Discussion

Over 200 industry advocates were identified during the surveillance activities in 2016/17. While there were mixed results within the regions in terms of industry engagement, in general the benefits of the program were positive overall and provided valuable insights.

Anecdotal evidence shows a higher level of learning and training is being sought by producers, with extension moving from simple awareness to more technical and specific information for their farming enterprise.

There was value in the by-catches for grower engagement as it provided insights into species composition within their own farming environments. Producers known to have a closely related Dermestidae species present in their farming system or operations, will hopefully help them to implement improved management practices and encourage extra vigilance in their operations.

Practice change especially around improvements to hygiene of grain storage was observed in many participants throughout the surveillance program.

In South Australia (SA), the programs significance was also acknowledged through additional industry funding (in the form of a SA grains industry trust grant to state diagnostics) as the extension of the program provided a unique opportunity to investigate the by-catches and the related native species composition in SA. The grant has allowed for further analysis, curation and permanent lodgement of reference material into a nationally recognised collection (Waite insect and Nematode Collection).

Biosecurity strategies emphasis the need for industry and community participation. Clearly this type of biosecurity surveillance program is a lot of work, expensive and time consuming, but has made a beneficial contribution in the collection of proof of absence data and industry awareness and education. Future engagement, cost effective resourcing, collaboration and value adding are required along with evaluating the real value of this type and source of surveillance data.

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Testing Wheat for Internal Infesting Insects with an Electrically Conductive Roller Mill

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Abstract

Although grain is always inspected for adult insects and insect damaged kernels upon shipping and receiving, immature insects living inside the kernels of grain cannot be readily detected. A laboratory roller mill was modified to measure and analyze the electrical conductance of wheat as it was crushed. The electrical conductance of normal wheat kernels is low and fairly constant. In contrast, the electrical conductance of infested wheat kernels produces a sudden change in the electrical signal. The peak height of the electrical spike depends on the size of the larvae and the resulting contact of the crushed larvae between the rolls. This instrument was designed to test wheat with moisture content of 13.5% or less. The laboratory mill can test a kilogram of wheat in less than 2 min. Hard red winter and soft red winter wheat samples were used in experiments. Known numbers of infested kernels were added to the wheat samples. The infested kernels contained larvae of rice weevils and lesser grain borers sorted into large, medium, and small size groups. The instrument detected ~8 of 10 infested kernels per 100 g of wheat with large-larvae (fourth instar or pupae). It detected ~7 of 10 infested kernels with medium-larvae (second or third instar) and ~5 of 10 infested kernels infested with the small-larvae (first or second instar). Under

reasonable grain moisture and careful sample handling, there were no non-infested kernels classified as insect infested. The mill can lead to rapid and automated detection of infested wheat.

Keywords: rice weevil, lesser grain borer, x-ray, insect fragment test

Introduction

Grain is commonly inspected for insect contamination using visual indicators such as sieving for adult insects or inspection of a 100-g sub-sample for insect-damaged kernels (GIPSA, 2009). However, internal infestations by insects such as *Rhyzopertha dominica*, lesser grain borer, and *Sitophilus* spp. are not easily detected with visual methods alone. With subsequent storage, these hidden infestations can lead to increased pest populations that require treatment such as fumigation and potentially contaminate resulting flour with insect fragments. Many methods of detecting infested wheat have been developed and are available, but all are relatively expensive and/or time consuming. Some of these methods include staining the wheat to detect weevil egg plugs (Milner et al. 1950), microphones for listening to insects feeding (Hagstrum et al. 1990), single kernel compression testing (Pearson et al. 2003), single kernel NIR measurements (Dowell et al. 1998, Perez-Mendoza et al. 2003, 2005), and x-ray imaging (Karunakaran et al. 2004; Haff and Slaughter 2004, Fornal et al. 2006).

X-ray images provide accurate determinations of infested seeds and larvae stages and number of internally infested kernels. However, x-ray systems are expensive and are only able to test a single layer of wheat and small sample sizes. NIR systems were able to correlate actual and predicted fragment levels over a range of 0 to 300 fragments. However, measurements below 100 fragments contained too much variability to clearly determine whether the flour is above the FDA (1988) defect level of 75 fragments from an average of six 50-g flour samples. Fragments in flour are estimated using a chemical method, AOAC 972.32.

The laboratory mill developed by Pearson and Brabec (2007) monitors electrical conductance through crushed wheat. The conductance mill can detect over 70% of the kernels infested with medium and large larvae and pupae, and is able to test 1 kg of wheat in about two minutes. If infested grain is detected, management could react by rejecting the lot, fumigating, storing the lot separately, or quickly milling the grain before insects have time to multiply. The objective of this study was to investigate the ability of the conductance mill to detect different size larvae in infested kernels (experiment 1) and to determine relationship between insect detections and subsequent insect fragment counts in milled flour (experiment 2).

Materials and Methods

A laboratory roller mill was fabicated and consisted of two, 8 cm diameter by 10 cm wide rolls which were mounted on a 2.5 cm diameter shaft. One mill-roll was electrically grounded through the gear motor. The slave roll was mounted into delrin bearings which made the roll electrically isolated. A 5 Vdc supply was electrically connected via a motor brush and contacted this roll. A schematic diagram of the system is shown in Figure 1.

Hard red winter wheat was obtained from a farm in central Kansas at time of harvest and stored in small barrels in a large refrigerator. This wheat was considered non-infested. The grain was cleaned by passing it through a Carter Dockage tester (Carter-Day, Minneapolis, MN) using the dockage configuration for wheat. The moisture content of the wheat was 12.0%. For experiments, two moisture contents were created: 11% and 13%.

Experiment 1. Approximately 250 *R. dominica* or *Sitophilus oryzae*, rice weevil, adults were added to ~500g of wheat which was tempered to 13% moisture and stored at 27°C for 4-5 weeks. This infested wheat was x-ray imaged (MX20-dc44, Faxitron X-ray Corp., Wheeling, Il.) and infested kernels with large, medium, and small larvae were selected based on the x-ray images (Fig. 2).

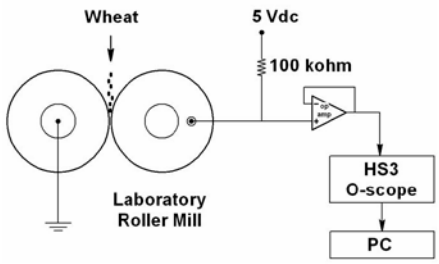

Fig. 1 Schematic diagram of the electrically conductive wheat mill and the associated circuit and basic data acquisition.

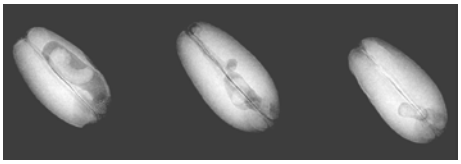

Fig. 2 X-ray image of infested kernels showing the large, medium, and small sized larvae from rice weevil.

Then 10 infested kernels of a given larvae size were added to 100g of sound wheat and crushed in the conductance mill. A micro-controller (Model EL, Tern Inc. Davis, CA) collected and processed the derivative of the conductance signal. The insect counts for a wheat sample were intermittent signal spikes above the baseline of the derivative signal (Fig. 3). The number of detects were recorded. Experimental variables were 11% and 13% moisture content wheat, rice weevil and lesser grain borer infestations, and three larval size categories.

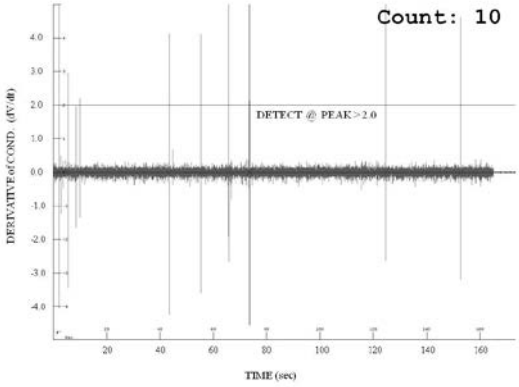

Fig. 3 Example of the software output and the derivative signal collected during the conductance milling of a 1 kg wheat sample. The signal spikes are from infested kernels containg large lesser grain borer larvae as they were compressed between the mill-rolls.

Experiment 2. To study the relationship between conductance detects and insect fragments in flour, infested kernels with lesser grain borers were prepared and added to 1 kg of sound wheat. A spoon of infested grain (~7.0 g) was taken from insect colonies and placed in small plastic bags, and x-rayed. The x-ray images were inventoried for infested kernels. For experiment #2, the initial infested kernels in the plastic bags had size distributions of ~60% large larvae and ~40% medium and small larvae. The original colonies were prepared and adults were removed after 3 weeks causing a bias in the larvae size distribution. The bags of infested kernels were added to the 1 kg of sound grain at three levels of infestation: 11-13 infested kernels (low), 23-25 infested kernels (medium), or 47-49 infested kernels (high) (Brabec et al. 2010). The infested grain samples were evaluated using the conductance mill at two times; day 0 and after six weeks of storage. After six weeks, any emerged adults were removed by sifting and the remaining grain was passed through the conductance mill.

Before each conductance test, a 300 g portion of clean wheat was passed through the conductance mill and the pre-sample was discarded. Then, the 1 kg test sample was passed through the conductance mill. The crushed wheat samples were bagged and stored at 7 °C until they were milled into flour for fragment testing.

Crushed samples were further milled using the Quadramat Jr. milling system (Quad Jr.) and AACC Experimental Milling method 26-50. Flour samples were sent to two U.S. cereal chemistry laboratories for insect fragment analysis. Both laboratories used acid hydrolysis methods. However, laboratory #1 performed the AOAC protocol (1996) using a five minute heating cycle in an autoclave at 121°C and 103 kPa. Laboratory #2 performed the AACC method 28-41b, using a 15 minute heating cycle in the autoclave. A single technician from each laboratory performed the wet chemistry and counted the fragments on filter paper using microscopy techniques.

Results

Experiment 1. The conductance mill is able to detect internal insects, but its ablility to detect varies with the size of the internal larvae. Small larvae (1st-2nd instar) were only detected on average ~50% of the time. The standard error of estimate for the small larvae was 1.5, thus for some samples with small larvae, only 2-3 infested kernels were detected. The large larve and pupae were detected ~80% of the time (Tab. 1).

Tab 1 Detection levels of the 10 infested wheat kernels within 100 g of wheat using the conductance mill for three different size classes of internally infesting larvae of *Rhyzopertha dominica* and *Sitophilus oryzae*.

	Number (+/-SE) of Infested Kernels Detected	
Larvae	***R. dominica***	***S. oryzae***
large	7.9 (1.4)	8.6 (1.1)
medium	7.1 (1.6)	7.7 (1.2)
small	5.5 (1.5)	6.3 (1.5)

Experiment 2. For the 1 kg samples prepared for the insect fragment testing, the infested kernels were a mixed population. At week 0, the lowest density infested samples had detection of ~75% of the infested kernels while the high density infested sample had detection of ~56% of the infested kernels. While accuracy was lower, detection of 28 infested seeds in a kilogram of wheat is already above the level that should raise concerns and therefore the reduced count accurary may be less of an issue. After samples were incubated for 6 weeks, the lowest infested sample went to 67 detects while the highly infested sample went to 120 detect. The insect fragment counts were significantly different between the two commercial laboratories. For laboratory #2, the week 0 samples all had insect fragments below the FDA threshold of 75. For laboratory #1, the fragment counts tended to be higher, even the control samples had fragment counts averaging over 15 counts.

Tab 2 Detection of infested seeds with the conductance mill for mixed infestions in a 1 kg sample of wheat. After the conductance milling, the crushed material was milled for flour and tested for insect fragments.

	Number (+/-SE) Detections	
Infested kernels	**0 wks**	**6 wks**
Control	0 (1)	2 (1)
Low 12	9 (1)	67 (11)
Med 25	16 (2)	88 (16)
High 50	28 (2)	120 (20)

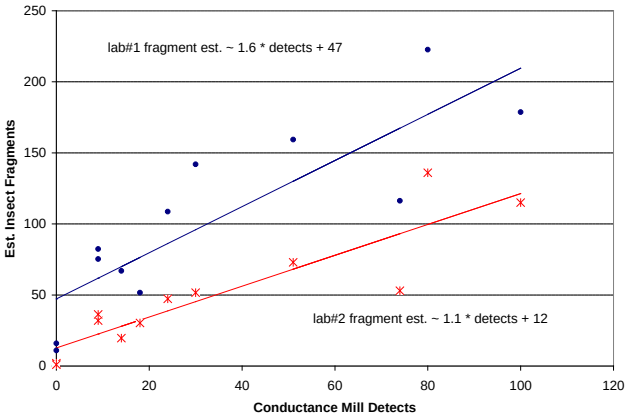

Fig. 4 Estimated insect fragment count verses the conductance mill detects. Two commercial cereal chemistry laboratories analyzed the samples; lab#1 and lab#2.

Discussion

The biggest challenge for sampling wheat for internally infesting insects is that detection is time consuming and that large amounts of wheat need to be sampled to detect low infestation levels. The conductance mill can process 1 kg in a couple minutes thus it is possible to quickly test wheat as grain is received from trucks or rail. Storey et al. (1982) studied over 2,000 wheat samples from many U.S. export grain terminals and found ~8% contained rice weevils and ~6% contained lesser grain borers after incubation, although less than 1% of their samples were graded as "weevilly". Perez-Mendoza et al. (2004) studied grain samples from eight rail cars, or 24 rail car compartments, at a grain processing facility. The study found that 20 of the 24 rail car compartments averaged less than one insect per kg of wheat. However, four compartments averaged 2, 6, 17, and 19 internal insects per 3 kg sample. Probing railcars and inspecting samples and storing samples for later insect emergence requires significant time and effort. Also, the visual sample obtained during inspection often did not match the internal infestation samples in terms of insect density. The conductance mill works well at detecting samples with lower infestation levels that are more realistitic in terms of what the industry needs to be able to detect. And the conductance mill can handle 15-20 kg of samples per hour as might be required while unloading railcars or truck.

There are different factors that can impact the accuracy of detection. False positive counts were caused by small clods of dirt in the wheat, so cleaning the wheat before processing by passing over some sieves is recommended. Also, any external moisture, such as rain or snow, could add signal noise, but usually this is not detected. Additionally, the conductance mill cannot detect internal infestations if the insects have died and are dried up, such as occurs after a fumigation and this will effect estimations of insect fragment levels in flour but will not be a factor in terms of estimating risk of insect population growth in a bin.

The conductance mill has also been test with rice and popcorn (Brabec et al., 2012, 2017). Rice is smaller than wheat and popcorn is larger than wheat, so each grain size needs appropriate mill gaps for the material to grind smoothly. For rice, the mill design included differential gearing during milling. Early test using 1:1 gearing and laboratory mill gaps of 0.018" and 0.028" show that detection decreased as roll gap increased, particularly from the small larvae (Pearson and Brabec 2007). Detection sensitivity was improved with the shearing action from differential rolls.

Acknowledgement

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Survey of *Trogoderma* species (Coleoptera: Dermestidae) Associated with International Trade of Dried Distiller's Grains and Solubles in the USA

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Abstract

Dried distiller's grains and solubles, DDGS, is a valuable commodity with substantial international trade. Vietnam discovered an infestation of *Trogoderma inclusum*, an actionable quarantine pest, in DDGS from the USA in 2012.

All subsequent shipments to Vietnam were required to be fumigated. A shipment to Vietnam from the USA 2015 was then discovered with *T. variabile*. We surveyed the presence and activity of *T. inclusum* and *T. variabile* at locations in the USA that provide DDGSs for shipment to Vietnam. Seven facilities in four states that either produced DDGSs or that facilitated bulk shipments were studied. Pheromone traps were deployed at each location and monitored for several weeks. *T. variaible* was trapped at all seven sites while *T. inclusum* was trapped at just five of these. *T. variabile* were captured in nearly every trapping period and at higher numbers than *T. inclusum* at five locations, while two locations captured more *T. inclusum* than *T. variabile*. Spatial variation seemed to occur within each site, but there was no common pattern among facilities. Substantial numbers of beetles were caught in the outdoor sticky flight traps for most locations, except for relatively low flight trap numbers at locations 1, 4 and 6. The results show that *T. variabile* and *T. inclusum* are commonly associated with DDGSs produced in the USA, that these beetles could infest product being shipped overseas, and provide information that can be used to develop risk assessment and pest management programs for the future.

Keywords: Coleoptera, Dermestidae, DDGS, Vietnam, quarantine.

Introduction

The United States Grains Council (USGC) learned in late 2012 that the Vietnamese government's Plant Protection Department (PPD) had discovered an infestation of the larger cabinet beetle, *Trogoderma inclusum*, an "actionable" quarantine pest for Vietnam, in a shipment of Dried Distillers Grains and Solubles, DDGS, from the US (USGC 2012). The Vietnam PPD required the infested shipment be fumigated and then re-exported. The PPD also required that all DDGS shipments from the US to Vietnam be fumigated before delivery from that time forward. The US DDGS industry complied with the required fumigation on all subsequent shipments. No infested shipments were reported in the subsequent three years, until a shipment of 12 containers of DDGS from Norfolk, VA on September 17, 2015 was inspected in Vietnam at arrival on October 27, 2015 and found to be infested with live warehouse beetles, *Trogoderma variabile*, a close relative to *T. inclusum*. It is presumed that this shipment had been fumigated at the time of export, as required by agreement. Assuming that fumigation was performed on the commodity before leaving the US, the infestation could have occurred via one of two ways: the fumigation was not entirely effective in completely disinfesting the shipment, or that infestation occurred after the Norfolk fumigation, but before the delivery in Vietnam six weeks later.

Both *T. variabile* and *T. inclusum* are stored grain insect pests that are commonly found in the US and around the world as part of a complex of many pest species that infest post-harvest agriculture products (Aitken 1975). Commodities infested by these species incude cereal grains, ground or milled grain products, nuts, dried fruits and numerous value-added food products (Hagstrum and Subramanyam 2009). *T. variabile*, the more common of the two, is reported in the scientific literature to occur in Vietnam. To our knowledge, *T. inclusum* has not been reported to exist in Vietnam, though it is reported in the entomology literature as occurring in Thailand. The Vietnam PPD considers *T. inclusum* to be an exotic pest subject to quarantine regulations that would involve inspection followed by some action if discovered. Quarantine action for introduction of *T. inclusum could include* disinfestation of arriving shipments via fumigation, return of infested commodity to the source country, or destruction of an infested shipment. All life stages (egg, larva, pupa and adult) of both beetle species can be effectively killed by properly fumigating with an effective gas such as phosphine or methyl bromide. Both species occur in the US, and it is likely that these species could feed on and reproduce in DDGS, but we have not found published reports of these species infesting DDGS. In any case, we know that both species are common in the US and in many other countries, and that these pests can probably infest DDGS and travel with shipments from the US to any of our trading partners.

We were contracted by the USGC in mid-2016 to assess the presence of *T. inclusum* and *T. variabile* in representative supply-chain contexts of DDGS production and commerce in the midwestern USA. Information on the occurrence and relative abundance of the target insects can be used to estimate the risk of infestation at DDGS facilities and then infer how that risk could lead to these pests being carried in shipments to Vietnam. It is hoped that the USGC and other trade or agricultural product

organizations could use such insect risk information to develop better pest prevention and mitigation practices for the DDGS industry. Specific objectives for us were:

- Select and engage DDGS companies in the north-central Midwest of the USA, including both ethanol plants and trans-loading facilities, to participate in the project.
- Make site visits to each of the cooperating companies to conduct a thorough inspection, interview key personnel, deploy insect traps for *T. variabile* and *T. inclusum*, and develop plans for continued trapping.
- Analyze all traps from each facility for the presence and numbers of the target species, with specific attention to relative numbers of insects trapped over time throughout the trapping season, and among specific trapping sites at each company.

Materials and Methods

Participating companies were in our geographic area of interest, whcih was the corn-growing region of the US at sites located in the states of Illinois, Indiana, Iowa and Missouri. These sites included five ethanol plants, numbered 1-5 in Table 1 below, and two trans-loading facilities, numbers 6 and 7. On-site visits were made to participating facilities during May, June, July and August of 2016. All facilities we studied were using corn as the grain to be distilled into ethanol and the manufacturing procedures at the ethanol plants were similar. Briefly, grain was delivered, stored, mashed with water, yeast and additives for fermentation, the ethanol separated and purified form the fermentation product distillation after which ethanol was prepared for delivery and the DDGS were dried, cooled and loaded for delivery. DDGS trans-loading facilities had a simpler lay-out compared to ethanol plants. The only activities at trans-loaders was to receive recently processed DDGS from ethanol plants and then load shipping containers for movement across the US, including to export terminals for shipment overseas.

Traps were deployed at four indoor locations and two outdoor locations at each of the ethanol and trans-load facilities in this study. We used traps baited with the synthetic pheromone attractant that is used by both *T. variabile* and *T. inclusum*. The lure is synthetic mimic of the female-produced sex pheromone that attracts males in nature. Two different traps were used: one known as the "Dome Trap" (Figure 1) for walking insects, and the other a "Storgard II" sticky trap (Figure 2) for flying insects. A single Dome trap was placed at each of four indoor locations such as fermentation, distillation, loading and one or more spots in the flat storage. A sticky trap was hung at about 2 m off the ground outdoors at the farthest east and west borders of each facility. Traps were deployed during the initial site visit to each of the cooperating facilities. One individual at each company was then responsible for collecting the traps after a two-week period, shipping the traps back to us at KSU, and then deploy a new set of traps sent by us for use at the same locations for another two weeks. Our trapping system therefore allowed for detection of the target species of beetles at four indoor and two outdoor locations at each of our study sites, and we had two or more trapping periods throughout the season to assess any change in insect populations or activity over time.

Fig. 1 The Dome trap (left) used for trapping crawling *Trogoderma* adults inside ethanol and trans-load facilities. Dome traps were placed at the bottoms of pillars or at floor-wall junctions at four places indoors (second and third from left) and then returned to the laboratory for processing (right).

Fig. 2 Storgard II sticky trap hung on a fence near the periphery of a research site (left). Beetles fly to the red rubber stopper that is slowly releasing the synthetic female sex pheromone, and then are stuck on the sticky trapping surface inside the trap (second from left). Adult *Trogoderma* are found in the trap (second from right), removed and cleaned in solvent prior to being identified to species and counted (right).

Results

There was a range of trapping periods across cooperators based on the dates we began trapping at a given facility and also due to time availability of cooperators to help with the project. Therefore, the number of trapping periods ranged from 2 trapping periods at facility 6, to 7 trapping periods at both locations 1 and 2.

Initial trap captures revealed numbers of beetles in traps ranging from no beetles upwards to over 100 in a two-week period. We soon realized that there were more than two species represented in traps at all the locations. Some insects that do not use the same pheromone as the lure used in our traps may still responded to the trap and be captured. Once we separated all members of the genus *Trogoderma* from others, we then gave special attention to accurate identification methods published by earlier researchers for these species to become proficient in the identification. Characters related to color of the elytra and diagnostic morphological features of the eyes, were critical for identification (Figure 3).

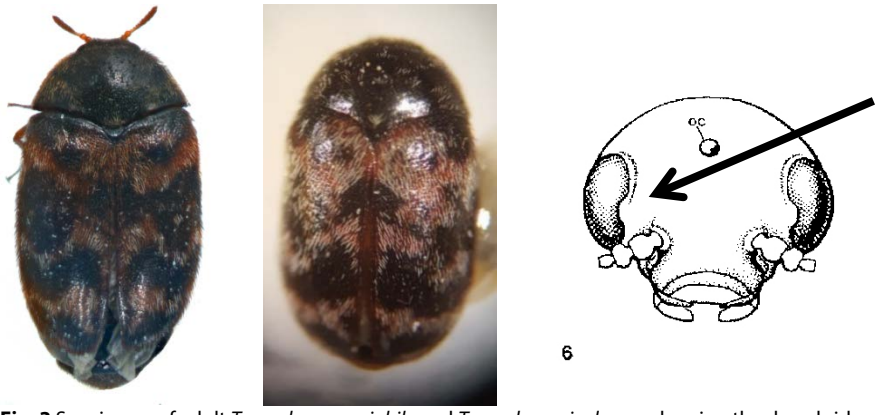

Fig. 3 Specimens of adult *Trogoderma variabile* and *Trogoderma inclusum* showing the dorsal sides. The species are very similar, but are separated by the black and brown bi-color appearance of the wing covers over which thin hairs were distributed in *T. variaible* (left) vs *T. inclusum* (middle) with just a black color under the white hairs. The best diagnostic character is the "notch" on the interior margin of the eye in *T. inclusum* shown at the arrow, and the lack of that notch, so that the margin of the eye is uniform and complete, in *T. variabile*. See ISPM (2016) for details.

Table 1 reports the numbers of adult beetles of both target species trapped at the indoor and outdoor locations for each of our seven study sites during the summer and fall of 2016. We have combined the numbers of beetles captured in the four indoor traps at each location, and these numbers are present in bold text in Table 1. Dome traps at all of our seven sites captured T. variaible,

while *T. inclusum* was trapped at five out of the seven sites, with none trapped at sites 4 and 7. Interestingly there were no *Trogoderma* trapped in our outdoor traps at locations 4 and 7, which suggests that these two species were in low or undetectable population levels at these places. *T. variabile* were captured during nearly every trapping period and at higher numbers than was *T. inclusum* at locations 1, 2, 4, 6 and 7. Traps at locations 3 and 5 captured more *T. inclusum* than *T. variabile*. Although Table 1 reports the sum of beetles from the four indoor traps in each time period, we found some trap to trap variation within and between facilities. For example, the trap in the fermentation area of site 1 consistently caught more *T. variabile* than did other locations at that facility. For the two trans-load sites that had Dome traps near the four corners of the flat storage, one corner seemed to consistently capture more beetles than any other. Spatial variation seemed to occur within each site, but there was no clear similarity between companies regarding which part of a facility had more beetles than another. Substantial numbers of beetles were caught in the outdoor sticky flight traps for most locations, except for relatively low flight trap numbers at locations 1, 4 and 6.

Tab. 1 Average numbers of adult *T. variabile* and *T. inclusum* captured per week in the indoor dome traps (sum of four trap), Inside, and in the two outside sticky flight traps to the west and east of the buildings, Out-W and Out-E, over a given number of trapping weeks at numbered ethanol plants and trans-loading facilities during the Summer-Autumn of 2016.

			T. variabile			T. inclusum		
Site	Type	Weeks	Out-W	Inside	Out-E	Out-W	Inside	Out-E
1	Ethanol	14	1.5	10.9	1.1	0	0.1	0
2	Ethanol	14	48.5	71.4	21.1	0	0.2	0
3	Ethanol	14	125.4	4.9	20.5	87.5	18.1	10.2
4	Ethanol	6	5.2	100.3	10.0	0	0	0
5	Ethanol	10	21.5	24.6	44.0	67.8	53.9	131.2
6	Trans-Load	4	6.8	2.3	1.0	8.8	1.0	0
7	Trans-Load	10	44.6	12.4	19.0	0	0	0

Discussion

The research reported here clearly shows that the beetles *T. variabile* and *T. inclusum*, the two species that were intercepted in Vietnam with DDGS from the US, commonly occur at ethanol plants and trans-load facilities that handle and market DDGS. This result met the expectation we had at the outset. Both species are very common in the US and previous studies have found that both can be trapped in many geographic regions of the US. Although we have data showing the occurrence of these species, we cannot report the density or absolute abundance of these species at each site. Pheromone trapping is an indirect sampling method that can only detect presence vs absence of a pest, and the relative numbers across locations and over time. Insects per unit of commodity (e.g. per bushel of grain or hundred-weight of DDGS) or per square meter of space would require more thorough and laborious methods to directly sample the pest populations. During our visits to cooperator sites we collected spilled DDGS and found no insects of any kind upon sifting these samples at our lab. Our trapping work clearly showed differences in relative captures of the two species, and also within species and across locations in a plant. It appears that numbers trapped at a given location in a facility could point to a need for sanitation or pest control to clean or disinfest areas with high trap captures. Captures of *Trogoderma* beetles at our outside traps indicate that beetles can be both outdoors and indoors, while the source location of trapped beetles is not confirmed.

Despite both beetle species being common and widely distributed, the risk of DDGS infestation by these pests and the risk that such pests may be transported with infested product, should vary in predictable ways. Trapping shows these species are common and thus could infest a suitable grain product at most times and places when weather and other environmental conditions are good for insects. However, these beetles can infest and persist in DDGS in only a few cases. Corn delivered to a site could be infested after harvest and through transport and storage periods. The longer grain

is stored, the more likely that infestation will occur. However, before becoming DDGS the corn is mashed and cooked, a practice that will kill all insects. The fermentation and distillation processes are fully insecticidal, and the temperatures during DDGS drying are extreme, over 600 F. The cooling period lasts about 24 h and during the majority of that time the DDGS would be too hot for infestation. DDGS should be susceptible to insect infestation when it is cool and handled in the flat storage for the 1-2 days prior to being loaded and shipped. Trapping has shown that beetles can be at all locations mentioned here, but access to suitable new DDGS would be only at the flat storage and also at the loading out location. Trans-loading facilities have no heating practices that can kill insects, and our trapping study shows that the target beetles can be present, but the product does not stay long before it is loaded into a container and shipped out. We were fortunate to encounter a man from the US Grain Inspection Service at one of our trans-load facilities who was taking timed samples of DDGS while they were being loaded into a container. He said that the samples were to be sifted for insects back at his office, and he told us that he had never found any insects in any samples like these he has taken in the past. Even if infestation of cooled DDGS occurs commonly, a buildup of detectable numbers would require several weeks under suitable conditions for substantial reproduction and increases in pest populations to occur. After leaving a trans-loading facility the DDGS may reach their ultimate destination within one day, or after several days or weeks for domestic rail service, or weeks to months for international ocean-going shipment. It is these time periods after drying and cooling that DDGS can be at risk for infestation.

Fumigation is the most effective and practical means to treat a potentially infested commodity to eliminate actionable quarantine pests before the commodity arrives at its destination (Myers and Hagstrum 2012). None of the seven facilties studied reported fumigating DDGS prior to any internatiaonl shipments, and all had discontinued shipping product to Vietnam at the time of our work. We interviewed one fumigation company about their practices with containers of DDGS. We were told they had fumigated containers near an export terminal with phosphine gas for 24 hours, and then the containers were ventilated and transported locally for loading onto a barge or ship destined for export. In our opinion this practice would not be the most effective to ensure a good kill of pests and quarantine security for the product (Hagstrum and Subramanyam 2009). The time after ventilation and prior to loading on a ship represents a period of susceptibility to pest invasion into the recently fumigated product. Also, the 24-hour fumigation may not give the most effective kill due to the short exposure time. Some pest species and certain lifes stages can be relatively tolerant to phosphine and a longer fumigation may be recommended. Many other variables can affect the efficacy of a 24-hour phosphine fumigation of shipping containers.

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Insect pest monitoring in museums - old and new strategies

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Abstract

Integrated Pest Management (IPM) is an important part of preventive conservation of museum objects made of wood, textiles, starch, paper, keratin and other organic materials. Long term monitoring data help us to discover new infestations and locate them in the building. Results from over 20 institutions (museums, storages, historic libraries and historic palaces) are presented and discussed how the monitoring can be improved, where active infestations were found, what treatment was done as a response and what new methods are used. What pests are the most abundant, which species are new for the indoor museum environment and when do we actually have active infestation and damage of museum objects? Monitoring and IPM in museums is also compared with the food storage industry. IPM is applied in many museum today, mainly to reduce the application of pesticides, for a long-term protection of the objects and collections and early detection of infestations. In this presentation, long term monitoring data with sticky blunder and pheromone traps for webbing clothes moth Tineola bisselliella is described. The analysis of the data show that in all museums and storages buildings with a monitoring in place different insect pest species are present, but only in few collections damage to museum objects was found. New pests like the grey silverfish Ctenolepisma longicaudata and Ctenolepisma calva - another species of Lepismatidae, are now found in many museums in Vienna, Austria. The odd beetle Thylodrias contractus was found recently in Austria, surprisingly in four different locations.

Remote monitoring of stored grain insect pests

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Abstract

A number of remote sensing methods were developed and tested in commercial grain warehouses; probe pitfall traps attached to vacuum lines, surface pit fall traps equipped with video cameras and white boards on grain surface monitored with video cameras. These methods were compared with detecting insects using grain samples. Warehouse trials by trapped methods were carried out in bins with 8520 t of wheat from 23 May until 8 August 2016.Grain temperatures were from 22.7 to 31.6℃. Psocids, *Liposcelis bostrychophila* Badonnel, were detected by grain samples, but there were higher number of pscoids trapped with the probe pitfall traps and pitfall traps than found in grain samples. *Plodia interpunctella* (Hübener), *Sitophlius zeamais* Motchulsky and *Cryptolestes ferrugineus* (Stephens) were detected by probe pitfall trap, but not in the grain samples. *S. zeamais* was detected by the pit fall traps. Using the remote controlled video camera in the warehouse head space, we were able to distinguish and count *S. zeamais*, *C. ferrugineus* and psocids on white boards. The video from pitfall traps can be sent to mobile phones. With all these methods, data can be collected remotely, and could be analyzed by imagine analysis allowing for rapid real time monitoring of insect pests.

Keywords: stored grain insect; monitoring; remote; feasibility

1. Introduction

Efficient sampling is a decisive factor for the timely and safe undertaking of measures in the management of stored products and foods (Buchelos and Athanassiou, 1999). Timely monitoring is necessary for pest management of stored grain, especially for wheat and paddy rice which can be stored for three to five years in China. The need for as many samples as possible, as frequently as possible, is a technical problem of conventional sampling methods (Subramanyam and Hagstrum, 1995). Sampling and sieving grain is a current method in stored insect monitoring as recommended in Chinese grain storage regulation. The grade of insect infestation of stored grain is decided by sampling, although this technique is effective primarily for detection of adults and some larvae.

Manual sampling of insects in stored grain is a laborious and time-consuming process (Flinn et al, 2009). Over the past few decades, many researchers have developed traps for use in store facilities as an alternative sampling method (Buchelos and Athanassiou, 1999). Probe traps, when compared to other trap types, have given satisfactory results in the trapping of many important Coleoptera and other stored product species; at the same time, they are easy to use and reliable even without the use of an attractant (Lippert and Hagstrum, 1987; Subramanyam et al., 1993; Fargo et al., 1994； Buchelos and Athanassiou, 1999). Pitfall traps were also developed for insects that are active on top, higher temperature, layer of grain bulk in summer. Automation of grain sampling and insect monitoring should help to increase the adoption of stored grain integrated pest management. A new commercial electronic grain probe trap (OPI Insector) has recently been marketed (Flinn et al, 2009). A probe pitfall traps system attached to vacuum lines had been developed ten years ago in China. The insects can be vacuumed from trap bottom through the line by remote control and then counted. Another approach is the use of a video camera in the headspace of grain warehouse which can be controlled remotely to capture insect pictures when they walk on a white board that was laid on surface of grain bulk. This method has been used in grain depots of Sino-grain. A surface pitfall trap equipped with video cameras was made and the captured pictures can be monitored by mobile phone. Here some insect monitoring results were reported for a number of remote sensing methods, including probe pitfall traps attached to vacuum lines, surface pitfall traps equipped with video cameras and white boards on grain surface monitored with video cameras. These methods were compared with sampling insects using grain samples.

2. Materials and Methods

2.1. Trial 1

The plastic probe pitfall traps, attached to vacuum lines (PPTAVL), consisted of probe with hole, on its wall were holes insects can go through, insect collecting chamber on bottom, after insect fall in, and vacuum line for sucking out the collected insects in the chamber. The vacuum line was connected with vacuum pump, insect collecting bottle, insect checking sensor, and remote controller (Fig. 1). The probe pitfall was inserted into grain mass so that the head was beneath the surface of the bulk.

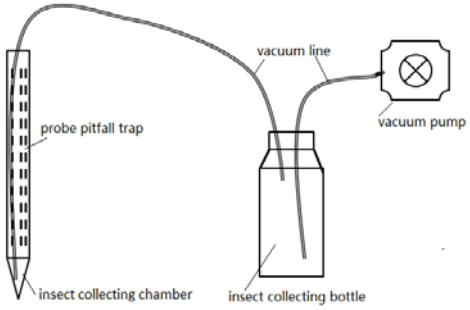

Fig. 1 Diagram of the probe pitfall trap with attached vacuum line.

The surface pit fall traps equipped with video cameras（SPTEVC） consisted of a disc contained radial channels, where insects can through, insect collecting chamber attached centrally below the disk, video camera right over the chamber, communication device with WiFi (Fig. 2). It was mostly made of plastic. Insect collecting chamber in the SPTEVC was inserted into top layer of bulk. The disk with the Insect going channels was laid on the level of bulk surface while monitoring. The insect trapped in the chamber were collected manually.

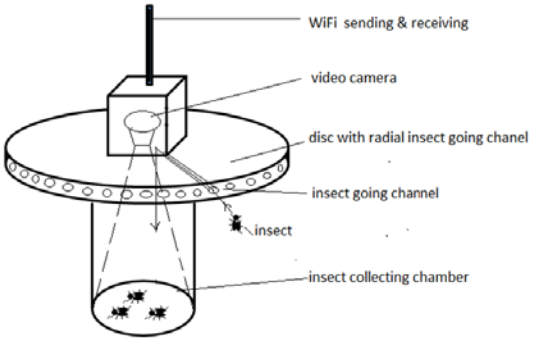

Fig. 2 Diagram of the surface pitfall trap equipped with video camera.

Sampling check was carried out using one kilogram grain samples for each monitoring time and position. The relative distance among between traps, grain sampling location, and warehouse walls, was one meter. There were five sets of traps and sampling points located in four corners and one central position in the storage which contained 8520 tons of wheat (Fig. 3). The stored wheat was loaded in June 2015 with 12.6% moisture content and 784 g/L test weight. The highest temperature average was 20.2℃ and and the lowest 0.1℃ in the winter of 2015. On the beginning of the trial, May 16th of 2016, insect density was zero per kilogram of grain by sampling method for beetle, moth and psocids.

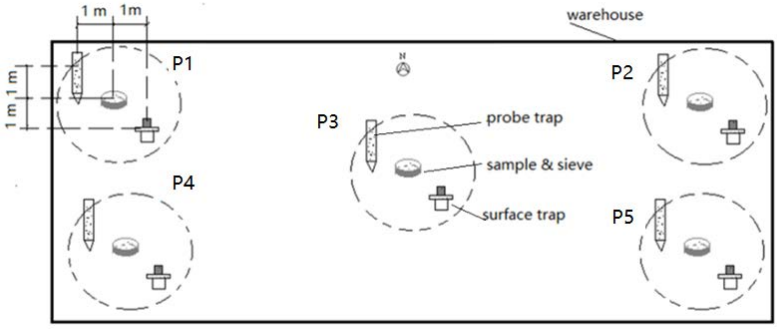

Fig. 3 Traps, sample position and five sets on grain bulk of the warehouse

2.2. Trial 2

White boards on grain surface were monitored with video cameras（WBMVC）to measure insect activity. One high definition video camera was set up in headspace of a warehouse, in which 7000 tons of wheat was stored in bulk. The video camera can scan whole surface of the bulk remotely to get clear figure of insect on grain surface, as is shown at Fig. 4. A white board with 1 cm grid pattern was laid on bulk surface. The insects that crawled on white board, even psocids, can be seen on screen of a remote control computer (Fig. 5).

Fig. 4 A picture of psocids on grain bulk surface captured by computer from high definition video camera

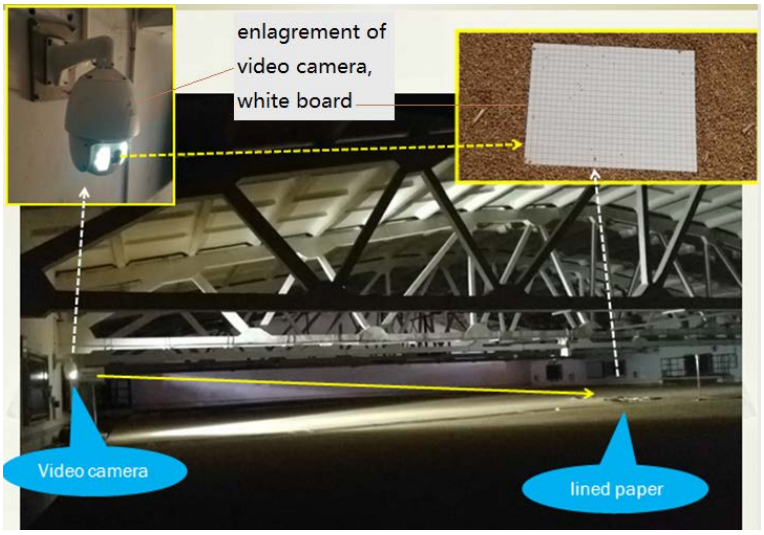

Fig. 5 The white board laid on surface and video camera in headspace of warehouse

2.3. Insect checking and handling

In trial 1, insects were monitored, and samples collected and checked, once a week. The number in traps was recorded as adults or larvae per week. The number in sample was recorded in adult per kilogram of sampled grain. All insects in traps were removed at each checking time. In trial 2, the picture was captured once a week. The dark spot in different sizes and outline shape of insects on white were checked and counted.

3. Results

3.1. The species detected by the different monitoring methods

During the monitoring time from May 16th to August 8th, *Liposcelis bostrychophila* Badonnel was trapped in PPTAVL and PTEVC and found in sieved samples. *Plodia interpunctella* (Hübener), *Sitophlius zeamais* Motchulsky and *Cryptolestes ferrugineus* (Stephens) were detected by probe pitfall trap, but not in the grain samples. *S. zeamais* was detected by the pitfall traps. The method of sample and sieve only detected the psocids even when beetles and moths were exiting in grain mass.The pitfall trap captured S. zeamais and *L. bostrychophila*, but not *P. interpunctella* and *C. ferrugineus* even though the probe pitfall trap can trap all the insects mentioned above.Due to the random distribution of insects in different monitoring locations, the probability of detection is very

different in the same monitoring method for the detected species. In these methods, however, the ability to detect insect species is obvious.

3.2. Comparison of number of captured insects using different methods

The insect number monitored by same method varied among the five positions at same checking time. The numbers at different checking times also varied for insect species and monitored methods in all trials. And the number of insects sharply varied among different methods at same positions (Table 1). For example, on May 23rd, 32 adults of *L. bostrychophila* in one week was trapped in PPTAVL which was obviously more than the 5 adults captured at the same time in PTEVC. There were eight *L. bostrychophila* adults sieved from grain sample. It means that the psocids can be checked or attracted by the three methods, but that they may be detected in greater numbers in probe pitfall trap.

The number of beetles and moths captured in two trapping methods was obviously different, although few insects were trapped in the trap trials. *P. interpunctella* number was 1-3 larvae per week detected by probe pitfall trap and zero per week captured in pitfall trap. The number of *C. ferrugineus* captured was 2-5 adults per week in probe pitfall trap and zero per week in pitfall trap. For *S. zeamais* was 1-3 adults per week in the probe pitfall trap and only one per week in pitfall trap. The pitfall trap set on surface of grain mass detected fewer beetles and moths than probe pitfall trap inserted into the bulk. Sampling and sieving method detected no beetles or moths, which indeed existed in the grain mass during June 20th to August 8th.

3.3 Insects on white board under of video camera

A picture from video camera in headspace of grain warehouse of the white board was captured on computer as shown in Fig. 6. The biggest dark spot was revealed as a *S. zeamais* adult, the middle sized dark spot was a *C. ferrugineus* adult, and the smallest dark spot indicated that *L. bostrychophila* crawled on the board. The species judging was based on dark spot size, picture outline and experienced knowledge. The picture captured by computer received from video camera can provide information about insect species and dynamic number during monitoring. Insect number or population dynamic can be known by counting the dark spots in 1 cm subsample or on whole white board at any time.

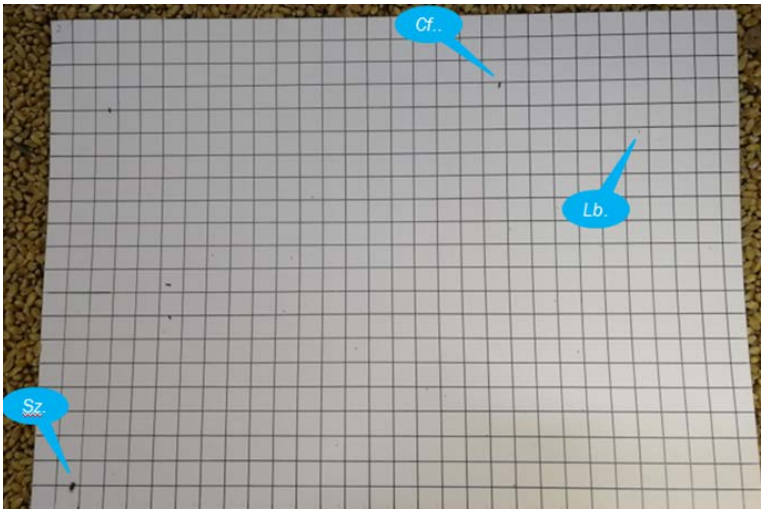

Fig. 6 Identification of insects on white board made from a image captured from a video camera (Sz for S. zeamais, Cf for *C. ferrugineus* and Lb for *L. bostrychophila*.

Tab. 1 Insect number captured by traps or recovered in grain sample.

Date /Month·Day	Temperature outside of warehouse /°C	Temperature in headspace /°C	Temperature of bulk /°C	*L. bostrychophila* in PPTAVL (adult/week)	*P. interpunctella* in PPTAVL (adult/week)	*S. zeamais* in PPTAVL (adult/week)	*C. ferrugineus* in PPTAVL (adult/week)	*L. bostrychophila* in PTEVC (adult/week)	*L. bostrychophila* sieved from grain (adult/kg grain)
5.16	20.0	25.0	20.8						2
5.23	26.0	28.0	22.7	32.0				5	8
5.30	24.0	28.0	21.8	80.0				10	30
6.06	22.0	27.0	23.2	100.0				15	30
6.13	26.0	31.0	23.6	180.0				15	25
6.20	27.0	35.0	25.5	190.0	1.0			10	30
6.27	24.0	32.0	28.8	130.0	1.0			5	25
7.04	24.0	34.0	28.9	85.0				3	23
7.11	28.0	33.0	29.4	67.0	3.0			5	27
7.18	25.0	32.0	28.9	76.0	2.0	1.0	2	4	33
7.25	29.0	36.0	30.3	65.0	2.0	3.0	2	6	40
8.01	29.0	38.0	32.5	5.0	2.0	3.0	5	1	2
8.08	26.0	35.0	31.6	15.0	1.0	3.0	2	8	10

4. Discussion and conclusions

Remote insect monitoring is being realized by insect sensors and remote information transfer which should be more convenient than manual methods of insect detection in grain storage. Accurate monitoring is needed and spatial analysis techniques are increasingly being used in entomological investigations (Liebhold et al., 1993; Trematerra and Sciarreta, 2004). These techniques apply specialized software to trap captures, interpolating the data from the sampled locations to generate data for a non-sampled location. All these data are subsequently represented in contour maps, from which a wide range of information can be obtained, notably the distribution of different pest species in space and time (Schotzko and O'Keeffe, 1989; Arbogast et al., 2000; Campbell et al., 2006; Trematerra and Sciarreta, 2004), their movements through facilities (Campbell and Hagstrum, 2002; Arbogast et al., 2002; Athanassiou et al., 2005). It is important to know the relationships for different species of insect between trapping and sampling & sieving under specific cases such as grain storage types, warehouse conditions, capacities of the bulk, temperature of grain mass and warehouse, quality of stored grain, status of insect infestation.

With the results in this research psocids can be found by traps and sampling. But there were higher number of psocids trapped with the probe pitfall traps and pit fall traps than found in grain samples during whole monitoring process. *P. interpunctella*, *S. zeamais* and *C. ferrugineus* were detected by probe pitfall trap, but not in the grain samples. *S. zeamais* was also detected by the pit fall traps. All detected information of insects was able to be transfered and controlled remotely. Using the remote controlled video camera in the warehouse head space in other trial, we were able to distinguish and count *S. zeamais*, *C. ferrugineus* and pscoids on white boards. All information can help us to improve pest management by indicating if it needed to kill the insect or not. It can also increase the effectiveness of treatments (Brenner et al., 1998; Blom et al., 2002; Campbell and Hagstrum, 2002) and reducing prospects for the development of resistance (Belda et al., 2011). Consequently treatments costs of insect monitoring may be reduced due to reducing on manual work.

Acknowledgement

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Can the DI-SPME gas chromatography mass spectrometer be a tool for identification of stored grain insects - fatty acids and sterols profiling

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Abstract

Identifying insect pests is essential for management, but these insects can only be reliably identified by a limited number of highly skilled taxonomists. Expert morphological determination can require dissection and slide mounting of specimens in order to examine distinguishing diagnostic features. Suspected insect pest specimens found in grain products usually consist of only the larvae or larval skins which are very difficult to identify to species, and sometimes impossible to diagnose morphologically. Adult specimens are usually scarce and more often damaged. Misidentification of species could lead to misled pest management practice.

Fatty acids (FAs) have long been recognised as biochemical markers for organism classification. The direct immersion solid phase microextraction gas chromatography-mass spectrometry (DI-SPME-GCMS) technology has been developed and validated for selectivity and accuracy by isolating fatty acids from natural fatty acid methyl esters. Seven different species of stored grain insect pests were analysed by using DI-SPME-GCMS method profiled fatty acids and sterols from insect extractions. Palmitic acid (C16:0), Stearic acid (C18:0) and Oleic acid (C18:1) were absorbed. The ratio of FAMEs/FAs (ME) were calculated and validated as a new biomarker for insect classification. Mid-

chain waxes, low boiling point semi-VOCs, and other lipid components can also be identified by the same method, which can be adopted to be an automated high-throughput method for insect classification, surveillance and quarantine purposes.

Keywords: direct immersion solid phase microextraction (DI-SPME), fatty acids & sterol lipids, biomarker, stored grain insect, insect morphology and identification.

Webbing Clothes Moth, Tineola bisselliella (Hummel) Sex Pheromone Transfer from Monitoring Lures to Textiles

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Abstract

The use of synthesized sex pheromone lures for the purpose of monitoring populations of webbing clothes moth, *Tineola bisselliella* (Hummel) in museum storage environments is typical in many museums. Questions about whether the pheromone incorporated in the dispensing lures could possibly transfer over to textiles that are in close proximity to the lures have been posed by museum conservators. Although some textiles may be naturally attractive to clothes moths, the concerns are that the textiles themselves may become even more attractive to insects due to exposure to the pheromone and that this could ultimately cause further damage to the collections. The focus of this study was to determine the degree to which textiles that have been exposed to pheromone lures absorb the pheromone and become attractive themselves. Based on the results of this study, the textiles observed here have little to no additional attraction to insect pests after focused exposure to synthetic pheromone lures over a two-week period.

Keywords: Webbing clothes moth, Tineola, sex pheromone, textile, monitoring.

1. Introduction

The webbing clothes moth, *Tineola bisselliella*, is a cosmopolitan pest that carries economic importance due to damage caused by their larvae feeding on objects that incorporate wool, feather, hair and hide (Krüger-Carstensen and Plarre 2011). Textiles that incorporate cotton, silk, linen, paper and synthetic fibers can also be damaged by *T. bisselliella* if these items have been soiled with urine, sweat, beer, milk, soft drinks, tomato juice or other substances that contain nutritional needs for the moths (Sloderbeck 2004).

Being one of the most common pests in museums in many parts of the world, this species of moth has caused severe damage to cultural heritage objects (Querner 2014). The use of synthetically produced sex pheromone monitoring lures specifically for *T. bisselliella* for the purpose of early detection and locating sources of infestation has become commonplace in some museum institutions to prevent this damage. The use of a pheromone lure within a sticky trap increases the rate of capture twenty-fold over a sticky trap with no lure (Cox et al. 1996) and is a key factor in determining increases in population density and economic thresholds (Plarre 2013).

Concern over the practice of pheromone monitoring was raised by a prominent museum conservation scientist and author who believed that the pheromone incorporated in the dispensing lures would transfer over to museum objects (Florian 1997). Following up on this, this same author made a statement in an online museum conservation listserv that suggested that the volatile fat-soluble pheromone can be adsorbed by materials of artifacts and thus make the artifacts themselves attractive to insect pests (Florian 2011). This posting suggests that even after monitoring lures are removed, the museum collections would continue to attract and draw-in damaging museum pests. The question that this study aims to answer is if pheromone transfer between the sex pheromone lures and a variety of textiles found in museum storage environments is occurring and if these pheromones are making the textiles themselves attractive to pests.

2. Materials and Methods

2.1 Exposing the Pheromone to the Textiles

In order to answer the question of whether textiles exposed to sex pheromone monitoring lures become more attractive to the insect pests themselves, it was first necessary to establish a means of exposure for the textiles so the theory could be tested. Pheromone plumes emanating from monitoring lures are typically carried by air currents out to the surrounding areas where they attract the insects back to the lure (Murlis et al., 1992). In order to ensure exposure of the textile to the pheromone in this study, a constant air current generated by electric fans was blown across the lures towards the textile at an air speed of 40 ± 1.5 meters/min for a 2-week period in controlled temperatures between 21.1° C and 22.7° C and within a relative humidity between 40% - 50%. This exposure system was set up using eight 30.48 cm long sections of 10.16 cm diameter corrugated polyethylene field drainage tile as a conduit for the air flow (Figure 1). The fans were placed 40.6 cm away from the corrugated field drainage tiles and were directed to blow air through the open center of the tiles. The airflow was calculated using a hand-held anemometer (#DCFM8906, General Tools & Instruments, Secaucus, NJ, USA). The pheromone lures used in this study were standard, commercially available webbing clothes moth Bullet® lures (Insects Limited, Westfield, IN USA). The lures used in the study had been manufactured within the previous month of the study, were frozen to ensure freshness and were then taken fresh from the package. These lures incorporate a pheromone dose of 4.5 micrograms per lure. This dose can be considered on the high end of commercially available pheromone lures for webbing clothes moth (Van Ryckeghem, 2014). The lures were suspended on the inside of the drainage tile using a flexible metal wire positioned at the opposite end from the fan. A screen mesh was placed over the open end of the drainage tile on this same side. This screen was set in place for the purpose of creating a physical barrier between the lure and the textiles being exposed, while still allowing air to flow freely across the lure and onto the textile. No direct physical contact between the pheromone lures and the textiles was made in any of our studies. The mesh screens were standard fiberglass insect screening with a 7 X 6 mesh count per cm and the fabric was 0.3 mm thick. The close-range exposure between the lures and the textiles was performed using only the screen mesh between them at a distance of 0.3 mm. A single set a data points was retrieved at the greater distance of 152 mm between the lure and modern synthetic pile carpet to give data that represents a distance that is more commonly found in a museum setting.

The five textiles that were chosen to be exposed in this study were selected as being textiles commonly found in museum storage settings. These textiles include:

- Antique Wool Pile Carpet (mid to late 19th century)
- Modern Synthetic Pile Carpet (late 20th century)
- Modern Synthetic Plain Weave (early 21st century)
- Antique Wool Plain Weave (mid to late 19th century)
- Antique Wool Flannel (late 19th century)

Relatively larger 30 cm^2 sheets of the various textiles were cut into smaller 50 mm X 50 mm squares for use in the exposure study. The textile squares were secured to the screen mesh using metal paper clips and were placed directly on the opposite side of the screen from the lures to ensure exposure to the pheromone. The textiles were handled only while the technician was wearing latex gloves to prevent any exchange of pheromone from person to textile. After an exposure period of two weeks to allow the sex pheromone to blow directly across the lures onto the textiles, the textiles were immediately taken and placed into 10.16 cm X 15.24 cm, 4 mil Metalized PET (Polyethylene terephthalate) Zipper Pouches. The zipper pouches were then sealed and placed into a standard upright freezer (-20°C) until they were used in the insect portion of the study. The PET is considered a barrier film for oxygen (Frounchi and Dourbash 2009). Since pheromones are larger molecules than oxygen, the PET pouches can also be considered a barrier for the pheromone that will retain

any pheromone absorbed onto the textile. Freezing the samples also slows molecular movement (Debenedetti and Stillinger 2001) and thus should slow any loss of pheromone out of the textile pouches and into the environment prior to use in the study.

After pheromone exposure of the textiles was performed, the second portion of this study was to determine if the adult clothes moths prefer pheromone-exposed textiles over non-exposed textiles of the same material. This determination was made with a choice test that included 4 different options for the adult moths to choose. The four options are:

1. Sticky trap containing a 50 mm X 50 mm square of textile that has been exposed to the pheromone and placed into the center of the base of the trap.

2. Sticky trap containing a 50 mm X 50 mm square of the same textile as above that has not been exposed to the pheromone and placed into the center of the base of the trap.

3. Sticky trap containing a pheromone Bullet lure specific for webbing clothes moths placed into the center of the base of the trap as a positive control.

4. Sticky trap with no textile or attractant inside, used as a control.

The pheromone lure option and the empty trap option were added as controls to the choice test to give comparative trap capture numbers: source moths are known to be attracted to (pheromone lure) and source that should have no attraction (empty trap).

The test arena that was used in the choice test was a 2.7 m X 4.0 m space that included a desk and storage cabinets (Fig. 2). This setup gave the moths plenty of hiding spaces other than the traps, if they preferred to not go to a trap at all. Moth colony jars, active with adult *T. bisselliella*, were opened on a platform 0.74 meters above the floor and at a distance of 2.06 meters from the wall where the traps were placed. The traps that incorporated a textile square on the inside for this study were prepared by taking a textile from the freezer and placing it into the center of a sticky trap. These traps were made of milk-carton stock, wax-coated cardboard with the interior base of the trap coated with a 1 – 2 mm layer of sticky adhesive (Flat Trap adhesive trap, Insects Limited, Westfield, IN, USA). The dimensions of the traps were 20.32 cm X 10.16 cm X 3.81 cm. All four traps were set on the floor and spaced at a distance of 53.34 cm apart from each other. These locations are marked 1 through 4 in Fig. 2.

Webbing clothes moth colonies were reared in 2-Quart (1.89 liter) screw-top canisters with 5 pin holes on the upper side of the canister. The pin holes were made to allow air exchange in and out of the canister. The diet consisted of a mixture of chicken feather meal with 1% brewer's yeast by weight. The colony jars were opened and placed on their side to aid in the release the adult moths.

After one week of allowing the moths to move out of the colony jar and enter the test arena, the individual trap captures were counted and recorded and the traps were rotated to a new trap location in the arena. Also, after one week, the existing colony jar of live moths was removed and a new colony jar with freshly emerged adult moths was opened in its place. A total of 4 repetitions, totaling 4 weeks of release for each textile, were performed. Each trap in the study would spend one week's time at each location of the four without duplication of location during that 4-week period. A total of six individual 4-week trials were run in this study. Five of those studies involved each of the different textiles exposed at the short 0.3 mm distance to the lure. The sixth trial was a single trial of the Modern Synthetic Carpet exposed to pheromone from the greater distance of 152 mm.

3. Results

Totals of captured moths after 4 weeks varied from 107 to 323 based on the number of adult moths in the colony jars at the time of release and the attraction of the traps. Throughout the six trials, a total of 913 *T. bisselliella* were captured in the different traps. It is estimated that a total of > 2000

moths were released through these studies. The results of the capture numbers for each individual textile, as well as the controls and pheromone lures can be seen in Fig. 3 – 7.

A statistical analysis was prepared using the Kruskal-Wallis *H* test (Microsoft Excel 2013). The Kruskal-Wallis test is a rank-based non-parametric method for one-way analysis of variance test that compares the samples even though they may have different sample sizes. The results of the Kruskal-Wallis test can be found in Table 1.

Fig. 1 Image of pheromone exposure to textiles with anemometer

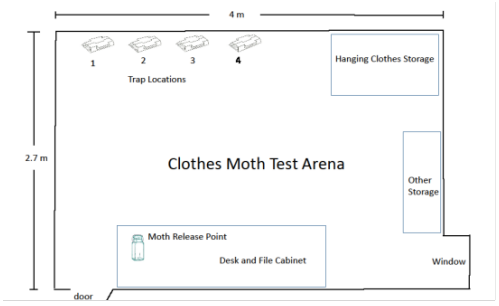

Fig. 2 Diagram of clothes moth test arena

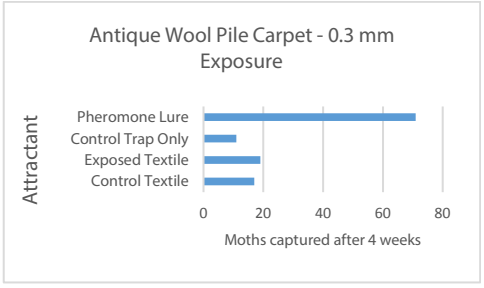

Fig. 3 - Choice test trap capture results for antique wool pile carpet exposed to pheromone at a distance of 0.3 mm.

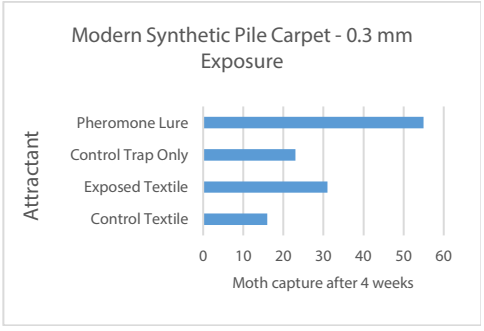

Fig. 4 Choice test trap capture results for modern synthetic pile carpet exposed to pheromone at a distance of 0.3 mm.

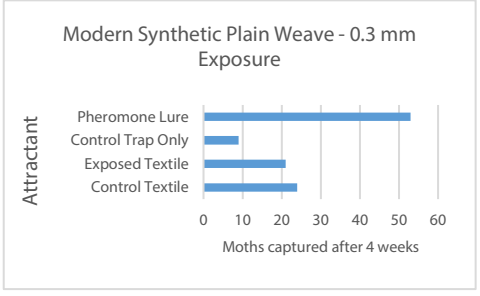

Fig. 5 Choice test trap capture results for modern synthetic plain weave exposed to pheromone at a distance of 0.3 mm.

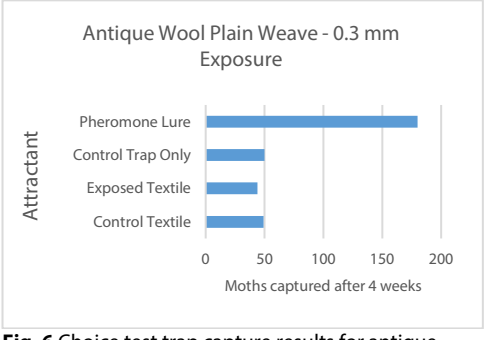

Fig. 6 Choice test trap capture results for antique wool plain weave exposed to pheromone at a distance of 0.3 mm.

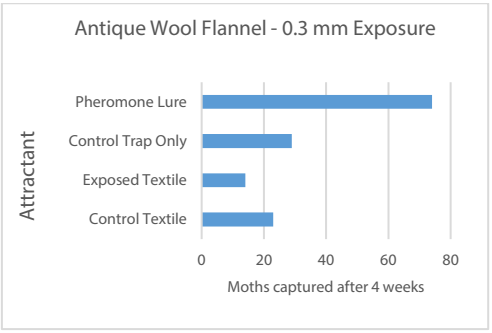

Fig. 7 Choice test trap capture results for antique wool flannel exposed to pheromone at a distance of 0.3 mm.

Tab. 1 Results of non-parametric Kruskal-Wallis tests comparing trap capture rates of combined textile types.

Pheromone Lure and Control Trap		Exposed Textile and Control Textile	
adjusted H	6.8182	adjusted H	0.0109
d.f.	1	d.f.	1
P value	0.0090	P value	0.9168
Control Textile and Control Trap		Exposed Textile and Control Trap	
adjusted H	0.0439	adjusted H	0.0982
d.f.	1	d.f.	1
P value	0.8340	P value	0.7540

4. Discussion

The procedures used to expose the textiles to the pheromones in this study represent what could be considered a worst-case-scenario for pheromone exposure in a real-world setting.

A lure with a relatively high dose of pheromone was used in this study. This is not always the case in a museum storage environment as pheromones with low dosages are commonly used. Also, pheromone traps are typically not placed in direct contact with museum objects as this could be detrimental to the object if it were to get stuck in the glue. Pheromone traps for pest insects and particularly traps for *T. bisselliella* are usually placed in open areas along the wall or on the floor so they can be inspected easily (Trematerra and Fontana, 1996). Even a pheromone trap that is in direct contact with a museum object is going to have the pheromone lure at a minimum of 1 cm away from the object due to the distance from the paper outside of the trap to the adhesive pad within where the lure rests. It may even be as high as 15 mm away depending on where the lure sits in the trap. These distances are considerably higher than the 0.3 mm that we used in this study.

Air currents are the mechanisms that translocate quantities of the sex pheromone from the lure into the air or onto a textile. In this study, a constant air flow was blown across the lure at a rate of approximately 40 meters/min ± 1.5 meters/min for a full 2-week period. This type of constant air flow across a lure and onto a textile is usually not seen in a museum storage setting unless the textile is placed directly between a return air vent and the pheromone lure or if the pheromone lure is placed directly in front of an air supply vent and the textile is positioned directly in the air path of that vent.

Although this study does represent a worst-case scenario for textile exposure to pheromone, this type of exposure theoretically could occur in a museum setting. Because of this potential, the questions of concern for this type of exposure need to be considered valid. Correlations between this study and other similar studies could not be done since other studies regarding the pheromone transfer from monitoring lures to textiles were not found in the available research.

There was a wide range of materials incorporated into the textiles that were studied here. Antique natural fibers were used in three of the five samples; wool pile carpet, flannel and antique wool plain weave. Also incorporated in some of these samples were synthetic materials that contained no

natural fibers at all. These were the new pile carpeting and modern synthetic plain weave. *Tineola bisselliella* larvae feed on a wide variety of dried material of animal origin (Griswold, 1944). This fact should theoretically make the woolen textiles more attractive than the synthetic textiles, at least to the female moths looking to lay eggs. When we look at the results from this study however, we find that only the antique wool carpet and the modern synthetic flat weave had apparently higher moth attraction than the control trap. No clear affinity for natural fibers over synthetic fibers could be found. It is possible that many of the females in the study were left unmated due to a large capture of the male moths in the traps. If this were the case, the unmated females were not looking for potential food sources to lay their eggs, so we did not see an affinity for the natural fibers. The addition of human sweat, urine or food stains to natural fibers can make these materials more attractive to *T. bisselliella* (Klass, 2010). A possible explanation for the low attraction is that the samples we used containing natural fibers did not contain any of these additional attractants.

5. Conclusions

The textiles in this study, whether exposed to pheromone or not, did not have greater captures than control traps (Table 1).

Given these results, it is unnecessary for museum staff to be overly concerned that they are making their textile collection objects more attractive to *T. bisselliella* if they are using pheromone monitoring traps within their collections storage. This study suggests that *T. bisselliella* monitoring traps are an effective, non-detrimental tool. The informational value gathered through use of the pheromone traps used to mitigate damage to collection textiles and objects, far outweighs any negative possibilities that the textiles themselves will attract pests into storage areas.

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Khapra beetle diagnostics

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Abstract

The khapra beetle, *Trogoderma granarium* Everts, is a serious pest of grains and stored dry food stuffs and is the subject of strict quarantine measures in many countries including Australia. Morphologically the khapra beetle can only be reliably identified by dissection by a limited number of skilled taxonomists. Suspect specimens found in grain products are usually the larvae or larval skins which are difficult to diagnose morphologically. Adult specimens are usually scarce and damaged. Due to their similarity, warehouse beetle (*Trogoderma variabile*) and other native *Trogoderma* spp. could be mistakenly identified as *T. granarium* with market access implicatons or could mask an incursion. Molecular diagnostic protocols have been developed for khapra beetle, but remain largely untested against other species of *Trogoderma*, some also capable of being pests. Western Australia has a broad large, poorly studied native *Trogoderma* fauna, many of which are still undescribed; their estimated number is possibly over 100 species. Occasionally native Australian species can occur in stored commodities. Their identification and at least separation from the pestiferous exotic *Trogoderma* presents a serious problem. The work in this paper has been undertaken in an attempt to distinguish *T. granarium* from Australian native *Trogoderma* and related Dermestid species by both morphological and molecular methods. Dermestid specimens were sourced mainly from a targeted survey around grain silos throughout Australia, using two trap types, inside and outside facilities. Khapra beetle specimens were sourced from different geographical locations around the world.

Keywords: *T. granarium*, PCR, native Australian *Trogoderma*, targeted survey, taxonomy.

Introduction

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is recognised as one of the world's most destructive pests of grain products and is the subject of strict quarantine measures in many countries. Khapra beetle is listed in the 100 "World's Worst Invasive Alien Species" by the Global Invasive Species Programme (Lowe et al 2000). Plant Health Australia has identified khapra beetle as one of the top 5 biosecurity threats to the Australian Grains Industry. By definition, khapra beetle does not occur in Australia, but there are occasional records of intercepts (Emery et al 2008; Day and White 2016). An incursion could lead to costly control and eradication efforts. Non-khapra beetle countries enforce quarantine restrictions on imported commodities from khapra beetle countries.

There are over 120 described *Trogoderma* spp. worldwide of which four are recognised as stored product pests, including *T. granarium*, *T. glabrum*, *T. inclusum* and *T. variabile* (Banks, 1994). In Australia there are over 50 described native *Trogoderma* species, and many more remain undescribed. None of these are pests but could accidentally get into grain stores and be misidentified. Due to their similarity, *T. variabile*, already in Australia, or native *Trogoderma* spp. could be mistakenly identified as *T. granarium* or could mask an early incursion of *T. granarium*.

Suspected *Trogoderma* specimens found in grain products are usually the larvae or larval skins which are difficult to diagnose morphologically (Banks 1994; Emery et al 1997). Adult specimens are usually scarce and damaged and need expert dissection for identification (EPPO, 2002; IPPC, 2012). Diagnostically the khapra beetle can only be reliably identified by a limited number of skilled taxonomists. Misidentification of *Trogoderma* and related Dermestids has the potential to seriously compromise Australian grain exports (Szito 1997).

The aim of this work was to develop a molecular diagnostic tool that could quickly discriminate between khapra beetle and native Australian *Trogoderma* fauna based on whole specimens or insect fragments.

The approach included a review and modification of a published diagnostic DNA method for khapra beetle (Olson *et al* 2014) as well as review and optimization of in-house protocols.

Thousands of native *Trogoderma* and related Dermestid specimens were used to verify the diagnostic components of this work. Native Australian *Trogoderma* specimens and related Dermestids were sourced from a national *Trogoderma* trapping program conducted throughout Australia between 2009-2011 at targeted sites around grain silos and ports. Khapra beetle material was sourced from overseas collections, of different geographical origin. The molecular approach included conventional PCR, real-time PCR and DNA sequencing methods. DNA was extracted from morphologically verified khapra beetle populations, field collected native Australian *Trogoderma*, warehouse beetle and other related pest Dermestids. Taxonomically verified target specimens were used to data mine for unique DNA sequence profiles.

1. Materials and Methods

Morphology – taxonomic verification of target species

Dermestid material from a national *Trogoderma* trapping program (2009 -2011) was a major resource for the project in terms of diversity of native *Trogoderma*, number of geographic sites and number of specimens (~17,000) in providing a broad range of *Trogoderma* species and *Trogoderma*-like species for DNA-validation work and generation of unique sequence profiles. A targeted trapping approach was used based on previous studies by Wright (1993) and Rees *et al* (2003) and data collected using hand held devices (PDAs) synchronised to desktop server database (Emery et al 2010). The survey involved setting two trap types at >70 seleted sites – both inside and outside grain silos around Australia (Botha et al 2012). The insect traps used in this study were commercially available products – Trece Storgard khapra beetle trap (Barak 2004), and a modified Lepidopteran wet trap (UniTrap) using *Trogoderma*-specific lures (Barak 1989). The survey was conducted between 2009 and 2011, with trap catch material collected on a monthly bassis and identified at the Department of Primary Industries and Regional Development (DPIRD), Western Australia.

Additional Dermestid material was provided by University of Western Australia collaborators (collected from Gnangara area of Western Australia). Ad-hoc specimens, specimens from smaller trapping projects, colony material and curated specimens were also used to build a diverse Dermestid collection for the project.

Khapra beetle specimens from different geographical locations were sourced through international contacts in Spain (colony, established 1956; origin: unknown), Canada (origin: Pakistan), Greece (origin: unknown, possibly Turkey), Germany (origin: Iran) and UK (Centre for Agriculture and Biosciences International (CABI); origin: unkown).

Morphological methodology included specialist insect handling, identification with chain-of-custody labelling for trace-back to collection site, date of collection, trap type etc. Thousands of specimens were pinned, labelled and data-based. Western Australian Department of Primary Industries and Regional Deveopment (DPIRD) taxonomists verified the specimens for the molecular development and verification in this project. Diagnostic image capture (photomontage) of the unique native *Trogoderma* identified was outside the scope of the project, nonetheless, some unique specimens were photomontaged and cross-referenced with specimen ID and DNA sequence codes.

Molecular diagnostics

The methodology included assessment of molecular (real-time PCR) khapra beetle protocols developed in previous Plant Biosecurity Cooperative Research Centre (PBCRC) projects (PBCRC20137,PBCRC60046), as well as testing a published DNA protocol (Olson et al 2014) on an extensive cohort of Australian native *Trogoderma* and khapra specimens from different geographical origin. Optimisation and development of new PCR primers for khapra beetle and

warehouse beetle (*T. variabile*) was also undertaken as part of this study. For DNA extractions and molecular procedures in the DPIRD Diagnostic Laboratory Service (DDLS), insect legs were removed from pinned, labelled adult specimens, or provided as larvae in etOH from multiple, labelled specimens, cross-referenced with DDLS codes for chain-of-custody.

The molecular protocols were tested for accuracy, specificity and reproducibility as outlined by the Australian Subcommittee on Plant Health Diagnostic Standards (SPHDS) instructions for National Diagnostics Protocols. The proposed research was designed to address the "International importance of accredited diagnostic laboratories using accepted diagnostic procedures" as written in the International Standards for Phytosanitary Measures (ISPM 27).

A 'blind-test' challenge using 30 insect specimens, including khapra beetle, warehouse beetle and a selection of related Dermestids (and non-Dermestids) was used to test the rigour of the protocol in a 'real-world scenario'.

Destructive and non-destructive methods for DNA extraction from larvae, adults and skin casts were tested. Below is a summary of molecular methods:

Modified Olson qPCR (Olson et al. 2014) for the detection of *T. granarium* specific mitochondrial 16S ribosomal RNA (16S rRNA) gene.

Conventional Folmer and Simon PCRs (Folmer et al. 1994, Simon et al. 1994) for the universal amplification and sequencing of the mitochondrial COI gene.

Conventional – 16SAr PCR (Simon 1994, Cognato & Volger 2001, Olson et al. 2014) for the amplification and sequencing of arthropod mitochondrial 16S rRNA gene

Universal Arthropod - 16SAr qPCR (Simon 1994, Cognato & Volger 2001, Olson et al. 2014) for the confirmation of successful DNA extraction from arthropod specimens.

Extraction options included:

A. Whole insects – remove 1–2 legs and transfer to a microcentrifuge tube containing 180 µL ATL buffer and 20 µL Proteinase K. Grind the sample using a sterile micropestle.
B. Larvae – a 'core biopsy' taken from the larvae using a fine gauged syringe and transferred to a microcentrifuge tube containing 180 µL ATL buffer and 20 µL Proteinase K.
C. Destructive – if the specimens are not required for further taxonomic work the entire larvae, adult or skin cast (or part thereof) may be homogenised in a microcentrifuge tube containing 180 µL ATL buffer and 20 µL Proteinase K using a sterile micropestle.
D. Non-destructive – place the entire larvae, adult or skin cast in a microcentrifuge tube containing 180 µL ATL buffer and 20 µL Proteinase K (larvae may be 'punctured' with a fine gauge syringe to aid extraction) and incubate at 56°C with gentle agitation for at least 1 hr (can be left overnight).

2. Results

Morphology – taxonomic verification of target species

The trapping program generated more than 17,000 Dermestid specimens, including at least 20 native *Trogoderma* species, which are yet to be formally described. In the project time-frame, 11 different native *Trogoderma* species have been identified, along with thousands of related Dermestid genera. Table 1 provides a summary of the Dermestid species collected and numbers that have been curated. Table 2 provides a summary of the non-dermestid species in the bi-catch trapped.

Tab. 1 Dermestid taxa recorded at grain storages in an Australian Dermestid trapping survey.

Dermestidae	Total numbers
Anthrenocerus	69
Anthrenus	24
Anthrenus verbasci	15
Attagenus	18

Dermestes	9
Dermestes maculatus	2
Orphinus	775
Phradonoma nobile	11
Thaumoglossa	81
Trogoderma (native)	3,793
Trogoderma variabile	12,111
Trogoderma granarium	0

Tab. 2 Non-dermestid Coleopteran taxa recorded at grain silos in an Australian Dermestid trapping survey.

Non Dermestidae
Anobiidae
Bostrichidae
Buprestidae
Carabidae
Chrysomelidae
Coccinellidae
Other Coleoptera
Cucujoidae
Curculionidae
Elateridae
Haliplidae
Hydraeinidae
Hydrophilidae
Laemophloeidae
Melyridae
Mycetophagidae
Nititulidae
Ptinidae
Tenebrionidae
Scarabeaidae
Silvanidae
Staphylindae

Molecular

The qPCR 'road-test'

A total of 1,618 *Trogoderma* and related Dermestid specimens underwent qPCR screening. The majority of the specimens consisted of 2-3 dissected insect legs, with the remaining insect pinned and labelled for reference. The khapra-specific 16S qPCR assay proved successful with a sensitivity of 100% and specificity of 97.20% when tested against the 1,618 specimens, including 61 known khapra isolates and 1,557 endemic beetles (Table 3). The performance of the 16S qPCR assay compared to the gold standard taxonomic identification is presented in Table 4. The performance of the modified Olson qPCR was within the recommended parameters of a validated diagnostic test.

Tab. 3 Total number of specimens tested by the Olson qPCR and the diagnostic sensitivity and specificity of the assay.

Total number of specimens	1,618
Total number of Khapra	61
Sensitivity	100%
Specificity	97.20%

Tab. 4 Confusion matrix detailing the performance of the Olson qPCR assay compared to the gold standard taxonomic identification. TP = true positive, TN = true negative, FP = false positive and FN = false negative.

	Taxonomic ID	
	Khapra	Non-khapra

PCR ID	Khapra	61 (TP)	43 (FP)
	Non-khapra	0 (FN)	1514 (TN)

Confirmatory sequencing

Sequencing of the DNA barcoding COI gene (mitochondrial gene cytochrome oxidase I) revealed >99% sequence homology with *T. granarium* specimens in GenBank. This result means that the qPCR test will rapidly identify positive khapra specimens, which can then be sent off for confirmatory sequencing at a third party laboratory, which is standard practice for NATA accredited Diagnostic Protocols, and current practice in the event of a 'real' incursion.

Molecular blind testing

The khapra beetle qPCR test correctly identified and discriminated khapra beetle specimens in the blind sample set (30 specimens), with no false positives or false negatives, with a results turn-around time of 2 days (non-urgent) (Table 5).

Follow-up sequencing to confirm the preliminary PCR diagnosis was undertaken by a third party facility (AGRF QEII Medical Centre) to simulate the diagnostic process that would occur in the event of a real incursion.

Sequencing results confirmed the positive khapra PCR test results, returning *Trogoderma granarium* partial 16S rRNA gene for all 4 khapra specimens. The four khapra samples were haplotyped as HT1 (Spanish 1956 colony); HT1 (Iran - German colony); HT2 (Pakistan – via Canada); HT2 (Pakistan via Canada).

A neighbour-joining tree for partial mitochondrial 16S rRNA gene sequences based on Olson-defined *Trogoderma* haplotypes was constructed (Fig. 1).

Tab. 5 Multiplex real-time PCR for the detection of *Trogoderma granarium* and *Trogoderma variabile* (in-house assay)

Species No.	Species ID	Species Description	*T. granarium*	*T. variabile*
0001	A1	*Trogoderma variabile*	-	+
0002	A2	Coccinellidae (native)	-	-
0003	A3	*Anthrenus* sp.	-	-
0004	A4	*Sitophilus oryzae*	-	-
0005	A5	*Trogoderma variabile*	-	+
0006	A6	*Anthrenus* sp.	-	-
0007	A7	*Anthrenus verbasci*	-	-
0008	A8	*Tribolium castaneum*	-	-
0009	A9	*Trogoderma* sp. (native)	-	-
0010	A10	*Trogoderma variabile*	-	+
0011	A11	*Anthrenus verbasci*	-	-
0012	A12	*Trogoderma granarium*	+	-
0013	A13	*Rhyzopertha dominica*	-	-
0014	A14	*Trogoderma variabile*	-	-
0015	A15	*Trogoderma variabile*	-	+
0016	A16	*Anthrenus* sp.	-	-
0017	A17	*Oryzaephilus surinamensis*	-	-
0018	A18	*Trogoderma granarium*	+	-
0019	A19	*Trogoderma* sp. (native)	-	-
0020	A20	*Trogoderma variabile*	-	+
0021	A21	*Anthrenus* sp.	-	-
0022	A22	*Trogoderma granarium*	+	-
0023	A23	*Cryptolestes pusillus*	-	-
0024	A24	*Trogoderma* sp. (native)	-	-
0025	A25	*Thaumoglossa* sp.	-	-
0026	A26	*Trogoderma granarium*	+	-
0027	A27	*Anthrenus verbasci*	-	-
0028	A28	*Anthrenus verbasci*	-	-
0029	A29	Coccinellidae (native)	-	-
0030	A30	*Trogoderma variabile*	-	+

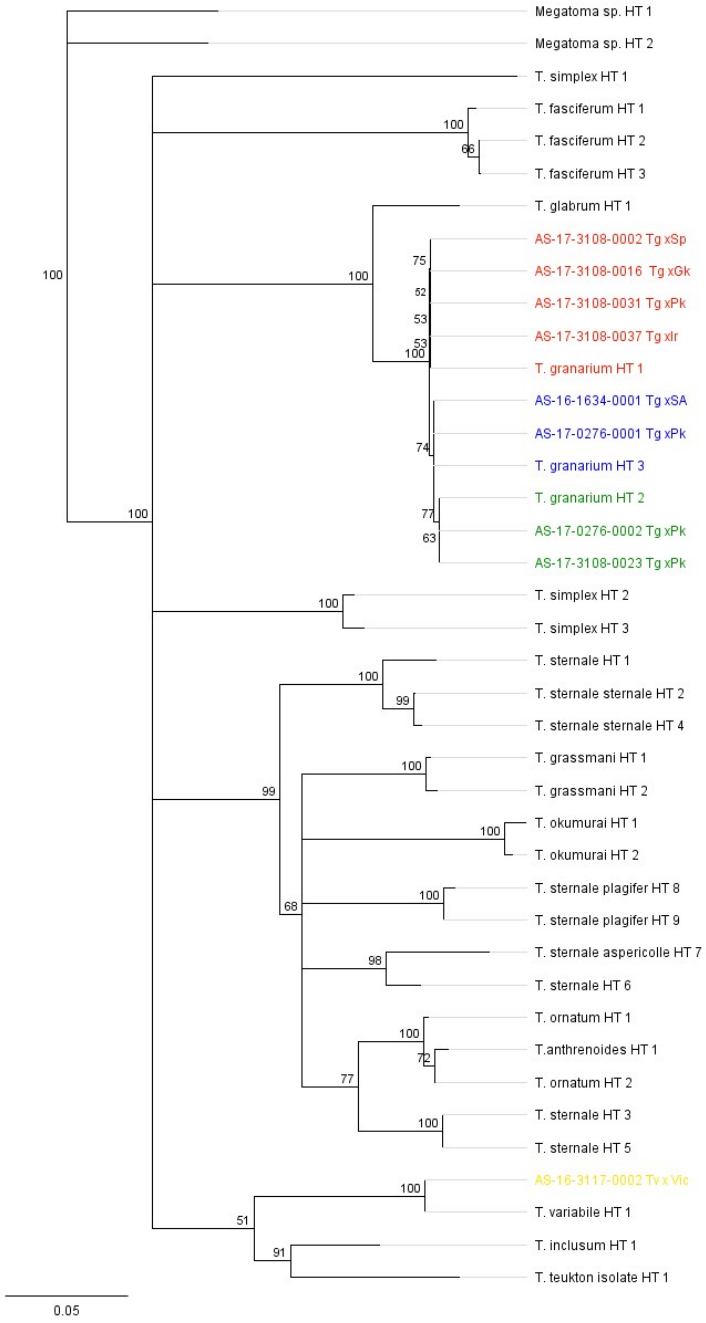

Fig. 1 Neighbour-joining tree for partial mitochondrial 16S rRNA gene sequences based on Olson-defined *Trogoderma* haplotypes (accessed via Genbank, NCBI). DPIRD sequencing results are denoted by laboratory accession numbers in colour (*T. granarium* HT 1 = red, HT 2 = green & HT 3 = blue and *T. variabile* in italics). Genetic distance was computed using the Tamura-Nei method. The tree is rooted to the outgroup species Megatoma sp. HT 1. The number on each node represents bootstrap probability based on 1000 replications.

Discussion

The project work has led to the development of a high-throughput qPCR diagnostic protocol for the species-specific detection of *Trogoderma granarium*. A second qPCR test for *Trogoderma variabile* was also developed. Additional 'universal' conventional (end-point) PCR assays form part of the diagnostic protocol which allow for the confirmation of identification via Sanger sequencing (if required). The protocol includes an optional qPCR method to quality control the DNA extraction process. This protocol is in routine use in our diagnostic DDLS facility, as a high-throughput Dermestid screening test.

As a result of this work, DPIRD has the capacity to undertake high-throughput PCR screening for the exotic khapra beetle which is absent from Australia. The PCR test offers a sensitive and specific quick 'first pass' screen for suspect khapra specimens adding to preparedness and planning options in the event of a pest incursion into Australia.

One of the advantages of the qPCR test is the ability to test insect fragments, damaged specimens and larvae that are almost impossible to identify morphologically. The project work has produced a Dermestid reference collection of more than 17,000 Dermestid specimens. This reference collection includes many previously unknown Australian native *Trogoderma* species and forms a unique and valuable legacy resource. Suspect Dermestid specimens can be tested in-house and their genetic sequences compared with in-house reference material, and against genetic reference profiles in publicly available databases (e.g. BOLD and GenBank). The diagnostic protocol developed for khapra beetle will be submitted shortly to the Subcommittee on Plant Health Diagnostics (SPHDS) in Australia for review as an accredited National Diagnostic Protocol for use throughout Australia (and Internationally). The Australian National Plant Biosecurity Diagnostic Network (NPBDN) publishes diagnostic protocols for priority pests online at:

http://plantbiosecuritydiagnostics.net.au/resource-hub/priority-pest-diagnostic-resources/.

It is anticipated that once approved by SPHDS, the khapra beetle protocol developed in this study will be published on the NPBDN website. The DNA sequences of Australian native *Trogoderma* and related Dermestids will be submitted to an internationally recognised genetic resource website at the conclusion of this study.

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Assessing drivers of maize storage losses in south west Benin using a Fractional Response Model

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Abstract

An assessment of drivers of maize storage losses was undertaken in south west Benin applying the Fractional Response Model on information collected from 400 smallholder maize farmers. Overall, respondents lose on average 10.3% of their harvest during the storage period. The average marginal effect obtained from the fractional response model of storage losses revealed that storage technologies, farmers' post-harvest attitudes, insects damage, the weather conditions and infrastructures played a significant role in the level of storage losses surveyed farmers have experienced. Farmers using bags and plastic containers have respectively reduced their storage losses by 6.7 and 7.8% compared to farmers using cribs. Considering the use of storage protectant, the results indicated that using ash, neem leaves, pepper or lemon lead to an increase of 4.1% of losses relative to

storing without any protectant. Drying after harvesting decreased by 1.9% the share of the quantity stored lost during storage. The percentage of maize lost increased by 5.1% for respondents who have reported insects as predators of their stored maize. Rain at harvest time increased the percentage of losses by 2.1%. A one-degree increase in temperature increased the percentage of maize loss by 4.4% and farmers who live at less than 26.5 km to the market have reduced by 0.17% of maize losses. Effective policies for a sustainable reduction of storage losses among maize farmers in the area should consider the need to discourage the use of cribs, ash, leaves, pepper and lemon as storage technologies. Farmers should avoid harvesting during times of rain, and should properly dry their produce after harvesting. Sustainable hermetic equipment should be promoted and farmers' access to markets facilitated.

Keywords: Maize; Storage equipment; Storage protectant; Storage losses; Fractional Response Model

1. Introduction

Each year, significant volumes of food are lost after harvest in Sub-Saharan Africa (SSA), the value of which is estimated at USD 4 billion for grains alone (World Bank, 2011). World Bank (2011) emphasizes that the high level of grain lost in developing countries after harvest, in addition to aggravating hunger, also leads to a waste of expensive inputs such as irrigation water, fertilizer and human labour. Storage is a critical stage in the food supply chain. In developing countries with hot climates, most smallholder farmers rely on sun drying to ensure that crops are well dried before storage. If unfavourable weather conditions prevent crops from drying sufficiently, such crops are subject to high losses during storage (Hodges *et al*., 2014). The need to deal with post-harvest losses and to undertake innovative and impact oriented PHL research is critical for achieving food security and reducing poverty in the sub region (Affognon *et al.,* 2015).

A major obstacle in the efforts to mitigate storage losses in developing countries is the lack of accurate knowledge on the magnitude of losses as well as the linkage between drivers of such losses. Outdated contextual estimates of these losses could lead to the implementation of bad policies (Affognon *et al*., 2015).

This paper offers a good understanding of the scope and nature of the problem of storage losses among maize farmers in south western Benin where maize is considered as an important food crop; mainly produced under rain fed agriculture by smallholder farmers and subject to important storage losses. The study is the first in Benin to assess drivers of storage losses in a multivariate setting. Planners and policy makers can rely on the results of the study to as early as possible in their decision cycle design appropriate and effective measures for storage loss reduction.

2. Materials and Methods

Data were randomly collected from over 400 farmers from September to October, 2016. Secondary information on temperature and rainfall pattern during 2015 were obtained from the local climate agency, known as ASECNA Benin/ Lokossa Station.

The dependent variable of interest in this study is the percentage of maize storage losses in south west Benin. The Fractional Response Model (FRM) has been defined for the first time by Papke and Wooldridge (1996) to deal with situations where the dependent variable is a proportion and its values are allowed to be zero or one. Authors have shown that the use of the Ordinary Least Squares (OLS), the censored regression (Tobit), or the transformed logistic normal model (the log-odds ratio of the dependent variable) in such cases are inefficient, as their error distributions will be heteroskedastic (Papke and Wooldridge, 1996; Kieschnick and McCullough, 2003). The Fractional Response Model is a non-linear model estimated using the Quasi-Maximum Likelihood Estimation (QMLE) method. The QMLE is asymptotically efficient and consistent compared to either OLS or Tobit. In the FRM model, a functional form for the dependent variable is chosen such that it imposes constraints on the response variable to ensure that predicted values will always lie within the closed interval [0,1].

The empirical FRM specification of storage losses retained in this study is:

$$E(Y_i/X_i) = G(X_i) = b_0 + \sum_{k=1}^{24} b_k X_{ik} + \varepsilon_i \quad (2.1)$$

$$E(Y_i/X_i) = G(X_i\beta) = b_0 + \sum_{k=1}^{n} b_k X_{ik} +$$

Where $0 \leq Y \leq 1$ correspond to the percentage of storage losses; X_i represent the explanatory variables for each observation *i* and $\varepsilon^{\varepsilon}$ represents the error term. *G(.)* is a distribution function similar to the logistic function.

Following Papke and Wooldridge (1996) and Wooldridge (2011), the generalised linear modelling (glm) was retained to fit the fractional response model for the percentage of storage losses in south west Benin.

3. Results

The volume of reported storage losses by maize farmers from the south western of Benin is on average 10.3% of the quantity harvested.

Storage equipment

The marginal effect computed from the fitted model showed that farmers using bags and plastic containers respectively have reduced their storage losses by 6.7 and 7.8% compared to farmers using cribs. There is however no difference between the predicted storage losses of users of rooms and cribs.

Storage protectant

Considering the use of storage protectants, the results indicated that using ash, neem leave, pepper or lemon leads to an increase of 4.1% of losses relative to storing without any protectant.

Drying

The results revealed that drying after harvesting decreased by 1.9% the share of the quantity lost during the storage period. Drying the harvest for a second time at home lowered the moisture content of maize and this significantly contributes to a loss reduction.

Insect attacks

The amount of maize lost during storage has increased by 5.1% for respondents who have reported insects as predators of their produce kept in stores.

Rains at harvest

The effect of rain at harvest time was significant and increased the percentage of losses by 2.1%. This result was expected, since rain at harvest time raises the issue of moisture content in harvested crops. The higher the wetness/moisture/dampness of the grain before storage, the higher is the likelihood of losing maize while being kept in stores.

Temperature

The temperature within the first three months of storage had a significant effect on the percentage of maize loss during the storage period. A one-degree increase in temperature increased the percentage of maize loss by 4.4%. The significant effect of temperature on losses is in line with the literature, where the climate conditions have been suggested as a factor in storage losses by Costa (2014). However, the study revealed a turning point of 26.8 over which the temperature contributes to losses reduction.

Market conditions

Market conditions have been tested through price and the distance to market. Prices do not significantly affect the percentage losses. However, the distance to market revealed a non-linear effect on the percentage losses. A one kilometre increase in the distance to market reduced by 0.2% of maize loss and this is true only when the distance to market is less than 26.5 km, the computed extremum. Beyond that, it contributes to storage losses. This result shows that distance to market remains an important issue when it comes to commercializing agricultural products.

4. Discussion

Cribs that are widely used are subject to storage losses. It suggests that awareness should be raised about the storage losses issue, as this is strongly related to the use of cribs in the region. The results show some limit within farmers' attitudes when it comes to preserving their maize product using storage protectant. The study revealed the irrelevance of using ash, neem leaves, pepper and lemon to store maize in south west Benin. The inefficiencies may be explained - without a proper investigation on the issue - by the fact that ash, pepper, lemon and neem leaves are commonly poured on the maize (especially in layers for neem leaves) with husk kept in stores. The fact that insects are damaging the grain itself and are even living inside the maize, the presence of husk between the used protectant and the stored product could prevent the effectiveness of the given protectant.

In the region, maize drying is commonly done in the field before harvest. However, some farmers reported drying their produce a second time before storage. This has contributed to storage losses reduction. Accordingly, dryer technologies with low fixed and operationalisation cost could be implemented in the region. This may help farmers reducing their losses by firstly harvesting after maturity of the crops and then drying adequately. Solar maize dryers could therefore be a better alternative.

Insect attacks remain a challenge for maize farmers. Insect infestation starts from the field when crops are not well treated and / or during the storage period. The effect of insects in damaging or destroying the edible part of the grain put in storage is well documented in the post-harvest literature (Hodges *et al.,* 2014), and that issue is not new. Unfortunately, insects continue to be a threat to maize farmers whose products are kept in stores. Recently, modern hermetic storage equipment have been suggested as a sustainable way to overcome the insect problem (Costa, 2014). Finally, farmers have to avoid harvesting during times of rain and their access to markets should be facilitated to effectively reduce losses that are likely to occur during storage.

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Insects and fungi in stored maize in Angola

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Abstract

In underdeveloped countries in Asia and Africa, non-effective post-harvest technologies and sometimes ideal environmental conditions for development of pests like insects, fungi, rodents and birds, can lead to damage of both raw or processed foods. Losses can achieve considerable proportions in dried vegetables used as food products, particularly in underdeveloped countries where food security problems are a daily basis routine. The major goal of the present study was the identification of insects and fungi associated with maize under local storage conditions in the Angola provinces of de Benguela, Bié, Cuando Cubango, Cuanza sul, Huambo, Huíla, Luanda, Malange and Namibe. A wide range of storage methods for cereals were sampled, from small containers of peasants and small farmers up to the large metal containers used by large agricultural companies and Estates. The achieved results will contribute for food security improvement in Angola and for the maintenance and preservation of good and healthy seeds at the traditional farmers' community level. The insect pests registered from the studied samples were *Cryptolestes ferrugineus, Gnatocerus maxillosus, Liposcelis bostrychophila, Oryzaephilus surinamensis, Rhyzopertha dominica, Sitophilus zeamais, Sitotroga cerealella and Tribolium castaneum*. The species *Prostephanus truncatus* was not found in the studied samples. Fungi in the genus *Aspergillus*, *Fusarium* and *Penicillium* were presented at a high incidence in all samples studied, although the relative abundance of different fungi species varied with the sample location.

Keywords: maize, insects, fungi, Angola, storage.

Introduction

In Angola, maize is the cereal with the highest production and one of the most consumed. An average maize yield of 640 kg/ha was reported for the period 2000-2010 (FAOSTAT, 2012). Although grain production in the country has increased, Angola still has a deficit of 3 million tons, achieving only 40% of consumption needs (INCER, 2014). Factors such as severe technical knowledge gaps, lack of incentives to producers, low fertility of soils, use of low-yielding varieties, non-application of technologies or lack of access to them, lack of access to production factors, lack of infrastructure for water management, lack of reliable storage structures, and low availability of credit resources greatly reduce the expected yields (Pacheco et al., 2011). There are a number of warehouse systems and warehouse types in Angola at the smallholder level. These warehouses are built with clay, sticks and covered with grasses or wood and grass. The poor condition of the warehouse structure, its hygiene and moisture control issues at the level of the small producer does not guarantee good phytosanitary status for the stored products.

Cereals storage is a specific agro-ecosystem, conditioned by several factors which are difficult to control, like temperature, relative humidity, water content, and oxygen availability (Barros, 1993). This is especially true in underdeveloped countries where technological innovations such as refrigeration and controlled atmospheres represent huge investments. Storage under deficient conditions can lead to insect or fungi attack, inducing organoleptic changes (taste, flavour and appearance), nutritional losses or even mycotoxin contamination. These cause significant economic losses and can represent serious health problems.

The insects of stored grains have certain preferences regarding temperature, relative humidity, water content and food. The interaction of these factors affect, directly or indirectly, the insects' proliferation rate and thus the possibility of causing damage and/or loss during storage of those products (Barros, 1996).

The objective of this work was to identify species of insect and fungi responsible for the deterioration of stored cereals present in samples of maize from Angola, and as a result contribute in some way to the improvement of the storage conditions of these products. Aim was not only to confirm the storage pests already identified in Angola in previous studies, but also to check for the presence of *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae). This species is a pest of stored maize and cassava, which became a major concern after being accidentally introduced in Africa.

This is pioneering and very important work for Angola. It covers approximately 50 % of the national territory evaluating the phytosanitary situation of stored cereals in the Benguela, Bié, Cuando Cubango, Cuanza Sul, Huambo, Huíla, Luanda, Malange and Namibe provinces, identifying all the insects and fungi present in the studied cereal samples.

Materials and Methods

Maize samples were collected randomly in Angola in different quantities and packed in plastic or paper bags, and transported to the laboratory in Lisbon. The samples were kept cool (0-4°C) after collection, during shipping and at the laboratry until being observed. These procedures took 7-8 days.

Samples originated from different sources, from small producers in the provinces of Benguela, Bié, Cuando Cubango, Huambo and Huíla, from the business sector, from the Pungo Andongo farm in the province of Malange and from the local markets of the provinces of Cuanza Sul, Luanda and Namibe. At the arrival of the samples in the laboratory they were cleaned and sieved for removal of stones, dust, crop pieces, excrement and insects. Then, using the Boerner divider the samples were subdivided for later entomological and mycological analyzes.

Insects analysis

The insects present in the maize samples, on arrival, were identified and recorded. The maize samples were then placed in glass bottles, identified with origin, arrival date and incubated at 27±1°C and 75-80 % relative humidity. The purpose of this procedure was to observe and identify the emergence of hidden adult insects inside the maize kernels.

Mycoflora analysis

Maize samples from six provinces of Angola were collected in sterilized containers and taken into the ISA laboratory. The maize samples were sub-divided into 110 kernel samples. These sub-samples were surface disinfected with 1 % sodium hypochlorite for two minutes, as describe by Pitt and Hocking (1997) and Magro et al. (2006).

Ten dried grains were placed in Petri dishes with 20 mL of Potato Dextrose Agar (PDA) medium with chloranphenicol (1 %). For each sample, ten replicates were made. Petri dishes with grains were incubated at 25°C for 7 days and then examined under a light stereomicroscope for fungal growth. Isolation of the colonies was made to obtain pure cultures. Slides of fungal growth were prepared and observed under a compound microscope for fungal morphology study. Identification was carried out using identification keys (Carmichael et al., 1980; Domsch et al., 1980; Onions et al., 1981; International Mycological Institute, 1991; Hanlin, 1997; Malloch, 1997; Pitt & Hocking, 1997; Barnett & Hunter, 1998; Samson et al., 2004).

Results

Insects

Table 1 shows a list of the insects identified in the maize samples from eight provinces of Angola. It was found that *C. ferrugineus*, *S. zeamais* and *S. cerealella* were present in all samples.

Tab. 1 Identified insects in stored maize samples from eight provinces of Angola.

Insect	Province							
	Benguela	**Bié**	**Cuanza Sul**	**Huambo**	**Huíla**	**Luanda**	**Malange**	**Namibe**
COLEOPTERA								
Cryptolestes ferrugineus	+	+	+	+	+	+	+	+
Gnatocerus maxillosus	+	+	-	-	-	-	-	-
Oryzaephillus surinamensis	-	-	+	-	-	+	+	-
Rhyzopertha dominica	+	+	-	+	+	-	+	+
Sitophilus zeamais	+	+	+	+	+	+	+	+
Tribolium castaneum	+	+	+	+	+	+	-	+
LEPIDOPTERA								
Sitotroga cerealella	+	+	+	+	+	+	+	+
PSOCOPTERA								
Liposcelis bostrychophila	-	-	+	-	-	+	+	+

Note: (-) without insects and (+) with insects.

Fungi

In this study, field and storage fungi were detected and identified in all samples. The field species isolated were *Diplodia maydis*, *Nigrospora* sp., *Rhizopus* sp., *Trichoderma* sp. and *Trichothecium roseum*.

The storage species isolated were *Aspergillus candidus*, *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. parasiticus*, *A. wentii*, *Fusarium moniliforme*, *F. oxysporium*, *Penicillium citrinum*, *P. funiculosum*, *P. furcatum*, *P. islandicum*, *P. purpurogenum*, *P. variabile* and *P. pinophilum*. The presence of different taxa in samples from the six provinces is presented in the Table 2.

Tab. 2. Frequency of identified fungi in stored maize samples from six provinces of Angola.

	Province					
	Benguela	**Bié**	**Huambo**	**Huíla**	**Malange**	**Namíbe**
High frequency	*Fusarium moniliforme* *P. citrinum*	*A. clavatus* *A. flavus* *A. ochraceus*		*F. moniliforme*	*A. flavus*	*Trichothecium roseum*
Frequent	*Rhizopus sp.*	*P. funiculosum*	*A. flavus* *A. fumigatus* *A. ochraceus* *F. moniliforme* *P. variabile* *P. pinophilum*	*A. flavus* *A. parasiticus* *P. purpurogenum*		*A. flavus* *P. citrinum* *P. variabile*
Low frequency	*A. clavatus* *A. flavus* *F. oxysporum* *P. islandicum*	*A. niger* *A. parasiticus* *F. moniliforme* *Nigrospora* sp. *P. pinophilum*	*A. niger* *A. parasiticus* *P. citrinum* *Trichoderma* sp.	*A. candidus* *A. niger* *A. wentii* *Diplodia maydis* *P. funiculosum* *P. furcatum*	*P. funiculosum* *P. furcatum*	*A. candidus* *A. niger* *F. moniliforme* *P. funiculosum* *Rhizopus* sp.

P. pinophilum
Rhizopus sp.

Discussion

The results show that most of the insects present in the studied samples belong to the Coleoptera order, confirming the results obtained by Amaro & Gouveia (1957), Carvalho (1984) and Matos (2004). Data support the conclusion of no differences in species in relation to the sample collection site; i.e., insect species found in samples collected in the silos are the same as those obtained in the local market.

The presence of the same insect species that have been identified in the other provinces is highlighted in Malange, which is somewhat worrisome given that the maize sample from this province belongs to a major agricultural company, which theoretically has good technical advice and practices, while the other samples are from small local producers and markets.

In all of the provinces where maize samples were collected the presence of *P. truncatus* was not detected. It is of paramount importance to continue this work by collecting a larger number of samples, for each province, for each type of storage, in maize, from the small farmer to the large storage companies, to detect the arrival of *P. truncatus*, a devastating pest already present in many African countries. Ensuring continuous training and implementation of pesticide regulation are also a priority for Angola.

Field fungi colonize maize grains only when the water activity (aw), temperature and relative humidity are high. However, as a result of an adaptation to low aw, fungi belonging to *Aspergillus* spp., and *Penicillium* spp., also designated as storage fungi, are able to invade the maize grains stored at aw levels considered as safe. They are frequently responsible for causing serious losses, even before they were visually detected. They affect negatively the product's appearance, flavour, odour and nutritional content. They also may produce mycotoxins with large impact on public health (Magro, 2001). It is important to emphasize that *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp., are potential mycotoxins producers. It is fundamental to improve and control the maize storage conditions as well as the cleaning process before any further grain processing.

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Automated detection and monitoring of grain beetles using a "smart" pitfall trap

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Abstract

A smart, electronic, modified pitfall trap, for automatic detection of adult beetle pests inside the grain mass is presented. The whole system is equipped with optoelectronic sensors to guard the entrance of the trap in order to detect, time-stamp, and GPS tag the incoming insect. Insect counts as well as environmental parameters that correlate with insect's population development are wirelessly transmitted to a central monitoring agency in real time, are visualized and streamed to statistical methods to assist effective control of grain pests. The prototype trap was put in a large plastic barrel (120lt) with 80kg maize. Adult beetles of various species were collected from laboratory rearings and transferred to the experimental barrel. Caught beetle adults were checked and counted after 24h and were compared with the counts from the electronic system. Results from the evaluation procedure showed that our system is very accurate, reaching 98-99% accuracy on automatic counts compared with real detected numbers of adult beetles inside the trap. In this work we emphasize on how the traps can be self-organized in networks that collectively report data at local, regional, country, continental, and global scales using the emerging technology of the Internet of Things (IoT). We argue that smart traps communicating through IoT to report in real-time the level of the pest population from the grain mass straight to a human controlled agency can, in the very near future, have a profound impact on the decision making process in stored grain protection.

Keywords: pitfall trap, sensors, Internet of Things, stored grain, beetle pests.

Introduction

Low tolerance of the presence of insect pests in stored grain requires the development and implementation of detection and monitoring methods that are sensitive enough to detect early pest infestation to prevent quality and economic losses (Trematerra, 2013). Today, the innovative uses of sensors and networks targeting animals are starting to be translated into new ecological knowledge (Portet et al., 2009). Traps equipped with a detection sensor and wireless communication abilities have some distinct advantages against manual monitoring. They can monitor insect populations 24 h a day, upon their entrance to the trap, every day of the year, in dispersed nodes across a variety of fields, simultaneously, and all counts and recordings can be permanently stored in a cloud service. Another distinct advantage is the determination of the precise onset of an infestation. Real-time reporting, opens new grounds in stored product research and mainly in crop protection as – besides a timely control action in response to a pest infestation – it can help in the evaluation of the impact of a control treatment and therefore reschedule future actions if necessary.

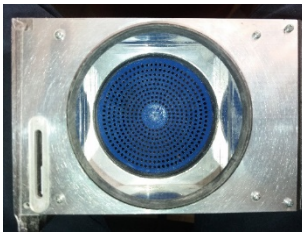
Pitfall traps are typically used for monitoring several species of stored-grain beetles (Coleoptera) in silos, warehouses and processing plants (Reed et al., 1991). They are placed inside the bulk grain near the external surface. The cone-shaped device is made of clear plastic and has a removable perforated lid, which allows insects to enter, but not escape. Various pheromone lures targeting different species may be used. Many destructive beetle pests of stored grain may be monitored by this type of trap: the flour beetles *Tribolium* spp. (Tenebrionidae), the grain weevils *Sitophilus* spp. (Curculionidae), the lesser grain borer *Rhyzopertha dominica* (F.) (Bostrichidae), the cigarette beetle *Lasioderma serricorne* (F.) (Anobiidae) and the khapra beetle *Trogoderma granarium* Everts (Dermestidae) (White et al., 1990; Neethirajan et al., 2007).

Our approach aims at reducing the necessity of human-in-the loop in any intermediate processing stage of the workflow and reserve the need of expert entomologists only for the highest abstraction layer: the interpretation of the data received (trap catches) normally presented in the form of georeferenced maps and the corresponding decision making and action planning based on pest Economic Injury Levels (EIL) population thresholds that are applied in the frames of Integrated Pest Management (IPM). Our work focuses on leveraging the quality of service of remote surveillance of pest populations to a better and cost-effective status than sparsely applied human inspection.

Materials and Methods

We have embedded our electronics into the Pitfall trap (EDIALUX, Bornem, Belgium) for monitoring populations of beetle pests of stored grain. There is always an emitter of light opposite to a receiver of light and the path of the incoming insect passes in between. The interruption of the path of light effects a voltage drop that exceeds a threshold and constitutes a count. Both receiving and emitting elements are deployed as 1D linear arrays that are long enough to cover the entrance to the trap. In the pitfall trap, an insect can enter from any hole of the lid. In order to avoid blind spots in the field of view we need to have a uniform field sensing insect sizes ≤ 0.5 mm. We used 16 LEDs and the same number of photodiodes and both emitter and receiver have a light diffuser. All sensors are operated in pulse mode i.e. there is no constant flow of light from emitter to receiver but a pulse train is emitted.

For the purposes of our study, a prototype (Fig. 1) equipped with a linear array of five Light Emitting Diodes (LED) opposite to 5 receiving photodiodes was evaluated. The prototype trap was put in a large plastic barrel (120lt) with 80 kg maize. Adult beetles of various species were collected from laboratory rearings and transferred to the experimental barrel. In order to ensure trap catches a large number of adult beetles was used resulting in an infestation level of more than 15 adults per kg maize. Caught beetle adults were checked and counted after 24h and were compared with the counts from the electronic system.

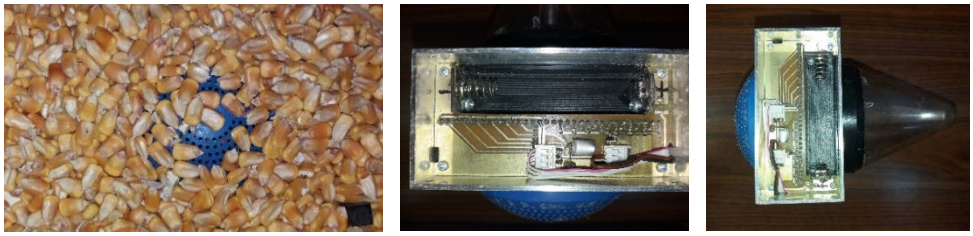



Fig. 1 The "smart" Pitfall trap. A sheet of light covers the lid entrance. Photo interruption due to a falling insect produces a voltage variation that is turned to a count. Counts as well as environmental parameters and a time stamp are transmitted wirelessly and uploaded to server.

Results & Discussion

Results from the evaluation of the prototype traps are presented in Table 1 and Fig 2. As it is clearly concluded from our data, our system is very accurate, reaching 98-99% accuracy on automatic counts compared with real detected numbers of adult beetles in each trap. The accuracy of our system in detecting adult beetle catches is also shown by the very high ($r > 0.99$ in all cases) correlation between the generated signals and actual numbers of insects caught in the trap.

Tab. 1 Number of actually detected (manual inspection) and automatically counted (electronic sensors) adult beetles in "smart" pitfall trap

Species	Actually Detected	Automatically Counted	Correlation coefficient (r)
	59	62	
C. ferrugineus.	45	49	0.9912
	67	74	
	31	34	
O. surinamensis	11	12	0.9978
	24	25	
	15	15	
R. dominica	23	24	0.9976
	24	26	
	21	21	
S. oryzae	32	36	0.9900
	29	30	
	13	13	
T. confusum	26	30	0.9912
	34	36	
	14	14	
R. ferrugineus.	45	49	0.9999
	67	74	

Single trap inside grain mass, insect density >15 adults / kg grain

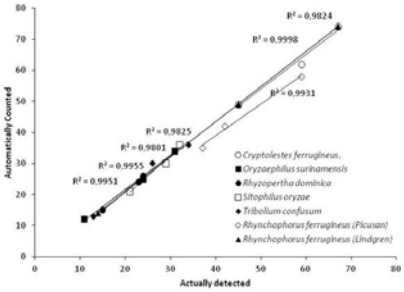


Fig. 2 Accuracy of the automatic counting in comparison with actual detection. The values of the linear regression coefficient R^2 prove that our system is 98-99% accurate (when detected and counted values are the same then R^2 equals to 1)

Only a few remote pest monitoring systems, based on wireless communication technology, have been evaluated in the past, with varying accuracy. The oriental leafworm moth *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) was effectively monitored by an ecological monitoring system combining GSM transmission technologies with mechatronics with accuracy ranging from 71 to 100 (Shieh et al., 2011). Average accuracies of 96.3% (Liao et al., 2012) and 94.9% (Deqin et al., 2016) were demonstrated by automatic monitoring systems counting the catches of the oriental fruit fly *B. dorsalis*. Other automated systems with image analysis technology also proved to be reliable in detecting mainly whiteflies and moths, with accuracies ranging from 70 to 100% (Xia et al., 2012; Ding and Taylor, 2016; Boissard et al., 2008; Lopez et al., 2012; Guarnieri et al., 2011). The accuracy of our system is higher than almost all of the abovementioned monitoring systems.

In this work we establish a connection between sensors' readings, pest population level and predictive models to ensure timely and effective control treatments. Acceptance of automated monitoring practices will raise doubts about the reliability of data collected without expert's intervention. The optoelectronics need to reduce their errors in order to reach comparable analysis to that done by experts. A long-term field operation is needed in order to identify the cause of possible false alarms and detection misses and sensor failures in sometimes harsh conditions before applying the output of such data-collection schemes to modeling and policy. We believe current results are sufficient to warrant further exploration on insect surveillance. Insect surveillance can provide insight into the effects of insecticide efficiency, reduce its use and shape our understanding of pest problems in agriculture. Provided we continue improving the reliability of devices and services and perform real-field, long-term trials we will upgrade automated practices to the level of being indispensable to farmers, policy makers and stakeholders.

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Detection and estimation of population density of bean weevils (Coleoptera: Bruchidae) in stored pulses via bioacoustic analysis

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Abstract

Stored product insects, produce acoustic emissions by moving, feeding or ovipositing inside the grain mass. These sounds can be used not only for detection purposes, but also for population density estimation. Acoustic emissions of adults of *Acanthoscelides obtectus* (Coleoptera: Bruchidae) and *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) were recorded infesting various pulses in varying population densities from 1 to 500 adults/kg product. The acoustic analysis system is being described. Population density, type of grain and pest species had significant influence on the number of sounds. The system was 100% precise in negative predictions and considerably successful in positive predictions. The system was very accurate (80-100%) in detecting insect presence even in the "critical" density of 1 adult/kg product, the most common threshold for classifying a stored mass as "infested" or "not infested". Our study suggests that automatic monitoring of the infestation state in bulk grain is feasible in small containers. This kind of service can assist reliable decision making if it can be transferred to larger storage establishments (eg. silos). Our results are discussed on the basis of enhancing the use of acoustic sensors as a decision support system in stored product IPM.

Keywords: Stored Pulses, Bioacoustic, Detection, bean beetles, Density Estimation

Introduction

More than 500 species of beetles have been reported to be associated with various stored grain products (cereals and pulses) and almost 100 of them may cause significant quantitative or qualitative losses. It has been estimated that between one quarter and one third of the world grain crop is lost each year during storage. The key for successful management of stored grain pests is not only early detection, but also an accurate population density estimation of the pest (Rajendran and Steve, 2005).

Acoustic detection is a very promising method for early detection of insects inside the grain mass (Eliopoulos et al., 2015; Hagstrum et al., 1996; Mankin et al., 2011; Potamitis et al., 2009 and others). Insects of stored grain generate sound by eating, flying, egg laying, or locomotion. Reliability and

efficacy of acoustic sensors has been greatly increased in the last few years as a result of the development of improved acoustic devices and signal processing methods (Mankin et al., 2011). Apart from detection, very few studies have evaluated the potential of the acoustic method in estimating the population density of the pest inside the grain mass (Hagstrum et al., 1988, 1990).

The aim of our study is to propose and evaluate an automated monitoring procedure for IPM implementation in grain handling and storing facilities. The main unit is composed of a piezoelectric sensor and a portable acoustic emission amplifier connected to a computer. The software analyses the vibration recordings of the piezoelectric sensor, performs signal parameterization and eventually classification of the infestation severity of adult beetles inside the grain mass in four classes, namely: Class A (densities ≤1 adult/kg), Class B (densities 1-2 adults/kg), Class C (densities 2-10 adults/kg) and Class D (densities >10 adults/kg). Our results are discussed on the basis of enhancing the use of acoustic sensors as a decision support system in stored product IPM.

Materials and Methods

For the purposes of our study, we used adults from two important beetle pests of stored pulses. We used the grain that each species is most commonly associated with in natural conditions. Specifically, we recorded acoustic emissions of the bean weevil *Acanthoscelides obtectus* (Say) (Bruchidae) on kidney and butter (giant) beans and the cowpea weevil *Callosobruchus maculatus* (F.) (Bruchidae) on broad (fava) beans.

Our system was adopted by Eliopoulos et al. (2015) and consisted of a 14cm long piezoelectric sensor mounted on the end of a probe that was pushed into the grain (hard wheat) and a portable acoustic emission amplifier (AED-2010L, Acoustic Emission Consulting, Inc., Fair Oaks, CA) connected with a computer. The experimental procedure (grain preparation, recording methodology etc) is described in detail by Eliopoulos et al. (2015). Each treatment (recording of the desired species and number of adults in the desired grain mass) was replicated 5 times. Recordings from uninfested pulses was used as control.

Various infestation densities were tested during the present study (1, 2, 10, 20, 50, 100, 200 & 500 beetle adults/kg grain). We proceeded into inserting the piezoelectric probe and taking 5 recordings per jar. We have grouped insects' density in 4 distinct classes: Class A (densities ≤1 adult/kg), Class B (densities 1-2 adults/kg), Class C (densities 2-10 adults/kg) and Class D (densities >10 adults/kg). We apply supervised learning techniques to our dataset as we know the class labels (i.e. we set the infestation densities). The task is given the counts/min of the unknown test jar the classification algorithm must predict the Class (i.e. severity) of the infestation.

In operational mode, the computer receives a vibration recording from the sensor which turns into counts of enumerated pulses (counts/min). From these counts/min it infers the distribution of probabilities over infestation severity classes A-D. By peaking the probability distribution the algorithm can output a single decision as well.

Our data (number of recorded sounds expressed as counts/min) were subjected to analysis of variance in order to evaluate the main effects. ANOVA was performed by using SPSS v.18.0. (SPSS Inc, 2009).

Results and Discussion

The mean numbers of counts/minute that were recorded during the present study are presented in Fig 1. The increase on population density (number of adults inside the grain mass) was always followed by an increase in recorded sounds. The differences were not always significant. The linear model was very effective in describing the relationship between population density and number of sounds given that values of R were high (>0.80) (Fig. 2).

The type of grain had notable influence on the number of sounds. This was observed in the case of *A. obtectus* in small (kidney) and large-sized (butter) beans. The number of sounds was significantly

higher when bean weevils were inside the kidney bean mass irrespective of population density ($F_{1,88} = 12.61$; $P = 0.0007$) (Fig. 1).

It has been well documented that acoustic sensors may be very effective in detecting insect presence in the grain mass (Eliopoulos et al., 2015; Hagstrum et al., 1991). However, there are only a few studies focusing on the estimation of pest density using bioacoustic technology. The first attempt was made by Hagstrum et al. (1988) using various densities of *R. dominica* larvae in wheat, and counting the produced sounds, with a piezoelectric microphone and earphones. They concluded that insect sounds increase with pest density and that the accuracy of estimation of insect densities with the acoustical method was comparable to that obtained with a standard grain trier. Following studies by the same research team revealed that acoustic sensors can be used for density estimation not only in experimental bins (Hagstrum et al., 1990, 1991) but also in real silos (Hagstrum et al., 1994, 1996) with very satisfactory results. Our results cannot be compared with those of the above mentioned studies because of the completely different methodology they used. For example, Hagstrum et al. (1996) correlated the number of sounds with pest density using 140 sensors on 7 cables in grain bins, checking each sensor for 10 sec 27 times per day.

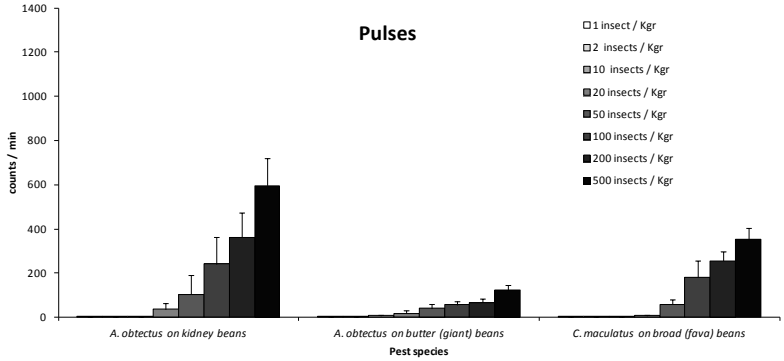

Fig. 1 Mean number of sounds recorded inside the pulses mass during the present study.

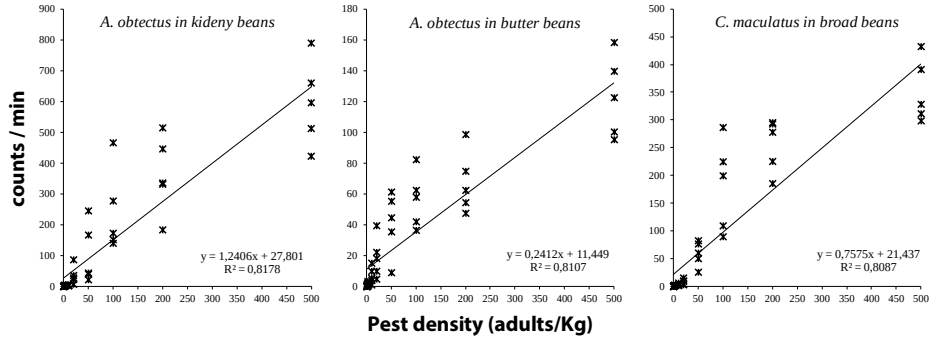

Fig. 2 Linear relationship of pest population density with number of sounds recorded in stored pulses

The type of pulse was an important factor that influenced the number of sounds. Although, sound is transmitted over longer distances in grains with a larger inter-kernel spacing, such as maize and butter beans (Hickling et al., 1997), we found that more sounds were generated when adult beetles were in small-sized grain like kidney beans. The reason for this should be that insects have smaller free space to move and they "interact" with kernels more often in small sized grains. Vick et al. (1988) also demonstrated that number of sounds produced by *S. oryzae, R. dominica* and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) varied significantly when they were in a different type of grain (rice, corn or wheat).

Our study suggests that automatic monitoring of the infestation state in bulk grain is feasible in small containers. This kind of service can assist reliable decision making if it can be transferred to larger storage establishments. Very soon and with further technological development (e.g. piezo electric sensors embedded in cables submerged in the grain) the acoustic methodology can provide a quick and easy way, not only of detecting, but also of estimating pest population density in larger establishments of stored grain facilities.

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PHID-Coleo - a database identification tool for wood-boring beetles in plant health interceptions

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Abstract

Recent examples for the introduction of wood-breeding beetles in Europe include the asian longhorn beetles *Anoplophora* spp. and *Aromia bungii* (red-necked longhorn beetle). These and other woodboring beetle species pose a high risk of economic damage to trees and wood products. Smaller beetles like the powderpost beetles from the families Bostrichidae and Lyctidae also have the potential for causing considerable damage. These are often not identified adequately during inspections of wood packaging materials, making it impossible to assess their risk for becoming invasive. This project will aim at closing that gap. Our project PHID-Coleo (= Plant Health Identification of Coleoptera) has the objective to develop new diagnostic tools for the identification of potentially invasive and economically important beetles that can be found in wood packaging materials. The identification methods include classical identification keys based on morphological characters as well as molecular methods based on DNA analysis by PCR (barcoding). The methods for species identification will be supplemented by molecular analyses of introduced populations to clarify within species variations. Such methods will make it possible to determine the taxonomic relationship of samples from different areas and to draw conclusions about the introduction pathways, resulting in more efficient monitoring of the invasive species and preventing their spread. PHID-Coleo will build a freely accessible database of relevant species which are potentially invasive.

Keywords: Cerambycidae, Bostrichidae, morphological diagnostics, molecular diagnostics, population analysis.

1. Global trade: introduction pathways for potentially invasive insect species

An increasing number of imported goods from all over the world are reaching European ports every day. These are often accompanied by untreated wood in the form of wood-packing material (WPM). The international transportation of WPM (e.g. as wooden dunnage, pallets or crates) is recognized as an important pathway for the introduction of non-native harmful insects. For this reason, specific international phytosanitary measures (International Standard for Phytosanitary Measures No. 15 or ISPM15) have been developed by the Food and Agriculture Organization of the United Nations (FAO) within the framework of the International Plant Protection Convention (IPPC) (IPPC, FAO 2013).

To prevent the introduction of harmful species this standard specifies that wooden materials (> 6 mm) used for shipment have to be free from living organisms, when being exported into a country following the ISPM15. In practice, this usually requires WPM to be debarked and then heat treated or fumigated. If such treatments have not been done adequately, wood boring insects may survive in WPM and then be introduced into the importing country. Therefore, inspections still need to be conducted at European ports. These inspections are aimed at an accurate identification of any intercepted species as well as preventing them from entering the country. But infested WPM are not always recognized properly and intercepted during the inspections at the import control and of course only in random samples. In such cases, non-native species can enter the country, may survive under suitable conditions (e.g. the availability of suitable hosts) and become established. This can result in serious damage to crops or forest trees. Recent examples of such introductions into Germany are the Asian long-horned beetle (*Anoplohora glabripennis*) (Mühleisen & Zimmermann 2016) and the rednecked long-horned beetle (*Aromia bungii*) (LfL Bayern 2018).

In case a new species is repeatedly found in the field, several possibilities need to be considered and distinguished, including whether a) the species is already established, b) there have been repeated introductions or c) the samples are related to each other. Identifying which of these possibilities apply with new molecular tools can help to improve the monitoring of invasive species and preventing their spread.

2. The problem of species identification and the necessity of pest risk analysis

The species identification is necessary, because it is the basis for pest risk analysis (PRA) conducted by the regulatory national plant health organization (e.g., the Julius Kühn-Institute for Germany). The PRA analyses the risks and possible consequences of the introduction of a new pest, under inclusion of biological (e.g. climatic tolerance, available hosts) and other scientific information. It also identifies the phytosanitary measures required to protect plant resources against a new

potential pest. In case of an introduction event, the PRA helps decision makers to react quickly and in the most suitable way (JKI - Julius Kühn-Institut 2018).

The morphological identification of non-native species is difficult, since identification keys are often unsuitable for the inspection teams, are unavailable, or are only available in a foreign language (Ohbayashi & Nisato 2007). This is especially the case for the immature stages of frequently imported groups of insects like Cerambycidae and Bostrichidae (Wang 2017, Geis 2002).

Another problem arises, when the sample is in a bad physical condition (e.g., a crushed larva) and identification traits have been lost. A molecular identification based on DNA barcoding analysis can help in such cases (Wu et al. 2017). For this, a genetic marker (a short sequence) of the specimen's DNA is compared with DNA references from an online database. If both DNA sequences match, the examined sample is most likely the species that has been deposited as reference in the database. Unfortunately, in databases such as NCBI there are error rates of up to 20%, because some species had been misidentified before they were barcoded (NCBI 2018). Such erroneous references must be recreated to meet quality standards and accreditation requirements in diagnosis.

At the same time other databases like the Barcode of Life Database, BOLD, with higher quality standards are yet often incomplete (Ratnasingham & Hebert 2007). Also, the Q-BOL Project which covered a broad range of organism groups of pests and diseases and resulted in the database Q-Bank, still does not include e.g. important Cerambycid beetles such as *Batocera lineolata* or *Saperda candida* (Q-Bank 2017). In Europe the important PM7 diagnostic protocols for identification are usually limited to some more relevant species that are already regulated (EPPO 2018). Available molecular references for species associated with WPM are currently very limited. Therefore, relevant species that have been found in WPM so far and are likely to be mistaken with similar species have to be barcoded to fill that gap and to build up reliable datasets for their use by diagnostic laboratories.

3. The project activities of PHID-Coleo

The project PHID-Coleo (**P**lant **H**ealth **ID**entification of **Coleo**ptera) has been designed in Germany as a cooperative project between the plant protection service of Baden-Württemberg in Karlsruhe (LTZ) and the University of Hohenheim (UHOH). The project was launched in 2017 and runs for three years until June 2020 (Bauer & Zimmermann 2018).

PHID-Coleo aims at developing new diagnostic tools for the identification of potentially invasive and economically important beetles which are associated with WMP. In addition, it aims to develop new molecular methods for the analyses of already established exotic species. Introduction pathways and relationships between existing populations of invasive species need to be investigated as fast as possible to predict the invasive potential and to prevent economical damages.

The activities of the project are divided into three sections, with sections one and three being implemented by the Centre for Agricultural Technology Augustenberg (LTZ), Department 33, Zoological Diagnostics in Karlsruhe, Germany and section two by the Departments of Live Stock Population Genomics and Applied Entomology at the University of Hohenheim, Germany.

Project activies - Section 1: Morphological and molecular key

The activities under section one aim at new identification tools for potentially invasive and economically important species of false powderpost beetles and long-horned beetles (Coleoptera: Bostrichidae and Cerambycidae, respectively). According to the European phytosanitary alert system EUROPHYT these are the most important groups of insects that can be found in WPM (EUROPHYT 2018). For this, classical identification keys based on morphological characters are being developed for the relevant species of these groups. The keys will not only consider the adult beetles but also the immature stages, because usually only the larvae are found in WPM.

The classical keys will be supplemented by molecular methods for species identification. This will make it possible to deal with the smallest tissue samples and physically damaged specimens. Thus, species can be identified quickly and easily in the laboratory.

In collaboration with entomologists and according to recently published species lists (Geis 2014, Eyre and Haack 2017), the project partners have identified more than 100 species, which were confirmed to be associated with WPM. These are currently being documented photographically and molecular references are being developed. The keys will also be available in a printed version and molecular data will be published as well in established online barcoding databases.

Project activies - Section 2: molecular analysis of insect populations

The activities under section two aims at developing a detailed understanding of the population dynamics of invasive beetles and their dispersal. *Anoplophora glabripennis*, the Asian long-horned beetle (ALB) is serving as a model example. Investigations of intraspecific nucleotide variations should help to understand the relationship between populations found at different infestation sites, e.g. in Germany.

Genetic markers for the molecular comparison of individual populations of invasive species will be selected and provided for diagnosis (Hasselmann et al. 2015). They should help plant protection services to trace and identify new infestation events and to understand introduction pathways. A higher resolution of the ALB population structure will be achieved by using a larger amount of genetic markers for mitochondrial and genomic DNA. A set of molecular tools such as sequencing, conventional microsatellite analysis and state-of-the-art single-nucleotide polymorphism screening (Nolte et al. 2005, Gruber et al. 2013) will provide a broader spectrum of genetic markers than available now. For the genotype analysis specific software for measuring genetical differences will be used (Pritchard et al. 2000).

The project partners are collecting genetical material of the ALB from different infestation sites in Germany, as well as from other countries for comparisons, including Europe and the native habitat of this species in Asia. Research results about the intraspecific genetic differences of ALB populations indicate that there are variations in the D-Loop region and the cytochrome oxidase subunits 1 and 2 of the mitochondrial genome, as well as in the microsatellite regions of the genomic DNA. A preliminary analysis with microsatellites in the PHID-Coloe project showed promising results. The genome of the ALB was ´screened´ and approximately 700 regions with tandem repeats have been found, of which 25 microsatellites had been tested, that have not been used in ALB-studies so far. After sequencing, eight of them showed considerable differences in length and in the number of so-called repeats in comparison to the provisional reference genome (McKenna et al. 2016).

Project activies - Section 3: open identification keys and an expert network for identifying beetles intercepted during plant health inspections

Under section three, the results obtained under the previous two sections will be published as booklets, printed identification keys, and as well as online in the form of a freely accessible database for interested scientists, plant health services and zoological diagnostic laboratories. Diagnostic workshops will also include training for companies that provide barcoding services. Workshops and single training will be offered during the project, but also beyond the term of the project. Interested parties may contact the PHID-Coleo partners.

A further aim of the project is to establish a long term collaborative network in the field of molecular pest diagnosis which will include plant protection professionals, entomologists, research scientists (e.g. from state institutes and museums) as well as commercial companies. This collaborative network should continue its activities after the end of the project for a quick and safe identification of future interceptions of unknown insect species.

Acknowledgement

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Visible Near Infrared Hyperspectral (VNIH) technique to differentiate Trogoderma variabile reared on different commodities

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Abstract

Under Trogoderma sp, some comes major stored grain pest, which are of economic concern and most of the times accurate identification becomes very difficult. Under this study a new diagnostic system using visible near-infrared hyperspectral (VNIH) imaging methods is developed to address identification gaps for T. variabile. This technique is useful because different materials have unique reflectance spectra, and this difference in reflectance spectra can be used to identify various constituents in an image. For this study both larvae and adult were studied for T. variabile fed on wheat, maize, canola, barley, oats and rice for more than 4 generations. Each individual insects killed by ethanol were scanned using a hyperspectral imaging system from 400 to 1000nm. Matlab 2016b was used to develop predictive model for hyperspectral image classification. Deep neural network approach gave more than 90% accuracy for both larvae and adult stages fed on different commodities. From this result we can say that T. variabile on the different host can lead to difference in VNIH reflectance spectra. This is one of most fundamental factors for development of robust VNIH technique as diagnostic tool.

In search of a new attractant for monitoring *Stegobium paniceum* L. (Coleoptera: Anobiidae)

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Abstract

Stegobium paniceum (L.) is a major pest for several stored products worldwide. Monitoring methods for this species, based on pheromone traps, are affected by the complexity and expensiveness of the chemical synthesis of the pheromone isomer [(2*S*,3*R*,1'*R*)-Stegobinone] and/or by its lost of efficacy after two weeks at room temperature. So other semiochemicals that can be exploited for monitoring this species are highly desirable. In this study was tested the behavioral response in two-choice olfactometer of *S. paniceum* adults to Volatile Organic Compounds (VOCs) collected from colonized substrate. The elution of the headspace collection from *S. paniceum* colony elicited attraction of both sexes. The GC-MS chemical analysis of the extract indicated the presence of several alkanes, alcohols, aldehydes and fatty acids, some of them already reported to attract other stored product coleopteran pests and promising candidates for further studies to test their attractiveness on *S. paniceum*.

keywords: drugstore beetle, monitoring, attractant, volatile organic compounds, headspace

1. Introduction

The drugstore beetle *Stegobium paniceum* (L.) (Coleoptera: Anobiidae), , is among the major pests for a wide variety of dry and durable stored agricultural products (Edde et al., 2012). Drugstore beetle females produce a sex pheromone that induces attraction behavior of males, with the highest responses 5–12 days after adult emergence (Kuwahara et al., 1975; Kodama et al., 1987).

However, the response to this synthetic pheromone in trapping experiments often had unsatisfactory results (Mahroof and Phillips, 2007). This can be due to the complexity and expensiveness of the chemical synthesis of the pheromone isomer [(2*S*,3*R*,1'*R*)-Stegobinone] at high purity grade and/or by its lost of efficacy after two weeks at room temperature (Kodama et al., 1987). For these reasons, it is important to investigate alternative attractants or pheromone synergists for monitoring and/or mass trapping *S. paniceum* adults. The *Volatile Organic Compounds* (VOCs) produced from the insect colonies of *S. paniceum* could be useful in order to develop an efficient and economically sustainable attractant for the drugstore beetle as positively exploited for *Lasioderma serricorne*, a related anobiid species (Buchelos and Trematerra, 1998; Mahroof and Phillips, 2007). To date, the olfactory responses of *S. paniceum* to VOCs of its colonies and their volatile chemical composition have never been investigated. In this study, the behavioral responses of *S. paniceum* adults to VOCs from colonized substrate were evaluated in a two-choice olfactometer and analyzed by gas chromatography-mass spectrometry (GC-MS).

2. Materials and Methods

Colony VOCs collection

VOCs from a *S. paniceum* colony (insects plus rearing substrate) were collected by a dynamic headspace method (pull system). To collect volatiles, 90 grams of the rearing substrate were placed into a cylindrical plastic container. About 200 unsexed adults of *S. paniceum* were added to the rearing media in the plastic cylinder. Volatiles were collected for 24 hours on adsorbent tubes filled with 40 mg of Tenax-TA and 40 mg of Carbotrap B, both Supelco (Bellefonte, PA, USA). The adsorbent materials were fixed in the adsorbent tubes using glass wool. To generate a flow rate of charcoal-filtered air (200 ml/min) a vacuum pump was used. Headspace samples from empty cylindrical plastic boxes and oven bags were used as controls. Volatiles trapped in the tubes were eluted with 500 µl of cyclohexane. All samples were stored in screw cap vials at -20 °C until chemical and behavioral studies.

Chemical analysis

GC-MS analysis of the colony headspace samples were performed on an Agilent 5890 GC system, equipped with a DB-5ms column, interfaced with a 5973 quadruple mass spectrometer. The GC oven temperature was set at 40°C for 5 min, then, increased by 10°C/min to 250°C. Identification of compounds was carried out by comparison of mass spectra and retention times with standard compounds purchased from Sigma Aldrich (Milan, Italy). When synthetic standard was missing, the identification was made by using the NIST 98 library and by Kovats retention indices (Adams, 2007).

Two-choice olfactometer bioassays

To validate the two-choice olfactometer used in our behavioural experiments, the sex pheromone [(2*S*,3*R*,1'*R*)-Stegobinone] was tested in preliminary experiments on *S. paniceum* adults. Subsequently, 200 µl of headspace elution was tested versus a blank headspace collection. The olfactometer consisted of a glass chamber (cm: 26 long × 17 wide × 13 high) covered by a glass lid and illuminated by a lamp positioned 1 m above the instrument. Each side of the chamber was covered outside with white printer paper to eliminate potential distractions to beetles and to diffuse light. Two pairs of white plastic cups (0.2 l and 0.3 l) were used as olfactometer arms. For each bioassay, one beetle was released inside the chamber through the entry-hole on the long side of the chamber. The presence of the beetle inside the olfactometer arm was verified after 24 hours. The position of the stimuli in the arms was switched after each replication to avoid the influence of the olfactometer position on beetle choices. Before each replication, to prevent the accumulation of odors, the collection cups and the ramps were changed while the glass chamber was cleaned with acetone and dried by paper towel and a hair-dryer.

3. Results

Chemical analysis

Overall, twenty-four VOCs were found in the GC-MS analysis of the elutions from the colony of *S. paniceum*. Among them, nineteen compounds were identified whereas five were unknown. The most abundant compounds were hexanoic acid (12.4%), heptanoic acid (9.5%), decane (9.1%), 4-methyldecane (8.5%), and nonanal (8%), contributing to 47.5% of the total composition. Other compounds detected were heptanal, 1-octen-3-ol, octanal, limonene, 3,6-methyl decane, 2-phenylethanol, tridecane, tetradecane and hexadecane. Stegobinone comprised 0.3 % of the total volatile profile.

Olfactometer bioassays

The results of the behavioral experiments are summarized in Fig. 1. The olfactometer was validated by the response elicited by the sex pheromone that attracted males ($\chi^2 = 6.3$, df = 1, $P = 0.01$, N= 30) but not females ($\chi^2 = 2.4$, df = 1, $P = 0.1$). The elution of the headspace collection from *S. paniceum* colony elicited attraction of both sexes (males: $\chi^2 = 11.6$, df = 1, $P = 0.0007$, N = 30; females: $\chi^2 = 5.1$, df = 1, $P = 0.02$, N = 35).

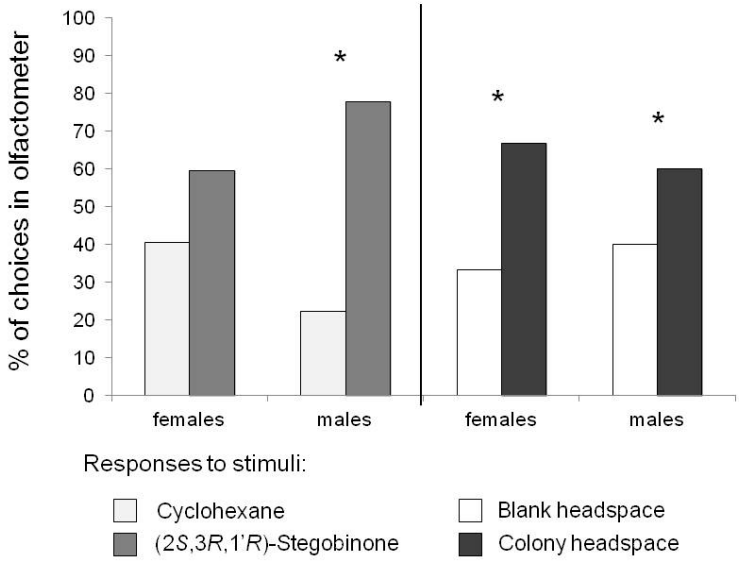

Fig 1. Percentage of choices of *S. paniceum* females and males to sex pheromone and to VOCs of the colony. * $P \leq 0.01$

Discussion

Our behavioural results showed that the elution from the dynamic headspace collection of the colony of *S. paniceum* elicited attraction for both adult sexes (Figure 1). Among the compounds identified in the chemical analyses of the colony VOC, several alkanes, alcohols, aldehydes and fatty acids have been already reported to attract other stored product coleopteran pests as *Oryzaephilus* spp., *Trogoderma* spp. and *Cryptolestes ferrugineus* (Levinson, 1978; Pierce et al., 1990, 1991). Interestingly, some of the chemicals detected in our analysis also co-occur in volatiles emitted from Chinese medicinal plant materials that elicit attraction toward *S. paniceum* adults (Cao et al. 2018).

Since *S. paniceum* sex pheromone attracts only males and loses its attractive capacity after two weeks at room temperature (Kodama et al., 1987), it is desirable to exploit alternative attractants for monitoring this pest. In anobiid beetles that feed on stored products, the use of food volatiles that, acting as kairomones, synergize the pheromone lures have been successful tested on *L. serricorne* (i.e. VOCs from *Capsicum* spp.) (Mahroof and Phillips, 2007). Similarly, the results of our study showed that the headspace elution, containing the VOCs from *S. paniceum* colony, is an attractant for both sexes of this pest species. In this context, our study gave the basis for the development of a new alternative and sustainable attractant for trapping the drugstore beetle. Further investigations are in progress aimed to identify which are the behavioral-active VOCs of the entire chemical composition involved in the attraction of *S. paniceum* adults.

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Field trials on attractiveness of the synthetic sex pheromone of the four-spotted bean weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae).

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Abstract

Quarantine pests of legumes pose a threat to many countries of the world including Russia. Pests that can enter the country as a result of the transportation of regulated articles (by sea, air, road, rail, etc.) pose a particular danger (Shutova, 1970; Dankvert *et al*., 2009). Monitoring and identification of legume pests is complicated by the fact that small beetles have a hidden mode of life. One of the most dangerous quarantine pest is the four-spotted bean weevil *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae), which is widespread throughout the world and can cause serious economic losses in agriculture of Russia. Research work on the identification, synthesis and laboratory evaluation of the synthetic sex pheromone of *Callosobruchus maculatus* was carried out at the All-Russia Plant Quarantine Center (Bykovo, Moscow region). Tests have shown that synthesized sex pheromone of *C. maculatus* has a high attractiveness for males. An effective dose of pheromone that attracts males of the four-spotted bean weevil has been found at the laboratory and is equal to 0.5 µg per

dispenser. Thereafter tests have shown that the concentration of pheromone above 2 µg does not cause behavioral response in beetles and doesn't result in contact with the stimulus. Dispensers with doses of pheromone from 4 to 8 mg have been used with a Delta trap in storage. The use of pheromone traps can help in pest identification, decreasing or complete avoidance of repeated treatments with chemicals at low pest population. The results of this study will be presented and discussed on the basis of laboratory and literature data.

Keywords: *Callosobruchus maculatus*, synthetic sex pheromone, pheromone trap, monitoring, plant quarantine.

Introduction

Polyphagous bruchids of genus *Callosobruchus* Pic (Coleoptera: Bruchidae) are the most dangerous pests of legumes. The most severely damaged crops are mung bean (*Phaseolus aureus*), chickpea (*Cicer*), pigeon pea (*Cajanus*), green pea (*Pisum sativum*), field bean (*Vicia faba*), cow pea (*Vigna*), soybean (*Glycine max*), lentil (*Lens*) and bean (*Phaseolus*) – in these crops seed damage can reach up to 100%. Beetles not only reduce the yield in fields, but also are imported to storages with harvested seeds and continue developing as storage pests without diapause. Therefore, pests drastically reduce the food value and crop quality in many legumes. Totally the genus *Callosobruchus* includes 15 species. In practice, 4 species of this genus are most often imported to Russia, namely: *Callosobruchus phaseoli* Gyll. – cosmopolitan seed beetle, *C. analis* L. – Graham bean weevil, *C. chinensis* L. – Chinese bean weevil and *C. maculatus* F. – four-spotted bean weevil (Sadomov and Mordkovich, 2004). All these species are similar in biology, harmfulness and habitats. Morphologically they clearly differ in adult form, but larvae are indistinguishable. Species of genus *Callosobruchus* are designated in the unified list of quarantine objects of the Eurasian Economic Union (approved by the Council Decision of the Eurasian Economic Commission dated November 30, 2016 № 158, entered into force from 1st of July 2017).

Fig. 1 Male imago of the four-spotted bean weevil *Callosobruchus maculatus* F.

Fig. 2 Male imago of the four-spotted bean weevil *Callosobruchus maculatus* F. feeds on a pea seed.

In practice, the Russia's federal service for veterinary and phytosanitary surveillance uses a complex visual method to identify the four-spotted bean weevil. But one may significantly reduce labor costs and improve efficiency of quarantine monitoring of beetles by using pheromone traps in the field and storages for monitoring and identification (Smetnik *et al*., 1986; Burov and Sazonov, 1987; Phillips *et al*., 1996). Synthetic pheromone was synthesized at the Department of Synthesis and Application of Pheromones in All-Russia Plant Quarantine Center in 2012. In laboratory tests with olfactometer synthesized pheromone has attracted males of *C. maculatus* effectively at dose of 0.5 µg. The experiments as well have shown that the concentration of pheromone above 2 µg does not cause behavioral responses by beetles and doesn't result in their contact with the stimulus (dispenser).

Field trials on attractiveness of synthetic sex pheromone of the four-spotted bean weevil were conducted on the basis of the "Nikola Pushkarov" Institute of Soil Science and Agroecology in Bulgaria as part of bilateral R&D work. Trials were carried out in order to determine the biological activity of pheromone of the four-spotted bean weevil *C. maculatus* F. for early detection in the area of its distribution.

Materials and Methods

Field trials

Field trials were conducted in 2014 from 15th of August till 14th of September. The experimental field is located in the village of Skryt (*41°23'53"N, 23°12'27"E*), which is suburb of Petrich, Blagoevgrad region, close to the border with Macedonia. The trial has been set at the field of *Vigna sinensis* L. in the time of the ripening of beans when the beetles cause the highest danger.

Delta traps with sticky inserts were used for trials, at the center of which the dispensers with correspondent doses of pheromone of *C. maculatus* were placed. Insect monitoring was carried out within a month from the date of installation, in total 6 records were carried out. Pheromone doses applied to the dispenser are the following: variant I (control)– 0 mg, II – 1 mg, variants III and IV – 2 and 3 mg, respectively. A rubber cork has been chosen as a material for the dispenser, which has the property of prolonged and gradual release of the chemicals for a long period of time. The number of replications over all variants was 3.

Storage trials

In 2015 and 2017, trials on the biological activity of the pheromone during storage of legumes were conducted in the storage at the Institute of Soil Science and Agroecology in Kostinbrod (Bulgaria). Trials were carried out in a facility with a total area of 25 m^2. In a storage, damaged leguminous crops infested by *C. maculatus* in the field were stored. *Vigna radiata* L. and *Vigna sinensis* (L.) Walpers were stored crops in 2015 and 2017 respectively, that had been grown in the southwest of Bulgaria in the territory of ecological agriculture and were not treated by pesticides during periods of cultivation and storage. In the course of trial, there were no other insect pests and diseases on beans. The average temperature in the storage ranged from 23°C to 25°C, and relative air humidity was 65-70%. The experimental traps were placed in a randomized way throughout the area of the storage at a height of 1.5 m at available places (Lebedev *et al*., 1984; Dospekhov, 1985). The distance between traps was not less than 2 meters.

In 2015, the doses of pheromone applied to the dispenser were the following: variant I – 2 mg, II – 10 mg, variants III and IV – 6 and 20 mg, respectively. Rubber cork was used as dispenser for variants I and III, and spongy material wicks for variants II and IV. A Delta trap with sticky glue insert was used for catching imago of *C. maculatus*. The research was carried out on *Vigna radiata* L. over 26 days (from 8th of August to early September). There were 5 replications and 6 surveys were done during the season. In 2017, an identical trial was set up during the storage period of beans of *Vigna sinensis* L. Walpers. The following doses of pheromone (by variants) were used: I – 2 mg, II – 4 mg, III – 8 mg and IV – 16 mg. There were 6 replications and 5 surveys were done during the season. Two types of traps were used: Delta and "Book" (storage trap type); dispenser with the appropriate dose of synthetic sex pheromone of the four-spotted bean weevil was placed to the center of the traps. Insect monitoring was carried out for 41 days (from 2nd of August to 11th of September). The recording and sampling of insects from traps was made every 10 days from the period of adult emergence and the beginning of insect flight.

Obtained data was processing by statistical methods and differences determined with least significant difference test (LSD).

Results

Field trials

Results of 2014 trial showed that the dynamics of imago flight to traps was extremely low, that was probably due to the low number of beetles in the field. Statistical differences (*LSD test, F_{05}, t_{05}*) between the tested variants were not revealed (Table 1). However, traps with the dispenser of variant II (1 mg of synthetic pheromone per dispenser) showed the highest attractiveness, and can be recommended for quarantine monitoring. Traps with dispensers III and IV (2 mg and 3 mg pheromone on the dispenser, respectively), and control traps (variant I without pheromone application) showed similar results (Table. 1). Variant II with 1 mg of pheromone had the highest results among the selected doses and control (*F*=4,76).

Tab. 1 Number of male insects caught during the period of flight.

variants	pheromone doses	average number of male beetles caught per one trap	significance
I (control)	0 mg	*1.4*	n.s.
II	1 mg	*2.4**	s.
III	2 mg	*1.1*	n.s.
IV	3 mg	*1.1*	n.s.

LSD_{05} = 0.94

Storage trials

Results of trials in 2015 showed that the largest number of caught male insects recorded in traps were with dispenser IV, with 27.3 individuals per trap during the flight period. Average number of male beetles per one trap in variants I, II and III were 12.7, 18.7 and 10.3 individuals, respectively (Table 2). At the same time, the average number for all variants (x_{avg}) was =17.25 male individuals per trap. The ratio of females (f) and males (m) in different variants was: I – 19 f:m 38, II - 5 f:m 56, III - 10 f:m 31 and IV - 17 f:m 82. The ratio of the total number of caught insects - 207 males and 51 females, i.e. 80.2% and 19.8%, respectively.

A long period of monitoring allowed us to estimate the dynamics of flight and the number of beetles of *C. maculatus*, it was stable and quite high.

Tab. 2 Trials in 2015: Number of male and female insects caught during the period of flight.

variants	pheromone doses	average catching of male beetles per one trap	significance	total number of caught males per one trap	total number of caught females per one trap
I	2 mg	*12.7*	n.s.	38	19
II	10 mg	*18.7*	n.s.	56	5
III	6 mg	*10.3*	n.s.	31	10
IV	20 mg	*27.3**	s.	82	17

LSD_{05} = 2.58

Statistical data processing has shown that there was a significant difference in insects caught among the tested variants. At the same time, variants I and II had the lowest attractiveness and number of caught insects was at the same level. Thus, for quarantine monitoring of the four-spotted bean weevil we can recommend the dispenser in the form of a spongy material wick with a dose of 20 mg of pheromone per dispenser.

Results of the trial conducted in 2017 did not reveal significant differences in the attractiveness among pheromone traps with tested types of dispensers: nearly the same number of insects was caught in variants with doses of 2, 4 and 8 mg (from 1.6 to 1.9 beetles per trap). The total number of captured insects was 67, of which 64 males and 3 females, representing 95.5% males and 4.5% females. The ratio of female (f) and male (m): I – 1:15, II - 2:12, and III and IV variants caught only

males, 16 and 10, respectively. At the same time average number for all variants was 2.3 individuals per trap.

Tab. 3 Trials in 2017: Number of male and female insects caught during the period of flight.

variants	pheromone doses	average catching of male beetles per one trap	significance	total number of caught males per one trap	total number of caught females per one trap
I	2 mg	*2,7*	n.s.	15	1
II	4 mg	*2,3*	n.s.	12	2
III	8 mg	*2,7*	n.s.	16	0
IV	16 mg	*1,7*	n.s.	10	0

LSD_{05} = 8.47

Most attractive type of trap was Delta with variants IV (8 mg) and I (2 mg). The "Book" type trap caught significantly fewer insects than Delta. Average number of caught insects in "Book" type trap was extremely low (0.3 males per trap for the entire period of flight), while the trap Delta consistently showed high results compared to the latter and the number of caught males on average for all variants was 2.3 individuals, that is 2 times more than for the "Book" trap type.

Traps with variants IV and I caught only males of *C. maculatus*, 16 and 15 individuals respectively. Pheromone dispensers with doses of 8 mg and 2 mg were the best attractive substance for catching insect males. In variants II (4 mg) and V (16 mg), the number of captured males was 12 and 10, respectively. It is possible to make the assumption that under conditions of closed spaces (storages) these dispensers may cause an effect of insect disorientation.

The attractiveness of all pheromone traps used in the trials was high enough throughout the research period; therefore, dispensers were not replaced.

Discussion

Trials were carried out according to the original method developed at All-Russia Plant Quarantine Center. It allowed us to draw a conclusion that the synthetic sex pheromone of the four-spotted bean weevil *C. maculatus* possesses biologically active properties and is attractive for males of this pest species. Based on results of the field trials in 2014, it can be concluded that variant II (1 mg of pheromone per dispenser) showed the highest results among the selected doses (F=4,76). It should be assumed that variant II is most effective for attracting individuals of the four-spotted bean weevil into traps in early flight of insects in the field during the ripening of beans.

Evaluation of attractiveness of various doses of pheromone showed the possibility of disorientation of the four-spotted bean weevil in storage during the trial in 2015. At the same time, in a closed room with a constant temperature and humidity the dose of applied pheromone may vary in dependence on dispenser type used in traps. For example, capture efficiency was the optimum when using Delta type traps with a wick and applied pheromone in dose of 20 mg rather than when using an insulin cork as dispenser.

Storage trial in 2017, taking into account the dynamics of male numbers of *C. maculatus* attracted by pheromone, Delta traps with variants 4 (8 mg) and 1 (2 mg) were the best options in terms of attractiveness. In almost all cases, the "Book" trap type attracted significantly fewer insects than the Delta. In all variants, average number of caught insects in the "Book" trap type was 2 times lower than for Delta trap.

A long period of monitoring allowed us to estimate the dynamics of flight and the number of beetles of *C. maculatus*, during the trial it was stable and quite high.

Acknowledgement

The authors extend sincere gratitude to their colleagues from the All-Russia Plant Quarantine Center (Bykovo, Moscow region) and the "Nikola Pushkarov" Institute of Soil Science and Agroecology (Kostinbrod, Bulgaria) for their help in organizing and conducting trials.

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A Multi-parameter Grain Detection System Based on Industry 4.0

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Abstract

A multi-parameter grain detection system based on industry 4.0 was used to map all kinds of sensors and devices into multiple network addresses through the integration of equipment, to realize the local visual perception and the network transmission of various grain data, using software plug-in architecture technology to build online extension of the software to achieve the corresponding grain multi-parameter monitoring plug-ins; setting sensor and device communication protocol standards to achieve remote monitoring of various grain situation data on the scene equipment Remote debugging and maintenance work to form a remote data center and equipment maintenance center. The system is compatible with a wide range of heterogeneous sensors and devices online and with a high degree of online scalability.

Keywords: grain detection system; Industry 4.0; the integration of equipment

Introduction

The grain detection system is a system that uses modern electronic technology to detect, store and analyze ecological parameters of stored grain. The current system has major problems such as single function, poor compatibility, poor extension capability, and low level of intelligence. The design architecture lacks systemic considerations. It is difficult for different manufacturers and different kinds of data to be integrated in one system. The system integration of different sensors and equipment is difficult and incompatible, and it is unable to achieve integrated collection, control and data transmission, and it is no longer adaptable to new demands.

The stored grain detection system implemented in this paper is an information system under the Industry 4.0 architecture. It relies on the heterogeneous sensor universal access hardware platform and integrated equipment deployed in the field to achieve real-time perception of multiple stored grain condition data and cooperative control field equipment, intelligent system based on this platform can achieve continuous evolution and upgrade of the system, use of big data technology to analyze the correlation relationship between sensor data, accurately extract characteristics of stored grain, and form an online extension and maintenance of the stored grain detection application system, the core of which is compatible with a variety of heterogeneous sensors and devices online, has a high degree of online scalability.

System implementation

Overall design structure of system

The system design adopts the idea of automatic evolution and divides the system into four subsystems: (1) universal access hardware platform and integrated equipment for the front-end granary; (2) software system for the grain depot monitoring center; (3) background data and maintenance center software system; (4) stored grain condition big data analysis application platform. as shown in Fig.1.

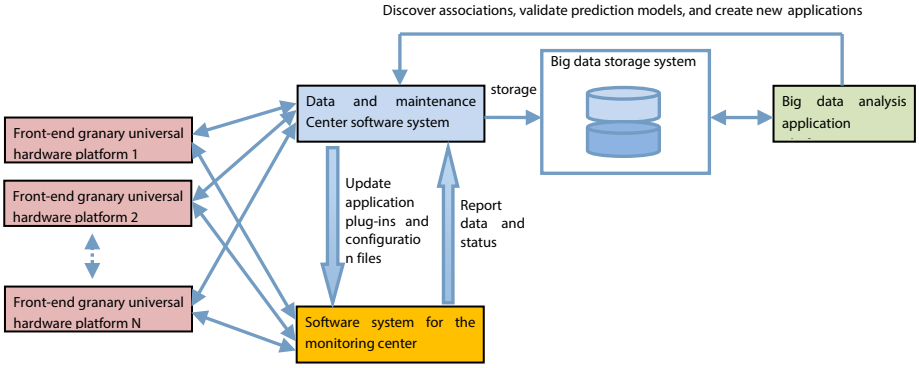

Fig.1. System Design

Each front-end general hardware platform and integrated equipment is installed in one granary, and is responsible for connecting different sensors and control devices according to user needs. Its design adopts the concept of Industry 4.0 and maps various on-site sensors and control devices into independent IP addresses. All sensors and devices can communicate over Ethernet, thus shielding the heterogeneity of various sensor and device communication physical layers. In addition, the heterogeneity of the communication protocols of different devices is packaged and transparently transmitted using the communication protocol packaging technology. This platform can also push data to different data terminals at the same time, and allows legitimate login clients to perform remote device debugging.

The software system for the grain depot monitoring center is a core software platform installed in the local control room of the grain depot. It adopts a full plug-in framework and can be upgraded to adapt to different sensors and devices through automatic plug-in upgrade. The service can be upgraded by authorizing download of the latest application.

The background data and maintenance center software is responsible for receiving and analyzing data from different sensors and electromechanical devices. It has a monitoring function and stores the data in a large data storage system. This software system is developed using a full plug-in framework and the system can adds and updates newly developed plug-ins and pushes the plug-in to the user software system for the grain depot monitoring center that purchased the application.

The stored grain condition big data analysis application platform is used to find out the relationship between sensor data in data analysis, develop prediction models, promote the development of new applications and plug-ins, and provide users with new services.

Design and Implementation of universal hardware platform and integrated equipment

"Universal hardware platform and integrated equipment" includes a general hardware interface platform and a field integrated equipment, as shown in Fig.2.

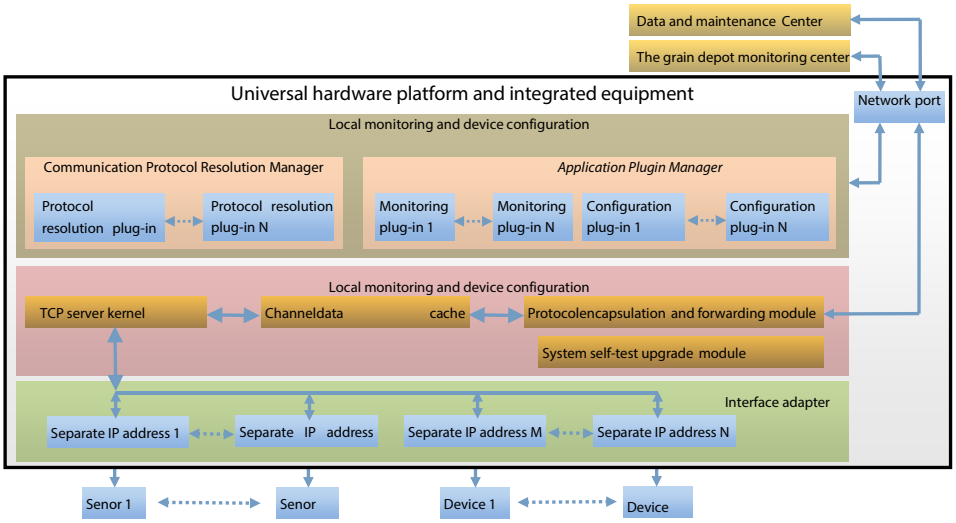

Fig.2. Universal hardware platform and integrated equipment Design

The universal hardware interface platform connects different sensors and control devices according to the user's needs. Its design uses the idea of the Internet of Things to map various on-site sensors and devices into separate IP addresses. All devices communicate through the network. It shields the heterogeneity of communication and physical layers of various sensors and devices, adopts the secondary packaging technology of communication protocols to transparently transmit data of different devices, and facilitates the rapid deployment of new sensors or devices in the future; while taking into account the local monitoring of stored grain conditions The data can also be pushed to different data centers at the same time to form a source node of big data information that can adapt to the future development.

The integrated equipment adopts an integrated industrial control computer running Windows operating system. The application program adopts a framework structure and plug-in mode. The developed application program has strong reliability, flexibility, and compatibility. It realizes the on-site visual display of stored grain condition data and system configuration and high-speed and flexible network data transmission. The software has an online upgrade function.

Software system for the grain depot monitoring center

"Software system for the grain depot monitoring center" is a software platform used by users. It adopts a full plug-in framework to develop and adapt to different sensors and equipments through automatic plug-in upgrade. At the same time, it can remotely apply for authorization to the data center and download the latest ones. The application of stored grain condition is used for online upgrades, as shown in Fig.3.

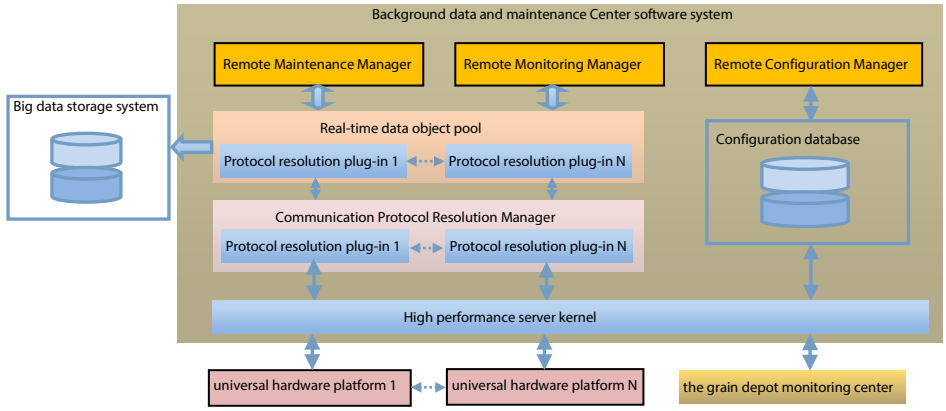

Fig.3. Software system for the grain depot monitoring center

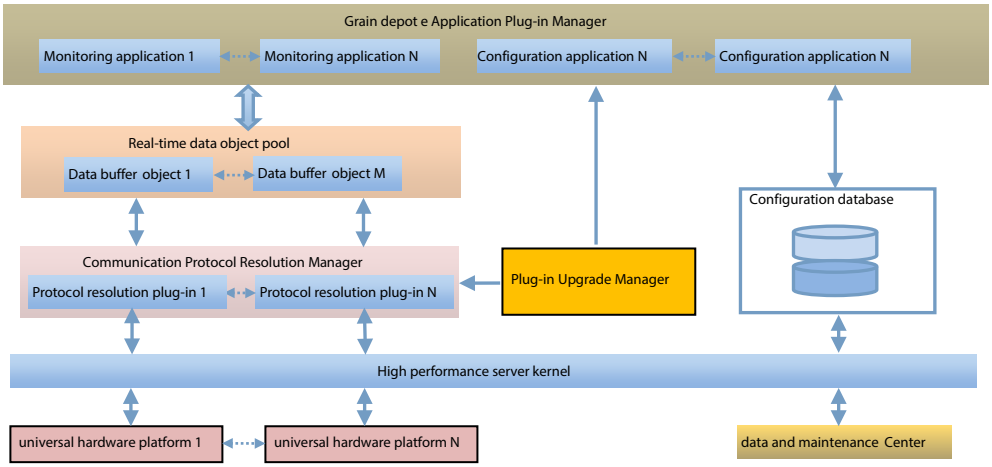

Fig.4. Background data and maintenance Center software system

Background data and maintenance center software system

"Background data and maintenance center software system" is a software platform installed in the data center. It is responsible for receiving and analyzing the transparent transmission data sent from different granaries of each grain depot. The system is developed with a full plug-in framework. Each plug-in can correspond to different field devices. In the data center, all the data information in the grain depot can be remotely monitored, and the equipment can be remotely commissioned and maintained in a transparent transmission mode to form a maintenance center. A variety of new application softwares are developed for application release on this platform; data from grain depot are stored in the big storage system to form an internal data center, as shown in figure 4.

Stored grain condition big data analysis application platform

"Stored grain condition big data analysis application platform" is used to find out the correlations among sensor data in the stored grain condition big data storage system based on hadoop, to develop prediction models, and to promote the development of new applications and plug-ins to form new high-additions to users. The value service is delivered to users through the " Background data and maintenance center software system ".

Conclusion

The system adopts a universal network platform design. Upgrading the front-end sensors and equipment will not affect the system. It only requires the development of relevant interface plug-in dynamic links. The system software of the data and maintenance center is developed by the plug-in architecture, and the system function expansion only needs to replace or add different dynamic connection blocks, which has good scalability. With the universalized front-end design, the integration process for installing or upgrading different sensors or devices will be standardized. With remote on-line device debugging capabilities, and the system is very maintainable. The system provides data mining tools based on historical data, finds the relationships among data, develops prediction models, and optimizes configuration information. A sustainable and intelligent evolutionary system is finally formed, which can generate value for users for a long time.

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Global establishment risk of stored products beetles

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Abstract

Stored-product beetles were regarded as some of the most important stored-product pests in the world. Predicting which one in hundreds of potential invasive stored-product beetles is the most likely to invade a region presents a significant challenge. A global presence/absence dataset, including 201 economically significant stored beetles in 143 countries/regions, was analysed using a Self-Organizing Map (SOM) to categorize regions based on similarities in species assemblages. This method is able to rank these stored-product beetles based on risk of establishment indices (values between 0 and 1). From the six countries/regions selected from each continent, we can have an overview of the global invasive risk of this group of beetles. We also found that those countries geographically close were clustered together by the SOM analysis because they have similar beetle assemblages and therefore represent greater threats to each other as sources of invasive stored-product beetles.

Keywords: stored-product beetles, Coleoptera, self-organizing map, establishment risk

The stored-product beetles (Coleoptera), include more than 300 species in 40 families and cause about 85% of stored pest damage (Zhang et al., 2015). These taxa are of quarantine significance since the beetles are usually small in size, have a broad host range, and have a high reproductive ability and dispersal capacity, and, in addition, the grain depot can offer a stable environment and abundant food for the establishment of alien species. For example, *Trogoderma granarium* originated from Asia, was first reported in California in 1953, where it caused 220 million dollars in loses amounting to 10% of income from agricultural products (Chu et al., 2008). Predicting which one in hundreds of potential invasive stored-product beetles is the most likely to invade a region is of significant importance for global trade policies such as China's 'Belt and Road' program.

A Self-Organising Map (SOM) (Kohonen, 1982), which is a type of Artificial Neural Network (ANN), has been used in the past to simultaneously rank and prioritize a large number of invasive species by their likelihood to establish in a region (Cereghino et al., 2003; Worner and Gevrey, 2006; Paini et al., 2010; Paini et al., 2011; Morin et al., 2013; Singh et al., 2013; Qin et al., 2015). We initially extracted the distribution data from the Crop Protection Compendium (CABI, 2018), Pest China dataset and monograph. Subsequently results of the presence (1) or the absence (0) of each stored beetles in each geographical area in the database comprised a 201×143 matrix (201 species in 143 countries). The analysis was performed by using Matlab and SOM Toolbox (version 2.0) (Laboratory of Information and Computer Science, Helsinki University of Technology, http://www.cis.hut.fi/projects/somtoolbox/). SOM indices were then extracted for all stored beetles for each country/region of the world.

Establishment likelihood lists of all the 201 beetles were generated for all 143 countries included in the analysis. The top 10 ranked species, which are currently absent in each country were extracted from the full lists of SOM indices and we present the top ten ranked species for six countries (China, USA, Nigeria, Chile, Italy, and Australia) (**Tab. 1**). We also examined how the SOM clustered the countries identifying which countries have the most similar stored beetle assemblages (**Fig. 1**). All 143 countries/regions were clustered into 66 neurons. We noted that many of the countries clustered together by the SOM analysis were also geographically close to each other, which suggests a species present in a country will be of greater threat to a neighboring country that is in the same cluster.

A SOM analysis could be used as an initial screening process to reduce the large numbers of potential invasive species to a more manageable number (Paini et al., 2011), the SOM indices for stored beetles currently absent from a country could be used to guide debate on which species should be listed for national surveillance needs to achieve early warning. More importantly, the SOM indices could provide a first screen of the beetles prior to going through more systematic risk analysis (Morin et al., 2013).

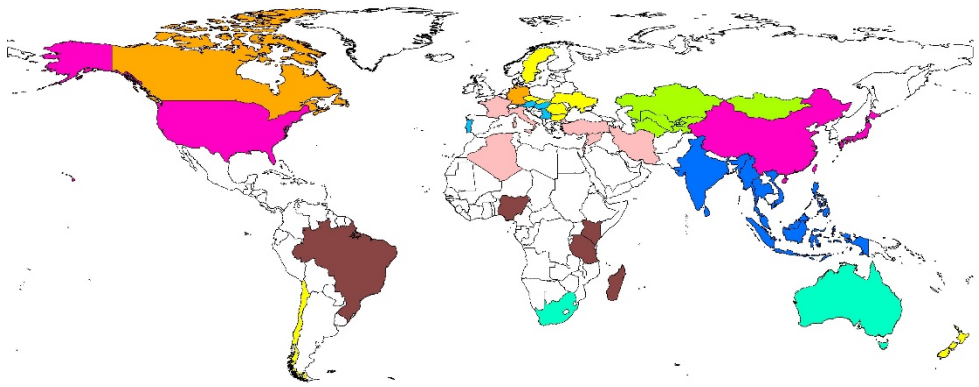

Fig. 1 Map of world showing country clustering (same color) based on stored-product beetle assemblages that were allocated to the same neuron in a SOM analysis and hence those countries that have the most similar stored beetle assemblages.

Tab. 1 Top 10 stored-product beetles species for each of six countries, representative of each continent (except for the Antarctic) that are currently absent but have the highest likelihood of establishment if introduced.

China	SOM Index	Nigeria	SOM Index	The United States	SOM Index
Trogoderma inclusum	0.57	*Callosobruchus analis*	0.77	*Gibbium aequinociale*	0.49
Dinoderus bifoveolatus	0.42	*Cylas formicarius*	0.76	*Dermestes coarctatus*	0.41
Callosobruchus ademptus	0.41	*Sinoxylon conigerum*	0.71	*Dermestes tessellatocollis*	0.41
Carpophilus mutilatus	0.37	*Urophorus humeralis*	0.65	*Dermestes vorax*	0.41
Reesa vespulae	0.35	*Gibbium aequinociale*	0.49	*Dermestes vorax var.albofasciatus*	0.41
Trogoderma angustum	0.35	*Dinoderus minutus*	0.40	*Dermestes freudei*	0.41
Bruchus brachialis	0.33	*Hylotrupes bajulus*	0.38	*Attagenus unicolor japonicus*	0.41
Trogoderma anthrenoides	0.31	*Cryptamorpha desjardinsii*	0.37	*Anthrenus nipponensis*	0.41
Bruchus signaticornis	0.31	*Xylopsocus capucinus*	0.37	*Orphinus japoonicus*	0.41
Prostephanus truncatus	0.29	*Minthea rugicollis*	0.37	*Atholus depistor*	0.41
Chile	**SOM Index**	**Italy**	**SOM Index**	**Australia**	**SOM Index**
Tenebroides mauritanicus	0.12	*Bruchus signaticornis*	0.48	*Attagenus unicolor simulans*	0.72
Hylotrupes bajulus	0.90	*Bruchus affinis*	0.47	*Orphinus japoonicus*	0.68
Tribolium castaneum	0.46	*Gibbium aequinociale*	0.44	*Bruchus rufipes*	0.65
Sitophilus oryzae	0.39	*Cryptolestes pusillus*	0.40	*Pseudeurostus hilleri*	0.64
Trichoferus campestris	0.32	*Bruchidius incarnatus*	0.33	*Mesomorphus villiger*	0.59
Trogoderma granarium	0.29	*Bruchidius trifolii*	0.33	*Thorictodes erraticus*	0.54
Cryptolestes pusillus	0.29	*Sapronus subnitescens.*	0.30	*Carpophilus delkeskampi*	0.53
Anthrenus polonicus	0.28	*Thorictodes heydeni*	0.28	*Cryptolestes ugandae*	0.42
Reesa vespulae	0.26	*Phradonoma nobile*	0.26	*Bruchidius terrenus*	0.36
Oryzaephilus mercator	0.19	*Ptinus clavipes*	0.23	*Holoparamecus signatus*	0.35

Acknowledgement

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Session 4
Engineering for Stored Product Protection and Pest Prevention

Bin coring: a simple practice for improving aeration performance and saving energy

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Abstract

The coring operation consists of removing the center portion of the grain mass, or core of the silosilo, to improve airflow distribution. Additional benefit of this practice is the elimination of a significant portion of the fine material, which is a source of fungal inoculum and feed for insects. The effect of coring on airflow distribution through a grain mass has been previously addressed, but the effect on energy savings was not fully quantified. Thus, the goals of this reseach were: 1) to quantify the airflow increase due to the coring operation of a silosilo full of wheat; and 2) to quantify the reduction on fan runtime and energy consumtion due to improvement in airflow distribution and airflow increase after coring. The effect of coring on airflow was quantified using the AireAr software, and the effect on aeration efficiency was studied through simulation using a specialized software (PHAST-FDM). For levels of coring (0%, 3%, 5% and 8% of total grain mass) and four levels of non-uniformity of airflow (center side difference) (30, 20, 10 and 0) were considered. Results indicated that the coring operation reduced the total time to achieve cooling, number of fan run hours, and fan power consumption. The main effect of the coring operation was the increase in specific airflow (up to 45% increase). Energy savings increased with coring, obtaining savings of 11%, 28% and 30% for 3%, 5% and 8% of coring, respectively. It was concluded that coring the silosilo by unloading from 3 to 8% of the stored grain mass is a recommendable practice, because it increases the specific airflow rate and airflow uniformity, reduces fan run hours and generates energy (and cost) savings.

Keywords: airflow resistance, airflow uniformity, simulation, fine material.

Introduction

One of the most frequent problems in storage facilities is the accumulation of fines in the center (core) of the silosilo. Fine material is defined as pieces of broken grains, foreign matter and weed seeds. Fine material occupies the void spaces in the grain mass, reducing the porosity of grain and increasing airflow resistance (Grama et al., 1984; Haque et al., 1981). When loading a silosilo through the center of the silosilo, fine material tends to concentrate in the center of the grain mass and increasing the airflow resistance in the core. Consequently, air velocity and specific airflow are lower in the core than in the periphery of the grain mass.

The coring operation is one of the most simple and recommended practices for improving the storability of the grain mass. The coring operation consists in removing the center portion of the grain mass, or core of the silosilo. When unloading a silosilo from the center opening in the floor, the first grain to come out is the grain of the core of the silo, which also contains most of the fines concentrated in that location of the grain mass (Bartosik and Maier, 2006). Removing most of the fines from the silo not only improves the airflow distribution, but also reduces the risk of developing insects and molds in that area or the silo.

Bartosik and Maier (2006) measured the concentration of fine material and air velocity at the center and periphery of the grain mass for 15 on-farm natural air/low temperature (NA/LT) in-silo corn drying and conditioning experiments. It was observed that the accumulation of fine material in the core was up to 232% higher than at the periphery. This accumulation of fines at the core of the silo resulted in non-uniform airflow distribution. It was observed that, on average, there was 74% more

airflow at the side (close to the silo wall) than at the center of the silo (ranging from 24 to 222%). Simulation was used to study the effect of non-uniform airflow caused by fine material accumulation at the center of the silo and the grain peak produced after loading the silo. They concluded that operators of NA/LT in-silo drying systems could reduce drying costs from 25 to 33% by leveling the grain peak after loading the silo. Additional reductions in drying costs from 18 to 22% could be achieved by installing effective grain spreaders or by coring the grain mass. Later, Lawrence and Maier (2011) developed a non-uniform airflow model using the finite volume method to predict air velocity for cored, peaked and leveled grain mass configurations.

Coring silos for long term storage, even though a known practice among elevator managers, it is not consistently implemented. Typically, during coring from 3 to 8% of the grain mass is unloaded. Cardoso et al. (2008) evaluated the fine material distribution in wheat silos and the effect on airflow. They found that unloading about 3% of the grain mass was required to remove most of the fines. Additionally, they concluded that the coring operation can increase not only the airflow uniformity but also the total airflow in the silo.

Simulation was used in the past to quantify the effect of fine material and non-uniform airflow on the performance of natural air/low temperature in-silo drying systems (Bartosik and Maier, 2006) with the PHAST-FDM model. However, not sufficient information was generated about the improvement in airflow distribution due to the coring operation, amount of grain to be unloaded during coring, reduction of the cooling time during aeration, and potential energy consumption reduction derived from this best management practice.

Thus, the goals of this reseach were: 1) to quantify the airflow increase due to the coring operation of a silo full of wheat; and 2) to quantify the reduction on fan runtime and energy consumtion due to improvement in the airflow distribution and airflow increase after coring.

Materials and Methods

Airflow estimation

The metal silo considered for this study had a cone botton with 30° inclination, 8.5 m diameter and 10 m heigh to the eave, with a centrifugal aeration fan of 2 HP (1.49 KW) (Chicago Blower, SQDA Agro-200 – 1410 RPM). The grain considered for the study was wheat at 14% moisture content (m.c.) and test weight of 76 kg/hl (0.76 t/m^3).

The effect of the coring operation on total airflow was evaluated with the AireAr software (http://online.inta.gov.ar:8080/aireAr/mainMenu). This software compares the performance curve provided by the fan manufacturer with the system airflow resistance curve computed with the Shedd equation and the set of parameters provided in the ASABE standard for wheat (ASAE, 1999) (a= 8,410, b= 2.72, Multiplier = 1.2) (Fig. 1), and estimates the resistance and the total and specific airflows in the silo (Bartosik et al., 2009).

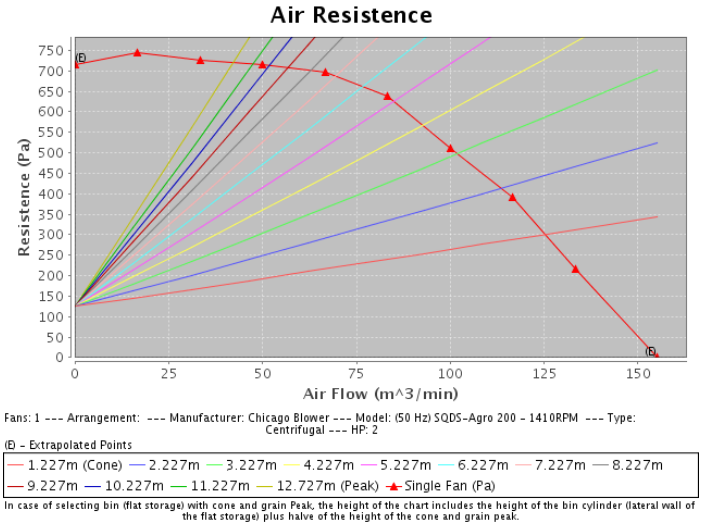

Fig. 1. Screen capture of the AireAr software showing the fan performance curve and the airflow resistance curves for the base condition.

The base silo configuration, before coring, had a grain peak of 3.0 m at the center with a total capacity of 509.6 t, and a multiplier of 1.2 was considered in the Shedd equation to account for the packing effect of the grain on airflow resistance. Three coring alternatives were evaluated (3%, 5%, 8%), which resulted with different differential height in the grain peak and different amounts of stored wheat. Additionally, the Shedd equation multiplier was proportionally reduced according to the coring percentage to account for the "loosening" effect in the grain mass, which reduces airflow resistance (Table 1).

Table 1. Height differential of the grain peak at the center of the silo, amount of unloaded grain and amount of remaning grain in the silo before and after coring.

Parameter	Base condition (Before coring)	After coring		
		3%	5%	8%
Shedd multiplier	1.2	1.1	1.05	1.0
Peak heigh differential (m)	3	1.95	1.2	0
Amount of grain (t)	509.6	494	484	468
Amount of unloaded grain (t)	-	15.6	25.6	41.6

Aeration simulation

The Purdue Post-Harvest Aeration and Storage Simulation Tool – Finite Difference Method (PHAST-FDM) is a numerical model that solves the heat and mass transfer during in-silo drying and conditioning in two dimensions (x, y) (Bartosik, 2005). To solve the problem of non-uniform airflow rate through the grain mass, PHAST- FDM simulates two grain columns: one for the core, and the other for the periphery. The heat and mass transfer equations are solved independently for each column, and the model assumes no interaction between them. PHAST-FDM accepts different non-uniformity factors (NUF) for airflow rates that can be entered by the user. The NUF was defined as the center-periphery difference with respect to the average airflow rate: (airflow periphery – airflow center / [(airflow periphery + airflow center) / 2] × 100). For instance, an NUF of 30% for an average airflow rate of 1 m/s indicates that the airflow rate is 0.85 m^3/(min t) at the center of the silo and 1.15 m^3/(min t) at the periphery. The PHAST-FDM model with the center-periphery differential airflow rates was validated for predicting MC changes in different grain layers for several on-farm NA/LT in-silo drying tests (Bartosik, 2005; Bartosik and Maier, 2006).

The initial average conditions for the cooling aeration simulation were 14% m.c. and 30°C temperature. It was assumed that the wheat was harvested on January first at Balcarce, South-East Buenos Aires province, Argentina. The final condition for the simulation was achieved when wheat reached 18°C average temperature and 19°C maximum temperature. The PHAST-FDM model was supplied with hourly temperature and relative humidity data of 13 different years. The silo considered had the same configuration as decribed in the previous section. The simulated aeration strategy turned on the fan whenever the ambient temperature was below 18°C. The airflow was obtained with the AireAr software as described in the previos section for four different coring percentages (0 (before coring); 3; 5 and 8%). Four NUF levels were considered (30 (before coring); 20; 10 and 0). A NUF of 30 represents the situation before coring, in which the difference in airflow between the center and periphery of the gran mass was the greatest, while a NUF of zero means that the airflow distribution was completely uniform (perfect coring).

Table 2. Combinations of specific airflow rates obtained for different coring percentages and non-uniformity factors considered for the aeration simulations with the PHAST-FDM model.

Non-Uniformity Factor	Coring percentage			
	0	**3**	**5**	**8**
30	X *	-	-	-
20	-	X	X	X
10	-	X	X	X
0	-	X	X	X

* Base line corresponding to the silo condition before coring

Energy savings

Energy savings due to the coring operation was computed taking into account the fan electrical power consumption (kWH) and substracting the electrical power needed to unload the silo for the coring operation. Fan power consumption was obtained by multiplying the fan runtime hours obtained for each simulation condition by the fan power (kW). For computing the electrical power consumption related to coring (unloading the silo) it was assumed a silo unloading auger of 5.5 kW and a bucket elevator of 11 kW were used with a conveying capacity of 60 t/h. The coring operation time was estimated as 0.0, 0.26, 0.49 and 0.63 hours for coring conditions of 0, 3, 5 and 8%, respectively, and the corresponding power consumption values were 0, 4.29, 7.04 and 11.44 kWH.

Results

Airflow estimation

Before coring, the silo full of wheat had a total capacity of 509.6 t, with a grain peak of 3 m at the center. Under that condition, airflow resistance against the fan was 720 Pa, which resulted in a total airflow rate of 42.1 m^3/min. Thus, before coring, the specific airflow was 0.083 m^3/(min t). The coring operation increased the specific airflow in three ways. First, after coring there is a lower grain depth, which reduces the airflow resistance and, hence, the total airflow provided by the fan increases. Second, the "loosen" effect of the coring further reduces the airflow resistance, which also increases the total airflow. Third, as the total amount of grain in the silo decreased, the specific airflow increased. Table 3 shows the total airflow, speficic airflow and airflow resistance for the different configurations considered in the study.

Aeration performance

The speficic airflows obtained in Table 3 were used as input in the aeration simulation runs carried out with the PHAST-FDM program for the four coring levels. Table 4 shows the average results of 13 years of simulation for each evaluated condition (coring % from 0 to 8%, and NUF from 30 to 0). In the base situation (before coring and NUF of 30), the total time to complete cooling from 30°C to less than 18°C was 1055 h (44 days). The accumulated fan runtime was 307 hours (fan was "on" 29%

of the time), and the aeration power consumption was 457.4 kWH. The final grain condition was 13.7% m.c. and 17.6°C.

Table 3. Total airflow provided by the fan, specific airflow and static pressure in the aeration system before coring and after different coring percentages.

Parameter	Base condition (Before coring)	After coring		
		3%	5%	8%
Total airflow (m^3/min)	42.1	47.6	51.4	55.9
Specific airflow (m^3/(min t))	0.083	0.095	0.108	0.12
Airflow resistance (Pa)	720	716	713	708

As the percentage of coring increased, the total time to complete cooling and the fan runtime hours decreased, while the energy savings regarding the base situation (no coring) increased.

Coring 3% of the grain mass resulted in a reduction of the total time to complete cooling to 909 hours and fan runtime hours to 269 hours. The aeration power consumption was 400.8 kWH, while the electrical power consumed by the unloading auger and the bucket elevator for the coring operation was 4.29 kWH. This resulted in an average energy saving of 52.3 kWH or 11% of the base condition.

For a coring percentage of 5%, the total time to complete cooling was reduced to 761 hours and fan runtime hours to 216 hours. The resulting aeration power consumption was 321.3 kWH, while the electrical power consumed by the unloading auger and the bucket elevator for the coring operation was 7.04 kWH. This resulted in an average energy saving of 129.0 kWH or 28% of the base condition.

For a coring percentage of 8%, the total time to complete cooling was further reduced to 727 hours and fan runtime hours to 207 hours. The resulting aeration power consumption was 308 kWH, while the electrical power consumed by the unloading auger and the bucket elevator for the coring operation was 11.44 kWH. This resulted in an average energy saving of 138.1 kWH or 30% of the base condition.

As the resulting airflow after coring became more uniform (NUF decreased from 30 to 0), the total time to complete cooling and the fan runtime hours decreased, while the energy saving regarding the base situation (no coring) increased. Across all percentages of coring, fan runtime hours and energy savings regarding the base condition (no coring and NUF of 30) for a NUF of 20 were 234 hours and 22.1%, respectively, while for a NUF of 0 (no airflow difference between center and side) the fan runtime decreased to 228 hours and the energy saving increased to 24.2%.

Table 4. Results of the PHAST-FDM simulation runs showing the time to complete cooling, fan runtime, aeration power consumption, and average final moisture content and temperature, and the computed coring electrical consumption and total energy saving due to coring for the four coring levels evaluated.

Coring	NUF	Time to complete cooling (hs)	Fan runtime (hs)	Aeration power consumption (KWH)	Coring power consumption (kWH)	Energy saving (kWH)	Final average m.c. (%)	Final average temp. (°C)
Before (0%)	**30**	1055	307	457.4	0	0	13.7	17.6
3%	**20**	916	272	405.2	4.29	47.8	13.7	17.5
	10	909	269	400.8	4.29	52.3	13.7	17.5
	0	902	266	396.3	4.29	56.8	13.7	17.6
	Avg	909	269	400.8	4.29	52.3	13.7	17.5
5%	**20**	770	217	323.3	7.04	127.0	13.7	17.6
	10	757	215	320.3	7.04	130.0	13.7	17.6
	0	757	215	320.3	7.04	130.0	13.7	17.6
	Avg	761	216	321.3	7.04	129.0	13.7	17.6
8%	**20**	752	213	317.4	11.44	128.6	13.7	17.5
	10	717	205	305.4	11.44	140.5	13.7	17.5
	0	713	202	301.0	11.44	145.0	13.7	17.6
	Avg	727	207	308	11.44	138.1	13.7	17.6

Discussion

The coring operation has a main effect of increasing the specific airflow for aeration. As a portion of the grain is unloaded, the total depth of grain is reduced, the path of the air through the grain mass is shortened, and the airflow resistance is reduced. An additional reduction in airflow resistance is obtained by the "loosening" effect of the grain mass. As a result of the reduction of airflow resistance, the fan total airflow increased (Table 3). The airflow increase depends on the characteristics of the fan (fan performance curve shape) and the operational condition of the fan. For instance, if the aeration fan has a performance curve that does not change much with static pressure (e.g., a high speed centrifugal fan), then the reduction in airflow resistance due to coring will have little effect on total airflow, and vice versa for an axial fan (with a fan performance curve that changes with static pressure). In addition to the increase in the total airflow, the specific airflow also increased due to the reduction in the amount of grain after coring. In this study, the increase on specific airflow was estimated up to 45%. Cardoso et al. (2008) reported an increase of 63% in the measured airflow after 3% of coring a silo with 700 tonnes of wheat.

The simulation of the effect of coring on airflow performance showed that the coring operation reduced the total time to achieve cooling, the fan runtime hours, and the fan power consumption. The reduction in fan power consumption was achieved through the reduction in the fan runtime hours. The electrical power consumption of the unloading auger and bucket elevator for coring the silo was always lower than the savings achieved, implying that coring always has an economical benefit (Table 4). The energy saving increased with coring, obtaining an energy saving of 11%, 28% and 30% for 3%, 5% and 8% of coring, respectively. Based on these results, 5% of coring was the most convenient, because this amount of coring had the larger marginal benefit in energy savings.

The improvement of airflow uniformity after coring also reduced the fan energy consumption, although to a lesser extent. For a NUF of 20 the total energy savings was 22.1% (across all coring percentage levels) while for a NUF of 0 (no airflow difference between center and side) the energy savings only increased to 24.2%. This implies that the main benefit of coring was through the increase in the specific airflow.

Additional benefts of coring, besides energy savings, also must be considered. A reduction in the time for achieving cooling objectives has consequences reflected in the final quality of the grain. For instance, total cooling time for the base condition was 1055 hours, while 5% of coring with a NUF of 0 shortened the total cooling time to 757 hours. Shortening cooling time by 12 days may provide important benefits preventing insect development (Navarro and Donahaye, 2005). Additionally, coring removes a significant proportion of the fine material from the silo (Cardoso et al., 2008), and fine material was reported to have higher mycotoxin concentration than whole grain (Abbas et al., 1985).

Thus, coring the silo by unloading from 3 to 8% of the stored grain is a recommendable practice, because it increases the specific airflow and airflow uniformity, reduces fan runtime hours and generates energy (and cost) savings. Additionally, by reducing the cooling time and eliminating the fine material reduces the risk of insect development and mycotoxins formation.

Acknowledgement

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Application of transverse ventilation in grain storage in China

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Extended abstract

In China, mechanical ventilation technology has been researched and applied since the 1950s. Beginning in 1998, large-scale grain warehouses started to be built with national government support. The mechanical ventilation technology, namely the "four-in-one" technology, was promoted enormously during this period. In the “four-in-one” system, the aeration technology was based on the vertical aeration system with ventilation ducts temporarily fixed on the floor of the warehouse. The airflow passed vertically through the grain bulk from the bottom to the surface or vice versa with air being pushed by fans, and the heat and moisture from the grain exchanged with the air during vertical aeration. This vertical ventilation system has been widely used for the last twenty years, but it is complex and inconvenient, and also air distribution is uneven.

To fix these problems, Chinese researchers developed a new transverse ventilation technology as shown in Fig. 1. In this system, aeration ducts are mounted along the opposite interior walls of the warehouse and air travels horizontally through the grain mass. A large number of pilot scale tests and warehouse applications have been done from 2010 to 2014.

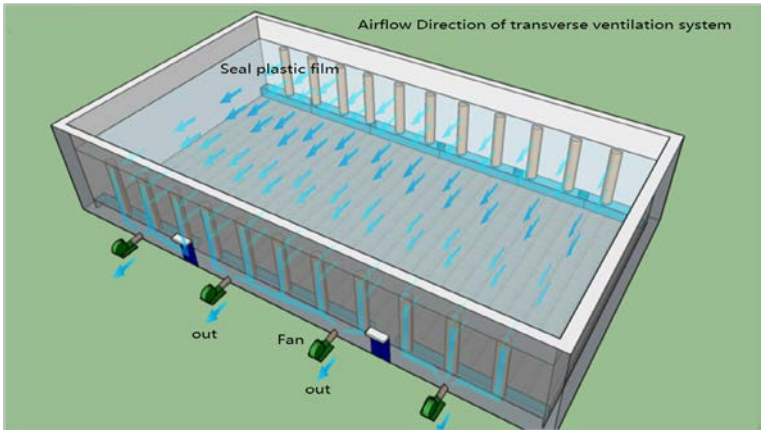

Fig. 1 The new transverse ventilation system.

The grain surface is sealed by plastic film during storage to prevent air from escaping through the surface layer during aeration and gas during fumigation. During aeration, the airflow is sucked from

one side of the aeration ducts and exhausted out the fans after horizontally passing through the grain bulk. With nearly five years of application, it has been demonstrated that the storage technologies in this new ventilation system, such as aeration, grain cooling, fumigation and controlled atmosphere treatment can be done effectively, ande grain moisture loss during ventilation can be reduced by 0.3-0.5 percentage points. Alos, the efficiency of loading and unloading grains can be increased by 100% as compared to the vertical ventilation system because on-floor ducts do not need to be removed during the unloading process.

Therefore, application of the granary transverse aeration system will obtain better economic and operational benefits as summarized in Tab. 1.

Tab. 1 Evaluation of vertical and transverse ventilation system.

No.	Evaluation index	Vertical ventilation	Transverse ventilation	Remark
1	Ventilation uniformity	80-85%	90-95%	Increase of 10%
2	Percent of moisture loss during ventilation	0.7-1.0%	0.2-0.3%	Reduced by 3-5 times
3	Capacity of grain load/unload/hour	50 t/hour	＞100 t/hour	Increase of 100%
4	The load/unload cost of per ton	5.0 ¥/t	3.0 ¥/t	Reduced by 40%
5	Labor cost	high	low	Reduced by 50%
6	Depreciation expense	high	low	Reduced by 20%
7	Labor intensity	high	moderate	
8	Mechanization level	low	high	

Until now, the transverse ventilation system has been applied in more than twenty provinces throughout China, and the quantity of stored gain has reached 3 million tons of warehouses storage capacity that is equipped with the new transverse system.

Technical and Economic Evaluation of Ambient and Chilled Aeration Strategies to Maintain the Quality of Paddy Rice During Storage in a Tropical Climate

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Abstract

Warm and moist conditions of some tropical climate regions make it difficult to use ambient aeration to cool stored grain, which contributes to pest problems and increases dependence on chemical control as part of grain management strategies. Grain chilling is a non-chemical alternative to cool grain stored under high risk climatic conditions. The objective of this research was to use computer simulation to evaluate the technical and economic viability of using grain chilling compared to four ambient aeration strategies developed for paddy rice stored under the tropical climatic conditions of the North Pacific coast of Costa Rica. The minimum grain temperature achieved through ambient aeration at the end of the six-month simulated storage period was 30.8°C, using an aeration strategy based on a grain-ambient temperature differential greater than 10°C. Grain chilling lowered the average grain temperature from 35°C to below 15°C in 117 hours and the maximum average temperature it registered after six months of storage was 15.5°C. The economic evaluation of the ambient aeration and chilling strategies determined that the operational costs of grain chilling were 1.83 US $/t lower than ambient aeration plus chemical control of pests. However, the initial cost of the grain chiller made the net present cost (NPC) of the grain chilling strategy 0.22 US $/t higher than the cost of ambient aeration plus

chemical control over a 10-year analysis. Several potential financial options were analyzed to make the grain chiller economically feasible for a rice miller in Costa Rica.

Keywords: Paddy rice, ambient aeration, grain chilling, economic feasibility.

Introduction

The conditions of high temperature and relative humidity (RH) during most of the year in most tropical climatic regions limit the cooling capacity of ambient grain aeration. This is why in this climate ambient aeration is used mainly to maintain the grain temperature and moisture content (MC) in equilibrium with the average ambient conditions, which avoids the development of hot spots and prevents condensation on walls and roofs (Lawrence and Maier, 2011; Noyes and Navarro, 2002).

A limited number of research studies have come up with strategies that give viable options for aeration in tropical climates. One of these studies was presented by Sinicio and Muir (1998), in which they determined that using a difference of 6°C between the average grain and ambient temperature, at an airflow between 0.08 and 0.16 m^3/min/t, provided the best storage conditions for wheat during eight month storage (only 0.1% shrink loss) under Brazilian conditions.

Aeration during night time or early morning hours has also been considered a technically viable option for tropical climates since lower temperatures during these hours have reasonable cooling effect on stored grain (Monroy and Valencia, 1978; Recio, 1999). However, the risk of rewetting grain is a restriction for using lower temperatures in this latitude, but according to Noyes and Navarro (2002), the RH is usually lower in the plenum due to the heat of compression produced by the aeration fans. According to Noyes and Maier (2002), for every ~248 Pa (1 in. of water column) of static pressure (SP) that is generated in the aeration system, the temperature of the air passing through the aeration fan can increase by ~0.5°C (1°F). According to Zeledon and Barboza (2000) (unpublished), the RH inside the plenum can be between 6 and 18 percentage points drier than the ambient air.

Grain chilling is an alternative to ambient aeration that allows cooling of grain under 20°C in weather conditions where otherwise it would not be possible. This helps limit or stop completely insect population growth (Fields, 1992). This technology has proven to be effective for cooling grain to below 17.5°C in relatively short periods of time (80-300 hours) in silos between 500 and 5000 metric tons (t), located in tropical regions of Argentina, Brazil and Israel (Calderon, 1972; Lazzari et al., 2010; Roskopf and Bartosik, 2009).

The high purchase price of grain chillers and the lack of economic studies that complement the technical studies has limited the implementation of this technology more widely in some tropical regions. One of the only studies that has made an effort to evaluate the true value of this kind of investment in the long term was developed by Rulon et al. (1999), in which they analized the economic feasibility of a grain chilling prototype developed by Purdue University using the Net Present Cost (NPC) methodology that analyzes the net cost of an investement through its life cycle. This study demonstrated that the grain chilling technology was highly competitive compared to the cost of using ambient aeration plus chemical control.

The obejctive of this resesearch study was to use computer simulation to evaluate the technical and economic feasibility of using grain chilling compared to four ambient aeration strategies developed for paddy rice stored under the tropical climatic conditions of the North Pacific coast of Costa Rica.

Materials and Methods

Ambient aeration and grain chilling computer simulation model

The ambient aeration and grain chilling strategies were analyzed using a finite element computer simulation model adapted from Lawrence and Maier (2011) and based on the storage conditions of paddy rice in the North Pacific region of Costa Rica, also called Guanacaste. For the development of

the computer model, five years of weather data (2010-2014) during the storage period of paddy rice in this region (November to May of next year) were collected. The initial conditions of the paddy rice were determined at 13% MC and 35°C, assuming it would go into storage directly from the dryer, and the physical properties such as bulk density, porosity, and thermal properties which were retrieved from ASABE standards D241.4 and D243.4.

The storage structure used in the computer model was a corrugated steel silo of 1500 t (diameter-to-height ratio of 1.0), which is commonly used for long term storage in this region. The aeration system of these silos consisted of one 20 HP centrifugal fan and a perforated false floor. Using these specifications, the airflow rate of the ambient aeration fan was determined to be 0.22 m^3/min/t (~0.2 cfm/bu) and the static pressure (SP) produced by the aeration system was determined to be 2070 Pa (~8.3 inches of water column) (Dickinson and Morey, 2013). This SP would cause an increase of the aeration air of approximately 5°C according to Noyes and Maier (2002) which was accounted for in the ambient aeration simulations.

Based on the analysis of the climatic conditions of the region and the structural conditions of the storage structure, the following ambient aeration strategies were proposed:

Run ambient aeration fan when ambient temperature is less than or equal to 24°C and ERH in the plenum is less than 70%.
Run ambient aeration fan from 6:00 a.m. to 8:00 a.m. and from 5:00 p.m. to 7:00 p.m.
Run ambient aeration fan from 5:00 a.m. to 9:00 a.m. and from 5:00 p.m. to 9:00 p.m.
Run ambient aeration fan whenever ambient temperature is 10°C lower than grain temperature in the top section of the grain mass.
The grain chilling strategy was programmed to start the grain chiller as soon as the paddy rice entered the silo and continue the cycle until the top section of the grain mass reached 15°C. The input data for the development of this strategy was collected from field trials developed on wheat storage in Kansas, U.S.A., during the summer of 2015 and 2016 (Morales-Quiros, 2017). The grain chiller used for these trials has a rated capacity to cool 100 to 170 t of grain per 24 hours of continous operation on silos of up to 1800 t, according to the manufacturer (Coolseed, 2016).

Net Present Cost economic model

The cost of the ambient aeration strategy with the best results from the previous section, based on fan run hours, MC, and final grain temperature, was compared with the cost of the grain chilling strategy using the NPC methodology developed by Rulon et al. (1999). The NPC economic model calculated the net cost of the investment over its life cycle (10 years for the grain chilling equipment), using factors like annual interest rate, tax rate, rate of return on equity and percent of business financed by debt. This information was collected from financial entities in Costa Rica.

The NPC of the ambient aeration strategy was calculated based on the power requirement of the aeration fan, maintenance labor, sampling labor and shrink loss. Due to the fact that it is not possible to control pests only with ambient aeration in this region, the cost of this strategy included fumigation cost, insecticide application, personnel safety equipment cost, application labor, among others. This information was collected from agrochemical companies and local rice milling industries.

The NPC of the grain chilling strategy was calculated based on factors like purchase price of the grain chiller (US $74700, according to the manufacturer), power requirement, installation and maintenance labor, sampling labor and shrink loss. Additionally, financial options for making the grain chilling technology feasible for the Costa Rican rice miller were also analyzed. Some of these alternatives were a leasing option, improving the capacity of the grain chiller, and premium sale price of paddy rice treated with the grain chilling technology. This information was compiled from previous field experience, financial entities and local rice milling companies.

The NPC calculations were based on a hypothetical rice milling company with six silos of 1500 t of paddy rice each, which is the average for the region, stored for six months.

Results and Discussion

Ambient aeration and grain chilling strategies

The results of the computer model demonstrated that it is possible to use low temperature, high RH air to aerate paddy rice since the temperature increase of approximately 5°C in the plenum will reduce the RH by approximately 20percentage points. This means that it is possible to use ambient air of up to 90% RH because in the plenum the RH will decrease to 70%. Similar observations were made by Zeledon and Barboza (2000).

The first ambient aeration strategy (Ambient temp. ≤24°C, plenum RH <70%) only reduced the average of the 5-year average temperatures of the grain mass by two degrees (35°C to 33°C), without noticeable MC variation.

The second and third ambient aeration strategies (2 and 4 early morning and night time fan run hours, respectively) showed adverse results because the temperature of the grain mass essentially remained unchanged but the average of the 5-year average MC was reduced dramatically due to the lack of restrictions for the conditions of ambient air that could be used in these strategies. The number of fan run hours were also excessive in these strategies (729 and 1458 hours, respectively). For this reason, the period of aeration was limited to between November and January of the storage period, which are the months with the lowest minimum temperature of the year. This helped to reduce the average of the 5-year average of rice temperature to approximately 33°C, without noticeable MC variation. This modification also reduced the fan run hours to 312 and 624 hours, respectively.

The fourth strategy (grain-ambient temp. difference ≥10°C) was the one that reduced the average of the 5-year average paddy rice temperature the most by the end of the six-month storage period, down to 30.8°C. This strategy increased the average of the 5-year average MC by 0.1% and required the least amount of fan run hours (214 ±43 hours) among all ambient aeration strategies analyzed (Fig. 1).

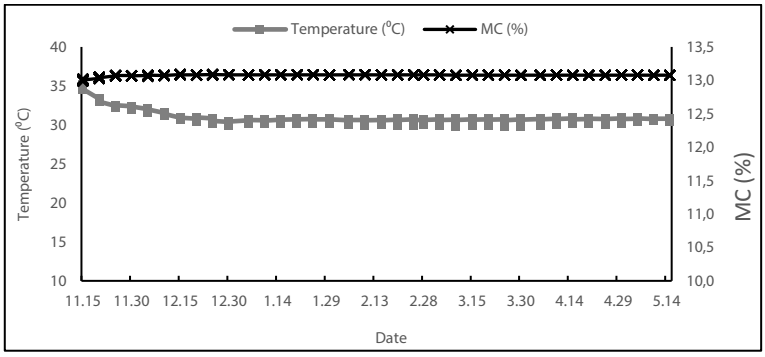

Fig. 4 Five-year average of the average grain temperature and moisture content (MC) profile of paddy rice stored from Nov. 15th to May 15th in Guanacaste, Costa Rica and aerated using Strategy 4.

The computer simulation of the grain chilling strategy showed that the average of the 5-year average grain temperature was reduced from 35°C to below 15°C in 117 hours of active chilling, and remained below 15.5°C for the six months of storage. Nevertheless, the average of teh 5-year average paddy rice MC increased by 0.2 percentage points with this strategy (Fig. 2).

Preserving paddy rice at low temperature demonstrated to be effective at controlling *R. dominica* and *Sitophilus* spp. for 60 days of storage in Brazil (Lazzari et al., 2006).

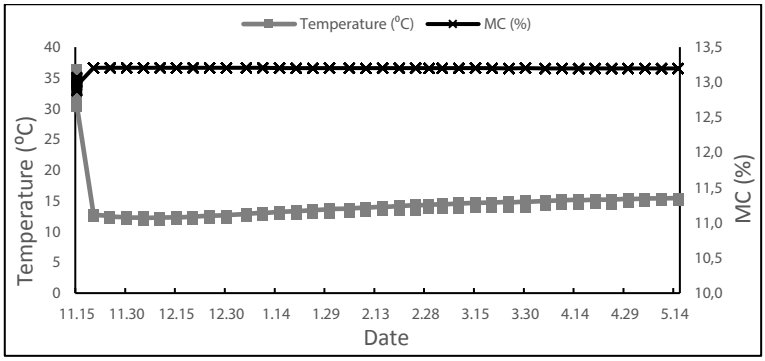

Fig. 5 Five-year average of average grain temperature and moisture content (MC) profile of paddy rice stored from Nov. 15th to May 15th in Guanacaste, Costa Rica and aerated using the grain chilling strategy.

NPC economic analysis

Since the fourth ambient aeration strategy was the one that required the least amount of fan run hours, and thus resulted in highest energy savings, and was also the one that maintained the lowest grain temperature throughout the six months of storage among all ambient aeration strategies, it was chosen for the NPC economic analysis. Its feasibility was compared against the feasibility of the grain chilling strategy.

The NPC economic analysis showed that, although the operational costs of running the ambient aeration fan in the fourth ambient aeration strategy were low, the added cost of the chemical control of pests increased the annual operational costs of this strategy up to 2.36 US $/t. On the other hand, the annual operational cost of running the grain chiller was only 0.53 US $/t, given that preserving the paddy rice at temperatures below 20°C in a climatic region, where otherwise it would not be possible, replaces the need for chemical control. Similar observations were made by Rulon et al. (1999).

Although the grain chilling strategy predicted to reduce the annual operational costs of the Costa Rican rice milling company, the high initial investment of the grain chilling equipment (US $74700) increased the total NPC of this strategy. It resulted in an annual amortized NPC of 1.51 US $/t, while the annual amortized NPC of the fourth ambient aeration strategy plus chemical control was 1.29 US $/t (Fig. 3), i.e., 14.5% lower.

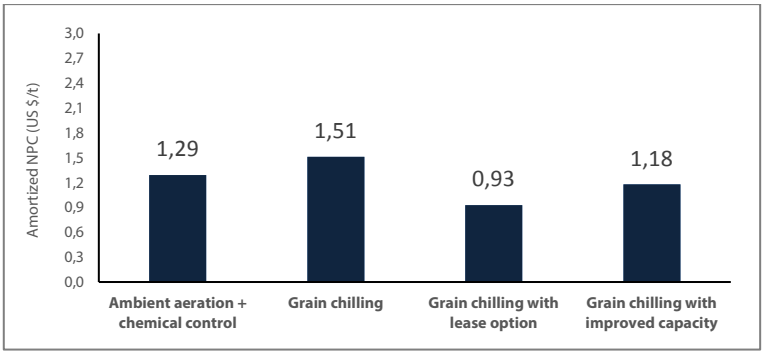

Fig. 6 Amortized Net Present Cost (NPC) of ambient aeration Strategy 4 and the grain chilling strategy with financial options for reducing the NPC.

In order to lower the NPC of the grain chilling strategy a leasing option was analyzed. It showed that leasing the grain chilling equipment for an annual rate lower than US $11,000 for a 10-year period

(assumed useful life time of the equipment), would reduce the NPC of chilling from 1.51 to 0.93 US $/t (Fig. 3), or -38.4%. Although this option would increase the annual opertional cost of the grain chilling strategy from 0.53 US $/t to 1.77 US $/t due to the addition of the annual leasing payment, this cost would still be lower than the operational cost of the fourth ambient aeration strategy plus chemical control, i.e., 2.36 US $/t.

Another feasible option for financing the grain chiller, according to the NPC economic analysis, was to increase the capacity or number of tons treated with the grain chilling technology, which would dilute the cost per ton of the grain chilling strategy. This analysis showed that increasing the number of silos of 1500 t treated with the grain chilling technology from six to eight would reduce the amortized NPC from 1.51 US $/t to 1.18 US $/t, i.e., -21.9% (Fig. 3). This seems like an achievable quantity for the rice milling industry of Costa Rica since there is usually more than one harvest per year. The rice companies are receiving paddy rice basically all year, which would justify the purchase of the grain chiller given that it would be utilized throughout the year, lowering the NPC further.

A third financial option for the grain chilling strategy, according to the NPC economic model, would be to sell the rice treated with the grain chilling technology as a value-added product because it would be free of residues from postharvest pesticides. If it were possible to incease the sale price of this product by US $0.50 to US $1.00 per ton, this would reduce the amortized NPC of the grain chilling strategy below 1.29 US $/t (amortized NPC of the ambient aeration plus chemical control strategy).

Conclusions

The ambient aeration strategy based on a grain-ambient temperature differential of 10°C or higher showed the best results on final grain temperature, moisture content and fan run hours; nevertheless, it was not possible to reduce the average temperature of the paddy rice below 30.8°C by the end of the six-month storage under the tropical conditions of Guanacaste, Costa Rica. On the other hand, the grain chilling strategy reduced the average grain temperature below 15.5°C in less than five days, and paddy remained below this temperature for the rest of the six-month storage period. This would potentially reduce insect populations and eliminate the requirement for chemical control.

The grain chilling strategy reduced the annual operational costs of the Costa Rican rice milling company, according to the NPC economic analysis, but the high initial cost of the grain chilling equipment made the amortized NPC of this strategy higher than the amortized NPC of the ambient aeration strategy plus chemical control. The leasing option of the grain chilling equipment at a reasonable price, increasing the capacity (number of tons treated) of the grain chiller, or charging a premium sale price of the paddy rice treated with the grain chilling technology, are all feasible options for reducing the amortized NPC of the grain chiller.

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CHILLING TEMPERATURE AND LOW MOISTURE CONTENT TO KEEP SOYBEAN GRAIN QUALITY DURING STORAGE

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Abstract

Soybeans are used as food, feed, oil and fuel. Losses may happen at harvesting, transportation, and mainly during storage. Moisture content (MC %) and temperature (T °C) of the soybean grain mass during storage are the main factors affecting quality, quantity and value of the product by favoring the development of microorganisms and insects. Large grain chillers have been used to maintain soybean quality and reduce insect infestation during storage. To evaluate the effect of MC and temperature on the quality parameters of soybean seeds, samples were stored at 58±2% RH, with five different MCs, at 15 °C (chilling temperature) and 30 °C (average temperature inside silos in Brazil) for 180 days. The following was observed: reduction in the MC at higher temperature; the weight of soybeans was maintained at either temperature when the MC was at about 12%; MC above 14% reduced the weight value independent of storage temperature; at 15°C the weight of 1,000 seeds was maintained during storage; low MC and temperature kept germination and vigor of the seeds at high rates; low MC and temperature reduced electrical conductivity; there was no noticeable influence of the storage temperature, regardless of the MC of the beans, on the free fatty acid content. In general, quality attributes tend to be reduced during storage, being more remarkable at higher temperature and MC of the seeds. In conclusion, the temperature of 15ºC, which simulates grain cooling conditions, favors the maintenance of quality, quantity and value of soybean for long-term storage.

1. Introduction

Soybean is one of the most important crops in Brazil and worldwide. It is used as food, feed, oil and fuel. Quality and quantitative losses in soybeans may happen during harvesting, transportation, and mainly in storage. Moisture content and temperature of the soybean mass during storage are the main factors that affect quality, quantity and value of the product.

The main cause of weight loss in stored soybeans is consumption of the dry matter (starch, proteins and fats) by storage fungi (Christensen and Meronuck, 1986). Lazzari (1997) stored soybeans for six months at 15 and 25 °C and water content varying from 13.9 to 22.1% wet basis (% w.b.). He concluded that the higher the temperature and the water content, the greater the fungi infection and consequently the dry matter loss which could range from 0.24 to 1.25% at 15 °C and from 0.39 to 36.6% at 25°C.

Teixeira (2001) mentions low temperature associated with drying of soybeans, allows a longer storage time without compromising quality during this critical period. Teixeira (2001) also mentions that grain with moisture content between 16 and 18.5% (% w.b.) can be stored safely for 3 to 18 months at cooling temperatures of 3 to 10°C, inhibiting the development of fungi, insects and the germination loss of seeds. It is important to consider that artificial cooling can be a cost effective alternative to aeration with ambient air. Considering the benefits, cooling soybean kernels during storage might be a valuable technology to reduce postharvest losses, although the effects of low temperatures and different water contents of soybean seeds need more complete evaluation.

In order to determine the benefit of cooling technology on the quality of soybean seeds, samples were stored at 58+2% RH, with five different water contents, at two temperature levels. The following parameters were evaluated: 1. variation in water content, 2. seed weight, 3. apparent specific mass, 4. weight of 1,000 kernels, 5. electric conductivity, 6. germination, 7. accelerated aging and 8. fat acidity.

2. Materials and Methods

The experiment was carried out in the Preprocessing and Storage of Vegetable Products laboratory, Department of Agricultural Engineering, University of Viçosa (UFV), Minas Gerais, Brazil. A 2 x 5 factorial experimental design was implementd in two climate-controlled chambers: one at 15°C simulating the cooling condition, and the other at 30± 2°C simulating storage temperature conditions prevalent in most areas of Brazil. The relative humidity was of 58+2% in both chambers. The subplots were five water content levels: 12, 14, 16, 18 and 20%, and five storage intervals (0, 45, 90, 135, and 180 days), for seven parameters evaluated, with three replicates.

Soybean seeds from the BIOAGRO/UFV experimental units were used, with initial water content of 22% (% w.b.). The seeds were dried in a fixed bed dryer, with air heated to 40°C using a LPG burner. The gravimetric process was used to obtain the water content of soybeans at 20, 18, 16, 14 and 12%. The variation of the mass of the evaporated water during drying from the initial water content was estimated by separating the fractions to obtain each of the desired levels. The final water content was measured after the seeds were in equilibrium at room temperature expressed as percentage wet basis (% w.b.). The soybean samples were packed in plastic bags measuring 0.40 x 0.45 m, with a capacity of 5 kg, and stored under the defined conditions, from April to October 2011.

Water content, weight of one thousand seeds, germination and accelerated aging were measured according to the methodology described in Brazil (2009). The electrical conductivity in the solution containing the soybean seeds was made using the "Glass System" (Vieira & Carvalho, 1994). The specific mass was determined using a weight scale with a capacity of 1 liter. The ethereal extract and total titratable acidity were performed according to the methodology described by Silva (2002).

3. Results

The temperature of the seeds remained practically uniform in most samples, with variations below 4°C at different points. The data indicate that the natural convection of the intergranular air was

minimal, reducing the mass transfer (water vapor) between the grains and the intergranular air and promoting stability in the water content of the grain (Fig. 1).

Water Content

At 15°C and 58+2 % RH, the values of the initial water contents were 12.6, 12.4, 15.9, 17.2 and 19.7% (Fig. 1A). After 180 days of storage, the water contents were 12.9, 12.8, 15.8, 17.0 and 19.7%, showing that seeds with water content lower than 13% suffered small increments in their humidity values, while those containing initial water contents of 15.9 and 19.7% did not present moisture content alteration (Table 1).

At 30°C, the initial water contents were 11.5, 13.6, 15.9, 17.8 and 20.0%. After 180 days of storage, the respective water contents were 11.2, 13.3 and 15.0%, without noticeable variation between the initial and final moisture contents. As expected, seed samples with the initial water contents of 17.8 and 20.0% were totally deteriorated by fungal activity at 90 days of storage (Fig. 1B and Table 1).

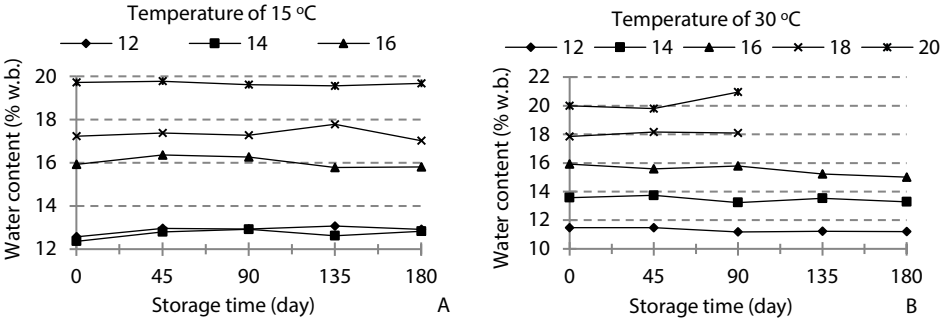

Fig. 1 Water content of soybean seeds stored at 15°C (A) and 30°C (B) for 180 days at 58+2% RH.

Seed Weight

For the seed weight variation (Table 1), it was found that at 15°C moisture gain was obtained for the drier seeds (12.6 and 12.4% initial water contents). Seeds with water contents equal to or greater than 15.9% lost water during the storage period.

Total soybean loss was found in the product stored at 30°C when its initial water contents were 17.8 and 20.0%. There was a slight gain in moisture when the product was stored with initial water contents of 12.6 and 12.4% at 15°C. Moisture losses of 24.4 kg t^{-1} and 21.4 kg t^{-1} occurred at 30°C with the initial water contents of 11.5 and 13.6%, respectively.

Apparent Specific Mass

When storing at 15°C, the highest value of apparent specific mass was 690.2 kg m^{-3} and the lowest was 636.6 kg m^{-3} for the seeds with initial water content of 12.9 and 19.7%, respectively. It was observed that soybeans with a lower water content had the specific mass unchanged after 90 days of storage (Fig. 2A). For soybeans with the other water contents, variation of the specific mass values was observed, which could be attributed to the tendency of adjustments related to hygroscopic equilibrium. However, soybeans with the initial water content of 19.7% had the specific mass reduced from 650.6 to 636.1 kg m^{-3}, indicating a mass loss for this qualitative attribute and that, even at the temperature of 15°C, this water content was too high for storing soybeans for 180 days. Storage fungi could grow in soybeans with moisture content above 16% and temperature of 15°C after 90 days in storage.

The results of the apparent specific mass reduction of the soybean seeds at 30°C with water contents ranging from 11.5 to 20.0% are shown in Fig. 2B. The seeds with water contents of 17.8 and 20.0% were badly degraded after 90 days in storage. The lowest observed value of the specific mass was 637.6 kg m^{-3} at 90 days of storage, when soybean seeds were infected by fungi of different species. The highest value was 691.7 kg m^{-3}, when soybeans had a water content of 11.2% at 135 days of storage.

Tab. 1 Initial and final water content, mass alteration and weight variation of soybean samples at 15ºC and 30ºC, at five levels of moisture content for each temperature, stored at 58+2% RH for 180 days.

Temperature (°C)	Water content (%w.b.)		Mass change (weight%)	Weight variation (kg t^{-1})
	Initial	Final		
15	12.6	12.9	(+) 2.70	(+) 27.0
	12.4	12.8	(+) 3.72	(+) 37.2
	15.9	15.8	(-) 0.81	81.6
	17.2	17.0	(-) 1.16	11.6
	19.7	19.7	(-) 0.25	2.5
30	11.5	11.2	(-) 2.43	24.4
	13.6	13.3	(-) 2.13	21.4
	15.9	15.0	(-) 5.71	57.2
	17.8	-	(-) 100.0	Total
	20.0	-	(-) 100.0	Total

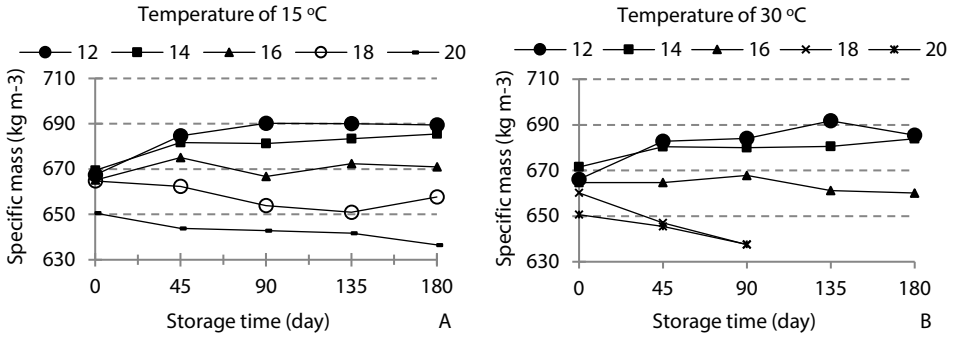

Fig. 2 Apparent specific mass (kg $m^{-3)}$ of soybean with different water content stored at 15ºC (A) and 30°C (B) for 180 days at 58+2% RH.

Weight of 1,000 Soybean Seeds

At 15°C, after 180 days the weight of 1,000 soybean seeds was between 188 and 181.8 g for the seeds with water contents between 16 and 20% (Fig. 3A). At 30°C, the weight was between 200 and 180 g for the samples between 16 and 20%, by the 90th day (Fig. 3B). In the beginning of storage, the weight of 1,000 seeds with water contents of 16 and 20% were of the order of 195 g and those with moisture of 12, 14 and 18% had a similar weight of 175 g. After 180 days of storage, only the seeds with a water content of 18% experienced weight reduction, resulting in a range of 180 to 185 g.

For the soybean samples stored at 30 °C, smaller dispersions were observed in the values of the weight of 1,000 seeds with the different water contents. However, these values were lower, ranging from 170.0 to 193 g, as compared to the samples stored at 15°C, after 180 days.

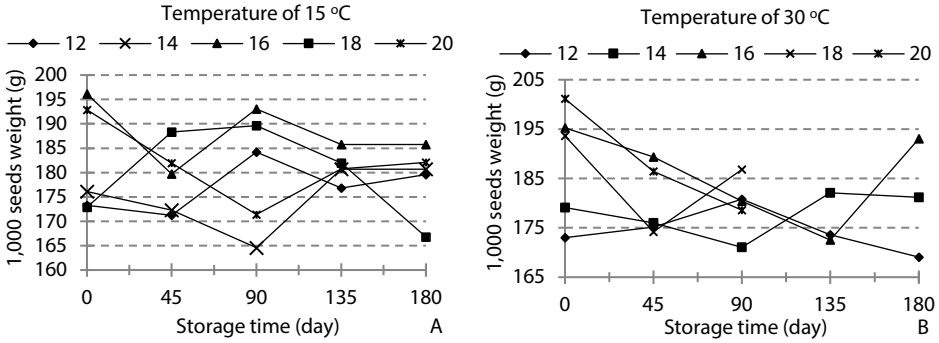

Fig. 3 Weight of 1,000 soybean seeds with five different water contents (% w.b.) stored at 15ºC (A) and 30°C (B) for 180 days at 58+2% RH.

Electrical Conductivity

The average values of the electrical conductivity of soybeans stored at 58+2% RH, 15ºC for 180 days, with water contents between 12 and 20%, ranged from 72.6 and 80.0 μS cm^{-1} g^{-1}, respectively (Fig. 4A). At 30°C, the average values ranged from 96.5 to 95.1 μS cm^{-1} g^{-1} (Fig. 4B).

It was observed during the storage period at 15°C that there was an increase in the electrical conductivity values of the order of 60 to 100 μS cm^{-1} g^{-1} (Figure 4A). For a water content of 20%, this value reached close to 140 μS cm^{-1} g^{-1}, indicating greater degradation of the product with higher water content after 180 days of storage. In soybean stored at 30°C, the damage was more intense at higher water contents. At a water content of 16%, the electrical conductivity ranged from 56.1 to 322 μS μS cm^{-1} g^{-1}. For a water content of 12%, the variation was from 68.8 to 141 μS cm^{-1} g^{-1}, and for a water content of 14%, it ranged from 62.0 to 191. μS cm^{-1} g^{-1}.

Germination

The average value of the germination index for soybean seeds stored at 15°C with a water content of 12% was above 98.7% from zero to 180 days of storage (Fig. 5A). With 14% water content, the variation was from 100 to 97.3%; at 16% it was from 99.3 to 91.3%; at 18% it varied between 99.3 and 88.7%, and at 20% between 100 and 55.3%. After 90 days of storage, there was a reduction in the germination index of the seeds with water content of 20%, from 100 to 86.7%.

In soybean seeds from the same samples stored at 30°C with the same water contents, higher deterioration rates were observed as compared to storage at 15°C. It was observed that after 45 days of storage the germination index of soybean seeds with water content of 18 and 20% reduced from 98.7 to 74% and from 100 to 24.7%, respectively (Fig. 5B). After this period, seeds with these water contents were totally degraded. The samples with 16% water content had the germination index reduced from 100 to 25.3% by the 90th day and to 0% by the 135th day. At 14%, the reduction in the germination index was from 99.3 to 10.7%, and at 12% from 100 to 92.7%,by the 180th day.

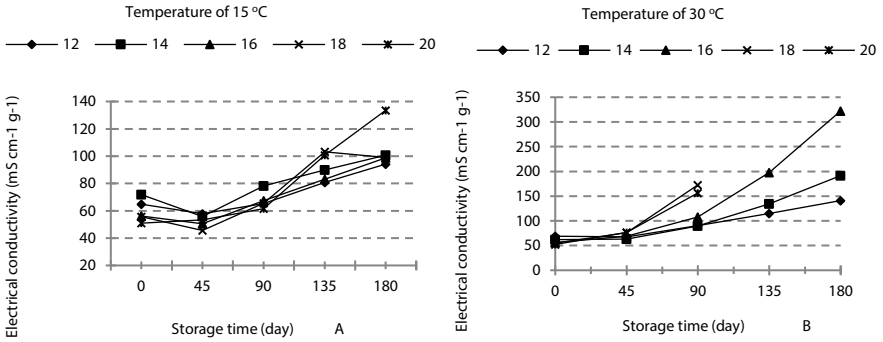

Fig. 4 Electrical conductivity of soybean seeds with different water contents (% w.b.) stored at 15ºC (A) and 30°C (B) for 180 days at 58+2% RH.

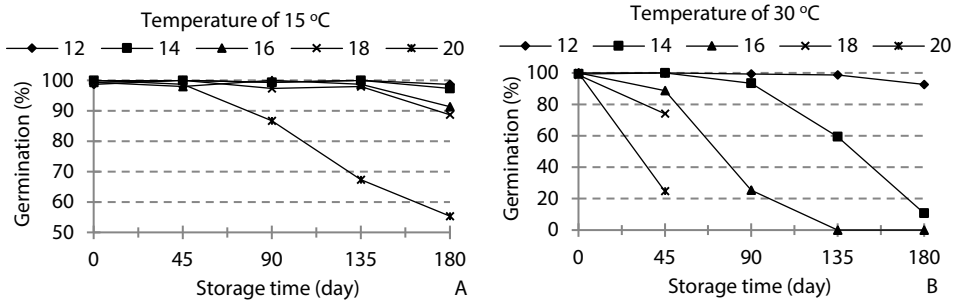

Fig. 5 Germination of soybean seeds with different water contents (% w.b.), stored at 15ºC (A) and 30°C (B) for 180 days at 58+2% RH.

Accelerated aging

Figures 6A and 6B show the deterioration of soybean seeds with different water contents measured by the vigor index (accelerated aging) when stored at 15 and 30°C.

For storage at 15°C (Fig. 6A) the higher the water content, the higher the seed degradation index. At 12%, the accelerated aging index varied between 100 and 99.3%; at 14% it stabilized at 100%; at 16% it was reduced from 97.3 to 77.3%; at 18% it was reduced from 99.3 to 72.7%, and at 20% it was reduced from 99.3 to 44.7%.

At 30°C for the same variety and water content, the seed degradation rate increased as compared to storage at 15°C. Reduction in the vigor index from 99.3 to 39.3% and from 98.7 to 9.3% was observed for the 18 and 20% water contents, respectively. At 45 and 90 days of storage the vigor index decreased further to 0%. For water content of 16%, the reduction was from 100 to 5.3% at 90 days, and to 0% at 135 days of storage. At 14%, the vigor reduction was from 99.3 to 68.7% at 90 days of storage, and to 0% at 135 days. At 12%, the reduction was from 100 to 63.8% after 180 days of storage.

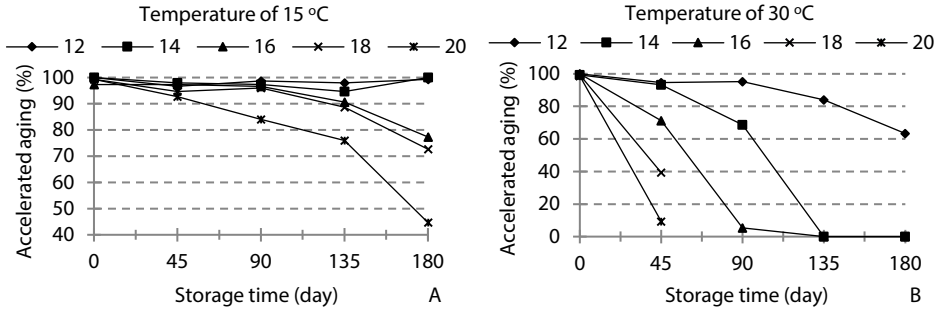

Fig. 6 Accelerated aging of soybean seeds with different water content (% w.b.) stored at 15°C (A) and 30°C (B) for 180 days at 58+2% RH.

Fat acidity

There was little variation in the fatty acid content during the entire storage period at 15°C, ranging from 0.94 to 2.32% at the beginning of the storage period. These acid contents had a noticeable increase for both temperatures after 135 days of storage. There was an accentuated decrease from about 4.0 at 135 days to about 1% at 180 days. At 30°C, variation between 0.48 and 1.2% was observed for soybeans stored at water contents between 12 and 16%. The seeds stored at 18 and 20% water contents were spoiled after 90 days of storage. Influences of temperature and water content on the fatty acid content were not observed.

Figures 7A and 7B show the variations in the fatty acid index of soybeans stored at different water contents and temperatures.

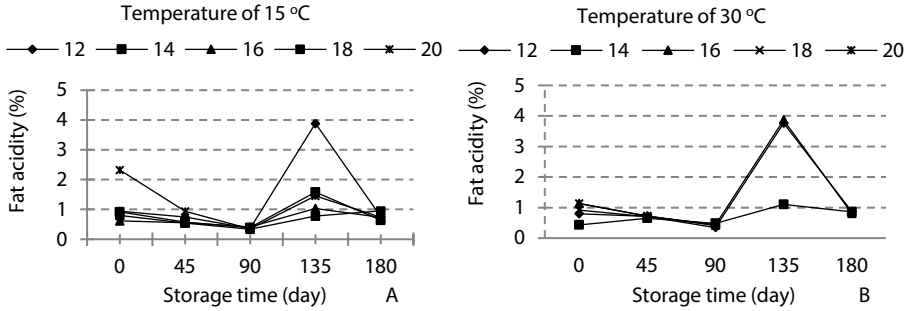

Fig. 7 Fat acidity of soybean seeds with different water content (% w.b.) stored at 15°C (A) and 30°C (B) for 180 days at 58+2% RH.

4. Discussion

The results showed that 15°C for each water content was a more adequate storage condition than 30°C for maintaining the initial seed characteristics including the sample with the highest water content. The higher temperature resulted in a greater reduction in water content of soybeans during storage. According to Christensen and Meronuck (1986) and Lazzari (1997), the higher the temperature and the water content, the greater the fungi infection and the resulting increase of dry matter loss and grain deterioration, as observed here in our experiment.

Our results demonstrated that for the same equilibrium relative humidity conditions at 15°C, the mass losses due to drying were lower than those at 30 °C. The specific mass values of soybeans stored at 15 and 30°C can be maintained when their water content was low (12 to 14%). For water content above 14%, there was a reduction in the specific mass values, independent of the storage

temperature. The main cause of the weight loss in stored soybeans was consumption of dry matter (starch, proteins and fats) by storage fungi (Christensen and Meronuck, 1986; Lazzari, 1997).

The higher the apparent specific mass value of soybeans, the lower its water content. The apparent specific mass of 12% soybeans is considered to be on the order of 750 kg m^{-3}. In our tests, after 90 days in storage soybeans with water content of 17% and above were so badly degraded due to infection by microorganisms that it was impossible to carry out the laboratory tests. On the other hand, the samples with lower water contents and lower temperature had the expected variation in the values of the specific mass indicated in the literature. Our results show stability of this quality attribute during 90 days at 15°C. However, a longer storage time of about 135 days resulted in lower apparent specific mass caused by fungi growth, despite the lower temperature.

According to Brazil (2009), the weight of 1000 soybean seeds varies according to their water content. Storage temperature of 15°C maintained the mass of 1000 seeds with a weight value higher than at 30°C. The variations observed in our tests, considering the studied range, may indicate the influence of possible dispersion of the values of water contents of individual soybean seeds in relation to the average value observed, and even a certain independence of the weight of 1000 kernels in relation to the water content. Petter et al. (2014) found mean values of 146 ± 14.2 g while in the study of Morais et al. (2014) they were in the range of 159.8 to 178.1 g, which are lower than those observed in the present study. These differences can be attributed to the agronomic characteristics of the varieties studied and the moisture content variation of individual soybean seeds (Lazzari, 1997).

The electrical conductivity test can be considered an auxiliary resource to assess early aging and possible damage to cell walls, allowing ionic solutions to be formed as a function of cell leakage. At both temperatures, the electrical conductivity increased with increasing storage days, indicating loss of soybean quality. For healthy soybeans, the values may vary depending on the variety studied; however, in the same variety, an increase in temperature and water content of soybeans results in greater damage to the cell walls of the seed. Researchers observed values of 56 μS cm^{-1} g^{-1} for the "Embrapa 48" soybean and 46 μS cm^{-1} g^{-1} for the "Paradise" variety. Low values of electrical conductivity indicate low leakage and consequently high physiological quality. The higher the temperature and water content of soybeans, the greater the increase in electrical conductivity and the resulting physiological damage, as observed in our tests, agreeing with Woodstock, cited by Simoni (2007), who mentions that seeds stored at low temperatures have less tissue deterioration.

Due to their sensitivity, the results of the germination test showed the importance of reducing the temperature and water content of the seeds in order to carry out storage safely, aiming at the physical, biochemical, nutritional and sanitary aspects of these seeds. Germination can be influenced by temperature, water content and length of storage. Under the same storage condition and for the same variety, the increase in water content resulted in a reduction in germination index at the end of the storage period for both temperatures, but it was considerably more accentuated at 30°C. Thus the lower the storage temperature, the higher the rate of germination and vigor during storage of soybean seeds. However, according to Lazzari (1997), even cool soybean seeds can be spoiled if stored with high water content.

The vigor index (accelerated aging) is another quality attribute that can be used to verify the physiological degradation of seeds. It was observed, at both storage temperatures, that there was a qualitative loss during storage as a function of the higher water content of the product. The higher the water content, the higher the seed degradation index. Our results indicate that soybeans with 14% is a moist product for storage in the natural environment. It was observed that for the same variety, even with low storage temperature, physiological degradation of the seeds may occur due to the higher storage water content. Thus, at a given temperature, high water content tends to reduce germination and vigor of the seeds. Also, time of storage reduces those two important parameters for stored seeds.

Another attribute of great importance for evaluating the quality of soybeans during storage is the acidity index. Soybeans are the main oil source for human consumption in Brazil and there is a maximum acidity limit for the commercialization of the product. In our tests, regardless of the water content of the seeds there is no noticeable influence of the storage temperature on the acidity of the soybean fat. The behavior of this parameter did not follow an expected pattern. According to Christensen and Kaufmann (1969), the vigorous development of fungi and their lipases at a specific moment of the deterioration of the seed increases the free fatty acids value. This explains the drop observed in our graphs, which could be the result of the consumption of portions of the fatty acids by fungi.

Overall, quality, quantity and value attributes of stored soybeans tend to reduce with storage time, being more remarkable at higher temperature and higher moisture content. One can conclude that the temperature of 15ºC, which simulates grain cooling conditions, favors quality maintenance of soybean seeds within a range of water content considered safe for storage. This range should be below 14%, because at or above this level of water content the soybean seeds are considered a moist product and may deteriorate during the storage period, unless the seed mass is stored under cooled conditions.

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Assessment of a mobile solar biomass hybrid dryer for insect disinfestation in dried maize grains

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Abstract

Considerable losses of stored food grains occur through insect infestation in tropical countries because climatic conditions are conducive for insect activity throughout the year. Studies have shown that in order to kill stored grain insects of all life stages temperatures above 50°C are required. However, grain simply laid in the sun or placed in a solar dryer does not reach such high temperatures. This study describes the use of a 1 tonne batch capacity mobile solar biomass hybrid dryer for disinfestation of infested maize and prevention of F1 progeny emergence in stored maize grains. To assess the effect of temperature and exposure period on mortality of maize weevils, infested maize in experimental cages were exposed for 3 and 6 hours of thermal disinfestation

treatment in the dryer. Comparing the heat generated in the dryer under hybrid mode operation where additional heat is generated by a biomass furnace in addition to solar, a mean temperature of 67°C was recorded compared to a mean ambient temperature of 36°C. Results showed that there was no significant difference ($p < 0.05$) in mortality of maize weevils during disinfestation treatment for 3 and 6-hour exposure periods. Mortality of 100% was obtained for samples disinfested in the highest tray (level 4) in the dryer. After 30 days of storage of disinfested maize grains, there was no emergence of F1 progeny from the maize grains exposed for 3 and 6 hours. Effect of ambient temperature and open sun exposure periods in the control set-up resulted in low mean percent mortality. Also, samples from the control set-up at both 3 and 6-hour exposure periods showed emergence of F1 progeny after storage. From this study, it can be concluded that an exposure period of 3 hours (or perhaps even less) in the solar biomass hybrid dryer could prevent damage by *Sitophilus zeamais* to stored maize grains after thermal disinfestation at a mean temperature of 67°C.

Keywords: Mobile solar biomass hybrid dryer; disinfestation; maize weevil, mortality,

Introduction

Maize is mostly destroyed by insects such as the maize weevil (MW), *Sitophilus zeamais* and the larger grain borer (LGB), *Prostephanus truncates*. Maize at harvest usually contains too much moisture (20-25%) which is a favourable environment for the growth of fungi and infestation of insects that normally cause grain damage (Folaranmi, 2008).

In Ghana, postharvest losses of maize occur in both the major and minor season which covers the period of April-August or Septmber and Septmber-December, respectively, especially in the middle belts of Ghana (Opit et al., 2014). Quantitatively, losses at harvest may be as high as 20% by weight of grains harvested by an average Ghanaian farmer (Ofosu, 1995 cited in Seidu et al., 2010). The major physiological, physical and environmental causes of postharvest losses are crop perishability; mechanical damage; excessive exposure to high ambient temperature, relative humidity and rain; contamination by spoilage fungi and bacteria; invasion by birds, rodents, insects and other pests; and inappropriate handling, storage and processing techniques (World Bank, 2011).

On the global scale, it is estimated that over two million tonnes of grains are destroyed annually by insects, moulds, rodents, birds and other pests (FAO, 2005). The MW is the most important insect pest of stored maize in tropical and sub-tropical countries (Ukeh, 2008). MW bores a hole through the grain kernel, consumes the endosperm, lays eggs in the holes and multiplies as their generation increases thereby causing vast damage to maize (Parker, 2008). In Ghana, out of an estimated total annual harvest of 250,000-300,000 tonnes of maize, about 20% is lost to MW (Obeng-Ofori and Amiteye, 2005). Therefore, it is important to mitigate these and other postharvest losses to ensure food security in Ghana (Opit et al., 2014). Infestation of maize by insects occurs mostly in the field due to delayed harvesting and also during maize storage. Postharvest activities such as timely harvest, shelling, drying and storage is a major concern because proper handling and storage generates more income to farmers. Grains can be stored for several purposes. Maize can be stored for short term (4-5 months), season-long (6-9 months), and long-term storage for more than 9 months (Mejia, 2008). Since storage is an important aspect of food security in developing countries (Kimenju et al., 2010), it is therefore important to store grains such as maize properly to prevent quality, physical, nutritional and biological losses which may occur.

Several techniques are employed in the storage of grains in developing economies. Some of these techniques include the use of traditional methods, botanical method, biological method, manipulation of drying and storage conditions, and use of synthetic chemicals. Synthetic chemicals are well known for insect pest control due to the important role these chemicals play in reducing storage losses. However, the disadvantages posed by the use of the chemicals such as risk to human health when inhaled and the toxic residues on food products, insect resistance due to its continuous use as well as high cost of these chemicals make them less attractive.

Aside from these synthetic chemicals and traditional methods, the use of non-chemical and low-cost technologies such as tapping the natural source of heat energy from the sun by the use of solar drying systems to heat the air that flows in the dryer, is a very effective, hygienic and efficient method for stored product protection. Solar dryers are specialized devices that control the drying

process and protect the agricultural product from being damaged by insects, pests, dust, rain and also from mould infection (Al-Juamily et al., 2007). According to Gatea (2009), the application of solar dryers in developing countries can reduce postharvest losses and significantly contribute to the availability of food.

Exposure to high temperatures to kill insects in stored food grains has long been known by farmers in developing countries where food grains are often laid in the open sun for thermal disinfestation. To achieve high mortality and destroy all life stages of insects, Hansen et al. (2011) reported that use of solar heating in excess of 50°C to disinfest stored commodities is possible. However, grain simply laid in the sun does not reach such high temperatures.

The present study describes the use of a developed mobile solar biomass hybrid dryer as a potential alterative for the eradication of insects in maize grains. Specifically, mortality of insects as affected by the high temperature and exposure period in the dryer was determined and compared to a control set-up using the open sun energy.

Materials and Methods

Experimental site and unit

The thermal disinfestation experiment was conducted using a developed 1-tonne capacity mobile solar biomass hybrid dryer (Fig. 1) designed and fabricated at the workshop of the Department of Agricultural and Biosystems Engineering, KNUST, Kumasi, Ghana.

Fig. 1 Developed mobile solar biomass hybrid dryer at KNUST, Kumasi, Ghana.

Description and operation of dryer

The mobile solar biomass hybrid dryer (SBHD) is based on a greenhouse structure design that utilizes locally available technology, materials and skills that make on-site construction possible. The dryer has two major parts; the drying compartment with overall dimension of 3 m x 1.8 m x 1.9 m totally enclosed with a 3 mm thick Perspex material. It has four layers of drying shelves (or racks) with a total holding capacity of 1 tonne. As shown in Fig. 2, the drying chamber is coupled to a biomass burner enclosed with a heat exchanger to raise the temperature of air that is blown into the drying chamber with a blower fan solely powered by an installed solar photovoltaic system which includes a back-up battery to store energy for off-peak operation and a DC bulb for night

operation. It integrates both solar and biomass energy to generate heat for drying crops or for thermal disinfestation of grain pests such as the MW. In operation, the dryer can rely on direct solar insolation during sunny days where trapped heat from the sun in the chamber is used for drying or disinfestation. Additionally, preheated air from the heat exchanger is forced/pumped into the chamber to affect drying or disinfestation (Fig. 2).

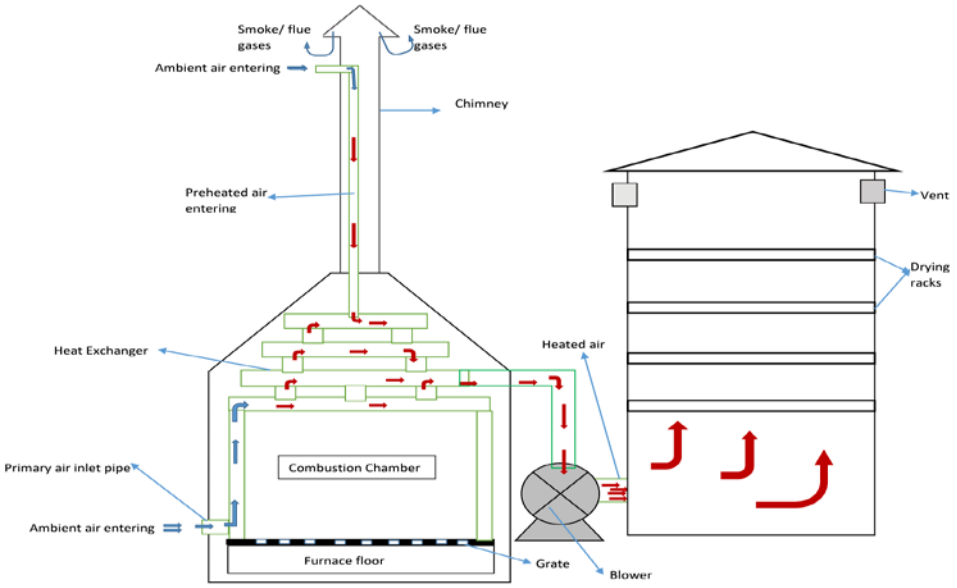

Fig. 2 Schematic view of the solar biomass hybrid dryer showing the air flow movement.

Methods

Culture of maize weevils

About 35 kg shelled maize (*Obatanba* variety) at 14% moisture content was obtained from the Agriculture Research Station at KNUST and used for the experiment. One kg of the dried maize grains was infested with adult *Sitophilus zeamais*. The infested grains were kept at room temperature for 10 days after which the insects were sieved from the grains. The sieved grains were thereafter kept in one litre Kilner jars at room temperature in the Entomology Laboratory of the Faculty of Agriculture at KNUST and cultured until the emergence of adult weevils. Emerged adult weevils served as the stock culture for the experiment.

Experimental set-up

The thermal disinfestation experiment was conducted on different days; 26th January 2017 (10:00 am to 16:00pm) where the heat source was from both solar and biomass energy (hybrid mode operation) and 7th March 2017 (11:00am to 17:00pm) where the heat for disinfestation was generated only from solar energy (insular mode). The experiment was set-up as a factorial experiment arranged in a Randomized Complete Block Design (RCBD). The effect of disinfestation period (3 and 6 hours) and heat source (solar and biomass combined; solar only) on weevil mortality were considered as treatments. Under each heat source application experiment, three replicate samples were set-up at each level in the dryer (four levels/blocks). The control samples were set-up in the open sun during the experiment. Under each experimental trial, 30 aerated cages were stocked with maize samples and the cultured weevils. Each level of the dryer had six cages (three

replicates for 3 hours of disinfestation period and the other three replicates for 6 hours). This brought the total cages in the dryer to 24 as shown in Fig. 3. The remaining six cages were set up in the open sun for the same thermal disinfestation period. The cages were fastened together and covered tightly to prevent any possible escape of the artificially introduced maize weevils after they were closed.

Fig. 3 Experimental cages for thermal disinfestation trials.

Disinfestation in solar biomass hybrid dryer

After stocking each cage with 500 g of maize, 20 of the cultured MW of different sexes and age were introduced into each of the 30 mesh-like 'cages' with forceps. The infested maize grains in the aerated cages were later placed on the drying racks/shelves in the dryer for thermal disinfestation at predetermined time intervals of 3 and 6 hours. The temperature profile in the dryer during the experiments under the different heat source applications (hybrid mode and solar only mode) was monitored using Tinytag data loggers (accuracy of ±0.01°C). The loggers were mounted at various levels in the dryer to record temperature conditions in the dryer and in the ambient environment. The loggers logged data at every 10-minute interval during the disinfestation period to account for weather fluctuations during the experiment.

Mortality rate

After the predetermined exposure periods (3 and 6 hours), the insects were separated by sieving the grains. The dead and live insects were counted and recorded. Similarly, the dead and live insects in the control set-up were also assessed. Inspected insects were confirmed dead when there was no response after pricking with the tip of the forceps. Mortality was estimated by counting the number of dead weevils and the mortality rate calculated using Equation 1 provided by Gazzoni (1998):

$$M_r = \frac{dw}{tw} \times 100 \quad \text{..... Eqn. 1}$$

M_r = mortality rate

tw= total number of weevils

dw= number of dead weevils

Emergence of F1 progeny (First Filial generation)

At the end of the thermal disinfestation process, the sieved grains were placed in Kilner jars covered tightly with calico cloths. The containers were kept in the Lab until 30 days after which the number of insects that emerged from each replicate sample were counted after sieving and then recorded. Likewise, the number of insects that emerged from the control were also counted and recorded. This continued daily until there was no emergence of F1 progeny.

Data analysis

Values obtained on weevil mortality were subjected to Analysis of Variance (ANOVA) using Statistix 9. A significance level of 5% was used for all analyses. The Least Significant Difference (LSD) was calculated where a significance was found between treatment means.

Results

Temperature profile in the dryer under hybrid and insular mode compared to ambient temperature

Results of the temperature profile in the dryer relative to the position of the experimental cages during the different heat source applications are shown in Figs. 4 and 5. For the 6 hour disinfestation period, it was observed that temperature conditions in the dryer under both heat source applications varied from the bottom level (L1) to the topmost level (L4) in an increasing trend.

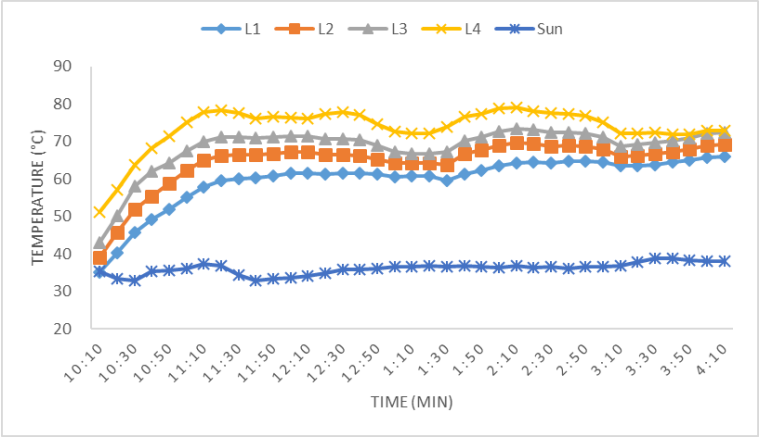

Fig. 4 Temperature variations in the dryer under hybrid mode test

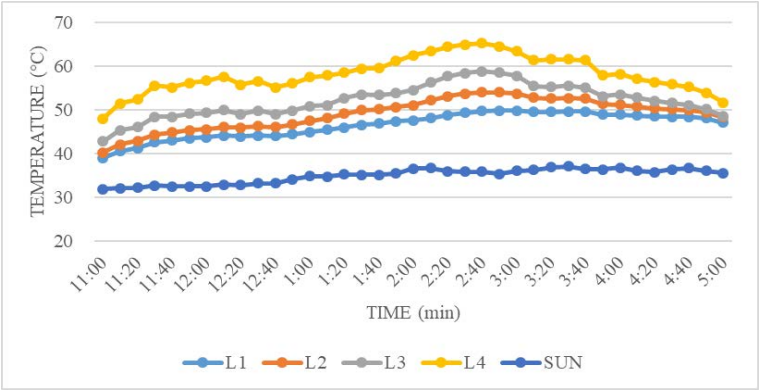

Fig. 5 Temperature variations in the dryer under solar only mode test

As presented in Tab. 1, mean temperature of 67°C was recorded in the dryer during thermal disinfestation using the combined heat source of biomass and solar (hybrid mode) while a mean temperature of 52°C was recorded using solar only as the heat source for disinfestation. Comparatively, the mean temperature inside the dryer was 31°C and 17°C higher than the ambient temperature during disinfestation under the hybrid and solar only modes, respectively.

Tab. 1 Mean temperature for insect disinfestation at 3 and 6 hour exposure periods.

Level	Mean temperatures (°C) at 3 and 6 hours (hybrid mode)		Mean temperatures (°C) at 3 and 6 hours (solar only mode)	
	3 hours	**6 hours**	**3 hours**	**6 hours**
L1	56.1	59.8	44.2	46.5
L2	61.6	64.5	46.5	49.1
L3	66.1	68.4	49.8	52.1
L4	72.4	73.6	56.5	58.1
Overall average in dryer	**64.1**	**66.6**	**49.3**	**51.5**
Sun	35.1	36.1	33.7	34.9

Mortality of adult *Sitophilus zeamais* and F1 progeny

Tab. 2 presents the results of mean mortality of MWduring thermal disinfestation for exposure periods of 3 and 6 hours under the hybrid and solar only mode tests. The mean emergence (F1 progeny) as affected by the temperature and exposure period in the dryer under the two experimental set-ups is also presented (Tab. 2).

Tab. 2 Effect of exposure period and temperature on mortality of *Sitophilus zeamais* and F1 progeny.

Exposure period and heat source	**Mean temperature (°C)**	**Average mortality (%)**	**Mean adult emergence (F1 Progeny)**
3 hours @ Solar + Biomass	64.1	84.9 ab	0
3 hours @ Solar only	49.3	65.4 c	0
6 hours @ Solar + Biomass	66.6	91.6 a	0
6 hours @ Solar only	51.5	78.2 b	0
3 hours @ Control (open sun)	35.1	13.3 d	6
6 hours @ Control (open sun)	34.9	41.2 e	5
LSD (5%)		**8.80**	

Within a column means followed by a different letter show significant difference ($p<0.05$).

Discussion

Effect of heat source on temperature trend

Temperature conditions in the dryer were significantly higher under the hybrid mode compared to when the heat for disinfestation was solely from the solar energy. However, there was a significant difference ($P < 0.05$) between the temperature conditions in the dryer under both heat source applications compared to ambient air temperatures and direct sun exposure. There was therefore a direct correlation between the energy input for thermal disinfestation and the temperature trend under the different modes of operation of the dryer. The increasing trend in temperature observed under the hybrid mode was due to the high energy input from both heating sources (solar and biomass). This was consistent with the work done by Okoroigwe et al. (2015) who reported that a hybrid heat source has an advantage over sole dependence on biomass or solar. Similar findings were also reported by Bolaji (2005), who designed and constructed a box type indirect solar dryer, where the drying chamber recorded a maximum temperature of 57°C at the time when the ambient temperature was 33.9°C. As clearly presented in Tab. 1, mean temperature conditions recorded in the dryer (hybrid or solar only modes) were above 50°C reported by Fields (1992) who suggested this temperature could cause death of insects within minutes of exposure.

Effect of exposure period and source of heat for disinfestation on mortality of adult *Sitophilus* zeamais and F1 progeny

From the results obtained, the exposure period of infested maize grains in the mobile solar biomass hybrid dryer is vital for thermal disinfestation of MW. Depending on the exposure period and the temperature profile in the dryer, insect infested grains could be controlled for long-term storage. There was significant difference ($p < 0.05$) in mortality of MW with respect to the exposure period and the source of heat used for disinfestation (Tab. 2) as compared to the control experiment.

During disinfestation under the hybrid mode, it was observed that (Tab. 2) mortality of adult weevils after 3 hours (10:10am to 1:10pm) and 6 hours (10:10am to 4:10pm) of exposure time showed no significant differences ($P>0.05$). There was, however, significant difference ($P> 0.05$) in adult weevil mortality during disinfestation relying only on solar energy as the heat source. Moreover, there was no significant difference ($P<0.05$) in MW mortality during thermal disinfestation at exposure periods of 3 hours (hybrid mode) and 6 hours (solar only). The highest mean mortality was achieved at the 6-hour exposure period in the hybrid mode although the results showed that thermal disinfestation under both experimental set-ups (hybrid and solar only modes) recorded a better effect on mortality compared to the control. Recorded mean temperatures were above 50 and 60°C in the dryer under both hybrid and solar only modes, respectively, and below 40°C for the control. Kitch et al. (1992) reported that exposing insects to temperatures above 45°C is known to be lethal to insects with most stored product insect pests known to succumb to death under such conditions. This agrees with Seidu et al. (2010) who reported that mortality of maize weevils exposed to varying temperatures and time in a conventional solar dryer required 120 minutes (2 hours) or more for achieve mortality. This suggests that shorter exposure periods of grains infested with the adult MW is required to achieve high mortality during thermal disinfestation in the hybrid mode while a longer exposure period of no less than 6 hours is required under the solar only mode to achieve the same efficacy.

Results on F1 progeny (Tab. 2) showed that disinfestation under both hybrid and solar only modes over 3 and 6-hour exposure periods was able to prevent the emergence of the MW by destroying all the developmental stages of the weevil during heat treatment in the dryer. However, there was some emergence of F1 progeny in the control under both 3 and 6-hour exposure periods. This is an indication that mean temperatures recorded in the dryer under both hybrid and solar only modes were high enough to kill adult weevils and destroy eggs they might have laid. This was evidence in the non-emergence of F1 progeny after the disinfested grains were stored in the lab for 30 days. However, there was re-emergence of F1 progeny in maize grains treated with the control set-up

(open sun disinfestation) where the ambient temperature had little effect on destroying all developmental stages of the MW including any laid eggs. This is corroborated by Agona and Nahdy (1998) who reported that the use of a low-cost solar dryer was effective in ensuring 100% mortality of adult beetles and the non-emergence of adults in solar treated cultures as well as eliminating all developmental stages of *Acanthoscelides obtectus* after exposure for 6 hours above 45°C. Similar trials have also recently been reported by Purdue University, USA researchers in which a similar method was used for killing cowpea weevils (*Callosobruchus maculatus*). They found that ambient temperature had little effect on the temperature developed inside the grain heater, provided that there was sunshine. Their results showed grain temperature of 62°C after 15 minutes of exposure and 100% insect kill after 3 hours. From the assessment of the hybrid dryer for potential use in thermal disinfestation, it was demonstrated that the dryer can be used in preventing damage by *Sitophilus zeamais* to maize grains. All life stages of the MW succumbed to death even at the lowest temperature achieved under the solar only mode within a disinfestation period of 3 hours and above. The successful use of the dryer for thermal disinfestation should be a motivation for farmers who could utilize the developed dryer for both drying their maize grains and controlling insects during storage to minimize postharvest losses and promote food security.

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Green Ecological Grain Storage Technology and Quality Control in China

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Abstract

Green ecological grain storage technologies (GEGSTs) are the means of controlling stored grain quality, and quality changes of stored grain are the basis of GEGSTs control. This paper introduces that GEGSTs are widely used in China, including monitoring and early warning of stored grain pest and mould, pest control by using food-grade materials, controlled atmosphere for pest control, ventilation for lowering and equalizing temperature, low and quasi-low temperature grain storage, treatment of hot spots, etc. And it introduces that grain processing enterprises' and market's request for grain quality, is called "quality control". It also clarifies that stored grain quality control is the purpose, and emphasizes that GEGSTs control is the process, so GEGSTs control should serve for quality control. Therefore, we propose that the technology application and the quality control of grain storage are equally important, and without the quality control, the technology application could be invalid, especially for sensitive areas in grain bulks. In the process of grain storage, special attention should be paid to quality changes in the sensitive areas, like real-time monitoring. Identify and utilize scientific and reasonable technology accordingly, including related technologies and equipment, to improve "overall" quality control level of stored grain bulks, and to gradually standardize them. By means of GEGSTs, positive ecological storage conditions are effectively utilized, which helps us achieve the purposes of safety, no pollution, high quality and nutrition during grain storage.

Key words: Storage Technologies, Grain consumption, Quality Control

Green ecological grain storage technologies (GEGSTs), based on the theory of grain bulk ecology, through the means of green ecological low-carbon, help us achieve the purpose of safety and quality control during grain storage. Grain storage technology control is a process, and grain storage quality control is the purpose, so grain storage technology control should serve for stored grain quality control. After harvest of grain, quality control in grain circulation involves three aspects, which are grain quality during consumption, warehousing and storage. Among them, stored grain quality is related to the warehousing quality and grain consumption quality, taking into account the two links of grain production and grain consumption. It is the key to do a good job in the convergence and coordination of these three aspects, to improve the technical level of grain storage management.

1. Green ecological grain storage technologies

GEGSTs are widely used in China, including pest control by using food-grade materials, ventilation for lowering and equalizing temperature, controlled atmosphere for pest control, low and quasi-low temperature grain storage, monitoring and early warning of stored grain pests and moulds, treatment of hot spots etc.

With the development of insect pheromones and different wavelength spectra to attract stored-grain pests, the density and insect situation of stored-grain pests in a granary could be monitored by using new trapping technology. Combined with the detection of grain condition, the population dynamics of stored-grain pests under different ecological conditions could be predicted, and thus the decision-making control technology was put forward. Integrated with grain storage information technology and other high-tech, a new core technology of comprehensive control of stored grain pests is formed.

Pest control technology by using food-grade materials is an upgrade of traditional inert-powder pest control technology with plant ash, diatomite and others. Insecticidal mechanisms of food-grade materials fall into the internode membrane of the insect body, which would lead to wear the

internode membrane during insects moving and adsorbing lubricating fluid and body fluids, thus resulting in pest death (Zidan Wu et al., 2011).

The gas composition in a sealed grain pile could be artificially changed, such as putting CO_2 and N_2 into the grain pile or reducing the oxygen concentration, so as to kill pests, inhibit the respiration and growth of the pests, prevent the occurrence of mold and delay the deterioration of stored grain quality. The main application technologies are related to natural hypoxia or artificial gas, which is essentially mechanical nitrogen-rich hypoxia process, to reduce oxygen in a granary.

Low temperature storage refers to the average grain temperature maintained at or below 15°C all year-round, and the partial maximum grain temperature is not higher than 20°C. Quasi-low temperature storage refers to the storage mode in which the average grain temperature is kept at and below 20°C all year-round, and the partial maximum grain temperature is not higher than 25°C. Grain warehousing temperature and moisture are the basis of low temperature and quasi-low temperature grain storage, which is generally divided into two cases: grain warehousing in high temperature seasons and grain warehousing in low temperature seasons. High temperature seasons are from May to October, and grain temperature is comparatively high when warehousing. In order to achieve low temperature and quasi-low temperature grain storage, cold ventilation technology must be employed, especially when the grain temperature is higher than 25°C. Once the granary is filled up, it is necessary to level off the grain surface, use horizontal ventilation technology to make outside air penetrate through the whole grain pile, or use uncovering-cloth ventilation technology for partial processing. These processes will help to not only eliminate the accumulated heat during grain warehousing, but also to eliminate the harmful gases released by the grain pile. Low temperature seasons are from November to March or April of the following year. During these times, grain temperature is low when warehousing ithe granary, so we just need to level grain surfaces.

2. Grain quality demand for grain consumption purposes

The quality demand of grain consumption varies according to the usage. The grain is eventually processed for humans, animals and industries in the market (Xiaohe Ma et al., 2008). Meeting the demand of the grain consumption market ensures grain storage rotation, higher prices for good quality, and good storage income and social benefits.

Human consumption (food grain) is the primary area of grain use, including mainly wheat and rice, and also maize and coarse cereals in small amounts. Food grain accounts for 50% of the annual grain consumption (Xiaohe Ma et al. 2008).

Requirements of the food grain are not only to eat enough, but also to eat well, to eat green, ecological and fresh food. In addition to providing products with good taste, color and smell, food grain processing must meet the standards of food hygiene, including prevention of heavy metals, mycotoxins, pesticide residues and other harmful substances in the product.

Grain provided for animals (fodder grain) is the second largest consumption area, supporting the development of the livestock and poultry industry and production of meat, eggs and milk. The fodder grain accounts for 33% of the annual grain consumption with consistent growth (Wei Jia et al., 2013).

In the process of development, the market demand for fodder grain quality is increasing and refining. Hygienical standards for feeds stipulate that the total number of mold in maize as well as wheat bran and rice bran is less than 40×10^3/g, and rules for maize with mold of $40\sim100\times10^3$/g is of limited use, and mold of more than 100×10^3/g is banned. Fodder corn standard stipulate that corn is divided into three levels, and the fatty acid value of the top-level corn is no more than 60 mg/100 g.

Grain provided for industries (industry grain), the third largest consumption area, accounts for 10% of the annual grain consumption, and the figure is predicted to be 13.9% in 2030. Industry grain is

used in a variety of products such as starch, modified starch, starch sugar, amino acid, organic acid, enzyme preparation, yeast and fuel ethanol, etc.

In recent years, some enterprises purchase and use poor quality grain in order to reduce the production cost of main raw materials, expecting to increase efficiency. But contrary to the expectation, low-quality grain leads to lower yield of main products, poor quality of by-products and poor market competitiveness.

3. Quality control requirements during takeover

Basic Requirements: "dry, full, clean"

Grain warehousing quality control is the source of grain circulation quality control. "Grain and Oil Storage Technical Specification" requires that quality of long-term stored grain should comply with the provisions of the Chinese national quality standards. Moisture content should not exceed the local safety moisture. Impurity content mixed with grain should not be greater than 1.0%, and when impurity content is higher, it should be cleaned out. At the same time, the stored grain quality indicators should comply with the "Stored Grain Quality Judgment Rules" and the relevant standards.

Quality Control Indicators

There are three main quality control indicators in the "Stored Grain Quality Judgment Rules", i.e., color and smell, fatty acid value, and taste score. Taste score, color and smell belong to sensory evaluation indexes determined in accordance with the conditions of the evaluation test by personnel with sensitive senses and identification ability usually in the laboratory environment. Fatty acid values are physical and chemical properties used for quantitative analyses.

Quality Control Key Points

The quality of newly stored grain is directly related to the appropriate storage degree, grain storage cycle and grain storage safety. On the basis of clearing impurities during warehousing to ensure "dry, full, clean", the fatty acid value of grain also needs to be strictly controlled.

A key link affecting fatty acid value changes is the process of grain drying. Because of the "labor problem", the number of post-harvest grain needing to be mechanically dried increases, and the number of natural air drying of grain systems decreases. The fatty acid value of mechanically dried grain is higher than that of naturally dried grain. The fatty acid value of newly harvested corn with natural drying is generally about 15 mg/100 g, and rarely exceeds 20 mg/100 g. The fatty acid value of mechanically dried corn reaches 29.7 mg/100 g ~ 45.3 mg/100 g, and among the samples with fatty acid values higher than 40 mg/100 g, there are more baked paste particles, more broken particles in the imperfect particles, and greater changes in the quality index in the mechanically dried corn than that with natural dryied (Chunlong Xia, 2008).

Technical Method of Quality Control

Before warehousing, we should pay more attention to controlling the fatty acid value. The lower the initial fatty acid value of grain is, the better. It is necessary for long-term storage of grain to take quasi-low temperature and low temperature control and other effective storage technology measures in order to control the rate of fatty acid increase and delay quality change; however, these measures will increase the cost of grain storage (Yurong Zhang et al., 2004).

In order to control the increase of fatty acid value, the drying process should be improved. In particular, the temperature of the drying air and the maximum temperature to which the corn is heated should be controlled to ensure the quality of the dried grain.

In addition, we should focus on developing grain drying technologies and devices that could be used and adopted easily by farmers. Farmers should be guided to carry out grain harvesting operations scientifically and reasonably, and do a good job at grain quality control.

4. Quality Control Requirements during Grain Storage

By taking advantage of good correlation between fatty acid and taste score, fatty acid value could be used as a sensitive indicator of daily monitoring of grain quality changes in order to monitor in a timely manner stored grain quality. We should pay special attention to parts of bulk grain, sensitive parts, and monitor in a timely manner, and to examine with reasonable scientific and technical measures, including related technologies and equipment, to improve "overall" quality control of stored grain.

5. Importance of Grain Quality Control

Guaranteeing grain quantity and quality are complementary. Guaranteeing grain quantity is relatively intuitive and tangible. However, maintaining quality, involving the biological and non-biological ecological environment of a grain bulk, is challenging. In order to control the physiology and biochemistry, molds, pests and other ecological factors of grain storage, it is necessary to strictly control grain quality during warehousing. At the same time, based on market oriented rules, we should strengthen the implememntation of proper grain storage technologies to achieve the requirementss of grain quality control, to meet the needs of grain consumption, and to ensure the high value of stored grain.

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A new approach to acoustic insect detection in grain storage

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Abstract

Insect pests in grain storages can cause severe financial losses. Infested grain needs to be treated and can be sold only with lower profit. Intense infestation can lead to contamination with mycotoxins and total loss of stock. Therefore, an early detection of insect storage pests is of great importance to farmers and storage keepers but is difficult to obtain in large amounts of grain.

Besides conventional detection methods such as insect traps and monitoring of temperature and relative humidity, acoustic monitoring can identify insect infestation. Insects in grain and other stored products produce sounds at a low level during movement and feeding activity. A new acoustic system was developed as part of the project "InsectTap" to increase the detectability of insect sounds. Highly sensitive microphones were installed inside a metal tube that increased the surface on which beetle signals could be detected. Additionally, the tube worked as a beetle trap recording all sounds from even one single beetle inside the trap.

The tube system was tested in 1 and 8 m^3 boxes filled with wheat. Infestation could be detected at a very early stage about 8 weeks before a temperature rise, or beetles at the grain surface indicated an infestation.

In the next step, this "Beetle Sound Tube"-System will be installed in different grain silos aiming for automatic early detection and specific identification of infestation. The information provided to the farmer or storage

keeper allows early and specific treatment to reduce losses. Additionally, the introduction of parasitoids via the tube system will be tested to increase the efficacy of biological control.

Keywords: Acoustic Detection, Monitoring, InsectTap, Beetle Sound Tube, *Sitophilus granarius*.

Introduction

Early detection of insect storage pests is important to reduce losses and preserve high quality food. Recognition of infestation in large amounts of storage goods is difficult, and in many cases it is only noticeable when the amount of insects increases considerably and causes a rise of temperature and relative humidity. At this stage, mites and mould can lead to major secondary losses.

Treatment of insect infestation has become more difficult due to a decrease of available chemical substances for storage protection, an increase of organic farming that cannot use chemical agents and the increasing disapproval of consumers to chemical treatments. Therefore, early detection is crucial to have a choice between different non-chemical treatments that are not suitable for mass infestation.

Besides measuring temperature and relative humidity, using traps or sieving samples, the detection of feeding and movement sounds is another way to discover insects in stored goods. A great advantage of acoustics is that even the sounds of hidden stages of insects can be detected (Leblanc et al., 2009). But very low amplitudes of signals and sound insulation properties of grain make it difficult to detect the sounds at distances of more than a few centimetres (Hagstrum and Subramanyam, 2006).

Another difficulty that devices for acoustic detection of insects face are settlement sounds of grain that can be mistaken for insect sounds. Therefore, a permanently installed acoustic system could have advantages (Hagstrum and Subramanyam, 2006) compared to mobile probes or acoustic test containers.

Aim of the project "InsectTap" funded by the Federal Ministry of Food and Agriculture (BMEL) was the development of an acoustic early detection system that allows detection and specific identification of insect infestation. Experiments under controlled conditions showed as a first result discriminability of a number of adult beetle species by sound (Kirchner et al., 2016).

Another part of the project that will be described in this paper were pilot plant scale experiments in 1 and 8 m^3 of stored wheat using high-sensitive microphones placed inside metal tubes to increase the detectability of insect sounds due to surface enlargement.

At the next step, this "Beetle Sound Tube"-System will be customized to the needs of farmers and keepers of small storage facilities and installed in different sized grain silos aiming at automatic early detection and specific identification of infestation. The information provided to the farmer or storage keeper allows early and specific treatment to reduce losses including the application of parasitoids via the tube system to allow easier access to the infestation.

Materials and Methods

Experimental set up

Two experiments were carried out using large wooden boxes of 1 or 8 m^3 filled with wheat. The boxes placed inside an about 77 m^2 storehouse were equipped with 17 to 20 data loggers (EasyLog EL-USB 2) to record temperature and relative humidity and 3-4 microphones for acoustic measurements.

During the first experiment using the 1 m^3 box (Fig. 1), three free field condenser microphones (PCB-378B02, PCB Piezotronics, Depew, USA) were used under different conditions. The microphones were either covered with a layer of PET rescue foil as dust protection and placed directly into the wheat or were suspended inside 0.75 m long galvanised steel tubes of 0.08 m diameter inserted into the wheat to focus sound signals from the surrounding substrate. While one of the tubes was a simple metal tube with a stainless steel lid at the bottom, the second tube was equivalent but with

a large number of 2.5 mm drilled holes and functioned as a beetle trap comparable to a WB probe trap (Barak et al., 1990) with a removable cup containing some grain at the lower end of the tube for accumulation of beetles. In the 8 m^3 experiment, four metal tubes of 1 m length and 0.1 m diameter were used from which one functioned as a trap.

The experiment in the 1 m^3-box was carried out between May and October 2016, while the 8 m^3 box was used from March to August 2017. At the beginning of both experiments, 200 wheat kernels containing larvae of the grain weevil (*Sitophilus granarius)* 25-28 days or 30-32 days after oviposition, respectively, were introduced at one position in the box. A data logger for temperature and relative humidity was placed directly above the position of insect infestation.

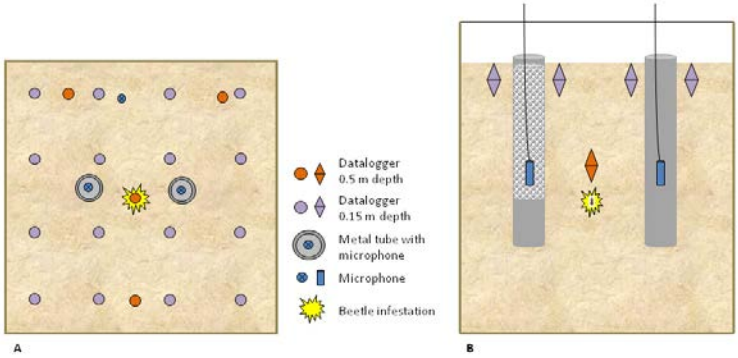

Fig. 1 Experimental set up of the 1 m^3 box. **A:** Top view of the box showing the positions of the three microphones, 20 data loggers and the position where the beetle infestation started. **B**: Lateral view with the two microphones in the two tubes (left tube functioned as a trap), data loggers and position of beetle infestation.

For the following months, temperature and relative humidity were logged every six hours. Acoustic data were recorded at the first 20 minutes of each hour using an IMC CS-3008-N High-resolution measurement device (imc Meßsysteme GmbH, Frankfurt, Germany) connected to a laptop using IMC Studio Pro 4.0 software. Additionally, the number of beetles in the trap was determined and the insects removed on a regular basis.

Acoustic evaluation

After it was checked that there was no daily rhythm in granary weevil activity, three times per day were chosen for acoustic evaluation (3 and 9 a.m., 9 p.m.). The times were chosen to include one recording during daytime, one during twilight and one during night-time. During daytime disturbance due to workers and traffic in the surroundings were common. In twilight there was less traffic, no working activity but natural sounds such as birds, while during night-time external noise was low unless the weather situation was rough.

The recordings were bandpass filtered (1000-12000 Hz using IMC FAMOS Professional 7.0) to reduce background noise. Four 15-second segments of the recording starting at minute 1, 6, 11 and 16 were acoustically evaluated by a trained person, counting the number of insect signals. In case of strong external disturbances the section for evaluation was moved to the next 15 undisturbed seconds of the recording. Therefore, 12 periods of 15 seconds were evaluated each day and the number of signals added up to a daily activity figure with standard deviation. Tab. 1 gives an overview about the duration of both experiments and the evaluated times.

In case of very frequent insect signals (more than 2.3 signals/second) an accurate count of signals was not possible and in those cases the result was given as >35 for the 15 second section. Results that are based on at least one 15-second section with more than 35 signals are indicated in the result section.

The increase of beetle activity inside the box as indication for increasing number of insects over time was evaluated at intervals of 1-11 but mainly 3-4 days.

Tab. 1 Overview of experimental duration and acoustically evaluated days.

	Duration of the experiment	**Evaluated days**
1 m³ box	148 days ≈ 21 weeks	24 days (72 hours)
8 m³ box	166 days ≈ 24 weeks	26 days (78 hours)

Modelling of beetle population

To estimate the size of the beetle population during the course of the experiment, the computer model "SITOPHEX" was used (Prozell et al., 2004). This model calculates the number of beetles and development stages over time considering temperature, relative humidity, reproduction rate and mortality rates of different stages.

Results

Temperature

In the first weeks of the experiments the temperatures inside the boxes rose slowly depending – with some delay - on the temperatures outside the box. The temperature outside the box and inside the storehouse was largely dependent on the ambient temperature.

After 12 weeks, the temperature inside the 1 m³ box just above the beetle-infestation rose quickly. During a period of 12 days, the temperature increased by 11°C, while the temperatures outside the box and the meteorological data remained at a lower level. The temperature increase inside the box was therefore not caused by external temperatures but biological activity (Fig. 2).

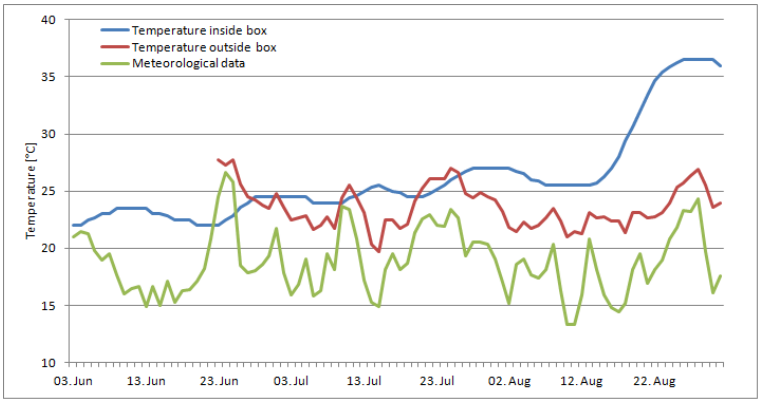

Fig. 2 Daily average temperature inside the 1 m³ experimental box above initial beetle infestation point compared with temperatures outside the box and data from a nearby meteorological station at about 1 km distance from the building.

The increase of temperature was most pronounced in the area where the beetle larvae were placed at the beginning of the experiment (Fig. 3) indicating a proliferation of weevils and larvae in the area.

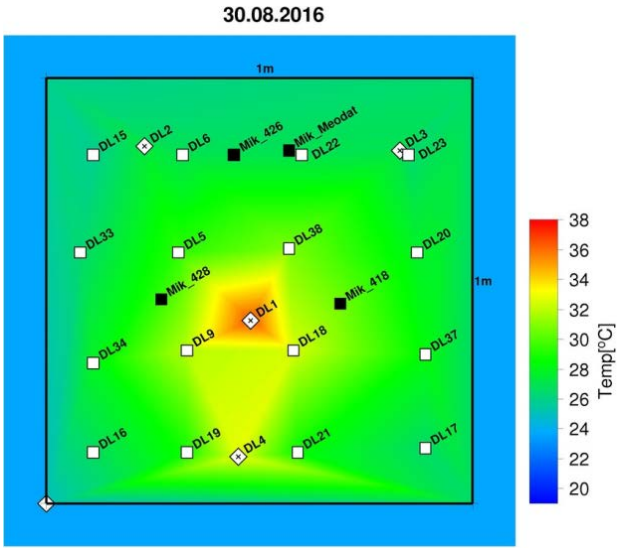

Fig. 3 Temperature in and outside the 1 m^3 experimental box measured with 21 data loggers on the 30th of August. DL1 is the data logger just above the centre of beetle infestation.

An increase of relative humidity could be observed simultaneously with the rise of temperature. While relative humidity rose by 7 percentage points in the first 10 weeks of the experiment, it increased steeply another nearly 3 percentage points in 5 days. Afterwards the relative humidity decreased again but stayed at a higher level.

The results of the 8 m^3 box were comparable, but the increase of temperature started after 122 days and therefore more than 5 weeks later compared to the 1 m^3 box. The reason for this delay is the much lower temperature in the second experiment. While the first experiment in the 1 m^3 box started in May with wheat temperatures of more than 20°C in the box, the second experiment in the 8 m^3 box started in March during very cold weather. Start temperature of the wheat was about 16°C and it took until the middle of May before the wheat reached a comparable temperature as at the start of the first experiment. This led to delayed development of beetles and therefore a later increase of temperature as an indicator for beetle infestation (Fig. 4).

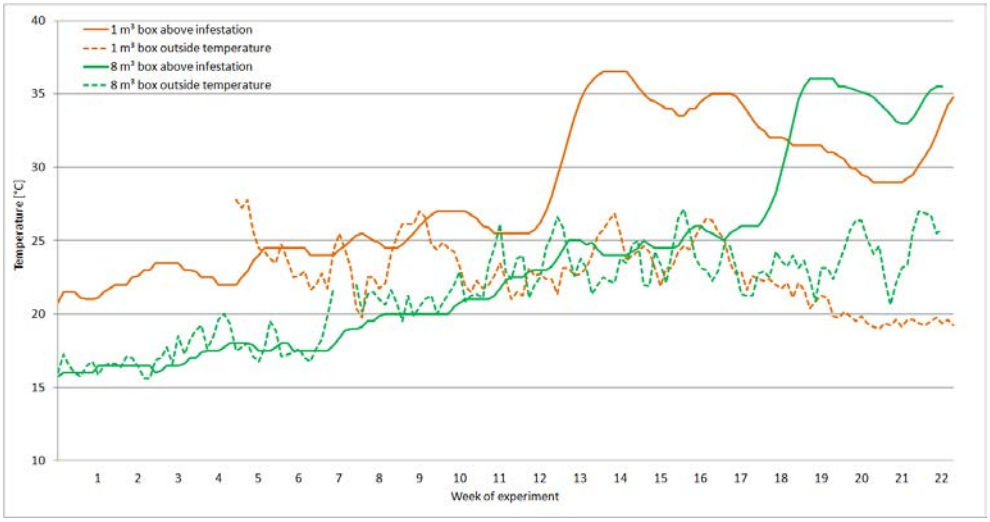

Fig. 4 Comparison of temperature in both experiments (1 and 8 m^3) over time. A temperature increase indicating insect infestation in the 1 m^3 box became obvious at week 12 while it took until week 18 in the 8 m^3 box due to lower wheat temperatures.

Modelling of beetle population

The population size of adult *S. granarius* calculated using "SITOPHEX" showed large differences between both experiments (Fig. 5). While the population rose from 200 to nearly 400000 adults in the 1 m^3 box in 21 weeks, it only reached about 150000 in 24 weeks in the 8 m^3 box due to the lower temperatures and therefore slower insect development.

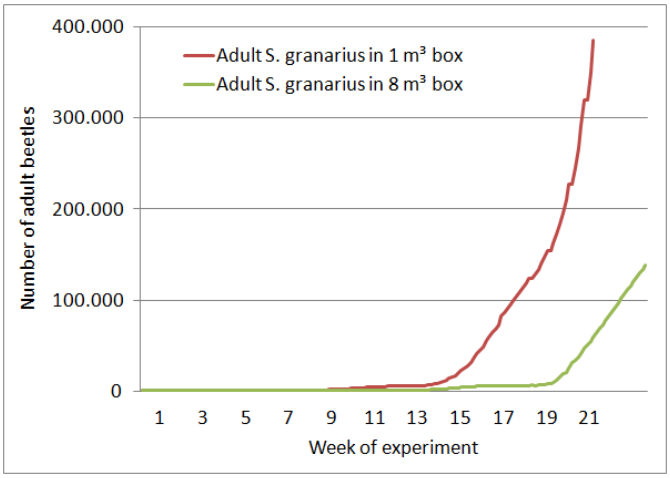

Fig. 5 Calculated numbers of adult *Sitophilus granarius* during the course of the experiments in 1 and 8 m^3 wheat based on the software "SITOPHEX".

Experiment in the 1 m^3 box

The evaluation of the recordings started before the first beetles were expected to hatch and ended at the end of August after the temperature increase indicated infestation. From the end of July (experimental day 69) onwards the number of signals picked up by the microphones in both metal

tubes exceeded the maximum countable number of 140 signals/minute, continuously. At the microphone placed directly into the grain, this point was reached after the 2nd of August.

Eleven days after the first beetles were expected to hatch, the first weak acoustic beetle signals could be detected with the microphone inside the tube trap at 0.22 m distance from the infestation start point while after 23 days the signals were strong and easy to detect. **Fehler! Verweisquelle konnte nicht gefunden werden.** shows the number of beetle signals during the course of the experiment from the day, when the first signals were detected, to the day, when the number of signals exceeded the maximum countable number of signals on all three microphones on the 6th of August.

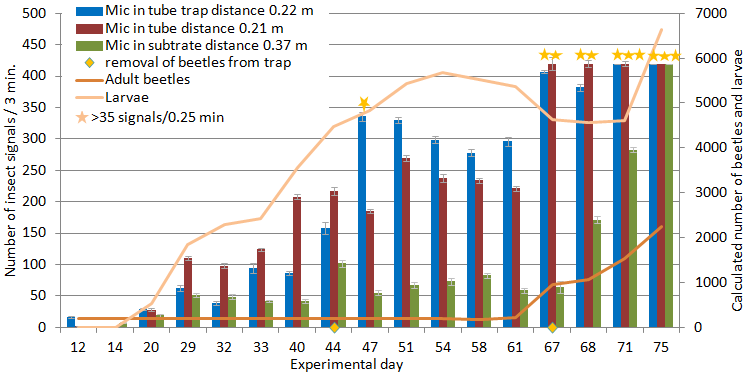

Fig. 6 Beetle signals recorded with three microphones inside the 1 m³ box. The columns show the sum of signals of twelve 15-second periods during an experimental day with standard deviation. Days on which the maximum countable number of 35 signals per 15 seconds was exceeded in at least one period are marked with an asterisk. Additionally, the calculated number of larvae and adult beetles inside the experimental box and the days on which beetles were removed from the trap are given.

All microphones showed an increase of signals with time corresponding to the increasing number of beetles and larvae inside the box. Already three weeks after the beginning of the experiment and about two weeks after the first adult beetles hatched signals could be counted regularly at all microphones.

A decrease of numbers of larvae was observed between experimental day 54 and 71 which resulted from the fact that all introduced larvae had the same age. Therefore, they pupated all at about the same time which led to a decrease of larvae before the number of adults increases. Afterwards, the number of larvae increased steeply after the young larvae of the next generation started to hatch.

Experiment in the 8 m³ box

First beetle signals could be detected inside the tube trap at the beginning of May more than 8 weeks after the first beetles hatched. Fig. 7 shows the number of beetle signals during the course of the experiment from the day when the first signals were detected to mid-August, when the experiment ended.

For more than 7 weeks, the microphone inside the tube trap was the only one recording signals. The high number of signals inside the tube trap was caused by few beetles inside the trap. After removal of three beetles from the trap on day 69, the number of detected signals decreased from 267 before to seven signals after removal. The next beetles were trapped in the tube causing the next peak on day 79. After the next generation of beetles hatched and the number of beetles increased inside the box, the trap-effect became negligible and the removal of beetles from the trap did not cause a clear decrease of signal numbers due to insects moving and feeding in the surrounding of the tube.

The microphone inside the tube at 0.62 m distance from the infestation recorded the first signals nearly 16 weeks after the first beetles hatched. The number of signals increased with the number of larvae and beetles in the box.

After nearly 21 weeks the first beetle signals were detected at a distance of 1.04 m, while it took only another 2 weeks until signals were recorded at 1.48 m distance from the infestation.

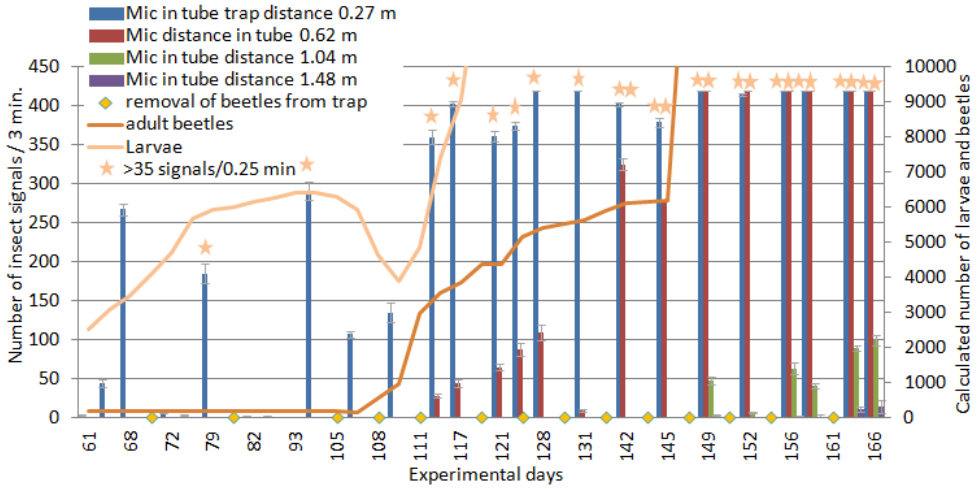

Fig. 7 Beetle signals recorded with four microphones inside the 8 m³ box. The columns show the sum of signals of twelve 15-second periods during an experimental day with standard deviation. Days on which the maximum countable number of 35 signals per 15 seconds was exceeded in at least one period are marked with an asterisk. Additionally, the calculated number of larvae and adult beetles inside the experimental box and the days on which the beetle were removed from the trap are given.

Comparison of temperature, insect detection and acoustic signals in both experiments

The results of both boxes are comparable apart from the fact that the development of beetle infestation was slower in the 8 m³ box due to lower temperatures. Fig. 8 shows the temperature above beetle infestation in both experiments as already given in Fig. 4 but time-displaced for better comparison. The day, when first signals were detected on the different microphones is displayed in the figure, showing that in both experiments an infestation of beetles could be discovered at least 8 weeks before an increase of temperature directly above the infestation was measurable and at least 6 weeks before beetles appeared on the surface of the substrate.

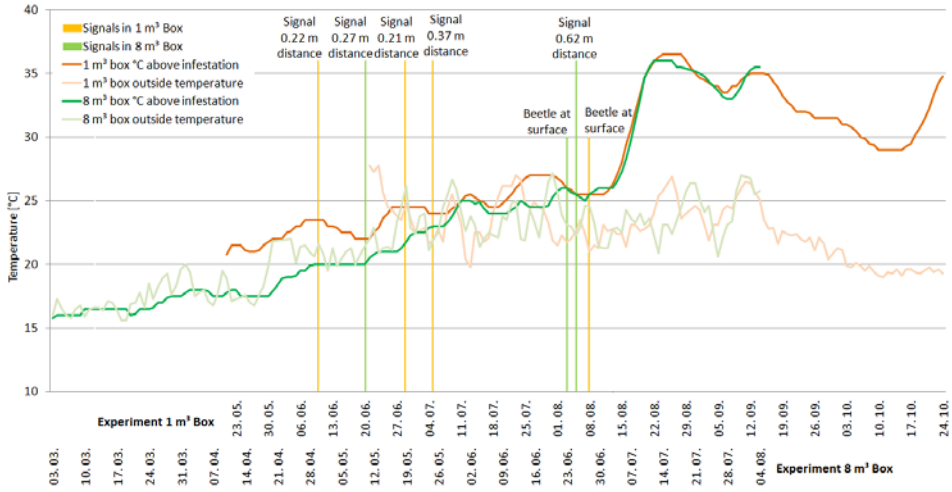

Fig. 8 Comparison of temperature in both experiments (1 and 8 m^3) displayed six weeks time-displaced. Both experiments show a very similar temperature curve related to the beetle infestation. Additionally, given are the times of the first signals recorded by microphones at different distances and of the first observation of beetles at the substrate surface in both experiments.

Discussion

Aim of the project was the development of a permanent acoustic early detection system for farmers and smaller storage keepers. The results showed that acoustic monitoring can provide much earlier detection of beetle infestation compared to conventional methods such as temperature measurements or surface traps. But it must be considered that in professionally managed storages the detection with conventional methods such as traps might be possible at an earlier stage as shown in the experiments and that in those cases the gap between acoustic and conventional detection might be smaller. But farmers and keepers of smaller storages often do not have the time for close inspections or the wheat is stored in silos that are not easy to access. In these cases, one could therefore benefit from early acoustic detection. In both tests, acoustic detection was possible many weeks before temperature rose. Of course, the distance between the initial point of infestation and the first acoustic device would determine how much earlier acoustic detection is possible in comparison to temperature probing or traps.

On the other hand, in the experiments the position of the infestation was known and the temperature measurements were taken exactly at the right position to detect an increase of temperature as quickly as possible. Under real-life conditions the temperature increase would likely be detected at a later stage due to a less perfect position of the sensor. Thus, temperature monitoring could be even slower than recorded here.

The beetles were detected at an early stage at distances of 0.22 to 0.27 m from the infestation and even at distances of 0.62 m from the infestation acoustic detection was earlier than by temperature.

During the 1 m^3 box experiment, the microphone inside the tube detected more signals than the one placed directly inside the wheat. This might be due to the larger surface of the tube that bundles the signals from a larger area. However, it could also be because the microphone in the tube was closer to the release point of beetles (microphone in tube 0.21 m, microphone in substrate 0.37 m). Additionally, it is not known how evenly the beetles spread from the position of the initial infestation and therefore how many beetles were close to which microphone when signals were detected. But since a microphone directly inside a stored grain mass would be very susceptible to

dust and tractive forces during grain loading and unloading, the tube would be useful to provide protection for the highly sensitive equipment and might also have acoustical advantages.

The tube trap greatly increased the detection as long as the number of beetles was small and even one beetle in the trap caused strong signals. At a later stage of infestation, the trap function was negligible, with still high numbers of signals after removal of beetles from the trap.

The calculated number of beetles for the experiments was important to get an impression about the population size and the differences between the two experiments. Since the program was not developed for experiments like the one described above, there is an important flaw. While it is possible to enter the number of beetles at the start of the experiment as a basis for the population, it is not possible to subtract the number of beetles removed from the trap. Especially in the first weeks of the experiment with only 200 adult beetles in the box, even small numbers of removed beetles will alter the size of the developing population. Therefore, the population size given in the results is likely to be overestimated.

The results indicated that the described acoustic system might be a suitable method for early detection of insects in storages. In a next step, the developed "Beetle Sound Tubes" will be installed in silos and tested with automatic signal detection software to provide farmers and storekeepers with detailed information about infestation and possible treatment.

Acknowledgement

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Controlling insects in stored grain by disturbing the grain

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Abstract

Insects can cause damage to stored grain, especially on smallholder farms in the tropics. *Sitophilus zeamais* (maize weevil, MW) and *Rhyzopertha dominica* (lesser grain borer, LGB) are often involved. Our objective was to determine, by four experiments, if physical disturbance of grain can control these pests. In Experiment 1, 2.6-L unsealed recycled coffee cans were each loaded with 1 kg of maize and 25 live adult MW/kg. Every 12 h, disturbed treatment cans were manually rolled through one circumference. After 160 d, live MW numbers had been reduced by 93% compared to undisturbed cans. In Experiment 2, MW-infested maize was placed in 20-L plastic cans and stored by farmers in Tanzania. Each farmer had three cans. Two were disturbed by shaking morning and evening and the third was left undisturbed. After 90 d, MW populations had increased in the undisturbed containers, but had decreased to zero in every disturbed container. In Experiments 3 (and 4), maize (wheat) infested with 25 adult MW/kg (LGB/kg) was placed in six boxes. Three of the boxes were disturbed every 12 h by use of Sukup motor-driven grain stirrers; the other three were undisturbed. After 120 days, MW numbers in undisturbed boxes had increased, but were zero in stirred boxes. In Experiment 4, 80-d samples showed increased numbers of LGB in undisturbed boxes but reductions of over 98% in stirred boxes. Quality of disturbed grain was similar or better than that of undisturbed grain. This work suggests that grain disturbance may be an effective non-chemical, non-hermetic physical approach for control of stored grain insects.

Keywords: maize, wheat, maize weevil, lesser grain borer, grain disturbance, postharvest loss

1. Introduction

About 70 million Mg of maize and 25 million Mg of wheat are grown in Africa each year (FAOSTAT, 2014; USDA, 2018). Postharvest dryweight losses for maize and wheat in Africa for 2016 are estimated at 18.8 and 13.6%, respectively (APHLIS, 2018). Without proper management, losses for an individual producer can reach 100%. A large contributor to the postharvest loss in maize is *Sitophilus zeamais*, the maize weevil (MW). Female maize weevils deposit eggs in holes bored into the grain and seal each hole with a protective gelatinous plug (Danho et al., 2015). Upon hatching, larvae feed on the endosperm of the kernel, and leave as adults through an exit hole. Maize weevils will over time totally destroy stored maize. One of the main contributors to postharvest loss in wheat is *Rhyzopertha dominica*, the lesser grain borer (LGB) (Government of Canada, 2013). Female grain borers deposit up to 500 eggs loosely onto kernels of grain and the egg stage lasts about 32 days. Larvae then eat into the wheat kernels where they complete their development. Adults emerge by chewing through the outer grain layers and can live up to 240 days (Akol et al., 2011). LGBs feed on the grain and leave behind empty husks and flour. Hermetic storage and use of insecticides are effective approaches to prevent or control insects in grain stored on smallholder farms, but each has their issues. Maintaining hermetic conditions in a container is difficult. Purchase of insecticides is a troubling recurring cost, toxic effects to people are possible due to misuse or residue, insect resistance can develop, effective insecticides may not be available, and fumigants may have environmental effects. Another approach that can be effective for smallholder farmers and others is physical disturbance that is an action such as tumbling or stirring that causes kernels to change position. This disturbance does not involve use of chemicals and it can be accomplished many different ways. Quentin et al. (1991), working with common beans infested with the common bean weevil, *Acanthoscelides obtectus* (Say), investigated the effect of disturbance by bean tumbling to control these storage insects. They hypothesized that when beans are physically disturbed numerous times, weevil larvae die due to exhaustion before gaining access to the cotyledon. The experiment consisted of tumbling storage containers loaded with beans and bean weevils every eight hours. A 95% or greater overall mean reduction in bean weevil population was achieved due to storage container physical disturbance. This paper describes four experiments carried out with the objective of determining the effectiveness of disturbance for control of maize weevils in stored maize and for control of lesser grain borers in wheat. Grain quality parameters (moisture content, fine material and test weight) were measured as part of each of the experiments, but only insect mortality is discussed in this paper.

2. Materials and Methods

Experiment 1 Materials and Methods

Recycled 2.6-L plastic ground coffee containers were used to hold the maize and weevils (Fig. 1). The containers had two internal baffles at approximately 120^0 apart as part of the container design. A third 1.5-x-1.5 x 10-cm wood baffle was affixed in by means of screws to ensure thoroughly mixing. One 10-cm diameter round hole was cut through each lid and screen was glued over the holes with silicon glue to allow air circulation while preventing escape of weevils. The lids with screens were held on the containers by two rubber bands per container. Commercial comingled bulk maize used in the experiment was purchased from West Central Coop Elevator (1095 T Ave, Boone, IA 50036).

Fig. 1. Experimental containers for Experiment 1 (Bbosa et al., 2014).

Each plastic container was loaded with 1.00 kg of 13.6% (w.b.) moisture maize that left approximately a quarter of the container volume unoccupied for thorough mixing while being turned. Maize weevils for this experiment *(S. zeamais)* were obtained from a supply maintained in maize of 10-14% (w.b.) moisture content at 27°C by the Department of Agricultural and Biosystems Engineering at Iowa State University. The experiment consisted of two treatments: undisturbed (control) containers and disturbed containers with three replications of each container, and four different storage times (40, 80, 120 and 160 days), totaling 2x3x4=24 containers. Twenty five live unsexed adult weevils were loaded into each of the containers, which were then randomly laid longitudinally in a chamber maintained at 27^0C. Humidity was not controlled in the chamber. Every 12 h, the disturbed treatment containers were manually rolled through one circumference (15.6 cm diameter or 49 cm). At 40, 80, 120 and 160 d, three undisturbed (control) containers and three disturbed containers were picked randomly from the experimental chamber for data collection. Weevil mortality and grain quality parameters were determined. A two-way ANOVA was performed and Tukey's means comparison was used to detect statistical significance in treatments at α=0.05 using JMP Pro 10.

Experiment 2 Methods and Materials

Experiment 2 was conducted over a three months period in three maize-producing regions (Manyara, Dodoma and Morogoro) of Tanzania. For each region, one major maize-producing district was selected. Then one ward was selected and from each ward, and three small-holder maize farmers were randomly chosen. Each farmer was given twelve plastic containers—nine for treatments and three for control. The study consisted of two treatments: disturbed and control. A total of 108 clean 20-L plastic containers (36 per region) were used. Each container was loaded with 10 kg of fresh white maize and 0.50 kg of white maize infested with mixed-aged adult *S. zeamais*. The initial numbers of *S. zeamais* were determined (Tab. 1). The disturbed containers were disturbed twice a day (12 hours apart), whereas the control containers were not disturbed until the end of the

study. At the end of each storage time (30, 60 and 90 days), three treatment containers and one control from each farmer were randomly opened and the number of live and dead *S. zeamais* were determined. Data collected were analyzed using the Statistical Analysis System (SAS) software using $\alpha = 0.05$.

Tab. 1 Initial numbers of *S. zeamais* in each region per 0.5 kg of infested maize for Experiment 2 (Suleiman et al., 2016).

Storage Time (days)	Dodoma		Morogoro		Manyara	
	Control	Disturbed	Control	Disturbed	Control	Disturbed
30	89	53	28	21	75	30
60	52	54	25	27	73	41
90	74	51	23	20	120	86

Experiments 3 & 4 Materials and Methods

These two experiments used the same equipment and procedure. Grain infested with 25 insects/kg was loaded in six 104 cm x 13 cm x 76 cm boxes in a 27^{0}C laboratory. Experiment 3 used maize and maize weevils; Experiment 4 used wheat and lesser grain borers. Three of the boxes in each experiment were disturbed by use of commercial Sukup electric motor-driven grain stirrers, i.e., one stirrer per box (Sukup Manufacturing Co., 2014) every 12 hours; the other three control boxes in each experiment were left undisturbed. Every 40 days, all the boxes were sampled using a grain probe.

Samples were analyzed for presence of live insects and for grain quality parameters.

3. Results

Experiment 1 Results

At 40 d, the live maize weevil mean declined from 25 to 11±1 in the undisturbed, and to 6±3 in the disturbed treatment, however this difference between treatments was not statistically significant (Tab. 2). By 80 d, the undisturbed population rebounded to 15±2, while the disturbed population dropped further to 1±2, where it remained through 120 d. The disturbed treatment population reached 3±2 at 160 d. It is unclear whether this slowly increasing trend would continue if the maize were stored longer. For 120 and 160 d storage periods, undisturbed containers showed a continued increase in the number of live weevils whereas in the disturbed containers numbers remained low. Live weevil means were not significantly different at 0 and 40 d between treatments but were significantly higher for the undisturbed treatment at 80 ($p=0.0016$), 120 ($p=0.0030$) and 160 d ($p=0.0006$). After 160 days, live weevil means in the disturbed containers were 7% of those in the undisturbed containers. An analysis of the results with time was also done for each treatment (Tab. 2). In the undisturbed treatment, there were no significant differences between 0, 40 and 80 days. Live weevil means were not significantly different between 120 and 160 days, but these values were significantly higher than those for 0, 40 and 80 days. The live weevil means in the disturbed treatment were significantly lower at all times after 0 day.

Tab. 2 Comparison of means of live weevils over time for disturbed versus undisturbed (control) treatments for Experiment 1 (Bbosa et al., 2014).

		Storage Time (days)				
Item	**Treatment**	**0**	**40**	**80**	**120**	**160**
Number of live weevils/kg	Undisturbed	25 ± 0^{Ab}	11 ± 1^{Ab}	15 ± 2^{Ab}	40 ± 8^{Aa}	44 ± 5^{Aa}
	Disturbed	25 ± 0^{Aa}	6 ± 3^{Ab}	1 ± 2^{Bb}	1 ± 2^{Bb}	3 ± 2^{Bb}

Each value within the table is the mean ± standard deviation of three replicates. Means not followed by the same upper case letter between treatments or not followed by the same lower case letter within each treatment indicate significant difference at the 0.05 level.

Experiment 2 Results

Tab. 3 shows the number of live insects throughout the study. For all control containers, insect numbers increased significantly between 30 and 60 days, and between 60 and 90 days. For the disturbed containers, there were no live weevils in any containers in any region after 90 days. Weevil numbers did not decrease significantly after 30 days in any region except Dodoma.

Tab 3. Number of live *S. zeamais* in maize for Experiment 2 (Suleiman et al., 2016).

Storage Time (days)	Control containers			Disturbed containers		
	Dodoma	Morogoro	Manyara	Dodoma	Morogoro	Manyara
30	20 ± 8^{c}	9 ± 2^{c}	12 ± 4^{c}	10 ± 2^{a}	2 ± 1^{a}	3 ± 1^{a}
60	68 ± 31^{b}	49 ± 35^{b}	77 ± 44^{b}	2 ± 1^{b}	5 ± 1^{a}	0 ± 0^{a}
90	109 ± 22^{a}	119 ± 35^{b}	152 ± 36^{a}	0 ± 0^{b}	0 ± 0^{a}	0 ± 0^{a}

Each value within the table is the mean ± standard deviation of three replicates. Means not followed by the same lower case letter in each column indicate significant difference at the 0.05 level.

Experiment 3 Results

After 40 days, live MW population means in unstirred control boxes decreased significantly to 1.7 per kg of maize but then rebounded significantly to 18 after 80 days (Tab. 4). No live MW were found in any of the stirred box samples after 40 or after 80 days. The experiment was terminated after 80 days. Stirring greatly reduced or eliminated maize weevils in the stirred boxes.

Experiment 4 Results

The 40-day samples of wheat from the three control boxes all contained multiple LGB, while there was a total of one LGB in the stirred box samples (Tab. 5). The mean of the control group was significantly greater than that of the stirring treatment. At 80 days, stirring was stopped and the stirred boxes were undisturbed for the next 40 days to see if eggs and larvae would emerge as adults. There were not significant differences found between the stirred and control treatments, although control box means are far higher than stirred box means. This is presumably because of the high standard deviations among the control replicates at both 80 and 120 days. Further analysis of these data is underway. Discarding of one or two outlier data points may be justified and may result in significant differences between treatments at 80 and 120 days.

4. Discussion

Assuming further analysis concludes there are significant differences between treatments after 80 days for Experiment 4, there is evidence from these four experiments that disturbance is effective in controlling maize weevil in stored maize and lesser grain borer in stored wheat. Further research will be needed to determine how disturbance can be carried out in larger grain containers. One untested possibility is to use grain stirring machines in conventional steel grain bins to carry out disturbance.

Tab. 4 Means comparison of live weevils for stirred versus unstirred (control) containers in Experiment 3 (Rau et al., 2018).

Item	Treatment	T=0 d	T=40 d	T=80 d
Number of live weevils per kg maize	Control	25 ± 0^{Aa}	1.7 ± 0.6^{Ab}	18 ± 4.0^{Ac}
	Stirred	25 ± 0^{Aa}	0 ± 0^{Ba}	0 ± 0^{Ba}

Each value within the table is the mean ± standard deviation of three replicates. Means not followed by the same upper case letter between treatments or not followed by the same lower case letter within each treatment indicate significant difference at the 0.05 level.

Tab. 5 Comparison of means of live lesser grain borers over time for disturbed versus undisturbed wheat in Experiment 4.

Item	Treatment	T=0 days	T=40 days	T=80 days	T=120 days
Number of live lesser grain borers/kg	Control	25 ± 0^{Aa}	11 ± 2.5^{Aa}	131 ± 110^{Aa}	91 ± 99^{Aa}
	Stirred	25 ± 0^{Aa}	1 ± 1.6^{Bc}	2 ± 1.7^{Ac}	8 ± 1.7^{Ab}

Each value within the table is the mean ± standard deviation of three replicates. Means not followed by the same upper case letter between treatments or not followed by the same lower case letter within each treatment indicate significant difference at the 0.05 level.

This technology is currently available. In all four experiments, quality of disturbed grain was similar or better than that of grain in undisturbed containers, except fine material in the stirred boxes which was higher than in the undisturbed boxes. In three of the experiments, we observed a drop in live insects from initial numbers in the control containers during the initial storage periods. Bbosa et al. (2014) also observed this decrease in an experiment with steel barrels. This decrease probably happens because some adult weevils die before adult weevils from eggs deposited in this new environment begin to emerge. All of these experiments employed a 12-hour disturbance interval, although we did not have a solid reason for choosing this interval. Quentin et al. (1991) found an eight-hour interval to be effective for control of bean weevils in stored beans. Additional research is needed to understand why disturbance is effective and to identify an optimum disturbance interval.

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The Adoption of Thermosiphon Powered, Ground Level Phosphine Application Systems in Australia.

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Abstract

Safe storage of grain on Australian farms requires a sealable silo to exclude grain insects and enable effective fumigation to avoid the development of insect resistance to phosphine.

A sealable silo must also be fitted with a pressure relief system that allows air to enter the silo rapidly, to avoid damage to the silo fabric, in the event of a sudden temperature drop and subsequent contraction of the internal atmosphere.

The most effective pressure relief system allows air into the headspace by a pipe attached to the silo wall, which is connected to an oil bath valve at ground level to facilitate servicing. The oil bath valve prevents grain insects entering and will allow air to bypass when the internal pressure exceeds or falls below 30 – 40 Pascals.

The addition of a pipe connecting the headspace pipe to the base of the silo creates a gas recirculation loop. Ambient temperature influences the air within the external pipe and Thermosiphon currents are created, circulating the internal atmosphere.

In 2004, a silo manufacturer in Western Australia proposed such a recirculation loop adding an aluminium phosphide (AlP) reaction chamber into the circuit at ground level. A ground-level application system removes the need to climb the silo, making fumigation simpler and safer.

Initial experiments in 2005 revealed that the phosphine gas would be extracted from the reaction chamber by Thermosiphon air movement, without building to dangerous concentrations.

Seven silo manufacturers across Australia have adopted the Thermosiphon recirculation system linked to a ground level AlP reaction chamber, producing nearly 12,000 transportable silos of 80 – 100t capacity in that period.

The development of the recirculation system and effectiveness of Thermosiphon gas distribution is discussed in this paper.

Keywords: sealed silo, Thermosiphon, recirculation, Aluminium Phosphide, Phosphine.

1. Introduction

Storing grain in Australia is characterised by the challenges of grain insect attack, similar to all warm climate countries in the world. The grain is harvested warm in early summer and remains warm in the silos unless aeration is used but this has limitations in the ability to cool the grain until winter occurs some 3 – 4 months later. Stored grain insects are endemic in the environment, and present a constant threat to stored food products.

To prevent insect attack and enable effective fumigations, sealed gas-tight grain stores have been a feature of the Western Australian grain industry since the 1980's when the central storage operators, Cooperative Bulk Handling of Western Australia, decided to seal all permanent grain storage (Newman, 2006). A 'nil-tolerance' of stored grain insects in grain delivered to the central system was established.

To meet this standard, higher quality of grain storage on farms was needed and assistance was co-opted from local silo manufacturers to produce sealable grain silos (Newman, 1997). The most common types of grain silos in Western Australia are <100t capacity, assembled in a factory and delivered on hydraulic trailers in one piece to the farm, ready to be set up for storage and fumigation. The factory construction process enables a high quality product to be manufactured and sealed to a gas-tight pressure test standard (AS 2628 - 2010).

A sealable silo must be fitted with a pressure relief system to avoid damage to the silo fabric. It allows air to enter the silo rapidly in the event of a sudden temperature or atmospheric pressure drop and subsequent contraction of the internal atmosphere.

The most effective pressure relief system allows air into the headspace via a pipe attached to the silo wall, which is connected to an oil bath valve at ground level to facilitate servicing. The oil bath valve prevents grain insects entering and will allow air to bypass when the internal pressure exceeds or falls below 30 – 40 Pascals. One silo manufacturer in Western Australia (WA) Moylan Silos, based at Kellerberrin, created an efficient pressure relief system using a 90 mm PVC pipe to the headspace coupled to a PVC oil bath valve at ground level (Fig. 1). Many thousands of these silos were produced and remain in use on farms.

Fig 1.

Fig 2

To initiate fumigation in these silos, fumigators attach a safety harness, carry the required amount of AlP to the top of the silo, fit on a personal air-purifying respirator, spread the solid formulation onto a wide tray in the headspace of the silo and close and seal the top hatch (Fig. 2).

2. Ground level phosphine application system proposed

In 2004, Mr Don Bird, owner of Bird's Silos, Popanyinning, WA proposed a recirculation loop, connecting a 90 mm headspace pipe to the base of the silo as a conduit for gasses and adding an AlP reaction chamber into the circuit at ground level (Boland, 1984). Ambient temperature influences the air within the external pipe and Thermosiphon currents are created, moving the gas up or down the pipe depending on the temperature gradient with the commodity. The ground level phosphine application system make the fumigation safer for the fumigator, removing the need to climb the silo. Simultaneously in 2004 a company in WA created a translucent, diesel resistant, Linear Low Density Poly Ethylene oil bath pressure relief valve (PRV) to fit to a 90 mm headspace pipe (Fig. 3). This enabled instant inspection of the oil levels in the valve to ensure the air entering will by-pass at a safe pressure and can also be used as a manometer for pressure testing.

Fig 3.

Fig. 4

3. Methods

In January 2005, a 90.9 m^3 elevated silo was prepared for a pilot trial of the Thermosiphon ground level phosphine application system on a farm at Yornaning, WA. A phosphine reaction chamber was constructed consisting of a metal box with a shelf to hold the AlP tablets and 32 mm internal diameter flexible tubing entering each side of the box to allow air to flow through. This phosphine reaction chamber was placed underneath the silo and the flexible tubing was connected into the base of the silo and to the headspace pipe (Newman et al., 2006). The gas concentrations were measured with a Spectros Non-dispersible Infrared Phosphine Monitor (Supplied by Fosfoquim of Chile), which could record phosphine concentrations up to 10000 ppm. The Thermosiphon pipe attached to the silo wall connecting the headspace to the phosphine reaction chamber was constructed of white PVC and included a translucent PRV.

Phosphine tablets at a rate of 1.5 g/m^3 (#130) were spread on solid trays in the phosphine reaction chamber. Peaks ranging up to 7000 ppm in the phosphine chamber were observed when the air ceased to move in the headspace pipe. This happened twice daily as the airflow direction reversed due to the change from diurnal or nocturnal ambient conditions and air moved up or down the pipe. There was a concern that the flexible tubing connecting the phosphine reaction chamber to the Thermosiphon pipe was restricting the airflow allowing higher concentrations of phosphine to occur.

The next experiment on the same farm in a similar silo incorporated a black painted PVC Thermosiphon pipe and an application rate of 1.1 g/m^3 (#100) using the same phosphine reaction chamber. The silo experienced lower peaks of up to 2300 ppm, which was due to a lower rate of application and faster air movement in the black Thermosiphon pipe.

In February 2005, airflow monitoring was conducted on a black coloured 90 mm PVC Thermosiphon pipe attached to a 90.9 m^3 capacity silo. The pipe to the headspace was connected to a 40 mm steel pipe into the base of the silo. Measurements were taken over 24 hours of the air flowing through the Thermosiphon system using a Kurz hot wire anemometer. The results showed the air moving

under favourable warm ambient conditions and stopping completely when the ambient and commodity temperatures were similar (Tab. 1).

Table 1.
Thermosiphon air speed test, February 26th 2005, E Popanyinning WA
Silo pressure test >180s, Barley @ 9.6% m.c. and 29°C

Weather	Time	Ambient °C	Ambient wind speed kph	Airspeed tube m/s (32 mm orifice)	Metres/s 90 mm pipe	Litres / min 90 mm pipe	m/3/hr
Fine	12:30	31	7.2	0.55	0.068	25.95	1.55
Fine	13:30	34	8	0.6	0.074	28.24	1.69
Fine	14:30	36	11	0.45	0.056	21.37	1.28
Fine	15:30	35.5	22	0.48	0.059	22.521	1.35
Fine	16:30	36	13	0.45	0.056	21.37	1.28
Cloud	17:30	35	16	0.29	0.035	13.6	0.8
Cloud	18:00	34	18	0.29	0.035	13.6	0.8
Cloud	18:30	33.5	15	0.21	0.026	9.9	0.59
Cloud	19:00	33	7	0.19	0.023	8.7	0.53
Cloud	19:30	32	6	0.11	0.014	5.3	0.32
Cloud	20:00	31	5.5	0.09	0.011	4.2	0.25
Cloud	21:21	30	0	0		0	
Cloud	22:00	29	0	0		0	
Cloud	23:00	28	0	0		0	
Part cloud	0:00	26	3	0		0	
Part cloud	2:00	24	6	0.04	0.005	1.9	0.11
Part cloud	4:00	24	5	0.06	0.007	2.67	0.16
Part cloud	6:00	23	0	0.1	0.012	4.6	0.27
Cloud	7:00	26	2	0.05	0.006	2.3	0.14
Cloud	8:00	28	8	0.08	0.01	3.8	0.23
Cloud	9:00	30	15	0.19	0.023	8.7	0.53
Cloud	10:00	31	20	0.15	0.018	6.8	0.41
Rain	11:00	27	9	0.04	0.005	1.9	0.11
Rain	11:30	27	7	0		0	

A fumigation was commenced in the same silo the following month when AlP tablets at a rate of 1.5 g/m^3 were placed on the sealing plate (Fig. 4) at the base of the silo. The space between the seal plate and the grain control 'butterfly valve', provided adequate space as a phosphine reaction chamber. In this experiment gas concentration reached a maximum of 3500 ppm in the phosphine reaction chamber and up to 1750 ppm in the headspace (Fig. 5). A test in a similar silo the following summer produced similar results with the characteristic high and low peaks in the phosphine chamber and even concentrations in other parts of the grain mass.

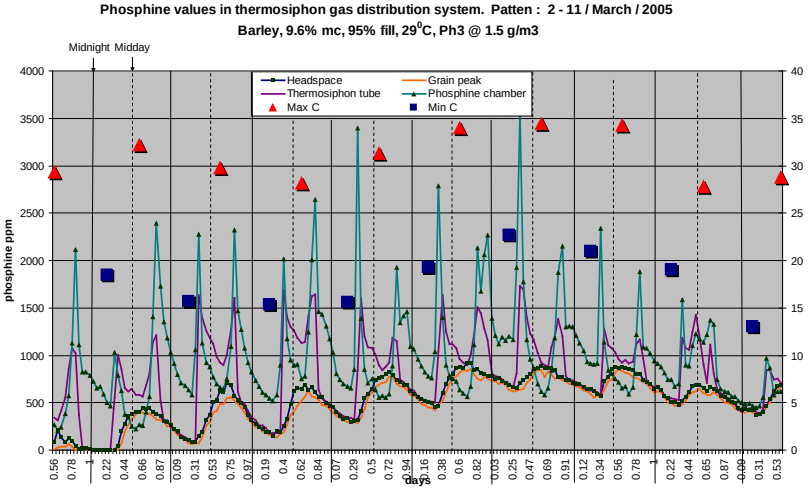

Fig. 5

The low concentrations in the phosphine reaction chamber shown in Tab. 2 are caused by the air flowing rapidly up the Thermosiphon pipe in the morning as the sun heats the pipe. The high concentrations in the evening are a result of the air moving down the pipe as the ambient temperature falls below that of the stored commodity. The even concentrations of gas in the grain mass demonstrate the mixing effect of the Thermosiphon induced air currents. This is in contrast to a 'top loaded' AlP fumigation where there is an initial high concentration in the headspace, which reduces over time as convection currents and diffusion carry the gas to the lower parts of the silo.

The results of these experiments demonstrated to Bird's Silos that the system was safe, efficient at moving released phosphine gas into the grain profile and removed the need to climb the silo to apply AlP into the headspace. The company modified the production line to produce all silos from their factory with the Thermosiphon powered ground level phosphine application system.

An important modification to the new silos was a deep bowl phosphine reaction chamber to hold a 'Bag Chain' formulation of AlP or a removable perforated steel plate in the base to hold the tablet formulation of AlP and allow the powder to drop through as phosphine gas is generated (Figs. 6a & 6b). In addition, the top lid on the silo was fitted with a cable and winch device, which is operated at ground level to open, close and seal the top lid also without having to climb the silo.

Fig. 6a &6b

4. Ground level phosphine application system adopted by other silo manufacturers

A silo manufacturer in South Australia showed interest in the system and a cooperative arrangement with Bird's Silos was established to share the technique. A silo company in Victoria also became interested in the system and came to an agreement with Bird's Silos to share the information. This company was part of a larger corporation controlling three independent manufacturing plants in three other Australian states who also adopted the ground level application system.

Modifications to the phosphine reaction chamber and connections have been made by silo factories across the country but retain the principle of the system with a 90 mm headspace pipe connected to the base of the silo. Fig. 7 shows one of the variations of the phosphine reaction chamber at ground level.

Six silo manufacturing plants around Australia have now adopted the translucent PRV and statistics provided by the manufacturing company indicate that up to December 2017 approximately 6500 silos have been fitted with the Thermosiphon powered ground level phosphine application system. In addition, Moylan Silos in WA, who first fitted the 90 mm headspace pipe, have also created a ground level phosphine application system but retained the PVC, PRV (Fig. 8) and retrofitted it to all silos produced since 2009. In that period, they have produced approximately 5400 silos. Across Australia there are now approximately 12,000 silos fitted with the ground level phosphine application system with Thermosiphon distribution.

Fig. 7

Fig. 8

5. Inert atmosphere application

The simple addition of three ball valves into the lower Thermosiphon pipe allows purging of the silo atmosphere at ground level through one of the valves while loading gas such as nitrogen (N_2) or carbon dioxide though one of the other valves (Fig. 9). The purging valve provides a convenient point at which to measure the composition of the internal atmosphere. When using inert gasses in a sealable silo, the halving pressure test must be elevated to a minimum of 5 minutes to avoid oxygen (O_2) ingress over the longer fumigation periods required.

Application of N_2 using a 30 m^3 per hour Pressure Swing Absorption N_2 generator (Fig. 10) into a 90.0 m^3 silo takes approximately 2.5 hours with an additional 0.5 hour the following day to remove the oxygen desorbed from the grain and retain the O_2 concentration at 1% (Newman, J – personal communication). Measurements over the succeeding 28 days showed even concentrations at all points as the gas was recirculated by the Thermosiphon pipe with a slight decay to ~3% O_2.

6. Testing of the Thermosiphon system at Kansas State University (KSU), USA

In 2015, a 63 m^3 Bird's Silo was transported from Popanyinning, WA to KSU, to conduct detailed analyses of the Thermosiphon ground level phosphine application system. The silo was shipped in pieces and assembled on site by a group of people including Mr. Don Bird and the author. Sealing on site was more complicated and the standard achieved in the factory could not be emulated in the field. In addition, a locally manufactured 71.9 m^3 SCAFCO silo was assembled on the site and sealed as it was being constructed; however, it was not designed to be sealed and required considerable innovation on site to achieve a seal (Fig. 11). The result was that both silos were sealed to a lower standard than required in Australia under AS 2628 (5 minutes halving pressure for a newly constructed silo).

Mr. S. Cook commenced experiments in August 2015 as part of a Master of Science degree (Cook, 2016). The experiments were conducted with solid formulation AlP, gaseous phosphine and sulfuryl fluoride. AlP tablets were applied in the ground level phosphine reaction chamber, gaseous

phosphine was applied via the Thermosiphon pipe and sulfuryl fluoride was applied through one of the monitoring lines directly into the grain. The silos were set up to include a ball valve in the lower pipe so that the gas recirculation could be studied with and without Thermosiphon.

Fig. 9

Fig. 10

Fig. 11

Experiments showed the Thermosiphon effect moving phosphine gas upward into the headspace during the period when the sun was warming the external pipe and concentrations rising in the lower parts of the silo in the cool evening as the air flow reversed and moved released gas out of the phosphine reaction chamber. When the Thermosiphon was turned off, the phosphine was forced to move upwards through the grain mass taking some time to reach lethal concentrations in

all parts of the silo, relying only on thermal convection currents. Air speed velocities in the Thermosiphon pipe were between 0.02 – 0.08 m/s under sunny conditions and 0.01 – 0.02 m/s in partly cloudy conditions (Cook, 2016).

7. Discussion

The development of the Thermosiphon ground level phosphine application system has made Australian grain silos safer for the fumigator and grain manager. Experiments in Australia on silos up to 1200 t have demonstrated that a Thermosiphon system provides effective recirculation without the use of electrically powered fans (Newman, 2012).

The addition of a Thermosiphon pipe to any silo ensures continuous mixing of the internal atmosphere and has been shown to be effective when used as the delivery conduit for a gaseous phosphine application. The gas is injected into the silo and the aeration fans operated with all seal plates in place to circulate the gas for 60 – 90 minutes, after which the aeration fans are turned off. The gas continues to circulate as powered by Thermosiphon alone, producing even concentrations throughout the silo for the remainder of the fumigation period (Ball, S personal communication).

Research at KSU demonstrated the effectiveness of the Thermosiphon powered ground level application system in distributing phosphine rapidly throughout the grain bulk. In that experiment, turning off the Thermosiphon air currents demonstrated the slower incorporation of phosphine by thermal air currents and diffusion alone. In comparison, with the Thermosiphon operating there was rapid mixing of the phosphine gas throughout the silo.

Future developments that could be explored to reduce the need to climb the silo include using the headspace pipe as conduit for extracting grain odours or carbon dioxide to determine grain quality and presence of mould or insects. Custom-made pheromone traps inserted into the headspace pipe at ground level would attract grain insects, providing a decision tool to initiate a fumigation procedure.

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Lessons learned for phosphine distribution and efficacy by using wireless phosphine sensors

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Extended abstract

1. Introduction

Phosphine is by far the most commonly used fumigant for disinfestation of stored grains, pulses etc. and also of dry processed commodities (Fields and White, 2002; Opit et al., 2012). For instance, approximately 80% of the grain production in Australia is fumigated with phosphine (Collins et al., 2001). It is a colorless, odorless and flammable toxic gas (Chaundhry, 2000). Phosphine is generally cheap, easy to apply for most durable commodities and it is effective for all life stages for nearly all the major insect pests, whereas it leaves minimal residues (Chaundry, 2000; Hasan and Reichmuth, 2004; Wang et al., 2006; Nayak and Collins, 2008). However, the extensive use of phosphine, in conjunction with low concentrations and poor sealing, has raised resistance issues and may lead to serious fumigation failures (Zeng, 1999; Collins, 2009). Currently, resistance by various storage insect populations is a reality in several parts of the world (Collins et al., 2002; Daglish, 2004). There are many traditional techniques available for monitoring gas concentration such as digital monitors that are placed outside of the treated area and glass tubes that are used to quantify concentration by sucking air from the treated substrate. Both methods are difficult in their use, often inaccurate and they need specialized personnel. Despite the fact that there are different types of electronic equipment that can be used to estimate phosphine concentration, the majority of them cannot be placed inside the treated area, due to the corrosiveness caused by this gas.

Recently, phosphine wireless sensors that can be placed inside the treated area have been developed and evaluated with success in storage facilities in Greece (Athanassiou et al., 2016). This initial work clearly indicated that gas concentration is uneven in the treated area, and that further experimental work is needed to evaluate its distribution. Moreover, it has been reported that inside a flour mill in the Czech Republic phosphine concentration varied remarkably, and the main factors for these variations were temperature and relative humidity gradients (Aulicky et al., 2015). Phosphine distribution in silos has been modelled by Isa et al., (2016) but there is still inadequate information regarding the effect of different biotic and abiotic factors towards this direction. At the same time, there are not many data available for the distribution patterns and spatio-temporal movement of phosphine in other commercial storage formations and facilities, such as containers, warehouses, silos and shipholds. Thus, the purpose of this study is to evaluate wireless phosphine sensors by estimating both gas concentration and kill rates of major stored product insects in "real world" tests.

2. Materials and Methods

2.1 Test insects

Adults of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), were used in the trials. The insects used were reared at the Laboratory of Entomology and Agricultural Zoology (LEAZ), Department of Agriculture, Crop Protection and Rural Environment, University of Thessaly, at 25°C, 65% relative humidity (r.h.) and continuous darkness. For each of the above species, two

populations were used in the experiment, one field and one laboratory population, namely GA6 *R. dominica*, ASC11 *O. surinamensis*, laboratory *R. dominica* and laboratory *O. surinamensis*. The laboratory populations are being reared for more than 20 years under laboratory conditions. The field populations were collected from different storage facilities from Greece and were characterized as tolerant to phosphine. From the above species, *R. dominica* was reared on whole wheat kernels, whereas *O. surinamensis* on oat flakes.

2.2 Experimental procedure

Plastic cylindrical vials (3 cm diameter and 8 cm in height) were the experimental units for the tests; the vial neck was covered with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent insects from escaping. Each vial was filled with 10 g of commodity, i.e., whole wheat grain for *R. dominica* and oat flakes for *O. surinamensis*. Then, ten adults of each species and population were introduced into each vial (separate vials for each species and population). In each fumigation trial, the vials were placed in different locations within each facility. For each species and population three vials were prepared per location and per facility. Separate vials with insects, placed in untreated areas of each facility served as controls. Then the vials were transferred to LEAZ and adult mortality was assessed. The vials were kept in incubators set at 25ºC, 55% r.h. in continuous darkness and progeny numbers were recorded 65 d later. Phosphine concentration monitoring was performed by the use of wireless sensors (Centaur Analytics Inc. CA, USA), and wireless signal amplifiers and receivers were connected to computers. The sensors were placed at various locations inside the treated area, including all the locations where insects had been placed.

2.3 Data analysis

All data, separately for each trial and insect species were submitted to Independent t-test, with insect mortality as the response variable. To determine the effect of location for each trial, data were subjected to an one-way ANOVA with insect mortality as the response variable and location as the main effect. Control mortality was generally low, so the data for control mortality were not used in the analysis. The same approach was also followed in the case of progeny production counts. Means were separated by using the Student's *t* and Tukey-Kramer HSD test at 0.05, whenever this test was considered necessary.

3. Results

Figures below show the results according to the fumigation treatment at different facilities, i.e., a warehouse, a container, two shipholds and two silos, with wireless phosphine sensors which were located in the fumigated area (Figs. 1, 2, 3, 4, 5, 6). In all cases, the mortality of control was generally low for all insect species and populations and did not exceed 10%. Regarding the fumigation which was carried out in the warehouse, complete control was detected only for the *O. surinamensis* laboratory population in contrast with the other three populations tested (Tab. 1). In that facility, the maximum level of phosphine concentration was 80 ppm for less than four days (Fig. 1). On the other hand, in the fumigated container, mortality reached 100% for all tested populations, while the concentration of phosphine was 2000 ppm for five days (Fig. 2). Furthermore, at the fumigated shipholds, where no forced recirculation system (J-system) was applied, mortality was complete (100%) only for the laboratory population of *O. surinamensis*. Moreover, progeny production in the treated substrate was lower when the J-system was applied, but parental survival could not be avoided. In these treatments, the concentration of phosphine reached 300 ppm for two days (Fig. 4). Regarding the fumigation which was carried out in the silo, the concentration of phosphine ranged between 200 and 600 ppm (Fig. 5), which clearly indicated that phosphine could not be distributed normally in the treated grain mass. The use of J-system in a silo showed that the phosphine concentration gradually increased (Fig. 6).

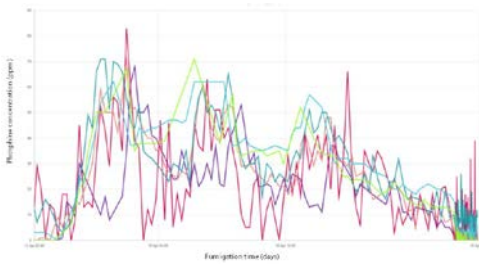

Fig. 1 Phosphine concentration during the fumigation inside a warehouse with six different wireless sensors (shown with different colors) placed at different locations.

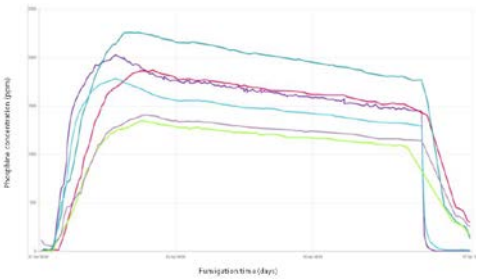

Fig. 2 Phosphine concentration during the fumigation inside a container with six different wireless sensors (shown with different colors) placed at different locations.

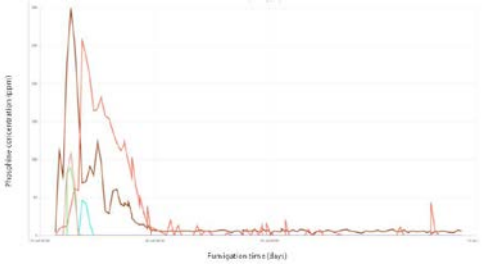

Fig. 3 Phosphine concentration during the fumigation inside a shiphold with five different wireless sensors (shown with different colors) placed at different locations.

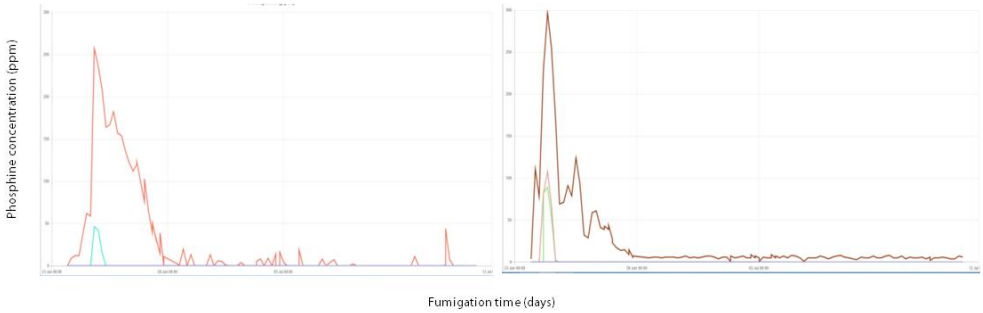

Fig. 4 Phosphine concentration during the fumigation in a ship hold with no use of recirculation system (left) with two different wireless sensors and in a ship hold with the use of a recirculation system (right) with three different wireless sensors (shown with different colors), placed at different locations.

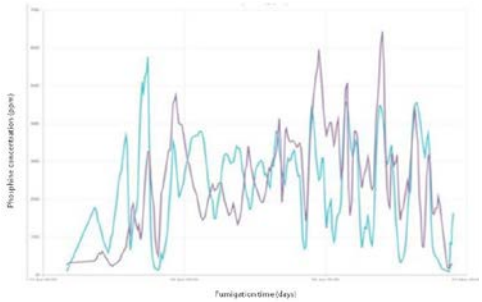

Fig. 5 Phosphine concentration during the fumigation inside a silo with two different wireless sensors (shown with different colors) placed at different locations without using forced recirculation system.

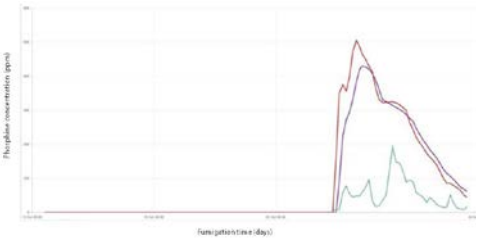

Fig. 6 Phosphine concentration during the fumigation inside a silo with three different wireless sensors (shown with different colors) placed at different locations by using forced recirculation system.

Tab. 1 Mortality (% ± SE) of parental adults for field and laboratory populations, in different facilities in which phosphine had been applied and the respective progeny production (number of adults per vial ± SE) 65 d later.

Facility	Insects	Mortality	Progeny production
Warehouse	ASC11 *O. surinamensis*	100 ± 0.0	0.0 ± 0.0
	Lab *O. surinamensis*	100 ± 0.0	0.0 ± 0.0
	GA6 *R. dominica*	100 ± 0.0	2.0 ± 0.7 a
	Lab *R. dominica*	100 ± 0.0	0.0 ± 0.0 b
Container	ASC11 *O. surinamensis*	34.2 ± 3.3 a	65.1 ± 11.4 a
	Lab *O. surinamensis*	100 ± 0.0 b	0.0 ± 0.0 b
	GA6 *R. dominica*	6.6 ± 2.2 a	48.4 ± 3.8 a
	Lab *R. dominica*	75.7 ± 4.1 b	12.6 ± 3.3 b
Shipholds	ASC11 *O. surinamensis*	not measured	0.3
	Lab *O. surinamensis*	not measured	0.0
	GA6 *R. dominica*	not measured	52.7 a
	Lab *R. dominica*	not measured	1.0 b

Within each trial and each species, means followed by different letters are significantly different. Where no letters exist, no significant differences are noted with Student's test at 0.05.

4. Discussion

In the fumigation treatments, we found high survival percentages of exposed adults and a considerable number of offspring in all cases, with the exception of the fumigations in the containers, in which complete control (100% mortality) was detected. This was partially due to the short duration of fumigation (approx. three to four days), in combination with low concentrations of phosphine in the warehouses, silos and shipholds. Phosphine leakage and sorption by the treated commodity are highly responsible for gas losses during fumigations (Bell, 2000, Aulicky et al., 2015). As a consequence, there was a sufficient number of insects that survived fumigation, and this number could gradually lead to resistance development. On the other hand, the fumigations in containers, which were the "best case scenario" here, clearly suggest that, if applied properly, phosphine can definitely lead to 100% efficacy levels. In the current trial, the container fumigation resulted in complete parental mortality, in conjunction with extremely low numbers of progeny production. In this context, for the same reasons noted above, fumigations in shipholds and silos are likely to fail due to increased leakage, which cannot be detected and quantified easily with the majority of phosphine detection techniques. In this regard, wireless phosphine sensors can be a valuable tool towards this direction (Athanassiou et al., 2016). Based on our results, in large areas, such as silos, distribution of phosphine was rather limited and thus, there were large areas within the grain mass that did not get enough gas in order to achieve a satisfactory insect mortality. The

adoption of a recirculation system in these cases can improve fumigation results. Summarizing, our tests clearly indicated that phosphine sensors were quite effective in measuring phosphine concentrations and can play an important role in the future in IPM-based programs during the post-harvest stages of agricultural commodities. Hence, sensors can be used as a "precision fumigation" tool and provide real-time estimates for insect control.

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Use of a 3D Finite Element Model for Post Fumigation Phosphine Movement Analysis

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Abstract

Phosphine is a dangerous gas commonly used in fumigations throughout the world. Grain that has not fully released the phosphine it absorbed during fumigation may continue to desorb phosphine into the headspace of a storage structure. U.S. OSHA standards for handling phosphine state the acceptable limit at 0.3 ppm. If this limit is exceeded grain handling may become dangerous. It is important to understand the process of phosphine

venting and desorption in order to ensure safe handling of fumigated grain in silos and during shipments. In order to achieve this, the venting and release of phosphine was studied on location in a well-sealed grain silo in Lake Grace, Western Australia, to serve as a set of data for verification of a computational model. This situation was then modeled using a 3D finite element model and compared to the real world results. Results were calculated using two fumigant desorption models based on previous literature, a reversed sorption model and an air-grain equilibrium model. Simulations reproduced accurate trends of desorption but did not accurately reproduce the quantity of fumigant, with a 55.5% error for the model based on reversed sorption equations and 86.3% error for the air-grain equilibrium based model. For both models, simulations were conducted to compare the effectiveness of existing grain venting regulations at producing grain that is within safe handling limits. These results highlight the necessity for continued desorption research and the importance of following venting guidelines.

Keywords. Finite Element Modeling, Fumigation, Phosphine, Stored Product Protection

1. Introduction

A successful fumigation relies on exposing each insect within a grain mass to the specific concentration of fumigant for a specified amount of time needed to kill all insects present at all life stages. There is a significant amount of literature available on the bio-efficacy of fumigants such as phosphine against a range of stored product pests at multiple life stages (Chaudhry, 2000; Price and Mills, 1988), however, information on the fumigant activity within the commodity during a fumigation is very limited. Therefore, modeling the behavior of gas fumigants in the interstitial air volume of the stored grain mass is helpful in determining what factors may cause fumigation failures, and how those factors can be affected by environmental conditions. A previous attempt at developing such a fumigation model was made by Isa et al. (2016) using the program Fluent instead of an independent computer code. They also simulated vertical gas flow in a silo using Fluent and Comsol (Isa et al., 2011). While using any of the available fluid dynamics software packages has several advantages, such as ease of use and ease of visualizing results, it has disadvantages as well. Their fumigation model simulates both sorption and leakage losses, but the leakage losses are not influenced by weather condition or operational variables which is not realistic. Since the boundary conditions are set inside Fluent, loss prediction was implemented with point losses only. The amount of loss was then controlled only by pressure half loss time. This strategy may be insufficient not only for fumigant loss that is affected by weather, but also in its inability to consider the combined effect of many small leaks over the entire external surface of the silo.

The M-L 3D finite element ecosystem model was previously developed to investigate stored grain environments and has the capacity to monitor chemical concentrations throughout the grain mass (Lawrence, 2010; Lawrence and Maier, 2011). In order for this model to accurately predict fumigant concentrations the model had to be improved with the added capacity to account for fumigant loss. The primary sources of fumigant loss are fumigant leakage from the silo and fumigant sorption into the grain.

Sorption of gas by grain was listed as one of the factors most likely to cause inadequate fumigation conditions in Australia (Darby, 2011). Wheat at higher temperature sorbed a greater amount of phosphine than lower temperature wheat. After 96 hours in a container with initially 1 mg/L phosphine, the fumigant concentration in the interstitial airspace of stored wheat at 35°C was below 0.1 mg/L, whereas in wheat at 15°C it was around 0.5 mg/L (Darby, 2011). That result was supported by Reed and Pan (2000), Sato and Suwanai (1974) and Dumas (1980) who reported phosphine sorption increased with higher grain temperature and moisture content. An increase of temperature also caused faster rates of sorption of phosphine in wheat independently from moisture content. An increase from 24°C to 35°C caused the sorption rate constant to increase from 0.0064 to 0.186 (Banks, 1986; Berck, 1968). Increased adsorption of phosphine to the surface of cereal grains with increasing temperature was also shown in Sato and Suwanai (1974).

There are a number of factors that may deter the efficacy of a fumigation where enough fumigant was applied to theoretically control the insects. According to Banks and Annis (1984) these factors are excessive overall loss of fumigant, inadequate fumigant dosage in localized regions, excessive

delay between application and fumigant reaching particular regions, or a combination of these factors occurring simultaneously. To observe whether any of these effects were occurring in a fumigation would be difficult and would require excessive monitoring of fumigant concentrations at a number of locations in the silo. Even with such controls, there could be regions that are not monitored and experience problems, or environmental conditions that are abnormal or unforeseen. How environmental factors and operational procedures influence a fumigation can be more easily and thoroughly investigated using a fumigant model that incorporates sorption and fumigant loss. A better understanding of such influences would allow applicators to take more effective corrective actions to prevent fumigation failures.

2. Materials and Methods

2.1 Effect of Sorption

To estimate sorption loss of phosphine gas in a grain silo, an equation for concentration as a function of time was obtained from Daglish and Pavic (2008). The equation is valid at a 1 mg/L application, and 0.75 fill ratio, resulting in an R^2 value of 0.96 at 25°C and 55% relative humidity. The equation presented in the literature was adjusted to fit the time step and units in the code, i.e., an hourly time step and units of kg/m^3. Additionally, to calculate the amount of phosphine lost due to sorption, the equation was modified by taking the derivative with respect to time. The resulting baseline sorption equation was:

$$C = 0.0000026e^{-0.0017t} \quad \text{Eq 1}$$

where,

C = fumigant concentration lost [kg/m^3], t = time [h]

Fumigant sorption also varies due to other factors that are important variables in our experiment, such as temperature and moisture content of grain. To account for these variables, Eq [1] was multiplied by factors dependent on temperature and moisture content. The effect of temperature on phosphine sorption was studied by Darby (2011) who determined sorption losses at 35°C were about five times as large as losses at 15°C, at a constant equilibrium relative humidity of 65%. Therefore, this result can be modeled with an exponential equation dependent on temperature, where the value at 35°C is five times the value at 15°C. The value for this expression is set to equal one when the temperature is at 25°C, because that is the temperature of the baseline equation from Daglish and Pavic (2008). This means that when the temperature equals that of the baseline equation, the overall equation should be unchanged. The effect of moisture content on the sorption of phosphine was studied by Reed and Pan (2000). They determined fumigant loss for several temperatures at two values of wheat moisture content, i.e., 11% and 13.5%. The sorption at the higher moisture content was 1.8 times greater than the sorption at the lower moisture content at 25°C. This was modeled with an exponential equation which was set to 11.5%, the equilibrium moisture content of the wheat from the baseline Daglish and Pavic (2008) equation. The resulting equation for fumigant loss due to sorption into the grain mass when modified to account for changing temperatures and moisture contents is therefore:

$$C = 0.0000026e^{-0.0017t} * 0.13365e^{0.0805T} * 0.067e^{0.235M} \quad \text{Eq 2}$$

where,

C = fumigant concentration lost [kg/m^3]

t = time [h]

T = temperature [°C]

M = moisture content [%], wet basis

Implementing this equation into the fortran computer code required that the fumigant concentration lost due to sorption is subtracted from the current fumigant concentration at each node for each time step but only if the current fumigant concentration at that node is higher than the sorption amount to be subtracted. If the phosphine concentration at a node is less than the concentration that would be lost to sorption, the phosphine value at that node is set to zero instead.

2.2 Effect of Silo Leakage

To estimate the amount of fumigant lost due to leakage from the silo, estimates for individual sources of leakage were taken from Banks and Annis (1984) along with additional information from the other sources to extrapolate estimates of fumigant loss as a summation of losses from various sources. The most significant sources of fumigant loss result from: (1) concentration differences between the inside of the grain silo and the ambient conditions, (2) chimney effects due to temperature differences, (3) chimney effects due to concentration gradients, and (4) wind effects.

To implement these equations into the computer code, the calculated fumigant concentration lost is subtracted from the current fumigant concentration at each node along the vertical silo wall at each time step but only if the fumigant concentration at that node is higher than the leakage amount to be subtracted. If the phosphine concentration is less than the amount to be subtracted, the concentration is set to zero.

The final equation to predict fumigant leakage from the silo due to effects of fumigant sorption and loss, and modified for changed environmental conditions is therefore:

$$C = \frac{5}{x} * \frac{Nn}{Nb} * (0.0002233e^{0.4621Sw} * Ci + 0.0000248\, e^{0.1386Tc} * Ci + 0.0000326e^{0.1386Td} * Ci + 0.0029962Ci^2) \quad \text{Eq 3}$$

Where,

C = fumigant concentration lost [kg/m^3]

X = pressure half loss time (minutes)

N_n = number of nodes

N_b = number of boundary nodes

Once equations were developed to predict phosphine loss from sorption and leakage from the fumigation in question from factors such as the effects of weather, they could be used to determine the sensitivity of fumigations to changing environmental conditions. The original 10-day fumigation was conducted from Aug 31 to Sept 9, 2015 in Manhattan, Kansas. Weather data for that time period was acquired from the Kansas State University Mesonet database (http://mesonet.k-state.edu/). This weather data was modified by changing hourly values of each key parameter (wind speed, ambient temperature, relative humidity) by +/- 25% and +/-50%. The modified simulations were compared to a base case that used the original weather data to simulate the fumigation described in Cook (2016).

2.3 Simulated Fumigation

A mesh with 2,587 nodes was created in the Abaqus finite element software for the simulation based on the dimensions of the silo supplied. While the dimensions were modeled precisely, the major discrepancy is that this silo contains a cone shaped bottom, and our model is limited to a flat bottom silo. For this reason, the cone was left off, but the extra distance may have provided space for more mixing of the fumigant before it arrived in the region of the simulation. For this reason, fumigant was applied across the entire lower boundary of the simulation, excluding boundary nodes. Weather data for the period in question was taken from the Kansas State University Mesonet database (http://mesonet.k-state.edu/). As phosphine cannot be directly input into the model, a

phosphine application method was implemented in which the base nodes of the silo, excluding edge nodes, were set at a starting amount that was held for 24 hours to approximate the phosphine release time in the experiment (Cook, 2016).

3. Results and Discussion

3.1 Simulation Accuracy

With model parameters such as loss and leakage quantified, the model was verified by comparison of the simulated fumigant concentrations to the experimental values measured by Cook (2016) with a gas release period of 24 h. Both the experimental and simulated results indicate a rapid increase of phosphine at the beginning of the fumigation, followed by a loss of phosphine that continually slows until the end of the fumigation, as seen in Fig. 1. While the trends are similar, the maximum average concentration is greater when only considering the points available in the experimental data. This effect demonstrates the potential for over predicting phosphine concentration when not measuring points along the sidewall of the silo.

The quantitative comparisons between the measured and predicted values are based on results reflecting the same locations and time readings. The root mean square error of this verification was 47.5 ppm, the average difference was 0.1 ppm, and the average of the absolute values of the differences was 38.6 ppm. The overall average experimental fumigant concentration was 283.3 ppm, therefore, the average percentage error compared to predicted values was 13.6%. The major discrepancy between the results seems to begin the evening of the first day of fumigation, around 1830 to 1930, when the increase in phosphine in the experimental data begins slowing and the predicted data do not. This coincides with the beginning of a decrease in night time temperature. This may be similar to the night time phosphine drop noted in Australian experimental data that was made available to the authors by the PBCRC (data not shown). This culminates in the largest difference between experimental and predicted data, at 1100 the next morning, after which the experimental phosphine readings begin to climb back to levels predicted by the simulation. The low temperature that night was 21.8°C, the afternoon highs for August 31st and September 1st were around 33°C. A similar effect appears to happen at a smaller scale the next night, with the values dropping slightly and then rebounding in the morning of September 2nd. The temperature that morning was around 25°C. After the second night, night time concentration drops are not seen in the experimental data, either because they did not happen, their effect was smaller due to lower phosphine values, or they were missed due to lack of night time phosphine sampling. The net effect, however, is that the predicted values appear to be a few hours ahead of the actual values. The night time decrease may also explain why the simulation slightly over predicted the amount of phosphine reported. This, along with an over prediction of the low phosphine levels seen late in the experiment, comprise the major differences between the simulation and experiment.

3.2 Temperature of Ambient Air

Shown in Fig. 2 are the predicted average concentrations of phosphine for five simulations with a varying ambient temperature, expressed as percentages of the ambient temperature for the model verification (i.e., 100%) in degree Celsius scale. As expected, when ambient temperatures were increased, total average phosphine concentrations in the silo decreased. The effect of increasing temperatures is not directly proportional, as the effects of temperature changes decrease as the temperature increases. When temperatures were decreased, total average phosphine concentrations increased by a higher amount. Halving the ambient temperature resulted in a phosphine concentration that when averaged over all locations and times was 26% greater than the concentration from the verification. At 1.5x the ambient temperature, overall average phosphine concentration was 27% less than the overall average concentration from the verification. Fumigations with lower ambient temperatures achieved higher maximum phosphine

concentrations (416, 410, 400, 380, and 364 for 50-150%, respectively). The scale of the effects builds with time, becoming larger as the simulation progressed (Fig. 1). By the end of the simulation the percentage differences from original were 102%, 57.3%, -47.1%, and -67.9%, for the 50%, 75%, 125%, and 150% ambient temperature situations, respectively. The temperature decreases caused 57% and 102% increases in phosphine concentration for the 75% and 50% temperature cases, respectively.

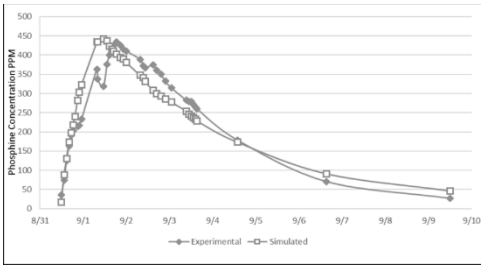

Fig. 1 – Comparison between average experimental and predicted phosphine concentration (ppm) results considering only data at the same locations and times from which data were recorded by Cook (2016) between August 31 and September 9, 2015, with a 24h fumigant release.

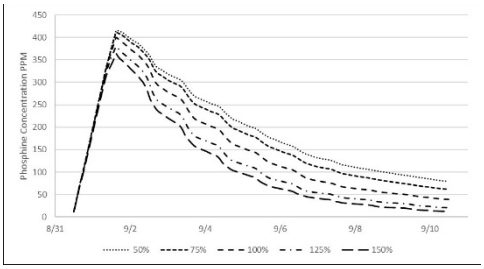

Fig. 2 – Overall average phosphine concentration (ppm) for five temperature conditions, expressed as a percentage of the temperatures (100%) used in the verification conducted between August 31 and September 9, 2015.

Increasing the ambient temperature by a factor of three (i.e., from 150% to 50%) decreased the overall average Ct-product by 71.5%, i.e., from to 47500 ppm-h to 27700 ppm-h (Table 1). Doubling the ambient temperature of the silo (i.e., from 75% to 150%) decreased overall average Ct-products by 39.1%, from 43800 ppm-h to 27700 ppm-h.

Table 1 – Cumulative average Ct-products (ppm-h) and the difference from original (%) for five ambient temperatures, expressed as a percentage of the ambient temperatures (100%) used in the verification conducted between August 31 and September 9, 2015.

	50%	**75%**	**100%**	**125%**	**150%**
ppm-h	47500	43800	37700	31400	2,700
Percent Difference	26.0	16.1	0.0	-16.6	-26.6

The primary reason for this relationship is found in the fumigant loss equation and its reliance on temperature. Based on baseline equations developed in Banks and Annis (1984), fumigant concentration loss increases as a function of grain temperature along the silo wall, and with increases in the difference between the silo temperature and ambient temperature. Additionally, temperature increases also increase the fumigant loss due to sorption as detailed in Daglish and Pavic (2008). Increasing and decreasing ambient temperature does not have an equal influence on overall fumigant concentrations. This is due to the previously discussed effect on increasing leakage from the silo. As the leakage rates increase at high temperatures, the effect diminishes because the leakage effect due to temperature difference comprises only two terms in the overall leakage equation, which is additive. This can be seen clearly in Table 1, as increasing from the 100% case to 125% had a larger effect than increasing from 125% to 150%, and decreasing from 100% to 75% had a larger effect than decreasing from 75% to 50%.

The effect of temperature is of particular interest in subtropical grain growing regions such as Australia, where temperatures are high and grain is commonly fumigated in the summer. Higher temperatures cause increased gas leakage, making sealing even more important. While high temperatures cause a decrease in phosphine concentrations in the grain mass, phosphine is more effective against insects at higher temperatures (Bond, 1989; Sun, 1946) in large part due to their increased activity and higher respiration rates. If, however, the silos were well sealed, increased

leakage caused by higher temperatures would be mitigated and insect susceptibility to the fumigant would be maximized.

3.2.3. Wind Speed

Shown in Fig. 4 are the predicted average concentrations for phosphine for five simulations with varying wind speeds, expressed as percentages of the wind speeds from the model verification (i.e., 100%). As expected, phosphine concentrations were higher for silos with lower wind speeds. Halving wind speed resulted in a phosphine concentration that when averaged over all locations and times was 10.4% greater than the concentration from the verification. At 1.5x, the same overall average phosphine concentration was 13.3% less than the overall average concentration from the verification. Fumigations with lower wind speeds achieved higher maximum phosphine concentrations (407, 404, 400, 393, and 386 for 50-150%, respectively), as leakage begins taking effect before the maximum values are reached. Percentage changes resulting from the five simulations are shown in Fig. 5. By the end of the simulation the percentage differences from original were 25%, 14%, -15%, and -29% for the 50%, 75%, 125%, and 150% wind speed cases, respectively.

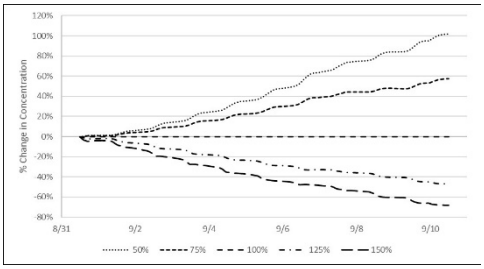

Fig. 3 – Change in overall average phosphine concentration (%) for five temperature conditions, expressed as a percentage of the temperatures (100%) used in the verification conducted between August 31 and September 9, 2015.

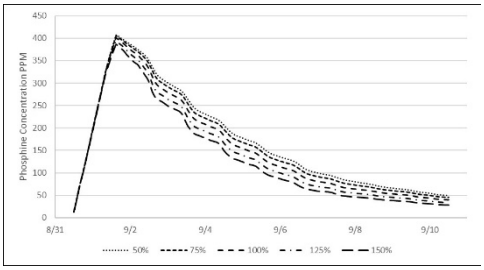

Fig. 4 – Overall average phosphine concentration (ppm) for five wind speeds, expressed as a percentage of the wind speeds (100%) used in the verification conducted between August 31 and September 9, 2015.

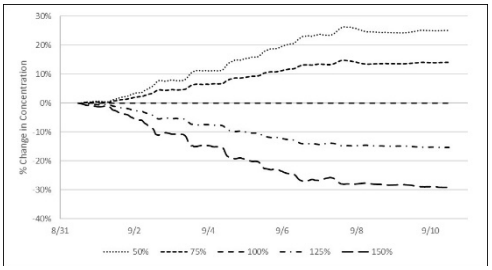

Fig. 5 – Change in overall average phosphine concentration (%) for five wind speeds, expressed as a percentage of the wind speeds (100%) used in the verification conducted between August 31 and September 9, 2015.

Increasing the wind speed by a factor of three (i.e., from 50% to 150%) decreased the overall average Ct-product by 27%, i.e., from 41600 ppm-h to 31700 ppm-h (Table 2). Doubling the wind speed of the silo (i.e., from 75 to 150%) decreased overall average Ct-products by 21%, from 39900 ppm-h to 31700 ppm-h.

Table 2 – Cumulative average Ct-products (ppm-h) and the difference from original (%) for wind speeds, expressed as a percentage of the wind speeds (100%) used in the verification conducted between August 31 and September 9, 2015.

	50%	75%	100%	125%	150%
Ct-product	41600	39900	37700	35200	31700
Percent Difference	10.4	6.0	0.0	-6.7	-13.3

For temperature and leakage effects, any change in conditions that resulted in an increased loss of fumigant had a smaller proportional change as higher amounts of loss were reached. In this case, the effect of increased wind speeds did not decrease at higher wind speeds, and in fact had a slightly larger impact on phosphine concentrations. The value in the exponent of the wind speed equation adapted for the model in Plumier et al. (2018) is more than three times the exponent in the temperature equations. Thus, the exponential effect of increasing wind speed will be much more pronounced than changes in temperature or leakage as seen previously. The exponential increase in fumigant concentration loss due to higher wind speeds was slightly more than enough to overcome the diminishing returns of increasing leakage, and will be more relevant given the likelihood that weather events can cause wind speed changes greater than those tested, which is unlikely for temperature. These results also agree with the results of Chayaprasert et al. (2015), which demonstrated increasing wind effects at higher velocities. The influence of wind speed on a fumigation is often more variable than the influence of ambient temperature, due to the overall percentage changes that occur. While the temperature effect continued to increase consistently over the course of the simulation, the wind effect was more dependent on varying weather conditions. The low wind speed conditions that were observed beginning on September 8 caused the effects of changing wind speed to level off, as seen in Fig. 5. These results indicate that weather events that cause high wind speeds are capable of having a large disruptive impact on phosphine concentrations in a silo. This points to the importance of best fumigation management practices such as seal testing a silo before a fumigation and monitoring gas concentrations during a fumigation. Monitoring phosphine concentrations helps detect increased fumigant loss due to sudden increases in wind speed.

4. Conclusions

- The verification demonstrates that the model effectively predicted the trend of phosphine concentrations, and predicted the overall Ct-product of the fumigation reasonably accurate.
- The accuracy of this fumigation model was found to be sufficient to use the model as a tool for conducting future simulations on predicting fumigant concentrations as a function of environmental conditions and operational variables.
- Increasing temperature and wind speed decreased phosphine concentrations, with temperature changes having a more significant impact overall than wind speed changes at tested levels. However, given the larger variability of wind effects possible beyond tested levels and the greater impact of increasing wind speeds relative to temperature, high wind weather events such as thunderstorms have the potential for substantial disruptive impact on phosphine concentrations.

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A Novel Engineering Design of Small Scale Metallic Silo for Food Safety in Rural India

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Abstract

Wheat is an essential component of the human diet for most of the world. In India wheat is an important staple food crop and it is used for the preparation of a diversity of products like *roti*, *parantha* (semi fried), *puri* (fried), bread, pasta, noodles, buiscuits etc. It has been reported that ~60-70% of wheat produced is stored at home or farm levels for domestic consumption. In order to understand the rural grain storage system, an extensive field study was carried out in villages of Haryana state (India). The field study revealed that ~95% of families store their grains in metallic silos of different sizes (300 to 2000 kg) and only Aluminium phosphide tablets (locally called sulfas) are used to protect grains from storage pests. Aluminium phosphide (AlP) tablets are used in an unscientific manner to control insect pest infestation, resulting in residues in stored grain. An experimental study of 12 months was carried out to identify the problems associated with pest management in conventional metallic silos. The storage period was divided into two parts, i.e., summer and winter, of 180 days each. Ambient temperature and relative humidity (RH) were recorded continuously for the entire period and temperature inside the silos was also recorded at different locations. The emergence of 'hot spots' was found during May-June when the temperature ranged from 37.6 to 42.7°C inside the silo during the summer season. During this period ambient temperature and RH ranged from 22.6-44.2°C and 37-82%. At this stage, convection current caused moisture migration at the top and bottom of the silo, whereas in the winter season moisture migration inside the silo was observed only at the top layer. Wheat samples from the topmost layer, in the vicinity of the "hot-spot" and from the bottom layers were collected and analyzed for various quality parameters.

The wheat samples near the "hot-spot" emergence were found most deteriorated in every aspect, for instance, in terms of protein content (decreased by 21.77%), fat content (decreased by 64.05%), germination capacity (decreased by 84.06%), thousand kernel weight (decreased by 22.09%), ash content (decreased by 41.96%), acidity (increased from 3.07-6.23 mm/gm) and insect-damaged kernels (increased by 80%). The results confirmed that even in a very small silo of 100 kg capacity if grains are stored without any fumigation treatment,

there exists the potential for moisture migration because of temperature fluctuation causing hot-spot formation, leading to grain quality deterioration.

Keeping in view the above aspects, an integrated engineering design of a double wall metallic silo with the special provision of a vertical perforated metallic tube in the centre was designed and fabricated. Tri-layer materials were tested for their thermal properties for fulfilling the needs of thermal insulation in the double wall silo. Wheat straw was found to be the best material in terms of thermal conductivity with a value of 0.040 W/mK. The special provision consisted of a removable string fitted with plates for keeping AlP tablets. To understand the function of the perforated tube in the centre of the silo for preserving stored wheat quality, 100 Kg of wheat (HD2733) was filled in this silo and after 12 months storage, wheat samples (at different depths inside the silo) were collected with the help of grain probes and mixed properly for quality parameter determination. Seed germination was determined before and after storage. It was found that germination decreased from 96% to 84%. Moisture content increased during storage from 9.8 to 12.7%. The initial kernel damage observed was 2-3% whereas after storage it was in the range of 13-15%. The initial lipid content recorded was 2.08% whereas after storage it was 1.4%. Also, the protein content decreased by 9.01%. Other parameters also showed quality degradation with time. The results were compared with the control (conventional) silo and it was found that the newly designed silo was better in terms of preventing insect infestation and quality deterioration. Also, the newly designed silo had a special provision for keeping AlP tablets suspended in the perforated tube to better control insects.

Future vision

The gap in technology transfer in India is increasing the chemical load on stored grain which can be minimized by incorporating small changes in the existing design of silos. To avoid the unnecessary repetitive use of AlP tablets, scientific knowledge should be developed and adopted, for example, on suitable wrapping/packaging material for AlP release at a slow rate over longer periods for effective control of insect pests in stored grains.

Keywords: Wheat, thermal conductivity, AlP, insect trap, design.

Food industry practices affecting Integrated Pest Management

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Abstract

Manufacturers of dry food products have a real challenge to exclude pests everywhere along the food chain because of the rather complex and different environments of food industry buildings. Current practices that influence pest presence and development in food industry facilities have been identified in the stages of food plant design, food ingredient reception and storage, processing or conditioning of finished food, and marketing. The preventive pest control measures in the food industry may be ineffective because of a non-observance of simple rules of good manufacturing practice (GMP), such as permanent control and monitoring of critical points or the ban of unsafe practices favourable to pest entry and infestation in food plants. The underutilization of methods for rapid assessment of pest presence and movement within food industry facilities, as well as the inability to rely on pest monitoring data for the economic damage threshold (EDT), are also underlined. Practical tools for processing data from pest monitoring systems should improve pest presence detection and alert. More realistic EDTs need to be proposed with direct links to decision-making support. More practical predictive models are also required for predicting the long-term efficacy and resilience of corrective control methods in food processing buildings, which should render the implementation of complex IPM programs easier.

Keywords: pests, food industry, manufacturing practices, food processing, IPM program

1. Introduction

Pest management practices in food industries are facing an important need to protect durable food products against pest infestation as many markets have very low pest-induced damage tolerance and are also subject to increasingly intense scrutiny through external inspections and audits. There are somewhat antagonistic trends such as less reliance on the use of residual pesticide treatments

and the demand for perfect food products, free of pesticide residues, which is becoming today one of the main challenges faced by the food industry in the field of pest management. However, food facilities typically are large complex structures with many locations vulnerable to insect pest infestation. They differ from each other in their activity or function (warehouse, mill, food processing plant, retail store, supermarket), in the concerned commodity (cereals, legums, animal-based materials, spices, dried fruits, cocoa), in the type of product generated (whole grain, flour, human food, pet food, confectionery, feed, etc.), in structure type (old or new, with variable construction material), in their equipment, in their geographic location and surrounding landscape, etc. This makes generalization about the pest infestation risks extremely complex and difficult: pest situation of a particular food industry facility has very specific characteristics for a given location.

Pest management in food facilities is a prerequisite for achieving food safety and food hygiene considering the scope of global quality assurance systems (HACCP). Recent regulations (EU Hygiene package and US Food Safety Modernization Act of FDA) aim at enforcing the application of HACCP systems to all food chains and in all plants, distribution centers and grocer's or retail stores. The main objectives of this paper consist in the analysis of the practices in the food industry affecting the risk level for both pest infestation risk and decision-making process for IPM related to food hygiene HACCP system conception and implementation, specifically adapted to the dry food industry sector.

2. Pest exclusion measures and sanitation in food industry facilities

Most buildings provide three main attractions for pests: shelter, food and warmth. It is commonly assumed that older buildings are more prone to infestation, but new buildings with enclosed roof spaces, suspended ceilings, wall cavities, panelling, raised floors, service ducts and lift shafts provide a large number of harbourages – with many interconnections – allowing a wide range of internal movement for pests. Most pests actually require very small amounts of food – an adult mouse for example, can survive on as little as 3 grams a day. A few degrees increase in temperature may be sufficient to encourage infestation, particularly in winter months. A master sanitation schedule is a vital component that influences pest management in the food industries. Importance of sanitation programs, and constant requirement for training personnel to implement sanitation practices are essential.

Elimination of pest refuges and pest colony "nests"

Harbourage of insect colonies

Constant monitoring of insects with different techniques and particular attention on behalf of staff prevent heavy infestations. This was accomplished by limiting Lepidoptera and Coleoptera populations by intensive trapping with pheromone and food traps, by examining tracks on dust left on floors or machine supports, substituting wooden structures with metallic ones, sealing cracks and crevices in walls and floors and replacing screw conveyors with pneumatic (fluid-lift) conveyors. Some elements in building structures and machinery needed to be changed or replaced (*e.g.,* gaskets). Crevices in which debris could accumulate must be sealed, wall edges and column floor junctions shoud be modified to avoid food particles accumulation.

Cleaning and hygiene maintenance

The removal of debris is more efficient than any localized chemical treatment. Only by controlling the entire processing cycle, from the purchase of raw material to the distribution of the finished product, will it be possible to reduce the risk of infestation. Nowadays, a few quality managers of food industries consider the problem of maintaining proper hygienic conditions as really important, although it represents the first step in reducing pest infestations. However, in many cases, standard cleaning procedures were modified but staff was not trained to clean the least accessible areas that are generally neglected. Therefore these are sure to be sources of infestation, and thus being considered as a potential critical control point. The most vulnerable points may be identified by

visual inspection of trained personnel, or better by an external audit carried out by a sanitation specialist. The attention of all staff should be drawn to the importance of cleanliness as it is their duty to adhere to these recommendations.

Influence of physical condition control

Site location and structure type design

Knowing that some pest infestation risks can originate from the proximate environment of any food plant, the perimeter around all structures and between structures should be kept free of vegetation and better with a concrete pavement of minimum one meter wide. The basement walls of food plant buildings should be "insect proof" at the junction with the steel cladding of the building wall. The repair of these damages creates critical entry points for pests that need to be quickly achieved and visual inspection of the exterior of the buildings should be easy all around. Where a new construction is being considered, an assessment of activities and the environment in the proximity to the proposed site must be made. Landfill sites, watercourses, marshlands, derelict sites, farms are examples of activities that regularly generate pest activity. When an old industrial building is re-used, the previous use of the site and its pest history must be considered. Thus, buildings that have previously been used in the food industry are most likely to have a pest history. Retrospective repair is far harder to accomplish once production has started and is running and when the construction company no longer has a presence on site. As a formal rule, no food should be allowed on to the site being constructed.

Temperature and air-conditoned manufacturing units

The population dynamics of stored product insect pest such as *Plodia interpunctella* or *Tribolium* spp. - which are common species in food industry facilities - is at their optimum in the range of 25-30°C. In factories producing cooked products (such as biscuits or bread), ambient temperature may be in this range all the year, especially in the rooms where cooking ovens produce heat. These areas have an increase risk of insect pest presence such as *P. interpunctella* which may lay eggs on the product after cooking. As an example of risky situations, when a belt covered with cooling biscuits stops (because of a technical issue), cooled biscuits are available to *P. interpunctella* female for egg deposition. One solution to this issue is to cool the food production areas with unprotected food flow to below the lower threshold of moving activity of flying insects (*P. interpunctella* or *Ephestia* spp. or *Stegobium paniceum*), *i.e.,* below 15°C. Below this lower limit, insects remain quiet and do not lay eggs on the produce before wrapping (*e.g.,* biscuits) and packaging. Consequently, air conditioning production areas to temperatures of 14-15°C or lower is a recommended practice that inhibits insect movements.

Internal and external lighting of the buildings

The type of lighting at a premise will, to a certain extent, determine the attractiveness of the site to flying insects. Most attractive types are mercury-vapour lamps and special fluorescent lamps used for perfect colour rendition. Next come "ordinary" commercial and household fluorescent tubes. The warmth of infrared light is also attractive to insects, although the area of attraction surrounding the source will probably extend only for a few meters. High-pressure sodium-vapour lamps, however, emit very little UV or IR and are currently thought to be the least attractive to insects. Unfortunately, these lamps give an orange light and cannot be used where the recognition of colours is important. It is recommended that an absolute minimum amount of lighting is physically attached to the building. Instead, position lights 5 or 6 meters away and direct lighting towards doorways. Apart from the obvious benefits of attracting insects away from the building, there are also benefits to be obtained in making the building less attractive to geckos, bats and birds that often roost and nest on such lighting structures due to their warmth. Lighting just inside doorways and in loading bays should be high-pressure sodium-vapour or low wattage incandescent bulbs.

White or light yellow surfaces of buildings should be avoided due to their ability to reflect UV light. Darker blue or green colours are preferable.

Exterior environment of food industry buildings

Perimeter security fences are generally of chain-link, wire mesh, weld-mesh or metal railing construction. These should be set into concrete footings to prevent mammals gaining entry under the fence. In the immediate building perimeter, concrete pathways are preferable to gravel pathways as gravel could be burrowed into by rodents despite of the ability of gravel to back fill on itself. Paving slabs are often laid on sand, which is conducive to infestation by ants and allows mole gallery digging.

Water drainage

Pooling water from overflow will encourage various pests, particularly flies. A readily available source of water is also a requirement for successful rat populations. Good drainage of land is required to avoid waterlogged soil. Certain insect pests (*e.g.,* cockroaches) rely on a water source for breeding. Grids should be designed so that waste materials can pass through easily and they can be removed easily for cleaning.

Increased risk of infestation by exterior environment

It is not advisable to plant trees or bushes near a food facility that will result in direct contact of tree leaves and branches with the exterior wall of the facility. This should be systematically avoided, because foliage provides excellent harbourage for many pest species. At a respectable distance from the walls, preference should be given to plants that shed the least seeds and fruits. Seeds and fruit may initially attract and then support insects, rats and mice, and various pest birds. Shrubs and trees should be of a coniferous type (releasing flavor repulsive for a range of food industry related insects). Leaf fall from deciduous trees that accumulates in guttering will restrict the run-off of rainwater and may give rise to localised infestations of insects that rely on standing water to breed, for example midges and mosquitoes. Leaves that accumulate along foundations provide harbourage and sheltered runs for rats and mice. Tree limbs and branches should be at least 2 m away from building exteriors (3 m if squirrels are a problem). Plants should not be planted too densely. Dense ground cover will provide cover and harbourage for rodent pests. Access in between shrubs is important for pest control inspection. Vegetation should not encroach within 5 m from any outside wall of a building. Rural vegetation can aggravate both rodent and insect pests. Climbing plants should not be planted against the walls of buildings. These could create entry routes for pest rodents, harbourage for pest bird species and entry routes for some insect pests. Grass should be kept closely cut at all times. Long grass will offer cover and harbourage for rodent pests. Raining water downspouts are easy ways for rodents to climb near the roof of the buildings to reach access to the space between the roof and the wall existing in numerous buildings.

Risks related to building structure design

Wall foundations must be taken down to a solid bottom at least 80 cm below ground level and concrete laid between the walls to prevent rodents burrowing into the building. The addition of a concrete curtain wall to a depth of 60 cm will protect the foundations against rodent ingress. It may be appropriate to apply a band of “non-friction” material 1 meter above ground level to prevent rodents climbing external walls. Airbricks supply ventilation to walled cavities but they also may allow mice and insect pests access. Pre-formed corrugated cladding should be avoided as corrugations are difficult to seal adequately against pest entry at the point where they meet conventional walling. An epoxy-resin type material should be considered. The external surface of walling should have no ledges because ledges may provide suitable day or nighttime roosts for pest bird species. For the same reason, over-developed external wall fascia should be avoided. The internal surface of walling should have no ledges. Ledges provide suitable areas for product residues

to accumulate and are difficult to access for cleaning. All drains should be accessible (from visit 'openings') and facilitate flushing and rodding. Special attention must be given to vertical ducts that pass between floors. Ducting may also allow rodent and insect pests free movement between floors (Troller, 1993).

Interior design of food plants and stores for pest-proofing

Floor, walls and ceilings design and colour

Tiled flooring is not recommended. All expansion joints should be well sealed and sealing material should be made from a material that allows for movement. Flooring under equipment (sur-elevated from the floor) should be completely smooth to allow thorough removal of waste material. Covings at wall to floor junctions reduce the accumulation of debris and facilitate effective cleaning. All cracks and crevices should be sealed to prevent the accumulation of product residues that provide insect breeding sites. Buildings are often designed in a way hard to reach for regular cleaning, for example roofs or very high ceilings, accumulate dust and debris and serve as a harborage for pests. So, one of the key industry practices that affect pest management is the building design.

Available access of pests to food and/or water inside food facilities

As rodents, birds and cockroaches rely on a supply of drinking water, sources of free water should be avoided. Any pools on concrete floors or on flat roofs have to be removed. Drainage channels should be sufficiently wide to accommodate expected volumes. They should be fitted with drainage grills that do not clog with waste and are easily removed for cleaning. The ends of drainage channels should be buttressed so that waste does not accumulate. Rainwater down pipes should be fitted externally; rodent entry into a down pipe from the ground can be prevented by the use of a back inlet gully. Pipes and cables, *i.e.,* gas, electric and water, must be tightly sealed where they pass through walls as rodents may gain entry via this route. Ducts can be sub-divided to prevent rodents gaining access along their length.

Doors, windows and portal apertures

Exit doors should be a good fit and self-closing; with a sensor to detect if the door has been properly open. Rats and mice can move around within a building via gaps that exist below doors. Roll-up doors should be fitted with a flexible bottom "seal" and T extensions to fit rail tracks. The use of strip curtain doors or rubber flap-back doors around external wall door openings should be avoided. Automatic high-speed roller doors are preferable but their timing needs to be adjusted so that they are open for the minimum amount of time. Vehicle loading ports should be adequately sealed once trailers have docked, and the port doors should not be opened until trailers are completely in position. Open loading ports equipped with lights will attract night flying and daytime flying insects. Installing doors that have hollow frames is not a recommended practice. Mice may use hollow doorframes as harbourage. Insects can breed in the accumulated food debris inside the base of the frame. Although opening windows can be adequately screened against flying insect ingress, air conditioning with light positive pressure inside the building is preferable. Nevertheless, a useful device to protect buildings from flying insect entry is the air curtain. Especially points of lorry loading openings, where doors are not very tightly closed, can be effectively protected from flying insects by this device. Outside air containing flying insects can be drawn into buildings that have negative pressures. Pest birds may use window ledges as day or nighttime roosts. Ceiling voids are potential harbourages for pests. Enclosed voids can also make inspection for pests difficult. GMP compliances for point of entries and common sense practices can eliminate pest infestation.

Storage of food products above ground level

Racking should be used to keep all goods off the floor. Raising goods will also allow effective cleaning. Adequate space around racking should be allowed. This will facilitate good pest control

inspection and allow for thorough cleaning. The pillars supporting the rack for pallets of raw food commodities are often protected from shocks by metallic shields that may house dust and food ingredients. These pods of pallet racks must be regularly cleaned to avoid insect colonies to establish in such protected locations. Adequate space between racking bays should be provided. This will allow for good pest control inspection and allow for thorough cleaning. Good stock rotation methods should be enforced. A minimum quantity of ingredients/packaging should be kept in stock; it is preferrable to have suppliers who are flexible enough to supply on demand. The use of pallets constructed of wood should progressively be replaced by the use of plastic pallets. Storage shelving should not have concealed cavities. If spillages cannot be cleaned easily, pests may make use of them to conceal their harbourages. Cleaning floors and wall basis must be regularly carried out and if possible each day.

Organization of food product chain

Food processing chain organization

The major principle of product flow organization in a food processing plant is that raw material and processed or finished produts should not be in close proximity. The strict separation of raw and processed product is essential to avoid contamination of any kind. The GMP recommendation for product flow direction in the process area is in compliance with the "go forward" principle, without raw ingredients that never cross processed or semi-processed food line. Because insect pest development cycles last a minimum of one month in indoor conditions, raw food commodities must be kept a minimum period of time in storage workshops. So, in all storage rooms, the product flow must comply with the "first in, first out" principle so that the stock rotation should be as short as possible. On the line for dry food product processing, there is a critical need to ensure a sanitary environment.

Cleaning material and equipment

The cleaning should focus on ingredients and dough fragments that have fallen down and have accumulated below the conveying belts or are sticking on belt support rollers. All residues in machinery should be removed at a regular interval (*e.g.,* each day) and the whole machinery should be thouroughly cleaned after each change in product type or before long shut-down periods. The food products waiting on a stopped conveyor for more than half an hour should be immediately removed and should not be stored in open containers close to the processing chain. Equipment which is to be taken out of production for a long period of time must be thoroughly cleaned to remove all food residues. All these cleaning practices are part of GMP and comply with the principles of proper sanitation in the food system sustained by recent regulations such as the Food Safety Modernization Act, enacted in 2011, or the EU Food Hygiene regulation package (Anonymous, 2004).

Underused packaging and food materials

Little used ingredients and packaging material are more likely to have pest activity develop in them and to be used by pests as harbourage. As an example, corrugated cardboard material temporarily stored in a food processing area may be a perfect refuge for migrant larvae of the Indian meal moth.

Isolation and treatment of infested commodities and out-of-use material

The construction of a quarantine building is recommended for the isolation of infested commodities or commodities that are being received from a suspect supplier. Returned goods should be stored in their own quarantine area away from ingredients, packaging and finished goods - ideally in a separate building unconnected to main production and warehousing areas. When food processing or packaging material is out of use, this equipment always remains attractive from food product or food dust deposit inside, which may attract flying pests. This "out-of-use" equipment should be

rapidly disposed off from workshops containing raw ingredients or processed food.

Packaging defaults (imperfect insect proofing)

Finished food produced from food processing plants is susceptible to quick infestation all along the marketing channels if packaging material is permeable to food flavor. This permeability to food flavor is a common weakness of a lot of cheap packaging films that are used to package finished food products. The result of such permeability generally is a rapid localisation of appropriate feed substrate by flying insects (*e.g., Plodia interpunctella*) or by rodents. Additionally, certain types of package (cardboard cassette and boxes with flexible pouring spout, or bags with wide aperture without resealing system after first use) are no more preventing insect entry after first aperture and the first pick up of food.

3. Early detection of pest presence and monitoring insect pest density

Identification of vulnerable situations for pest in food industry

Visual inspection "corridor" between products, machinery and walls

In storerooms, stacking of goods should be about 30 – 50 cm away from walls to allow free access to the area behind for inspection and cleaning. Strict segregation is required between raw materials, food processing areas, finished food products, and packaging zone to prevent cross-contamination. Plant and other equipment must be free of infestation before being brought on site. Rubbish storage areas must be kept tidy, using only close-fitting containers regularly emptied.

Management of waste

Waste areas should be sited more than 10 m away from the main building in order that any pests that may be attracted are kept at a distance. All waste bins should have tight fitting lids which must be kept closed at all times. If individual bins or skips are not covered, then the area should be enclosed within a mesh cage to prevent access by birds. Waste skips should be placed on a concrete pad to prevent rats burrowing underneath and be situated on rails of a height that will allow for thorough cleaning below.

Factors limiting IPM strategies implementation in the food industry

Full implementation of the IPM approach requires more effort than other types of control programmes, but once in place, it can be used to make more reliable pest management decisions. Unfortunately, many of the studies reported in the literature have been achieved under laboratory conditions, so there is limited information on their integration under field conditions. The IPM strategy is based on corrective intervention in dependence on EDT.

Self-determination of EDT and decision support tools use

Relationship between monitoring data and pest infestation level prediction

Many of the components of an IPM programme are known and are available for use, but our understanding of how to optimally integrate and target these tactics as part of IPM is limited. An IPM program is an evolving process that applies local intelligence and responds to changing needs (Pinniger and Child, 2002). Adoption has also been hindered by: i/ a poor understanding of pest population displacement in the spatially and temporally complex landscapes where food is processed and stored; ii/ the difficulty of evaluating pest populations; iii/ the limited information on structure treatment efficacy, and iv/ how to optimally select and combine management tools. Many questions remain about the use of these tools: from the very practical issues such as how many traps are needed and which types work best, to fundamental issues concerning the relationship between trap captures and pest population density, distribution and level of infestation.

- **Strengthening pest monitoring programs for food industry**

Insect monitoring is a primordial component of pest management in food processing plants (Campbell et al., 2002). Economic losses due to insects and unnecessary pest management expenses can be avoided using insect monitoring and decision-making tools related to risk prediction by the assessment of EDT, predictive models of pest populations density changes over time, and expert systems to determine the best time and way to suppress pest populations (Arthur and Phillips, 2003; Fleurat-Lessard, 2011). Computer simulation models can be used to compare the effectiveness of different pest management methods, alone or in combination, for stored-product insects. These models can also be used to evaluate the effectiveness of different implementation options, and to optimise the timing of pest management programs for stored-product insects (Fleurat-Lessard, 2011; Campbell et al., 2012). Currently, computer simulation models are available primarily for insect pests of stored grain, but in the future such models should be particularly useful in decison-making for pest management strategies for dry food processing and marketing chains (Trematera, 2013).

4. Modern tools to be integrated in IPM programs for pest risk minimization

As stated by Adam et al. (2006) in the case of implementation of IPM in stored-grain, many quality managers of food plants have not yet adopted IPM practices for many reasons: additional cost or personel implication, minimum required knowledge, difficulty to adopt a new technology by the managers, pressure of pesticide supplier or fumigation company, etc. Limited acceptance of IPM in food facilities is partially explained by a combination of the costs of corrective pest control interventions, difficulties in sampling properly, unreliable data, and difficulties encountered in the calculation of meaningful EDT. Precise treatment thresholds and economic injury levels have not been completely established for operational practice, and standards and rejection criteria are inconsistent and difficult to apply. As a result, treatments based on an economic threshold are not typically performed and control strategies are often applied preventatively, even when using tactics that do not have any residual effect. In current practice, many locations still rely on calendar-based pesticide applications and have little understanding of the basis of pest management. Nevertheless, most of the risks of infestation of food industry plants by noxious pests listed above may be controlled by customized application of IPM programs covering the four components of dry Food Quality and Safety Assurance from raw commodities to finished food products (Tab. 1). Combining and integrating different management tools and careful selection and timing of different approaches, together with an understanding of pest behaviour and ecology, should result in a greater effectiveness and more accurate solutions to pest presence in finished food.

Peculiarities of bulk-stored commodities

For bulk-stored commodities, and particularly in commercial elevators, it is often difficult to adequately monitor large grain bulks due to the need to directly sample the large volume of grain and detect relatively low densities of insects. Collecting samples may only give information on insect presence when relatively high densities are present. The lower limit of density that can be expected from bulk sample examination is evaluated at one insect per 2 kg of raw material (as grain) (Fleurat-Lessard, 2011). This is already a high level of infestation and much higher than most of the tolerable EDT (more often fixed at one insect per 5 kg of grain). As grain products move from bulk storage to processing and milling facilities, then through distribution and marketing channels to consumers, the concept of EDT becomes more difficult to apply. When there is 'zero tolerance' for insects, controls become more preventative, but it is not very realistic. More often with bulk raw commodities, there are no precise damage thresholds or injury levels, and it may be difficult to adequately determine pest levels or to estimate all of the direct and indirect costs of corrective interventions. In this context, there is reluctance or lack of interest on the part of the food grain storage and handling industry to move away from calendar-based pesticide treatments to a more integrated approach, based on prevention rather than control after EDT is reached. This is due, in

large part, to a justifiable concern about making mistakes with pest control in an industry with an extremely low pest threshold requirement.

Difficulty of applying biocontrol agents in the food industry buildings

The artificial nature of food chain environments and low tolerance in many situations for the presence of insects, means IPM relies less on promoting population regulation using natural enemies and puts greater focus on modifying the environment to make it less favourable for pest establishment and persistence. The exception to this is bulk storage, where biological control shows more potential for success since some insects can be tolerated in many situations and natural enemies can be cleaned out of the material before processing (Schöller and Flinn, 2000; Phillips and Throne, 2010).

A summary of the more promising modern tools that may be integrated to IPM programs for the food industry is described in Tab. 1. The IPM concept is a whole system based on risk prevention, monitoring and prevision including pest resistance management, use of selective chemical treatments, use of corrective intervention thresholds, and promoting environmental sustainability.

5. Further research needs for larger implementation of IPM in the food industry

Research should optimise or further develop other semiochemicals (attractants and repellents) to aid in the monitoring of some stored-product insects and to provide new biocontrol tools. In this regard, future stored-product protection combinations of repellents and attractants may also find use in push-pull tactics (Cook et al., 2007). Push-pull strategies involve the behavioural manipulation of insect pests and their natural enemies via the integration of stimuli that act to make the protected resource unattractive or unsuitable to the pest (push) whilst luring them towards an attractive source (pull) from where the pests are subsequently removed. Deterrent or repellent semiochemicals can be used to discourage pests from entering a premise, while at the same time, attractants or stimulants can encourage pests to congregate in an adjacent area where they can be controlled more effectively and safely by chemical pesticides or biocontrol agents. Computer, smartphone and touchpad applications affording a practical and user-friendly support in building IPM specific programs and on-line advice for risk assessment and prevention should become accessible to food industry quality managers in the near future.

Tab. 1 IPM more recent tools that may be integrated in IPM programs for the food industry.

IPM component	Actions for risk management	Alternative tool	Main advantage	Main constraint
Identification of critical pest entry points in food industry facilities	Identification of critical points by which insect pests can penetrate into the facility	Interpretation of trap network data with geographical positioning system (GPS)	Accurate detection of the core of infestation	Each trap bar-coding and GPS positioning of each trap
		Localisation of loci of pest infestation by contour mapping from trap catches	Accurate localisation of infested goods	Weekly trap data processing
Pest exclusion measures for risk prevention	Sanitation measures especially at pest entry points and regular inspection and surveillance of identified CCP. Regulation of physical conditions	Low temperature and RH in working areas when free-access food is on the chain	Corrective treatment never needed	Air conditioning of all rooms
		Pest-proof packaging film and structure for finished food products for sale	Protection from pests in the marketing channels	Bioassay to carry out for food bag or box testing insect proof properties
Permanent monitoring for risk prediction	Identification of infestation locations inside the building, processing equipment and machinery	Enhanced strategies of pheromone use: mass-trapping and attract-and-kill strategies	Effective means of surveillance for flying insects	Not adapted to limit crawling beetles populations
		Permeation of food facility atmosphere with pheromone for mating-disruption or auto-confusion	Effective against flying insects	Slow reduction of pest population expensive renewal of dispensers
		Use of electronic devices detecting very low level of insect density in bulked commodities	Early detection especially for grain insect pests	Only useful for insect detection in bulked commodities
		Prevision of pest density changes over time by predictive models from physical-chemical parameters or conditions	Calculation of safe storage time before EDT reaching	Collection of daily data of temperature and RH for model feeding
Application of pest control measures (when EDT is reached)	Selection of non-chemical solutions rather than chemical disinfestation means develop the use of biocontrol or beneficial agents	Pheromone trap use for auto-inoculation-release of a microbial pesticide	Self-function device	Expensive and slow in action
		Improvement of efficacy of registered pesticides by combination with mineral products or biorationals	Lower risk of chemical residues in food	Preventive action; weak curative effect
		Replacement of surface or space treatments with chemicals by bio-control agents or biopesticides	Targeting more specific pest species than chemical pesticides	Difficulties to register for use in food processing plants
		Use of physical treatment as alternative to fumigation (microwave heating, temporary freezing, controlled- and modified-atmospheres)	Complete disinfestation process with neither persitence nor residual effect	Competitive only for high value commodities (e.g., medicinal plants and spices)
		New fumigants for whole structure, plant or warehouse disinfestation (SO_2F_2, methyl iodide, ethyl formate ...)	Complete disinfestation of food plants or stores in a sigle fumigation	Minimal airtightness of buildings required; manager reluctance
		Use of natural pesticides of microbial or fungal origin, a vegetal extract or an essential oil (EO)	Short period of remanence (activity and smell) for the most volatile EO	Difficulty to register fomulations from a few number of active substances
		New formulation or conditioning of phosphine controlled-release phosphine gas by automatic equipment	More practical implementation and control of fumigant doses	Managers reluctance to regularly use toxic gas at a high concentration
		Replacement of fumigation of food-processing plants by heat disinfestation	Complete disinfestation through one application	Stop of the working activity during one day minimum

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Abbreviations

EDT=Economic Damage Threshold; EU=European Union; FDA US=Food & Drug Administration; GMP =Good manufacturing practices; IPM=Integrated Pest Managment; IR=Infra-red radiation; HACCP=Hazard Analysis Critical Control Point; IMM=Indian meal moth; UV=Ultra-violet light

Static and Dynamic Stress Analysis of Flat Bottom-Bamboo Reinforced Concrete Silo for Rough Rice Storage

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Abstract

Concrete silos are one of the most robust and reliable structures for grain storage in tropical countries. This study analysed the structural behavior of a low cost, flat bottom bamboo-reinforced concrete (BRC) silo for rough rice storage. This research included the design and development of a BRC silo in accordance with the guidelines mentioned by the Indian Standard (IS) codes. The Finite Element Method was employed to develop the stress profile in the silo walls under "grain at rest" and "grain filling" conditions. The results obtained were further compared with experimental results, classic silo theories (Janssen's theory) and standards of different countries in the world. The numerical technique gave stress magnitudes very close to those of the experimental results. The classic theories as well as the standards of different countries predicted an over estimation of the magnitude of stresses in the BRC silo. This would result in extra cost of construction of BRC silos. The study also suggested that the vertical stresses were most predominant under static and filling conditions. Maximum stresses were developed at the silo bottom. This study is expected to aid the development of economical silos with minimum wall thickness and material requirement which are ideal for on-site construction and use by smallholder farmers.

Keywords: Bamboo reinforced concrete silos, rough rice, finite element method, stress profile

1. Introduction

Food grain silos are highly efficient in storing bulk grains for a long period of time and safe from deteriorating agents such as rodents and insect pests. Modern silos are generally made of materials such as galvanized steel, reinforced cement concrete, painted Aluminum, plastic etc. If designed properly, these structures provide hermetic conditions ideal for safe storage of food grains, ensuring

minimal storage induced losses and improved food security. Bamboo based grain storage structures have been popular among Indian farmers since time immemorial. These include traditional bamboo baskets, mud plastered bamboo bins, bamboo-reinforced concrete bins etc. Bamboo based concrete structures have gained popularity in the present time and have been judged as an environmental friendly and sustainable technology (Holani, 2001).

In spite of several studies being performed on grain storage silos, the structural behavior of certain designs is still unclear. The complexities associated with different types of grain silos can be mainly attributed to the nature of the bulk material being stored (moisture content, internal friction, bulk density, grain shape etc), the nature of the containment structure (material, shape, dimensions etc) and the interactive effects between the stored material and the structure walls. Classic silo theories such as Janssen's and Reimbert's theories fail to address the effect of these critical factors. International standards for silo designs such as the Indian Standard codes, Eurocode and Australian Standards are also designed based on these classic theories. While experimental trials are the most accurate method of studying silo behavior during grain storage, the high cost associated with their construction and automation discourages researchers from utilizing them.

Recent studies have reported numerical methods such as finite element method (FEM) and discrete element method (DEM) as reliable techniques for modelling of grain silo phenomena such as silo filling and discharge (Gallego et al., 2015), granular flow patterns (Wang et al., 2013), buckling (Zaccari and Cudemo, 2016) and development of innovative silo shapes (Anand et al., 2008). While continuum based FEM is ideal for the prediction of stresses developed on silo walls, the discretization based DEM has been found to be suitable for modelling granular flow of grains in and out of silos (Rotter et al., 1998). FEM is highly efficient in developing a realistic elastoplastic behavior of stored grains as a continuum medium and predicting its effect on the silo body. More recent research has occurred applying FEM for modelling stresses developed in silos with different planforms (square, rectangular), construction material (steel, concrete, polymethylacrylate), eccentric discharge, and flat or hopper bottoms. Advances have been made in simulating the interactive effects between grain-silo walls from node-node contact to surface-surface contact algorithms.

This study aimed at designing and developing a bamboo reinforced concrete silo of 1000 kg capacity to be used for rough rice storage at the farm level. A 3D FEM model was developed for the prediction of wall pressures in this intermediate slenderness silo, under static and filling conditions of rough rice. The predicted pressure values have been further compared with the results of classic silo theories, standard codes as well as experimental values.

2. Materials and methods

2.1 Stored granular material

The paddy to be stored inside the BRC silo was procured fresh from the experimental farm of the Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur. A high amylose, long variety of paddy, IR-36, was chosen for the present study, considering its popularity and ease of availability in the region. The paddy was thoroughly cleaned to ensure the best quality. The paddy was tested for its moisture content prior to storage.

2.2 Experimental determination of FEM input parameters

The results of the finite element method is highly dependent on the input parameters associated with the particles/bodies involved in the system concerned. The physical properties of the granular material was directly obtained from the works done at the Central Institute of Agricultural Engineering, Bhopal (Reddy and Chakraverty, 2004). The poisson's ratio of the paddy was determined from the K_o test (Moya et al., 2002), wherein, K_o, is the lateral pressure ratio. The modulus

of elasticity of the paddy grains were obtained from the triaxial test described by the same group of authors. The values of various parameters used in this study have been tabulated below:

Tab. 1 Input parameters for paddy.

Material parameter	Values	Source
Grain unit weight (kN/m^3)	5.638	Experimental procedure
Angle of repose (˚)	42·35	Reddy and Chakraverty (2004)
Wall friction coefficient	0.5	IS 4995: 1974- Part 1
Modulus of elasticity (kPa)	10,000	Moya et al. (2006)
Poisson's ratio	0.2	Moya et al. (2006)

Tab. 2 Input parameters for concrete and bamboo reinforcement.

Material parameter	Values	Source
Concrete (M20 grade)		
Concrete unit weight (kN/m^3)	22.55	IS 456:2000
Modulus of elasticity (kPa)	2.23 x 10^7	IS 456:2000
Poisson's ratio	0.18	IS 456:2000
Bamboo		
Bamboo specific weight (kN/m^3)	8.825	Agarwal et al. (2014)
Modulus of elasticity (kPa)	2.44 x 10^7	Agarwal et al. (2014)
Poisson's ratio	0.28	Agarwal et al. (2014)

2.3 Finite Element Method

Material models

The stress profile developed on the BRC silo walls was developed in ANSYS software (ANSYS, 2018) reproducing the real silo dimensions and geometry. The reinforcing bamboo strips were generated in the design modeler and their properties were assigned. A linear elastic material model was used to describe the concrete as the non-linearity arises only under high levels of stresses. The granular material stored in the silo was described using an elastoplastic material model, wherein the elastic portion was described using the isotropic linear elastic model, while the plastic portion was described by the Drucker-Prager model (Drucker and Prager, 1952). The material parameters involved in these models have been selected according to Gallego et al. (2010). The Coloumb friction model was selected to model the interaction between paddy grains and the silo walls, wherein the wall friction coefficient was the most relevant parameter.

Types of ANSYS elements

The ANSYS elements are highly effective in modelling the physics of the problem and in the present study different elements have been assigned to the individual silo system components. The silo body, made of concrete was assigned the element type SOLID186 while the reinforced bamboo strips were modelled using LINK180 elements. The SOLID186 element is a 3D homogenous structural solid with 20 nodes supporting features such as plasticity, stress stiffening, and mixed formulation capability for simulating deformation of nearly incompressible elastoplastic material. The LINK180 element on the other hand is a 3D spar, tension-compression element with nodes having three degrees of freedom each. The grain body was assigned the element type SOLID187, a 3D 10 node element with the ability to model irregular meshes. The contact algorithm between the silo walls and grains were described using pair based contact elements (CONTA173 and TARGE170). The CONTA173 can model contact and sliding between a 3D rigid body and a deformable body.

Boundary conditions

Only two boundary conditions were employed for modelling the stress profile in BRC silo walls:

- Fixed support at the silo bottom (constrained rigid body motion) and
- Bulk unit weight of the stored paddy.

During the filling process, all nodes at the silo bottom were restrained from motion in any direction (Gallego et al., 2010). Prior studies by the same group of authors had reported that progressive filling yields better results than en masse filling. The grain filling process was modelled using the sequential activation of subsequent grain layers through the birth and death feature of ANSYS (Gallego et al., 2015). The Newton Raphson procedure was adopted to solve the set of nonlinear equations during the entire process.

3. Results and discussion

3.1 Description of silo design

An intermediate slenderness, bamboo-reinforced concrete (BRC) silo of 1000 kg capacity as shown in Fig. 1 was developed at the Indian Institute of Technology Kharagpur. It was comprised of a domical roof, silo body and a base support. The grain is loaded from the top opening (40 x 40 cm) on the domical roof and the discharge done through an inclined chute (15 cm φ) at the bottom of the silo wall. The domical roof was made of concrete of 8 cm thickness. The cylindrical silo body was made of a bamboo framework reinforced in cement concrete. The frame work consisted of vertical and circumferential bamboo strips with trapezoidal cross section (2 x 1 x 0.5 cm). The bamboo strips were coated with a layer of araldite and sand spray to ensure better adherence with concrete. The thickness of the silo wall was limited to 12 cm. The entire silo was supported on a circular base of 40 cm height and a diameter of 2 m. This ensured protection from water seepage and inaccessibility to rodents.

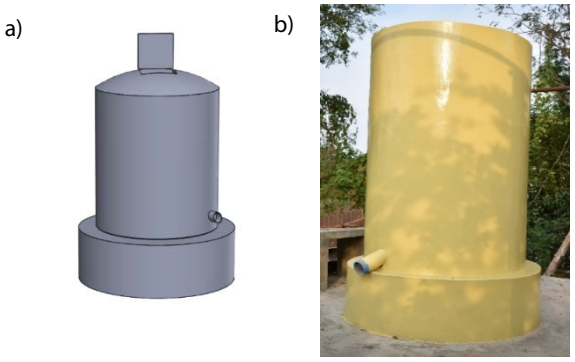

Fig. 1 Bamboo-reinforced concrete silo for rough rice storage: a) 3D representation b) Silo erected on the field.

3.2 Lateral and vertical pressures along the BRC silo walls under static condition

The lateral and vertical pressures prevalent in the BRC silo under static state are depicted in Fig. 2. The wall pressure values predicted by the FEM model were lesser than those estimated using the design codes or Janssen's equation. This could be due to the differences in the input parameters of the material models used. Several studies have reported that the material model adopted has the least effect on the pressures developed in a flat bottom silo. However, the poisson's ratio (ν), lateral pressure ratio (k) and wall friction coefficient (μ) employed in the models significantly influence the pressure profile obtained. For instance, the k values adopted in the Indian standard code and Eurocode (EN1991-4) are 0.5 and 0.63, respectively, while one adopted in the numerical model was 0.47. The pressure values were normalized in order to reduce the redundancy in the data values. Finite element analysis suggested a peak pressure at the silo bottom, which was not accounted for in any other method of estimation. Varying the mesh density at the silo base did not affect this peak

pressure. The results suggested that the wall thickness of 15 cm for concrete grain silos, suggested in the Indian Standard codes, is an over estimation and would result in additional costs for economically constraints farmers. A wall thickness of 10 cm was sufficient to take the grain loads, static and dynamic, which were well within the permissible strength of concrete. However, this finding has to be supplemented with studies related to the life cycle assessment of the BRC silo.

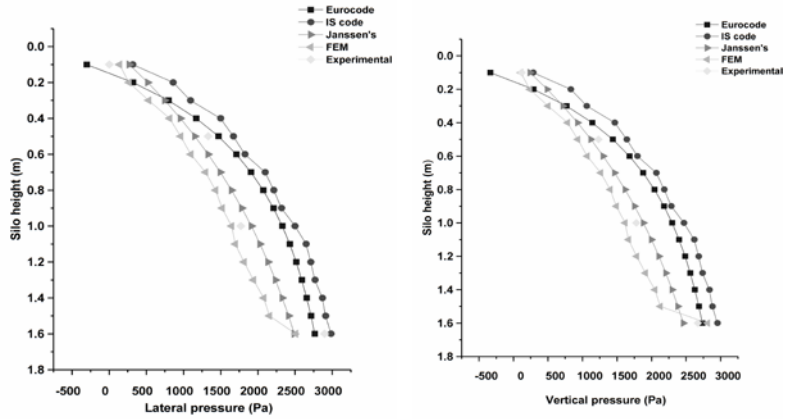

Fig. 2 Lateral and vertical pressures acting on the BRC silo wall under static conditions.

3.3 Lateral and vertical pressures along the BRC silo walls under filling conditions

The pressure profile of the silo walls is illustrated in Fig. 3. Significant distortions were observed in the pressure values predicted by FEM. These values were normalized and the modified values were represented in the chart. These distortion could be a result of numerical issues, roughness of meshes, or differences developed in the subsequent grain layers during filling. Previous studies have reported similar kind of leaps in FE values during grain filling in metal silos (Gallego et al., 2010). These issues needs to be addressed in depth to obtain better pressure distribution profiles in grain silos. The study also suggests that during the filling of grains the vertical stresses are more predominant than the normal pressures.

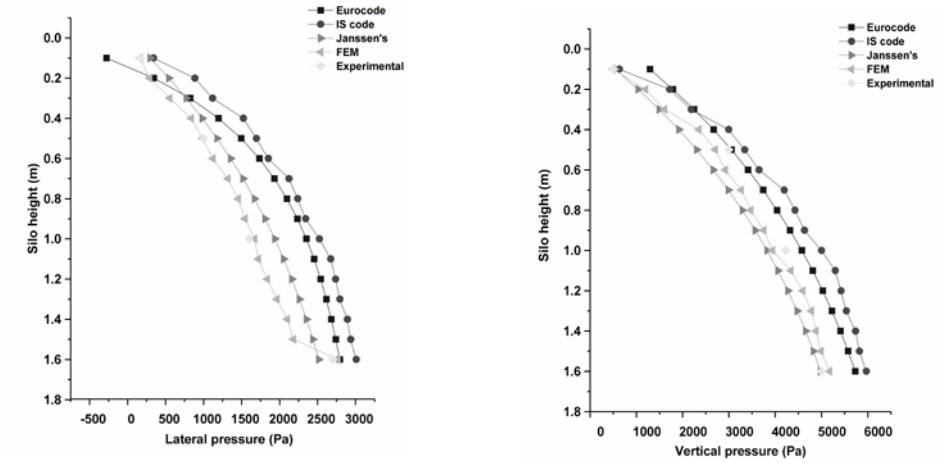

Fig. 3 Lateral and vertical pressures acting on the BRC silo wall during progressive filling of grain.

4. Conclusion

The study dealt with the development of a low cost bamboo reinforced concrete silo for farm level storage of rough rice. The structural safety of the developed structure was analyzed using the finite element method and the results were compared with Janssen's theory and design codes. The numerical method has been adjudged as one of the most reliable and versatile tools for development of innovative structures of various scales. The selection of input parameters for the FE model is a critical process affecting the prediction of the method. Dynamic pressures developed during filling of grains have been quantified in this study.

Acknowledgements

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Increase of Paddy Moisture with Automatic Aeration in a Warehouse Guided by Adsorption Equilibrium Absolute Humidity Equation

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Abstract

An automatic bulk monitoring and aeration controller was programmed with an adsorption equilibrium absolute humidity (CAE)equation and was used to aerate paddy with the aim to increase moisture content (MC)

and preventing fissuring. The ventilation control window for rewetting paddy was developed according to two conditions: (i) the average grain bulk temperature (t_g) is higher than the dewpoint temperature (DPT_a) of the atmosphere; and (ii) the equilibrium absolute humidity (EAH_g) of grain moisture content plus 1 percentage point is lower than the absolute humidity (AH_a) of the atmosphere. The ventilators were turned on when the atmosphere state point was within the ventilation windowand turned off outside that window. In a humid subtropical monsoon climate, during Oct. 8th to Nov. 1st, 2013, the system was used for a paddy depot of 1035 t in Dianjiang, Chongqing province. The natural humid air was introduced into the paddy bulk by negative pressure suction aeration during the 10-12 h night time period and allowed to equilibrate with grain kernels during the 12-14 h day time period. Aeration increased grain MC by 0.6 percentage points with two 1.5 kW axial flow ventilators and power consumption of 209 kW·h. The unit energy consumption was 0.336 KW·h (1% moisture·t)$^{-1}$. The broken milled rice percentage was decreased by 2-3 percentage points. In the warm temperate semi-humid monsoon climate, during April 13th to June 16th, 2017, the system was used to rewet japonic paddy in a 2489 t depot in Qihe, Shandong province. The conditions for running two 0.85 kW axial flow fans were: (i) when the atmosphere relative humidity (RH_a) is ≤80% and its temperature (t_a) is <28°C, t_g>DPT_a, and EAH_g<AH_a; and (ii) when RH_a >80% and t_a <28°C. Whenever t_a was >28°C, the two fans were switched off. This rewetting aeration increased grain MC from 13.5% to 14.0%, and the unit energy consumption was 0.455 kW·h (1% moisture·t)$^{-1}$. The percentages of average head rice yield and damaged grains after aeration were 71.7% and 7.7%, respectively.

Keywords: Paddy, EMC, moisture adsorption, increasing moisture, automatic aeration.

1. Introduction

Rice is the staple food for approximately 65% of the Chinese population. China is the world's largest rice producer with annual production over 144 million metric tons (FAO, 2014), and due to its large population, about 40% of its production is assigned to store for two years in the form of paddy with deterioration controlled largely through moisture content (MC) and temperature. For improving physical control in paddy storage, sound knowledge of the relationship between equilibrium moisture content (EMC) and equilibrium relative humidity (ERH) is essential (Jayas & Mazza, 1991; Sun, 1999; Li et al 2010; Li & Jiang, 2014). After two-year storage, paddy in China usually has moisture losses over 1.5-2.0% wet basis (w.b.). In order to increase the head rice yield and milled rice quality, rewetting up to 14% w.b. 1-3 months before retrieving from storage is needed. The present study investigated increasing paddy moisture with automatic mechanical aeration in a flat warehouse based on an adsorption equilibrium absolute humidity equation (CAE) with the aim to determine suitable aeration rewetting conditions.

2. Materials and Methods

2.1. Using the CAE equation in a computer controlled grain aeration system

In a computer controlled grain aeration system (Wu, 1987; Wu and Li, 1994), the parameters known as the CAE model for paddy adsorption (Li et al., 2014) was used to make curve graphs for determining the equilibrium relative humidity (ERH_g) of paddy kernels with particular MC at certain temperature. The following equation (1) was used to make the curve graphs for determining the equilibrium absolute humidity (EAH_g) of paddy kernels with particular MC at certain grain temperature and dewpoint temperature (DPT_g) of grain at this absolute humidity:

$$\mathrm{EAH_g} = \exp\left\{\frac{\left[\frac{D}{222}\left(\exp\left(\frac{B_1-MC}{A_1}\right)-\exp\left(\frac{B_2-MC}{A_2}\right)\right)+0.9845\right]\cdot\left(1737.1-\frac{474242}{273+t_g}\right)+D\left[1-\exp\left(\frac{B_1-MC}{A_1}\right)\right]-68.57}{87.72}\right\} \quad (1)$$

where EAH_g is grain bulk equilibrium absolute humidity (mm Hg), MC is grain moisture content (% w.b.), t_g is grain temperature (°C). A_1, A_2, B_1, B_2, D are five parameters of the CAE equation.

The dewpoint temperature (DPT_gin °C) of the grain bulk was calculated by equation (2):

$$\mathrm{DPT_g} = \frac{474242}{1872.7-89.1\lg(\mathrm{EAH_g})} - 273 \quad (2)$$

The atmosphere absolute humidity (AH_a) and dewpoint temperature (DPT_a) were respectively calculated with equations (3) and (4):

$$\mathrm{AH_a} = 100\exp\left\{\frac{87.72\lg(\mathrm{RH_a})+0.9845\left(1737.1-\frac{474242}{273+t_a}\right)-270.57}{87.72}\right\} \quad (3)$$

$$DPT_a = \frac{474242}{\frac{474242}{273+t_a} - 89.1\lg(RH_a) + 410.34} - 273 \quad (4)$$

where AH_a is atmosphere absolute humidity (mm Hg), RH_a is atmosphere relative humidity (%), and t_a is atmosphere temperature (°C), DPT_a is atmosphere dewpoint temperature (°C).
The relative humidity or absolute humidity in equations (1) - (4) was calculated on the basis of sea level atmospheric pressure. The values of DPT_g and DPT_a were used in characterizing whether dew condensation would occur with a decrease in temperature.

2.2. Aeration window controlling ventilator operation

The ventilation window for increasing grain moisture was constructed according to two conditions of aeration control: (i) the grain bulk temperature (t_g) is higher than the dewpoint temperature (DPT_a) of the atmosphere; and (ii) a condition in the Grain Industry Standard LS/T 1202-2002 of the PRC was modified as follows: the equilibrium absolute humidity (EAH_g) of grain moisture plus 1 percentage point is lower than the absolute humidity (AH_a) of the atmosphere, and not the grain moisture content plus 2.5 percentage points. Whenever both conditions were true then the axial flow ventilators were switched on. Fig. 1 shows the aeration rewetting window. If the grain state (13.5% MC) has an adsorption equilibrium absolute humidity of 10 mmHg and grain temperature of 15.8°C, and the atmosphere has an equilibrium absolute humidity of 12 mmHg and dewpoint of 14°C, airflow with lower temperature and higher humidity could increase the moisture of the paddy bulk, and thus the axial flow ventilators would be switched on.

The aeration controlling system included the hardware such as ventilator-controlling module, digital humidity transmitter, new type temperature measuring cable, and protective filter cover for humidity sensors. This system automatically detected grain bulk temperature and the air temperature and relative humidity of the headspace in the warehouse every 15 min, and the atmosphere temperature and relative humidity outside of the warehouse every 5 min. An aeration window was constructed by the curves of paddy adsorptive equilibrium absolute humidity and the saturation absolute humidity. When the atmosphere state point lied within the aeration window, the axial-flow ventilators were turned on to increase paddy MC. When the atmosphere state point was outside the aeration window, the axial-flow ventilators were turned off.

2.3. Two in-situ experiments for remoisturizing aeration in flat warehouses

2.3.1. The remoisturizing aeration in a warehouse in Dianjiang, Chongqing

The first experiment was carried out at the Dianjiang State Grain Reserve Depot, Dianjiang, Chongqing, China. Dianjiang lies in a basin (30°N, 107°E, 450 meters of average altitude) with a subtropical humid monsoon climate. The experimental No. 12 warehouse made of steel frame, concrete wall and tile roof, is 31.4 m in length and 14.12 m in width. It has six ground cage-channels equipped with two axial-flow ventilators, each ventilator responsible for three channels. The gap between two channels is 4.7 m, the percent of aperture in each cage-channel at the beginning and the end are 25% and 35%, respectively. The ratio of longest to shortest pathway of air is 1.5. The indica paddy of 1330 tonne was garnered in January 2012 and had a 5.12 m bulk height and 0.6% of foreign material. During April to September 2013, 295.5 tonnes of paddy was sold and the rest of the 1034.5 tonnes of paddy were used for the rewetting experiment starting on October 7th. The warehouse doors were closed, and its four windows in the sides above the grain surface were opened. Aeration used negative suction pressureto draw air into the warehouse through the windows then passed downward through the layers of the grain bulk, and was exhausted from the ground-level ventilators. The two ventilators (SFG4-2 type, 1.5 kW power) generate 320/220 Pa of full/static pressure, 11000 m^3/h of air volume, and 2800 r/min of rotational speed, thus the calculated airflow rate is 10.6 $m^3h^{-1}t^{-1}$. In order to accurately determine the electricity consumption, an intelligent electricity meter was used for aeration manipulation. The actual power consumption was 0.567 kW·h (1%moisture·t)-1.

2.3.2. Rewetting aeration in a flat warehouse in Qihe, China

The second experiment was carried out at Shandong Grain Reserve Depot for Army Provision, Qihe, Shandong province, China. Qihe lies in a basin (36.8°N, 116.8°E, 20 meters of average altitude) with warm temperate semi-humid monsoon climate. The experimental No. 13 warehouse is 39.8 m in length and 20.4 m in width. It has five U-shaped air channels equipped with two axial-flow fans, each fan is connected to 2.5 U-shaped air channels. The ratio of longest to shortest pathway of air is 1.4. The japanic paddy of 2489 tonne from the northeast China was garnered in December 2016, with 5.0 m bulk height, 14.0% MC and 0.6% foreign material. After levelling the grain bulk surface, the equalizing-temperature aeration decreased the average grain bulk MC to 13.5% during January 2017. Aeration was negative suction pressure suction with air entering the warehouse through five vents and then passed upward through the paddy bulk layers, and finally exhausted from the two fans fixed on the windows in the roof structure. The warehouse doors were closed, its five vents in the warehouse side bottom were opened, and the two axial-flow fans fixed on the windows at opposite sides above the paddy surface were opened. The two fans (FTA-75 type, 0.85 kW power) have 320/220 Pa of full/static pressure, 13800 $m^3\ h^{-1}$ of air volume, and 2300 r min^{-1} of rotational speed, thus the calculated ventilation rate is 8.9 $m^3\ h^{-1}\ t^{-1}$. The No. 13 warehouse of paddy was used for the reweeting experiment with natural humid air during April 13th to June 16th, 2017.

2.3.2.1. Protocol for rewetting paddy in No. 13 warehouse

Firstly, the local daily 24-h data of atmosphere temperature and RH during April to June in 2015 and 2016 were collected from Qihe County Bureau of Meteorology. A paddy desorption equilibrium moisture equation (eqn. 5) was used to predict paddy static moisture content near the warehouse vents:

$$EMC_p = 36.953 \cdot RH^3 - 48.528 \cdot RH^2 + 30.791 \cdot RH + 0.03859 \cdot RH^2 \cdot t + 0.006744 \cdot RH \cdot t - 0.08611 \cdot t + 5.089 \quad (5)$$

where EMC_p is the predicted EMC (%w.b.) of paddy, RH and t are the relative humidity (%) and temperature (°C) of the atmosphere, respectively.

Secondly, the aeration channels in the paddy warehouse were used for automatic rewetting aeration. The temperature of the grain bulk was similar to the atmoshperic temperature thus the atmospheric RH should be 25% higher than the RH of the grain bulk. Thirdly, the grain bulk temperature and grain moisture near the vents and at the bulk surface were checked regularly. The percentage of head rice yield and damaged grains from sampling sites were determined.

3. Results

3.1. The rewetting aeration in No. 12 flat warehouse in Dianjiang, Chongqing

3.1.1. Change in the BCDE area of rewetting aeration window

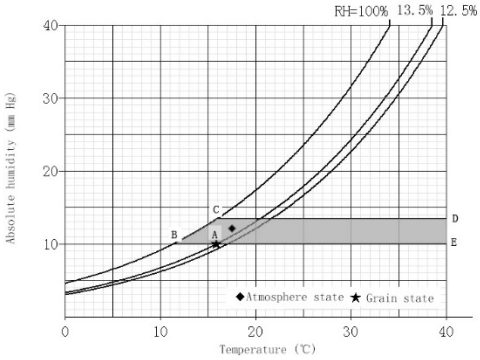

Fig. 1 The operating window for aeration to rewet paddy rice.

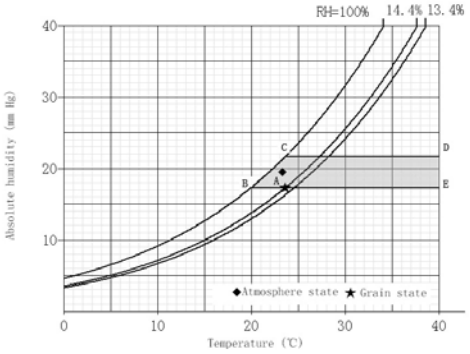

Fig. 2 The ventilators were running at 23:31 on October 8th, 2013.

At 23:31 on October 8th, 2013, the automatic detection system showed average grain bulk temperature (t_g) of 23.6°C in No. 12 warehouse, atmosphere temperature of 23.3°C, and atmosphere RH of 92%. The moisture of grain bulk was determined to be 13.4% using a LSKC-4B type moisture meter (Wuhan Electronic Devices Second Factory, China). The t_g was higher than the dewpoint temperature (DPT_a, 21.93°C) of the atmosphere. The atmosphere state point was within the BCDE area of the rewetting aeration window (Fig. 2), and the ventilators were turned on.

At 19:01 on October 20th, 2013, the moisture of the grain bulk was 13.8%, the automatic detection system showed average grain bulk temperature (t_g) of 16.5°C, atmosphere temperature of 18.0°C, and atmosphere RH of 91%. The t_g was equal to the dewpoint temperature (DPT_a, 16.5°C) of the atmosphere. The atmosphere state point was at the edge of the BCDE area of the rewetting aeration window (Fig. 3), thus the rewetting aeration condition was not satisfied and the ventilators were turned off.

At 22:00 on October 21th, 2013, the moisture of the grain bulk was 13.8%, the automatic detection system showed average grain bulk temperature (t_g) of 16.8°C, atmosphere temperature of 17.3°C, and atmosphere RH of 92%. The t_g was higher than the dewpoint temperature (DPT_a, 15.98°C) of the atmosphere. The atmosphere state point was within the BCDE area of the rewetting aeration window (Fig. 4), thus the rewetting aeration condition was sufficient and the ventilators were turned on.

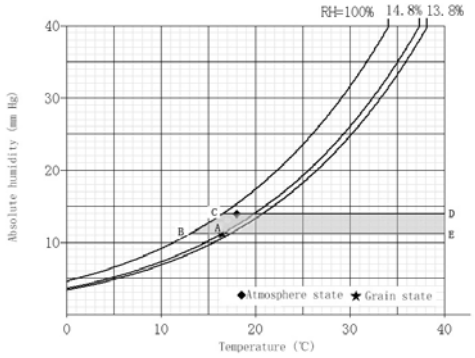

Fig. 3 The ventilators were turned off at 19:01 on October 20th, 2013.

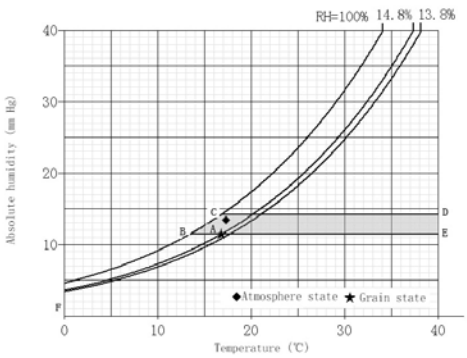

Fig. 4 The ventilators were running at 22:00 on October 21th, 2013.

At 11:14 on October 31th, 2013, the moisture of the grain bulk was 14.1%, the automatic detection system showed average grain bulk temperature (t_g) of 15.6°C, atmosphere temperature of 15.8°C, and atmosphere RH of 95%. The t_g was slightly higher than the dew temperature (DPT_a, 15.01°C) of the atmosphere. The atmosphere state point was outside of the BCDE area of the rewetting aeration window (Fig. 5), and the ventilators were turned off.

The rewetting aeration in No.12 warehouse was ended on November 2nd, 2013. Table 1 shows the data of automatic bulk detection. At 10:29, the grain bulk had a maximum temperature of 16.3°C, a minmum temperature of 15.8°C, and a mean temperature of 16.0°C. The temperature gradient in the grain bulk was ≤1°C m^{-1} grain layer. The air temperature and RH above the grain bulk surface was 16.0°C and 91%, respectively; the temperature and RH of the atmosphere were 16.3°C and 95%, respectively. The moisture of the grain bulk was 14.2%.

3.1.2. Energy consumption and profit anlysis for automatic paddy aeration

The rewetting manipulation was being carried out while the grain bulk was retrieved from storage. The moisture of the outbound grain was 14.1-14.2%. The moisture content in the remaining 612 t of paddy was 14.1% on November 2nd, and rewetting aeration was stopped. The total output grain was 1324 t until November 12th, and grain loss was 5.85 t. The aeration system ran 181.5 h, and power consumption was calculated to be $181.5 \times 2 \times 0.576$=209.1 kWh The annual mean grain loss in the depot was 0.8%, the grain loss for 1330 t paddy should be 10.64 t. Therefore, the increase in grain weight by rewetting was 4.79 t. The price for output paddy was 2.16 yuan kg^{-1}, the sale of 4.79 t of paddy was 10346.4 yuan. The electricity charge per kW·h was 0.92 yuan, and electricity cost was 192.37 yuan, thus the net profit was 10154.03 yuan. The unit energy consumption [kW·h (1%moisture·t)$^{-1}$] was $\frac{209.1}{10346.4\times(14.1-13.5)\%} = 0.3336$.

Tab. 1 Detection data in No. 12 paddy warehouse before and after rewetting aeration**.**

Time	Grain layer	Mean grain layer temp. (°C)	Min. grain temp. (°C)	Max. grain temp. (°C)	Mean grain bulk temp. (°C)	Temp. above bulk surface (°C)	Atmo-sphere temp. (°C)	RH above bulk surface (%)	Atmo-sphere RH (%)
23:31 Oct. 8th, 2013	1	23.6	23.5	23.8	23.6	23.3	22.3	92.0	92.0
	2	23.6							
	3	23.7							
	4	23.8							
10:25 Nov. 2nd, 2013	1	16.0	16.3	15.8	16.0	16.0	16.3	91.0	95.0
	2	16.1							
	3	15.9							
	4	15.9							

Tab. 2 The running time of two ventilators.

Date	Running time	Date	Running time
Oct.8th-9th	9 h 43 min	Oct.22th-23th	12 h 20 min
Oct.9th-14th	Turned off	Oct.23th-24th	11 h 10 min
Oct.15th-16th	14 h 19 min	Oct.24th-25th	10 h 54 min
Oct.16th-17th	19 h 52 min	Oct.25th-26th	10 h 42 min
Oct.17th-18th	9 h 59 min	Oct.28th-29th	10 h 21 min
Oct.18th-19th	9 h 44 min	Oct.29th-30th	19 h 30 min
Oct.20th-21th	12 h 10 min	Oct.30th-31th	14 h 30 min
Oct.21th-22th	9 h 34 min	Oct.31th-Nov.1st	8 h 30 min
		Amount	181 h 38 min

3.2. The rewetting aeration in No. 13 warehouse in Qihe, Shandong province

3.2.1. Predicting paddy EMC from meteorological data

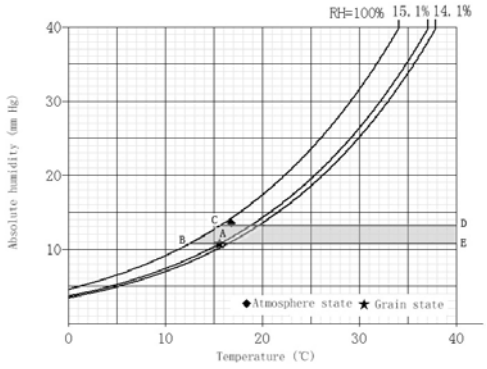

Fig. 5 The ventilators were turneded off at 11:14 on October 31th, 2013.

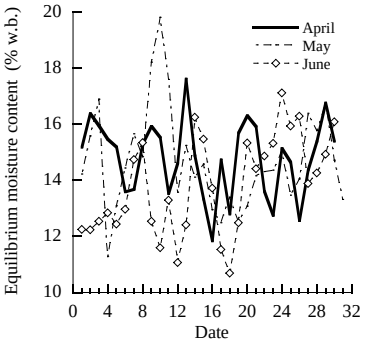

Fig. 6 The predicted EMC of paddy in Qihe, Shandong province.

The daily atmosphere RH and temperature during 17:00 to 8:00 from April to June in 2015 and 2016 was reviewed to predict the EMC of paddy. The number of days that the predicted desorption EMC is above 13.5% w.b. was 25, 22, and 16 in April, May, and June, respectively. The mean desorption EMC in April, May, and June was 14.81%, 14.71%, 13.83%, respectively, yielding an average of 14.45%. This indicated that the local atmosphere RH and temperature could be used to rewet paddy to 14% MC with automatic aeration.

3.2.2. Efficacy of automatic rewetting aeration

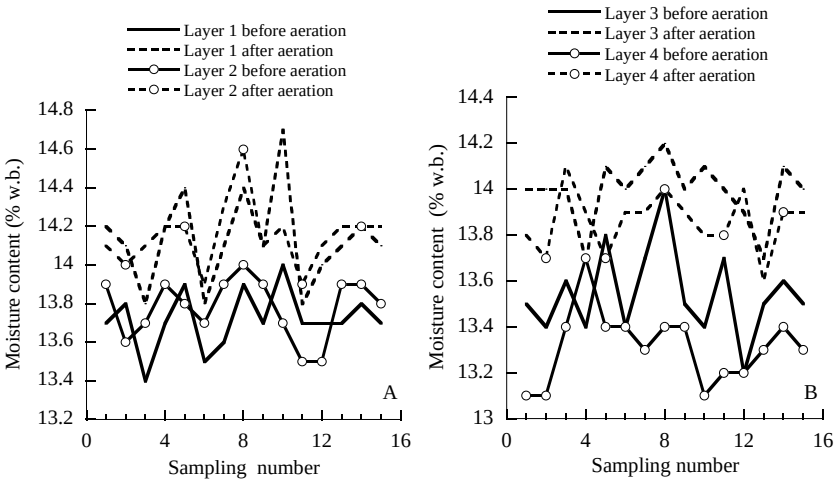

Fig. 7 Change in sample MC in paddy with automatic rewetting aeration.

Sampling number shows the average value of four bulk layers.

Tab. 3 Effect of controlled rewetting aeration on percentage of head rice yield in samples.

Sampling site	Before aeration				After aeration			
	Layer 1	Layer 2	Layer 3	Layer 4	Layer 1	Layer 2	Layer 3	Layer 4
			Head rice		yield (%)			
1	67.4	69.9	67.5	-	70.6	73	71.9	-
2	68.1	66.4	-	-	71.3	70.8	-	-
3	68.2	-	69.5	-	72.1	-	72.8	-
4	-	70.1	-	68.3	-	71.8	-	71.4
5	-	68.1	-	66.3	-	70.6	-	72.2
6	-	69.1	68.8	70.5	-	71.2	72	71.9
7	-	67.7	67.5	68.2	-	71.2	72.1	70.4
8	67.5	-	66.2	-	71.6	-	70.8	-
9	68.3	67.8	-	-	72.2	71.3	-	-
10	-	68.5	67.7	-	-	71.6	70.9	-
11	-	67.9		68	-	71.2	-	70.7
12	70.1	69.5	68.8	68.2	71	72.4	71.9	71.5
13	67.5	67.2		69.3	72.2	71.4	-	72.6
14	-	68.1	69.4	-	-	70.9	72.4	-
Average	68.2	68.4	68.2	68.4	71.6	71.8	71.9	71.5

The No. 13 warehouse in Qihe depot was chosen for rewetting on April 13th, and the treatment ended on June 16th, 2017. During automatic aeration over two months, three conditions were set: (i) when the atmosphere relative humidity (RH_a) is ≤80% and its temperature (t_a) is <28°C, the average grain temperature (t_g) is higher than the dew temperature (DPT_a) of the atmosphere, and the equilibrium absolute humidity (EAH_g) of grain moisture plus 1 percentage point is lower than the absolute humidity (AH_a) of the atmosphere, the two axial-flow fans were turned on; and (ii) when RH_a >80% and t_a <28°C, the two axial-flow fans were turned on. Whenever the t_a is above 28°C, the two fans were switched off.

This rewetting aeration increased grain moisture from an initial moisture content of 13.5% to 14.0% (Fig. 7) within the accumulated power consumption of 566.1 kWh using two 0.85 kW axial-flow fans. The unit energy consumption was 0.455 kW·h (1% moisture·t)$^{-1}$. The percent of average head rice yield in the whole depot after aeration was 71.7% (Tab. 3), significantly higher than that (68.2%) of

the non-rewetted paddy. The Chinese national standard of paddy (GB1350-2009, China) stipulates that the percentage of head rice yield of first grade japanic paddy should be higher than 61%. Tab. 4 shows the percent of damaged grains at each sampling location. It had some difference among different sampling sites in each bulk layer, but its mean values among four layers were not significantly different, indicating even moisture distribution in the whole paddy bulk. The percent of damaged grains in the whole warehouse was 7.7±1.8%.

Tab. 4 The percentage of damaged kernels in samples after controlled rewetting aeration.

Sampling site	1	2	3	4	5
Layer 1	10.01±2.89b	4.22±0.98ab	5.80±4.39ab	2.01±1.22b	10.41±4.64ab
Layer 2	5.77±5.34bc	4.13±1.41ab	6.54±0.44a	2.99±1.42b	14.50±1.97a
Layer 3	0.45±2.01c	6.22±2.06a	0.82±2.07b	10.92±4.61a	8.08±1.21b
Layer 4	13.34±0.42a	3.45±0.48b	10.47±3.68a	13.45±0.86a	7.38±2.34b
Sampling site	6	7	8	9	10
Layer 1	5.34±1.34a	3.20±1.58b	1.76±1.38c	3.62±2.01b	2.99±0.96b
Layer 2	7.94±3.78a	8.41±3.36a	16.82±0.84a	9.34±1.34b	9.27±2.81a
Layer 3	4.03±1.27a	5.76±2.11ab	10.08±4.65b	16.65±4.37a	0.50±2.23b
Layer 4	3.41±2.41a	10.04±4.34a	21.20±4.11a	7.73±4.47b	9.34±0.84a
Sampling site	11	12	13	14	Average
Layer 1	5.92±1.27b	10.75±4.81bc	5.83±0.53a	5.34±0.15b	5.52±2.98a
Layer 2	2.22±1.79c	7.52±1.98c	0.61±2.27b	6.47±1.81b	7.24±4.58a
Layer 3	11.13±1.64a	24.92±4.11a	5.83±1.11a	9.62±1.17a	8.22±6.68a
Layer 4	9.97±0.15a	11.20±0.91b	8.36±3.21a	8.15±6.91ab	9.83±4.43a

The damaged kernel was determined as described by Li et al. (2016). The different small letters in the same column show significant different (p<0.05) at LSD-test.

4. Discussion

Banaszek and Siebenmorgen (1990) reported that air conditions of 12.5°C/RH 50%, 15°C/RH 50% and 12.5°C /RH 90% were not obtainable with the RH and temperature control unit used for their adsorption EMC experiment with rough rice. The reason is not clear. We found that for "Longyang" variety japanic paddy samples from northeast China with 13.57% initial MC and under 65% ERH it had moisture adsorption at 10°C, but had moisture desorption at 20 to 35°C. Below 86% ERH condition, it had moisture adsorption at 10 to 35°C (Li, et al., 2015). These results suggest that paddy samples with 13.5% MC could be rewetted at 80% RH and 10-25°C of ambient condition.

Acknowledgements

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Drying Ginger and Preserving 6-Gingerol

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Abstract.

Ginger rhizome (*Zingiber officinale*) is widely used as a spice or as a medicinal plant. The major bioactive compound in fresh ginger rhizome is 6-gingerol and it is known for having a number of physiological effects. This compound is heat-sensitive and during cooking or drying will transform into 6-shogaol. Hence, the 6-gingerol content is used to evaluate the quality of dried ginger. The content of 6-gingerol during drying was measured using HPLC. Several factors that could affect the 6-gingerol content were considered and a predictive model for changes in 6-gingerol has been developed from the experimental data. The predictive model includes a single term drying model that predicts the changes of moisture content during drying. Drying time and relative humidity (ranging from 10% to 40%) impacted 6-gingerol content whereas drying air temperature (ranging from 30°C to 60°C) had a lesser effect. It was also found that the 6-gingerol content in fresh rhizomes was highly variable and thus required thorough testing prior to drying to be able to make the prediction more accurate.

Keywords: ginger, air drying, 6-gingerol, HPLC, predictive model.

1. Introduction

Background

Ginger, with a scientific name of *Zingiber officinale* Rosc, is a member of the tropical and sub-tropical family Zingiberaceae. It originates in tropical rainforests in southern Asia and spread to Mediterranean regions by the 1st century. In ancient Rome, ginger was a popular spice used to make delicacies. Throughout the history of global trade, ginger has been traded longer than most other spices. In the ancient world, it was regarded as a costly herb for its medicinal merits and nutritional value.

Over the long history of ginger trading around the world, ginger has been planted on most continents. Given different growing environment, ginger has developed into several cultivars. In commercial trading, ginger is often designated by the country where it originates from, such as Chinese ginger, Indian ginger, Australian ginger or Jamaican ginger. However, ginger has a large cultivar diversity, so that even in one country, there could be dozens of cultivars. Generally, a cultivar comes from a specific growing place, and hence many cultivars were named after their growing place.

Chemical composition of ginger

Ginger rhizomes contain a variety of compounds. Researchers have found more than one hundred compounds which can be classified into three groups: essential oils, gingerol and diarylheptanoids.

Essential oils are hydrophobic liquids, containing volatile chemical constituents. Distillation and extractions are the most common ways to isolate the essential oils. The major components of essential oils are the terpenoids, including monoterpenes and hemiterpenes. Most compounds from these two groups have a strong volatile aroma and biological activity, which are important ingredients in medicine, cosmetics and food production.

Gingerols are major pungent constituents of ginger which are made up of several different compounds. Gingerols have a 4-hydroxy-3-methoxyphenyl group in the chemical structure, varying according to different aliphatic chains attached to the main group. Gingerols can be classified as gingerol, shogaol, gingerdione and gingerdiol.

Gingerols are thermally labile due to the presence of a β-hydroxy keto group in the structure and produce corresponding shogaols via a dehydration reaction (Bhattarai, 2001). The dehydration process will be affected by drying air temperature and residence time. It is reported that raising the reaction temperature and extending time significantly increased the conversion of 6-gingerol to 6-shogaol (Kou et al., 2017). Among gingerols, 6-gingerol has been studied more thoroughly compared to other gingerols such as 8-gingerol and 10-gingerol. This is because the proportion of 6-gingerol in fresh ginger is the highest among all gingerols.

Effects of ginger on human health

Among all of the compounds in ginger, essential oils play an important role in improving consumers' mood. The benefits of ginger essential oils include offering a warm, spicy aroma which enhances feelings of vitality, promotes feeling of physical well-being, and helps improve body blood circulation. It is a frequent addition to blends for massage, arthritis and muscle aches and pains. Ginger oil is commonly used to soothe, comfort and balance digestive discomfort. Gingerols may make a contribution towards human health effects in medical applications of ginger. Studies have shown antitumor activity of 6-gingerol（Parket al., 1998), analgesic and anti-inflammatory effects (Young et al., 2005), and 10-gingerol and 12-gingerol have antibacterial activity against periodontal bacteria (Park et al., 2008). The content of gingerols varies significantly with ginger varieties and cultivating locations.

Use of ginger and derived products

Ginger is used as a main ingredient in many products throughout the world. Fresh ginger roots are juicy with a mild taste and can be used as spices for sweet or salty food such as soup, meat, vegetable, seafood, pickle, curries, drinks and cake. However, due to the strong pungent flavour of fresh ginger root, fresh ginger is normally dried, and used to produce ginger powder, which makes the spicy flavour weaker. Yet, the major use of ginger is in the pharmaceutical area. In many Asian countries, especially China, India and Japan, ginger is treated as one of the additives in traditional medicine, rather than as herb. Therefore, ginger is considered as herbal medicine with strong health benefits.

Dried ginger and dried ginger products account for the largest amount of ginger consumption around the globe, as fresh ginger is mainly produced in tropical and subtropical countries. Dried ginger significantly lowers the cost of transporting and storing. Generally, dried ginger is produced from fresh mature ginger rhizomes whereas immature ginger rhizomes are processed to make preserved ginger, as the mature rhizome has stronger flavour and aroma.

Aim of this study

The aim of this study was to investigate the ability of a two-layer drying model to predict the optimum conditions for drying fresh ginger rhizomes for the maximisation of the retention of 6-ginerol in the dried ginger.

2. Materials and Methods

Ginger samples

The samples of fresh ginger rhizomes were obtained from Buderim Ginger Pty Ltd in Yandina, Queensland, Australia. They were shipped to Sydney in a refrigerated container and then placed in

a freezer at -20°C until being used for experiments. The day before the experiments the samples were defrosted overnight in a fridge at +4°C.

Defrosted ginger rhizomes were peeled and sliced to 5 mm thickness using a slicer. A sample of about 10 g of slices was used for determination of initial moisture content. Duplicate samples were placed in a convection oven at 105°C for 24 h.

Drying

The drying experiments were conducted in a cabinet dryer constructed in the workshop of the School of Chemical Engineering of the University of New South Wales. The cabinet dryer (see Fig. 1) had an electric heater (15 kW) fitted with a PID controller and a fan (0.75 kW). The airflow was parallel to the tray on which the drying samples were placed in a thin layer. The temperature and relative humidity were monitored and recorded with a datalogger. The weight loss was recorded with an electronic balance placed under the sample holding tray.

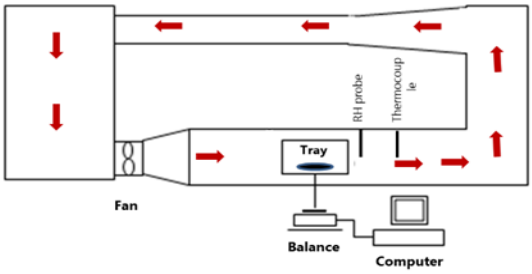

Fig. 1 Cabinet dryer.

The drying conditions in the experiments were either a constant temperature and relative humidity (RH), as in Runs 1-3, or a changing temperatue and RH, as in Runs 4-11 (see Tab. 1).

The reason for this experimental design was that drying temperature was expected to affect the drying process. Since the properties of the ginger samples were changing during the drying process, a change of the drying conditions in the dryer was allowing for investigation of the effects of the changed conditions on the drying behavior of the samples.

The wet sample of sliced rhizome was placed on the tray as quickly as possible in order to reduce the influence of ambient conditions on those in the drying chamber.

After the weight of the sample became steady, i.e., the sample reached the equilibrium moisture content corresponding to the drying conditions (temperature and RH), the drying run was finished. A duplicate dried sample (10 g each) was taken and used for the determination of the final moisture content.

The remainder of the dried sample was weighed and preserved in a vacuum-sealed bag. It was kept in a cool place for the determination of the 6-gingerol content.

Determination of 6-gingerol

The 6-gingerol standard (≫ 98% purity) was obtained from Sigma-Aldrich. Methanol of HPLC grade was purchased from Burdick & Jackson. Water for HPLC analysis was purified with a Milli-Q water system. Agilent vials for HPLC with caps were used. Whatman filter paper had a pore size of 0.45 µm.

Dried ginger slices were pulverized using a grinder and passed through a 40 mesh (0.42 mm) sieve before extraction. In contrast, the fresh ginger was crushed with a mortar and pestle prior to extraction. The sample of ginger powder or paste (1 g) was dissolved in 25 mL HPLC-grade methanol and sonicated for 30 min. The mixtures were centrifuged at 10000 rpm for 10 min and supernatant

was filtered through Whatman filter paper. Then the supernatant was diluted with water to reach a final concentration of 10% methanol and 90% water. Extracts of ginger were transferred to the HPLC vials and capped. All the extracts were kept at 4°C until being used.

Tab. 1 Summary of drying conditions.

Run number	Temperature (°C)	RH (%)
1	40	30
2	50	20
3	60	10
4	60	10
	50	20
5	60	10
	50	30
6	60	10
	50	40
7	60	10
	40	30
8	60	10
	30	40
9	50	30
	40	30
10	50	20
	60	10
11	40	30
	50	20

From Run 4 to Run 11, the drying conditions were changed from those in the 1st rows to those in the 2nd rows when the sample had lost 50% of its weight.

For the calibration curve of 6-gingerol, a stock solution of 5.0 mg/mL of standard in HPLC-grade methanol was prepared. Serial standard dilutions were made from the stock solution to obtain concentrations of 5.0, 10.0, 20.0, 40.0, 60.0 and 80.0 µg/mL. All dilutions of 6-gingerol standard were capped and stored at 4°C.

The HPLC system used in this study was from Shimadzu, model Prominence LC-20AD. The separation of the compounds was conducted in a C18 column (XTerra), 3.5 µm, and 2.1×150 mm. Water (A) and methanol (B) constituted the mobile phase for the separation. The following linear gradient was used: 0-5 min, 50% B; 5-10 min, 50-60% B; 10-15min, 60% B; 15-25min, 60-80% B; 25-30 min, 80% B; 30-35min, 80-50% B; 35-50 min, 50% B. The injection volume was 20 µL and the flow rate was 0.2 mL/min. The detection wavelength of the UV detector (0~1000 nm) was set at 281 nm and the column temperature was maintained at 30°C. The tests were done in triplicate.

3. Results

Content of 6-gingerol in dried samples

Prior to the drying experiments, 6-gingerol content of fresh ginger rhizomes was determined and found to be on average 0.59 ± 0.06 µg/mg 6-gingerol on dry matter basis.

Tab. 2 shows the summary for three constant drying conditions and multiple comparisons while Tab. 3 shows the results of the statistical analysis of the samples (ANOVA). There was a considerable variability within the results of each treatment as indicated by the value of the coefficient of variation (CV). It appears that the highest 6-gingerol content in a dried sample (0.456 µg/mg) was obtained after drying at 50°C and 20% RH. This was far below the initial content in fresh sample (0.5 µg/mg). From the ANOVA test, see Tab. 3, the 6-gingerol content from the three runs was significantly different from each other. Run 2 showed a higher 6-gingerol content than Run 1. This was expected since the drying temperature in Run 2 was higher and the drying time shorter.

However, a considerably shorter drying time in Run 3 did not necessarily result in a higher 6-gingerol content.

Tab. 2 Characteristics of samples subjected to different drying treatments with constant temperature and relative humidity.

Run	Drying conditions	Drying time (min)	6-gingerol content* (µg/mg)	CV (%)
1	40°C 30% RH	314	0.337	9.52
2	50°C 20% RH	293	0.456	4.57
3	60°C 10% RH	154	0.444	3.43

*Average of three experiments

Tab. 3 ANOVA test between constant drying conditions runs

Run comparison	P value
1 vs 2	0.000
1 vs 3	0.000
2 vs 3	0.360

ANOVA, P=0.05

Tabs. 4 and 5 show the summary for eight changing drying conditions and multiple comparisons. In general, the following trend in 6-gingerol content was observed: higher drying temperature and longer drying time resulted in lower gingerol content. This corresponds to the conclusions from the study of Bhattarai et al. (2001) that higher temperature results in rapid and faster dehydration of 6-gingerol and forming of the degradation product, 6-shogaol.

It is clear that there was no significant difference between Runs 4 and 10, Runs 5 and 9, and Runs 7 and 8. Runs 4 and 10 were conducted under similar conditions but Run 10 was 72 min longer, which indicated drying time had less impact on 6-gingerol reduction. There was a significant difference between Runs 4, 5 and 6, in which samples were dried at the same temperature but at different RH and with a different drying time. This showed that RH could have played a role in the decrease of 6-gingerol.

There was no significant difference between Runs 7 and 8. However, Run 8 had a lower average temperature and longer drying time. The reason for this result could be due to the combined impact of drying temperature and drying time.

Tab. 4 Characteristics of samples subjected to different treatments with changing conditions

Run	Drying conditions	Drying time (min)	6-gingerol content* (µg/mg)	CV (%)
4	60°C 10% RH to 50°C 20% RH	207	0.349	3.53
5	60°C 10% RH to 50°C 30% RH	133	0.578	4.79
6	60°C 20% RH to 50°C 40% RH	283	0.448	2.79
7	60°C 10% RH to 40°C 30% RH	253	0.401	2.47
8	60°C 10% RH to 30°C 40% RH	275	0.403	4.56
9	50°C 30% RH to 40°C 30% RH	231	0.588	4.40
10	50°C 20% RH to 60°C 10% RH	279	0.356	9.08
11	40°C 30% RH to 50°C 20% RH	282	0.497	0.98

*Average of three experiments

Tab. 6 shows ANOVA test results between constant drying conditions and changing drying conditions. Four groups showed no significant difference, which were Runs 1 and 4, Runs 1 and 10, Runs 2 and 6, and Runs 3 and 6. Runs 1 and 4 both obtained a lower gingerol yield while Run 4 was conducted at a higher temperature and lower humidity. The reason for this could be the much longer drying time of Run 1 (107 min longer). Similar situation happened in Runs 1 and 10, while Run 1 lasted 35 min longer than Run 10. Runs 2 and 6 had a similar drying time. However, Run 6 was conducted at a higher temperature and RH. This result again suggested that RH could affect 6-gingerol content in drying process.

Tab. 5 ANOVA test between changing drying conditions runs

Run	P value	Run	P value
4 vs 5	0.000	6 vs 8	0.000
4 vs 6	0.000	6 vs 9	0.000
4 vs 7	0.000	6 vs 10	0.000
4 vs 8	0.000	6 vs 11	0.000
4 vs 9	0.000	7 vs 8	0.863
4 vs 10	0.519	7 vs 9	0.000
4 vs 11	0.000	7 vs 10	0.000
5 vs 6	0.000	7 vs 11	0.000
5 vs 7	0.000	8 vs 9	0.000
5 vs 8	0.000	8 vs 10	0.000
5 vs 9	0.713	8 vs 11	0.000
5 vs 10	0.000	9 vs 10	0.000
5 vs 11	0.000	9 vs 11	0.000
6 to 7	0.0 00	10 vs 11	0.000

ANOVA, P=0.05

No significant difference between Runs 3 and 6 showed that drying at a higher temperature, shorter time and lower relative humidity results in a similar gingerol content as a run at lower temperature, longer drying time and higher humidity, which indicates that interactions between drying temperature, time and RH are likely to have an impact on 6-gingerol content.

Tab. 6 ANOVA test between constant and changing conditions.

Run	P value	Run	P value
1 vs 4	0.483	2 vs 8	0.006
1 vs 5	0.000	2 vs 9	0.000
1 vs 6	0.000	2 vs 10	0.000
1 to 7	0.001	2 vs 11	0.027
1 vs 8	0.001	3 vs 4	0.000
1 vs 9	0.000	3 vs 5	0.000
1 vs 10	0.301	3 vs 6	0.790
1 vs 11	0.000	3 vs 7	0.023
2 vs 4	0.000	3 vs 8	0.028
2 vs 5	0.000	3 vs 9	0.000
2 vs 6	0.662	3 vs 10	0.000
2 vs 7	0.005	3 vs 11	0.005

ANOVA, P=0.05

Predictive model for 6-gingerol

Drying time and drying temperature are considered as the two main factors affecting gingerol content (two-factors model). The prediction model function of 6-gingerol in this case is expressed by equation (1):

$$G = G_0 \exp(-k_{G_0} t) \qquad (1)$$

where G is the final 6-gingerol content, G_0 is the initial 6-gingerol content, k_{G_0} is the 6-gingerol rate constant and t is the drying time.

Combining equation (1) with the single term drying model for ginger we obtain equation (2):

$$G = G_0 \exp\left(-k_{G_0} \exp\left(\frac{h}{RT}\right) t\right) \qquad (2)$$

where h is the activation energy (J), R is the gas constant and T is the product absolute temperature (K).

Tab. 7 shows the experimentally determined value vs the model calculated 6-gingerol content for each of the drying treatments.

Tab. 7 Summary of 6-gingerol content from different drying treatments: experimental and model calculated values.

Run	Stage	Drying time (min)	6-gingerol content (µg/mg)	
			Experiment	Model
1		282	0.336	0.402
2		293	0.454	0.413
3		154	0.444	0.479
4	1	35		
	2	172	0.348	0.463
5	1	32		
	2	101	0.578	0.510
6	1	53		
	2	231	0.448	0.419
7	1	33		
	2	220	0.401	0.436
8	1	54		
	2	219	0.403	0.423
9	1	60		
	2	170	0.587	0.448
10	1	49		
	2	230	0.356	0.421
11	1	77		
	2	204	0.494	0.420

"-" means constant drying conditions. 1 and 2 means before changed drying conditions and after changed drying conditions.

The quality of the fit between the experimentally obtained values and the model calculated values was evaluated by the coefficient of determination (R^2) and root mean square error (RMSE). For the results shown in Tab. 7, they were R^2=0.4994 and RMSE= 0.0713.

The reasons for this relatively poor fit may have been due to the fact that there was a considerable level of variability in the initial moisture content of ginger rhizomes. Furthermore, the model did not take into account the RH of the drying air. In order to study the effects of RH, anew model (see equation (3)) was developed including RH:

$$G = G_0 \exp\left(-\left(k_{G_0} + A * RH\right) exp\left(\frac{h}{RT}\right) t\right) \qquad (3)$$

where A is a constant, RH is the relative humidity (decimal). Equation (3) is based on equation (2), and is the simplest modification to this model for including the effect of relative humidity on 6-gingerol depletion.

The new models showed an improvement of the fit having a higher coefficient of determination (R^2=0.5923) and a lower RMSE (0.0675).

4. Discussion

Combining a single term drying model and a three factors model for predicting gingerol content allows for devising the most appropriate drying conditions to obtain a maximum 6-gingerol yield.

A shorter drying time, a lower drying temperature and a higher RH reduce the 6-gingerol depletion in dried ginger.

The results suggest that the optimum drying condition of ginger is: air temperature of less than 40°C; RH of 40% at the late stage of drying, drying time not exceeding 300 min.

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Numerical modeling of the horizontal flow and concentration distribution of nitrogen within a stored-paddy bulk in a large warehouse

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Abstract

The insect population in grain stores can be kept under control by maintaining a high concentration of N_2 gas throughout the grain bed. The development of controlled atmosphere storage technology for insect control requires an accurate prediction of the distribution of introduced gases in bulk grain. In this paper, based on the convective-diffusion model, the horizontal flow of N_2, which was introduced into the paddy bulk in a large warehouse by means of the horizontal ventilation system, are modeled as fluid flow in a porous medium. The experimental data for N_2 transfer and flow through ducts and bulk paddy were used to validate the model. The equations were solved using the finite difference method, and the predictions from the proposed model were in good agreement with the experimental results. The concentration distribution and flow uniformity of nitrogen in stored paddy were also analyzed during the nitrogen-filling procedure for CA storage. It was shown that it is feasible and practical to introduce nitrogen into stored bulk grain in a large warehouse by means of the horizontal ventilation system.

Keywords: numerical modeling, stored paddy, concentration distribution, nitrogen-filling procedure

1. Introduction

Chemical control methods such as fumigation with phosphine are effective against insect pests, but have disadvantages including residue problems and development of tolerance by insects (Banks et al.,1990). In the recent past, the use of controlled atmosphere (CA) as a safe residue-free alternative to chemical fumigants and protectants has gained popularity for controlling insects infesting stored grain. Controlled atmosphere with low oxygen (O_2) and high nitrogen (N_2) in storage by injecting nitrogen into the storage displacing the oxygen is just one of a number of methods that can be used for controlling pests in stored products.

Controlled atmosphere storage for insect control involves the alteration of the proportion of the normal atmospheric gases, i.e., N_2, carbon dioxide (CO_2) and O_2, to create an atmosphere that is lethal to the insects. The success of CA and fumigants in killing stored product pests depends on the movement of gas through and uniform distribution of the gas in the stored grain, and maintaining a lethal gas concentration level for the required exposure period.

Three-dimensional heat, mass and momentum transfer models with concentration species were developed by Singh et al. (1993), Lawrence et al. (2013a, 2013b) and Mat Isa et al. (2014) for predicting fumigant concentrations in a rectangular domain or cylindrical silo. These models need to be validated under a wider range of conditions, and can then be used to evaluate causes of fumigation failures and to develop best management practices to prevent the failures. Although

the Lawrence et al. model was not validated, it included gas sorption and insect extinction models which were empirically based.

A mathematical model of the three-dimensional movement of CO_2 in stored wheat was solved using the finite-element method (Alagusundaram et al., 1996a, b). The model simulates the initial bulk flow of CO_2 as it sublimates and expands from a dry ice source. The mean relative errors between the predicted and measured CO_2 concentrations in a bin with an open top surface were 24% at 3 h and 16% at 21 h. The model developed by Alagusundaram et al. (1996c) handles the loss of CO_2 by modifying boundary conditions. It can predict the CO_2 concentrations at any point in a grain bulk stored in any shape or size structure and can be used by engineering designers to determine the best location for adding CO_2, the approximate amount of CO_2 needed, and the expected level of insect mortality. The boundary conditions for gas transfer models in stored grain can be quite variable (Jayas, 1995). The CO_2 leaving the grain surface will diffuse rapidly into the head space air. If the head space is not well ventilated by wind through openings in the roof and wall, the CO_2 concentration may rise above the atmospheric level. The top surface could be covered with a gas impermeable sheet, resulting in zero flow across the boundary.

Uniform and fast application of N_2 to all parts of the grain storage is fundamental to effective pest management. However, relatively few studies have been reported on the movement of N_2 through the stored grain and uniform distribution of N_2 in the stored grain. This study investigated the flow and concentration distribution of nitrogen inside a grain storage structure during the nitrogen-filling procedure. The specific objective was to model the flow uniformity of nitrogen within a stored-paddy bulk in a large warehouse using computational fluid dynamics. In this paper, the horizontal flow, concentration distribution and uniformity of nitrogen were evaluated and verified within a stored-paddy bulk in a large warehouse during the nitrogen-filling procedure by means of a ventilation system.

2. Materials and Methods

2.1. Controlled atmosphere (CA) grain storage model

The simulation used a 3-dimensional model of a stored paddy warehouse with dimensions of 18 m wide, 50 m long and 5.0 m flat height. The flat top surface of the stored paddy was covered by a gas impermeable sheet of polythene (Fig. 1). The stored grain was represented by a porous zone. Nitrogen from a nitrogen generator was introduced into the stored paddy at the main horizontal and branch vertical perforated ducts on the north side, then cross-flowed the stored paddy to the main and branch perforated ducts on the south side using suction fans which exhausted the gas to the outside of the warehouse.

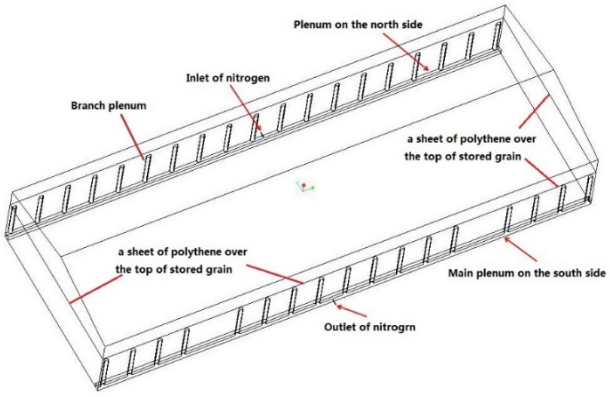

Fig. 1 Schematic diagram of the horizontal ventilation system.

2.2. Mathematical Model and CFD Model Parameters

2.2.1 Mathematical model of nitrogen flow and convection-diffusion in stored grain mass

The physical processes that occur in bulks of stored grain obey conservation laws. These phenomena were captured mathematically by a partial differential equation of the form:

$$\varphi \frac{\partial C}{\partial t} + u_j \frac{\partial C}{\partial x_j} = \frac{\partial}{\partial x_j}\left[D_{eff} \frac{\partial C}{\partial x_j} \right] \quad (1)$$

where φ is the grain bulk porosity (φ =0.55), u_j is the component of velocity in the j[th] direction (m/s), t is the time (s), C is the nitrogen concentration (species fraction) in stored grain, D_{eff} is the nitrogen effective diffusion coefficient in stored grain (D_{eff} =2.46 * 10^{-5} m^2/s) (Thorpe, 2008).

2.2.2 CFD model parameters

ANSYS Fluent was used to solve the transient species transport model. For the porous zone, a porosity of 0.55 for paddy and a computed viscous loss coefficient of 2.037 x 10^8 $/m^2$ were used as inputs; the inertial loss coefficient was 26767 ($Pa \cdot s^2/kg$) (Thorpe, 2008). Nitrogen inlet was given a fixed velocity of 5.0 m/s, i.e., the volume flow rate of nitrogen at the inlet was 60 m^3/h. The concentration of nitrogen at the entrance was 0.998 (species fraction). The initial temperature was assumed 25°C. The walls were considered no-slip wall boundary conditions. The initial species mass fractions were assumed 0.78 for N_2 and 0.22 for O_2.

3. Results

3.1 Numerical simulation results and analysis

Fig. 2 shows the streamlines of nitrogen flow in the stored paddy. It can be seen that the nitrogen uniformly entered from the ducts on the north side, then cross-flowed through the stored paddy with a superficial velocity of 6.67×10^{-5} m/s to the ducts on the south side. Fig. 3 shows nitrogen continues to move forward horizontally in the grain mass covered by a sheet of polythene. The average concentration of nitrogen in the grain mass was above 0.99 (species fraction) when the time of the nitrogen-filling procedure was about 61 hours (above 2.5 days) (Fig. 4), while nitrogen concentration of the outlet reached 0.978 (species fraction). According to the standard of CA with nitrogen in China (more than 0.97), the nitrogen-filling process can be stopped at this time.

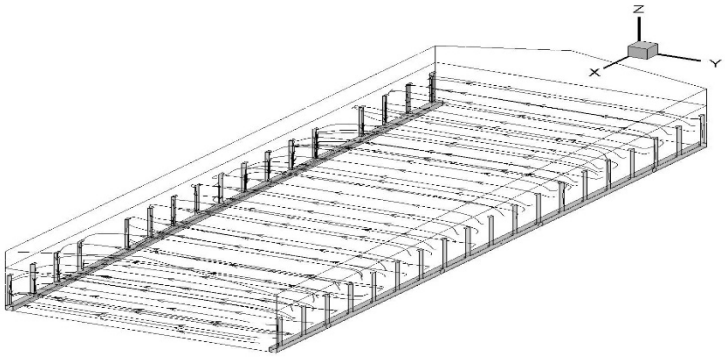

Fig. 2 The streamlines of nitrogen in the stored paddy inside the storage warehouse.

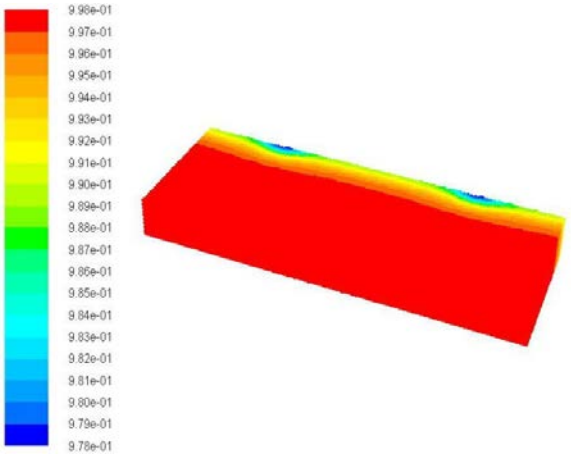

Fig. 3 Distribution of nitrogen concentration at 61 hours during the nitrogen-filling procedure in stored paddy.

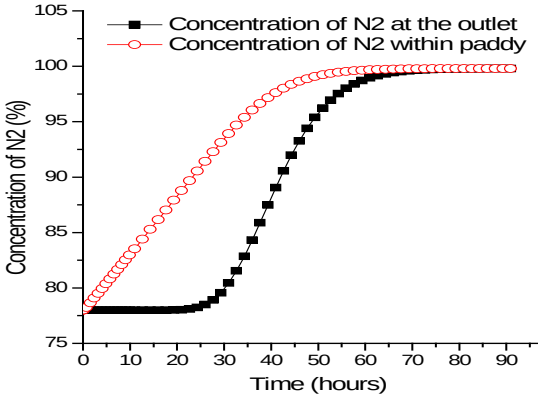

Fig. 4 Variation of nitrogen concentration in stored paddy with the time of nitrogen-filling.

3.2 Evaluation of uniformity of nitrogen concentration in the grain mass

The uniformity index was used to evaluate the uniformity of nitrogen concentration distribution in the stored paddy during nitrogen-filling. The formula of uniformity presented by Weltens (1993) was used to calculate the value of uniformity of nitrogen concentration inside stored paddy. Fig. 5 is a layout of the monitoring points of nitrogen concentration at each level in the stored paddy. For monitoring of the nitrogen concentration, 27 monitoring points at three levels were placed inside the stored paddy, and each layer had nine points. The heights of the monitoring points for the upper, middle and bottom layers were 1, 2.5 and 4 m, respectively, away from the warehouse floor. Each monitoring point was 1 m away from the inner wall of the warehouse. The evaluation of observed and simulated uniformity values of nitrogen concentration distribution inside the stored paddy is summarized in Tab. 1. It was found that the nitrogen gas was distributed uniformly in the stored paddy after 61 hours of nitrogen-filling. A good agreement between the observed and simulated nitrogen concentrations indicated that the model and the parameter values used in the model are applicable for predicting nitrogen gas concentration in stored grains.

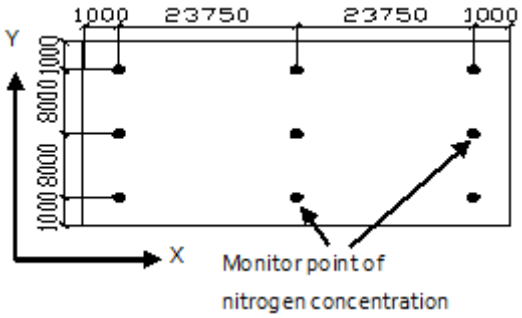

Fig. 5 Layout of monitoring points of nitrogen concentration at each layer in stored paddy.

Tab. 1 Comparison of uniformity values of nitrogen concentration distribution from a field observation and simulation.

	Uniformity of nitrogen concentration in X direction			Uniformity of nitrogen concentration in Y direction			Uniformity of nitrogen concentration in Z direction		
Location (m)	X=1.00	X=24.75	X=48.50	Y=1.00	Y=9.00	Y=17.00	Z=1.00	Z=2.50	Z=4.00
Value of monitoring (species fraction)	0.9911	0.9897	0.9916	0.9890	0.9997	0.9882	0.9948	0.9936	0.9987
Value of simulation (species fraction)	0.9997	0.9997	0.9997	0.9999	0.9999	10.000	0.9997	0.9997	0.9996

4. Conclusions

Application of controlled atmosphere technology in grain storage using nitrogen is highly efficient, environmentally friendly and safe. It is essential to investigate the movement of gas through the grain mass and the uniformity of gas distribution in the stored grain in order to maintain a lethal concentration level for the required exposure period. In this paper, numerical simulation of the horizontal flow and concentration distribution of nitrogen within a stored-paddy bulk in a large warehouse was conducted. The concentration distribution and flow uniformity of nitrogen were also analyzed during the nitrogen-filling process for CA storage. The following specific conclusions were drawn from this study:

(1) Numerical modeling can accurately predict the flow and concentration of nitrogen inside a stored grain mass during the nitrogen-filling process.

(2) It is feasible and practical to introduce and distribute nitrogen gas into a stored grain bulk in a large warehouse by means of the horizontal ventilation system of the warehouse.

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Study on Rapid Detection of Degree of Freshness of Paddy Rice in China

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Abstract

This paper describes research results and progress of rapid detection of the degree of freshness of paddy. We studied the changes of degree of freshness, fat acidity value and taste evaluated value of paddy under different storage conditions in the laboratory. The correlations between the degree of freshness, fat acidity value and taste evaluated value were analyzed. The results showed that there was a significant negative correlation ($p < 0.01$) between the degree of freshnessand fat acidity value. The correlation coefficient was -0.845. The degree of freshness was significantly positively correlated with the taste evaluated value, and most of the correlation coefficients were above 0.9. The nationwide investigation result of paddy's degree of freshness showed that there was an obvious distinction in the degree of freshness between newly harvested rice and rice harvested in previous years. The degree of distinction of indica rice achieved 85%. Due to its special reasons, japonica rice had a lower degree of distinction, but it also reached 75%.

Keywords: paddy, degree of freshness, fat acidity value, taste evaluated value.

1. Introduction

Rice is a staple food for more than 60% of the world's population, especially in China (Wei et al., 2007). As a primary dietary source of carbohydrates, rice plays an important role in meeting caloric requirements and nutrient intakes (Yang et al., 2006). Aging during storage results in numerous changes in the chemical and physical properties of rice (Patindol et al., 2005; Singh et al., 2006; Sodhi et al., 2003). These changes in pasting properties, color, flavor, and composition affect rice cooking and eating quality (Srikaeo K et al., 2013; Park C E et al., 2012). The fresh rice is prefered in the market in China. So it is particularly important to detect the degree of freshness rapidly during acquisitions, and during daily or long-term storage of paddy rice.

Since 2013, we have developed an instrument which could detect the paddy freshness rapidly for the degree of freshness. The higher the degree of freshness of paddy is, the fresher it is. The detection principle of the degree of freshness of paddy is that milled rice is mixed with special reagent, according to the different contents of ketones and aldehydes, the solution reveals different color. Analysing the spectrum of these colors, we can quantify the degree of freshness of paddy. The instrument is easy to use, and the results are objective and accurate.

In nearly two years, the research on rapid detection of degree of freshness has made new progress. We studied the changes of paddy freshness qualities during different conditions of storage in a

laboratory and the correlations between the degree of freshness, taste evaluated value, and fat acidity value. The result proved that the degree of freshness is a sensitive index which can reflect its freshness quality in the aging process. We have detected the degree of freshness of paddy rice of different producing areas, varieties and production years for three years across the country. A total of 9381 samples were statistically analyzed, yielding the standard of determination and evaluation of degree of freshness of paddy.

2. Materials

2.1 Materials of Storage Experiment

Fourteen samples of fresh japonica rice and indica rice were selected from 6 provinces which included Jiangsu, Heilongjiang, Jilin, Jiangxi, Zhejiang and Anhui. The japonica rice samples were numbered from 1 to 9, and the indica rice were numbered 10 to 14.

2.2 Nationwide investigation of paddy degree of freshness

The producing area of indica rice samples covered 14 provinces and the harvest years were from 2012 to 2016. The number of samples of indica rice was 3106. There were 2097 new havest samples in 2015 and 2016, and 1032 samples harvested in previous years from 2011 to 2014.

The producing area of japonica rice covered 6 provinces and the harvest years were from 2011 to 2016. The number of samples of japonica rice was 1612. There were 1177 new havest samples in 2015 and 2016, and 357 samples harvested in previous years from 2011 to 2014.

3. Instruments and Equipment

Degree of freshness tester of paddy rice: JCXD10, Beijing Dongfu Jiuheng Instrument Technology Co., Ltd.

Rice hulling machine: JDMZ, Beijing Dongfu Jiuheng Instrument Technology Co., Ltd.

Rice mill: JNM - Ⅲ, Chengdu Shitewei Technical and Development Company.

Hammer Cyclone mill: JXFM110, Shanghai Jiading Grain and Oil Instrument Co., Ltd.

4. Experimental Method

4.1 Grain storage and sampling method

After packing and sealing, experimental paddy samples were stored at indoor constant temperatures of 25℃ and 35℃, respectively. Three hundred and fifty grams of paddy were sampled periodically and the specific sampling times are shown in Tab. 1.

Tab. 1 Sampling Time.

Sampling Frequency	Sampling Time	
	25℃/(W)	35℃/(W)
start	0	0
first	8	2
second	20	4
third	60	7
fourth	92	12
fifth		24
sixth		32
seventh		64

W: means per week

4.2 Degree of freshness determination

In accordance with LS/T 6118-2017 Inspection of grain and oils-Determination and evaluation of degree of freshness of paddy.

4.3 Fat acidity value determination

According to the appendix A of *GB/T 20569-2006 The determination rules of rice quality in storage.*

4.4 Taste evaluated value determination

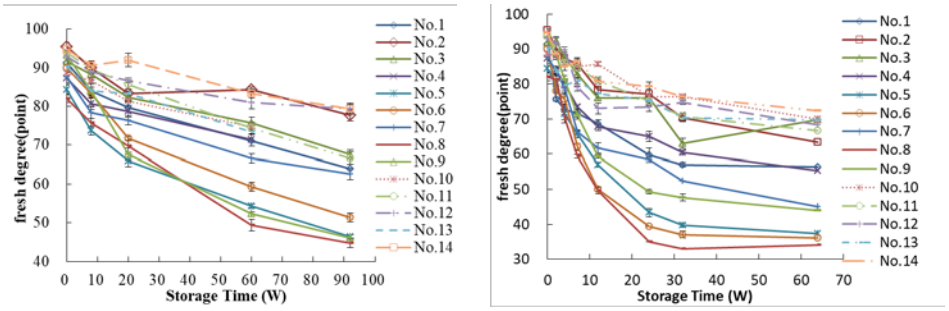
According to the appendix B of *GB/T 20569-2006 The determination rules of rice quality in storage*.

5. Results and Analysis

5.1 Results of storage experiment

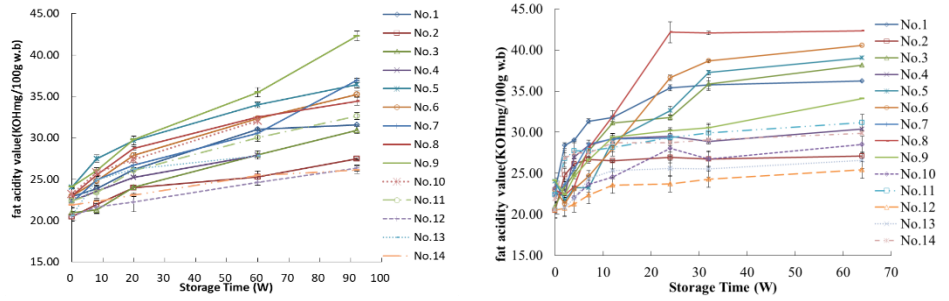
5.1.1 Change of each quality indicators

The change in degree of freshness, fat acidity value and taste evaluated value in fourteen paddy samples stored respectively under 25℃ and 35℃ constant temperature conditionsare as shown in Figs. 1, 2 and 3 over lngth of storage time.



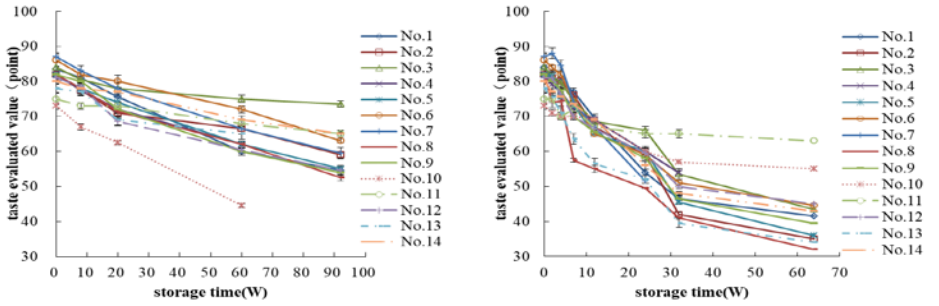
a 25℃ b 35℃

Fig. 1 Change in degree of freshness during storage time.



a 25℃ b 35℃

Fig. 2 Changein fat acidity value during storage time.



a 25℃ b 35℃

Fig. 3 Change in taste evaluated value during storage time.

These figures show that paddy degree of freshness and taste evaluated value decreased over storage time, while fat acidity value increased. Thus, these three indicators can accurately reflect the degree of deterioration of paddy's quality during time in storage.

5.1.2 Correlation between degree of freshness and fat acidity value

The density ellipse of degree of freshness and fat acidity value of storage samples at a confidence level of p = 0.95 is shown in Fig. 4.

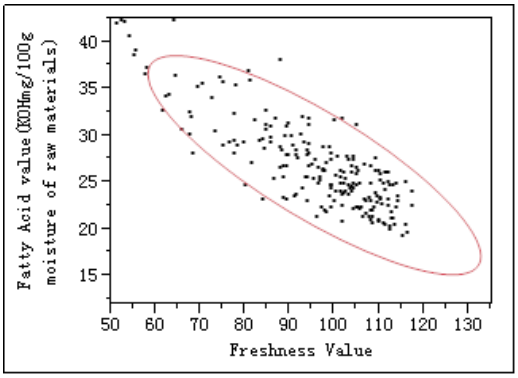


Fig. 4 Correlation of the degree of freshness and fat acidity value of storage samples at a confidence level of p=0.95.

The correlation and significance test of degree of freshness and fat acidity value of storage samples are shown in Tab. 2.

Tab. 2 Correlation and significance test of the degree of freshness and fat acidity value of storage samples.

variable	mean value	standard deviation	correlation r	significance probability p	quantity
Degree of freshness	91.22	14.78	-0.845	＜.0001*	162
Fatty acids value	27.68	4.96			

The correlation coefficient of degree of freshness and fat acidity value was -0.845, and the significant probability was smaller than 0.0001. These values suggest that the degree of freshness of paddy is significantly negatively correlated with its fat acidity value.

5.1.3 Correlation between degree of freshness and taste evaluated value

Due to the great influence of paddy varieties on the taste evaluated value, the correlation analysis between the degree of freshness and the taste evaluated value was carried out separately for each sample. The results are shown in Tab. 3 and Tab. 4.

Tab. 3 Relationship between degree of freshness and taste evaluated value of paddy stored at 25 °C.

Number	Indicator	Storage time (w)					Correlation coefficient
		0	8	20	60	92	
1	TAV	84	81	76	60	55	0.98
	FD	91	84	80	71	64	
2	TAV	82	78	71	67	59	0.97
	FD	95	90	83	84	78	
3	TAV	84	81	78	75	74	0.97
	FD	92	88	82	76	68	
4	TAV	82	78	72	62		0.98
	FD	87	81	79	71		
5	TAV	82	78	74	62	55	0.98
	FD	84	74	66	54	46	
6	TAV	86	82	80	72	63	0.96
	FD	90	83	72	59	51	
7	TAV	87	83	78	67	60	0.97
	FD	88	78	76	67	63	
8	TAV	82	78	72	62	53	0.98
	FD	82	76	70	49	45	
9	TAV	82	80	72	60	54	0.98
	FD	93	84	68	52	46	
10	TAV	73	67	63	45		0.98
	FD	89	87	81	75		
11	TAV	75	73	73	68	65	0.99
	FD	94	90	86	74	67	
12	TAV	81	79	69	61	55	0.98
	FD	93	89	87	81	79	
13	TAV	78	77	69	65		0.87
	FD	90	84	83	73		
14	TAV	80	78	77	69	65	0.99
	FD	95	91	92	83	79	

Tab. 4 Relationship between degree of freshness and taste evaluated value of paddy stored at 35 °C.

Number	Indicator	Storage time (w)								Correlation coefficient
		0	2	4	7	12	24	32	64	
1	TAV	84	80	80	77	70	54	47	42	0.90
	FD	91	76	73	72	69	60	57	56	
2	TAV	82	81	80	76	67	59	42	35	0.97
	FD	95	90	87	86	78	77	70	63	
3	TAV	84	82	80	75	69	66	54	44	0.92
	FD	92	92	88	83	76	76	63	70	
4	TAV	82	79	77	72	69	60	54		0.97
	FD	87	84	80	73	68	65	60		
5	TAV	82	82	76	73	65	60	46	36	0.94
	FD	84	80	71	66	57	43	40	37	
6	TAV	86	84	82	75	65	59	51	45	0.96
	FD	90	78	76	62	50	39	37	36	
7	TAV	87	88	85	74	67	52			0.93
	FD	88	84	79	66	62	58			
8	TAV	82	74	75	58	55	50	41	32	0.95
	FD	82	81	70	59	50	35	33	34	
9	TAV	82	80	78	70	66	58	47	40	0.95
	FD	93	88	81	71	59	49	48	44	
10	TAV	73	71	70	70	69	60	57	55	0.98
	FD	89	88	86	85	86	76	76	70	
11	TAV	75	75	70	72	67	65	65	63	0.96
	FD	94	90	86	85	81	75	71	67	
12	TAV	81	81	76	70	67	60	50	45	0.90
	FD	93	92	90	79	73	73	75	68	
13	TAV	78	76	71	64	57	52	40	34	0.95
	FD	90	84	80	81	77	75	70	70	
14	TAV	80	77	76	74	69	56	48	43	0.93
	FD	95	89	85	86	81	79	76	72	

Note: TAV means taste evaluated value. FD means degree of freshness

These experiments were terminated once insects and mould appeared in individual samples during the later period of storage.

The data in Tabs. 3 and 4 show that the degree of freshness of paddy was positively correlated with the taste evaluated value, and most of the correlation coefficients were above 0.9

5.2 Results of nationwide investigation of paddy degree of freshness

5.2.1 Analysis and results of indica rice

The results of degree of freshness of 3106 indica rice samples were analysed, and the distribution of degree of freshnesss of the samples is shown in Fig. 5.

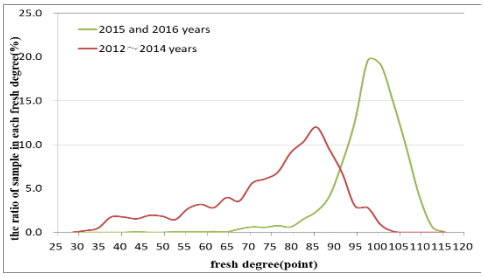


Fig. 5 Distribution of the degree of freshness of indica rice samples.

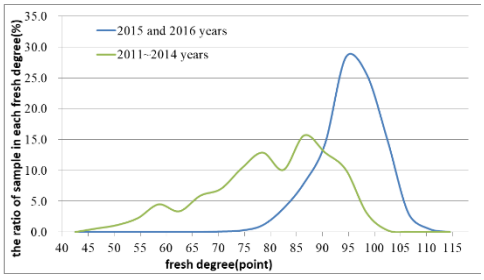


Fig. 6 Distribution of degree of freshness of japonica rice samples.

As shown above, there was an obvious distinction of degree of freshness between new harvest rice and rice harvested in previous years. With data analysis, the discrimination of new harvest samples in 2015 and 2016 and the rice harvested in previous years was close to 85%, ranging from 89 to 90 points.

5.2.2 Analysis and results of japonica rice

The distribution of the degree of freshnesss of 1612 japonica rice samples is shown in Fig. 6.

The results of statistical analysis showed that the discrimination of the degree of freshness of new harvest samples in 2015 and 2016 years and the samples harvested in previous years was close to 76%.

The discrimination of japonica rice was lower than that of indica rice, mainly because of good storage conditions of low temperature and humidity in its production area. Meanwhile the samples of japonica rice with good quality was larger than indica rice during the previous year's production.

Discussion

Above all, the degree of freshness is a rapid detection indicator that can accurately reflect changes of quality of paddy (fresh or not) and has significant correlations with fat acidity value and taste evaluated value. There was a significant negative correlation ($p < 0.01$) between degree of freshness and fat acidity value with a correlation coefficient of -0.845. However, degree of freshness was significantly positively correlated with taste evaluated value. The correlation coefficient was above 0.9.

According to the results of **a** nationwide investigation, there was an obvious distinction between degree of freshness of new harvest indica rice and indica rice harvested in previous years. The degree of distinction achieved 85%. For pecial reasons, japonica rice had a lower degree of distinction, but it also reached over 75%, which conforms to preserving quality according to China's legale storage requirements.

The above results show that the rapid detection technology of paddy's degree of freshness has great applicability to distinguish fresh and non-fresh paddy in China.

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Fumigation with Ph3 using automatic generation - Presentation of results of recent trials

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Abstract

Fumigation is essential part of preservation of grains, other edible commodity and perishables. Phosphine is most commonly used fumigant since more than 65 years. It is now practically the only fumigant and most commonly used. While fumigating with conventional metal phosphide formulations most common problems or concerns are operator safety, laborious to apply, gas retention in structure, uniformity of gas concentration in the structure, solid residues left in the commodity, limitations in ambient conditions to apply the fumigant and others. Bad fumigation practices lead to failed fumigations. These are blamed on insect resistance. Scientists have noted higher tolerance levels, but not resistance to phosphine. To address all the concerns referred, and limitations of conventional fumigants, we have developed a Phosphine generator and a suitable formulation for use with the same. This is a fully automatic machine. The formulation is granular and dust free. Those using our generators have stopped using conventional formulations of phosphine. The paper presents merits of technology, results of trials in various locations and on different commodity. This is the only system, which ensures uniform distribution of gas in entire structure to give 100 % guaranteed fumigation results.

Browning Mechanism and Process Optimization during MaizeMaize KX7349 Drying

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Abstract

Browning of KX7349 maize during drying occurred mainly in the pericarp layer. Browning was caused by oxidation of water soluble matter in the pericarp layer. Moisture content had no significant influence on browning rate. Drying temperature, drying time and drying method (vacuum drying or hot-air drying) had significant influence on the browning rate. Through lab research, a prediction model for the relationship between browning rate and drying air temperature was developed. Total drying time is $y=-13.086+0.289X_1+1.045X_2$, where y is the browning rate（%）, X_1 is drying temperature（℃）, X_2 is total drying time（h）, the value range of X_1 was 30～80, the value range of X_2 was 2～10. The concurrent and counter current dryer was applied in Nenjiang to optimize the drying process. The hot air temperature in each drying stage was reduced. When the hot air temperature of the 1st, 2nd, 3rd drying stage was reduced to 95℃,75℃,60℃

respectively, the browning rate was reduced to 15%～16%. Keeping the hot air temperature constant at each drying stage, by drying twice, the browning rate was reduced to 4%～6%.

Keywords: KX7349, drying, browning mechanism

1. Introduction

Maize is one of the most important cereal grains in the world. It is cultivated worldwide. America and China are the main producers of maize, which yielded 57.5% of maize production in 2015 in the world. In China, maize is cultivated widely. According to geographical and climatic features, there are six maize planting areas. Among them, the northern spring maize region accounts for 30% of maize production in China. This area includes Heilongjiang, Jilin, Liaoning and Inner Mongolia. When the maize is harvested in this area, the temperature decreases rapidly, maize cannot be sun-dried sufficiently. The moisture content is high. Especially in Heilongjiang province, on some occasions, the MC is up to 30% wet-basis. Rapid moisture-removal technology is needed to achieve a safe stoage moisture level and to inhibit the growth of microorganisms. Hot-air drying is an appropriate approach.

Maize KX7349 is a variety bred by KWS, a German seed company. It is planted widely in Inner Mongolia, Heilongjia and Jilin. After drying, many maize kernels undergo browning, which results in a rapid drop in price. It is rejected by the food industry if premium quality is needed.

This phenomenon motivates us to find the reasons and explore methods of improving the process of drying. Studies were conducted to determine the factors affecting browning and the separation and extraction of browning materials.

2. Materials and Methods

Materials: KX7349 maize, harvested in 2014, provided by Heilongjiang province.

Main instruments: DHG-9146A electric constant temperature drying oven (Shanghai Yiheng Science Instruments Co., Ltd.); DZF-6090 vacuum drying oven (Shanghai Yiheng Science Instrument Co., Ltd.); AL204 electronic scales (Shanghai Mettler-Toledo Instruments Co., Ltd.); HSNT25 concurrent and counter current dryer (COFCO engineering Co., Ltd.); HPLC-ELSD analyzer with a sugar analysis column (Agilent Technologies Co., Ltd.).

Main methods:

- Browning rate

Browning rate (%) = Weight of browning shelled maize×100/ Total weight of shelled maize

- Main factors affecting browning rate

The KX7349 shelled maize at 30% MC was dried by the electric constant temperature drying oven at 30℃, 40℃, 50℃, 60℃, 70℃ and 80℃, respectively. The browning rate was tested every two hours.

The KX7349 shelled maize with original MCs of 14%, 20%, 25% and 30% was dried at 80℃. The browning rate was tested every half an hour to analyze the effect of MC on the browning rate.

KX7349 maize growing in different areas was tested to evaluate the effect of growing area on browning rate.

Also, two different drying methods, namely hot-air drying and vacuum drying, were compared.

- Separation and extraction of browning materials

The maize pericarp was peeled off the kernel. The maize pericarp and the kernel were dried at 100℃ for 30 min to test the colour changes. The pericarp was also treated in distilled water for 2 hours and then dried for 30 min at 100℃. Browning and non-browning shelled maize were analyzed by the HPLC-ELSD analyzer equipped with a sugar analysis column.

- Drying process optimization

The KX7349 shelled maize at 30% MC was dried by the electric constant temperature drying oven at 30℃, 40℃, 50℃, 60℃, 70℃ and 80℃, respectively. The browning rate was tested every two

hours.Maize

The concurrent and counter current dryers were applied in Nenjiang to optimize the drying process. The initial MC of maize was 27% ~ 31%. The drying process included two concurrent flow stages, one counter current flow stage and one cooling stage.

3. Results

- Main factors affecting browning rate

Table 1 shows the results of browning rate of KX7349 maize being dried at different temperatures and drying times. The browning rate increased as both drying time and temperature increased. At 80℃, after 10 hours the browning rate was highest at 19.99%. At 80℃, the browning rate increased much more rapidly as compared to drying at the other temperatures. The analysis showed that the drying time and temperature significantly affected the browning rate ($p < 0.01$).

Table 1. Browning rate (%) at different drying temperatures and times.

	2 h	4 h	6 h	8 h	10 h
30℃	1.13±0.12	1.47±0.05	2.01±0.02	3.00±0.02	5.01±0.01
40℃	1.51±0.09	2.00±0.09	3.05±0.15	4.13±0.05	5.81±0.09
50℃	3.96±0.13	6.96±0.14	7.84±0.05	8.94±0.14	11.93±0.07
60℃	6.07±0.14	7.98±0.03	9.99±0.09	13.87±0.06	17.87±0.12
70℃	7.96±0.22	10.02±0.07	12.97±0.06	16.96±0.06	19.98±0.10
80℃	9.93±0.14	13.05±0.09	16.82±0.06	19.87±0.12	19.99±0.02

Table 2 shows changes in the browning rate of different original MC maize being dried at 80℃. Regardless of the original maize MC, the browning rate increased with drying time. maizeAlso, the analysis showed that the moisture content of maize had no effect on the browning rate (p=0.647).

Table 2. Browning rate (%) of maize with different MCsmaize.

MC (%)	0.5 h	1 h	1.5 h	2 h
14	2.56±0.09	5.40±0.01	7.24±0.05	9.23±0.06
20	2.44±0.04	5.33±0.12	7.29±0.09	9.29±0.07
25	2.56±0.15	5.26±0.11	7.31±0.05	9.23±0.09
30	2.59±0.12	5.33±0.09	7.28±0.03	9.30±0.04

Table 3 shows the browning rates of maize from two different areas being dried at 90℃. At the same drying conditions, the browning rate of the maize from Nenjiang area was much higher than the maize from Qitaihe area. The analysis showed growing area of maize had a significant effect ($p < 0.01$). Because the annual accumulated temperature was different in these two places, the maturity level was different for maize from these two places.

Table 3. Browning rate (%) of maize from different areas.

Drying method	Nenjiang	Qitaihe
Hot-air drying 90℃, 30 min	43.73±0.42a	6.70±0.13b
Hot-air drying 90℃, 1 h	72.50±0.19a	25.12±0.15b

Table 4 shows the browning rates from the different drying methods. After 30 min of drying, the browning rates by vacuum and hot-air drying were 6.24% and 43.72%, respectively. After 1 h, the browning rate by vacuum drying was 6.03%, while the browning rate by hot-air drying was 72.50%. Compared with hot-air drying, vacuum drying could obviously inhibit maize browning. This indicated the maize underwent browning due to the participation of oxygen.

Table 4. Browning rate （%） for maize dried by two different methods.

Drying method	Browning rate (%)
Vacuum drying 90℃，30 min	6.24±0.24
Vacuum drying 90℃，1 h	6.03±0.12
Hot-air drying 90℃，30 min	43.73±0.42
Hot-air drying 90℃，1 h	72.50±0.19

- Separation and extraction of browning materials

Fig. 1. Before drying.

Fig. 2. After drying.

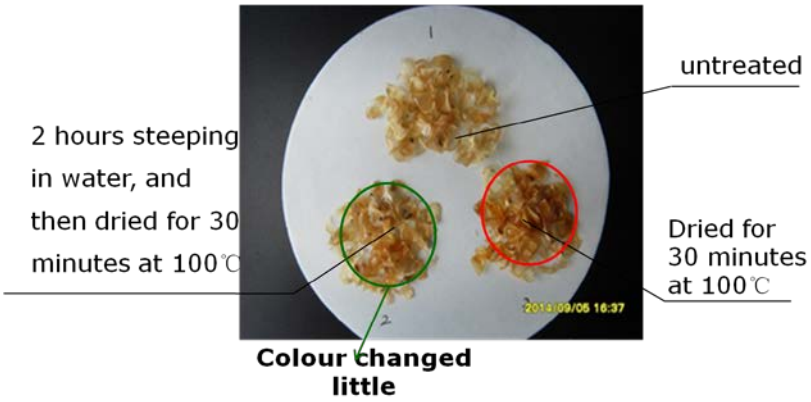
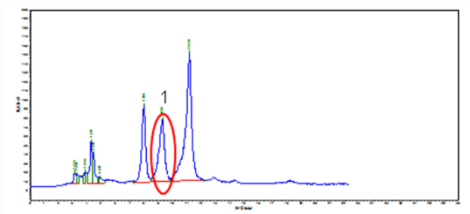


Fig. 3. Dissolution characteristics of browning materials.

The maize pericarp was peeled off the kernel. After drying, the maize pericarp underwent browning, however, the endosperm and embryo changed only slightly (Fig. 1 and Fig. 2). This indicated that most of the browning materials were in the pericarp. The pericarp was treated in distilled water for 2 hours, and then dried for 30 minutes at 100℃. Compared with the untreated pericarp after being dried at the same condition, the colour changed slightly, indicating that the browning materials were soluble in the water (Fig. 3).

From the HPLC fingerprint spectrums (Fig. 4), Peak 1 was found. The retention time of Peak 1 corresponded to that of glucose. The results showed that the glucose content of the browning in maize was twice as much of the non-browning maize. From this point, we deduced that browning might be caused by Mailard reaction. In addition, we concluded the following new findings: 1. browning materials were mainly in maize pericarp and soluble in water, 2. Browning was induced by oxidation of the water-soluble materials and 3. glucose content was higher in browning maize than that in non-browning maize without drying.



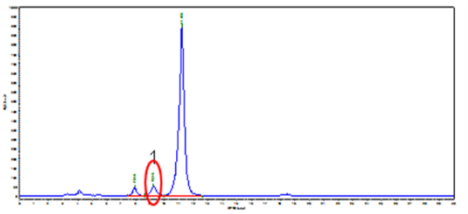


Fig. 4. Sample of browning

Fig. 5. Sample of non-browning

- Drying process optimization

From the results of the above analysis, we found that main influence factors of browning rate were drying temperature, drying time and drying method. Because vacuum drying has not been applied commercially in practice, drying temperature and drying time became the most important factors determining the maize browning rate.

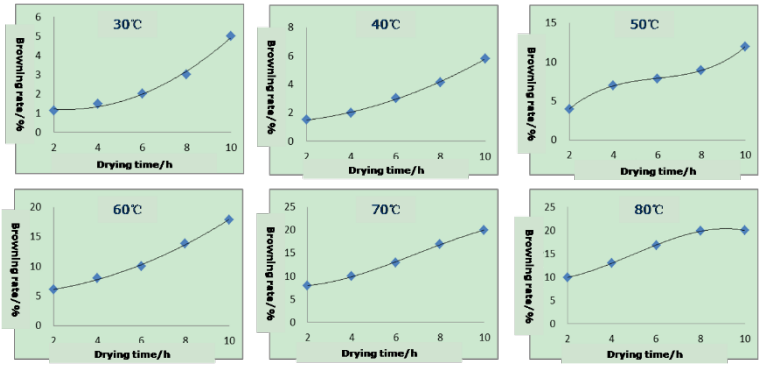


Fig.6. Changes in browning rate with drying time and temperature.

Fig. 6 shows changes in browning rate with drying time and drying temperature. The browning rate increased with drying time for every drying temperature. During the early drying period, the slope of the curve was very steep for the higher temperature, compared with the lower temperature, suggesting that drying temperature had more obvious effect on browning rate than drying time.

Fig. 7. Concurrent and counter current dryer.

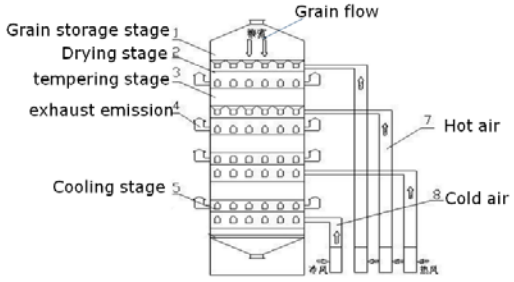


Fig.8. Schematic drawing of concurrent and counter current dryer.

The concurrent and counter current dryer (Fig.7) was applied in Nenjiang to optimize the drying process. In the process, the hot air temperature in each drying stage was gradually reduced. When the hot air temperatures of the 1st, 2nd and 3rd drying stages were reduced to 95°C, 75°C and 60°C, respectively, the browning rate was reduced to 15% ~ 16% (Table 5). Keeping the hot air

temperature constant at each drying stage, by drying twice, the browning rate was reduced to 4%～6%.

Table 5. Browning rate (%) of MaizeKX7349 maize.

1st drying stage		2nd drying stage		3rd drying stage		Draining maize (Hz)	Browning rate (%)
Hot air (℃)	Grain (℃)	Hot air (℃)	Grain (℃)	Hot air (℃)	Grain (℃)		
120	40	100	52	70	45	10	25 ~ 30
110	30	95	50	70	43	9	22 ~ 28
100	30	80	40	70	41	8	17 ~ 20
95	26	75	37	60	45	7	15 ~ 16
95	24 25	75	35 40	60	42 43	15 16	(drying twice) 4 ~ 6

This can be explained by the fact that between the first and second drying stages, there was a sufficiently long tempering stage. During the long tempering stage, moisture transported from the interior of a kernel to the surface, and consequently, the water near the surface could be removed easily when subjected to drying conditions again. This not only reduced the drying time but also the energy consumption.

4. Discussion

Drying temperature, drying time and drying method were the main influence factors of the browning rate. Initial MC of maize hardly influenced browning. Increasing drying temperature and drying time led to an increase in the browning rate. The analysis showed drying temperature and time significantly affected the browning rate. This finding had important implications in optimization of the drying process.

Regarding the browning mechanism, the following new findings were concluded: 1. browning materials were mainly in maize pericarp and soluble in water, 2. the browning was induced by oxidation of the water-soluble materials, and 3. glucose content was higher in browning maize than in non-browning maize.maizemaizemaize

The concurrent and counter current dryer was applied in Nenjiang to optimize the drying process. The hot air temperature in each drying stage was gradually reduced. When the hot air temperatures of the 1st, 2nd and 3rd drying stages were reduced to 95℃, 75℃ and 60℃, respectively, the browning rate was reduced to 15% ~ 16%. Keeping the hot air temperature constant at each drying stage, by drying twice, the browning rate was reduced to 4% ~ 6%.

Acknowledgement

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Session 5
Physical and Biological Control

Temperature: Implications for Biology and Control of Stored-Product Insects

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Extended Abstract

Insects are affected by temperature in all aspects of their biology, ecology, reproduction, behaviour, physiology and biochemistry (Fields, 1992; Beckett et al., 2007). Stored-product insects reproduce between 15 and 35°C, with maximum reproduction occurring at approximately 33°C. Above and below these temperatures insects can move, but cannot complete their development. Temperatures below 5°C and above 40°C insects cannot walk, and will eventually die. Between -15 and -25°C insects freeze and die instantaneously. There are significant changes to these general patterns depending upon species, life stage and acclimation. For example, insects can become 10 times more resistant to cold if acclimated at cool temperatures (5-15°C) before being exposed to sub-zero temperatures.

Temperature also effects trapping (Fargo et al, 1989). The speed and direction of movement is affected by temperature (Flinn and Hagstrum, 1998). Insects move faster at higher temperatures, so that if the populations are the same, more insects will be trapped at higher temperatures. Insects will move towards warm temperatures, and avoid temperatures that are too hot.

In general insecticides work better at higher temperatures (Kenaga, 1961; Iordanou and Watters, 1969; Fig. 1), but some insecticides have only a small increase in efficacy (methyl bromide), others have a large increase in efficacy (carbon tetracholoride), whereas others have a decrease in efficacy (pyrethrins) with higher temperatures. Contact insecticides degrade faster at higher temperatures (Desmarchelier, 1977).

Given the many effects of temperature on every aspect of stored-product insects, researchers should carefully design experiments to avoid unseen effects by temperature, understand its effect on their area of study. Grain managers need also to be aware of the many ways, sometimes not obvious, that temperature can affect storage.

Keywords: insecticide, degradation, behaviour, heat, cold

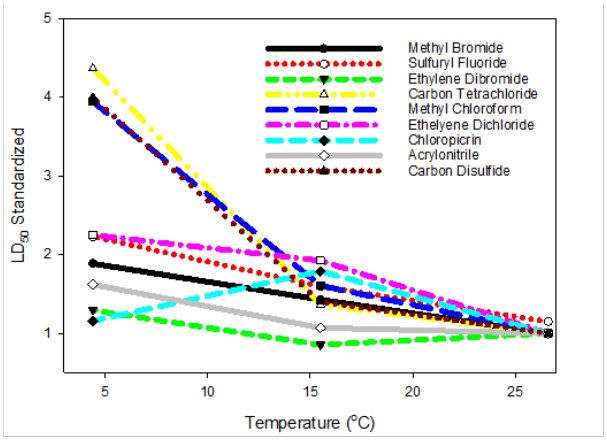

Fig. 1 Effect of temperature on efficacy of fumigants on *T. confusum*. LD_{50} are expressed as a proportion of LD_{50} at 26.7°C, data from Kenaga, 1961.

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Evaluation of insecticidal efficacy and persistence of Nigerian raw diatomaceous earth against *Callosobruchus maculatus* (F.) on stored cowpea

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Abstract

The insecticidal efficacy and persistence of Nigerian raw diatomaceous earth (DE) were evaluated in the laboratory on cowpea against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). The raw DE was applied to 1.5 kg lots of cowpea seeds at 0 (untreated control), 250, 500, 750, 1000 and 1500 mg/kg, and a commercial DE formulation (Protect-It®) applied at 1000 mg/kg was included in the test as positive (treated) control. The treated cowpea seeds were kept under ambient laboratory conditions (26 - 34°C and 24 - 93% RH. Bioassays were conducted on samples taken from each treatment at the day of storage and every 30 d for 6 consecutive months. Adult *C. maculatus* were exposed for 3 and 5 d to the samples and adult mortality was assessed over this exposure interval and progeny production and seed damage were assessed after additional 30 d. On freshly treated cowpea, both the raw DE and Protect-It® were highly effective against *C. maculatus* causing 100% adult mortality following 5 d of exposure. In general, the raw DE was less persistent on cowpea providing complete adult mortality only for two months. Protect-It® on the other hand was stable over the 6-month period of storage causing 95.8 to 100% adult mortality. None of the treatments completely inhibited progeny production after 2-3-moths storage period. The results of this study indicated that Protect-It® may provide suitable protection for 6 months against *C. maculatus*, but the raw DE in its present state is not suitable for long-term protection against this insect pest.

Keywords: *Callosobruchus maculatus*, raw diatomaceous earth, cowpea, residual activity

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most economically and nutritionally important indigenous African grain legumes produced throughout the tropical and subtropical areas of the world (Abate et al., 2011). It is a source of relatively low cost, high quality protein, and for many West and Central African farmers a major cash crop (Langyintuo et al., 2003). As production and consumption do not occur simultaneously, producers and traders need efficient storage systems to ensure year round cowpea availability for consumers. Consumers, on the other hand, want to buy cowpeas at the cheapest cost without compromising quality characteristics (Ndong et al., 2012).

Nigeria is the largest producer and consumer of cowpea, accounting for about 45 percent of world's production (Lowenberg-DeBoer and Ibro, 2008) and a per capita consumption of 25 - 30 kg per year (Nurudeen and Rasaki, 2011). The major storage pest of cowpea in Nigeria is *C. maculatus* (Adedire, 2001). As a field to-store pest, the attack, which starts before harvest and intensifies during storage, may cause total losses (Faroni and Sousa, 2006). The damage by *C. maclatus* is caused by oviposition on the surface of grains or pod and subsequent larval penetration into the grains. The attack results in weight loss, nutritional value, reduced level of product hygiene (presence of droppings, eggs, and insects), reduced seed germination resulting in decreased retail value (Almeida et al., 2005). According to Singh et al. (2002), a 5% annual production loss to this bruchid in Nigeria alone would cost about $100 million USD, or a loss of over 40,000 tonnes of cowpea. Fumigants, chiefly phosphine and dichlorvos are the major synthetic insecticides used in controlling *C. maculatus* in Nigeria. The storage conditions available to most farmers enable re-infestation, increasing the frequency of insecticide use. These chemicals may result in deleterios effects ranging from cowpea poisoning, environmental contamination, residues in grain, development of genetic resistance due to improper usage, and hazards to workerIn addition, the high costs of chemicals may also make it difficult for small-scale farmers to access (Lowenberg-DeBoer and Ibro, 2008), accompanied by increased infestation and losses.

The search for alternatives to synthetic insecticides in stored-products for insect pest management has been intensifing. One alternative is the use of diatomaceous earth which has received considerable attention, and are considered among the most promising alternatives to synthetic residual insecticides in stored-grain protection (Athanassiou et al., 2003).. During the last 20 years, DE has been the subject of several review papers with the numerous references cited within each of review. Also DE is now registered as a grain protectant or for structural treatment in several countries (Korunic, 2016). The mode of action of DE is different from the synthetic insecticides. DE absorbs the insect's cuticular waxes, and insects die from desiccation (Korunic, 2013). The advantages of using DE are its low mammalian toxicity, its stability, leaving no toxic residues on grains, control of the synthetic insecticide resistant pests and applied using the same technology for conventional grain protectants (Vayias et al., 2006).

Regional deposits of DE have been shown to be effective against local populations of stored-product insect species. For example varying deposits exist in Croatia (Korunic et al., 2009; Liska et al., 2015), Greece and Romania (Athanassiou et al., 2016) and Iran (Ziaee et al., 2013; 2016). There are also sevaral deposits of DE in Nigeria, however, their insecticidal efficacy has not been widely investigated. Kabir et al. (2011) first reported the insecticidal efficacy of Bularafa DE against *Tribolium castanem* (Herst) then against *Rhyzopertha dominica* (F.) (Kabir et al., 2013). Later, Nwaubani et al. (2014) reported the efficacy of Bularafa and Abakire diatomites agaisnt *R. dominica* and *Sitophilus oryzae* (L.). Information on insecticidal efficacy is important for commercial development of Nigerian DE deposits for use as grain protectant. The objective of this research was to evaluate the insecticidal efficacy and residual activity of Bularafa raw DE to control *C. maculatus* in stored cowpea.

Materials and Methods

Test Insect

Callosobruchus maculatus were obtained from laboratory culture, wich were maintained on cowpea for about a year. Adult insects were used to establish new insect cultures for the experiments. Two (1 litre capacity) glass jars were filled with 400 g of cowpea grains and 100 mixed-sex adults of the test insects were introduced into culture medium to oviposit. Each jar was covered with nylon mesh and secured with rubber bands. Parent insects were removed five days after introduction and the resulting F_1 progeny aged 0-2 days were used for the bioassay. New cultures were set up monthly to ensure availability of adult insects throughout the experiments.

Cowpea seeds

Insecticide free cowpea grains (Var. Borno Brown), were obtained from Borno State Agricultural Development Programme (BOSADP) Maiduguri, Borno State. The grains were cleaned and disinfested according to Kabir (2013), then equilibrated with laboratory condition for 10 days.

Diatomaceous earths

The raw diatomaceous earth (RDE) in the form of soft chalky rock was obtained from mines located 6 km North of Bularaffa village (Latitude: 11^0 8′ 48″ and Longitude: 11^0 49′ 17″ E) in the Gujba Local Government Area of Yobe State, Nigeria. The DE was oven dried, ground and put through a 63 µm sieve. Its pH and tapped density were analyzed in accordance with methods described by Korunic (1997) while its mineral composition was analyzed in the Geology Laboratory, Ahmadu Bello University, Zaria, Nigeria. It has the following properties: tapped density- 312.5 g/L, pH-9.2; mineral composition: SiO_2 - 80.43%, Al_2O_3 -5.02%, CaO – 0.48%, Na_2O – 0.07%, K_2O -0.14%, Fe_2O_3 -0.17%, ZnO - 0.01%, and MnO - 0.01. The commercial formulation of DE (Protect-It®) was obtained from Diatom Research and Consulting Inc., Toronto, Canada. It is an enhanced DE that contains approximately 83.7% amorphous SiO_2, 5.6% Al_2O_3, 2.3% Fe_2O_3, 0.9% CaO, 0.3% MgO and 1.9% other oxides e.g. TiO_2 and P_2O_3), and 3-5% moisture content (m.c.). The median particle size is between 5 and 6 µm with 10% silica aerogel (Athanassiou et al., 2009).

Bioassay Procedure

Adult *C. maculatus* adults were bioassayed at RDE doses of 0 (untreated control), 250, 500, 1,000, 1500 mg/kg RDE and Protect-It at 1000 mg/kg . De's were applied to cowpea grains under ambient conditions (31-34° C and 24 - 30% R.H.). For the acute toxicity test, the appropriate amounts of DE were applied to 50 g of cowpea and placed in 150 ml glass bottles that were tumbled manually for 5 min to achieve an even distribution of the DE on the grains. Then, 30 mixed-sex adult insects were introduced into each bottle, capped with perforated plastic lids and kept on a laboratory shelf. Each treatment combination was replicated four times. Adult mortality was recorded on 3 and 5 d after exposure, while progeny production and grain damage were assessed 40 days after infestation (DAI). The residual toxicity was assessed on 1500 g lots of cowpea grains treated with above mentioned doses and stored in plastic containers for 180 days (from April to October) under laboratory conditions (26-32° C and 33-93% RH). Similar bioassay procedures and observations as described above were conducted at 30 days intervals

Data Analysis

Where necessary, mortality data obtained were first corrected for control using Abbott's (Abbott, 1925) formula and together with data on grain damage were arcsine transformed. Data relating to number of F1 progeny were square root $\sqrt{(x + 1)}$ transformed. All were then subjected to Analysis of Variance (ANOVA Statistix 8.0). Differences between treatment means were separated using Tukey-Kramer Honestly Significant Difference (HSD) test at ($P \leq 0.05$).

Results

There are significant ($P<0.05$) variations in mortality levels of *C. maculatus* adults caused by different doses of RDE, when exposed for 3 days (Fig. 1). Irrespective of storage period, adult mortality increased with increase in raw DE dose. Protect-It was the most effective DE causing 100% adult mortality following 3 days of exposure to freshly treated cowpea grains and on those treated and stored for upto 60 days. With the RDE, similar effects were achieved, however, only on freshly treated seeds. Within each month there were significant declines ($P<0.05$) in mortality levels among raw DE doses.

Adult mortality increased with extended exposure period (Fig. 2). After 5 days of exposure mortality levels recorded for all RDE and Protect-It dosages significantly ($P<0.05$) increased irrespective of

post-treatment storage period. The RDE applied at 1500 mg/kg caused 100% adult mortality only on freshly treated cowpea and after 30 days of storage, whereas with Protect-It caused complete adult mortality for upto 90 post-treatment days of storage. Efficacy of both RDE and Protect-It declined with an increase in post-treatment storage period. In the case of RDE applied at 1500 mg/kg, adult mortality level decreased from 85% after 90 days 58.3% after 180 days post-treatment; and a similar trend was observed for other doses. With Protect-It, however the minimum mortality level caused (92.5% adult mortality) was recorded at 120 days post treatment and did not significantly change thereafter (Fig. 2)

Both DEs had significant impact on progeny production of *C. maculatus*. Effect on progeny production was significantly (P<0.05) influenced by DE dose and storage interval. Throughout the post treatment period, the untreated control supported significantly (P<0.05) higher number of progeny than the treated grains, except on those treated at 250 mg/kg after 60 days post-treatment. Furthermore increase in raw DE dose resulted in increased progeny suppression. Even the highest dose of RDE could not prevent progeny development, although in allcases the number of progeny was less <10. Protect-It was more effective in progeny inhibition inducing complete suppression on grain freshly treated or treated and stored for 30 days. The progeny that emerged thereafter was <3 per bottle (Fig. 3).

Progeny development in all treatments were drastically reduced after 90 days post-treatment. The percent of damaged seeds followed the same trend with number of progeny produced. Significant (P>0.05) differences in grain damage were noted among RDE doses and storage periods (Fig. 4). Higher grain damage was record in the untreated control and grains treated at 250 mg/kg of RDE, where differences were not significant except on freshly treated grains and after 30 post-treatment.

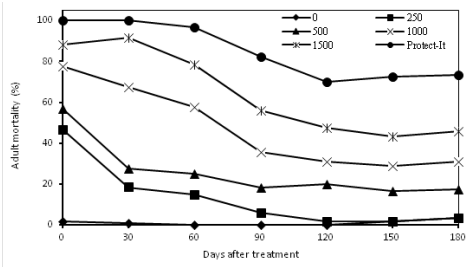

Fig. 1. Mean mortality of *C. maculatus* adults after three days of exposure to cowpea treated with different doses of DE and stored for various periods

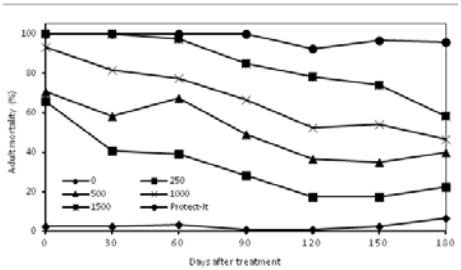

Fig. 2. Mortality of *C. maculatus* adults after five days of exposure to cowpea treated with different doese of DE and stored for various periods

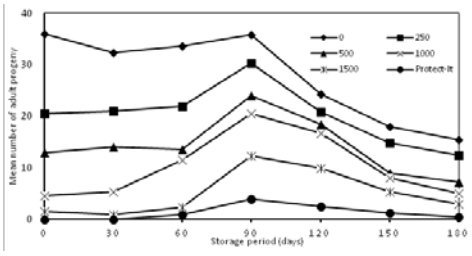

Fig. 3. Mean number of *C. maculatus* F1 progeny after 40 DAI on cowpea treated with different doses of DE and stored for different periods

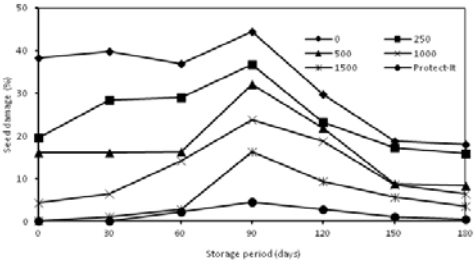

Fig. 4. Seed damage caused by *C. maculatus* 40 DAI on cowpea treated with different doses of DE and stored for different periods

Even on grain treated at 1500 mg/kg grain, damage could not be contained. After about 90 days post treatment, at all DE doses and the untreated control, there was a slight but significant increase in grain damage. Protect–It prevented grain damage on freshly treated seeds and after 30 days post-

treatment; and even where grain damage was recorded they were less than 3% and differences between storage periods were not significant ($P>0.05$).

Discussion

Adult progeny emerged in all DE treatments except in Protect-It treated (freshly treated and 30 days post treatment) grains, possibly because oviposition occurred before the adults died before exposure to the DE (Subramanyam and Roesli, 2000). However, in all treatments and the untreated control, progeny production significantly increased at 90 days post-treatment. This being the period coinciding with, middle of the rainy season in Maiduguri. This period is characterized by lower ambient indoor temperature (26 - 29ºC) and higher relative humidity (>80%) as compared to the first three months of the experimentation (May-July, when the r.h. was bet ween 24 and 58%). DE efficacy is related to relative humidity, temperature and changes in physical proprieties of treated grain (Athanassiou et al., 2005). During this period, it is likely the DE absorbed moisture from the atmosphere (Stathers et al., 2004). Other Studies have also shown that an increase in relative humidity reduces DE efficacy (Fields and Korunic, 2000; Rojht et al., 2010; Beris et al., 2011). Given that DE efficacy is reduced by higher moisture, there are direct consequences of DE effectiveness for grains stored in ventilated structures, especially in humid areas. On the other hand, the relatively higher efficacy of the RDE and Protect-It during the first 60 days of storage (May and June) which coincided with a period of high temperature (32) could be attributed to the fact that at higher temperatures insects are more mobile (Arthur, 2000) increasing contact with the DE particles, thus resulting in greater damage of the insect cuticle and water loss (Athanassiou et al., 2005; Wakil et al., 2010; Athanassiou et al. 2016). These results suggest that raw DE could be more effective in the Sudan and Sahel savannah regions, characterized by long dry season, high temperature and low relative humidity than in the humid areas. Another interesting finding of this study is the general reduction in progeny production including the untreated control after 90 days of storage (Fig. 3). The reason could not be explained. Perhaps cowpea grains became unsupportive of the pest's reproduction. This hypothesis needs to be verified by experiments.

One of the major drawbacks limiting the widespread use of DE is its reduction in efficacy under high moisture storage conditions (Korunic et al., 2016). This limitation could be overcomed in humid areas by thorough grain drying before storage and limiting moistuture equillibration with the sorrounding by using hermetic storage sructures.

In conclusion, this study indicated that the Nigerian RDE may not be suitable for long-term storage of cowpea grains against *C. maculatus* when applied at a dose rates of 1500 mg/kg; perhaps 2000-2500 mg/kg may be effective. Given that DE efficacy decreased during the months with high relative humidity, it is necessary to store DE treated grains in airtight structures or modify storage structures to limit moisture absorption from the surrounding environment in order to increase the benefits of DE treatments. Further studies on different particle sizes, higher dose rates and enhancement of Nigerian RDE are recommended.

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Thermal disinfestation of stored grains by solar energy

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Abstract

Chemical control especially fumigants is the most commonly used method to control stored-grain pests. A safer alternative for disinfestation is by heating up grains to a temperature of 50-60 °C. However, this alternative consumes high thermal energy due to the relatively high temperature required to achieve the required goal. Using solar energy as heat source for low temperature applications has become a viable mean for heating applications. Heating of grains using solar energy requires special design of grain storage system as well as development of efficient heat transfer mechanism to increase grain temperature over a limited period of time. The main objective of the current study is to use thermal disinfestation as a non-chemical, safe control method for grain management. A heating system based on solar energy has been developed as heat generator to control stored-grain insects. The target temperature range is 50-60 °C, which is enough to kill most of stored-grain insects. The grain hopper heating system relies on hot water supplied from a solar collector. The temperature of grains can be controlled based on the amount of grains contained in the hopper and the amount of energy transmitted to grains inside the hopper. The effectiveness of the system will be measured by reaching the best temperature and time combination for each insect species without affecting the seeds quality. The best temperature and time combination for cowpea beetles will be discussed in more details.

Retrospect, insights and foresights: Biological control of *Anobium puntcatum* with *Spathius exarator*

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Abstract

Biological control using beneficial organisms is getting more and more important in Integrated Pest Management. An effective strategy in the fight against the most common timber pest species, the furniture beetle *Anobium punctatum*, is based on the parasitoid wasp species *Spathius exarator*. This braconid wasp parasitizes its host species by piercing its ovipositor directly through the wood surface followed by oviposition onto the beetle larva. After feeding on the larva and pupation, the adult wasp emerges through a tiny 0.5 mm wide wood hole, which can be clearly distinguished from the 2 mm wide hole of *A. punctatum*. This enables us to observe easily the treatment success as each new *S. exarator* exit hole is equivalent to one killed beetle larva.

Between 2012 and 2017, the braconid wasps were introduced into about 80 *A. punctatum* infested buildings. At least twelve treatments over a period of up to three years were performed. On exactly defined areas, the newly emerged exit holes of *A. punctatum* and *S. exarator* were counted and the parasitisation rate was calculated. Here we present pooled data of 29 *A. punctatum* infested churches, successfully treated and monitored over a period of one to five years. Furthermore, as a representative sample, we show the results of one church over a period of six years.

We demonstrate the biological control of the common furniture beetle with this braconid wasp as an efficient, sustainable alternative to conventional residual methods. However, after a period of up to three years intensive treatment, a continuous monitoring-program with necessary additional single treatments should follow.

Key words: biological control, wood pest, cultural heritage, common furniture beetle, parasitic wasps

1. Introduction

Many chemical products for wood preservation are currently in a review process and possibly will be restricted from the European Biocidal Product Regulation. Thus, the expansion of alternative methods for pest control will be required like physical treatments or biological control. Physical methods like heat treatment or anoxia for controlling wood boring insects are well established and have a long tradition in practical experience. Biological control using natural enemies, on the other hand, had not been applied so far, although many antagonists against common wood boring species are known (Haustein, 2010; Schmidt 1952) and some reports of laboratory research or practical experience had been published (Lygnes, 1956; Haustein, 2010). Advantages in using parasitoids for biological control are the exclusive feeding on their host individual and the pinpointing of their hosts in hidden places, even at low densities (Schöller & Prozell, 2011). Described enemies of the common furniture beetle *Anobium punctatum* are amongst others the checkered beetles (Cleridae) *Opilo domesticus* and *Korynetes caeruleus* and the parasitoids *Spathius exarator*, *Sclerodermus domesticus* (Schmidt, 1952) and *Cerocephala cornigera* (Becker, 1942). So far, laboratory breeding experiments with predators in the family Cleridae revealed less success in mass rearing, thus making them currently unsuitable candidates for biological control (Haustein, 2010). Further practical monitoring (Ott, 2005; Paul et al., 2008; Schöller et al., 2008) confessed, that the braconid wasp *S. exarator* is one of the most common natural enemies of the furniture beetle in historic buildings in Germany. With the scientific knowledge of Becker (1942) and Lygnes (1956), the innovative pest control company APC AG from Nuremberg bred *S. exarator* as a commercial biological control method against *A. punctatum* (Auer and Kassel, 2014). After successful mass rearing, first results of laboratory as well as several praxis tests were published (Kassel and Auer, 2015; Biebl and Auer, 2017).

This publication shows the practical use of the parasitoid *S. exarator,* presenting pooled data from 29 infested objects, as well as one representative church, selected from currently more than 80 *S. exarator* treated objects.

2. Material and Methods

Biology of Spathius exarator

The hymenopteran wasp *S. exarator* has a body size of up to 9 mm, with females possessing an ovipositor about their body length. A female wasp localizes its host feeding within timber by its movements and gnawing. After drilling the ovipositor through the wood, it paralyzes the larva and lays a single flexible egg onto it. At a temperature of 20°C and humidity of 60%, the *S. exarator* larva hatches after 3 to 5 days and feeds from its host larva. After pupation, the adult wasp hatches 28 to 30 days after oviposition from the wood through a self-gnawed exit hole (diameter up to 0.5 mm), which can easily be distinguished from the exit holes of *A. punctatum* (diameter 1-2 mm).

General treatment and monitoring procedure

Depending on intensity of infestation, at least twelve treatments over a period of up to three years at a building temperature of >15°C were performed by the company APC AG. About 100 bred *S. exarator* were assembled for each defined infested area inside the building. Usually, a total of 500-800 wasps were released per object and treatment. After a period of up to three years of intensive treatment, monitoring was continued and, if necessary, further single treatments were conducted.

On exactly defined areas, the new exit holes of wasps and furniture beetles were counted and documented after each treatment. From these data, the reduction of newly hatched *A. punctatum* beetles per year was calculated. As data of hatching beetles in the year before the treatment are lacking, a real blank value is missing. Thus, additionally the parasitisation rate using the following formula was calculated:

$$\frac{\text{no. of } S.\ exarator \text{ exit holes}}{(\text{no. of } S.\ exarator \text{ exit holes}) + (\text{no. of } A.\ punctatum \text{ exit holes})}$$

Pooled Data

Presented data compare the basic parasitisation rate at the day of the first treatment with the parasitisation rate after the last monitoring of 29 buildings treated with *S. exarator* up to five years, using Mann-Whitney U-test. Furthermore, we show the data of the decline in newly appeared *A. punctatum* exit holes and the parallel mean cumulative increase of *S. exarator* exit holes in these objects. From 53 treated objects up to 2016, 24 could not be included in the analyses since monitoring modalities have changed (n=5), basic parasitisation rates could not be calculated because of missing data (n=6) or monitoring data were not collected (n=13).

Chapel P. (Bavaria)

In the Chapel P. in Bavaria, eight, six and eight treatments were performed in the years 2012 to 2014. In 2015 and 2016 two treatments per year and in 2017, one treatment was done. At each treatment, about 500 braconid wasps were released at infested foot stools and the altars of the chapel.

Statistics

Statistical analyses were conducted using the software PAST: Paleontological Statistics software package for education and data analysis (version 2.12; Hammer et al., 2001). Figures were made with Microsoft Excel or the Microsoft Excel add in SSC-Stat (Statistical service center, University of Reading, UK).

3. Results

Results from five years of practical application in 29 *S. exarator* treated objects showed promising results. The mean number of treatments per year were 5.8, 5.4, 3.7, 0.7 and 1.7 for treatment years one to five, respectively. Parasitisation rates in the monitored areas increased after treatment with *S. exarator*: Before the onset of applications, parasitisation rates ranged from 0 to 0.276 (0.085±0.088; mean ± standard deviation; n=29). Parasitisation rates in objects treated for one to five years were significantly higher, ranging from 0.017 to 0.565 (0.206±0.114, mean ± standard deviation; n=29; Mann-Whitney U-test, p<0,001; Fig. 1).

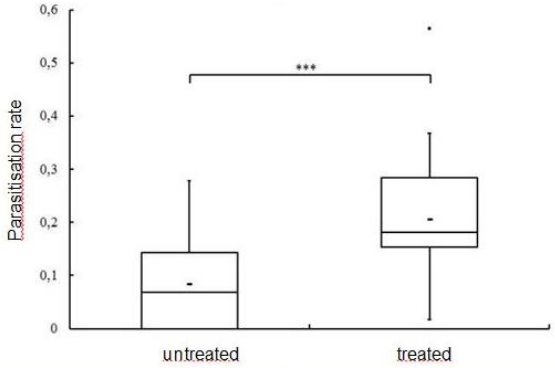


Figure 1. Mean parasitisation rates ± SD of 29 churches. Untreated: before first treatment; treated: during last monitoring.

Asterisks indicate significant differences (Mann-Whitney U-test, p≤0.001). Rhombus: outlier.

In table 1, an overview is given on the number of treated objects, the mean number of treatments per year as well as the mean number of newly hatching adult furniture beetles and wasps over treatment years one to five.

Number of newly hatched adults of *A. punctatum* continuously decreased over the first three years of treatment, as indicated by the declining number of newly appeared exit holes of *A. punctatum*. In the second year, an overall reduction by 61.22% was reached, and in the third year, a significant

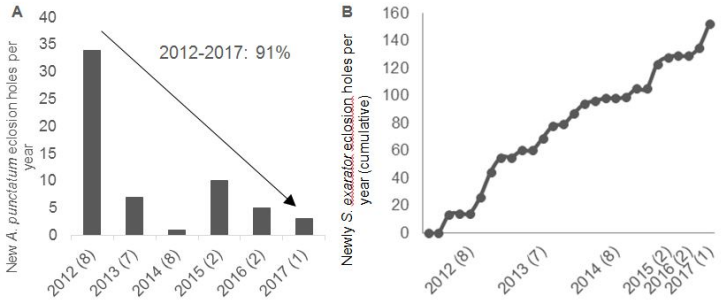
reduction of even 92.61% was achieved (Mann-Whitney U-test, p=0.016). After three years of treatment, the number of adult *A. punctatum* slightly increased. Compared to the first year, the overall decline of newly hatched *A. punctatum* still was 74.14% in the fourth and 67.68% in the fifth year. However, only few objects were treated over a period longer than three years (n=3, year four and five, respectively) and the number of applications (0-2) in these objects was rather small.

Simultaneously to the decreasing number of newly appeared *A. punctatum* exit holes, the number of *S. exarator* exit holes continuously increased over the treatment years (table 1).

Table 1. Number of annually new eclosion holes of *A. punctatum* and the cumulative number of eclosion holes of *S. exarator* in treatment years 1 to 5; SD: standard deviation

Year of treatment (n=number of objects)	Mean number of treatments per year	Mean number of *A. punctatum* eclosion holes (±SD)	Mean number of *S. exarator* eclosion holes (cumulative) (±SD)
1 (n=29)	5.8	15.47 (±17.74)	37.10 (±30.35)
2 (n=17)	5.4	6.00 (±7.13)	59.12 (±51.53)
3 (n=7)	3.7	1.44 (±0.83)	61.43 (±47.69)
4 (n=3)	0.7	4.00 (±4.33)	116.33 (±34.08)
5 (n=3)	1.7	5.00 (±4.08)	123.67 (±40.27)

In Figure 2A, the number of newly hatched furniture beetles in the Chapel P. in Bavaria is shown, as indicated by the number of their eclosion holes. From 2012 to 2014, after repeated treatments each year, a strong decline was measured (34, 7 and 1 new exit holes, respectively). However, a slight increase after the fourth treatment year was found (10 new exit holes). In the following treatment years, a decline in newly hatched furniture beetles could be observed to an overall reduction of 91% compared to the first year (2016: 5 and 2017: 3 new exit holes).



Figures 2 A–B: Eclosion holes by (A) *A. punctatum* and (B) *S. exarator* in foot stoles in Chapel P.

A. Number of newly found *A. punctatum* eclosion holes per year; B. Cumulative number of newly found *S. exarator* eclosion holes; Numbers in brackets indicate numbers of treatments per year

Figure 2B shows the amount of newly hatching *S. exarator*, represented by the number of their eclosion holes. In 2012 before first treatment, no *S. exarator* exit holes were found. At the end of 2012, after eight treatments, we found 55 new exit holes. This amount continuously increased over the treatment years up to a cumulative amount of 152 newly occurring adult wasps until now.

4. Discussion

The monitoring of the treated objects, presented in this publication, showed promising results. In all treated churches, the decline in newly hatched adult furniture beetles can be attributed to parasitisation by the released wasp *S. exarator*, as indicated by the increased number of their

eclosion holes. Thus, *S. exarator* appears to be an efficient and sustainable biological control method against the common furniture beetle. Furthermore, the success of a treatment can be estimated easily, as the eclosion holes of *S. exarator* and *A. punctatum* can be distinguished just by their size.

Larvae of the parasitic wasp *S. exarator* need larvae of *A. punctatum* for their development and, in consequence, each exit hole of *S. exarator* represents a parasitized and killed larva of *A. punctatum*. Over a period of five years of treatment, a mean number of 123.67 new *S. exarator* wasps hatched in the narrow-monitored areas of each treated church, representing as much killed furniture beetle larvae in this area. Corresponding to that, the number of annually newly appeared *A. punctatum* exit holes continually decreased with an overall reduction of 92.61% between the first and the third treatment year. However, in the fourth and fifth year of treatment, the number of newly eclosed furniture beetles slightly increased. However, only few objects were treated over a period longer than three years (n=3, year four and five, respectively) and additionally, the number of application (0–2) in these objects was rather small. Thus, monitoring objects over a longer period will reveal further insights in the population dynamics of *A. punctatum* and *S. exarator*.

It has to be noted that the amount of new *A. punctatum* exit holes in the first treatment year is already a reduced value. We started the first treatment about two months before the yearly one-time eclosion of *A. punctatum*. Thus, the wasps had been able to parasitize the beetle larvae for about two months until we documented the number of hatching *A. punctatum* beetles for the first treatment year. For a better estimation of the infestation level, it might be useful to monitor the eclosion of *A. punctatum* for one year before the onset of the treatment period.

As shown in literature (Ott, 2005; Paul et al., 2008; Schöller et al., 2008; Haustein, 2010), *S. exarator* appears to be a naturally occurring parasitoid of *A. punctatum*. Our own observations confirm that, since we found eclosion holes of *S. exarator* in two third of the 29 *S. exarator* treated churches before first treatment. After collecting data of exit holes of *A. punctatum* and *S. exarator* in untreated objects for two years, Haustein (2010) calculated a prey-predator relationship of 26.5 yearly eclosed *A. punctatum* per *S. exarator* (1:26.5). Even despite the knowledge of different data sets, our data show clearly reduced relationships of 1:1.6 after one year of treatment and 1:0.2 after the second treatment year (Auer and Kassel, 2017). Due to natural predator-prey relationships, the use of a natural antagonist in controlling pest organisms will not result in a 100% elimination of the pest (Graf, 1992; Querner, 2017) but in a decline of their population under a predefined minimum infestation level (Haustein, 2010). This is approved by our data, as the number of newly found eclosion holes of *A. punctatum* in treated objects slightly increased after a short period with a reduced number of treatments or without releasing *S. exarator*. Consequently, a continuous monitoring program with a well-adjusted treatment protocol is strongly recommended. This is even more important in the light of the relatively long development period of *A. punctatum* which can take up to 5 years (Pinniger, 1996).

Additionally, the parasitisation success of *S. exarator* also depends on various factors like paintings on infested objects (Biebl and Auer, 2017), previous insecticide treatments, type of wood and infestation level and should be investigated in further laboratory experiments as well as practical experience. By revealing the effects of these parameters, an elaborated application program adapted to the respective conditions in the treated objects can be developed and enhances the efficiency of the biological control of *A. punctatum*.

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Prospects of Entomopathogens in Post-Harvest Integrated Pest Management

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Abstract

In these exploratory experiments, entomopathogenic nematodes and fungi were investigated for the management of the populations of postharvest insect pests. Nematodes were screened for pathogenicity to *Plodia interpunctella* (Hübner), while nematodes and fungi were investigated for virulence to the maize weevil, *Sitophilus zeamais* (Motschulsky). Adults and larvae of *P. interpunctellea* were screened for susceptibility to the following six nematodes: *Heterorhabitis bacteriophora* Poinar (HP88, Lewiston and Oswego strains); *H. indica* Poinar, Karunakar and David (Homl strain); *H. marelatus* Liu and Berry (Point Reyes strain); *H. megidis* Poinar, Jackson, and Klein (UK211 strain); and *H. zealandica* Poinar (NZH3 strain). The nematodes that had the highest virulence to larvae and adults of *P. interpunctellea* were *H. indica*, *H. megidis*, and *H. marelatus*. Six strains of nematodes were studied, namely *H. bacteriophora*, *H. indica*, *H. georgiana* (K22), *Steinernema feltiae* SN and *S. carpocapsae.* All strains of fungi, *Beauveria bassiana* (GHA) and *Metarhizium brunneum* (F52) were evaluated for infectivity to adults of *S. zeamais*. The two strains of Steinernematidae nematodes and a strain of fungus, *B. bassiana* were found to cause significant mortality of the weevils compared to the rest of the entomopathogens and the control. To demonstrate the practical application of entomopathogens, wettable dust of *B. bassiana* were dispensed on jute bags after which weevils were exposed to the treated surfaces for 30 min. The exposed weevils recorded between 90 to 100% mortality 14-d after exposure. Additional study demonstrated that the parasitoid, *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) could be integrated with entomopathogenic nematodes. These experiments demonstrate the potential usefulness of entomopathogens in the management of stored product Lepidopteran and Coleopteran pests.

Keywords: entomopathogens, nematodes, fungi, parasitoid, virulence.

1. Introduction

Stored product Lepidopteran and Coleopteran pests are cosmopolitan pests that cause severe postharvest losses of grains and processed commodities. In the past, chemical pesticides have been used to disinfest commodities of storage pests. Integrated pest management (IPM) strategies in postharvest systems based on chemical pesticides pose health, legal and financial risks due to pesticide residues that may occur in foods (Monaco et al., 2002). Furthermore, there are dramatic restrictions in the use of synthetic pesticides to control pest populations in storage facilities. Natural enemies present alternative methods to overcome the potentially harmful effects of chemical pesticides especially in the management of postharvest pests since natural enemies are mostly environmentally safe and do not pose any dangers to humans or the environment. Natural enemies employed in postharvest IPM have been mostly parasitoids. Other forms of biological control such as use of entomopathogenic nematodes and fungi have recently started to attract attentions. This study investigated the potential of entomopathogens in the management of stored product pests. *Plodia interpunctella (Hübner*) (Lepidoptera: Pyralidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Dryophthoridae) were the test insects in this study.

Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae spp.) have the potential to control a broad range of arthropod pests including stored product insects. Nematodes kill their insect hosts with their mutualistic relationship with bacteria *Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae (Gram-negative Enterobacteriaceae) that inhabit the intestinal lumen of nematodes as symbionts (Boemare, 2002). Free living and infective juveniles (IJs) or third stage juveniles of nematodes enter the hemocoel of the host insects through the natural openings such as mouth, anus and spiracles or respiratory system, and release their pathogenic bacteria that propagate rapidly and kill insects within 48 hours (Poinar, 1990). The nematodes develop and complete 2 to 3 generations before leaving the host insect. Mbata and Shapiro-Ilan (2010) investigated the pathogenicity of different spp. of entomopathogenic nematodes to *P. interpunctella* larvae and reported *Heterorhabditis indica* (HOM1) strain to be most virulent of all the strains tested. In a laboratory experiment, strains of Steinernematidae and Heterorhabditidae showed higher virulence for the larvae of *Ephestia kuehniella* Zeller, *Tenebrio molitor* (Linnaeus) and adults stages of *Acanthoscelides obtectus* (Say) compared to *Sitophilus zeamais* and *S. oryzae* (Barbosa-Negrisoli et al., 2013). In another study, *H. indica* was reported to be pathogenic to several stored product pests including *S. zeamais* (Maketon et al., 2011). Ramos-Rodriguez et al. (2006) reported that *T. molitor*, *Oryzaephilus surinamensis* (Linnaeus) and *Tribolium castaneum* (Herbst) were found to be susceptible to *Steinernema riobrave* Cabanillas, Poinar and Raulston.

Entomopathogenic fungi have also been demonstrated to be a promising alternative to chemical pesticides for biological control of arthropod pests. Pathogenicity of two strains of *Purpureocillium lilacinum* to *T. confusum, R. dominica* and *S. zeamais* has been reported (Barra et al., 2013). The pathogenic effect of ten strains of *Beauveria bassiana* and two of *Metarhizium brunneum (Metschnikoff) treated with 1 x* 10^9 *conidia/ ml against S. zeamais resulted in weevil mortality in the range of 53 to 93% (Ruelas-Ayala et al., 2013). In contrast, Trevisoli et al. (2015) reported that S. zeamais is less susceptible to the fungal strains of B. bassiana*, *M. brunneum and Isaria fumosorosea.* Thus, susceptibility of the maize weevil to *B. bassiana* and *M. brunneum* requires further elucidation.

The studies reported here comprised of results from exploratory investigation into the potentials of entomopathogenic nematodes and fungi for biocontrol of two stored product pests, P. interpunctella and S. zeamais. In addition, combined application of Braconid wasp, H. hebetor, with entomopathogenic nematodes were compared with applications of nematodes or wasp alone to determine if the two biocontrol agents could be integrated for the management of P. interpunctella populations.

2. Materials and Methods

Rearing of insects and natural enemies

Plodia interpunctella stock culture was originally obtained from USDA-ARS, Grain Marketing and Research Laboratory, Manhattan, KS in 2001 and had since been maintained on the artificial moth diet at 28 ± 1.5 °C, 70 ± 5% RH and a 16h: 8h photoperiod in Biology department of Fort Valley State University (FVSU), Fort Valley, GA.

Foundation culture of *S. zeamais* was obtained in August, 2006, from the University of Georgia's center for Invasive species and Ecosystem Health, Department of Entomology, Tifton, GA. Populations of *S. zeamais* have been maintained in the insectary of FVSU.

Wasp culture was originally collected from the Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK. Wasps were reared on 50 late instars of *P. interpunctella* in 1000 mL glass rearing jars kept in experimental chamber maintained at 28 ± 1.5 °C, 70 ± 5% RH and a 16: 8 (L:D) photoperiod.

Nematodes were reared at ~ 25ºC in last instar of greater wax moth, *Galleria mellonella* (Linnaeus) following a procedure described by Woodring and Kaya (1988). The larvae of *G. mellonella* were obtained from Webster's Waxie Ranch (Webster, WI). Nematodes were stored at 13 °C for 15 d or less before being used for experiments.

Cultures of *H. bacteriophora* (HP88) and *H. megidis* were obtained from the MicroBio Group of Becker Under Wood (West Sussex, UK), *H. bacteriophora* (Lewiston) and *H. indica* from Integrated BioControl Systems (Lawenceburg, IN), *H. bacteriophora* (Oswego) from Dr. Elson Shields (Cornell University, Ithaca, NY) and *H. marelatus* and *H. zealandica* from P. stock (University of Arizona, Tucson, AZ). *H. indica* Poinar, Karunakar, and David (Homl strain), *H. georgiana* (K22), *Steinernema feltiae* (SN), and *S. carpocapsae* (All) were obtained from USDA-ARS culture collection in Byron, GA.

Entomopathogenic fungi, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (GHA strain) and *Metarhizum brunneum* Petch (F52 strain) were originally obtained from Stefan Jaronski (USDA-ARS) and cultured on Sabouraud dextrose agar supplemented with 0.2% yeast extract according to a procedure described by Goettel and Inglis (1997). Established cultures of fungi were stored at 4 ºC for one week before experimentation begins.

Pathogenicity of entomopathogenic nematodes to third and fifth instars of Indian meal moths

Virulence of the nematodes was tested at 150 and 480 dose rate of IJs per larva in plastic cups (3-4 cm internal diameter, 3 cm deep; Bioserv Frenchtown, NJ) at ~28 ± 1.5 °C and 70 ± 5% RH against 10-d third and 18-d-old fifth instars of *P. interpunctella*. One larva with 3.5 mL moth rearing medium was placed in each cup that was covered with plastic lid. Inoculum of IJs in 0.5 mL water was added to each cup 1-d after the introduction of larva and incubated until the emergence of adults and assessment of mortality. Moisture content of the medium was ~14% after adding 0.5 mL water. Four replicates of 10 cups per treatment of nematode strain and untreated control (water) of third and fifth instar of *P. interpunctella* were set up and two trials were conducted for both larval stage. Mortality of third and fifth instars was recorded after 10-d and 21-d respectively in treated and control cups.

Influence of combined application of nematodes and parasitoid versus single of either nematodes or parasitoid

This laboratory experiment assessed the virulence to *P. interpunctella* and interaction among biological control agents was conducted in 1 l rearing jars (7.4 cm diameter and16.8 cm height). A set of four jars were set up with two host larval densities, 20 and 40. Treatments consisted of *H. indica* and *H. hebetor*; 8000 IJs with 2 mL water (200 and 400 IJs/insect) were applied to larvae in one treatment, three pair (three males and three females) of adult parasitoids exposed to larvae in

second treatment, combination of exposure of 2 mL of water consisting of 8000 IJs and three pairs (2-d old males and females) of adult *H. hebetor* in treatment three and in treatment four, control was set up with 2 mL water. All jars were covered with filter paper and maintained at 28 ± 1.5 °C, 70 ± 5% RH and a 16: 8 (L:D) photoperiod. After 3-d period, mortality of host larvae and parasitoid was observed and afterwards all jars containing larvae with parasitoids, IJs or to both were transferred to incubator until the complete development of F_1 parasitoids. Each treatment was replicated four times and three runs were conducted.

Pathogenicity of entomopathogenic nematodes to *Sitophilus zeamais*

The protocol for inoculating the weevils with entomopathogenic nematodes followed a method used in screening *P. interpunctella* for susceptibility to entomopathogenic nematodes (Mbata and Shapiro-Ilan, 2005). Dose-response evaluation of the nematodes was carried out with infective juveniles (IJs) of *H. bacteriophora* Poinar (VS) and *S. carpocapsae* (All). Infective juveniles of nematodes in aqueous solutions were inoculated onto 7 cm filter papers (Whatman grade 40) placed in petri dishes (6 cm diameter) with 0.35 µL of nematode suspensions at the rates of 100 IJs/cm^2 (2400 IJs/0.350 uL), 200 IJs/cm^2 (4800 IJs/0.350 µL) or 400 IJs/cm^2 (27458 IJs/0.350 µL) to determine the rate of application that was infective to *S. zeamais*. Based on dose-response experiment, the application rate of 400 IJs/cm^2 was selected as the effective rate for screening of the six nematodes for virulence to *S. zeamais*. The controls were set up as described above but consisted of 0.35 µL of tap water sprayed onto 6 cm filter papers in petri dishes. Ten *S. zeamais* adults (1-3 d old) and a kernel of maize were transferred to each of the petri dishes. The experiment was organized in a completely randomized design with nine replicates of 10 weevils each per treatment and control that were grouped into three sets for examination of weevil mortality 3, 7 and 14 dpi (days post inoculation), consecutively. The experiment was conducted over four consecutive trials with new generations of weevils. The petri dishes were kept in a controlled chamber maintained at 25 °C and uncontrolled but high relative humidity due to the moist filter paper.

Pathogenicity of entomopathogenic fungi to *Sitophilus zeamais*

Beauveria bassiana (GHA strain) and *M. brunneum* (F52 starin) were investigated at three different concentrations designated as low (1 x 10^7 conidia), medium (1 x 10^8 conidia) and high (1 x 10^9 conidia) doses. The experimental design involved 7 treatments consisting of three different doses of each of the fungi and the control. Nine petri dishes for each of the strains, three for each dose, were prepared with filter paper and each petri dish was added with a different concentration of the fungal suspension from the cultures at the rates specified above that corresponded to 6857 (low), 13714 (medium), and 27482 (high) conidia/mL. One maize kernel, and 10 adult weevils were added to each petri dish and the dishes were sealed with parafilm. Observations of the weevils for mortality were carried out 7 and 14 ds post inoculation. The experiment was repeated over two consecutive trials with new generations of weevils.

Infectivity of fungal spores applied to jute bags against *Sitophilus zeamais*

The more virulent entomopathogenic fungus to *S. zeamais* determined in the test described above, *B. bassiana*, was investigated further for the protection of bagged maize grain by applying the wettable powder to jute bags used in the postharvest storage of maize. At this stage, it is reasonable to investigate the fungi further since they do not require moist surface for survival and dispersal[29]. Wettable powder of *B. bassiana* (GHA strain) 4.4 x 10^{10} conidia/g (Botanigard 22wp® WPO) was obtained from BioWorks (Victor, NY).

Fungal application during the experiment was carried out under a biosafety cabinet to contain the fungal powder and prevent contaminating the control weevils. The jute bags (surface area = 2.06 x 10^5 mm^2) were each treated with one of three rates of the wettable powder comprising of 2.13 x 10^7 conidia/mm^2, 1.07 x 10^7 conidia/mm^2, and 0.5 x 10^7 conidia/ mm^2. Control bags were not treated with any powder. Appropriate quantities of the wettable *B. bassiana* powder were weighed out for

each of the treatments and transferred into containers or bins (L 50.8 x W 47.8 x D 15.2 cm). Jute bags were placed in each of the containers dispensed with the wettable *B. bassiana* powder. Following the replacement of the lids of the containers, the containers were shaken vigorously to ensure even distribution of the powder through the surface of the jute bags. Each treatment and the control were replicated three times. Twenty weevils per bag were released to crawl on bags for a 30 min period and were transferred thereafter to 60 mm petri dishes lined with filter papers moistened with 0.35 mL of tap water. A kernel of maize that served as food for the weevils was placed in each of the petri dishes. The lids on dishes were taped down on to trays to prevent insects from escaping. Survival of insects was determined 7 and 14 d post-exposure. The entire experiment was repeated two times.

3. Results

Mortality of third and fifth instars of *P. interpunctella* exposed to entomopathogenic nematodes

Mortality differences in the 3rd and 5th instars of *P. interpunctella* exposed to nematodes were observed between all treatments and the control (Tab. 1; $F = 6.61$; df = 12, 67; $P < 0.0001$). Third instar larvae were found to be more susceptible at the rate of 480 IJs/insect than 150 IJs/insect. At the higher exposure rate of 480 IJs/larva, *H. marelatus* (Point Reyes), *H. megidis* (UK211), *H. indica* (HOM1) and *H. marelatus* strains caused higher larval mortality than the control whereas at the rate of 150 IJ/larva *H. zealandica* and *H. indica* caused greater mortality than the control (Tab. 2).

Influence of combined versus separate application of nematodes and parasitoid

Moth larvae exposed to nematodes at the rate of 200 (4000 IJs/2 mL) and 400 (8000 IJs/2 mL) IJs per larva had significantly different larval mortality (at 200 IJ/larva: F = 276.23; df = 3, 36; $P = 0.0001$; Fig. 1 and at 400 IJ/insect: F = 110.02; df = 3, 36; $P = 0.0001$; Fig. 1). Combined treatment of nematodes at the dose rate of 200 and 400 IJs per larva with *H. hebetor* (3 pairs of males and females) caused higher *P. interpunctella* larvae mortality.

Chi-square value (χ^2) indicated that combined effect of nematodes and parasitoids on the larval mortality were not antagonistic but could possibly be additive or synergistic because at both exposure rates of nematodes with *H. hebetor*, high mortality of host larvae was achieved (for 200 IJ/larva: $\chi^2 = 1.81$; M = 0.87; $P < 0.05$ and for 400 IJ/larvae: $\chi^2 = 2.26$; Me = 0.84; $P < 0.05$).

Pathogenicity of nematodes to *Sitophilus zeamais*

No significant differences were observed on the survival (%) of maize weevils following 3 and 5 dpi with nematodes compared to the control but survival of adult S. *zeamais* at 7 dpi was significantly lower than the control. (Tab. 3: $F = 2.78$; $df = 6, 41$; $P < 0.0001$). Maize weevils treated with *S. carpocapsae* (All) exhibited the highest susceptibility with survival rate at 28.3%. Pathogenicity of *S. carpocapsae* against weevils was significantly higher than the rest of the nematode strains except *S. feltiae*.

Pathogenicity of entomopathogenic fungi to *Sitophilus zeamais*

Survival of maize weevils was significantly reduced at 14 dpi ($F = 6.05$; df = 6, 41; $P \leq 0.0004$; Tab. 4) following exposure to fungi but at 7 dpi survival of weevils exposed to fungi was not significantly different from the control. Survival of weevils exposed to low dose of *M. brunneum* (6857 conidia/mL) was significantly different from the survival of weevils exposed to medium fungi doses (13714 conidia/mL) at 14 dpi. Significant reduction in the survival of maize weevils was obtained at low, medium and high rates of *B. bassiana* compared to *M. brunneum* and control.

Fungal strains infectivity to jute bags against *Sitophilus zeamais*

Wettable *B. bassiana* powder applied to jute bags at 25, 50 and 100 g highly affected the survival of weevils at 14 dpi (Tab. 5: F= 76.16; df = 3, 15; $P < 0.0001$) compared to 7 dpi (Tab. 5 : F= 40.75; df = 3, 15; $P < 0.0001$) and the control. No significant differences were observed on the percentage survival of maize weevils exposed to low dose (25 g) of *B. bassiana* and the control weevils. Higher rate (100 g) of *B. bassiana* generated 100% mortality of treated weevils at 14 dpi.

Tab. 1. Mortality of third instar of *Plodia interpunctella* after 10-d exposure to nematodes strains at rates of 480 and 150 IJs/larva.

	Mean ± S.E.	
Nematode strain	**480 nematodes/larva**	**150 nematodes/larva**
***H. bacteriophora* (HP 88)**	4.11 ± 0.68bc	2.78 ± 0.43cd
***H. bacteriophora* (Lewiston)**	4.22 ± 1.23bc	2.44 ± 0.88cd
***H. bacteriophora* (Oswego)**	4.00 ± 1.00bc	2.67 ± 0.60cd
***H. indica* (Homl)**	5.56 ± 1.12ab	3.78 ± 0.92c
***H. megidis* (UK 211)**	5.44 ± 1.20ab	2.11 ± 0.75d
***H.marelatus* (Point Reyes)**	6.22 ± 1.04a	2.00 ± 0.71d
***H. zealandica* (NzH3)**	3.22 ± 0.88cd	4.22 ± 0.92cd
Control	1.33 ± 0.44d	1.50 ± 0.46d

Mean ± SE (out of 10) within a column followed by same letter are not significantly different (Tukey's test, $P < 0.05$).

Tab. 2. Effect of strains of entomopathogenic nematodes on the mortality of fifth instars of *Plodia interpunctella*.

Nematode strain	Mean ± S.E.
***H. bacteriophora* (HP 88)**	3.6 ± 0.33a
***H. bacteriophora* (Lewiston)**	4.1 ± 0.43a
***H. bacteriophora* (Oswego)**	4.2 ± 0.36a
***H. indica* (Homl)**	4.1 ± 0.41a
***H. megidis* (UK 211)**	3.9 ± 0.31a
***H.marelatus* (Point Reyes)**	4.1 ± 0.46a
***H. zealandica* (NzH3)**	4.3 ± 0.42a
Control	1.4 ± 0.22b

Means within a column followed by same letter are not significantly different (Tukey's test, $P < 0.05$)

Tab. 3. Percentage survival of adult *Sitophilus zeamais* after exposure to entomopathogenic nematodes for 7-d. Control consisting of water only.

Nematode strain	**Mean ± S.E.**
Control	100 ± 0.00a
H. georgiana	78.33 ± 3.07b
H. indica	60.00 ± 10.95bc
***H. bacteriophora* (Lew)**	56.67 ± 2.10bc
***H. bacteriophora* (Osw)**	50.00 ± 7.30c
S. carpocapsae	28.33 ± 7.40d
S. feltiae	41.67 ± 4.01cd

Different letters within a column are significantly different (Student-Newman-Keul's test, $P < 0.05\%$).

Tab. 4. Survival (%) of maize weevils exposed to entomopathogenic fungi for 14-d. Control is with water.

Fungal strain	**Mean ± S.E.**
Control	90.00 ± 6.32a
Bb-lo	65.00 ± 12.58bc
Bb-med	61.67 ± 9.45bc
Bb-hi	38.33 ± 10.13c
Met-lo	75.00 ± 12.58ab
Met-med	78.33 ± 7.03ab

Met-hi	48.33 ± 6.00bc

Different letter (within column) is significantly different at 5% significant level (Student-Newman-Keul's test).

Tab. 5. Seven and 14 dpi survival (%) of maize weevils exposed for 30 min. to jute bags treated with different rates of wettable *B. bassiana* powder. Control was water only.

Dose of Fungi	7 dpi	14 dpi
Untreated control	98.33 ± 1.66a	76.66 ± 3.80a
Bb-25 g (low)	71.67 ± 2.10b	8.33 ± 4.01b
Bb-50 g (medium)	64.17 ± 5.83b	0.83 ± 0.83c
Bb-100 g (high)	72.50 ± 2.14b	0.00 ± 0.00d

Different letter (within column) is significantly different at 5% significant level (Student-Newman-Keuls test).

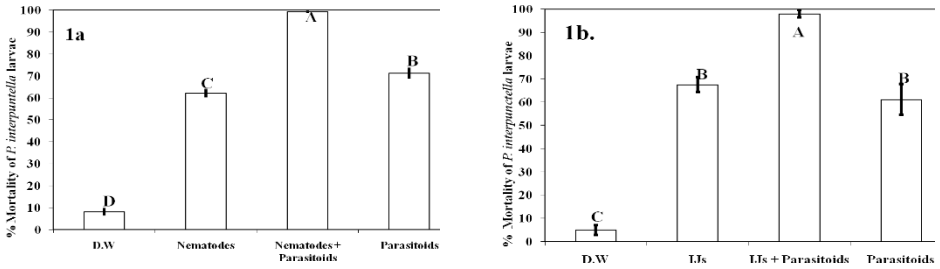

Fig. 1. Mortality (%) of Indian meal moth larvae at the exposure rate of 200 (1a) and 400 (1b) IJs of *Heterorhabitis indica/moth larva*, parasitoids (*Habrobracon hebetor*) or combination of *H. indica* and parasitoids. D.W. = Distilled Water for control, IJs= Infective juveniles nematodes. Different letter above bars indicate significant difference (Student-Newman-Keuls test, $P < 0.05\%$).

4. Discussion

Heterorhabditis marelatus (Point Reyes), *H. megidis* (UK211), and *H. indica* (HOM1) showed more pathogenicity and caused higher mortality in Indian meal moth larvae. These nematodes have unique characteristics that recommend them for consideration for year round management of *P. interpunctella*. *H. marelatus and H. megidis are considered to be cold tolerant (Grewal et al., 1994, Berry et al., 1997) while H. indica is heat tolerant (Shapiro and McCoy, 2000). This implies that these three nematodes could be used at different times of the year to regulate populations of P. interpunctella.*

Combination of nematodes and parasitoids enhanced the mortality of *P. interpunctella*. Dillon et al. (2008) observed that the interaction between the nematodes *H. downesi* or *S. carpocapsae* and the parasitoid *Bracon hylobii* enhanced the mortality of the pest host, *Hylobius abietis*. Interaction between entomopathogenic nematodes and the parasitoid could possibly be additive or synergistic.

Maize weevils were more susceptible to Steinernematid strains particularly *S. carpocapsae* (All) and *S. feltiae* (SN) compared to the Heterorhabditidae. Entomopathogenic fungi, *B bassiana*, exhibited strong virulence against adult maize weevils compared to *M. brunneum* (Vega and Hofstetter, 2014). High concentration of wettable powder of *B. bassiana* applied to jute bag surface caused 100% mortality in maize weevils 14 days after inoculation. This implies that a path exists by which entomopathogens could be integrated in the IPM of postharvest arthropods.

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Chilled Aeration to Control Pests and Maintain Grain Quality During the Summer Storage of Wheat in North Central Region of Kansas

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Abstract

Chilled aeration allows to cool grain, independent of ambient conditions, to "safe" temperatures where insect, fungi, and spoilage is reduced to the minimum. The objective of this research was to evaluate the advantages of using grain chilling to preserve the quality of grain and reduce post-harvest losses, compared to conventional

aeration and storage strategies used during the summer storage of wheat in Central Kansas, U.S.A. The research trials were developed in two 1,350 metric ton (t) steel silos in a Farmer's Cooperative during the summer and fall of 2015 and 2016. One of the silos was chilled and the other was used as a control managed by the Cooperative. Variables evaluated were: grain temperature, moisture content (MC), grain quality, insect development and reproduction rate. The chilling treatment reduced the grain temperature from 28°C- 39°C to a minimum of 17°C-17.6°C in less than 250 hours. Grain temperatures below 25°C were not possible during the summer using ambient aeration. Minimum variation of MC was observed in the Chilled silo while ambient aeration reduced the MC by 0.5%. Reproduction rates of RFB and LGB were significantly reduced by chilled temperatures lower than 17°C. Lower temperatures also reduced insects discovered in probe traps and insect damaged kernels (IDK). The energy cost of the grain chiller was between 0.26 US $/t- 0.32 US $/t higher than ambient aeration.

Keywords: Ambient aeration, Grain chilling, Summer storage, Post-harvest losses, Wheat.

Introduction

Grain that is harvested during the summer season of the Northern Hemisphere presents the inconvenience that it is collected when the ambient temperature is high (26°C to 40°C). In these conditions, the grain goes into storage at a high temperature, which makes it prone to immediate insect infestation and mold growth that can affect its quality. Therefore it is imperative that the grain temperature is decreased as soon as possible (Reed and Arthur, 2000). Nevertheless, cool ambient conditions may be limited during this part of the season, thus the use of chilled air could be considered as a solution. Chilled air refers to aeration air that is cooled before it comes contact with the grain by passing through an evaporator coil of a grain chilling unit (Maier and Navarro, 2002). When the chilled air comes in contact with the grain, it lowers the temperature of the grain, independent of ambient conditions (Maier and Navarro, 2002). This technology makes it possible to cool down grain temperature between 20°C and 15°C immediately after summer harvest, which reduces insect populations and consequently the need for chemical control (Navarro et al., 2002).

Based on field tests using chilled aeration on low-moisture wheat stored in Michigan, Maier (1992) simulated chilling in the Midwestern region of the U. S. The computer simulation showed that chilled aeration was capable of lowering the temperature of 579 metric tons (t) of wheat from 30°C to 15°C in just one week. Continuous ambient aeration took 1.5 times longer to cool the grain down to 10°C, which caused higher dry matter losses (DML). Other grain chilling field trials developed in 2,500 t wheat silos in Central Kansas determined that the cost of chilling the grain from 32°C- 35°C to 15°C– 17°C in six days was less than 0.16 US $/t, while the cost of fumigating and turning the non-aerated silo was 0.67 US $/t, plus the additional shrink loss cost of approximately 7.5 t from the bulk (Hellemar, 1993).

Maier et al. (1996) compared eight combinations of ambient aeration, fumigation and chilled aeration strategies in three different locations of the U.S. through computer simulations. Chilling the grain below 17°C in a short period of time proved to be the best strategy to avoid DML and reduce the populations of maize weevil (MW) *Sitophilus zeamais*.

While the strategy of chilling grain is the effective control of insects, there are other benefits that come from the grain chilling technology, such as the possibility of storing damp grain for a limited time, predictable drying capability and better preservation of end-use quality (Hellemar, 1993; Maier and Navarro, 2002).

The objective of this research was to evaluate the advantages of using grain chilling technology to preserve the quality of grain and reduce post-harvest losses caused by insects and fungi, compared to the conventional aeration and storage strategies used during the summer storage in North Central Kansas.

Materials and Methods

This research was conducted at Farmer's Cooperative in the North Central region of Kansas, U.S.A., from August to November 2015 and from June to September 2016. The research trials were conducted in two 1,350 t steel silos of 11.3 m in diameter and 16.8 m in height from the bottom to the eave, filled almost completely with hard red winter wheat (HRW) harvested in the summer of

2015 and 2016. Before each harvest, the silo walls were cleaned up to 6 m from the bottom and the remaining grain on the floor of the silo was vacuumed out. Attached to these silos there were two centrifugal fans in parallel, each with a 10 HP (7.5 kWh) motor (Baldor Electric Co., Fort Smith, AR). One of the silos was chilled (Chilled silo) and the other one was used as a treatment control (Control silo) managed by the Cooperative using their regular grain quality management strategies.

Grain chiller setup and monitoring of air and grain conditions

The grain chiller GCH-20 used in this project was facilitated by the Brazilian company Coolseed (Santa Tereza do Oeste, Brazil). This equipment has the rated capacity to chill 100 to 170 t per 24-hour continuous operation in silos of up to 1,800 t, according to specifications of the manufacturer.

The grain chiller was connected to the grain silo through thermally insulated ducts that were connected into the two inlets of the aeration fans that where removed from the (Figure 1).

Figure 7. Grain chiller GCH-20 setup: (a) Insulated duct connected to the chiller's outlet at one end and to a "T" connector at the other, (b) Two ducts attached to the fan transition parts of the aeration fans that were removed.

The conditions inside the Chilled and Control silo were monitored through three temperature cables (TSGC Inc., Spirit Lake, IA) in each silo, that were attached to the roof and the floor of the silo. Additionally, temperature and relative humidity (RH) sensors were placed in the fan transitions, outside of the silos to record ambient conditions. In the 2016 trials, additional sensors were placed in the fan outlet of the grain chiller and inside the insulated ducts.

The wheat moisture content (MC) was measured using a GAC 2500-UGMA (Dickey John, Auburn, IL) every 30 days. Grain samples were taken at four different depths and three depths in each of the silos. The samples collected per location were put together and homogenized to make up a composite sample per location in each of the silos. The composite sample from each location was considered a replication for the calculation of significant differences between sampling dates. Statistical analysis was performed using the SAS statistical software (SAS Institute Inc., NC). Statistically significant differences were analyzed with Tuckey's test ($p < 0.05$).

Insect pest population monitoring and quantification

Insect bioassays

The effect of chilled aeration on the survival rates of insects was quantified using insect bioassays with the species Lesser Grain Borer (LGB) *Rhyzopertha dominica* and Red Flour Beetle (RFB) *Tribolium castaneum*. The bioassays consisted of plastic jars of 0.2 L with holes on the bottom and top, covered with wire mesh and filled with an exact number of adults of each species, together with a mix of flour, yeast and broken kernels for insect feeding.

In each of the silos, a bioassay of each species was located in the center of the silo and next to each temperature cable, and buried 0.3 m below grain surface. A fifth bioassay per species was located

in one of the fan transition parts. In 2016, three jars per location were put inside the grain mass and transition parts. One jar from each location was taken out every 28 days.

When the jars were taken out of the silos in each sampling date, the number of dead and live adults were quantified and then discarded, and the larvae, pupae and eggs (if any) were kept in a growth chamber and counted 28 days after as adults. The total progeny number was calculated by the total insect count (initial dead and live insects when jar was pulled out of the silo plus the progeny number after 28 days in the growth chamber) minus the original number of insects put into the jar. Statistical analysis was performed using the SAS statistical software. Statistically significant differences were analyzed with Tuckey's test ($p < 0.05$).

Endemic insect population sampling

Insect populations inside the silos were quantified by placing five perforated insect probe traps model Storgard W.B. Probe II (Trece Inc., Adair, OK) of approximately 0.6 m in length in the North, South, East, West, and Center sections of the silos, approximately 1.5 m from the walls. Insects inside the probe traps were checked every 28 days and identified (up to the genus level). Adults of the main insect pests of stored-products were counted.

Grain quality analysis

Grain samples for these analysis were collected using the same procedure described to collect the MC grain samples. In 2015, the samples were only collected in the first two months of the trial, while in 2016, the sampling period was expanded for one more month.

For the grain quality analysis, only one composite 2,500 g composite sample was taken per silo each sampling date. This composite sample was sent to the Kansas Grain Inspection Service (KGIS) in Topeka, Kansas, for grading.

Electrical cost of chilled and ambient aeration strategies

The energy consumption during the chilling treatment was measured using a kWh counter that was installed at the of the power inlet of the grain chiller. The energy consumed by the aeration fans in the Control silo were calculated according to the hours of operation reported by the Cooperative. The costs of the ambient and chilled aeration process were calculated based on the energy consumption, using an average cost of 0.084 $/kWh (obtained from the local electrical service provider), and considering additional charges for basic service and consumption fees.

Results and Discussion

Ambient and chilling aeration trials

Trial of 2015

The grain chilling treatment started on August 22nd, and the cool air front reached the top of the grain mass after 175 hours of active chilling at an airflow rate of 0.07 m^3/min/t and at a temperature of approximately 17°C (initial grain temperature: 28°C). Due to technical difficulties with the grain chiller during certain periods, the equipment was left running longer to tests its capacity, until September 14th, 2015, for a total of 314 hours (Figure 2).

The ambient aeration strategy applied in the the Control silo by the Farmer's Cooperative was based on turning on the fans when the ambient temperature was below 27°C in the summer, and below 18°C during the fall. The total active aeration time was 308 hours, at an average airflow of 0.11 m^3/min/t. Temperatures inside the Control silo remained over 17°C until mid-November, which was about two months after this temperature was reached in the Chilled silo. According to Hagstrum and Subramanyam (2006), for every month that cooling is delayed, populations of insects can grow 5- to 25-fold their original frequency.

During the trial, the ambient air fluctuated between 8°C and 37°C, with an average of 23°C. The average ambient RH was 63.5% with a minimum of 27.4% and a maximum of 93.1%.

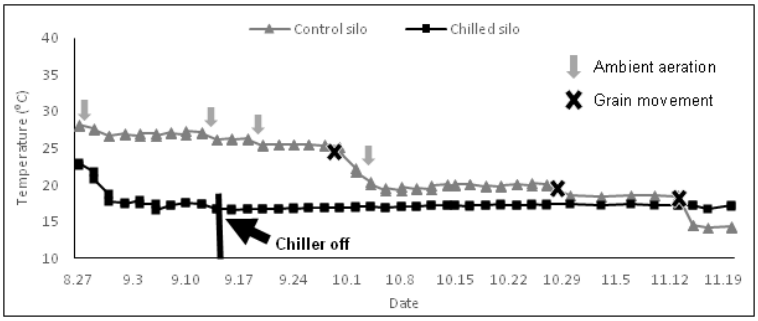

Figure 8. Grain temperature profile (°C) of the grain mass inside the Control and Chilled silo from Aug. 27th to Nov. 20th, 2015 in Farmer's Cooperative, Kansas, U.S.A.

In 2015, the average MC inside the Chilled silo was 11.4% and did not change significantly, while in the Control silo the average MC decreased significantly from 11.1% to 10.5% in the last two months (October and November) of evaluation.

Trial of 2016

In 2016, the initial grain temperature in the Chilled silo was higher than the previous year (39°C). The grain chilling trial started on June 21st, and reached the top of the grain mass after 245 hours of active chilling at aproximately the same airflow rate and temperature as the previous year. Once again, there were some issues with the grain chiller so the equipment was left running longer until July 12th, 2016, for a total of 384 hours (Figure 3).

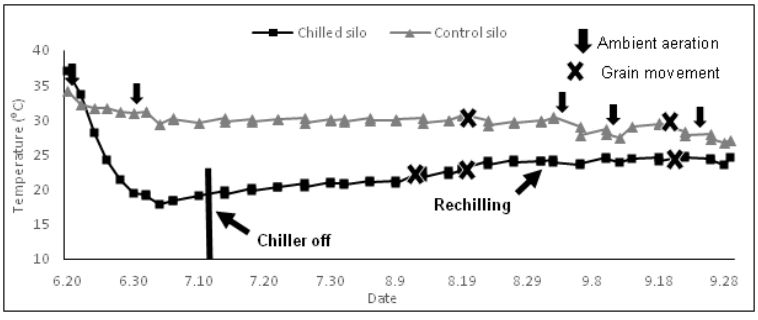

Figure 9. Grain temperature profile (°C) of the grain mass inside the Control and Chilled silo from June 20th to Sep. 29th, 2016 in Farmer's Cooperative, Kansas, U.S.A.

The additional temperature/RH sensors placed in the chiller outlet and insulated ducts in 2016 showed that the temperature of the chilled air coming out of the grain chiller was 12.5°C and increased by an average of 3°C in the transition parts. As well, the RH coming out of the grain chiller was 85% and decreased by an average of 13% in the transition parts.

During the chilling period, the average ambient temperature was 26°C, with 16.5°C and 38°C as minimum and maximum, respectively. The average relative humidity was 63.8%, with 22.7% and 91.2% as minimum and maximum, respectively.

Due to the high ambient temperature during most of July (over 32°C), the issues with the grain chiller, and the constant movement of grain, it was difficult to maintain the temperature of the grain below the optimum insect development threshold (25°C to 33°C) (Fields, 1992), so a rechilling

treatment was proposed by early-September, but the issues of the grain chiller persisted and the rechilling cycle did not have much of an effect on the grain temperature.

In the Control silo the initial grain temperature was 34°C. The aeration fans were activated using the same criteria as the previous year. The total fan run hours were 371 and the lowest temperature achieved was 25°C by late September.

In 2016, the average MC inside the Chilled silo was 10.2% and did not change significantly, while in the Control silo the average MC decreased significantly from 10.6% to 10.0% in the last sampling date (September). This was about the same tendency observed in 2015.

During the night of September 29th, one of the eaves from the Chilled silo cracked and a side of the silo split open. Given the incident it was decided to terminate the trial on this date.

Effect of grain chilling on insect reproduction and survival

During the 28 days the bioassays were inside the silos in 2015, the average temperature in the grain surface and fan transition part of the Chilled silo was 19°C and 17°C, respectively, while in the Control silo it was 27°C and 25°C, respectively. The cooler temperatures inside the Chilled silos significantly slowed down the total progeny development of LGB and RFB compared to the progeny observed in the Control silo (Table 1).

The temperatures of 17°C and 19°C in the top of the grain mass and transition part of the Chilled silo, respectively, are considered "safe" since population growth is almost insignificant at these temperatures (Navarro et al., 2002).

Table 2. Total progeny number (mean±SE) of adults of LGB and RFB for 2015 bioassays located 0.3 m below grain surface and in fan transition parts of the Chilled and Control silo for 28 days.

Year	Insect species	Location in the silo	Silo	
			Chilled	Control
2015	LGB	0.3 m below grain surface	2.3 ±0.7^{B}	974.3 ±33.7^{A}
		Transition	1.0	768.0
	RFB	0.3 m below grain surface	5.3 ±1.4^{B}	21.3 ±5.6^{A}
		Transition	0.0	7.0

[A, B] Mean values with the same letter within the same line are not significantly different by Tuckey's test (p>0.05).

The difficulty to maintain temperatures below 20°C in the Chilled silo during 2016 due to issues with the grain chilling unit, higher ambient temperatures and constant loading of warm wheat from the field during the trial, did not allow a significant difference to be observed between the insect progeny of the Chilled and Control silo. The average temperatures inside the Chilled and Control silo during the 68 days the bioassays were inside the silos were 23°C and 31°C, respectively. Although the average temperature inside the Chilled silo was lower, the fast temperature rise due to several issues previously mentioned caused an acclimation effect that basically eliminated the cooling effect on the development rate. According to Burks et al. (2000), if the temperature increases after the insect has been exposed to non-lethal cold temperatures, it may recover from the mild cold-injury effect.

Insect populations in the Chilled and Control silos

The main insect pests found in the probe traps of both silos were: flat grain beetle (FGB) *Cryptolestes* spp., flour beetle (FB) *Tribolium* spp. The populations of these genera increased faster in the Control silo than in the Chilled silo in both years (Table 2), even though the temperature difference between the silos was narrower in 2016.

Table 3. Total number of insects of main stored-product pests found in probe traps of Chilled and Control silo on Aug. 15th, Sep. 22nd and Nov. 20th, 2015, and Aug. 2nd, Sep. 20th and Sep. 30th, 2016.

Silo	Insect species	2015			2016		
		08/15	09/22	11/20	08/02	09/20	9/30[x]

	FGB	27	84	131	9	171	¥
Chilled	FB	1	80	74	5	78	¥
	WEV	0	10	270	1	29	¥
	FGB	33	3280	1236	44	719	328
Control	FB	4	1350	142	13	722	1241
	WEV	1	0	1	0	8	12

¥Probe traps lost when the Chilled silo cracked.

ˣTrial terminated earlier due to the accident in Chilled silo.

The main internal insect pest found in the probe traps were weevils (WEV) of the genus *Sitophilus* spp. More individuals of this genus were found in the Chilled silo than in the Control silo and the reason could have been that this genus was in competitive disadvantage with the high populations of FB and FGB.

Grain quality evaluation

The grain quality results indicate that there was no change in grade throughout the trials in either of the silos (Table 3). This means that there was no noticeable quality deterioration of the wheat during the duration of the trials.

In 2015, one IDK was identified in the Chilled silo in each sampling date which indicates that, although this damage was present before the grain chiller was turned on, it did not increase, probably due to the chilled temperatures (Table 2).

Table 4. Grain quality analysis of wheat stored in Chilled and Control silos from samples taken on Aug. 15th to Sep. 22nd, 2015, and July 1st to Sep. 27th, 2016.

Year	Sampling date	Chilled silo			Control silo		
		July	August	September	July	August	September
2015	**Insect Damaged Kernels (#/100 g)**	-	1.0	1.0	-	0.0	0.0
	Grade	-	1	1	-	1	1
2016	**Insect Damaged Kernels (#/100 g)**	0.0	0.0	0.0	0.0	0.0	1.0
	Grade	1	1	1	1	1	1

In 2016, IDK increased in the Control silo after three months of storage, while there was no detection in the Chilled silo. This means that, although internal-feeding insects were detected in both silos according to the results of the probe traps, it seems like the slightly lower grain temperature in the Chilled silo discouraged the insect damage.

Power consumption and cost analysis

The total power consumption and cost per ton of the ambient aeration and grain chilling trials are shown in Table 4.

In both years, the cost of grain chilling nearly doubled that of ambient aeration. These results agree with those reported by Quirino et al. (2013). Nevertheless, it has to be taken into consideration that the temperature of the Chilled silo was taken down to levels considerably lower (approximately 17°C) in only 175 hours in 2015 and 245 in 2016, with basically no considerable shrinkage loss. It also has to considered that the cost analysis did not include fumigation cost as these were not required during the trials, but previous research trials have demonstrated that grain chilling is economicallly feasible compared to the use of ambient aeration plus fumigation. Maier et al. (1997) determined that the annual operating cost for chilling wheat from 25°C- 27°C to 15°C- 17°C in 182-240 hours would lower the costs by 1.48 $/t compared to in-house fumigation combined with ambient aeration.

Table 5. Power consumption (kWh) and metric ton ($/t) for running chilling and ambient aeration in 2015 and 2016.

Year	Silo	Average Load (kWh)	Hours of Operation	Total Energy Consumption (kW)	$/t[3]
2015	Chilled	28[1]	314	8,794	0.54
	Control	15[2]	308	4,620	0.28
2016	Chilled	28[1]	384	10,752	0.66
	Control	15[2]	371	5,565	0.34

[1] Average load of system: 1 centrifugal fan of 7.5 kW+ 2 axial fans of 950 W/ea+ 2 compressors of 9.325 kW/ea.
[2] Two centrifugal fans of 7.5 kWh/ea. connected to the Control silo.
[3] Based on an average cost of 0.084 $/kWh

Conclusions

The grain chiller GCH-20 was capable of lowering the temperature of 1,350 t of wheat from 28°C-39°C to approximately 17°C in less than 250 hours. The shrinkage loss with the grain chilling treatment did not significantly increase in either of the trials. Using ambient aeration, the average grain temperature inside the Control silo remained over 25°C all summer during both years and there was a significant shrink loss of approximately 0.5%.

The stable low grain temperatures of 17°C in the Chilled silo in 2015 significantly slowed down the development rate of RFB and LGB, but in 2016, the increasing trend of the grain temperature in the Chilled silo from 17.6°C to more than 25°C avoided this effect to be observed.

The lower grain temperatures in the Chilled silo decreased drastically the progeny development of FGB and FB in both years. The most common internal-feeder found in the probe traps was the WEV, although proof of increasing levels of IDK were only found in the Control silo in 2016.

The cost analysis of the trials, based only on the power consumption of both aeration strategies, showed that the cost of grain chilling is between 0.26 $/t- 0.32 $/t higher than ambient aeration.

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Does it really work? 25 years biological control in Germany

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Stored-product protection, museum environments as well as protection of materials are growing fields of application of macro-organisms for biological control in Central Europe during the last 25 years.

Material destroying pests

Stored-product pests may destroy materials as well, either on their way to pupation sites or because the materials contain ingredients suitable for development. This initiated the interest in biological control of these pests in museums and other environments with cultural heritage items, as well as research in specific natural enemies of museum pests.

Spider beetles are mainly scavengers feeding equally on plant or animal materials. Beside their natural habitats, a number of species infest historic houses feeding on organic insulation materials and become a nuisance in residences (Howe, 1959). Moreover, spider beetles were found to infest historic books and herbaria (Gamalie, 2006). A number of spider beetle species were found to be suitable hosts for the larval parasitoid *Lariophagus distinguendus*, such as *Ptinus* spp. (Kaschef, 1955), *Gibbium psylloides* (Czenpinski, 1778) (Kaschef, 1961) and *Niptus hololeucus* (Faldermann, 1835) (Schöller and Prozell, 2011). Spider beetles are difficult to control in houses because the larvae develop hidden within walls and in dead floors, and no monitoring devices are available. In recent years, *L. distinguendus* was released against the hump beetle *G. psylloides* and the golden spider beetle *N. hololeucus* in Germany by pest control companies and became a regularly applied control technique (Kassel, 2008).

Larder beetles (Dermestidae) are among the cultural heritage pests most difficult to control by chemical means. Two approaches for biological control were tested so far, the control by a parasitoid naturally occurring in houses, and the control by a generalist predator transferred from the stored-product environment. The parasitoid *Laelius pedatus* (Say, 1836) (Hymenoptera: Bethylidae) is a gregarious ectoparasitoid of several larder beetle species including *A. verbasci* and *T. angustum*. The shiny black wasps measure 2 to 3 mm in length. During its life span a female wasp paralysed 74 ± 20 larvae of *A. verbasci* (Al-Kirshi, 1998). The average number of eggs per female wasp and day was 1.42 ± 0.2 if larvae of *T. angustum* were used as host. Most egg-laying activity was observed at temperatures between 25° and 28°C, while no oviposition occurs at 15°C. A mated female lives 6 to 8 weeks at room temperature (Al-Kirshi 1998). This parasitoid is occuring spontaneously in Central Europe in buildings, but there are not studies on the biological control potential of laboratory-reared wasps in field trials.

Stored product pests

Biological control in stored products is commercialized since 1998. Most applications were against stored-product moths in bakeries, food processing industries, retail trade and private households, and against weevils in grain on farms. Fifty percent of the types of application are control of pyralid

stored-product moths. The reasons for this might be the fact that biological control of pyralids was the first commercialized application and is best known in the public, and/or the fact that *Trichogramma* spp. are hardly visible under practical conditions due to their small size. The adults of these egg-parasitoids are 0.3 mm long. They lay their eggs into lepidopteran eggs, preferring freshly-laid eggs. Upon hatching, the wasp larva consumes the content of the egg. It pupates inside the egg and emerges as an adult wasp. Adult wasps mate shortly after emergence. A female wasp will parasitize approximately 50 eggs in her life-span of 3 to 14 days. While foraging for moth eggs, the females are usually walking. Typically parasitized eggs fixed to a card are applied (Prozell & Schöller, 2003). These cards are placed on shelves and palettes. The cards can be stored at 8 to 12°C in the dark for seven days.

Habrobracon hebetor is a cosmopolitan idiobiont gregarious ectoparasitoid. It develops on larvae of many Lepidoptera, mainly members of the family Pyralidae (Schöller, 1998). Actually the number of hosts even increased, but this is probably due to the presence of different strains in fields and warehouses (Heimpel et al., 1997). Today, *H. hebetor* is recommended for biological control and it has been studied from the biological and demographical point of view (Eliopoulos and Stathas, 2008; Akinkurolere et al., 2009).

Anisopteromalus calandrae is one of the most frequently found parasitoids in stored grain, and it is widely distributed. It has been reported as natural enemy of the following pests: *S. granarius*, *S. zeamais*, *Rhyzopertha dominica* (F.), *Stegobium paniceum* (L.), *L. serricorne*, *A. obtectus* (Say), and *Callosobruchus maculatus* (F.) (Williams and Floyd, 1971; Arbogast and Mullen, 1990; Ngamo et al., 2007). *A. calandrae* is a primary, idiobiont ectoparasitoid attacking the late larval stages and early pupae of beetles inside seeds and cocoons (Shin et al., 1994).

Lariophagus distinguendus has been reported as potential agent for biological control for a wide number of beetles that infest stored agricultural products (Steidle and Schöller, 1997): *S. oryzae* (Lucas and Riudavest, 2002), *S. granarius* (Steidle and Schöller, 2002), (Wen and Brower, 1994), *R. dominica* (Menon et al., 2002), *L. serricorne*, *S. paniceum* and *A. obtectus*. It is a solitary ectoparasitoid of larvae and prepupae.

In the meantime biological control was adopted by the conventional sector after its start in the organic market. Moreover, many pest control operators are using natural enemies. On the one hand, customers are demanding pesticide-free solutions and products, on the other hand the evaluation of non-chemical alternatives prior to the application of synthetic insecticides is regulated by law.

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Storage of Mungbean in Hermetic PVC Tank

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Abstract

This research was carried out to evaluate the effect of hermetic storage on quality of mungbean. About 260 kg of mungbean samples were stored in an especially design 350 L capacity hermetic PVC tanks (hermetic tank) and non-hermetic PVC tanks (control tank). Hermetic PVC tanks were closed air-tightly. All tanks were randomly placed in a warehouse. Each hermetic and control PVC tanks were artificially infested by 50 unsexed *Callosobruchus chinensis* kept in 4 glass jars containing 100 g of mungbean and jars were dipped in four different depths. The gas concentrations in the tanks were monitored up to 6 months intervals. Percentages of germination, moisture content, and grain damage were evaluated at the end of the storage. The oxygen content of hermetic samples was dropped to 11±1.2% and carbon dioxide content was increased up to 7±0.7% within 6 months of storage. Live insects of *C. chinensis* were not found in hermetic samples after 6 months but abundant population of *C. chinensis* was found in the control PVC tank just after one month. After 6 months, germination percentage of the mungbean samples stored in hermetic tanks had decreased from 95±3% to 82±4%, whereas it was decreased from 95±3% to 47±7% in control tanks due to grain damage. Percent grain damage of the hermetic sample was only 4.5±1% compared to the heavy insect damage of the control samples. Moisture content of hermetic samples remained unchanged compare to the control.

Keywords: Hermetic storgae, PVC tank, Mungbean, *Callosobruchus chinensis*

Introduction

Nearly 30-40% of cereals and grain legumes harvested in Sri Lanka are stored by farmers for consumption, seeds and future sale for a period of three to nine months (Adhikarinayake, 2006). Mungbean, cowpea, black gram, and soybean are major legume grain grown in Sri Lanka. These grains are mainly stored in polybags causing insignificant postharvest loss about 15% within 3-4 months (Sartaj and Ekanayake, 1991), but grain damage can be high as 68% after 4 months of stored in polysack bags (Prasantha et al., 2014a). Mungbean (*Vigna radiate* (L.) is cultivated around 9760 ha mainly by dry-zone farmers in Sri Lanka are yielding around 14000 MT per annum. Legumes are an inexpensive source of dietary protein supplement for more than 67% of Sri Lankans that consume them as an alternative to animal protein. However, local production is insufficient for local consumption and more or less 7000 MT is imported to Sri Lanka every year. Munbean and cowpea are highly susceptible to bruchids damage from pests such as *Callosobruchus chinensis* (L.) and *Callosobruchus maculates* (Fabricus) which are commonly known as southern cowpea weevil and cowpea weevil respectively. *C. chinensis* is the most common bruchid species that infests stored grain legumes in Sri Lanka. Mostly under poor storage conditions, *C. chinensis* attacks on stored grain legumes cause substantial losses to both quality and quantity. Although the infestation of grain legumes by bruchids begins in the field before seed maturation (Huignard et al., 1985), they reproduce rapidly in poor storage condition. New generation of weevil immerges in every 28 days (Prasantha et al., 2002) and may cause losses up to 12-15% in 2 months of storage. If infestation of weevil is not controlled, complete grain damage (100%) of mungbean could occur within 6 months where mungbean stored in common storage (in polybag) condition (Prasantha et al., 2014a). As a result, farmers try to sell their grains at low prices or apply hazardous insecticides to protect their stored grains soon after harvesting. Phosphine fumigation is not recommended at the farm level due to risk and safety issues in the application. Quality deterioration of mungbean is unavoidable under common storage in polybag. Hard-to-cook (HTC) defect is well-known quality deteriorating problem of mungbean which is related to the increase time of cooking due to poor storage (Prasantha et al., 2014a). The other problem is the loss of stored grain viability or percentage of seed germination due to insect infestation and development of HTC characteristics. Therefore, an effective storage method is necessary to prevent the insect infestation and avoid the development of HTC.

Hermetic storage is an airtight grain storage technique for controlling stored-product pests and avoid the development of HTC (Sanon, et al., 2011; Prasantha et al., 2014a). The respiration of insects, microorganisms and grains hinder the growth of insects as a result of creating high carbon dioxide (CO_2) and low oxygen (O_2) in the storage environment (Murdock, 2012). This indicates the importance of hermetic storage where early infestation can be avoided without substantial damage to the stored grains. According to previous studies hermetic storage of grain legumes in PET bottles containers and plastic bags can successfully control the damage of legume grains by bruchids (Murdock, 2012; Guenha et al., 2014; Prasantha et al., 2014b) more than 6 months. Although it is a relatively simple method of storage of mungbean, farmers are reluctant to adopt the method due to lack of appropriate plastic bags and handling problems of the bags. However, the farmers are preferring to use type of larger storage tanks where they can store larger quantities of grain with minimum space and low cost of larger numbers of bag handling.

The other major problem is the lack of information on final quality of stored mungbean such as germination and cooking quality. Therefore, it is important to study the applicability and effectiveness of hermetic storage on preservation of mungbean. This research was carried out to evaluate the suitability of bin type PVC hermetic tank for storage of mungbean to minimize postharvest losses and thereby to improve the seed germination and minimizing the HTC characteristics of mungbean.

Materials and Methods

The research was carried out at the "Palvehre" seed farm, Department of Agriculture, Sri Lanka. Mungbean samples (*Vigna radiate* (L.) Wilczek) were obtained directly from the field 2-3 weeks after harvesting and sun dried to moisture content about 12±1% (w.b) before storage. Approximately 260 kg of mungbean sample stored in an especially design 350 L capacity hermetic PVC tank (hermetic tank) and non-hermetic PVC tanks (control tank). Hermetic tank was air-tightly closed using thread seal with airtight PVC lid and covered by high vacuum silicon grease. Control tank was closed without hermetic sealing by PVC lid (Fig. 1). The control tank was also allowed to infest naturally similar to the common aerated storage.

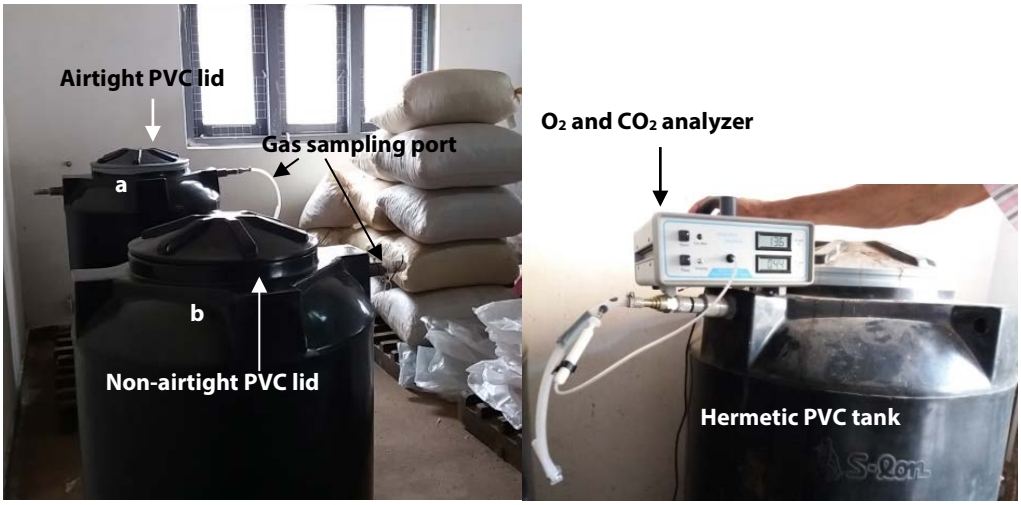

Fig 1. Storage of mungbean in PVC tanks (a) hermetic tank (b) control tank and (c) method of measuring of internal gas content

Biological tests

Prior to the experiment, 5 kg of mungbean sample was stored in a freezer (-18 °C) for about 2 weeks to destroy any hidden infestations of insects. Adults of *C. chinensis* were obtained from the same store and cultured on mungbean at the Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka, for 3 months. About 500 g of mungbean samples was obtained and stored in a 750 ml glass jar. The sample was artificially infested with 50 unsexed freshly emerged (1-3 days) adults of *C. chinensis* and the jar was covered with fine wire mesh. The samples were kept one week at ambient condition (26±1 °C and r.h. 78±3%) for egg laying and then weevils were removed from the samples through sieving. During grain loading, 3 artificially infested jars were buried inside each PVC tank at 3 different depths (i.e. bottom, middle and top) of each PVC tank. Number of adults emerged from the samples were determined by sifting three sample jars after 196 days of storage in hermetic and control tanks.

Hermetic storage

A rubber septum was glued onto the gas sampling rubber tube (10 mm internal diameter) as gas sampling port immerging from the centre of the PVC tank (Fig 1). Gas sampling port of the PVC tank pierced with a needle connected to the gas analyser (Quantek modle-902D, USA) to determine the percentage of O_2 and CO_2 contents. The initial O_2 and CO_2 content (atmospheric) were adjusted as 20.7% and 0.2% respectively. All experiments were conducted for 196 days in grain storage warehouse conditions. The temperature and relative humidity (r.h.) in these storages were 28±2 °C and 73±5%, respectively. Gas samples were measured almost at every 30-40 days intervals over the

period of 196 days. About 3-4 gas samples were withdrawn from each PVC tank in every test. This study was repeated 3 consecutive times during 2015-2017 at the same period (November-May) of each year. Altogether six PVC tanks were used in equal numbers for the control and hermetic study.

Storage grain quality

The m.c, of the initial, control and hermetic samples was determined (% w.b) by forced-air oven drying at 105 °C for 24 h. Grain germination of the initial, control and hermetic mungbean samples was tested after 196 days (ISTA, 2006). Samples of 100 mungbeans from each storage method were germinated on wet paper towels. Percent germination was calculated as the number of grains showing plumule and radicle emergence after 24 h of incubation at room temperature of 28±2°C. The HTC characteristics was evaluated using minimum cooking time (Singh et al., 1991). Two grams of mungbean samples were taken into a boiling tube and cooked by adding 20 ml of distilled water in a boiling water bath. The cooking time was determined by removing few grains at different time intervals during the cooking. The gains were pressed in between two glass slides until uncooked core was disappeared. This experiment was repeated for 4 times.

Storage losses

Percent grain damage was estimated using 50 g samples (Boxall, 2002) at the end of storage method using the following equation. Altogether 30 replicates were used to estimate the storage loss of this study.

$$Grain\ damage\ \% = \frac{N_d}{N_d + N_u} \times 100$$

Where;
Nd = Number of damaged grains in the sample
Nu = Number of undamaged grains in the sample

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SAS (1990) statistical package using PROC GLM procedure. Duncan's multiple range tests was used to separate means when ANOVA showed significance at $P < 0.05$. Other descriptive statistics and graphical methods were used to present the data with time and storage when appropriate.

Results and Discussion

Biological tests

After one month of storage, the large number of insects emerged from the control tank were identified as *C. chinensis*. It is important to note that there was no other species of bruchids were found in the samples. Artificially infested mungbean samples stored in the control tank were found with high number of insect emergence holes and an abundant number of *C. chinensis* progeny. However, 0.5±0.25% grain damaged was observed in the initial samples obtained from the field (Table 1). This indicates that mungbean samples obtained directly from the field had already been infested by *C. chinensis*. Generally, mungbeans are highly susceptible to damage by bruchids. According to Mutungi et al. (2014) ineffective methods of storage cause a substantial loss in quality and quantity of grains.

Storage losses

Grain damage had increased up to 98.5±1.5% in the control samples after 196 days of storage. It was remained significantly low at 4.5±1% ($P<0.05$) in the hermetic samples, which was a 96% reduction of grain damage compared to that in the control samples. Mortality of *C. chinensis* was

recorded at 100% in mungbean sample stored in hermetic conditions for 196 days. The number of progeny emergence of weevils was significantly lower (11±3.0) in the artificially infested samples stored in a hermetic tank. Prasantha et al. (2014b) noted more or less similar results of hermetic storage of mungbean.

Storage grain quality

Initial moisture content of mungbean was 12.2±0.1% (w.b.). Control samples showed comparatively higher moisture content than hermetic samples but no significant difference (P>0.05) was noted in the moisture content between hermetic and control mungbean samples. Comparatively high moisture content detected in the control samples may be related to the accumulation of metabolic moisture (both weevils and grains) and the absorption of atmospheric moisture.

Germination of the initial mungbean sample was 95±3% and it was significantly reduced ($P < 0.05$) to 46.8±7% in the control samples compared to the 82±3.8% germination remained in the hermetic samples after 196 days (Table 1). However, there was a 14% reduction of germination observed in mungbean stored in the hermetic tank compared to initial samples. Adikarinayake et al. (2006) reported that paddy stored in a hermetically sealed bin has completely lost its germination percentage after six months. Similar to this study, Prasantha et al. (2014a) also showed that germination of mungbean stored in the hermetic condition decreased slightly compared to initial sample after 6-12 months. Hamel, (1989) reported that high CO_2 storage can reduce the seeds viabilities of wheat, rape seed, soybean, and onion. The possible reason for this reduction might be the lowering of physiological and biochemical activities in mungbean due to development of HTC characteristics with ageing.

Table 1. Number of adults emerged, moisture content, percent germination, percent grain damage and minimum cooking time of initial, control and hermetically stored mungbean samples in PVC tank under ambient conditions.

		PVC tanks	
Test parameters	**Initial**	**Control**	**Hermetic**
Number of progeny	0	TNC[†]	11±3.0
Moisture (w.b %)	12.2±1.0[a*]	13.4±1.2[a]	12.7±0.7[a]
Germination (%)	95±3.0[c]	46.8±7.0[a]	82±3.8[b]
Grain damage (%)	0.5±0.25	98.5±1.5[b]	4.5±1.0[a]
Cooking time (min.)	25±1.2[a]	33±2.0[b]	26±1.0[a]

All data represent the mean ± SD of three-five replicates

*Values followed by the different small letters in each raw significantly different at $P < 0.05$

[†]TNC = Too numerous to count

Cooking time of hermetically stored mungbean samples did not show any significant change ($P > 0.05$) compared to the initial samples (Table 1). Cooking time of control samples significantly increased ($P < 0.05$) from 25±1.2 min to 33±2.0 min which was about 35% increase compared to the initial cooking time. Gradual development of high cooking time with storage is indication of grain hardness development and it is commonly known as HTC characteristics. Kon and Sanslulck (1981) reported that cooking time of common beans increased by about 5-fold when bean sample was stored at high r.h. and high temperature conditions. In contrast to finding of this study, Nasar-Abbas et al. (2008) reported HTC characteristics of faba bean increased significantly when beans stored in airtight bags. However, this study has revealed that hermetic storage can successfully delay the development of HTC in mungbean at least by 6 months.

Hermetic storage

A significant reduction of ($P < 0.05$) of O_2 and increase of CO_2 was observed in the hermetically stored mungbean samples in PVC tanks (Fig 2). Initially, the O_2 and CO_2 contents approximately dropped below 11% and increased more than 6% respectively, within the first 38 days after storage in

hermetic tank. Throughout the storage period, the O_2 content dropped to an average of 11.2±1.2% % and CO_2 increased to 6.8±0.7% in the hermetic PVC tank. There were no weevils found in the hermetic samples at the end of the storage period. The drop of atmospheric O_2 content in the hermetic PVC tank was approximately 48% compared to control tank sample, and there was no change in the gas composition detected in the control PVC tank. Similar results were observed by Murdock et al. (2012) and our previous studies of hermetic storage (Prasantha et al. 2014a and 2014b). Although the respiration of mungbean is low, but high metabolic activity of weevils and their developing immature stage (larval/ pupal) inside the mungbean were the reasons for lowering the O_2 and raising the CO_2 contents of inter-granular atmosphere of hermetic stored grains (Murdock et al., 2012; Navarro, 2012). The death of weevils may have occurred due to the low O_2 content and reduction of O_2 partial pressure within the inter-granular space during the storage period (Mbata et al., 2005). According to the data, successful developments of hermetic condition in the PVC tank without changing the gas composition during 196 days indicate that the suitability and sustainability of the hermetic PVC tank as a storage method for direct field application.

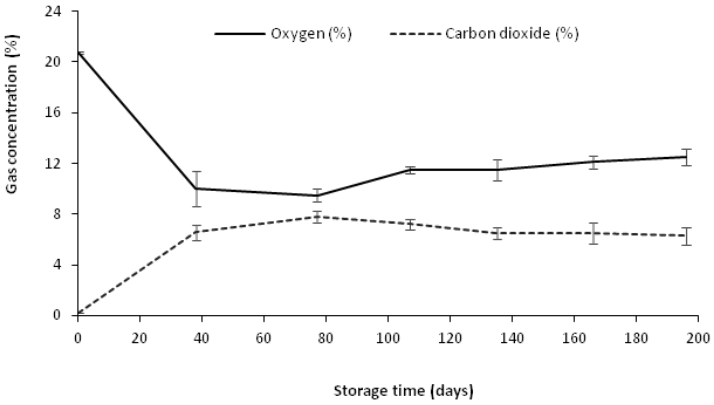

Fig 2: Changes in gas concentration (%) of hermetically stored mungbean samples in PVC tank. Data used are means ± SD of three tanks.

Conclusions

Storage damage of mungbean was mainly caused by *C. chinensis*. Hermetically storage of mungbean in PVC tack was successfully reduced the weevil development and grain damage. Although a slight change in percentage of germination was observed in the hermetically stored samples, moisture content and cooking time of beans did not change with the storage in hermetic tank. The increase of CO_2 and drop of O_2 contents in hermetic samples indicated the successful development of the hermetic condition within the stored mungbean in hermetic PVC tank. We conclude that hermetic storage can prevent the development of HTC characteristics and postharvest loss of mungbean.

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Combination of Mating Disruption and parasitoid Habrobracon hebetor against Plodia interpunctella in a chocolate factory

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Abstract

A field experiment of 4 years' duration was carried out to evaluate the efficacy of combining the mating disruption (MD) formulation Dismate ZETA (9Z,12E-tetradecadienyl acetate), with the parasitoid *Habrobracon hebetor* against the Indianmeal moth *Plodia interpunctella* in a chocolate factory. The experimental period began early in 2011 and ended in late 2014. Began Dismate dispensers were placed in the facility from 2011 to 2014 and *H. hebetor* was released in 2014. Pheromone-baited traps were used to monitor the flight activity of the male

moths and oviposition Petri dish cups were placed to assess the progeny production of *P. interpunctella* females. Following the start of MD, a decrease in the number of *P. interpunctella* males caught in monitoring traps was observed from 2011 to 2013. A further decline in the moth population was noted in 2014, when MD was combined with the release of parasitoids. The presence of larvae in the oviposition cups was occasionally observed throughout the monitoring period, from 2011 to 2014. This study demonstrates that the combined system of MD and parasitoids is an effective and reliable technique that can be used to successfully control *P. interpunctella*.

Keywords: food processing, Integrated Pest Management, *Plodia interpunctella*, mating-disruption, *Habrobracon hebetor*

Introduction

The use of pheromones for suppressing pest populations through mating-disruption (MD) has been widely studied for stored-product Lepidoptera. For stored-product moths such as *Ephestia* spp. and *Plodia interpunctella* (Hübner), a single pheromone compound, known as TDA [(9Z,12E)-tetradecadienyl-acetate] can act as a male attractant (Trematerra, 2012; Trematerra et al., 2013). The enclosed environment of storage facilities provides an ideal area for the application of MD, given that the sources of external infestation in this environment are limited (e.g., the introduction of infested raw materials or the immigration of mated females from untreated areas) (Trematerra and Fleurat-Lessard, 2015). For instance, in a recent study, Trematerra et al. (2011) illustrated the reduction in the population of various Pyralidae following the use of MD dispensers in several areas and facilities in Europe, which clearly indicated that this technique can be of widespread use.

MD can only lead to a reduction in chemical treatments, which however, remain necessary, because males of moths such as *P. interpunctella* can inseminate on average 6 females in their lifetime. Moreover, females need to be mated only once per lifetime to produce their full number of viable eggs. Consequently, a few successful copulations in an environment under MD treatment will guarantee the survival of the population. Therefore, the application of pheromone-based IPM tools should be accompanied by additional nonchemical measures, such as cleaning and sanitation, especially in critical areas, such as the inside of the machinery and around raw materials and packaging areas. More recently, it has been suggested that combining and integrating different management tools and the careful selection and timing of different approaches, together with an understanding of pest behaviour and ecology can result in greater effectiveness (Trematerra, 2013). Another approach to overcome the challenges faced by the stored-product industry due to stored-product insects is the integration of pheromone-based IPM tools with biological control, like the parasitoid wasp *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) (Ghimire and Phillips, 2010). *Habrobracon hebetor* can parasitize all larval instars of *P. interpunctella*, but significantly fewer early instars are paralyzed and reproduction requires last-instar larvae (Akinkurolere et al., 2009). In the past, various applications of *H. hebetor* in stored products have been investigated, for example, in grain spillage (Press et al., 1982), packaged products (Cline et al., 1984; Adarkwah et al., 2014), bulk peanuts (Brower, 1990) and bulk grain (Adarkwah and Schöller, 2012).

MD targets *P. interpunctella* adults, whereas the parasitoid *H. hebetor* should target *P. interpunctella* larvae. The purpose of this 4-year study (2011–2014), conducted in a chocolate factory, was to analyze the effectiveness of MD for the first time and to evaluate the combination of MD and the release as parasitoid *H. hebetor*.

Materials and Methods

The chocolate factory is located in Southern Italy, in the area of Ospedaletto d'Alpinolo (Campania region). In the factory, dried fruits were stored and processed and chocolate food was produced. The factory was constructed from concrete, and contained 3 floors with the first floor being dedicated to food production and having an area of about 2000 m2 and a height of 5 m (about 10000 m3 in total).

MD treatments

From 2011 to 2014, the pheromone component TDA [(9Z,12E)-tetradecadienyl- acetate] (Dismate, Russell IPM, Deeside, UK), was used for MD. Each cellulose acetate MD dispenser was baited with 100 mg TDA. In 2011, MD dispensers were placed in a small area of the chocolate factory (of about 320 m^2), but covered the entire chocolate factory (2000 m^2) from 2012 to 2014.

In 2011, MD dispensers were placed from May to December; in 2012 and 2013, MD dispensers were installed all-year round. In 2014, MD dispensers were put in place between July and December, during P. interpunctella adult flight activity. In 2011, 6 MD dispensers were installed. The MD dispensers were replaced with new ones every 2 months; in 2012, the number of MD dispensers was increased to 25, resulting in one dispenser per 50 m2 throughout the entire chocolate factory area.

Parasitoid release

During 2014, adults or pupae of *H. hebetor* (from Biologische Beratung Ltd., Berlin, Germany) were released within the chocolate factory. Starting from April 2014, 40 units containing 30 pupae or 30 adults each were released monthly (April 7, May 9, June 25, July 21, August 20, September 22, and October 19). Consequently, about 1200 pupae or adults were released monthly, representing a total of ca. 8400 individuals of *H. hebetor*.

Monitoring of *P. interpunctella* adult males

P. interpunctella adult males were monitored (as a control) by 7 pheromone-baited sticky traps (X-lure R.T.U. Combo 4, Russell IPM), each baited with 1 mg TDA. The traps were checked every 7 d. The TDA lures were replaced at 2 monthly intervals. The traps were placed at a height of 2.0–2.5 m above the ground. Monitoring started on March 31, 2011 and ended in December 2014. The following monitoring periods were analyzed: from April 11 to December 3, 2011; from May 14 to October 14, 2012; from June 10 to December 15, 2013; and from February 17 to November 9, 2014.

Monitoring of *P. interpunctella* oviposition

A Petri dish cup containing 15 g crushed peanuts was placed near each pheromone monitoring trap, which was used as a control oviposition trap. Seven oviposition Petri dish cups were placed throughout the entire facility (Cups: Cu1–Cu7). The cups were replaced on each trap-check date (i.e., at 7-d intervals), and the old cups were brought to the laboratory, where they were placed in incubators at 27.5 °C and 70% relative humidity after 45–60 d, the Petri dish cups were examined for emerging individuals in most cases, larval stages and usually last-instar larvae.

Data analysis

he number of *P. interpunctella* males caught in the traps during 2011–2014 was analyzed using the Duncan test and compared using Kruskal–Wallis one-way analysis of variance on ranks, and the means were separated using the Tukey's Test ($P < 0.05$) (using SigmaStat software, San Jose, CA, USA).

Results

The number of *P. interpunctella* males that were trapped in different areas of the chocolate factory between 2011 and 2014 is reported in Figures 1 and 2. Figure 2 and Tables 1 and 2 depict the capture trends from 2011 to 2014 in the pheromone monitoring traps. The catches obtained during the 4 years differed, and a progressive decrease in the number of *P. interpunctella* caught from the second to the fourth year of monitoring was observed. In all years of the experiment, an increase in the number of individuals caught during the hottest months (late spring and summer) was observed. During the cold months, low temperatures negatively affected the population of *P. interpunctella*, resulting in fewer trapped adults. From 2011 to 2014, the monitoring traps caught 2127 moths, and

during 2011, 2012, 2013, and 2014, pheromone traps caught 706, 784, 416, and 221 moths, respectively.

The mean catches of individual traps from 2011 to 2014 are presented in Table 3. There was a significant effect of trap location on the number of moths caught (Kruskal– Wallis one-way analysis of variance on ranks, $H = 23.568$, $df = 6$, $P < 0.001$). However, only traps 2 and 3 caught significantly more moths than trap 7. Trap 2 caught the highest number of *P. interpunctella* individuals (mean ± SD 127.50 ± 41.66), whereas trap 7 caught the fewest individuals (20.25 ± 1.66). This might be due to their location in the chocolate factory; trap 2 was located in the packaging area, which was characterized by windows and a door that can facilitate the migration of moths inside, which are attracted by odors of the sweet products. Traps 7 and 4 were placed in the production area of nougat, a seasonal sweet; when the machinery is operational, the temperature is high enough to impede colonization of the area by moths.

The number of *P. interpunctella* individuals trapped during the whole period of MD and MD + parasitoid experimentation in 2011–2014 decreased in all traps. The overall trend of the weekly catches from 2011 to 2014 is shown in Figure 2. Following the start of MD, a decrease in the number of *P. interpunctella* moths caught in monitoring traps was observed and the number ranged from 706 in 2011 to 416 in 2013. A further decrease in the population was observed during 2014, when MD was combined with the release of *H. hebetor* parasitoids, with 221 moths being caught in the monitoring traps.

In 2011 and 2013, the number of *P. interpunctella* individuals captured was extremely high before the beginning of the MD programme, with a rapid decrease in adults observed soon after the placement of new MD dispensers. That situation was less evident during 2014 when *H. hebetor* was released, in addition to MD activity (Figure 2).

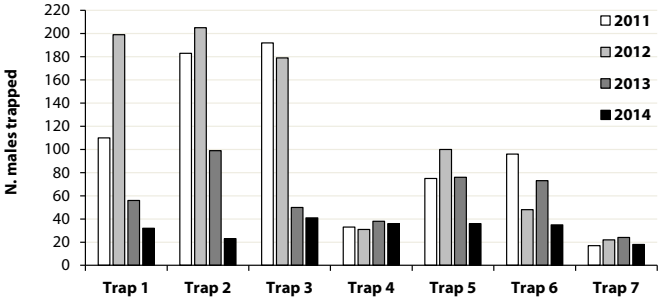

Fig. 1 *Plodia interpunctella* males caught by different pheromone traps 1-7 from 2011 to 2014.

The percentage reduction in moth captures between 2012 and 2013 reflected the increase in the number of dispensers (from 6 MD dispensers to 25 MD dispensers) and was 46.94% (Table 3). During the MD period in 2012, captures ranged from 22 (trap 7) to 205 (trap 2); in 2013, captures per trap ranged from 24 (trap 7) to 99 adults (trap 2) (Table 1). During 2014, when control with MD was only performed from July to November, and was associated with H. hebetor parasitoids, the number of trapped moths was lower, with a 46.87% reduction compared to that in 2013 (Table 4). In 2014, the moth captures per trap varied from 18 (trap 7) to 41 (trap 3) (Table 1). In 2014, the mean number of *P. interpunctella* individuals caught by each trap was significantly lower than at the beginning of the experiment (2011–2012) (Table 2).

The presence of larvae in the oviposition cups was observed occasionally during the monitoring period from 2011 to 2014. Except for positions Cu1, Cu3 and Cu5, all positions were infested at least once (Table 4).

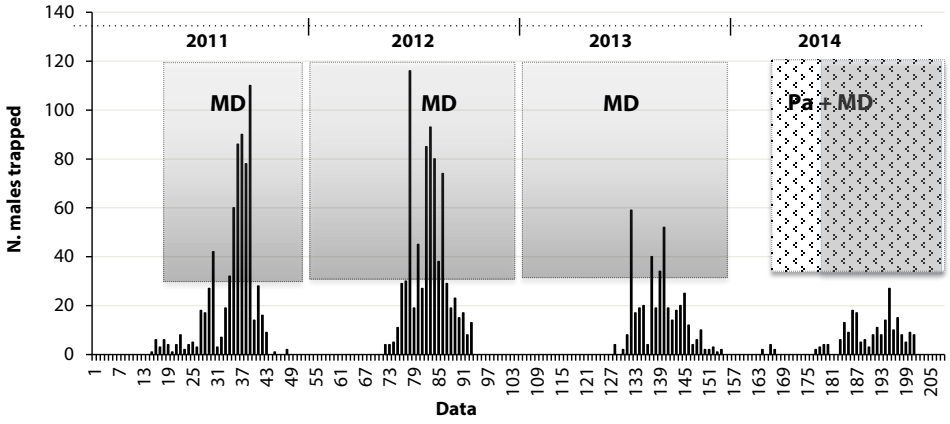

Fig. 2 Number of *Plodia interpunctella* males caught during 2011-2014 with indication of mating disruption treatments (MD) and parasitoids release (Pa).

Discussion

A reduction in the number of *P. interpunctella* moths in pheromone-baited monitoring traps was observed after placing MD dispensers at the beginning of the annual flight period of *P. interpunctella* (in May) and also after increasing the density of MD dispensers from a localized application to a general application (Figure 2). After placing the MD dispensers according to the protocol of 1 dispenser per 50 m2, the number of moths caught in the monitoring traps was drastically reduced and this trend was evident throughout the entire experimental period, particularly from 2012 to 2014. All sites showed a decrease in the number of trap catches after the introduction of MD. At 2 sites with low initial moth catches (trap 4 and trap 7 areas), the trap catches after the introduction of MD remained constant. In contrast, in areas with a higher initial population density, the reduction in trap catches was initially very low, especially in 2011, when MD dispensers were placed only in a small area of the chocolate factory. In 2013 and 2014, there was a clear reduction in the number of individuals caught in traps 1, 2, 3, and 5, especially in 2014, when *H. hebetor* parasitoids were used against *P. interpunctella* larvae, in addition to MD treatment; it is known that MD is more effective at low population densities (Trematerra et al., 2011; Trematerra, 2012).

Tab. 1 Mean number (± SD) of *Plodia interpunctella* adults caught in pheromone traps 1–7 from 2011 to 2014, per trap.

Trap	Area	Mean number of trapped moths (± SD)
1	Almond brittle	99.25 ± 37.04 ab
2	Packaging	127.50 ± 41.66 a
3	Dried fruit	115.50 ± 40.55 ab
4	Nougat	34.50 ± 1.56 ab
5	Ovens	71.75 ± 13.25 ab
6	Cooling	63.00 ± 13.54 ab
7	Nougat	20.25 ± 1.66 b

Data followed by a different letter differ significantly (Tukey's test, $P < 0.05$).

Tab. 2 Mean number (± SD) of *Plodia interpunctella* male adults caught in pheromone traps 1–7 from 2011 to 2014.

Year	Mean number of trapped moths (± SD)

2011	100.86 ± 25.58 b
2012	112.00 ± 30.71 b
2013	59.43 ± 9.57 ab
2014	31.57 ± 3.08 a

Data followed by a different letter differ significantly (Duncan test, $P < 0.05$).

Tab. 3 Changes in mean adult males trapped from 2012 to 2014 after MD and parasitoids (Pa)+MD treatments.

	Adult males trapped					
Traps	**Years**			**MD vs. MD**		**MD vs. Pa+MD**
	2012	**2013**	**2014**	**2012 vs. 2013**	**2012 vs. 2014**	**2013 vs. 2014**
1	119	56	32	- 52.94	- 73.11	- 42.86
2	205	99	23	- 51.71	- 88.78	- 76.77
3	179	50	41	- 72.07	- 77.09	- 18.00
4	31	38	36	+ 22.58	+ 16.13	- 5.26
5	100	76	36	- 24.00	- 64.00	- 52.63
6	48	73	35	+ 52.08	- 27.08	- 52.05
7	22	24	18	+ 9.09	- 18.18	- 25.00
1-7	784	416	221	- 46.94	- 71.81	- 46.87

Tab. 4 Chocolate factory 2011–2014, control dates with *Plodia interpunctella* infestation in oviposition cups (Cu1-Cu7), all other dates without infestation.

Petri dishes		Cu1	Cu2	Cu3	Cu4	Cu5	Cu6	Cu7
Data								
2011	4.XI	-	-	-	yes	-	-	Yes
25.XI		-	-	-	-	-	-	-
2012	13.I	-	-	-	yes	-	-	-
17.II		-	-	-	yes	-	-	-
4.V		-	-	-	-	-	-	Yes
8.VI		-	-	-	-	-	yes	-
2013	12.VII	-	yes	-	-	-	-	-
6.IX		-	-	-	yes	-	-	-
2014	1.VIII	-	-	-		-	yes	-

During the MD application period (2011–2014), the moth density, overall moth activity and degree of infestation was remarkably lower than in previous years without such treatment. In addition, the chocolate factory manager reported that fewer customer complaints were made in 2014 than in 2011.

In Europe, stored-product moths are among the most serious pests in stored grain, in the retail trade, mills, the food processing industry and private households. The use of pheromones to control populations of stored-product moths has been demonstrated (Trematerra, 2012) and MD has proven successful against stored-product moths in commercial field settings (Ryne et al., 2001, 2006, 2007; Burks et al., 2011; Trematerra et al., 2011; Burks and Kuenen, 2012; Trematerra and Savoldelli, 2013). In addition, other methods that involve pheromones in mass trapping (Phillips et al., 2000; Trematerra & Gentile, 2010), attract-and-kill methods (Trematerra and Capizzi, 1991; Phillips et al., 2000; Nansen and Phillips, 2004; Campos and Phillips, 2013, 2014) and the auto-confusion system (Trematerra et al., 2011) have been successfully used to manage stored-product moths. However, in these cases, complete elimination of the moth infestations was never achieved. The monitoring of pyralid moths using pheromone traps is likely to be affected by MD. One alternative to pheromone traps for monitoring is the use of water traps; however, these have also been shown to have a control effect (Trematerra and Savoldelli, 2013). In this study, oviposition cups were used as an alternative approach to pheromone traps; however, the number of infested cups was too low to analyze the population trends based on the data.

In the present experiment, the release of the parasitoid *H. hebetor* against *P. interpunctella* larvae helped to reduce the residual moth population in the chocolate factory in 2014 (Trematerra et al., 2017).

In Italy, the use of parasitoids in food facilities is rare, and our study represents one of the first applications. In Europe, and particularly in Germany, parasitoids have been evaluated in commercial food-processing facilities since 1995, and they have been commercially available since 1998. In the United States, insect natural enemies were technically designated as insecticides, in order to be regulated, and then they were exempted from a requirement for a tolerance level in food.

Recently, Trematerra (2013) suggested that combining and integrating different management tools and the careful selection and timing of different approaches, together with an understanding of pest behavior and ecology can be more effective in pest control.

According to our results, the combination of MD and biological control can be used to control *P. interpunctella* infestation in a chocolate factory. These techniques must be considered as part of an Integrated Pest Management programme and should not be considered in isolation. MD is effective against *P. interpunctella* adults, but cannot control larval and pupal stages; in contrast, *H. hebetor* can attack larval instars, and could also be used against overwintering larvae in warehouses or food facilities, when environmental conditions are suitable.

The use of insect natural enemies and MD in an integrated approach against stored-product moths is an opportunity for the biologically based management of storage pests. MD combined with natural enemies has been shown to be promising, and might be especially relevant in the organic food industry when *P. interpunctella* and other pyralid moths are the target pests.

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Host-age preference of *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae), a larval parasitoid of the lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) and the cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)

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Abstract

The pteromalids (Hymenoptera: Pteromalidae) *Anisopteromalus calandrae* (Howard), *Dinarmus basalis* (Rondani), *Lariophagus distinguendus* (Förster), *Pteromalus cerealellae* (Ashmead) and *Theocolax elegans* (Westwood) are solitary larval ectoparasitoids used to suppress several species of stored-product insects that infest storage grains. We investigated host-age preference of *T. elegans* using no-choice laboratory experiments. Lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) larvae (9, 11, 13, 15, 17, 19, 21 and 23 days-old) in wheat grain kernel and cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) larvae (5–19 days-old) in cowpea beans were exposed to neonate *T. elegans* mated females to lay their eggs for two days. Our results showed that the highest number of parasitoids emerged from 23 days-old *R. dominica* larvae. The numbers of parasitoids emerged from 19, 21 and 23 days-old *R. dominica* larvae were statistically significantly different in experiments (F-test, 0.05). Progeny of *T. elegans* reared from *R. dominica* and *C. maculatus* larvae were either fully-winged (macropterous), short-winged (brachypterous) or wingless (apterous). Female *T. elegans* were rarely host-feeding on *C. maculatus* larvae. *Theocolax elegans* progeny were emerging from 14 days-old *C. maculatus* larvae only. We discussed insectary mass production of *T. elegans* for biological control.

Keywords: Biological control, *Callosobruchus maculatus*, host-age preference, *Rhyzopertha dominica*, stored-product insects

Introduction

Stored products such as grain, flour, legumes, tobacco and dried fruits have a value of more than one billion United States Dollar (USD) in developing countries (Eliopoulos *et al*., 2002). However, these commodities frequently lose quality and quantity due to the action of stored-product insect pests. The important problems are damages by stored-product insect pests (Coleoptera) such as the larger grain borer *Prostephanus truncatus* Horn (Bostrichidae), rice weevil *Sitophilus oryzae* (L.) (Curculionidae), maize weevil *Sitophilus zeamais* Motschulsky (Curculionidae), khapra beetle *Trogoderma granarium* Everts (Dermestidae), rusty grain beetle *Cryptolestes ferrugineus* (Stephens) (Laemophloeidae) and saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Silvanidae) and some moths such as Angoumois grain moth *Sitotroga cerealella* (Oliver) (Gelechiidae), rice moth *Corcyra cephalonica* (Stainton) and Indian meal moth *Plodia interpunctella* (Hübner) (Pyralidae) (Arthur, 2010; Flinn*et al*., 2006; Hayashi *et al*., 2004; Johnson *et al*., 2000; Plague *et al*., 2010). Many countries have controlled stored-product insect pests using fumigation with methyl bromide and phosphine. However, the first mentioned compound has been banned (Credland, 2010) and resistance towards the second mentioned have been detected (Nayak*et al*., 2003).

Biological control is an alternative, environmentally friendly method of insect pest management. Parasitoids and predators are used to reduce stored-product insect pests because the natural enemies are not harmful to the environment or the user (Schöller*et al*., 2006). Nevertheless, natural enemies such as the hymenopterous parasitoids *Anisopteromalus calandrae* (Howard) and *Lariophagus distinguendus* (Förster) (Pteromalidae), *Cephalonomia tarsalis* (Ashmead) and *Holepyris sylvanidis* (Bréthes) (Bethylidae), and *Venturia canescens* (Gravenhorst) (Ichneumonidae) are well-known to suppress stored-product insect pests.

Theocolaxelegans (Westwood) (Pteromalidae) is a solitary ectoparasitoid used for biological control. *Theocolax elegans* attacks several stored-product insect pests that develop inside the grain kernel such as *S. zeamais* (Wen *et al*., 1994), cigarette beetle *Lasioderma serricorne* (Fabricius) (Anobiidae) (Hayashi *et al*., 2004) and lesser grain borer, *Rhyzopertha dominica* (F.) (Bostrichidae) (Flinn *et al*., 2006). The female parasitoid parasitizes host larvae within infested grain by using her ovipositor (Sharifi, 1972). Gordh (1979) found different morphs in *T. elegans*, i.e. winged and wingless morphs both in males and females. However, little is known concerning host-age preference of *T. elegans* with *R. dominica* and *C. maculatus* host larvae.

Materials and Methods

Mass rearing of insects

Callosobruchus maculatus, R. dominica, *S. zeamais* and *T. elegans* were reared at the Post-harvest and Processing Research and Development Division, Department of Agriculture, Chatuchak, Bangkok, Thailand under conditions of 24–30°C, 70–73% RH and 12L:12D/natural photoperiod. The mass-rearing method used a glass container holding 50 g of brown rice (Poaceae). One hundred unsexed adults of *S. zeamais* were placed in a glass bottle (5.5 cm diameter and 15 cm height) for oviposition on brown rice. Then the glass bottle was covered with a filter paper. After five days *S. zeamais* adults were sieved off from the brown rice. *Sithophilus zeamais* larvae (21 d old) were used as hosts for *T. elegans*. *Callosobruchus maculatus* and *R. dominica* larvae developed on cowpea bean (Fabaceae) and wheat grain (Poaceae), respectively, they were used as hosts in our further laboratory trials.

Host-age

Callosobruchus maculatus and *R. dominica* were mass reared on cowpea bean and wheat grain, respectively 50 g in each glass bottle as host species. Twenty unsexed adults of *C. maculatus* and *R. dominica* were sustained on grains in our no-choice experiment. The adults of two host species were mated and laid eggs for five days. *Theocolax elegans* parasitized different ages of *C. maculatus* larvae (5–19 d old) and *R. dominica* larvae (9, 11, 13, 15, 17, 19, 21 and 23 d old). A neonate mated *T. elegans*

female was released into a glass bottle for parasitism. The experiment was replicated 30 times. The number of progeny and sex ratio of wasps in the bottle were recorded.

Statistical Analysis

Analysis was performed using the software IBM SPSS version 20.0. The numbers of *T. elegans* progeny were fully-winged, short-winged or wingless morphs that emerged from different host-ages depending on host species was estimated using the mean±SD. The total numbers of *T. elegans* progeny were determined using one-way analysis of variance to compare mean via an F-Test in completely randomized design (CRD).

Results

Our experiments showed that *T.elegans* progeny were either fully-winged, short-winged or wingless morphs (Tab. 1). The numbers of progeny produced on *R. dominica* (9, 11, 13, 15, 17, 19, 21 and 23 d old) and on *C. maculatus* larvae (5–19 d old) was different. The highest number of *T. elegans* fully-winged progeny emerged from 23 d old *R. dominica* larvae including both females and males (Tab. 1), suggesting that ovipositing females were fertilized.

Tab. 1 *Theocolax elegans* (mean±SD) progeny emerging from different host-ages on *C. maculatus* and *R. dominica* larvae.

Host-ages in different host species	*T. elegans* ♀			*T. elegans* ♂		
	Fw	Sw	Wl	Fw	Sw	Wl
14 d *C. maculatus*	0.5±0.9	0.0±0.0	0.1±0.3	0.1±0.4	0.0±0.0	0.0±0.0
19 d *R. dominica*	1.7±2.5	0.0±0.0	3.1±4.2	0.3±1.3	0.0±0.0	1.0±2.1
21 d *R. dominica*	3.3±4.1	0.1±0.3	1.6±1.9	1.0±1.7	0.0±0.0	0.4±0.8
23 d *R. dominica*	8.3±6.7	0.1±0.4	1.1±1.5	1.9±1.4	0.1±0.4	1.3±1.2

Fw = Fully-winged, Sw = Short-winged, Wl = Wingless

The number of *T. elegans* progeny was statistically significantly different (P value<0.05) depending on host species. The highest number of progeny emerged from 23 d old *R. dominica* larvae (Fig. 1). Sex ratio of *T. elegans* progeny produced from 19, 21 and 23 d old *R. dominica* larvae was 3.7:1.0, 3.5:1.0 and 2.9:1.0, respectively. However, the results showed no progeny on 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18 and 19 d old *C. maculatus* larvae. Female *T.elegans* preferred to parasitize *C. maculatus* larvae only at an age of 14 d (Fig. 2). Sex ratio of *T. elegans* progeny emerged from *C. maculatus* larvae was 5.7:1.0.

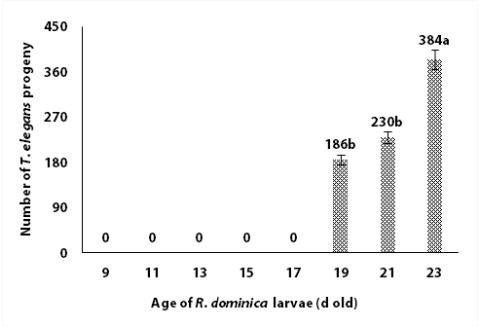

Fig. 1 Progeny of Theocolax elegans emerging from Rhyzopertha dominica larvae at different host-ages.

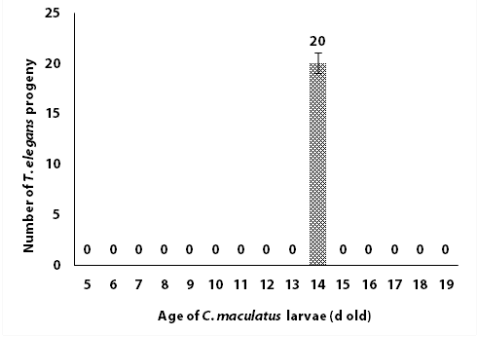

Fig. 2 Progeny of Theocolax elegans emerging from Callobruchus maculatus larvae at different host-ages.

Discussion

Theocolax elegans was reared on larvae of *R. dominica* and *C. maculatus*, respectively at different larval ages. Godh (1979) reported that fully-winged, short-winged and wingless morphs exist both in female and male. We found the number of *T. elegans* progeny produced on *R. dominica* larvae was higher than on *C. maculatus* larvae (Tab. 1). In this study, we infer that *T. elegans* prefers to parasitize *R. dominica* larvae compared to *C. maculatus* larvae. Similarly Shin et al. (1994) found that the Pteromalidae parasitoid *L. distinguendus* parasitized more *S. oryzae* larvae than *Callosobruchus chinensis* (L.) (Chrysomelidae) larvae. Odours may have different influence on parasitoid activity. Steidle *et al*. (2001) reported that parasitoids were affected by odours which emanate from host plants. Volatiles from faeces originating from *C. maculatus* were different to those from *Sithophilus granarius* (L.) (Dryophthoridae) and *R. dominica* (Steidle *et al*., 2001).

The results showed that the number of *T. elegans* progeny emerged from 23 d old *R. dominica* larvae was highest (Fig. 1). The optimal host-age for *T. elegans* to parasitize was 23 d old *R. dominica* larvae. Similarly, Choi and Ryoo (2002) reported that *A. calandrae* preferred to parasitize older host larvae more than young hosts. However, our no-choice test showed that *T. elegans* females prefer to parasitize *C. maculatus* larvae at 14 d only. The other ages of *C. maculatus* larvae were never parasitized (Fig. 2). Host numbers per kernel decreased with increasing host size (Wen *et al*., 1995). The larval instar of *S. oryzae* affects the progeny sex ratio of *A. calandrae* (Choi and Ryoo, 2002). Charnov (1982) and Ji *et al*. (2004) reported that female parasitoids assign daughters on large hosts and sons on small hosts. Host-age or host size also influenced the sex ratio (Wen *et al*., 1995). Type of grain also influences pteromalid parasitoid growth (Smith *et al*., 1995). The resource of food as nutritient is important to parasitoid progeny (Godfray 1994).

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Phytochemical-Based Nano Emulsions for Stored Grain Protection

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Abstract

Stored grain losses caused by pest insects contribute significantly to the global food crisis. Currently, there are two main chemical control methods against stored product insect pests: fumigation with very toxic gases and grain protection by residual contact insecticides. Today, the global tendency is to prevent/reduce the wide use of insecticides, which have high toxicity to humans and harm the environment. Therefore, there is an urgent need to develop alternative eco-friendly approaches for stored insect pest control.

Essential oils from *Micromeria fruticosa* and *Mentha rotundifolia* (Fam. Labiatae) and their main constituent pulegone which previously were shown by us as very active against stored product insect pests, were encapsulated into coarse and nano emulsions. The insecticidal activity of the developed formulations against primary internal insect rice weevil (*Sitophilus oryzae* L.) and secondary external pest red flour beetle (*Tribolium castaneum* Herbst) was evaluated in laboratory and pilot experiments.

It was found that the phytochemical-based nano emulsions offered significant advantages and provided powerful and prolonged biological activity compare with the coarse formulations. The developed nano emulsions could serve as a natural, effective, low-toxify for human, and environmentally preferred method for protection stored grain and dry food products from pest insects.

Keywords: essential oils**,** nano emulsions, pulegone, stored product insects, stored product protection.

Introduction

Insect damage in stored grain contributes significantly to the global food crisis (Philips and Throne, 2010, Nopsa et al, 2015). Today, the use of fumigants and protectants are common chemical control methods for stored product protection against pest insects. In spite of their high efficacy, both these methods have well known disadvantages (Kostyukovsky and Shaaya, 2012, Opit et al, 2012, Nayak et al, 2013, Daglish et al, 2014). The use of plant essential oils (EOs) and their constituents may be one of alternative eco-friendly approaches for stored insect pest control (Shaaya et al., 1991, 1993, Shaaya and Kostyukovsky, 2006, Kostyukovsky and Shaaya, 2011, 2012). However, for the implementation of the essential oils, suitable formulations are needed.

Encapsulation of essential oils allow even distribution of the active agents, slow evaporation of volatile compounds, and avoid the undesired odors (Onvulata et al, 2012, Majeed et al, 2015). Oil-in-water emulsions are utilized to introduce the water insoluble ingredients into aqueous solutions. In addition to the coarse emulsions, nanoemulsions can be prepared utilizing high or low energy methods. Due to a higher ratio of droplet surface area per mass unit, nano emulsions typically have increased kinetic stability and higher encapsulation capacity (Silva et al., 2012, Salvia-Trujillo et al., 2015).

Essential oils extracted from the *Micromeria fruticose* and *Mentha rotundifolia* (Labiatae) plants content 70-98% of monoterpenes (pulegone and SEM respectively), which were found to possess excellent insecticidal activity, include against stored product insect pests (Shaaya et al., 1993, Franzios et al., 1997, Kostyukovsky and Shaaya, 2011). However, their effective use is limited by the absence of suitable formulations.

In this research, we aimed to develop an appropriate formulation for effective delivery of EOs, the nature-sourced pest-managing agents for the grain storage. For this purpose, EOs were encapsulated into coarse and nano emulsions. The prepared emulsions where characterized by spectroscopic and microscopic methods and their stability and release ability at various environmental conditions were examined. The insecticidal activity against *S. oryzae*, the primary insect pest, and *T. castaneum*, the secondary insect pest, was studied under the laboratory and pilot conditions.

Materials and Methods

Oil-in-water emulsions were formed by stirring pulegone/SEM with sunflower oil in 1:1 ratio at various concentrations with Tween 80 (1.0% v/v) and double distilled water at 1000 rpm for 30 min. Microemulsions were homogenized for 3 min using Power Control Unit homogenizer (Kinematica, Switzerland). Nano emulsions were prepared by ultrasonicating the coarse emulsion with Vibracell ultrasonicator (Sonics&materials, Inc. Newtown, CT, USA) at 70% intensity for 30 min. The average droplet size and polydispercity index (PDI) were measured by dynamic light scattering (DLS) on a Zetasizer Nano ZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK) working at 633 nm at 25°C and equipped with a backscatter detector (173°), which is appropriate for measuring submicron particles (Brar and Verma, 2011). Emulsions with pulegone concentrations of 1, 5, 10% and an emulsifier concentration of 1%, were placed at room temperature and checked over the period of a month to examine changes in particle size (published by Golden et al., 2018). The reported values represent an average of three measurements and standard deviation.

The adults of coleopterans *S. oryzae* and *T. castaneum* served as the test insects. The insects were reared in the Volcani Center, Department of Food Quality and Safety under the controlled climatic conditions: the air temperature 28 ± 1°C, the air relative humidity 65 ± 5% in prolonged darkness. After the laboratory treatments, the tested insects were maintained in an incubator under the same controlled conditions. The cultures of the tested insects have been reared for many years without any contact with insecticides. For rearing of *S. oryzae*, the whole wheat grains of 12% moisture content were used. *T. castaneum* was reared on the wheat flour (egg laying) and on the ground wheat grain.

The laboratory experiments were conducted in the incubator at the temperature of 28°C. The glass chambers of 600 ml volume were filled with 100 g of wheat grains with the moisture content of 12%. To each chamber, 200 µl emulsions containing pulegone/SEM at concentrations of 0.1, 1, 5, 7.5, 10 and 20% were added (2-400 ppm on the wheat kernels). Coarse and nano emulsions were examined. The chambers were closed and the grains were mixed for a few minutes. Ten unsexed adults of each test insect 10-15 days old were introduced into the chambers and the chambers were closed hermetically. The control grains were treated with an emulsion without pulegone/SEM. The insect mortality was checked weekly. The grains were sieved by the 10-mesh sieve and were re-infested with the new adults of the same species. Before grain sieving, the concentration of carbon

dioxide (CO_2) was measured by Oxybaby instrument (Witt-gasetechnik-Germany). The experiments were conducted in three replicates and were continued until the loss of the emulsions efficacy.

The pilot experiments were conducted in the bins of 60 l volume with 45 kg of the treated and untreated wheat grain. The tested insects were placed in cages with holes in three depths locations. The nano and coarse emulsions contained 10 or 20% pulegone/SEM were tested. The insect mortality was checked every two weeks and the grain was re-infested with the new adults of the same species.

Statistical analyses were done using the ANOVA and TUKEY student range test, which were performed with the JMP 13 software (Statistical Discovery™ from SAS, Cary, NC).

Results

The optimal content for the stable emulsions was istablished. The concentrations of SEM/pulegone of 5 and 10% v/v and Tween 80 of 1% v/v resulted in stable emulsions with insecticidal activity. The droplet size of nanoemulsions was depended on the pulegone concentration and stabilized after a week. The initial amount of the pulegone released from the nano formulation (after 4 days) was 3-3.5 times higher than that of the coarse emulsion. The nano emulsions provide significantly higher amounts of pulegone for prolonged period in comparison to coarse emulsion (0.15-0.35 mol/l vs 0.05-0.1 mol/l). After 200 days, in 10% pulegone at 32°C, coarse emulsions released all the pulegone whereas from nano emulsions the pulegone was still released (published by Golden et al., 2018).

The nano emulsions were found much more active compared with the coarse formulations. The total mortality of *S. oryzae* adults was recorded for nano emulsion of 10% pulegone for 5 weeks (exposure of a week) compared with one week in the coarse emulsion. The same tendency was observed in *T. castaneum*. Mortality of above 90% was observed for over 5 weeks, with 10% pulegone nanoemulsion compared with one week in the coarse emulsion (published by Golden et al., 2018).

In the case of 10% SEM emulsions, the high efficacy against *S. orysae* was recorded for 5 months with nano- and for 2 months with coarse emulsions, and against *T. castaneum* for only 1 week (nano).

In parallel to the re-infestation process, the levels of CO_2 were checked weekly. At the first few weeks, concentrations of CO_2 in the control reached 4-14%, elevating to 15% and more, that are lethal for the insects. In contrast, in the pulegone/SEM coarse and nano emulsions that caused insect mortality, the concentrations of CO_2 were at low levels of 0.1-4%.

In the pilot experiments, 10% pulegone emulsions were active against *S. orysae* for 3 months, against *T. castaneum* for a month with the advantage for nano emulsion. 10% SEM emulsions were active against *S. orysae* for 6 and 5 months (nano and coarse), against *T. castaneum* for two weeks.

Discussion

The release studies show the advantage of nano emulsions, and the effective prolonged release of pulegone. These properties are necessary when considering slow release and prolonged exposure of food storage insects to the pest-managing agent. In the most treatments, the quantity of the released pulegone/SEM from nanoemulsions were higher for prolonged period compared to the coarse emulsions. Nanoemulsions have a higher surface area in comparison to coarse emulsions and therefore can release a higher amount of pulegone. They are more stable than coarse emulsion (Arnon-Rips and Poverenov, 2016). After 200 days of application, the pulegone concentration in nanoemulsions was still high.

In the most experiments, pulegone/SEM nano emulsions provided prolonged efficacy against both primary and secondary grain pests *S. oryzae* and *T. castaneum* compared to coarse emulsions. The high efficacy of the emulsions was observed for longer periods against adult stage of *S. oryzae* compared with *T. castaneum*. This finding consist with our earlier experiments (Shaaya et al, 1993) in which *T. castaneum* was found the most tolerant insect to a wide range of essential oils and their

constituents, including pure pulegone and SEM, compared with the other stored product insect pests.

EOs-based emulsions applied to wheat grain may serve as both fumigant and protectant. The insects inhale released from the kernels fumes, contact with the treated kernels and ingest them.

EOs have good properties for fumigation and may be applied for a wide range of insect pests (Shaaya and Kostyukovsky, 2006, Isman and Akhtar, 2007, Rajendran and Sriranjini, 2008, Kostyukovsky and Shaaya, 2011, 2012). On the other hand, Germinara et al (2017) performed topical application of EO and reported a high mortality rate of adult granary weevils due to contact activity of the EOs.

The current global tendency is to decrease the wide use of toxic chemicals in food. EOs-based nanoemulsions applied to stored wheat grain may serve as an alternative eco-friendly approuch for stored product insect control.

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Anti-termitic properties of Jatropha (Jatropha curcas L.) on wood termites (Macrotermes bellicosus (Smeathman)

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Abstract

The efficacy of *Jatropha curcas* in the management of wood termites, (*Macrotermes bellicosus*) was carried out in the Teaching and Research Farm of the Department of Forestry and Wildlife Resources Management. University of Calabar. The experiment consisted of 5 levels of *J. curcas* oil (0, 0.5, 1.0, 1.5 and 2.0) and a corresponding quantity in powder, replicated 4 times and arranged in Randomized Completely Block Design (RCBD). Each concentration was tested on 20 unsexed adult wood termite placed in grave yard of 8cm x 8cm. Data on mortality rate was taken at 12 hourly up to 72 hours. The result from the experiment showed that *J. curcas* oil was significantly efficacious compared with *J. curcas* powder both in the field and in the laboratory. It was observed that there was progressive increase in mortality rate due to increased concentration and time duration. The management of termite using *J. curcas* should be encouraged due to its environmental friendliness and should also be incorporated into integrated pest management (IPM).

Key words: Jatropha curcas, Macrotermes bellicosus, oil, powder, mortality

Introduction

Termites (*Macrotermes bellicosus)* are social insects living in colonies, they are sometimes called white ants but are not ants, because the true ants belong to the order hymenoptera, while termites belong to the order isoptera (Grimaldi and Engel, 2005). Termites occur in all temperate an d tropical countries of the world, many of which cause extensive damage to wooden structure and to manufactured goods made of wood, paper and cloth. Occasionally, they cause significant damage to growing trees such as teak and agricultural crops such as cotton (Solomon, 1995). The economic importance of a few pest species is so dramatic that the importance of termite breaking down woody tissue and returning nutrient to the soil is obscured (Truman and Robinson, 1982). Termites are responsible for some of the degradation of wood and other cellulose material in the terrestrial environment, mainly in the tropics and subtropics (Bulthman, 1979). Cellulose being the principal food of termites, wood and wood product such as paper, fabrics and wood structures are consumed and destroyed by termite, and hence a constant effort is directed towards their control. Field and laboratory test indicated that some woods are not resistant, but are susceptible to attack by African wood termite causing significant damage. Factors affecting wood consumption by termites are numerous and complexly related. Among the most important of these factors are: wood species, hardness of the wood Presence of toxic substance, feeding inhibitors or deterrents, Presence or absence of fungi or fungal decay, Moisture content of the wood and soil (Carter *et al*., 1974; Peralta *et al.,* 2004).

Termites of the *Macrotermes* spp are fungus growing termite belonging to the family rhinotermitidae, they are mostly mound builders and are the largest termite species (Osipitan and Oseyemi, 2012). The species of the termite under the genus *Macrotermes*, impact the economy negatively by causing damage to various agricultural crops, rangeland, wooden portions of buildings, furniture, books, utility poles and fences in several parts of Africa (Wong *et al.,* 2001*;* Mitchell, 2002; Cox, 2004). It has been reported that *Macrotermes* causes a complete damage of between 80 to 100 % on stored products (Michael, 2000; UNEP and FAO, 2000; Sekematte, 2001;

Nyeko *et al*., 2010). In some part of Africa, *Macrotermes* do cause a yield loss of 30-60% (UNEP and FAO, 2000). In east Africa, the loss caused on various crops and tree species due to termite vary ranging from 50-100% (Sekematte, 2001; Nyeko *et al*., 2010). Pests are organism which are invasive or detrimental, notorious, troublesome, destructive to either plants or animals, or which constitute nuisance to livestock, and humans (Sharma *et al*., 2011). Termite are serious pest of arable crops such as wheat, sugarcane, groundnut, paddy rice causing significant yield loss and also to perennial crops such as forest trees and wooden structures in buildings especially in semi-arid and sub-humid tropics of the world. They are very destructive insect as they feed on both dead wood and living plants. They can eat through the timber of wooden houses and can even attack hard wood such as *Tectona grandis*. Also, they eat furniture, books, boxes and other products of wooden origin. It has been observed that termites conveniently build their nest in fallen logs, stumps of trees, wooden buildings or pieces of wooden debris on the ground, some termite even live the heartwood of large trees (Cox, 2004).

Pesticides play an important role in the integrated pest management (IPM) on agricultural production and productivities (Logan, 1990). For controlling termite, certain synthetic termiticide such as DDT, BHC, aldrin, heptacor, and organochlorinated hydrocarbon have been used for the management of termite but were banned due to the harmful effect on humans, non-targeted spp and the environment (Mulroney *et al* 2005; Soomro *et al*, 2008; Sileshi *et al.,* 2009). As a result of the negative impact of the use of persistent and deleterious synthetic pesticide on the environment, research on the identification of eco-friendly and locally available alternative tool for the control has been the agenda of entomologist. The use of plant materials in the management of insect pest, including termite has been an old strategy in Africa and among many botanicals used in insect pest management plants such as neem (*Azadirachta indica*,), garlic (*Allium sativum*), *Clausena anisata* and have been successfully used to control termite. **(**Owusu*,* 2001**;** Doolittle *et al.,* 2007; Dubley *et al.,* 2008, Muhammad, 2009).

Bio-Pesticides are pesticides that are derived from natural live forms such as plants, bacteria, fungi and nematodes and others (Copping, 2009). They are often important component of integrated pest management (IPM) and used as a component of integrated pest management program, these pesticides can greatly decrease the use of conventional pesticides hence, improve the quality of timber as well as crop production. Bio-pesticide control pests and diseases either selectively or with broad spectrum approach. Bio pesticides are generally target specific and affect only the targeted population (EPA, 2012). Control of termite has been through the use of synthetic insecticides such a DDT, BHC, aldrin, heptacor, which are environmentally hazardous. There is therefore the need to assess the efficacy of non synthetic insecticides which are environmentally friendly.

Materials and Method

Location of the study area

This experiment was conducted at the teaching and research farm of the Department of Forestry and Wildlife Resources Management, University of Calabar, Nigeria

Collection of insect sample

Plastic rubbers, 30 cm in length and 15 cm in diameter were buried in a moist soil that surrounds the termite infested trees. Soil was introduced into the plastic rubbers and pieces of rolled carton were placed inside the rubbers and the rubbers were left in the soil for 3-4 weeks. After that, the plastic rubbers were checked if they were infested with termite and the cartons containing termites were incubated under dark condition with high humidity. The termites were fed with sawdust to ensure their survival. Over 2000 population of wood termite were collected for the experiment.

Preparation of bio-pesticide

Seeds of *Jatropha Curcas* were sourced from the tree, shade-dried for two weeks and made into powder using an electric blender and stored in a cool and dry environment till when needed.

Preparation of plant extract

Alcohol extract: Fifty grams (50 g) of *J. curcas* powder each was taken using a rolled filter paper and placed in a soxhlet extractor in 50^0C with 200 ml of ethanol added to it and kept for 24 hours. This procedure was repeated many times in order to get enough amount of extract. The extract was dried in an oven in 45^0C for one hour and kept for use.

Extraction of essential oil from *Jatropha curcas:*

Fifty grams of the same powder was introduced into a flask containing 500 ml of distilled water and exposed to source of heat. The rising steam from the sample was condensed by condenser connected with a glass cylinder to collect the resultant water of the evaporation. The oil layer accumulating on the surface of water was obtained by separating funnel. The oil was kept in the refrigerator till when needed.

Experimental design

A grave yard experiment of 8 cm x 8 cm was measured, thereafter, 0, 0.5, 1.0, 1.5 and 2.0 g each of the powder and a corresponding quantity of the oil were thoroughly mixed with saw dust and introduced into the grave yard and left for one hour and then 20 unsexed adult termites were introduced into the grave yard. Each treatment and the control was replicated four times and arranged in a Randomized Completely Blocked Design (RCBD). Similar experiment was conducted in the laboratory with four replications in a completely randomized design (CRD).

Data collection

Data were collected 12 hourly in each case after administering of the bio-pesticide. Parameters assessed include mortality at 12, 24, 36, 48, 60 and 72 hours after application, respectively.

Data analysis

Data collected were subjected to analysis of variance (ANOVA) using Statview statistical software and significant means were separated using Duncan Multiple Range Test (DMRT) at 5 % level of significance.

Result

Result of the insecticidal properties of *J. curcas* oil and powder showed significant effect on wood termites at ($P< 0.05$) Five levels each of *J. curcas* oil and powder 0, 0.5, 1.0, 1.5, and 2.0mls and the same concentration of the powder were applied to determine their efficacy on the mortality at 12, 24, 36, 48, 60 and 72 hours period of exposure. Generally at 12, 24, 36, 48, 60 and 72 hours of exposure, the mortality rate of termites was higher in oil when compared to powder. At 12 hours of exposure, *J. curcas* oil at 2.0 mls was highly effective compared to other levels and recorded 30% mortality rate. However, 2.0 g of *J. curcas* was as effective as 1.0mls and recorded 22.5% (Fig1). Similarly, at 24 hours of exposure, application of 2.0mls of *J. curcas* oil was effective and recorded significantly higher mortality rate of compared with other levels. Application of 0.5mls was as effective as 1.0ml of *J. curcas* oil in management wood termite. There was no significant difference that existed between 0.5, 1.0 and 1.5 g of the powder. However, the application of 2.0 g was as effective as 1.5 mls of *J. curcas* oil (Fig 2). Similar trends were observed at 36 and 48 hours of exposure. 2.0 mls of *J. curcas* oil was significantly efficacious compared to 0.5, 1.0, 1.5 mls and also the untreated. Similar result were also obtained in the application of the *J. curcas* powder with 2.0 g recording a better performance compared with other levels of application. There was no

significant difference between 1.5ml and 2.0 g (Fig 3&4). At 60 and 72 hours of exposure, application of 1.0 and 1.5 mls were as effective as applying 2.0mls of *J. curcas* oil. Application of 1.5g of *J. curcas* powder was as effective as 2.0 g at both 60 and 72 hours of exposure and were significantly different from 0.5 g, 1.0 g and the untreated. However, application of 1.5 g and 2.0 g were significantly different from 0.5 ml (Fig. 5 & 6). Generally, the mortality rate of the wood termite increased with increase in both hours of exposure and concentration of bio-pesticide.

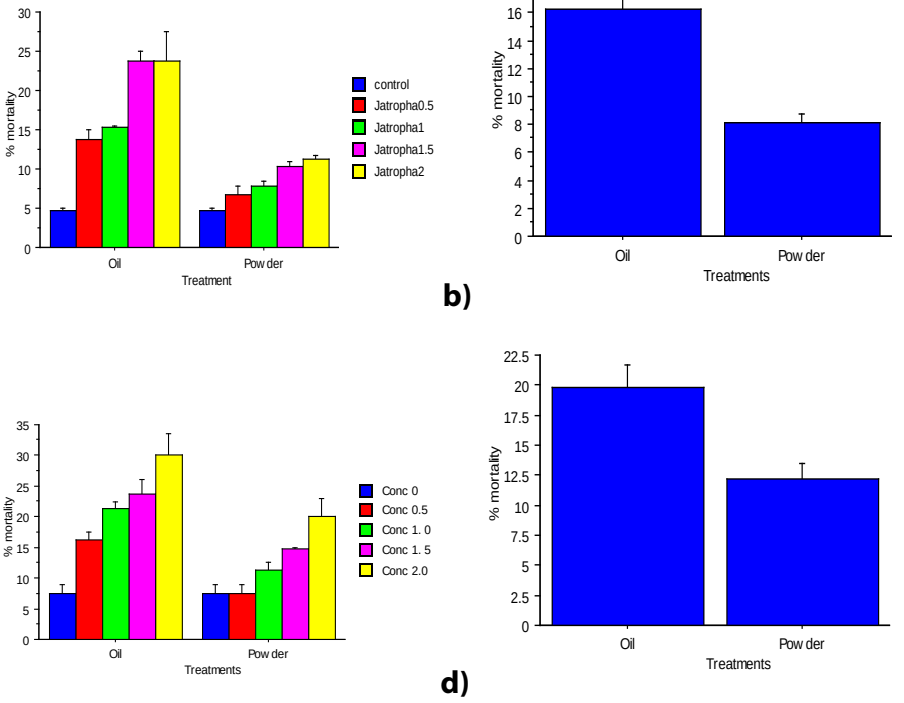
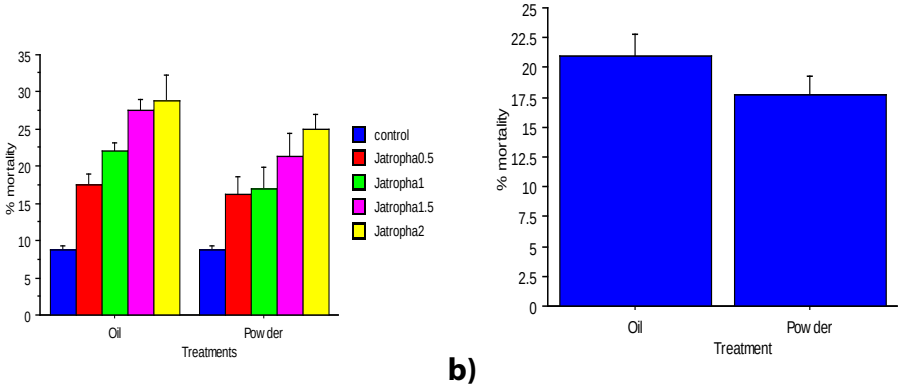


Fig 1: Effect of *J. curcas* oil and powder on percent mortality at 12 hours of exposure; a & b = laboratory result; c & d = field result



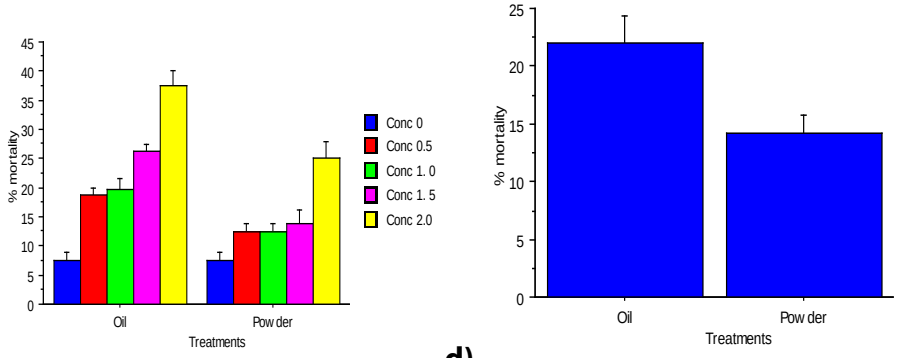
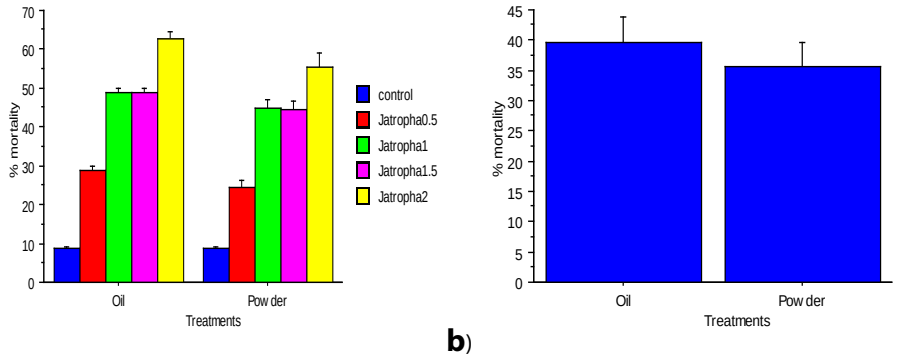


Fig 2: Effect of *J. curcas* oil and powder on percent mortality at 24 hours of exposure, a & b = laboratory result; c & d = field result



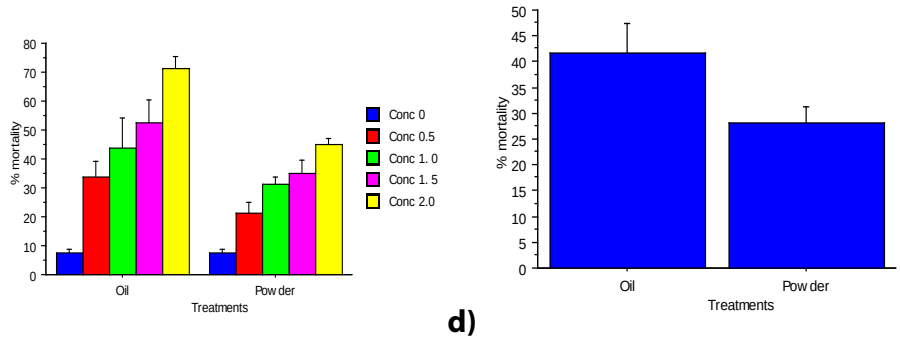


Fig 3: Effect of *J. curcas* oil and powder on percent mortality at 36 hours of exposure; a & b = laboratory result; c & d = field result

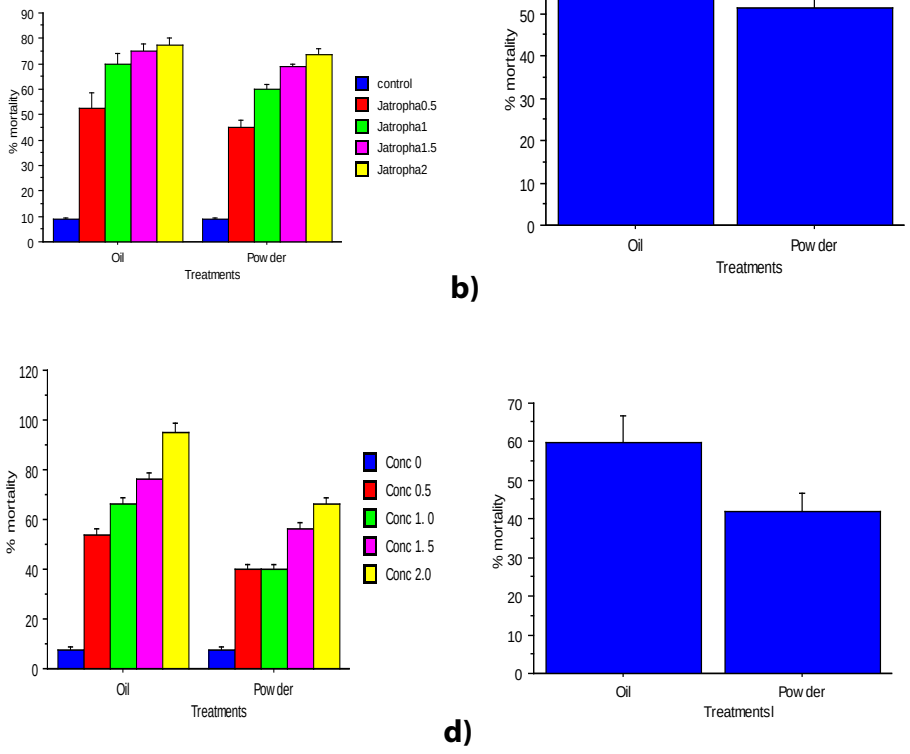
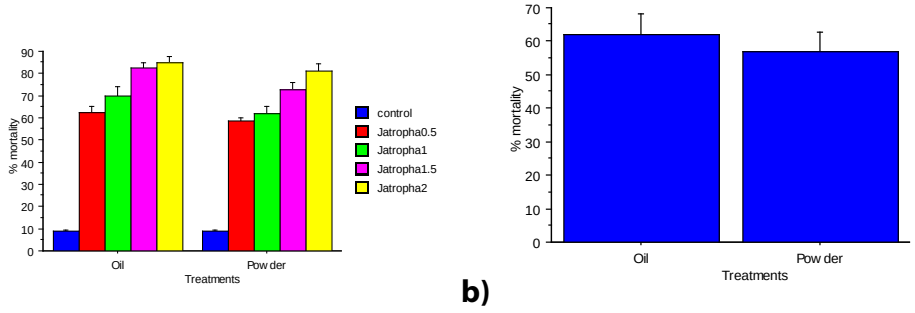


Fig 4: Effect of *J. curcas* oil and powder on percent mortality at 48 hours of exposure, a & b = laboratory result; c & d = field result



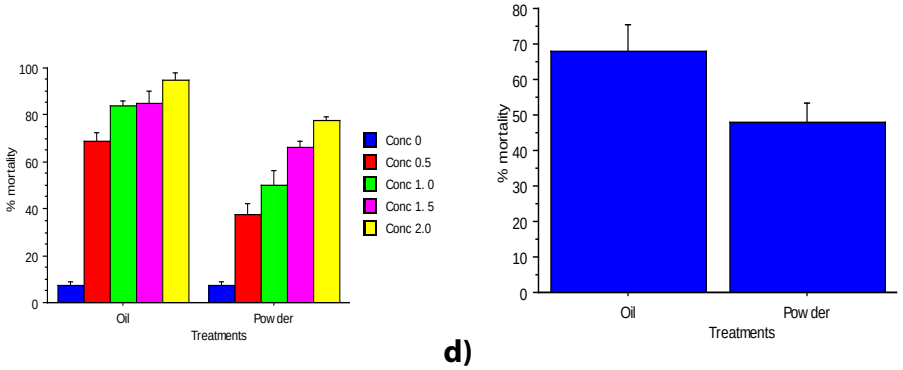
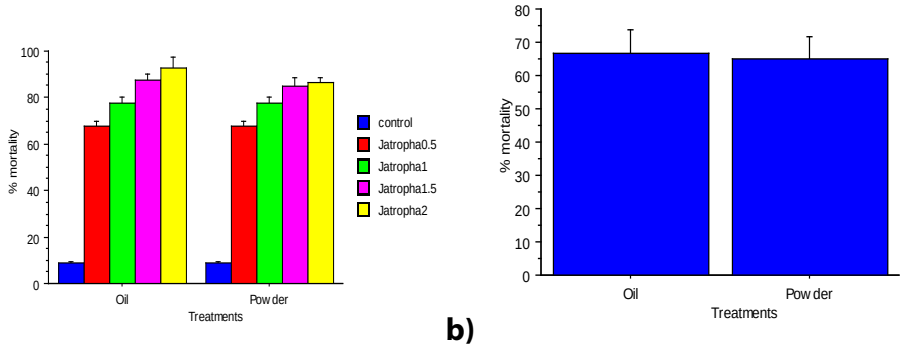


Fig 5: Effect of *J. curcas* oil and powder on percent mortality at 60 hours of exposure, a & b = laboratory result; c & d = field result



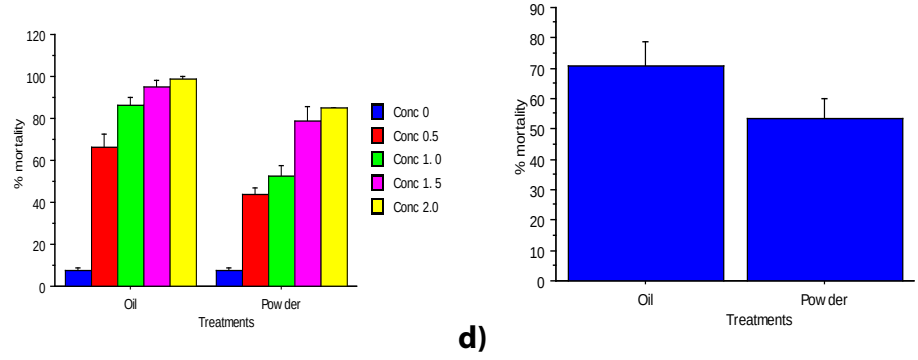


Fig 6: Effect of *J. curcas* oil and powder on percent mortality at 12 hours of exposure, a & b = laboratory result; c & d = field result

Discussion

Jatropha curas has been shown to poses bio-pesticidal properties that works against many pests. Previous works have reported the insecticidal activities of *J. curcas* oil against *Busseola fusca* and *Sesamia calamistis* (Makhar *et al.,* 2007, *Helicoverpa zea* (Olapeju *et al*., 2008), termite (Acda, 2009), mites (Juliet *et al*., 2012), desert locust (Bashir & Shafie, 2013) and *Sitophilus zeamais* (Ojiako *et al.,* 2014). This study demonstrated the toxic effect of *J. curcas* oil and powder in the management of

wood termite. The plant extract generally increased the mortality rate of termite and oil of *J. curcas* was found to be significantly efficacious in the management of wood termite when compared to the powder and there was an increase in the mortality rate of termite in the application of the oil than the powder. This experiment is in accordance with Habou *et al.,* (2011) who reported that *J. curcas* oil was effective against many insect pest associated with cowpea under laboratory and field condition. Also, Adebowale and Adedire, (2006) conducted a similar experiment in the laboratory on *C. maculatus* Fabr devastating insect of cowpea in Nigeria. They observed a significant reduction in egg laying of all tested concentrations and a total inhibition of eggs and larvae. The number of eggs laid by *C. maculatus* females was also reduced due to the application of *J. curcas* oil. Markkar *et al.,* (1998) reported that the *J. curcas* oil contained more phorbol esters which exerted potential insecticidal effect on *Busseola fusca* and *Sesamia calamistis.* A higher mortality rate of (70%) was recorded after 36th hour of exposure. This may be due to the breakdown of protective barriers of the insect and the active ingredient of the plant extract. Plants extract are slow to act and degrades easily in the environment. Earlier research findings therefore recommended their application at higher rates and at an increased frequency to achieve effective pest control (Ewete *et al*, (1996). At 72 hours of exposure, all the levels of *J. curcas* oil were highly effective and 2.0 ml recorded 100% mortality and this is in accordance with (Boateng, 2008) who reported that the susceptibility of *Callosobrunchus maculatus* to the *J. curcas* seed oil was highly toxic at 72 hours of exposure. *Jatropha curcas* oil being more effective than the powder and resulting in higher mortality rate in this research was due to the extraction of the bio-pesticide using ethanol. This is in conformity with the work of (Goel *et al*., 2007) who reported that enhancing the phorbol ester or curcin extract using ethanol indicated significant improvement in insecticidal and molluscicidal properties of the plant. Various methods like heat and chemical had been found to render other toxins in the plant inactive except phorbol esters. This study has revealed that treatment of wood products with bio-pesticide will protect wood from destruction by termite infestation. The bio-pesticide used in this study had a lethal effect on wood termite and has shown to be highly effective towards the management of termites in agronomic and forest crop, as well as domestic materials. *Jatropha curcas* is readily available, biodegradable and has proven to be environmentally friendly. It could serve as a valuable alternative to synthetic insecticide in the management of wood termite.

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The use of essential oils for the control of *Callosobruchus subinnotatus* (Pic) in stored *Vigna subterranea* L.

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Abstract

Studies were conducted in the Crop Science laboratory, University of Calabar to evaluate the insecticidal actions of essential oils (EOs) of *Xylopia aethiopica, Dennetia tripetala, Pysostigma venenosum* and *Senna hirsuta* in the management of *Callosobruchus subinnotatus*. The EOs were extracted using soxhlet apparatus with n-Hexane as the solvent. Four concentrations (0.25%, 0.50%, 1.00% and 2.00 %) and n-Hexane as control were laid out in completely randomized design with three replications. Parameters assessed included repellency, fumigant action, weight loss as well as Lethal concentration (LC_{50}) of the treatments to the beetles at the lowest concentration of 0.25%. The EO of *Senna hirsuta* treated samples generally resulted in significantly ($P > 0.05$) lower weight loss than n-hexane treated samples. LC_{50} computation revealed that *D. tripetala* and *P. venenosum* (LC_{50} 0.22 at 48 hrs) were most efficacious against C. *subinnotatus*. The result supports the use of the test plants by small scale farmers in the protection of stored *V. substerranea* against *C. subinn*

Key words: Insecticidal action, repellency, contact toxicity, fumigant action, LC50, weight loss.

Introduction

There are various estimates of crop losses caused by storage insect pests which range from 10 - 40% in the hot, humid regions of the world (Phillips and Throne, 2010). Losses caused by insects include not only the direct consumption of kernels, but also accumulation of exuviate, webbing and cadavers. High levels of insect detritus may result in grains that are unfit for human consumption and loss of the food commodities, both in terms of quality and quantity (Meikle *et al*., 2002; Ukeh and Mordue, 2009). The major pests of stored grains and pulses of the sub-Saharan African region include those capable of penetrating and infesting intact seeds (grains) and have immature stages developing within the grains and secondary pests which feed on broken kernels, debris, and grains damaged by primary pests. Important among the primary pests are the pulse beetles, *Callosobruchus maculatus* and *C. subbinotatus* (Coleoptera: Bruchidae), the maize weevil, *Sitophilus zeamais* and rice weevil, *S. oryzae* etc. (Rees, 2004). The adults or larvae feed on the grains and eat the albumen or germ or both of them. The attack on the endosperm results in weight loss of the grains, reduction in nutrients and overall deterioration of their quality (Ukeh *et al*., 2010). Synthetic insecticides are often used in controlling insect pest in stored grains (Duke *et al*., 2003). The use of these pesticides is usually regarded as the panacea to pest problems in stored grains in order to feed the alarming human population growth. However, these insecticides pose health hazards to mammals and the environment. Their use is further limited by the lack of technical know-how of farmers, traders and consumers in handling these poisonous insecticides. There is thus, the need to search for alternative methods of pest control in all stages of agricultural production including storage. There is need for the application of less hazardous and safe alternatives that are locally and readily available in nature, cheap and affordable to farmers, simple and convenient to use, specific to the target species and are generally environmentally friendly (Isman, 2006; Umoetok *et al*., 2009). The objectives of the study were to investigate the mode of action (eg. Repellent, contact toxicity and fumigant action) of Guinea Pepper (*Xylopia aethiopica*), Pepper Fruit (*Dennettia tripetala*), Stinking Cassia (*Senna hirsuta*), Calabar Ordeal Bean or "Eseri" Bean (*Physostigma venenosum*) against *Callosobruchus subinnotatus* in *V. substerranea* L.

Materials and methods

Description of the study location

The research was conducted under laboratory conditions in the Department of Crop Science, University of Calabar, Cross River State, Nigeria. The study area is located at geographical coordinate of Latitude 4^0 57^1N of Equator and Longitude $8^0$$19^1$E of Greenwich Meridian, with an altitude of 37m a.s.l (Iloeje, 2001). Calabar is characterized by two distinct moist tropical climates of wet (April-November) and dry (December-March) seasons, respectively. It has an annual rainfall of between 2200 – 3700 mm, average relative humidity of 89%, an annual air temperature range of 26–30 ^{0}C

and lies along the humid coastal region of South Southern Nigeria (Iloeje, 2001).

Collection of plant materials and extraction of essential oils

The plant materials used for the trial were; Guinea Pepper (*Xylopia aethiopica*), Pepper fruit (*Dennettia tripetala*), Calabar bean (*Physostigma venenosum*) and Stinking Cassia (*Senna hirsuta*). The fruits of *Xylopia aethiopica D. tripetala* were obtained from Akpabuyo Local Government Area of Cross River State, while seeds of *S. hirsuta* and *Physostigma venenosum* were collected from fallow lands in Calabar Municipal area in Cross River State. The plant materials were cleaned, air dried under shade for 3 days and preserved in the freezer until they were needed for the various experiments. Fifty grams (50 g) of the dried portion of each plant material was ground and oil extracted with n-Hexane (50nml).

Fifty millimeters (50 ml) of N-Hexane was measured into 500 ml round bottom flask containing the plant powder and extracted using Soxhlet. The plant extract was put into a beaker and evaporated in water bath at 80 ^{0}C. The process was repeated until the required volume of oil needed was obtained.

Insect Culturing of insects

Callosobruchus subinnotatus was cultured on 500 g dry untreated Bambara groundnut seeds which were obtained from local farmers in Yala Local Government Area of Cross River State. The adults of *C. subbinotatus* were obtained from the laboratory stock culture maintained in the Department of Crop Science, University of Calabar, Calabar. About 100 g of Bambara ground seeds were put into kilner jars. Twenty unsexed adult *C. subinnotatus* were introduced into each jar. The covers of the jars were replaced with wire mesh to facilitate air circulation. The insect culture was maintained at room temperature in the laboratory. The adults were allowed to oviposit in the containers for 3 days, after which they were removed. The culture was left for 35 days and the new beetles emerging from each culture jars were sieved out for the experiment.

Repellence bioassays

The repellency test was adopted from the method of McDonald *et al.* (1970) as modified by Talukder and House (1995) and reported by Liu *et al.* (1999). Each plant essential oil was tested for its repellence activity against *C. subinnotatus*. Four concentrations of the four plant essential oils (0.25, 0.50, 1.00 and 2.00%) were obtained and prepared for use in the experiment by diluting each essential oil at 0.05, 0.10, 0.20 and 0.40 ml in 20 ml of n-hexane, respectively. Whatman No. 1 Filter paper (9cm diameter) was cut into two equal parts and placed in the Petri dishes (diameter 8cm) at 2 cm apart. Half part was treated with the plant essential oils and the remaining half treated with the solvent (n-Hexane). Ten (10) unsexed adults each of *C. subinnotatus* were introduced at the center of the Petri dishes, in between the filter papers and the Petri dishes were arranged on the laboratory bench in a Completely Randomised Design (CRD), replicated three times, under a relatively dark environment to minimize the effect of light and the high activity of *C subinnotatus*. The number of beetles on both sides of the filter papers were recorded from each petri dish after 30mins, 1 and 2 hrs treatment application. Based on the number of insects which stay on the treated and untreated sides of the filter, repellency was determined. Percentage repellency was calculated by the equation:

$$\text{Repellency (\%)} = \frac{(C - T)}{(C)} \times 100$$

Where C = No. of insects collected from the untreated filter papers

T = No. of insects collected from the treated filter papers

Fumigant toxicity bioassay

The various concentrations of the plant essential oils were obtained as described earlier. Strips of filter papers were soaked in the different concentrations of the four plant essential oils (i.e. 0.25, 0.50, 1.00 and 2.00% V/V) in beakers. The treated filter paper strips were allowed to dry for 3 minutes and then placed against the wall of a 100 ml flat bottom glass flask. Ten *C. subinnotatus* adults were introduced separately into the treated paper strips in the flask and the bottle sealed with screw caps. Filter papers soaked with n-hexane only served as control. The 18 treatments were laid out in a completely randomized design (CRD) with 3 replications. Mortality was determined at 1.5 hrs. and 3 hrs after treatment. Adults were considered dead if appendages did not move when probed with a Carmel hair brush. Percentage mortality was calculated and probit analysis used to estimate the lethal concentration (LC_{50}) values.

Weight loss bioasay

Hundred grams (100 g) each of maize seeds and Bambara groundnut were weighed out into separate transparent plastic containers. The seeds in each container were treated with 20 ml of each essential oil at different concentrations (0.25, 0.50, 1.00 and 2.00% V/V). The seeds and essential oil were thoroughly mixed. Twenty unsexed 3-day old *C. subinnotatus* were introduced into the admixture of oil and the seeds. Similarly, containers with seeds mixed with 20 ml n-hexane only served as controls. Thus, in each experiment, there were eighteen treatments replicated 3 times to give 54 experimental units laid out in a Completely Randomized Design (CRD). The containers were covered with nylon mesh and their perforated lids screwed in place to ensure confinement of the insects. Data on weight loss were taken cumulatively for 12 weeks. On each occasion, the insects and the produce were separated from the powder emanating from each container due to insect activities, the seeds weighed with a top load electronic weighing balance. Percentage weight loss was obtained by using the formula:

$$\frac{(W_I - W_S)}{(WI)} X\ 100$$

Where W_I = Initial weight of grain before storage
W_S = Weight of grain after storage at a specified time.

Results

Repellency of the four essential oils to *C. subinnotatus*

The repellent effects of the different plant essential oils (Eos) evaluated at different concentrations and exposure time on *C. subinnotatus* are presented in Table 1. Exposure of the beetles for 30 minutes to the different concentrations of oils resulted in an increase in repellency which was dose-dependent. It was observed that, an increase in the concentration of *P. venenosum* and *S. hirsuta* from 0.25% to 0.50% significantly ($p< 0.05$) resulted in higher repellence to the bean beetles. However, when the concentration was increased from 1.00% to 2.00%, *S. hirsuta* caused higher repellence than the other oils. A hundred percent (100%) repellence was obtained with *P. venenosum* at 2.00% concentration. The repellence of the beetles for an exposure period of 1 hour followed a similar trend as when it was exposed for 30 minutes. There were no significant ($p>0.05$) differences in repellency among the plant EOs at the lowest concentration which resulted in increase in percentage repellence with the exception of *P. venenosum*. Increase in EO concentration from 0.25% to 1.00% significantly increased percentage repellence of the bean weevil. *Xylopia aethiopica* EO at 0.50% was as effective in repelling *C. subinnotatus* as other plant essential oils at 1.00%. There were no significant ($p>0.05$) differences in repellence among the different oils when applied at the highest concentration of 2%. No significant ($p>0.05$) difference was also observed when the test insect was exposed to all the essential oils at 2 hours. Percentage mortality of adult *C. subinnotatus* exposed to varying concentrations of different plant essential oils applied as fumigant. The results of the fumigant toxicity of the plant essential oils (EOs) on the mortality of

bean weevil (*C. subinnotatus*) at different exposure period are presented in Table 2. When the bean weevil was exposed to the EOs for 1.5hrs, there was no significant ($p>0.05$) difference in weevil mortality between *X. aethiopica, D. tripetala* and *S. hirsuta* at both 0.25% and 0.50% concentrations respectively. The fumigant test showed that higher concentrations (1.00 and 2.00%) of the plant oils resulted in most cases to no significant ($p>0.05$) percentage mortality of the weevil than at lower levels of 0.25 and 0.50% respectively. No significant ($p>0.05$) mortality was observed when *X. aethiopica* and *D. tripetala* were applied at 0.25% and 0.50% and *D. tripetala* at 0.50% and *S. hirsuta* at 0.25%. Generally, the fumigant toxicity effect increased with increase in the period of exposure of the weevil to the different concentrations of the plant EOs. However, total mortality of the bean weevil was not achieved in any of the plant EOs within the 3.00hrs exposure period. (Table 2).

Variation in LC_{50} values of essential oils by contact toxicity

The LC_{50} values of the different plant essential oils against adults *C. subinnotatus* at different period of exposure by contact toxicity are shown in table 3. The LC_{50} values consistently decreased with increase in the time of exposure to *P. venenosum*. However, for the other plant materials the trend was not consistent from 3 to 6 hrs exposure period but from 12 to 48hrs, there was consistent decrease in the LC_{50} values. At 3 hrs of exposure, the least LC_{50} values was recorded when the bean beetles were tested against *X. aethiopica,* however, at 6 hrs the essential oil of *S. hirsuta* had the least LC_{50} value. At 24 and 48 hrs, *P. venenosum* EO had the least LC_{50} value. Generally, at 48hrs of exposure, the LC_{50} value for all the plant EOs was low with *D. tripetala* and *P. venenosum* having the lowest.

Effect of plant essential oils on weight loss of treated Bambara groundnut by *C. subinnotatus*.

The results on the evaluation of the efficacy of plant essential oils in reducing weight loss in Bambara groundnut is presented in Fig 1. The same trend were observed at 12 weeks after application (WAA) when higher percent weight loss was recorded on the control as against samples treated with essential oils. Generally, there was a reduction in percent weight loss with increase in both concentrations of the EOs.

Table 1: Repellent effects (%) of different plant essential oils (EOs) against *C. maculatus* at different time of exposure

			Time of exposure (Hours)	
Plant essential oils	Conc. (%) 0.5		1.00	2.00
X. aethiopica	0.25	33.33e	22.22e	11.11ed
D. tripetala		49.20e	22.22e	65.74abc
P. venenosum		33.33e	33.33de	73.67bc
S. hirsute		41.27e	22.22e	41.27bcde
X. aethiopica	0.5	73.67bcd	69.05abc	30.16cde
D. tripetala		69.05cd	61.11bc	84.25a
P. venenosum		49.20e	33.33de	49.20abcde
S. hirsute		49.20e	49.20cd	57.14abc
X. aethiopica	1.0	79.63abcd	75.00ab	49.20abcde
D. tripetala		84.25abcd	69.05abc	77.38ab
P. venenosum		88.88abc	69.05abc	55.16abc
S. hirsute		60,05d	69.05abc	55.16abc
X. aethiopica	2.0	96.29ab	92.59a	61.11abc
D. tripetala		96.29ab	87.96a	69.44abc
P. venenosum		100.00a	88.88a	59.79abc
S. hirsute		92.59abc	92.59a	69.05abc

Means within a column followed by the same letters are not significantly different according to Duncan's

Table 2: Percent mortality of adult *C. subinnotatus* exposed to varying concentration of different plant essential oils applied as fumigant

		Time of exposure (Hours)			
Plant essential oils	Conc. (%)	1.5		3.00	
X. aethiopica	0.25	3.33	(6.13 f)	3.33	(6.13 g)
D. tripetala		10.0	(15.00 ef)	16.67	(19.93 fg)
P. venenosum		33.33	(34.93 cde)	56.67	(48.93 cde)
S. hirsuta		13.33	(21.13 def)	20.00	(26.07 efg)
X. aethiopica	0.5	3.33	(6.13 f)	6.67	(12.27 fg)
D. tripetala		63.33	(53.07 abc	86.67	(72.80 abc)
P. venenosum		40.00	(38.87 bcd)	60.00	(51.13 bcde)
S. hirsuta		60.00	(50.87 abc)	36.67	(37.13 def)
X. aethiopica	1.0	40.00	(39.77 bcd)	63.33	(53.33 bcde)
D. tripetala		76.67	(61.73 ab)	93.33	(81.13 ab)
P. venenosum		70.00	(57.00 abc)	86.67	(68.87 abc)
S. hirsute		83.33	(66.13 a)	83.33	(70.07 abc)
X. aethiopica	2.0	66.67	(54.80 abc)	66.67	(60.00 abcd)
D. tripetala		96.67	(60.00 abc)	96.67	(83.87 a)
P. venenosum		90.00	(51.00 abc)	100.00	(90.00 a)
S. hirsute		90.00	(71.60 a)	10.00	(90.00 a)
Control		0.00	(0.00 f)	0.00	(0.00 g)
Hexane		0.00	(0.00 f)	0.00	(0.00 g)

Means within a column followed by the same letters are not significantly different according to Duncan's

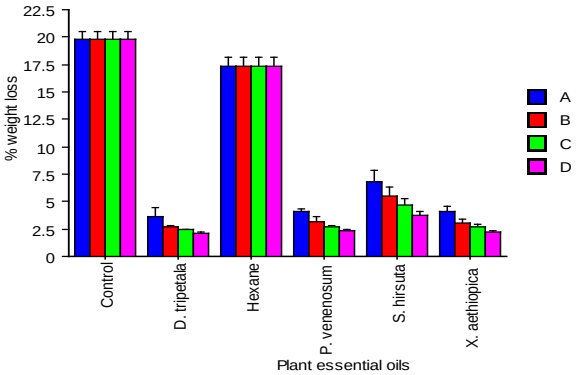

Fig.1: Effect of different plant essential oils on percentage (%) weight loss of Bambara groundnut infested with *C. subinnotatus* at 12 Weeks after application.
Key: A = 0.25%, B = 0.50%, C = 1.00%, and D = 2.00%

Table 3: Percentage variation in LC_{50} values with respect to the duration of exposure of *C. subinnotatus* to the essential oils of different plants during contact toxicity test

	Duration of exposure (hrs)				
Plant materials	3	6	12	24	48
X. aethiopica	1.84	11.76	2.65	1.04	0.50
D. tripetala	2.59	19.32	5.32	0.78	0.22
P. venenosum	18.87	4.32	2.58	0.36	0.22
S. hirsute	2.53	5.17	2.29	1.69	0.37

Discussion

The results obtained from this study showed that the fruits of *X. aethiopica and D. tripetala* and the seeds of *P. venenosum* and *S. hirsuta* had varying levels of insecticidal action against *C. subinnotatus*.

When compared with the control, all the plant essential oils were effective in reducing the population and activities of *C. subinnotatus* under laboratory condition. Their effectiveness was dependent on cconcentration and exposure period except in repellence bioassay where short exposure period resulted in higher repellency than prolonged exposure. This explains why mortality was at highest concentration of 2.00% at 2hrs, for repellency bioassay and 12 wks for weight loss evaluation respectively. Dose related mortalities in similar treatments have been reported by earlier authors (Kieta *et al.,* 2000; Law-Ogbomo, 2007; Ukeh *et al*., 2012). Ogunwenmo *et al*. (2007) reported that plants have phytochemicals that act as chemical defense against other organisms thus, the strong odour produced by the oils of these plants and their chemical composition may have been responsible for the mortalities observed on the insects (Lee *et al.,* 2007; Kouninki *et al*., 2007). The bioactivity of these plant essential oils were due to fumigant action and contact toxicity of the oils to the insects. At higher concentrations, the oils may have blocked the insect spiracles thus, disrupting respiration and resulting in suffocation (asphyxiation) and death as reported earlier by Oparaeke and Kuhiep (2006). The results of the present study confirm reports by Lajide *et al*. (1995), Ejechi and Akpomedaye (2005), Adedire and Akinkurolere (2005), Rayapakse (2006), Kouninki *et al*. (2007) and Asawalem *et al*. (2012) that *Dennitia tripetala*, *Xylopia aethiopica* and other tropical plants species have strong anti-feedant and anti-survival effects on different storage pest including weevils and beetles. Phytochemical screening and isolation of *X. aethiopica* according to Lopez-Martin *et al*. (2002) revealed the presence of alpha-pinene, beta-pinene, 3-carene and terpinene-4-ol, while that of *D. tripetala* revealed the presence of beta-phenylnitroethane, alkaloids, dennettine, three phenanthrine alkaloids (identified as uvariopsine), stephenanthrine, argentinine, phenolics and vanillin. The presence of these anti-oxidant and semio-chemicals must have been responsible for the acute toxicity of the essential oils (EOs) to the bean beetles. These findings on the effect of contact toxicity on *C. subinnotatus* are consistent with other reports on essential oils that exhibited insecticidal activity on stored product pests (Hall and Harman, 1991; Adedire *et al*., 2011). The results of percentage mortality of adult *C. subinnotatus* exposed to varying concentrations of plant EOs applied as fumigant revealed that, all the plant essential oils resulted in significantly ($P<0.05$) higher percentage mortality than the control treatment with hexane. The EOs of *D. tripetala* and *S. hirsuta* at 0.50% concentration were as effective as the highest concentration (1.00 and 2.00%) of *X. aethiopica*. These agree with other researchers on the use of plant essential oils as fumigants in the control of stored product pests (Kieta *et al.,* 2000; Law-Ogbomo, 2007; Adedire *et al*., 2011; Ukeh *et al*., 2012). The results of this study showed a very good potential for the use of the four plant essential oils as fumigants. Repellents in the form of essential oils, powders or distillates have the potential for the exclusion of stored-product pests from grains and they have been used to prevent insects from feeding and oviposition (Asawalem *et al*. 2012; Ukeh *et al.,* 2012). The presence of certain chemical compounds in the essential oils which altered the behaviour of *C. subinnotatus* as a result of the effect of the oils on the olfactory sensilla of the insect's antennae, Several workers including Javid and Poswal (1995), Talukdar and Howse (1995), Tapondjou *et al*., (2002) and Ukeh *et al.,*(2009) had reported that n-hexane or ethanol extract of *D. tripetala* could individually result in 40.1–100% repellence when used in protecting stored dry fish or grains from beetles. All the plant essential oils tested in this study were highly repellent to the bean beetles. Omar *et al.* (2007) reported feeding deterrence of *Cosmopolites sordidus* due to *D. tripetala* extract while anti-feedant effect was reported by Lajide *et al.* (1995). On weight loss, results obtained from the experiment showed that there was a significant ($P<0.05$) increase in weight loss in the untreated (control) and hexane treated samples irrespective of exposure time.. Kieta *et al*. (2003), Tripathi *et al*. (2002) and Singh and Yadav (2003) reported that various oils used as seed treatments against storage pests are effective in reducing damage (weight loss). The different effects between the plant oils used in the present study may be due to the type of plants and the composition of their different active ingredients, but all the tested plant oils were effective in reducing weight loss of stored Bambara groundnut.

The results of the LC_{50} values of different plant essential oils indicated that with prolonged exposure time, the potency of the EOs are increased. Also, it is apparent that the efficacy of the essential oils

varied among the plant materials. The active ingredients in these plant materials and the physiological mechanism of interference in the insects may possibly have accounted for this variations. From the LC_{50} values obtained, fumigation of the grains with the plant essential oils should be considered compared with spraying or just rubbing with the oils. It is possible that the volatile active components of the oils could easily permeate and penetrate the grains vis-a-avis the bean beetle, than when it is rubbed or sprayed. It was observed that the efficacy of the botanicals were dose-dependent with higher doses resulting in higher mortalities of the *C. subinnotatus*. Graphs of percentage mortality versus log of concentrations were constructed and the LC_{50} was computed for each essential oil. Results of the LC_{50} revealed that *D. tripetala* and *P. venenosum* (LC_{50} 0.22 at 48hrs) were the most efficacious against *C. subinnotatus*. Photochemical screening from literature revealed the presence of several active compounds in the essential oils of these plants and may probably be responsible for the bio-insecticidal properties of these oils and the observed mortalities.

Conclusion

Results obtained from this research revealed that the essential oils *X. aethiopica, D. tripetala, P. venenosum* and *S. hirsuta* were toxic and effective in controlling Bambara groundnut beetle, *C. subinnotatus*. They could therefore be incorporated into the integrated pest management practices by the local farmers to reduce damage caused by insect pests.

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Influence of Abiotic Factors on the Efficacy of Insect Growth Regulators Against *Trogoderma Granarium* (Everts)(Coleoptera: Dermestidae)

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ABSTRACT

Present study was designed to investigate the effects of different combinations of three temperatures (20, 25 and 30°C) and three relative humidity levels (55, 65 and 75%) on the efficacy of three synthetic IGRs i.e., pyriproxyfen, lufenuron and buprofezin at concentrations of 1, 5 and 10ppm on fecundity and adult emergence inhibition of *T. granarium* under controlled laboratory conditions. This study was conducted at Grain Research Training and Storage management Cell, Department of Entomology, University of Agriculture, Faisalabad, Pakistan. All the treatments were replicated three times using Completely Randomized Design. Larvae of *T. granarium*were exposed to IGRs at different levels of temperature and relative humidity. F_1 adult emergence results showed that at temperature 20°C, the highest percent reduction in adult emergence (84.38, 70.65 and 79.94%) was recorded after exposure to lufenuron, buprofezin and pyriproxyfen treated diet, respectively. At 75% relative humidity, lufenuron, buprofezin and pyriproxyfen caused 77.53, 80.00 and 80.32% reduction in adult emergence, respectively. Adults were exposed to IGRs at different temperature and relative humidity to evaluate the oviposition inhibition. The results revealed that at temperature 20°C, maximum percent reduction in fecundity (87.95, 80.45 and 70.55%) was recorded after exposure to buprofezin, pyriproxyfen and lufenuron treated diet, respectively. At 75% relative humidity buprofezin, pyriproxyfen and lufenuron caused 86.73, 83.72 and 69.11% reduction in fecundity, respectively. It is concluded that temperature and relative humidity play an important role in the effectiveness of insect growth regulators.

Key words: Temperature, Relative Humidity, *Trogoderma granarium,* Insect Growth Regulators, Efficacy

Introduction

About 9-20% post-harvest losses by stored grain pests had been reported in the developed and developing countries (Phillips and Thorne, 2010). Insect pests cause alterations in the chemical structure of the products by destroying the quality and quantity of food commodities. Among the insect pests of stored cereals, the Khapra beetle *Trogoderma granarium* (Everts) (Dermestidae: Coleoptera) is a serious pest of stored grains and their products (Burges, 2008; Ali *et al*., 2012). In case of severe infestation, quality and quantity of grains are reduced by feeding and contamination with shed skin. Hairs of larvae may adversely affect human health (Hosseininaveh *et al*., 2007; Ahmedani *et al*., 2009).

Excessive use of conventional synthetic pesticides (Organophosphates, Pyrethroids) to protect stored cereals has resulted in the development of insecticide resistant strains, handling hazards, insecticide residues on food, threat to human health and serious environmental issues (Bell, 2000; Benhalima *et al*., 2004; Desneux *et al*., 2007). There is the need to replace synthetic chemical insecticides with safe grain protectants (Silver, 1994). Insect Growth Regulators (IGRs) are one of the best alternatives to conventional synthetic pesticides that are highly effective against pests of stored grain commodities because they have low mammalian toxicity, little environmental and health hazard effects (Kostyukovsky *et al*., 2000; Mondal and Parveen, 2001; Ishaaya *et al*., 2007). IGRs affect metamorphosis and molting by simulating juvenile hormone (JH, juvenile hormone agonists) or interfering JH activity (ecdysteroid agonists) or by disturbing the cuticle formation (chitin synthesis inhibitors) (Oberlander *et al.,* 1997). In contrast to traditional insecticides, IGRs are less toxic to higher animals. They inhibit the chitin synthesis of insects by causing abnormal endocuticular deposition and absorptive molting (Post and Vincent, 1973). IGRs are used to manage a wide range of insect species by interfering with their process of growth and development (Yu, 2008).

Lufenuron (CSI) is a new synthetic insect growth regulator. It is highly effective for controlling lepidopteron and coleopteron larvae on maize, cotton, vegetables, rust mites and citrus whitefly on citrus fruits. Buprofezin has become successful IGR to manage insect pests in various countries.The reproduction ability of adult females is reduced by feeding on buprofezin treated diet. (Uchida *et al*., 1985; Izawa *et al*., 1985; Konno, 1990). Pyriproxyfen is an IGR that strives for juvenile hormone binding position, juvenile hormone mimics and thus retaining an immature stage (Sullivan and Goh 2008). It is a safer compound for non-target organisms and used for management of public health pests (Miyamoto *et al*. 1993). Adult emergence and embryogenesis suppression are also ascribed to pyriproxyfen (Ishaaya and Horowitz 1995).

Toxicity of an insecticide is affected by several factors including temperature, insect species, insecticide type and nature of the food on which insect develops (Kljajic and Peric, 2007; Liang *et al*., 2007). Integration of temperature with other control measures is a modern pest management strategy for stored grain insect pests (Dowdy, 1999). Similarly, temperature and relative humidity play a significant role in the efficacy of spinetoram, which becomes less effective at higher dose (Vassilakos and Athanassiou, 2013).

Keeping in view the above mentioned facts, the study sought to determine the effect of insect growth regulators on percent reduction in fecundity and adult emergence of *Trogoderma granarium*; the impact of relative humidity and temperature on the effectiveness of IGRs against test insects and the influence of relative humidity and temperature on dose and response.

Materials and Methods

Insect Rearing

Mixed population of *Trogoderma granarium* was collected from grain market and godowns of Faisalabad district, Punjab Food Department of Pakistan. Under laboratory conditions in the Stored Grain Management Cell (SGMC), department of Entomology, University of Agriculture, Faisalabad,*T.*

granarium was reared on whole wheat grains in an incubator (SANYO-MIR-254) at 30±2°C and 65±5% relative humidity according to the procedure used by Ali *et al*., 2012. Briefly the grains (200g) were sterilized at 70°C for 15 minutes in an oven and then put in separate glass jars (250g capacity). Fifty adults of mixed sex were released into the jars. The mouth of the jars was tightly covered with muslin cloth using rubber band to prevent the escape of adult beetles. After three days the parent beetles were sieved out from culture. The wheat grains having freshly laid eggs were put into separate glass jars of 250g capacity and kept in cooled incubator (SANYO-MIR-254) at optimum growth conditions 30±2°C and 65±5% relative humidity to get homogenous population. Five days old grubs were used in further series of experiments.

Insect Growth Regulators (IGRs)

Locally available three synthetic insect growth regulators, (1) pyriproxyfen (Peradigm®) 10.8EC, (2) lufenuron (Lufenuron®) 5EC and (3) buprofezin (Buprofezin®) 25WP were obtained from FMC United (Pvt) Limited and used in the bioassays at the concentrations of 1, 5 and 10 ppm.

Grain treatment with IGRs

Untreated whole hard wheat (*Triticum aestivum* L.) with moisture contents 10 %, (as determined by Dickey John moisture meter) was used in the tests. Lots of 1.5 kg of grains were equally sprayed with IGRs at concentrations of 1, 5 and 10 ppm using volume at the rate of 100 ml of formulated spray per kg (150 ml of formulated spray per 1.5 kg of wheat grains). Additionally 1.5 kg lots of grains were sprayed with distilled water and used as the control treatment. After treatment application, the jars containing IGR treated diet were allowed to dry at room temperature for 30 minutes in order to evaporate the solvent.

Effect of IGRs on adult emergence of *T. granarium*at different temperatures and relative humidity levels

Five-days old larvae of *T. granarium* were placed into each plastic vial of 50ml capacity, with separate vials for the three IGRs. Different combinations of three temperatures regimes (20, 25 and 30℃) and three relative humidity levels (55, 65 and 75%) were maintained to evaluate the efficacy of pyriproxyfen, lufenuron and buprofezin, at concentrations of 1, 5 and 10 ppm on the inhibition of adult emergence of *T. granarium.*The vials were placed in separate incubators(SANYO-MIR-254) with saturatedsalt solutions at the bottom in order to maintain the relative humidity at the desirable level. After 42 days, adult emergence was observed for *T. granarium*. Percent reduction in adult emergence was calculated using the following formula (Sagheer *et al*., 2012).

Percent reduction in adult emergence $= 100 \times (1\text{-}t/c)$

Where

t = Number of adults in treated diet

c = Number of adults in control

Effect of IGRs on fecundity of*T. granarium*at different temperatures and relative humidity levels

Three plastic cylindrical vials (3 cm in diameter, 8 cm in height) were used as replicates. Each vial was filled with 20 g of treated grain and 20 adults of *T. granarium* were placed in each vial. The vials were placed in separate incubators (SANYO-MIR-254) with saturatedsalt solutions at the bottom in order to maintain three temperatures (20, 25 and 30℃) and three relative humidity levels (55, 65 and 75 %). The relative humidity in the plastic containers was continuouslymonitored by digital Hygrometer. Fecundity (the number of eggs laid) of exposed beetles was assessed after 4 days. It was calculated using the formula (Sagheer *et al*., 2012).

Percent reduction in fecundity $= 100 \times (1\text{-}t/c)$

Where

t = Number of eggs in treated diet

c = Number of eggs in control

Statistical analysis

Data were subjected to statistical software Statistix 8.1 for analysis of variance. The means of significant treatment were compared using Tukey's Honestly Significant Difference (HSD) test at 5% level of significance.

Results

A significant variation in the inhibition of adult emergenceof *T. granarium* was observed at different temperature regimes (F=3.26; P<0.05), relative humidity levels (F=14.63; P<0.001) and concentrations (F=20.01; P<0.001) after buprofezin treatment. The inhibition of adult emergence varied with different temperature regimes (F=8.35; P<0.001), relative humidity levels (F=6.81; P<0.05) and concentrations (F=21.08; P<0.001) after exposure to pyriproxyfen treated diet. Similarly, temperature (F=16.82; P<0.001), relative humidity (F=21.23; P<0.001) and lufenuron concentrations (F=14.16; P<0.001) caused significant variations in the reduction in adult emergence of *T.granarium*.

At temperature 20ºC, the maximum percent reduction in adult emergence, (70.65, 79.94 and 84.38%) was observed after exposure to buprofezin, pyriproxyfen and lufenuron treated diets, respectively (Table 1). At 75% relative humidity, the highest inhibition of adult emergence (80.00, 80.32 and 77.53%) was recorded on exposure of buprofezin, pyriproxyfen and lufenuron, respectively (Table 1). Maximum reduction in adult emergence (81.46, 86.39 and 82.45%) was observed at 10ppm concentration of buprofezin, pyriproxyfen and lufenuron, respectively (Figure-1).

Tab. 1 Impact of temperature and relative humidity on effectiveness of insect growth regulators against percent reduction in adult emergence of *Trogoderma granarium*

IGRs	Temperature (°C)			Relative Humidity (%)		
	20	25	30	55	65	75
		Mean±S.E			Mean±S.E	
Buprofezin	70.65±2.04a	54.75±1.09b	63.15±2.55 ab	46.31±2.50c	62.24±1.91b	80.00±2.23a
Pyriproxyfen	79.94±1.57a	72.60±2.48ab	62.57±1.30 b	64.88±3.65b	69.91±2.36b	80.32±1.39a
Lufenuron	84.38±2.23a	56.01±1.66b	54.42±2.50 b	43.16±2.99b	74.12±3.92a	77.53±3.90a

Percent reduction in adult emergence is calculated by formula=100×(1-t/c), where "t" is the number of adults in treated diet, and "c" is the number of adults in control treatment.

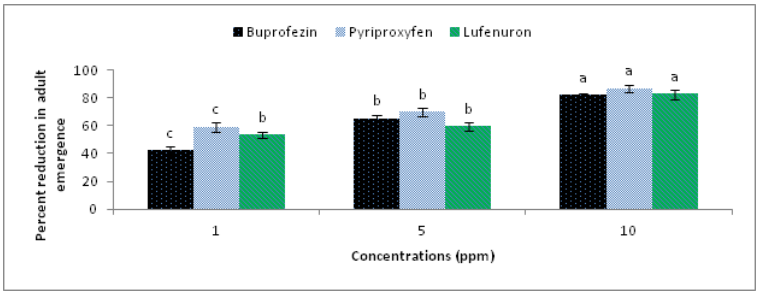

Fig. 1 Impact of various concentrations of insect growth regulators against percent reduction in adult emergence of *Trogoderma granarium*

The interaction effect of temperature and relative humidity caused maximum reduction in adult emergence (86.77%) of *T. granarium* at 25ºC temperature and 75% relative humidity after buprofezin treatment (Table 2); while highest adult emergence inhibition (90.74%) was recorded in

both pyriproxyfen and lufenuron treated diet at 30ºC temperature and 75% relative humidity (Table 2).

Tab. 2 Interaction effect of temperature and relative humidity on activity of Insect Growth Regulators against percent reduction in adult emergence of *Trogoderma granarium*

IGRs	Temperature (ºC)	Relative Humidity (%) 55 Mean±S.E	65 Mean±S.E	75 Mean±S.E
Buprofezin	20	64.26±3.33 ab	67.02±1.76 ab	80.66±2.21 ab
	25	27.19±2.35 c	50.28±2.51 bc	86.77±1.22 a
	30	47.48±2.18 bc	69.43±3.43 ab	72.55±3.01 ab
Pyriproxyfen	20	31.33±3.56 b	35.07±2.38 b	76.07±3.96 a
	25	63.92±1.85 ab	77.72±2.43 a	80.55±2.32 a
	30	68.81±2.57 a	70.51±3.01 a	90.74±3.89 a
Lufenuron	20	27.11±1.84 b	26.29±2.82 b	76.07±1.96 a
	25	63.88±1.84 a	77.94±3.46 a	80.55±3.32 a
	30	68.51±2.48 a	73.33±2.95 a	90.74±1.89 a

A significant variation in oviposition inhibition of *T. granarium* was observed at different temperature regimes (F=6.01; P<0.05), relative humidity levels (F=8.49; P<0.001) and concentrations (F=12.06; P<0.001) of buprofezin treated diet. Reduction in fecundity varied with different temperature regimes (F=3.72; P<0.05), relative humidity levels (F=16.57; P<0.001) and concentrations (F=12.06; P<0.001) of pyriproxyfen treated diet. Temperature (F =15.94; P<0.001), relative humidity (F=11.53; P<0.001) and lufenuron concentrations (F=6.38; P<0.001) caused significant variations in fecundity reduction of *T.granarium*.

At temperature 20ºC, the maximum percent reduction in fecundity (87.95, 80.45 and 70.55%) was observed after exposure to buprofezin, pyriproxyfen and lufenuron treated diet, respectively (Table 3). At 75% relative humidity, the highest inhibition in oviposition (86.73, 83.72 and 69.11%) was recorded on exposure of buprofezin, pyriproxyfen and lufenuron, respectively (Table 3). The maximum reduction in fecundity (74.78, 84.43 and 72.85%) was observed at 10ppm concentration of buprofezin, pyriproxyfen and lufenuron, respectively (Figure 2).

Table 3. Impact of temperature and relative humidity on effectiveness of insect growth regulators against percent reduction in fecundity of *Trogoderma granarium*

IGRs	Temperature (ºC) 20	25 Mean±S.E	30	Relative Humidity (%) 55	65 Mean±S.E	75
Buprofezin	87.95±1.24a	83.30±1.02b	86.61±1.42ab	86.29±1.22a	84.87±1.46a	86.73±1.00a
Pyriproxyfen	80.45±2.10a	75.65±2.34ab	72.66±3.49b	67.32±3.65b	77.72±1.52a	83.72±1.54a
Lufenuron	70.55±0.43a	54.40±3.35b	67.27±2.86a	55.75±2.01b	67.37±1.29a	69.11±1.44a

Percent reduction in fecundity is calculated by formula=100×(1-t/c), where "t" is the number of eggs in treated diet, and "c" is the number of eggs in control treatment.

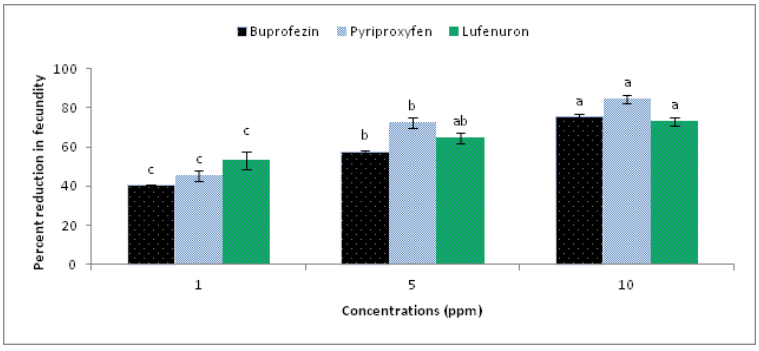

Fig. 2 Impact of various concentrations of insect growth regulators against percent reduction in fecundity of *Trogoderma granarium*

The interaction effect of temperature and relative humidity caused maximum reduction in fecundity (91.92%) of *T. granarium* at 30°C temperature and 55% relative humidity after buprofezin treatment (Table 4); while at 20°C temperature and 55% relative humidity highest oviposition inhibition (71.66 and 70.88%) was observed in case of pyriproxyfen and lufenuron treated diet, respectively (Table 4).

Table 4. Interaction effect of temperature and relative humidity on activity of Insect Growth Regulators against percent reduction in fecundity of *Trogoderma granarium*

IGRs	Temperature (°C)	Relative Humidity (%) 55 Mean±S.E	65 Mean±S.E	75 Mean±S.E
Buprofezin	20	84.68±2.41 abc	88.61±2.11 ab	90.5±1.41 a
	25	82.26±1.07 bc	79.86±1.08 c	87.8±1.90 ab
	30	91.92±1.01 a	86.15±3.12 abc	81.8±1.41 bc
Pyriproxyfen	20	71.66±0.68 a	70.22±0.75 a	70.55±1.02 a
	25	32.11±3.15 b	64.72±2.15 a	68.61±2.53 a
	30	68.66±3.49 a	67.16±3.02 a	68.16±3.51 a
Lufenuron	20	70.88±0.38 a	70.52±0.75 a	70.55±1.02 a
	25	29.88±2.75 b	64.72±2.15 a	67.61±2.53 a
	30	66.50±2.62 a	64.16±3.02 a	68.16±3.51 a

Discussion

In this series of experiments,the larvae and adults of *T. granarium* were exposed to different concentration of IGRs treated diet at various levels of temperature and relative humidity. IGRs significantly prolonged the larval duration of *T. granarium*at different temperature and relative humidity levels. At 20°C temperature and 75% relative humidity the highest reduction in adult emergence was observed. Subsequent pupal development and adult emergence was completely prohibited. These results are similar to the findings of Sagheer *et al*., (2012). It has been reported that IGRs reduced body weight of insects (Smagghe *et al*., 1996; Parveen, 2000). Meola *et al*. (1999) reported that due to lufenuron treatment in fleas, larval hatching was prevented by raptures in the cuticle, which opened during eclosion resulting in the loss of hemolymph and desiccation of the larva.

It has been found that short term exposure to different levels of temperature had positive effects on toxicity of insect growth regulators. The lowest mortality was recorded at higher level of temperature due to decomposition of active ingredients of insecticides. These results confirm the findings on impact of high temperatures on efficacy of hydroprene applied to control *T. castaneum* (Arthur and Dowdy, 2003). Among the abiotic factors, temperature, grain moisture contents and gas compositions play a vital role in insect growth and development (Hagstrum and Milliken, 1988; Muir, 2000). The interaction of temperature and relative humidity has been studied extensively with often inconsistent results (Arthur, 1999; Fields and Korunic, 2000; Fang and Subramanyam, 2003).

All the three insect growth regulators showed reduction in fecundity of *T. granarium*. It was observed that different levels of temperature and relative humidity showed a significant variation in effectiveness of IGRs against egg laying capacity of *T. granarium*.Significant variations in response of insect were found between temperatures at different levels of relative humidity. At 20°C temperature insect growth regulators were highly effective in percent reduction in fecundity of *T. granarium*compared to other levels of temperature, while at 75% relative humidity the highest reduction in fecundity was observed. These results confirm the findings ofthe effect of temperature and relative humidity on the efficacy of spinetoram for the control of three stored product beetle species (Vassilakos and Athanassiou, 2013).

When the adults of *T. granarium* were released to oviposit on untreated and treated diet; fecundity was reduced significantly on treated diet compared to control treatments. These results showed

resemblance to transovarial activity of CSIs that caused reduction in fecundity in treated diets. Similar results that the adults of insects reared on treated diet lay fewer eggs compared to untreated adults have been reported by several workers (McGregor and Kramer, 1976; Nickle, 1979; Saxena and Mathur, 1981; Elek, 1998a; Parveen *et al*., 2001). It has been reported that insect growth regulators affect the embryogenesis partially or fully (Mian and mulla, 1982).

Furthermore, in this study IGRs did not kill the adults of *T. granarium* but induced suppression in egg laying capacity of treated insects compared to untreated insects. These results are similar with other findings (Carter, 1975; Faragalla *et al*., 1985; Ammar, 1988; Elek and Longstaff, 1994; Kostyukovsky and Trostanetsky, 2006). It has been found that chitin synthesis inhibitors showed a strong insecticidal activity by foliar application against Colorado potato beetle and reduced oviposition (Cutler *et al*., 2005).

Our study reveals that temperature and relative humidity have significant effecton the efficacy of the three insect growth regulators tested. Maximum control of stored grain insect pests was observed at lower temperature (20ºC) and higher relative humidity (75%), because at high temperature insecticides start to degrade. Overall control of stored grain insect pests depends on biological and physical factors such as insect species, temperature, relative humidity, dose rate and time period for which insects were exposed to insecticides. In addition, some other factors that may affect the effectiveness of insecticides are grain type, grain moisture contents and methods of insecticide application.

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Efficacy of pheromones for managing of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, in food and feed processing facilities

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Abstract

In recent years, considerable progress has been made in the monitoring and control of Lepidoptera, by pheromones also used in mass-trapping, attracticide (lure and kill), mating-disruption, auto-confusion methods. In context of IPM "insectistasis" can be readily achieved by continual supervision of environments by traps in combination with a limited number of preventive and curative measures appropriately timed. In the present paper are reported some promising results offering efficient control of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, populations in food and feed processing facilities based on pheromones and line up a number of remaining questions to be answered to improve the reliability and competitiveness of the methods used. These field researches show potential for successful pheromone-based suppression methods for Mediterranean flour moths in practical applications.

Keywords: Mediterranean flour moth, *Ephestia kuehniella*, pheromones, monitoring, mass-trapping, attracticide method, mating-disruption.

1. Introduction

Pheromones and other semiochemicals have been identified for more than 40 species of stored-product insects over the past four decades. In recent years, considerable progress has been made not only in monitoring but also in direct control of stored-product insects by different techniques (Burkholder, 1990; Chambers, 1990; Phillips, 1997; Trematerra, 2002 and 2012; Phillips *et al*., 2000; Cox, 2004; Anderbrant *et al*., 2007; Campos and Phillips, 2010 and 2014; Savoldelli and Trematerra, 2011; Plarre, 2013; Athanassiou *et al.,* 2016; Trematerra *et al*., 2017).

In the present paper are reported the main results obtained in the control of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, populations by means of mass-trapping, attracticide (lure-and-kill), mating-disruption, and auto-confusion methods applied in food and feed processing facilities.

2. Mass-trapping method

As it is know any attempt to suppress the population by mass-trapping would require a sufficient number of trapped males so that nearly all females would be unmated. Theoretical considerations of mass-trapping males take into account the density of males in the population and the potential number of matings that a male is able to secure in its lifetime. If a male can mate with 6-10 females in a lifetime, as is the case of the Indian meal moth, *Plodia interpunctella* (Hübner), then up to 90% of the male population can be trapped without affecting the number of mated females as well as the subsequent larval generation (Brower, 1975).

Early attempts of mass-trapping were conducted by using pheromone blends of many target insects species. A major problem was the quantification of the number of traps necessary per unit area to achieve an effective control. Proper experiments of mass-trapping are not easy to conduct due to inadequate controls or poor replication, especially in commercial food/feed storage and processing facilities.

In practice the effectiveness of the mass-trapping technique can be reduced by factors such as: inefficient trap design, saturation of traps especially in situations of high pest density, poor pheromone release or duration, attraction of only one sex, inappropriate positioning of traps and the extensive immigration of new pests from outside the area treated with pheromones (Trematerra and Gentile, 2010).

Food lures used in combination with pheromones may offer a way of enhancing the effectiveness of mass-trapping system for stored product pests trapping males and females of a target species (Chambers, 1990; Toth et al., 2002; Cox, 2004; Trematerra, 2012).

Recent studies have investigated the potential of pheromone based mass-trapping methods to control indoor populations of *E. kuehniella* (Trematerra, 1990; Süss *et al*., 1996; Trematerra and Battaini, 1987; Athanassiou *et al*., 2003; Anderbrant *et al*., 2007; Trematerra and Gentile, 2010). In

particular, Trematerra and Battaini (1987) demonstrated that integrated control of *E. kuehniella* could be achieved by mass-trapping. Furthermore, Trematerra (1990) reported results obtained by the practical application of mass-trapping to control an infestation of *E. kuehniella* in a flour-mill.

Subsequently Trematerra and Gentile (2010) presented the 5 years results of applying the mass-trapping method to contain *E. kuehniella* populations infesting a large traditional flour-mill in Central Italy. The study also investigated the effectiveness of mass-trapping, combined with other pest control techniques, at improving the procedures applied to combat *E. kuehniella* infestations using an IPM approach. Over 5 years pheromone funnel-traps baited with 2 mg of (*Z,E*)-9,12-tetradecadienyl acetate (TDA) attracted a total of 54,170 males. The constant presence of the traps caused a marked decrease of *E. kuehniella* populations. The results of the study have shown that the population density of the moth can be effectively reduced and maintained at a low level by means of mass-trapping techniques accompanied by localized insecticide treatments, and careful cleaning of various mill areas and equipment (Figure 1).

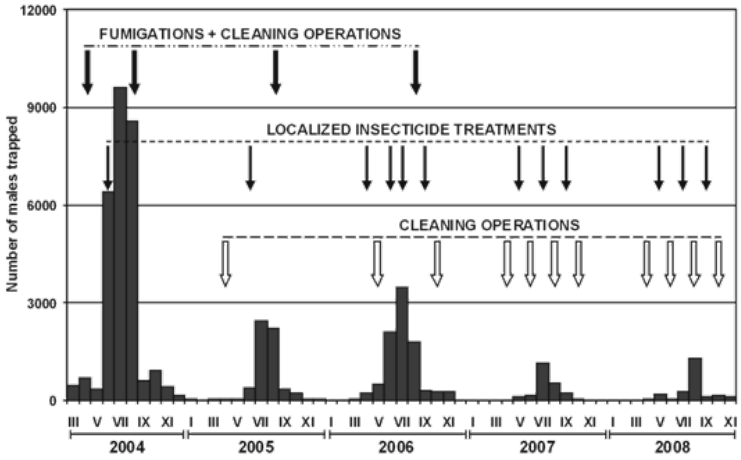


Fig. 1 Mass-trapping, cumulated montly trap catches of *Ephestia kuehniella* males inside a flour-mill.

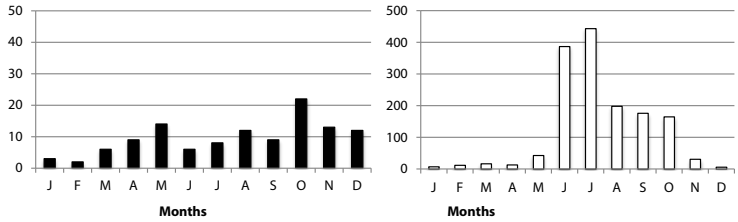
3. Attracticide (lure-and-kill) method

Attracticide (lure-and kill) method is in some ways analogous to mass-trapping, although many more insects are affected because the attracticide is broadcast over a large area and the killing effect is not limited to individual traps. There are various promising results on the use of the attracticide concept in flour-mills and confectionary industries in the control of *Ephestia cautella* (Walker) and *E. kuehniella* in Italy (Trematerra and Capizzi, 1991; Trematerra, 1995). Preliminary results on attracticide for *Plodia interpunctella* (Hb.) also have been reported more recently in the United States (Nansen and Phillips, 2002 and 2004; Campos and Phillips, 2010, 2013 and 2014).

In Italian flour-mills, *E. kuehniella* males were successfully lured to laminar dispensers (2 cm x 2 cm), baited with 2 mg of TDA (daily release of 13 µg) and treated with 5 mg of cypermethrin; this caused a marked decrease in moth population. Trematerra and Capizzi (1991) performed behavioural tests involving olfactometer, electroantennogram, and insecticide efficacy evaluations in order to clearly determine the effectiveness of pheromone and toxicant in the attracticide method. In field tests in a practical application was conducted to determine the degree of control of *E. kuehniella*. In the olfactometer tests 80 to 90% of males responded to pheromone plus insecticide dispensers, confirming the low repellency of cypermethrin in sexual behaviour. In this context the possible interaction between optical and pheromone stimuli was also studied.

Later encouranging results were obtained by Trematerra (1995) using attracticide applications in

flour-mills placed at 220 to 280 m^3 intervals. This experiment was undertaken in Central Italy in a large mill of 16,000 m^3 that produced flour and semolina. During 2 years of application the attracticide method removed males from *E. kuehniella* populations preventing an increase in the residual population. The prolonged presence of the treated dispensers in the four-mill, particularly during periods when the moths were able to breed, led to a reduction throughout the flour-mill, including areas where no processing occured. After the two years of using the attracticide method, the usual second fumigation of mill proved to be unnecessary. The continuous presence of attracticide dispensers in the mill caused a marked decrease in the *E. kuehniella* population also during the third year.



Figs 2-3 Dynamic population of *Ephestia kuehniella* males in a traditional flour-mill protected by attracticide method (2) and in untreated traditional flour-mill (3).

4. Mating-disruption methods

The mating-disruption technique generally requires use of much larger quantities of pheromones than those employed for mass-trapping and attracticide method. Therefore, the mating-disruption may be practical if the pheromones are inexpensive. In addition, there may be concerns about possible contamination of the stored products in situations where high concentrations of pheromone come in direct contact with the product. Therefore, the application of mating-disruption requires the simultaneous development of a reliable system to monitor if the method is effective for the intended time period. The response of females in the presence of high concentrations of pheromones must be evaluated. The low level of matings and the pheromonal substance present inside the treated area could induce females to leave such areas in favour of outdoor areas and could also stimulate dispersal (Trematerra, 1994; Shani and Clearwater, 2001; Trematerra *et al*., 2013). Also, it has been suggested that insects may evade the effect of mating-disruption by progressive elevation of pheromone production and response threshold or through a change in pheromone composition over consecutive generations to compete with the background pheromone. Studies carried out in field indicate that there is a significant correlation between some of these factors and the spatial distribution of several Lepidoptera in food-processing facilities (Nansen *et al*., 2003; Trematerra and Sciarretta, 2005; Trematerra and Gentile, 2010; Athanassiou *et al*., 2016).

Another disadvantage of mating-disruption is that the method does not prevent mated female immigration from adjacent areas, thus oviposition and subsequent infestation are likely to still occur (Cardè and Minks, 1995; Jones, 1998). Hence, monitoring of female activity and/or oviposition is essential when developing a mating-disruption-based control program (Savoldelli and Trematerra, 2011).

The component pheromone (*Z,E*)-9,12-tetradecadienyl acetate attracts males of several Pyralid moths, thus, this 'multi-species pheromone' has been used successfully for mating-disruption in stored-product facilities. Particularly the use of mating-disruption against pyralid moths, in stored-product facilities has been evaluated, with promising results, in both laboratory and field tests. Several studies from many parts of the world have shown more or less similar results for *E. cautella*, *E. kuehniella*, and *P. interpunctella* (Phillips, 1997; Trematerra, 2002; Plarre, 1998; Ryne *et al*., 2001, 2006 and 2007; Anderbrant *et al*., 2007 and 2009; Sieminska *et al*., 2009; Mueller, 2010; Trematerra *et al*., 2011; Campos and Phillips, 2014).

However, there are consistent methodological problems with evaluating mating-disruption in practice, such as defining what a replicate is and estimation of control based on trap captures (Anderbrant *et al.*, 2009; Sieminska *et al.*, 2009). Ryne *et al.* (2007) compared two adjacent storage rooms, one that was treated with mating-disruption and one that was not, and found using electrophysiological recordings (male antennal response) that there was leakage of pheromone into the untreated room. Mating-disruption-based experiments usually use a single or low number of treatment and control rooms. Each food processing and storage facility is unique that makes finding a 'control facility' which is similar with the treated facility extremely difficult (Sieminska *et al.*, 2009). As a result, there is still inadequate information on mating-disruption effectiveness under different microclimates and in different types of facilities.

Sieminska *et al.* (2009) present results from long-term monitoring of *E. kuehniella* populations in two similar flour mills in Poland. One mill was treated with pheromone for mating-disruption for two years, whereas the other mill was untreated. Thirty pheromone dispensers (one per 100 m^3 factory volume), each releasing about 2 mg TDA per day, were used. The reduction in trap catch during the mating-disruption treatment was about 90% or more, compared with the untreated mill or pre-treatment periods in the mill where mating-disruption was practiced. The reduction was larger during the second year of mating-disruption than during the first year. One of the basic drawbacks of mating-disruption method is that oviposition by mated females that enter areas under treatment from untreated areas can still occur (Jones, 1998; Athanassiou *et al.*, 2003; Campbell and Arbogast, 2004; Trematerra and Gentile, 2010). Consequently, the number of captured males in monitoring pheromone-baited traps may not be a clear indicator of mating-disruption.

To avoid these methodological issues, Trematerra *et al.* (2011) conducted a two-year, large-scale experiment that included eight facilities located in Czech Republic, Greece and Italy. The facilities were flour-mills, food and drug storage rooms, and warehouses storing organic foods, pasta, raisins, or wheat. Dispensers of cellulose pad, each with 50 mg of TDA were placed at a rate of one dispenser per 9 m^2 (or one dispenser per 54 m^3). Based on the results reported in some storage facilities and trap-check dates, the suppression of captures in the mating-disruption-treated areas was <95% in comparison with untreated areas, suggesting that some mating may have occurred.

Generally, there is no clear indication that the moth species made a difference in mating-disruption program effectiveness, so Trematerra *et al.* (2011) proposed that the mating-disruption method had the same efficacy level for *E. cautella*, *E. kuehniella* and *P. interpunctella*. The use of a single pheromone component [(*Z,E*)-9,12-tetradecadienyl acetate] to accomplish simultaneous suppression of more than one pest species is an additional advantage for using mating-disruption in storage facilities (Anderbrant *et al.*, 2009).

In large-scale experiments with mating-disruption dispensers, the 'untreated' areas may not serve accurately as 'controls' because of the potential air permeation from the treated. Also mating-disruption may have a cumulative effect after multiple years of implementation. Historical data from previous years, concerning both adult captures and larval presence for the target facilities, may serve more accurately as 'controls' because it can also reflect seasonal patterns in activity.

Oviposition and/or immature emergence should be monitored, in conjunction with adult activity in pheromone-baited traps, to indicate if successful mating-disruption is occurring. In this regard the pheromone effect on population growth or decrease could be measured by the presence of spermatophores in females (Trematerra and Savoldelli, 2013). Also in this case, one of the most important factors impacting the efficacy of mating-disruption is the population density.

Three years of field trials (from 2007 to 2009) were carried out in Central Italy by Trematerra and Spina (2013) to evaluate MD of the Mediterranean flour moth, dispensers containing the pheromone TDA were placed in two traditional flour mills. Pheromone-baited funnel traps were used to monitor the population fluctuations of moth males throughout the entire experimental period; female oviposition was assessed by placement of petri cups containing wheat germ–semolina flour bait. According to the results, the use of MD dispensers does not interfere completely

with the reproduction of *E. kuehniella*. However, looking at the overall data, there was a significant reduction in both adults and larvae in treated mills after the MD application. According to hazard analysis and critical control point procedures (HACCP), treatment should be accompanied by general cleaning of the facilities, including corners and inside machinery, where insects can hide and reproduce.

In integrated pest management programs, the use of MD can lead to a drastic reduction in the need for chemical treatments, with improvement in food quality.

5. Auto-confusion

A particular method of mating disruption is auto-confusion, Baxter *et al.* (2008) and Hugget *et al.* (2010) reported preliminary laboratory studies to examine behavioural effects of auto-confusion on virgin male *P. interpunctella*. The method used TDA, combined with a patented electrostatic powder delivery system to disrupt mating and interrupt the lifecycle of several moth pests. Laboratory flight tunnel studies showed that contact with SP-Tab auto-confusion significantly reduced the ability of male *P. interpunctella* to locate females for up to two days.

These males could increase the confusion effect by becoming competitive attractive point sources for other males (Huggett *et al.*, 2010). Using auto-confusion Pease and Storm (2010) presented preliminary practical trials that were conducted in two flour-mills in UK and in a spice factory in Netherlands. Populations of *E. kuehniella* and *P. interpunctella* were monitored. In all cases populations were reduced compared to the same area in the previous year and compared to untreated control areas in accordance with local pest control practices.

Preliminary results of the SP-Tab auto-confusion system for mating-disruption of *P. interpunctella* in 2008 and 2010 was reported from United States by Campos and Phillips (2014).

Trematerra *et al.* (2013) applied Exosex SPTab dispensers that contained the Entostat powder, at a 5x5 m grid, in three facilities, one feed-mill in Italy and two retail stores in Greece. In the feed-mill, the most abundant pyralid species was *Ephestia kuehniella*. Monitoring through pheromone-baited traps in this facility indicated that the application of the Exosex SPTab dispensers decreased the number of captures 2 months after the initial application. In the case of the facilities in Greece, the most abundant species was *Plodia interpunctella*. In these facilities there was a continuous monitoring of moth populations from January 2008 until February 2011, with pheromone-baited traps and Petri dishes with semolina, which served as oviposition traps. In both facilities, the presence of *E. kuehniella* and of *P. interpunctella* males in the pheromone-baited traps was reduced after the placement of the Exosex SPTab dispensers, in comparison to captures for the same interval from the previous years. At the same time, the number of emerging individuals in the oviposition traps was notably reduced after the Exosex SPTab dispensers placement, in comparison to the previous monitoring interval. Our study documents that the auto-confusion system is an effective and reliable technique that can be used with success against stored-product Pyralidae, to retail stores and feed-mills (Figure 5).

6. Future prospects

As previously reported, there are consistent methodological problems in assessing the efficacy of mass-trapping, attracticide, mating-disruption and auto-confusion methods in practice. Each food storage and processing facility is unique and therefore finding a comparable 'control facility' is difficult when pheromone-based control methods are deployed. As a consequence, interpreting effectiveness of pheromone-based control measures in various facilities, at different insect densities, and microclimates becomes difficult. The available data indicate that many factors influence both male and female behaviour.

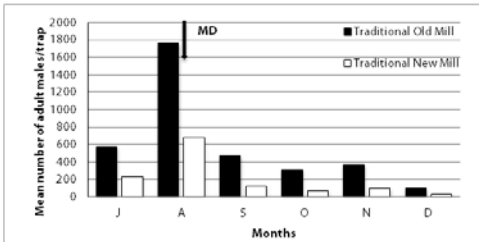


Fig. 4 Mating-disruption: Italy, traditional old and traditional new mill, 2008: mean number of *E. kuehniella* adults/trap caught in the monitoring period.

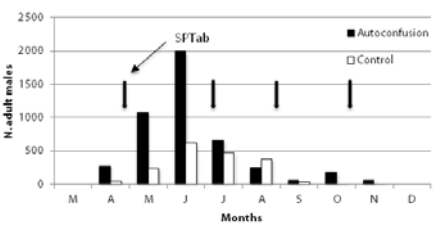


Fig. 5 Dynamic population of *Ephestia kuehniella* males in feed-mill protected with auto-confusion method.

The relative importance of these factors varies among species and among populations of the same species, undoubtedly reflecting the different ecological conditions to which they are normally subjected (Ryne *et al*., 2007; Trematerra *et al*., 2011 and 2012; Campos and Phillips, 2014).

In stored-product moth control, the pheromone efficacy was evaluated using the following parameters: male capture in pheromone traps, oviposition and larval emergence from eggs, incidence and frequency of mating as measured by spermatophores in females. The number of captured males or the absence of males in pheromone traps may not be a clear indicator of mating suppression and female oviposition. Oviposition and/or immature emergence should be monitored, in conjunction with adult activity. One of the most important factors, impacting the efficacy of pheromone control is the population density. Historical data from previous years, concerning both adult captures and larval presence in the target facilities, may serve as internal 'control' because such informations shows seasonal patterns of insect activity. The necessity of better controls and adequate replications need to be emphasized. In this regard the pheromone effect on population growth or decrease could be measured by the presence of spermatophores in females' *bursa copulatrix*, which is a good indicator of mating activity (Trematerra and Savoldelli, 2013). The female dissection to count spermatophores, as an estimate of mating reduction, is a more direct method than reduced trap catch or reduced oviposition in diet cups and should probably be used more.

In stored-product protection the increased use of pheromones will help reduce the number of chemical treatments with consequent economic and qualitative advantages. Pheromone-based methods need to be considered as a part of an overall IPM program in food systems (Trematerra, 2013; Trematerra *et al*., 2017). In the future, more efficient formulations of pheromones and other semiochemicals are needed, coupled with research under real-world conditions, for effective management of stored-product insects (Trematerra and Fleurat-Lessard, 2015).

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Influence of low doses of gamma irradiation on cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae)

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Abstract

Phytosanitary irradiation for food commodities has been widely accepted in recent years. Gamma irradiation has been used as a phytosanitary treatment against microbial diseases, insect infestation and food spoilage. The goal of the current study was to determine the lowest possible dose of gamma irradiation that will induce long-term sterility of insects through generations. The effect of four gamma irradiation doses examined were; 20,40, 50 and 70 Gy. Irradiated males were crossed with normal females. For the cowpea beetle *Callosobruchus maculatus*(F.), adult fecundity, hatchability, adult emergence, sterility% was investigated. 100% adult mortality was achieved by 70 Gy dose. Fecundity, hatchability, number of adults emerged, sterility% were significantly reduced when males exposed to 20, 40, and 50 Gy compared to the control. The effect of parental irradiated males exposed to 20 Gy on F2 generation was also studied. Fecundity, hatchability, number of adult emerged, sterility% were significantly reduced in F2 compared to F1 and control progeny. Interestingly, for F1 generation, the effect of gamma rays on adult emergence% exhibit a hermetic effect response although it was not significant. These results demonstrat that pulse irradiation relying on low-doses of gamma radiation induce inherited semi-sterility through generations and is a very promising phytosanitary food technology for post-harvest treatments.

Keywords: inherited sterility, gamma radiation, low-dose effect, sterile male technique, sterile insect technique, hormesis.

Introduction

Inherited sterility (IS) has evovled as an alternative to the standard sterile insect technique(SIT) (IAEA, Ibrahim *et al.*2017). Male insects irradiated with sub-sterilizing doses of gamma or x-ray radiation and mated with virgin females resulted in F1 generation with more sterility than their parents. Previous sutdies have been shown that IS has a long-term impact on pest control programs against many insects such ad moths and mosquitoes (JANG *et al.*, 2012 and Shetty *et al.*,2016).

On the other hand, food irradiation has been widely used as a phytosanitary treatment and insect disinfestation methods. It is an environmentally friendly safe treatment and it has been used against many pests as an alternative to chemical control (Ibrahim, *et al.*, 2017, Ihsanullah and Rashid, 2017).

The original data for this paper has been previously published (Ibrahim, *et al.*, 2017). Here, the effect of low doses gamma radiation on the cowpea beetle *Callosobrocus maculatus* and the inherited effect on F1 and F2 generations were examined. The first objective was to determine the lowest gamma radiation dose to be used for insect disinfestation. The second one was studying the long-term effect of low doses gamma radiation on IS through generations.

This work will hopefully increase the effectiveness of using SIT and other releasing biological control programs by induced IS across different generations.

This work also expands on the importance of the potential for radiation hormesis effect that could appear as a result of using low-doses in food irradiation.

Materials and Methods

Insect rearing

The insect used was cowpea beetle, *C. maculatus*. The insect culture was maintained on cowpea seeds (*Vigna unguiculata* (L.) Walpers) in Entomology Department, Ain Shams University for many years. . Insects were maintained at 27 ± 2oC and relative humidity of 60 ± 5%.

Preliminary test

It has been reported that the highest dose of gamma radiation permitting reproduction was 50 Gy and the sterilizing dose was 70 Gy (Hasan and Khan 1998). Therefore, five doses of gamma radiation (0,20 ,40, 50, 70 Gy) were selected to determine the lowest dose that permitting reproduction and induce IS for both F1and F2 generations. No. of eggs, no. of hatched larvae, no. of adult emerged, adult emergence% were determined for F1 offsprings

From the insect rearing jar and prior to adult emergence, each 1-egg-seed was transferred to an Eppendorf tube to prevent any mating prior the experiment.

Irradiation technique

Newly emerged adult males (1-day old) were irradiated with one of five doses of (0,20 ,40, 50, 70 Gy) using ^{60}Co Indian gamma cell (Gy 4000 A), located at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The dose rate was 0.713 Rad/s.

Biological assay

For biological assay a 20 Gy dose was selected because it was the lowest dose that caused IS (Ibrahim, 2017) and at the same time produce sufficient individuals to study long-term effect in F1 and F2 progeny. After exposure to radiation, the newly emerged adult males were mated with virgin normal females in 9 mm Petri dishes with cowpea seeds. Each replicate has two males and two females. Five replicates were made for each treatment, 20 Gy and the control one. Insects were also maintained at 27 ± 2oC and relative humidity of 60 ± 5%. No. of eggs, no. of hatched larvae, no. of adult emerged, adult emergence%, sex ratio, and sterility% were recorded per each treatment for both F1 and F2 resulted form irradiated male parents.

The percentage sterility was calculated according to Chamberlain's formula (1962):

% sterility=100 -((a ×b)/(A ×B) ×100)
Where:
a = number of eggs per female in the treatment.
b = Percentage of hatched eggs in the treatment.
A = number of eggs per female in control
B = Percentage of hatched eggs in control.

Statistical analysis

For preliminary test, adult emergence % data were analysied using non-paramitric tests. Otherwise, the results were analyzed with ANOVA and followed by posthoc analysis using LSD-test with the help of SPSS program. The level of significance used was $p< 0.05$.

Results

Preliminary test

There was an overall significant effect between treatments for both number of eggs and number of hatched larvae ($P< 0.05$, Fig. 1). All doses significantly reduced the number of eggs and the number of hatched larvae compared to the control group (Fig. 1). There was also an overall significant effect between different doses for number of adult emerged ($P< 0.05$, Fig. 2). On the other hand, results of adult emergence% were not significantly different from each other ($P= 0.06$, Fig. 2). Interestingly, adult emergence% for those exposed to 20, 40, and 50 Gy seemed to be better than that of the control group although this effect was not significant ($P= 0.06$, Fig. 2).

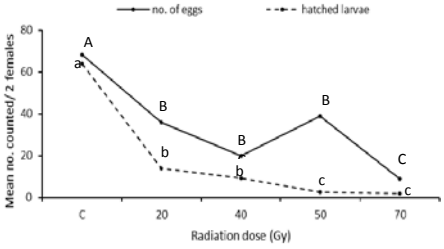

Fig. 1 Number of eggs and number of hatched larvae after crossing irradiated males of C. maculatus with normal females at 0, 20, 40 and 50 and 70 gamma radiation doses. Different letters indicate significant differences, P< 0.05. Capital letters for no. of eggs laid and small letters for no. of hatched larvae.

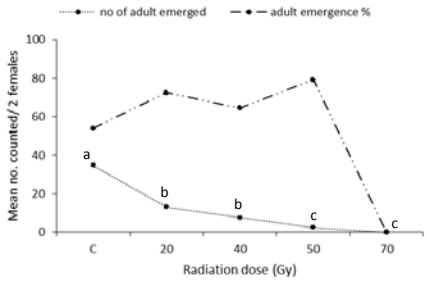

Fig. 2 Number of adult emmerged and Adult emergence % after crossing irradiated males of C. maculatus with normal females at 0, 20, 40 and 50 and 70 gamma radiation doses. Different letters indicate significant differences between no. of adult emerged among doses, P< 0.05.

Biological assay

There was an overall significant effect between treatments for number of eggs, number of hatched larvae, hatchability %, no. of adult emerged and adult emergence % (For all, $P< 0.05$, Table 1).

Tab. 1 Biological aspects of the F1 and F2 progenies resulted from irradiated parental males *C. maculatus* with 20 Gy dose of gamma radiation and crossed to normal females. Different letters indicate significant differences among different treatments, $P < 0.05$.

Conc.	No. of eggs	No. of Hatched larvae	Hatchability %	No. of adult emerged	Adult emergence %
0 GY	68.25±1.89 a	64.00±1.87 a	93.77±0.8 a	34.75±2.1 a	54.14±1.76 a
20 Gy F1	35.80±11.2 b	13.80±4.75 b	40.93±9.2 b	13.25±4.9 b	72.54±9.46 b
20 Gy F2	5.60±2.16 c	5.00±2.35 c	68.91±16.1 c	2.50±0.05 c	22.92±14.0 c

The male sex ratios were38 ± 1.00%, 42 ± 3.00%, and 17 ± 17% in the control, F1, and F2 progenies, respectively with no significant difference ($P = 0.162$). The adult percentage sterility was significant between the control (0.00 ± 0.00) and the individuals of F1 (70.87 ± 8.70) and F2 (88.31 ± 7.01), ($P = 0.000$). There was a significant difference in percentage sterility between the F1 and F2 generations ($P = 0.049$)

Discussion

Preliminary test

The present data shows that doses of 20, 40, and 50 Gy gamma radiation significantly reduce the number. of *C. maculatus* progeny compared to the control group. A dose of 70 Gy completely inhibits *C. maculatus* production. These data in agree with previous work that showed that complete sterility is achived by 70 Gy and the highest dose that permits *C. maculatus* reproduction is 50 Gy (Hasan and Khan 1998).

Data illustrating *C. maculatus* adult emergence %, demonstrate that low-doses of 20, 40, and 50 Gy seems to have a hermetic dose effect. In dose-response relationships, the hermitic effect or hormesis is a biphasic effect. Some doses of certain stimuli, whether it chemicals or radiation or even any stressors have a positive (stimulatory) effect on an organism at low doses compared to normal individuals (the control groupwhere high doses have an inhibitory effect and concurs with Baldwin and Grantham, (2015).

Biological assay

A low-dose of 20 Gy induces IS and has a long-term effect on both F1 and F2 progenies for *C. maculatus*. The same effect of low- doses of gamma or X-rays radiation has been reported for other insects (JANG *et al*., 2012 and Shetty *et al*.,2016).

Conclusion

This paper showed that irradiated male C. maculatus with low-doses of gamma radiation induces inhirited sterility through F1 and F2 progenies. This is a very promising new technique for insect control and could be an alternative to SIT and other chemical control programs. At the same time, attention should be directed to the potential of the hormesis effect that could exist. Otherwise, low-doses induces IS might have very dramatic results instead.

Finally, hormesis effect should be investgated more in relation to different stimuli such as insecticides, radiation, physical and even environmental stressors.

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Radio Frequency Heat Treatment for Controlling Cigarette Beetle in Dried Tobacco

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Abstract

Tobacco (*Nicotiana tabacum* L.) is one of many agricultural commodities produced in Thailand. There are Virginia (flue-cured tobacco) and Burley (air-cured tobacco) typesand Cigarette beetle, *Lasioderma sericorne* F. is the most important insect pest that attacks dried tobacco. The efficacy of radio frequency (RF) heat treatment at 27.12 MHz was examined to control cigarette beetle on dried tobacco. Various growth stages of cigarette beetle were prepared within samples of dried tobacco and were exposed to RF at 55, 60, and 65 °C for 1, 2 and 3 minutes. The results showed that pupal and adult stages of cigarette beetle were the most tolerant stages to RF heat treatments. The RF treatment at 65 °C for 3 minutes is able to cause complete mortality of egg, larval, pupal and adult stages of cigarette beetle4.

Keywords: dried tobacco, *Lasioderma serricorne,* tolerant stage, heat treatment

Introduction

Virginia and Burley tobacco production in Thailand is found in 10 provinces mostly in the north and northeast of Thailand covering about 21,000 ha and is valued at approximately 2 billion US dollars in 2016-2017 cigarette sales. In Virginia and Burley tobacco storage, cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) is the most damaging insect pests of dried tobacco and grain products such as corn, beans and dried herbs in Thailand. Insecticides have been used as the main control measure for managing stored tobacco pests. To avoid chemical contamination in the commodity other control measures such as storage sanitation and pest exclusion to remove sources of infestation should be considered. Several studies have been performed using radio frequency (RF) energy to control stored product insects. Mitcham et al. (2004) tested RF energy to control Codling moth (*Cydia pomonella),* navel orangeworm (*Amyelois transitella*, and Indianmeal moth (*Plodia interpunctella),* at 55°C for 5 minutes and was able to gain complete control.. Lagunas-Solar et al. (2007) also reported that using RF at, 10 kHz to 1050 MHz resulted in >99% mortality of Anguomois grain moth (*Sitotroga cerealella*).

The use of radio frequency, as a heat treatment, has been investigated to control many kinds of insect pest associated with agricultural products (Nelson, 1996). Experiments have been conducted to control many stored product insects to determine the tolerant stages of insects to the RF heat (Table 1.). For heat treatment with radio frequency, commodities are allowed to increase temperature rapidly due to the vibrationof water molecules. Nutapong (2012) tested the efficacy of RF to kill cigarette beetle on packaged dried tobacco which was infested with cigarette beetles in all stages. The results showed that the adult stage was the most tolerance to RF heat treatment at higher temperatures (104 °C) for complete control Radio frequency also has less effect on various kinds of grains compared with conventional heat (Srikam et al., 2014; Wangsapa et al. 2016; Zhou

et al. 2015). There is very little research work of RF treatment on dried tobacco. Therefore, this experiment is aimed to examine the reduction of temperatures that causes mortality of cigarette beetle with RF to save energy costs and to reduce the effect on commodity quality.

Table 1. The exposure time of radio frequency heat on insect pests associated with various agricultural commodities which resulted for 100% morality of tolerant stages. (The Pilot scale of 27.12 MHz radio frequency was provided.)

Commodities	Insect pests	Tolerance stages	Temp (°C)	Time exposure	References
Corn	*Sitophilus zeamais*	Adult	92	4 min	Faikrajaipuen et al. (2011)
Feed	*Tribolium casteneum*	Pupa	70	1 min	Bualoi (2009)
Milled rice	*Corcyra cephalonica*[*]	Egg	60	3 min	Luechai (2008)
Milled rice	*Orhyzaephilus surinamensis*	Adult	70	2 min	Srikam et al. (2014)
Milled rice	*Rhyzopertha dominica*	N/A	70	3 min	Janhang (2005)
Milled rice	*Rhyzopertha dominica*	Adult	70	2 min 30 sec	Sumetha et al. (2009)
Mung bean	*Callosobruchus maculatus*[*]	Egg, larva, pupa	74	3 min 40 sec	Na Pijit et al. (2011)
Rough rice	*Sitotroga cerealella*[*]	Egg, pupa	50	3 min 40 sec	Buapud et al.(2012)
Rough rice	*Sitophilus oryzae*	Adult, pupa	65	2 min	Wangsapa et al.(2015)
Tobacco	*Lasioderma sericorne*	Adult	104	4 min	Nutapong (2012)

[*] Adult was not in the test
N/A =not available

Materials and Methods

Insect rearing and efficacy test of RF treatment to control cigarette beetle

Cigarette beetles were cultured with green leaf tobacco or dried tobacco in flue cured Virginia tobacco (bright tobacco) at 28-32°C with 75% rh in saturated salt solution box in Stored Product Laboratory, at Department of Entomology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand. Cigarette beetle eggs at 2-4 days old while larvae, pupae, and adults were 20-25, 30-32, 35-40 days old respectively after oviposition were placed on Virginia tobacco at approximately 12% mc. Fifty insects were put in dried tobacco in small plastic cups and tempered to 75% rh to acclimate insects The cup was put in an arena test chamber that is a cylinder of 16 cm in diameter with 5 cm height and was used to be exposed to RF heat t. Pilot scale radio frequency at 27.12 MHz was used to treat all stages of cigarette beetle, at temperatures of 55, 60, 65 °C and for 0, 1,2, and 3 minutes (9 combination treatments). The pilot scale of radio-frequency unit was developed and built from the Institute of Agricultural Engineering, University of Göttingen, Germany.

The effect of RF to cause insect mortality was examined after 42, 22, 12, and 3 days on egg, larval, pupal and adult stages, respectively. The number of survivalors was calculated for the insect mortality by deduction from the total. In the untreated control, insects were not treated with RF but maintained in tobacco leaves as were those exposed to treatments. Each treatment was replicated 4 times with 50 insects per replication. Insect mortality in untreated controls was corrected using Abbott formula (Abbott, 1925). All combinations of treatments between temperatures and time exposure were calculatedusing analysis of variance and LSD test.

Results

Eggs of cigarette beetle showed 79.8% mortality at the treatment using 55°C for 1 minute. The experiment showed complete mortality using RF treatments at 65°C for 1, 2 and 3 minutes. Although in each experimental unit was run individually the longer exposure times also showed

serially increasing mortality. The mortality of eggs exposed at 65°C in 1, 2 and 3 minutes showed significantly differences from others at 55 and 60°C (Table 2.). Both temperatures alone and time exposure alone caused significantly difference of insect mortality.

Table 2. Mortality of eggs of cigarette beetle exposed to different temperatures from radio frequency at 27.12 MHz for 1,2 and 3 minutes.

Temperature from RF treatment (°C)	Average mortality of eggs			Total[2/]
	1 minute[1/]	2 minutes	3 minutes	
55	79.79 ± 2.76 c	86.33 ± 3.33 bc	90.65 ± 3.13 b	85.59± 3.16 Y
60	80.00 ± 3.87 c	88.41 ± 2.69 b	93.04 ± 1.97 b	87.15± 3.82 Y
65	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 X
Total[3/]	86.60 ± 6.70 B	91.58 ± 4.25 A	94.56 ± 2.80 A	

[1/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=6.73).
[2/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD = 3.88).
[3/]Means in the same row followed by the same letter are not significantly different at P≥0.05 (LSD = 3.89).

At 1 minute exposure time of 55°C- RF heat treatment, the mortality of cigarette beetle found 41.27 % with increasing of exposure time. This heat treatment caused 100 % mortality on larvae for either of 60°C for 3 minutes or 65°C for 2 minutes. There were both showed significant difference exposure time and temperature (Table 3).

Table 3. Mortality of larvae of cigarette beetle exposed to various temperatures from radio frequency at 27.12 MHz for 1, 2 and 3 minutes.

Temperature from RF treatment (°C)	Average mortality of larvae			Total[2/]
	1 minute[1/]	2 minutes	3 minutes	
55	41.27 ± 3.62 e[1/]	46.89 ± 1.95 de	52.29 ± 3.66 cd	46.82 ± 3.18 Z[2/]
60	56.81 ± 2.17 c	93.62 ± 0.95 b	100.00 ± 0.00 a	83.48 ± 13.46 Y
65	90.28 ± 0.78 b	100.00 ± 0.00 a	100.00 ± 0.00 a	96.76 ± 3.24 X
Total[3/]	62.79 ± 14.46 C	80.17 ± 16.74 B	84.10 ± 15.90 A	

[1/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=5.67).
[2/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD = 3.27).
[3/]Means in the same row followed by the same letter are not significantly different at P≥0.05 (LSD = 3.27).

For pupae stage, the mortality also was checked from the survival of insect after exposing to RF heat treatment. The mortality was present in the same pattern as in larval stage but only one treatment which achieve to kill insects all compared to in larval stage. It means the pupae required higher temperature of RF heat than in larval stage. Only 65°C for 3 minute- treatment showed completely control to pupae of cigarette beetle (Table 4).

Table 4. Mortality of pupae of cigarette beetle exposed to various temperatures from radio frequency at 27.12 MHz for 1, 2 and 3 minutes

Temperature from RF treatment (°C)	Average mortality of pupae			Tota[2/]
	1 minute[1/]	2 minutes	3 minutes	
55	70.19 ± 1.21 g	74.11 ± 1.17 f	91.20 ± 1.47 d	78.50 ± 6.45 Z
60	85.61 ± 1.56 e	89.80 ± 1.31 d	96.44 ± 1.19 bc	90.62 ± 3.15 Y
65	94.72 ± 0.79 c	98.37 ± 0.88 ab	100.00 ± 0.00 a	97.70 ± 1.56 X
Total[3/]	83.51 ± 7.16 C	87.43 ± 7.10 B	95.88 ± 2.56 A	

[1/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=3.12).
[2/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD = 1.81).
[3/]Means in the same row followed by the same letter are not significantly different at P≥0.05 (LSD = 1.82).

Adult mortality was checked after insect exposed to heat for 3 days. The results were similar to those in pupal stage. Only 65°C for 3 minute- treatment showed completely control to pupae of cigarette beetle (Table 4). There were significant differences among temperature treatments (55, 60 and 65°C).

Table 5. Mortality of adults of cigarette beetle exposed to various temperatures from radio frequency at 27.12 MHz for 1, 2 and 3 minutes

Temperature from RF treatment (°C)	Average mortality of adults			Total[2/]
	1 minute[1/]	2 minutes	3 minutes	
55	51.38 ± 2.87 d	81.17 ± 2.78 b	87.16 ± 2.26 b	73.24 ± 11.06 Z
60	69.07 ± 5.79 c	88.26 ± 2.65 b	98.93 ± 0.57 a	85.42 ± 8.74 Y
65	81.09 ± 4.71 b	98.95 ± 0.56 a	100.00 ± 0.00 a	93.35 ± 6.14 X
Total[3/]	67.18 ± 8.63 C	89.46 ± 5.17 B	95.36 ± 4.11 A	

[1/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=8.66).
[2/]Means in the same column followed by the same letter are not significantly different at P≥0.05 LSD = 5.00).
[3/]Means in the same row followed by the same letter are not significantly different at the P≥0.05 (LSD = 5.00).

4. Discussion

Yu et al. (2011) and Conyers and Collins (2006) reported that eggs of cigarette beetle were the most tolerant to air dry heat and complete mortality of eggs required 50°C for 18 hours. Thus RF heat treatments would be an alternative control measure for solving the problem of using large amounts of energy. Nutapong (2012) demonstrated, larval and egg stages of cigarette beetle were more susceptible to RF heat than pupal and adult stages exposed to the radio frequency at 420 watts for 60 seconds. The rank of RF tolerance is: adult>pupal>larval= egg stages. Other stored product insects such *as* adult of *S. oryzae, S. zeamais* and *Rhyzopertha dominica* have demonstrated tolerance to RF heat (Faikrajaipuen et al. 2011; Sumetha et al., 2009; Wangsapa et al., 2015). On the other hand, eggs of Angoumois grain moth, *Sitotroga cerealella* (Buapud et al., 2012) and the cowpea weevil, *Callosobruchus maculatus* (Na Pijit et al., 2011*)* which both lay eggs on rice kernels and mung beans are RF heat tolerance and is likely due to their adherence to the seeds.

The treatments; 60°C for 3 minutes, and 65°C for 2 and 3 minutes caused 100% mortality of larvae. These results can be associated with greater water content in larvae which would cause higher temperature to kill insects due to high oscillation of water molecules or polar molecules as presented by Wangsapa (2016) who reported that moisture contents of *S. oryzae* larvae (66.53±0.8% mc) was greater than pupae(64.21±0.9%) and adults (46.96±0.4% mc), respectively.

Adult of cigarette beetle showed 51% mortality when exposed to RF at 55°C for 1 minute while the pupal stage showed 71.19%, but both adult and pupal stage were completely killed when exposed to treatments of 65°C for 3 minutes. This experiment would confirm that pupal and adult stages of cigarette beetle are the most tolerant to RF heat treatment. From previous experiment done by Nutapong (2012) the result also showed adult and pupal stages were the most tolerant to heat greater than egg and larval stages compared to the heat of 104°C for 180 seconds. Since the treatment of 65°C for 3 minutes caused 100% mortality of tolerant stage it can be recommended for commercial scale treatment. Thus, this result was able to minimize the heat of RF on controlling of all stages of cigarette beetle with 100% of the insect control.

While no work on the effects on progeny were performed in this work, Wangsapa (2016) found that thirty rice weevil, *S. oryzae* treated with 27.12 MHz in laboratory condition were able to produce 13.25±4.5 insects compared with the untreated control insects that produced 26.75±0.8 offspring. Srikam et al. (2014) also determined the effect of RF treatment on sawtoothed grain beetle, *O. surinamensis* infested rice. Their results found that when insects were treated with RF, the number of progeny produced from survivors was significantly less than in untreated controls. If adult of *O. surinamensis* were completely killed by RF heat treatment, there was no progeny production found.

This indicates that even if insects have the opportunity to lay eggs before the heat treatment was completed, all adults and their eggs would die when exposed to heat.

In dried tobacco process, there is the lamina re-drier process of tobacco which consisted of 4 continuous drier chambers at 80-88°C for 3 min in each chamber (personal contact), so this treatment temperature would have less effect ton tobacco quality. The data was supported by research done in Nutapong (2012) which treated dried tobacco with 27.12 MHz of RF energy at 104°C for 3 min caused slightly changed for Burley green leaf tobacco (B2F grade). In dried tobacco treated with RF the nicotine content was 4.13% significantly higher than in untreated control (3.18%). The chemical and physical properties of RF-treated tobacco were remained in Burley green leaf tobacco standard. According this experiment, the death profile of cigarette beetle with 65°C for 3 minutes has potential to apply on dried tobacco with less effect to tobacco quality.

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Lethal effects and mechanism of infrared radiation on *Sitophilus zeamais* and *Tribolium castaneum* in rough rice

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Abstract

The objective of this study was to investigate the characteristics of adult *Sitophilus zeamais* and *Tribolium castaneum,* and the 21.1% dry base (d.b.) MC of rough rice by ATR-FTIR spectra, and determine the theoretical optimum infrared (IR) heating temperature of the tested samples. In laboratory experiments, a ceramic IR drying device was used to heat infested rough rice to research the mortality of *Sitophilus zeamais* and *Tribolium castaneum*, the drying characteristics of rough rice, and milling quality. The theoretical calculation optimum temperature of IR heating was 300 ˚C according to the results of FTIR spectra. In addition, the effects of the different IR radiation intensities and heated rough rice temperatures on mortality of insects, moisture removal, and milling quality were determined in this text. A high insect mortality, heating rate and corresponding high moisture removal were achieved by using IR heating. After heating, tempering process significantly increased insect mortality when the heated tempered rice temperature was less than 55 ˚C, and improve moisture removal and milling quality of rough rice during nature air cooling. When the rice heated under the IR radiation intensity of 2780 W/m^2 for 110 s, the rice temperature reached 60.2˚ ± 0.5˚C, 100% mortality of *S. zeamais* and *T. castaneum,* and 3.97 percentage points of moisture removal during the heating period after tempering and natural cooling. In addition, the high rice milling quality can be achieved after tempering treatment. Therefore, it can be concluded that the optimum conditions of simultaneous disinfestation and drying were 60 ˚C rice temperature under the IR radiation intensity of 2780 W/m^2, followed by tempering and natural cooling.

Keywords rough rice; infrared radiation; *Sitophilus zeamais*; *Tribolium castaneum;* disinfestation; milling quality

Introduction

Rough rice is a major source of food for both human and animals as one of the three main grain varieties. During storage, infestations by stored grain pests may occur if the internal and external conditions are suitable (Lee et al., 2001). It was estimated that 10-40% of worldwide annual production of grain (Mishra et al., 2013) and 27% of the milled rice was lost due to the infestation of pests (Alfonso-Rubí et al., 2003). Various methods of pests control have been implemented to protect the rough rice, in which the chemical fumigation is the most widely used method for disinfestation (Ogendo et al., 2010). However, the effect of chemical fumigation is becoming less effective due to the increase of resistance of insect pests. Chemical application may affect the environment and grain, which could potentially affect the health of human beings (Vadivambal, et al., 2010). Therefore, it is urgent to research and develop alternative technology to chemical fumigation methods.

Infrared radiation (IR) is an efficient and safe physical process method, with wavelengths range from 0.75 to 100 μm. IR can be directly transferred to the material without medium, and converted into heat after the absorption of the electromagnetic wave. In the 1940s, gas-fired IR technology was firstly used for grain disinfestation. Frost et al. discovered that the mortality rate increased with the temperature rise of insect pests (Frost et al., 1944). In the 1960s to 1980s, gas-fired IR method began to be investigated to kill different immature stages and adults of stored-grain pests (Cogburn 1967; Cogburn et al., 1971; Kirkpatrick and Tilton, 1972b; Tilton et al., 1983). Tilton et al. (1963) exposed three species of insects commonly found in grain to IR and achieved 100% of mortality with the temperature of grain ranging from 65 ˚C to 70 ˚C. It was also reported that adult *Rhyzopertha dominica* was killed by IR at 57 ˚C (Kirkpatrick et al., 1972a). The infrared generators in the above

study had an open flame with temperatures exceeding 926 ˚C (Kirkpatrick and Cagle, 1978). Such high temperatures are not suitable for grain processing because of potential safety hazard. In recent years, a sort of flameless catalytic infrared technology was developed for various drying applications, and these catalytic emitters generated temperatures of less than 500 ˚C. Khamis et al. (2010) treated *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum* by the flameless catalytic infrared, and proved that there was significant correlation between mortality and temperatures of the 3 pests according to logistic regression statistics. Pan et al. (2008) concluded that rough rice was heated to 60 ˚C followed by tempering and slow cooling can achieve simultaneous drying and disinfestation with high rice milling quality. However, the effects of different IR intensity on the mortality of insects and its theoretical mechanism of insect lethality were unclear. Therefore, the objectives of this research was to investigate the effect of IR on rice disinfestation and rice quality, and analyze the differences of rice and insect heating rate to reveal the relevant mechanism.

Materials and methods

Preparation of insect and rough rice samples

As one of the main cultivated japonica rice in Jiangsu province, freshly harvested No.5 Huaiyin japonica rough rice was selected for this study, which was obtained from Shibuqiao Grain Reserve Depot, Nanjing, Jiangsu province. The moisture content of rough rice was 21.1 ± 0.5% in dry basis (d.b.). All moisture content was determined by the standard air oven method at 130 ± 2 ˚C for 24 h (ASAE, 1995) in an electric dry oven (Model 101-3AS, Sujin Instrument Factory, Shang Hai, China) and was expressed as percentage in dry mass basis with triplicates.

Two major stored-grain insects, *Tribolium castaneum* and *Sitophilus zeamais* obtained from Chengdu Grain Storage Research Institute, Chengdu, Sichuan province, were used for the study. The *T. castaneum* samples were grown in laboratory on wheatmeal and yeast extract (Oxoid Lid, Wade Road, Basingstoke, Hants, UK) and maintained at 28 ± 2 ˚C and a relative humidity (RH) of 64 ± 3% (Kirkpatrick 1975). The *S. zeamais* was maintained on organic whole wheat (Beidahuang Qinmin Organic Foods CO., LTD) in incubator at a temperature of 30 ± 2 ˚C and a RH of 70 ± 2%.

ATR-FTIR spectra of insects and rough rice

IR heating results in the vibration and rotation of atoms and molecules and leads to a rise in sample temperature. In order to research the IR absorption characteristics of insect and rough rice, the spectral information of live adults and rough rice were collected by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) (Tensor 27, Bruker Corporation, Germany). A single adult or rough rice kernel was placed at the center of the ATR ZnSe crystal (Pike, USA) using a pair of forceps. The background spectrum of the air was scanned before determination of the sample. Through ATR-FTIR application, the spectral region of sample ranged from 4000 to 600 cm^{-1} The number of scans was 64, and the resolution was 4 cm^{-1}. All reported data were means of triplicates. The results of samples exposed to ATR-FTIR spectra were calculated to evaluate the optimum radiation temperature during IR heating following Wien displacement law with equation (1).

$$T = 2898/\lambda_{max} \quad (1)$$

Where T is blackbody temperature, K. λ_{max} is the peak wavelength of blackbody radiation energy.

Infrared heating treatment

A laboratory-scale ceramic infrared drying device provided by Maybo Innovation (MB-EHR12/10KW, Zhen Jiang) was used to treat the rough rice and insect samples. The infrared drying device consists of an IR emitter, a circulating fan, a sample holder and a control panel. The IR emitter was the source of IR radiation (HTS, Elstein-Werk, Germany), and it had a surface temperature of about 630 ˚C and

corresponding peak wavelength of 3.2 µm, assuming a blackbody. The sample holder was a steel reticulation of dimension 50 cm ×35 cm.

The samples, made up of a mixture of rough rice and insects were placed in a single layer on a tray with a loading rate of 2.08 kg/m^3 at a 20 cm distance under the IR emitter. The mixture samples were separately heated to 50, 55, 60 and 65 ˚C under different IR emitter temperatures (200, 300, 400, and 500 ˚C), which corresponds to the IR radiation intensity of 2125, 2780, 3358 and 3974 W/m^2. The temperature of IR emitter was controlled using the control panel. The radiation intensity of heated rice was measured with Ophir thermal excimer absorber head (FL205A, Ophir, Washington, USA) under the different temperature of IR emitter. The temperature of heated rice was measured by using a type-K thermocouple (RDXL4SD, OMEGA Engineering Inc. Stamford, USA), which can be monitored in real time by contact of the thermocouple to the rough rice. The experiments were implemented using rough rice samples and insects with a proportion of 50 insects/100 g rough rice (Yan et al., 2014). The single layer of infested rough rice was uniformly distributed on the holder, insects were randomly distributed.

Long period of IR heating without tempering would affect milling quality of rough rice due to the high heating rate of rice (Ding et al., 2015a). To improve the rice milling quality, treated rough rice was immediately transferred into sealed containers and placed in an incubator with the same temperature of heated rice for 4 hours (Khir et al., 2011). The tempered and non-tempered samples were prepared for analyzing the influences of tempering on disinfestation and milling quality. After the tempering or no-tempering treatment, the samples were placed on a laboratory bench for 40 mins of natural cooling to room temperature of 23 ±1 ˚C.

Determination of temperature distribution of infested rough rice

Water molecules are highly polar and easily absorb the radiation energy into heat. The moisture content of insects is much higher than that of rough rice, and results in the heating rate of insects higher than that of rough rice under IR treatment. In order to analyze the temperature distribution of rough rice and insects, a series of temperature monitoring experiments were conducted. Based on the theoretical calculation optimum temperature of IR heating was 300 ˚C. This is according to the results of FTIR spectra, and the spectra of adult *S. zeamais* and *T. castaneum* were similar. Thus, the adult *T. castaneum* samples were placed in the circular area uniformly in a thin layer (3 mm thick). The mixture of rough rice and adult *T. castaneum* were placed in single layer on tray at a 20 cm distance under IR emitter, and were separately heated under the IR emitter radiation intensity of 2780 W/m^2 for 160 s. After IR heating, the temperatures distribution photographs of heated rice and insects were captured by infrared thermal imager (TiX580, Fluke Corporation. USA). The image results could be analyzed and processed by its own software (FLUKE SmartView 3.7).

During the IR heating experiments, the insects number were much lower than rough rice and they tend to move to the bottom of rice layer, resulting in the temperature determination of insects difficultly. To determine the temperature distribution of insects by IR heating, the heated insects were concentrated in a circle area without rice samples, and the radius of the circle area was about 3 cm.

Assessment of mortality of Sitophilus zeamais and Tribolium castaneum

Treated rough rice samples were placed in jars and placed in incubator at 28 ˚C and 70% RH to allow development of any surviving *S. zeamais* and *T. castaneum*. Adult mortality was determined after 24 h. Mortality of eggs, larvae and pupae was based on the number of adults that emerged from those stages (Khamis et al., 2010). The values were compared with adult emergence in untreated rough rice samples.

Determination of rice milling quality

Milling quality is is an important factor for the processing and sale of rice, the most important rice milling quality indices are total rice yield (TRY) and head rice yield (HRY). To evaluate the effects of IR heating treatment, the 400 g rice samples were dehulled and milled using Yamamoto Husker (FC-2K) and Yamamoto Rice Mill (VP-222N, Yamamoto Co. Ltd., Japan). The rice samples were milled three times to achieve well-milled rice as defined by the standard GB 1350-2009. The settings of throughput and whitening were 1 and 4 during the first two millings and 1 and 5 during the third milling process (Ding et al., 2015b; Pan et al., 2007). TRY was the percentage of milled rice weight divided by the weight of untreated rough rice (GB/T 5495-2008). HRY was determined by GB/T 21719-2008. All reported data of milling quality indicators are means of triplicates.

Statistical analysis

Analysis of variance (ANOVA) was performed in a completely randomized design, data of the mortality of insects and rice milling quality were statistically analyzed with PASW 18 (IBM SPSS Statistics, Chicago, IL, USA) at a 95 % confidence level. One-way ANOVA with Duncan's multiple comparisons test were applied to compare the data among different radiation intensity and different exposed time. Significance was reported at $p < 0.05$ for all data.

Results and Discussion

Characteristics of the ATR spectra of insects and rough rice

The average ATR-FTIR spectra of adult *S. zeamais*, *T. castaneum*, and rough rice as shown in Fig. 1. The spectra form of adult *S. zeamais* and *T. castaneum* were similar. There are two significant absorption region of adult *S. zeamais* and *T. castaneum*, that was mainly distributed in the wavenumber range of 3600-3000 and 1800-800 cm^{-1}. The rough rice has a similar significant absorption in the wavenumber range of 3600-3000 cm^{-1}. The spectrum of the rough rice also has a higher absorption in the wavenumber range of 1250-800 and 1700-600 cm^{-1}, which was different from the fingerprint region in 1800-800 cm^{-1} of the *S. zeamais* and *T. castaneum*. Since the *insects* consist of higher amount of proteins and lipids than rough rice that mainly absorb the IR with wavelength ranges from 5.71 to 10 µm (corresponding wavenumber were 1751 to 1000 cm^{-1}) (Pan and Atungulu, 2011). Therefore, the spectra of adult *S. zeamais* and *T. castaneum* could absorb higher IR within 1800-800 cm^{-1}.

The absorption peak around 3600-3000 cm^{-1} is related to vibration of O-H, with the relevant component of O-H being water and/or carbohydrates in the samples (Ionel 1992). The maximum absorbance of adult *S. zeamais* and *T. castaneum* in this range was 0.23 and 0.16, which are higher than that of the rough rice (0.05). This is may be due to the higher MC of adult *S. zeamais* and *T. castaneum* than that of the rough rice. The corresponding temperature of the spectra region were range from 869 ˚C to 1043 ˚C according Wien displacement law, and this is too high for large-scale commercial processes.

The maximum IR absorbances of adult *S. zeamais* and *T. castaneum* were 0.2 and 0.3 at 1632 cm^{-1}, and were significantly higher than that of rough rice (0.15 at 1020 cm^{-1}). Obviously, the IR absorption ability of insects is higher than that of rough rice. To improve the efficiency of IR drying and disinfestation, the strong absorption wavelength range should be applied during IR treatment. According Wien displacement law, the corresponding temperature of the wavenumber of 1800 cm^{-1} was 248 ˚C. As practical materials, the IR absorption rate of *S. zeamais*, *T. castaneum* and rough rice were less than that of blackbody. To facilitate the experiment operation and commercial process requirements, the IR radiation temperature of 300 ˚C should be more effective with low energy consumption.

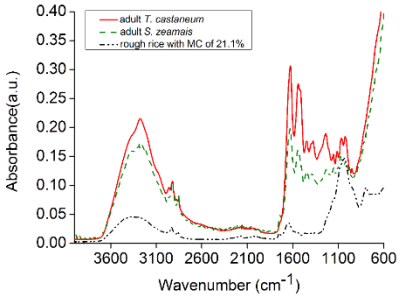

Fig. 1 Average attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra of adult *S. zeamais*, *T. castaneum* and rough rice with MC of 21.1%.

Mortality of insects

Mortality of Sitophilus zeamais

The mortality of egg, larvae, pupae, and adult stages of *S. zeamais* is given in table 1. The control mortality of the four stages of *S. zeamais* was zero. After the rice samples were heated to temperatures of 50, 55, 60 and 65 ˚C, the mortality of adult *S. zeamais* was 81±4.6, 98±3.5, 99±0.6 and 100% at the radiation intensity of 2125 W/m^2 with non-tempering treatment, respectively. The mortality of *S. zeamais* was positively correlated with the heated rice temperature, and above 96 % when the rough rice temperature exceeded 55 ˚C.

After the rough rice temperature heated to 50 ˚C, regardless of tempering, the four stages of *S. zeamais* were still living. The results revealed *S. zeamais* could not reach the complete death when the rough rice temperature heated to 50 ˚C in a short time (110 s). However, the mortality of adult *S. zeamais* at the radiation intensity of 2125, 2780, 3358 and 3974 W/m^2 was 81±2.6, 76±3.5, 73±3.5, and 93±3.1 % when the rough rice temperature of 50 ˚C without tempering, respectively. Only 7±3.1 % adult *S. zeamais* survived at the radiation intensity of 3974 W/m^2 without tempering treatment. Similar ascend trends of the mortality of four stages of *S. zeamais* were observed along with the increase of IR intensity at same heated rough rice temperature. The *S. zeamais* temperature rose faster at higher radiation intensity, and the temperature of *S. zeamais* increased rapidly in a short time, which could result in dramatic changes in *S. zeamais*. It was also shown that the mortality of adult *S. zeamais* was lower than that of other stages at the same experimental condition when the heated rice temperature was 50 ˚C without tempering treatment. For example, the mortality of *S. zeamais* egg, larvae, pupae, and adult stages was 87±4.6, 83±4.0, 81±4.5 and 76±3.5 % at the radiation intensity of 2780 W/m^2 and the heated rice temperature of 50 ˚C without tempering treatment, respectively. This might be due to the fact that the adults move to the lower temperature area when heated by IR.

The mortality of all life stages of *S. zeamais* was 100% when the rough rice was heated to 65 ˚C, regardless of tempering, and 55 ˚C with tempered rice that achieved 100% mortality. The results obtained agreed with reported results that the time to mortality of the insect was less than 40 s when they heated to temperature above 65 ˚C (Kirkpatrick and Tilton, 1972b). In addition, the non-tempered samples, especially at low rice temperature, had fewer insects survived than the samples with tempering. This could be because the lethal temperature of *S. zeamais* was about 56 ˚C (Schroeder and Tilton, 1961), the lethal rate would significantly increase when *S.zeamais* for a long time (4h) at the rough rice temperature beyond 50 ˚C.

Mortality of Tribolium castaneum

The mortality of egg, larvae, pupae, and adult stages of *T. castaneum* is given in table 2. The control mortality for the four stages of *T. castaneum* was zero. There are many similarities to the results of *S.*

zeamais mortality. For instance, the mortality of *T. castaneum* adult is the lowest among the four stages. In addition, at the 65 ˚C of the rice temperature, regardless of tempering, 100% mortality were found in all the samples under such treatment. Moreover, tempering treatment could effectively improve the mortality of insect. However, the mortality of egg, larvae, pupae, and adult stages of T. *castaneum* was 75±2.1, 73±2.6, 64±3.2 and 56±2.1 % at the rice temperature of 50 ˚C without tempering, respectively, which was lower than that of four stages of *S. zeamais* (by 87±4.6, 83±4.0, 81±4.5 and 76±3.5 %) at the same treatment conditions. The results were especially obvious when heated rice temperatures were 50 and 55 ˚C. This shows that *T. castaneum* has a stronger heat resistance than *S. zeamais* at these temperatures. In addition, 100% mortality was observed in all samples for the four stages of *T. castaneum*,at rice temperatures of 60˚C.

Based on these results, it can be concluded that insects tested can be effectively killed by using IR, especially with the tempering treatment. It is recommended that the rice temperature of IR heating be controlled at close to 60 ˚C, and followed tempering treatment, which could ensure that the mortality of *S. zeamais* and *T. castaneum* was 100%.

Temperature distribution of rough rice and insects

The rough rice sample temperatures changes during different heating durations under different radiation intensities were shown in Fig. 2. It was proposed that the heated rice temperature of 60 ˚C was the suitable drying parameter of IR drying for rough rice (Ding et al., 2015b). In this research, after 30 s of IR heating with intensity of 3974 W/m^2, the temperature of the single layer of rice samples with MC of 21.1±0.5% achieved 60.5±1.8 ˚C. For the IR intensity of 2125, 2780 and 3358 W/m^2, the rice temperatures increased to 10.7+23.2 ˚C, 20.5+23.2 ˚C and 39.0+32.2 ˚C after 60 s of heating, respectively. It was obvious that the IR heating could rapidly increase the rice temperature. Because IR is an electromagnetic wave with wavelength of 0.75 to 1000 μm (Carlomagno et al., 2002). When the infrared electromagnetic wave acts on the rice surface, the electric, vibrational, and rotational states of atoms and molecules in rice will be changed accordingly, and the IR energy absorbed by rice will be transformed into thermal motion of molecules allowing rice to heat and evaporate the water for drying (Matsuoka 2011).

Under the same loading capacity and thickness of rough rice, when the radiation intensity was 2125 W/m^2, the temperature rough rice temperature rose to 60 ˚C after 260 s exposed time. In general, the rough rice temperature was linear with the exposed time, it means that the average heating rate of rough rice was 0.14 ˚C/s. Similarly, under the radiation intensity was 2780 and 3358 W/m^2, the temperature rose to 60 ˚C after 110 s and 60 s exposed time, and the corresponding average heating rate of rough rice was 0.34 ˚C/s and 0.62 ˚C/s. The results revealed that the heating rate of rough rice and radiation intensity is positively correlated, and suggests that IR intensities may achieve a higher heating rate for rough rice. This is because that the higher energy could cause stronger vibration of molecules in rough rice, which leading to faster heating rate of rough rice. However, when the radiation intensity was 3974 W/m^2, the temperature of rough rice was 72.3±1.3 ˚C after 40 s of heating, and the average heating rate of rough rice was 1.23 ˚C/s, the heating rate of rough rice was high and easily cause gelatinization and denaturation of rough rice starch and protein, even lead to the burning of heated rough rice. Therefore, in order to avoid the harm of high IR radiation intensity and improve heating efficiency for commercial drying processing of rough rice, the radiation intensity of 2780 and 3358 W/m^2 were more operable and reasonable among the four radiation intensities.

Table 1 Mortality of life stages (egg, larvae, pupae, adult) of *S. zeamais* expose to IR radiation intensity of 2125, 2780, 3358 and 3974 W/m^2 with or without tempering.

Data (mean ± SD) in each column with different letters have significant differences ($p<0.05$).

Radiation intensity(W/m^2)	Rice temperature (°C)	Tempering	Mortality of different life stages of *S. zeamais* (%)			
			egg	larvae	pupae	adult
2125	50	Yes	94±2.5ac	95±2.0a	92±2.3a	92±4.5a
	50	No	88±3.2b	86±3.2b	82±2.6b	81±4.6b
	55	Yes	100d	100e	100d	100c
	55	No	100d	100e	100d	98±3.5c
	60	Yes	100d	100e	100d	100c
	60	No	100d	100e	100d	99±0.6c
	65	Yes	100d	100e	100d	100c
	65	No	100d	100e	100d	100c
2780	50	Yes	93±2.6a	91±3.8c	88±2.3c	83±4.6b
	50	No	87±4.4b	83±4.0d	81±4.5b	76±3.5d
	55	Yes	100d	100e	100d	100c
	55	No	100d	100e	99±0.6d	98±2.5c
	60	Yes	100d	100e	100d	100c
	60	No	100d	100e	100d	99±1.5c
	65	Yes	100d	100e	100d	100c
	65	No	100d	100e	100d	100c
3358	50	Yes	96±2.1c	94±3.6a	89±2.6c	82±3.2b
	50	No	87±3.0b	85±3.1bd	78±2.1e	73±3.5d
	55	Yes	100d	100e	100d	100c
	55	No	100d	100e	100d	96±2.9c
	60	Yes	100d	100e	100d	100c
	60	No	100d	100e	100d	100c
	65	Yes	100d	100e	100d	100c
	65	No	100d	100e	100d	100c
3974	50	Yes	100d	100e	100d	100c
	50	No	100d	100e	95±1.2f	93±3.1a
	55	Yes	100d	100e	100d	100c
	55	No	100d	100e	100d	98±2.6c
	60	Yes	100d	100e	100d	100c
	60	No	100d	100e	100d	100c
	65	Yes	100d	100e	100d	100c
	65	No	100d	100e	100d	100c

Table 2 Mortality of life stages (egg, larvae, pupae, adult) of T. *castaneum* expose to IR radiation intensity of 2125, 2780, 3358 and 3974 W/m^2 with or without tempering.

Radiation intensity(W/m^2)	Rice temperature (°C)	Tempering	Mortality of different life stages of *T. castaneum* (%)			
			egg	larvae	pupae	adult
2125	50	Yes	84±3.6ab	82±2.5a	78±3.8a	74±5.7a
	50	No	67±4.4c	68±2.5b	57±6.5b	52±9.5b
	55	Yes	100f	100h	96±3.6cd	92±3.5cd
	55	No	92±4.6d	93±3.2c	88±4.6ef	80±3.2ef
	60	Yes	100f	100h	100d	100g
	60	No	100f	100h	98±1.5cd	93±2.1cd
	65	Yes	100f	100h	100d	100g
	65	No	100f	100h	100d	100g
2780	50	Yes	91±2.1d	85±1.5d	78±4.0a	72±7.6a
	50	No	75±2.1e	73±2.6e	64±3.2g	56±2.1b
	55	Yes	100f	100h	100d	100g
	55	No	98±1.5f	96±1.7f	91±1.5fh	84±3.2f
	60	Yes	100f	100h	100d	100g
	60	No	100f	100h	100d	96±2.5dg
	65	Yes	100f	100h	100d	100g
	65	No	100f	100h	100d	100g
3358	50	Yes	87±2.5b	87±3.5d	79±3.2a	75±2.3a
	50	No	78±2.9e	75±1.2g	67±4.0i	62±2.6h
	55	Yes	100f	100h	100d	96±2.1dg
	55	No	100f	96±1.7f	94±2.1ch	89±3.2c
	60	Yes	100f	100h	100d	100g
	60	No	100f	100h	98±1.5cd	97±2.6dg
	65	Yes	100f	100h	100d	100g
	65	No	100f	100h	100d	100g
3974	50	Yes	92±3.6d	91±1.5c	85±3.2e	78±5.9ae
	50	No	83±4.6a	78±3.8g	72±3.8j	65±5.5h
	55	Yes	100f	100h	100d	98±1.7dg
	55	No	100f	100h	96±2.3cd	93±1.5cd
	60	Yes	100f	100h	100d	100g
	60	No	100f	100h	100d	100g
	65	Yes	100f	100h	100d	100g
	65	No	100f	100h	100d	100g

Data (mean ± SD) in each column with different letters have significant differences ($p<0.05$).

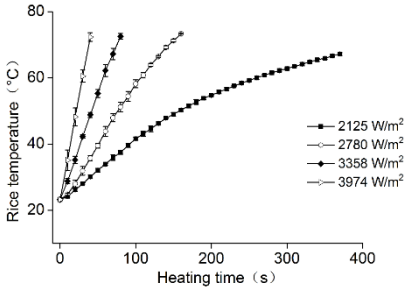

Fig. 2 Heating curve of rough rice under IR radiation intensity of 2125, 2780, 3358, 3974 W/m^2.

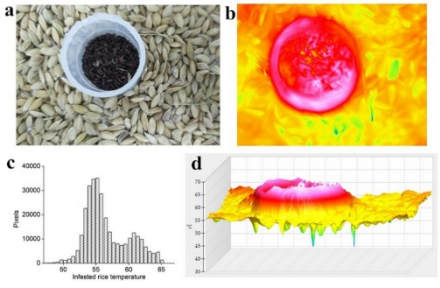

Fig. 3 Thermal (a) and visual (b) images of infested rough rice heated for 160 s under IR radiation intensity of 2780 W/m^2. The rectangle area represented rough rice, and circular area represented adult *Tribolium castaneum*.

Thermal and visual images of infested rough rice heated for 160 s under IR radiation intensity of 2780 W/m2 were shown in Fig. 3a and Fig. 3b. In thermal image of infested rough rice, the red part indicated that the temperature was high, and the temperature range from 50 ˚C to 80 ˚C. The adult T. castaneum temperature was 74.3±2.4 ˚C while the rice temperature was only 71.6±3.6 ˚C. Based on the analysis of FTIR results, when the IR radiation intensity was 2780W/m^2, the maximum IR absorbance of adult *T. castaneum* was 0.3 at 1632 cm^{-1}, and was higher than that of rough rice (0.15 at 1020 cm^{-1}). Obviously, the IR absorption ability of adult *T. castaneum* was higher than that of rough rice. In addition, the temperature of rough rice heated using IR was uniform in Fig. 3a. The results also confirmed the previous finding that the uniformity of IR heating was higher than that of microwave heating (Kirkpatrick et al., 1972).

Moisture removal

The MC of rice samples during IR heating after tempering and non-tempering with different radiation intensities were shown in Fig. 4. The MC of the rough rice that heated to 50 ˚C under 3358 W/m^2 had decreased 1.1 percentage point, which was the least moisture removal (MR) samples of all. Even though, IR heating could effectively remove the moisture in rough rice. The reason for high drying efficiency of IR was that the moisture molecules in rough rice could absorb IR easily and transfer the electromagnetic energy into intermolecular friction, which may lead to the temperature rising and moisture evaporation. Ding et al. (2015b) reported that the drying rate of studied IR heating process for rough rice was 21 and 186 times of that of hot air drying and ambient air drying, respectively.

For the rice samples dried to the same temperature under IR heating, higher moisture removal was usually achieved by those dried with lower IR intensity, which may due to the low energy efficiency of process under high IR intensity. The MR of rice was strongly positively correlated with heated rice temperature. Under the radiation intensity of 2125 W/m^2, the MC of non-tempering samples varies 19.3% to 15.6% in the tested temperatures range from 50 ˚C to 65 ˚C. The higher rice temperature means that the more IR energy were absorbed, which could result in the molecule friction and moisture evaporation of rough rice.

It was also found that the MR after tempering treatment was higher than that without tempering. For instance, when the rice was heated to 60 ˚C, the MR of rice after tempering was 1.2, 1.2, 1.3, and 1.5 percentage points lower that the heated rice after non-tempering, which showed that tempering treatment significantly improved the MR during cooling. The tempering process could reduce the moisture gradient in rice kernels and allow the moisture to distribute uniformly before natural cooling. For the heated rice without tempering treatment, there was a significant moisture gradient in the rough rice kernels and low MC near the rice surface, which led to less moisture removal during cooling. Therefore, tempering process is an essential step to increase the moisture removal during cooling, especially when the rice was heated to a high temperature. The results were similar to those reported by other researchers (Aquerreta et al., 2007; Dong et al., 2010; Pan et al., 2008).

Rice milling quality

The TRYs of infrared dried rough rice were shown in Fig. 5a. Compared with the untreated rough rice, the infrared drying without tempering have negative effects on the milling quality of rough rice. In contrast, the TRYs of tempered rice by using IR were higher than that of non-tempered rice. For instance, when the radiation intensity of 2780 W/m^2, the TRYs of rice dried by IR heating followed with tempering treatment were 2.6 percentage points higher than the untreated rough rice when the rough rice heated to 60 ˚C. The TRYs of the rough rice heated to 60 ˚C under IR intensity of 2125, 2780, 3358 and 3974 W/m^2 with tempering were 69.4±0.5%, 70.2±0.7%, 69.5±0.6% and 68.6±0.9%, which were higher than other groups of rough rice that heated to 50, 55 and 65 ˚C. However, the rice samples treated under 3974 W/m^2 with tempering had lower TRYs than the other rice samples

heated to 50, 55 and 65 ˚C with tempering, which revealed that the high radiation intensity would decrease the TRY of rough rice.

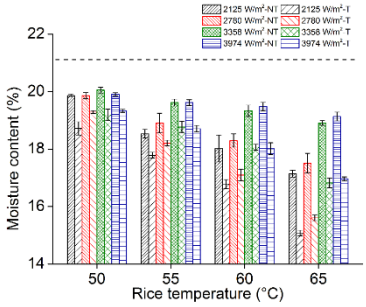

Fig. 4 Measured moisture content under IR radiation intensity of 2125, 2780, 3358, 3974 W/m^2 with and without tempering for rough rice after heating to various temperatures. (T- Tempering, NT- No tempering.) In addition, the dotted line indicated the control group value (untreated samples).

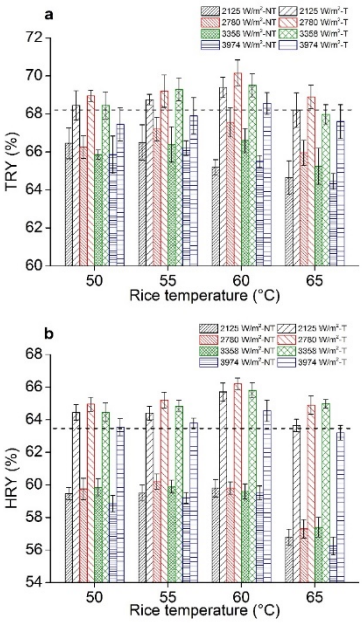

Fig. 5 Total rice yields (TRY) (a) and head rice yields (HRY) (b) of rough rice under IR radiation intensity of 2125, 2780, 3358, 3974 W/m^2 for rough rice after heating to various temperatures. (T- Tempering, NT- Non tempering.) In addition, the dotted line indicated the control group value (untreated samples).

Similar results were also found for the HRYs in Fig. 5b. The rough rice heated using IR with non-tempering had significantly lower HRYs than the untreated rough rice. For example, the HRYs of non-tempered rice that heated to 65 ˚C were 6.2 to 7.4 percentage points less than the untreated rice. However, the HRYs of the rice heated using IR with tempering were higher than untreated rice except for the rice sample dried under the IR intensity of 3974 W/m^2. The maximum HRY was 66.2±0.4% that was heated to 60 ˚C under the intensity of 2780 W/m^2 with tempering.

Based on the milling quality results, the highest milling quality was achieved by IR heating to 60 ˚C followed with tempering and natural cooling. Conssen (2002) reported that the glass state in rice may transfer into a rubbery state at 60 ˚C, and may result in a large amount of moisture removal and well maintaining of milling quality. Since the IR could penetrate the single layer of rough rice, the internal moisture in rice kernels could uniformly absorb IR as the moisture distributed close to the surface. Therefore, the temperature and moisture gradient were kept in same direction (from inside to outside) which could reduce the moisture gradient increase between internal to surface in rice kernels, and contribute to the good rice milling quality. In addition, tempering treatment plays an essential role for maintaining the rice milling quality. During cooling process, based on the glass transition hypothesis, the surface temperature and moisture of rice changed first and starch reached glass state during cooling. However, the center temperature and moisture in the rice kernel were still in rubbery state. Due to the different stage of starch in the rice kernel, stress and fissure were generated that resulting in low TRY and HRY. Therefore, tempering process is important for rough

rice IR drying that could maintain the uniformity of temperature and moisture in the rice kernel and preserve the quality effectively.

Conclusions

IR heating was used as an alternative disinfestation method of *S. zeamais* and *T. castaneum* in rough rice. The theoretical calculation optimum temperature of IR heating was 300 ˚C according to the results of FTIR spectra, which corresponding to the IR intensity of 2780 W/m^2. After the IR radiation intensity of 2780 W/m^2 for 110 s with tempering, the mortality of *S. zeamais* and *T. castaneum* achieved 100 % while the rice temperature reached 60.2˚ ± 0.5 ˚C. Besides, 3.97 percentage points of moisture were removed and rice milling quality were well maintained. When the rough rice was heated to 65 ˚C, the insects could be completely killed regardless of tempering in all samples. Although the higher mortality of insect could be achieved with the increase of heated rice temperature, the higher IR heating intensity and long exposure time might reduce the rice milling quality. Therefore, the tempering treatment after IR heating is important to achieve high insect mortality and moisture removal with good rice milling quality. It can be concluded that the rough rice heated under IR of 2780 W/m^2 to 60 ˚C with tempering and cooling is a feasible processing method for rough rice disinfestation and drying.

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Effect of passing *Beauveria bassiana* through alkane based media on the adult mortalities of *Rhyzopertha dominica* and *Sitophilus oryzae*

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Abstract

Entomopathogenic fungi have been investigated for management of stored product pests as alternatives to chemical control. *Beauveria bassiana* is commonly considered and thus increasing its efficacy has also been studied. The purpose of this study is to evaluate the effect of passing two *B. bassiana* cultures (wild and single-spore cultures) through n-hexadecane and n-octocasane based media on *Rhyzopertha dominica* and *Sitophilus oryzae* adult mortalities. For each Petri plate, 2 ml of 10% alkane was spread, let to evaporate and fungus was inoculated. After sporulation, spores for pathogenicity tests were produced by solid fermentation method on rice. Pathogenicity tests were conducted by application of 500 ppm (w/w) spores in wheat on 20 adults at 25±2°C, 65±5 r.h. in darkness with five replications. The efficacy of wild culture towards *R. dominica* adults was enhanced in both treatments. Mortality in 7 days increased from 35% to 55 and 69% when n-hexadecane and n-octocasane were used, respectively. Similarly, these treatments increased 14-day mortalities from 65% to 77 and 87%, respectively. Treatment of single-spore culture, however, either showed no change or reduced mortality. Passing both cultures through both alkane based media did not statistically affect the activity against *S. oryzae*. This study illustrated that increasing the virulence of *B. bassiana* is possible for *R. dominica* and increase depends both on the starting fungus culture and alkane used. Starting with a wild fungus culture with a wider genetic diversity, and using n-octocasane can produce a better enhancement.

Keywords: microbial control, biological control, virulence, entomopathogen.

1. Introduction

Cereals are produced throughout the world as nutrition for both humans and livestock. These commodities generally require storage for at least a short time and need to be protected against

insect and mite pests. Unprotected stored grains usually lead to quantitative and qualitative loss of grain and reduction of seed germination (Moino et al., 1998; Padin et al., 2002; Haq et al., 2005; Stejskal et al., 2015). Although synthetic insecticides have been used to control stored product pest populations (Athanassiou & Palyvos, 2006), they have various negative consequences such as residue accumulation in products (Ferizli et al., 2005), hazardous effects to humans and the environment (Michalaki et al., 2007), and pest resistance (Arthur, 1996). Therefore, there have been increasing efforts to find environmentally friendly and nontoxic ways to control these pests. Entomopathogenic fungi have been one of the considered alternatives (Moino et al., 1998; Michalaki et al., 2007; Sewify et al., 2014; Wakil & Schmitt, 2014) because they are natural and safer for humans and the environment (Moore et al., 2000). Bioinsecticide potential of entomopathogenic fungi against various insect pests of stored products have been established with a number of studies (Cherry et al., 2005; Wakil & Ghazanfar, 2010; Shams et al., 2011; Barra et al., 2013; Khashaveh & Chelav, 2013; Sewify et al., 2014). They have also been considered potential in combination with diatomaceous earth (Athanassiou & Steenberg, 2007; Athanassiou et al., 2008; Wakil et al., 2011; Riasat et al., 2011, 2013; Shafighi et al., 2014). Enhancing the pathogenicity of a potential fungal isolate would increase its value as biocontrol agent and has been the subject of many studies (Ortiz-Urquiza et al., 2015). One way of doing this is the modification of culturing media by using alkanes as carbon source (Crespo et al., 2002; Pedrini et al., 2011; Barra et al., 2015). In this study, the effect of passing two *B. bassiana* cultures (wild and single-spore cultures) through n-hexadecane and n-octocasane based media was tested to increase their efficacy against *Rhyzopertha dominica* and *Sitophilus oryzae* adults.

Materials and Methods

Insect cultures

Rhyzopertha dominica and *Sitophius oryzae* cultures have been maintained in our laboratory. Starting insects had been originally obtained from surrounding storage facilities. Durum wheat with 12% moisture content was used for the cultures. Glass jars of 1 Lt capacity with 250 gr of wheat were used. Adults of mixed sex were placed into the jars and kept for three days for oviposition. After removing the adults, the cultures were incubated for the emergence of new generation adults. One week old adults were used for the bioassays. All the cultures were maintained at 26±2 ºC and 65±5% relative humidity in darkness.

Fungus cultures and spore production

In the study, two *B. bassiana* cultures were used; one wild culture (151138) and another one that was obtained after single-spore selection (5-4) (Er et al., 2016). The fungi were grown on potato dextrose agar and their spores were suspended in %0.02 Tween 80. After determination of concentration by using Neubauer hemacytometer, spore concentration was adjusted to 10^6 spores/ml by dilution. 200 µl of spore suspension was spread on deficient media agar (DMA) containing alkane (Crespo et al., 2002). 10% n-hexadecane and 10% n-octacosane were prepared using hexane and 2 ml of required alkane was spread on DMA and evaporated prior to spore inoculation. In order to see any effect of the solvent hexane, DMA with only hexane was also tested. These cultures were kept at 25±2ºC for 14 days and grown fungi were used for spore production following mass production procedure described by Barış (2016). 100 g of rice was soaked overnight with tap water and the excess water was drained. The rice supplemented with 1.5 gr of $CaSO_4$ and $CaCO_3$ was sterilized in a polyethylene bag (25 cm x 38 cm). After cooling, it was inoculated with 10 ml of spore suspension ($2x10^7$ spores/ml) and sealed. Following fungal growth at 25±2°C, 12/12 photoperiod for 14 days the culture was dried at 25±2°C. Spores were separated from the substrate by using a 500 µm sieve.

Pathogenicity tests

Centrifuge tubes of 50 ml capacity each with 40 g of wheat were used for the tests. Wheat in each tube was mixed with 20 mg of spores producing a final concentration of 500 ppm (w/w) by shaking for 5 minutes. Twenty adults were released in each tube and kept at 25±2ºC, 65±5% relative humidity in constant darkness. Wheat kernels without spores were used as control. The experiment had five replicates.

Results

The efficacy of wild culture (151138) of *B. bassiana* towards *R. dominica* adults was enhanced when the fungus was passed through both n-hexadecane and n-octocasane based media. Mortality in 7 days increased from 35% to 55 and 69% when n-hexadecane and n-octocasane were used, respectively. Similarly, these treatments increased 14-day mortalities from 65% to 77 and 87%, respectively. Treatment of single-spore culture (5-4) of *B. bassiana*, however, either showed no change or reduced mortality. Passing both *B. bassiana* cultures through both alkane based media did not statistically affect their efficacies against *S. oryzae* adults. Using hexane alone did not change the effects of the *B. bassiana* to either of the species.

Discussion

This study illustrated that increasing the virulence of *B. bassiana* against *R. dominica* adults is possible by passing the fungus through media having n-hexadecane or n-octocasane as carbon source. Increase in mortality was reported by Crespo et al. (2002), Pedrini et al. (2011) and Barra et al. (2015) when fungi were grown on media containing these alkanes. In the case of *S. oryzae*, this procedure did not enhance the efficacy. This may be due to differences in the cuticular components of two insect species. In the previous studies, host insects were treated with spores that had been harvested directly from media with alkanes. However, in the present study the fungi were passed through media with alkanes and then cultured by mass production procedure using rice as substrate. Therefore, the results of this study indicate that the increase in virulence against *R. dominica* adults was due to a selection of fungi that can use n-hexadecane or n-octocasane as carbon source. This was also supported by the mortality levels caused when single-spore culture (5-4) of *B. bassiana* was used after passing through the alkanes. Starting with a wild fungus culture with a wider genetic diversity, and using n-octocasane can produce a better enhancement.

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Bio-nanosilver synthesized by the entomopathogenic nematode-symbiotic bacterium as bio-insecticide for the red flour beetle (*Tribolium castaneum)*

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Abstract

Biological control can be another important way to manage post-harvest insect pests. Some organisms that showed biological control activity against some soil pests are insect-parasitic nematodes. There are two different species of nematodes, steinernematids and heterorhabditids, who carry within their bodies insect-pathogenic bacteria. *Xenorhabdus* spp are bacteria which infest steinernematids and *Photorhabdus* spp. bacteria infect

heterorhabditids. The study aimed to develop pesticide alternatives by synthesizing silver bio-nanoparticles (AgNPs) using *Xenorhabdus indica* bacterial filtrate. The nanoparticles synthesized by the bacterial strains were purified and its cytotoxicity and bioactivity was examined against the larvae of the *Tribolium castaneum*. AgNPs were characterized by Scanning Electron Microscopy and X-Ray diffraction analysis, and the results revealed that the obtained nanoparticles are nanosilver with sizes ranging from 30 to 70 nm, with spherical shape and non-smoothed surface. Insect larvae were initially exposed to descending concentrations (100, 50, 25, 10 and 5 µg/ml) of the biosynthesized nanosilver for 48 hours. Results of the bioassay showed that mortality of treated larvae was concentration-dependent with LC_{50} of 25 µg/ml. Higher mortality percentage (89%) was observed with the concentration 100 ug/ml and the lower one was obtained by the concentration 5 ug/ml (60%). Subsequently, data of the present study suggest these bio-AgNPs-bacterial filtrate complexes could be used as potentially effective eco-friend bio-control candidates. However, testing other types of bio-synthesized nanomaterials, and its vital effect as bio-insecticide for storage insect species are still under investigation.

Keywords: Entomopathogenic bacteria, biocontrol, nanosilver, *Tribolium* sp., *Xenorhabdus* sp.

Introduction

Stored commodities are vulnerable towards attack of insects (Ukeh *et al*., 2012) and a possible infestation can deteriorate the quality as well as the quantity of the attacked commodity (Nadeem *et al.,* 2012). This will resulted in significant decrease in volume, nutritional value, substantial weight loss and reasonable germination damage (Nadeemet al., 2012, Phillips et al., 2010). It was reported that *Tribolium castaneum* is a common pest found in granaries, mills, warehouses, especially in wheat flour, which causes serious damages to all kinds of stored grain products (Prakash *et al.,* 2008), but also feeding on different stored-grain and grain products (Weston and Rattlingourd, 2000). Being polyphagous and cosmopolitan, a number of insecticides had been used for successful control of this pest (Islam and Talukdar, 2005).

Synthetic insecticides have been successfully used to protect stored grains from insect infestation (Sighamony. *et al*., 1986). *T. castaneum* is affected by both the quantity and quality of synthetic insecticides such as malathion, pirimiphos-methyl, chlorpyrifos-methyl, deltamethrin and the fumigant phosphine. These are currently the main products used to protect stored grains from insects (Bond, 1984). Increased public concern over the residual toxicity of insecticides applied to stored products, the occurrence of insecticide-resistant insect strains, and the precautions necessary to work with traditional chemical insecticides stress the usage of e.g. botanicals to control insects of stored product (Su, 1991).

In the present decade, nanotechnology is a promising field that introduces an excellent chance for research and is expected to give major impulses to technical innovations in a variety of industrial sectors in the future. Benelli (2016) reported that the biosynthesis of AgNPs is an arising tool for fighting mosquito vectors. Nanoparticles of noble metals like silver and gold exhibited remarkable physical, chemical and biological properties from their bulk counter parts (Priya and Santhi, 2014).

Microbial and endo-toxin insecticides based on *Bacillus* spp. bacteria, as well as decreasing of breeding habitat can achieve considerable IPM program goals (Rydzanicz *et al*., 2009). In this context, Adams and Nguyen (2002), found that *Xenorhabdus* and *Photorhabdus* gram negative symbiotic bacteria accompanied with the entomopathogenic nematodes *Steinernema* and *Heterorhabditis*, are injected into the haemocoel of target insect hosts. A variety of these toxins have been characterized and classified into four major groups (Rodou *et al*., 2010). The toxin complexes (Tcs) are one of these four major groups which attracted attention from the fact that some of their complexes showed a high potential toxicity towards insects after oral application, suggesting potentiality as insecticides (Waterfield *et al*., 2001).

Results and Discussion

The results revealed that the obtained nanoparticles are nanosilver with sizes ranging from 30 to 70 nm, with spherical shape and non-smoothed surface. Insect larvae were initially exposed to descending concentrations (100, 50, 25, 10 and 5 µg/ml) of the biosynthesized nanosilver for 48 hours. Results of the bioassay showed that mortality of treated larvae was concentration-dependent

with LC_{50} of 25 µg/ml. Higher mortality percentage (89%) was observed with the concentration 100 ug/ml and the lower one was obtained by the concentration 5 ug/ml (60%). Subsequently, data of the present study suggest these bio-AgNPs-bacterial filtrate complexes could be used as potentially effective eco-friend bio-control candidates.

The cause of insect death could be *via* binding the nanoparticles to proteins containing sulphur in the intracellular space or phosphorus in the DNA, which leads to enzyme and organelle degradation. Basically, cell death is mainly caused by decreased membrane permeability and disturbed proton motive force which leads to cellular function loss. The pathogenicity of these toxin complexes upon releasing into the host heamolymph causes histopathological lesions and septicaemia leading to host death. Moreover, the high larvicidal activity of AgNPs can be attributed to their lower particle size which increases the surface area to volume ratio, and thus, increases its action against insect.

Conclusion

Subsequently, data of the present study suggest these bio-AgNPs-bacterial filtrate complexes could be used as potentially effective eco-friend bio-control candidates. However, testing other types of bio-synthesized nanomaterials, and its vital effect as bio-insecticide for storage insect species still under investigation.

Tab 1: The effect of the culture filtrate and the biosynthesized nanosilver on insect mortality compared with a commercial insecticide.

		Mean mortality± SE					
	Concen-	*Time* ***(hrs)***					
	tration ***(%)***	***3hr***	***6hr***	***12hr***	***24hr***	***48hr***	***72hr***
Water	*Cont.*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*
Culture	*1*	*0.33±0.577*	*15.0±10.0*	*23.33±12.58*	*30.0±10.0*	*35.0±15.0*	*50.0±10.0*
filtrate	*10*	*0.00±0.00*	*26.67±2.9*	*31.667±2.89*	*41.67±2.89*	*51.667±2.89*	*65.0±5.0*
	20	*66.67±18.9*	*75.0±22.9*	*78.33±20.82*	*81.667±15.*	*83.33±12.58*	*90.0±10.0*
Insecticide	*1*	*0.0±0.0*	*1.67±2.89*	*5.0±0.0*	*13.3±2.887*	*16.667±2.89*	*26.67±2.9*
	10	*0.0±0.0*	*3.3±2.887*	*13.33±7.638*	*23.33±2.887*	*30.0±0.0*	*50.0±5.0*
	20	*56.667±16.*	*66.67±16.*	*75.0±17.32*	*78.3±15.28*	*80.0±18.028*	*86.7±12.5*
	1	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*6.67±2.887*	*11.667±2.89*	*21.67±2.9*
Bio-	*10*	*11.667±7.6*	*23.33±10.*	*35.0±13.229*	*53.33±10.4*	*60.0±13.229*	*73.33±10.*
synthesized	*20*	*90.0±5.0*	*90.0±5.0*	*93.33±2.887*	*95.0±5.0*	*98.33±2.887*	*100.0±0.0*
nano silver							

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Insecticidal Effect of Central Anatolian Region Diatomaceous Earths Against Confused Flour Beetle (*Tribolium confusum* Du Val.) on Stored Paddy

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Abstract

In this study, insecticidal efficacy of different local diatomaceous earth (DE) deposits obtained Central Anatolian Region in Turkey and commercial DE deposit (German origin), Silicosec® were evaluated against substantial pest on stored grain as *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) at five different concentrations of 100, 300, 500, 900 and 1500 ppm on stored paddy. Mortality of the exposed adults was assessed after 7, 14 and 21 days of exposure. Also progeny productions were assessed after 65 days The tests were carried out at 25±1 oC temperature, 55±5% R.H. under dark conditions. The most effective DE in a short time were assessed AG2N-1 which caused 97% mortality of *T. confusum* adults at 1500 ppm concentration after 7 days of exposure in paddy. Complete mortality of *T. confusum* adults was recorded on AG2N-1 at 900 ppm for 14 days and treatments of AG2N-1, BGN-1, CBN-1 for 21 days at 500, 900 and 1500 ppm respectively whereas 87% mortality rate was determined for 21 days exposure of Silicosec® at the highest concentrations on paddy. In conclusion, this study indicated that Turkish DE deposits, AG2N-1, BGN-1 and CBN-1 had high insecticidal efficacy in comparison with the commercial Silicosec® and would have potential to be used against insects in the pest management of stored paddy.

Keywords: Turkish diatomaceous earths, *Tribolium confusum*, toxicity, paddy, Silicosec

1. Introduction

Currently, the control of insect pests in durable stored food products, such as grains and legumes, is based on the use of chemical methods such as fumigants and residual insecticides. However, the use of these substances is directly related with toxic residues on the final product, as well as serious environmental hazards. These factors, along with the consumers' demand for residue-free food and the development of resistance by several insect pests, have made essential the evaluation of alternative, low-risk and environmentally-friendly control methods. One of the most promising alternatives over the use of traditional pesticides in durable stored products is the use of diatomaceous earths (DEs). DEs are composed by the fossil skeletons of phytoplanktons, also known as diatoms, which occur in fresh and salt water since the Eocene period and produce a soft sedimentary rock, which is composed mainly by amorphous silica ($SiO_2 + H_2O$). The DEs currently mined vary remarkably in their insecticidal activity, depending upon species composition, geological and geographical origin as well as certain chemical characteristics, such as SiO2 content, pH and tapped density (Korunic 1997). DEs are probably the most efficacious natural resource-based dry materials that can be used as insecticides (Korunic 1998). DEs act in the insects' exoskeleton (cuticle) causing rapid desiccation resulting in death through water loss. They are non-toxic to mammals (rat oral LD50>5000 mg/kg of body weight), leave no toxic residues on the product and

according to the US EPA they are classified in the category of GRAS (Generally Recognised As Safe) since they are used as food or feed additives (FDA 1995). Regarding their insecticidal use, DEs can be applied with the same application technology with traditional grain protectants, which means that no specialized equipment is required (Athanassiou et al. 2005). Several DEs, based on natural deposits, are now commercially available, and have proved very effective against stored grain pests (Subramanyam and Roesli 2000, Athanassiou et al. 2011). However, the investigation for newer, naturally-occurring DEs that are more effective in insect control is still in progress, especially in areas rich to silicaceous rocks. Based on the first evidence and preliminary samplings, it seems that Turkey is considered rich to natural DE deposits, and there is clear evidence for the existence of large DE deposits at some areas (Özbey and Atamer 1987, Mete 1988, Sıvacı and Dere, 2006, Çetin and Taş 2012). Diatomite reserve of Turkey is 125 million tons. However, there is limited information on the efficiency of local DEs from these areas in Turkey against stored grain insects. In this study, efficiency of three local diatomaceus earth formulations against Confused Flour Beetle (*Tribolium confusum* Du Val.) on paddy, was investigated under laboratory conditions.

2. Materials and Methods

2.1. Test Insects

Confused Flour Beetle adults used in the bioassays were taken from a culture that was kept at the Namık Kemal University, Department of Plant Protection, Toxicology Laboratory on whole wheat at 26 ± 1ºC, 65 ± 5 %. All individuals used in the tests were <2 wk old.

2.2. Diatomaceous earth formulations

The three DE formulations used in biological tests were of three Turkish diatomaceous earth formulations (AG2N-1, BGN-1 and CBN-1) and commercial diatomaceous earth (Silicosec®). Turkish diatomaceous earth formulations were collected from diatomite reserve at middle Anatolia of Turkey.

2.3. Experimental procedure

Exposure studies were carried out at 25 ± 1ºC, 55 ± 3 % RH, and five dose rates of four Turkish diatomaceous earth formulations and commercial diatom earth (Silicosec®) (100, 300, 500, 900 and 1500 ppm) on paddy (*Oryzae sativa* L. variety of Osmancık 97) with %13 moisture content. For each trial (DE formulation-dose combination), five samples of 50 g paddy were taken. Each sample was placed in a small glass jar that was closed, and that was covered with organtine for sufficient ventilation. The samples were treated individually with the respective DE quantity and then shaken manually for 5 min to achieve equal distribution of the dust in the entire product quantity. Five additional tubes, containing untreated paddy, served as control in each case. Subsequently, 20 adults of *T. confusum* were introduced into each tube. The tubes were then placed in incubators, set at above mentioned temperature and relative humidity level. Dead adults of both species were counted after 7, 14, 21 d. After the last count for mortality, all adults (dead and alive) were removed from the DE-treated jars, and the jars were left in the incubators for an additional period of 65 d. Then, the emerged *T. confusum* individuals were counted.

2.4. Data processing and analysis

Generally, the control mortality was very low, but where it was considered necessary the mortality counts were corrected by using the formula of Abbot (1925). The data were analyzed, separately for each species, by using the Anova test of SPSS (SPSS,2009), with insect mortality as the response variable and type of DE formulation and dose rate, as the main effects. For the progeny production counts, with number of progeny as the response variable and type of DE formulation, and dose rate as main effects. Means were separated by using the Anova test at $P<0.05$.

3. Results

Efficacy of Turkish DE's represented different values according to exposure times and concentration at the end of the experiment (Table 1,2 and 3). Complete mortality of *T. confusum* adults was recorded after 14 days of exposure at 900 ppm treated with AG2N-1 (Table 2) and 21 days of exposure at 500, 900, 1500 ppm treated with AG2N-1, BGN-1 and CBN-1 respectively whereas 87% mortality rate were recorded for Silicosec® after 21 day exposure at the highest concentrations on paddy (Table 3). According to the results of the biological tests carried out on the paddy, F1 values of *T.confusum*, including the control group, was not seen in the adult exit. This result wold be caused by confused flor beetle one of the important secondary insect species in the stored product pests and was not able to eat whole kernel of the paddy.

Table 1. Mean percentage mortality (±SE) of *Tribolium confusum* adults exposed to 5 different concentrations of 9 DEs after 7 days

	DE Formulation						
Concentration (ppm)	**Silicosec®**	**BGN-2**	**BHN-1**	**AG2N-1**	**CBN-1**	**F** value	**P** value
1500	3±2 Ac*	25.3±4.3 Ab	0±0 Ac	96.9±2.1 Aa	19.2±4.5 Ab	$F_{4,20}$=105.52	P<0.0001
1000	1±1 Ac	16.2±2.6 Ab	1±1 Ac	82.3±4.5 Ba	1.8±1.8 Bc	$F_{4,20}$=87.38	P<0.0001
500	0±0 Ac	5.3±1.7 Bb	0±0 Ac	54.2±5 Ca	0±0 Bc	$F_{4,20}$=111.33	P<0.0001
300	0±0 Ab	2.4±1 Bb	1±1 Ab	32.3±4.4 Da	1.6±1 Bb	$F_{4,20}$=32.96	P<0.0001
100	0±0 Ab	1.6±1 Bab	1±1 Ab	4.2±2.1 Ea	0±0 Bb	$F_{4,20}$=3.99	P=0.015
Control	0±0	1±1	0±0	4±1.9	1±1		
F value	$F_{4,20}$=1.708	$F_{4,20}$=15.303	$F_{4,20}$=0.5	$F_{4,20}$=74.503	$F_{4,20}$=17.621		
P value	P=0.188	P<0.0001	P=0.736	P<0.0001	P<0.0001		

Table 2. Mean percentage mortality (±SE) of *Tribolium confusum* adults exposed to 5 different concentrations of 9 DEs after 14 days

	DE Formulation						
Concentration (ppm)	**Silicosec®**	**BGN-2**	**BHN-1**	**AG2N-1**	**CBN-1**	**F** value	**P** value
1500	39.4±4.2 Ad	89.8±4.6 Ab	15.2±2.9 Ae	100±0 Aa	80.6±4.1 Ac	$F_{4,20}$=77.96	P<0.0001
1000	5.5±2.7 Bd	66.3±9.2 Bb	4.4±2 Bd	100±0 Aa	25.5±4.4 Bc	$F_{4,20}$=72.54	P<0.0001
500	4.6±3 Bc	21.4±4.7 Cb	0±0 Bc	94.3±1.8 Ba	1.2±0.7 Cc	$F_{4,20}$=97.46	P<0.0001
300	2.6±1.8 Bc	16.3±6.4 Cb	3.6±2.7 Bc	72.7±4.5 Ca	3.9±1.8 Cc	$F_{4,20}$=30.01	P<0.0001
100	1.8±1.8 Bb	2.4±0.6 Dab	1.6±1 Bb	8.4±3 Da	1.2±0.7 Cb	$F_{4,20}$=2.21	P=0.103
Control	1±1	2±1.2	1±1	12±3.7	2±1.2		
F value	$F_{4,20}$=13.74	$F_{4,20}$=33.82	$F_{4,20}$=7.33	$F4_{,20}$=135.58	$F_{4,20}$=75.73		
P value	P<0.0001	P<0.0001	P=0.001	P<0.0001	P<0.0001		

Table 3. Mean percentage mortality (±SE) of *Tribolium confusum* adults exposed to 5 different concentrations of 5 DEs after 21 days

	DE Formulation						
Concentration (ppm)	**Silicosec®**	**BGN-2**	**BHN-1**	**AG2N-1**	**CBN-1**	**F** value	**P** value
1500	86.9±3.4 Ab	100±0 Aa	56.6±3.4 Ac	100±0 Aa	100±0 Aa	$F_{4,20}$=140.39	P<0.0001
1000	29.3±6.6 Bc	100±0 Aa	23.2±8.2 Bc	100±0 Aa	72.6±3.1 Bb	$F_{4,20}$=75.52	P<0.0001
500	11.3±4.6 Ccd	83.3±1.9 Bb	5.7±3.8 Cd	100±0 Aa	14.7±5.9 Cc	$F_{4,20}$=78.53	P<0.0001
300	2.6±1.8 Cc	50±8.2 Cb	5.5±2.7 Cc	94.2±2.6 Ba	7.4±2.7 Cc	$F_{4,20}$=51.93	P<0.0001
100	2.6±1.8 Cb	4.2±1.3 Db	2.4±1 Cb	33.3±7.4 Ca	1.1±1.1 Db	$F_{4,20}$=15.76	P<0.0001
Control	1±1	4±1.9	1±1	13±4.1	5±2.7		
F value	$F_{4,20}$=44.42	$F_{4,20}$=181.57	$F_{4,20}$=15.41	$F_{4,20}$=70.61	$F_{4,20}$=140.14		
P value	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001		

*Two-way variance analysis (ANOVA) was applied to the data and the differences between the averages were based on the 5% significance level. The different uppercase letters in the same column and the different lowercase letters in the same line are statistically different.

Discussion

The most effective DE in a short time were assessed AG2N-1 which caused 97% mortality of *T. confusum* adults at 1500 ppm concentration and 82% at 900 ppm concentarition after 7 days of exposure on paddy. Other diatoms showed low toxicity on all concentrations. Complete mortality of *T. confusum* adults was recorded on AG2N-1 at 900 ppm for 14 days and treatments of AG2N-1,

BGN-1, CBN-1 for 21 days at 500, 900 and 1500 ppm respectively whereas 87% mortality rate was determined for 21 days exposure of Silicosec® at the highest concentrations on paddy. Athanassiou et all. (2004), reported that Silicosec was't reached complete mortality at 750,1000 and 1500 ppm concentration on rye, oats and triticale againt *T.confusum* after 21 days application but decrased to number of insect on F_1. In a similar study conducted on rice with *T. confusum* adults, Alagöz (2016) found that Silicosec® commercial diatom was found to be 20% at the end of 7th day, 75% at the end of 14th day and 99% at the end of 21st day, at the end of the day, did not find a new generation of adult outbreaks, including the control group, in all diatoms used in experiments. Ziaee et al. (2012), a study conducted on wheat with *T. confusum* adults, found 51% mortality with Silicosec after 2days at 2000ppm concentration and complete mortality was recorded at 1000,1500 and 2000 ppm after 7 days and more. In conclusion, this study indicated that Turkish DE deposits, AG2N-1, BGN-1 and CBN-1 had high insecticidal efficacy in comparison with the commercial Silicosec® and would have potential to be used against insects in the pest management of stored paddy.

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Twelve years (2005-2017) of scientific and professional work in the field of stored products pests protection in Slovenia

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Abstract

Scientific and professional work in the field of stored products pests protection in Slovenia began in 2005, when we tested the efficacy of entomopathogenic nematodes against the granary weevil (*Sitophilus granarius*) and the sawtoothed grain beetle (*Oryzaephilus surinamensis*) adults under laboratory conditions. In 2007, we participated as partners in the project SEE-ERA.NET "Development of a non-toxic, ecologically compatible, natural-resource based insecticide from diatomaceous earth deposits of South Eastern Europe to control stored-grain insects pests" (coordinated by C. Athanassiou), and we thus became acquainted with the research work in the field of investigation the efficacy of diatomaceous earth in controlling beetles from the *Sitophilus* genus. We have continued the research of different aspects of diatomaceous earth (the influence of geochemical composition and abiotic factors on its efficiency, the effects of individual and combined application, the effects on various harmful insect pests, etc.). In search for comparable substances to diatomaceous earth (regarding the efficacy), we have studied insecticidal effects of quartz sand and entomopathogenic nematodes from Slovenia,

plant powders and essential oils on various harmful beetles. In the recent years, our research work has been mainly dedicated to studying the efficacy of wood ash and zeolites as natural insecticides, which have demonstrated sufficient efficiency in suppressing *Sitophilus* beetles. In the same period, we studied the seasonal dynamics of the Indian mealmoth (*Plodia interpunctella*), the Mediterranean flour moth (*Ephestia kuehniella*) and the Angoumois grain moth (*Sitotroga cerealella*) in cereal stores, where we were also searching for possible indigenious natural enemies of stored product insects pests. We have confirmed the occurrence of two parasitoids, *Anisopteromalus calandrae* and *Dibrachys microgastri*. In 2017, we have organized the 11th Conference of the IOBC/wprs Working Group on Integrated Protection of Stored Products (Ljubljana, 3-5 July), which was attended by 136 participants from 25 countries. We also transfer knowledge to Slovenian agricultural specialists about the harmfulness and possible ways of controlling stored products insects pests. In 2014, we have organized a workshop on this topic ("From Technological Maturity to Storing of Cereals and Legumes"). In 2015, we have hosted C. Athanassiou as an invited lecturer at the 12th Slovenian Conference on Plant Protection with international participation in Ptuj. In recent years, we have been working with experts from other countries with the aim of studying the efficacy of environmentally acceptable insecticides (spinosad, spinetoram) and the influence of cereal production technologies on grains' susceptibility to attack by *Sitophilus* beetles. Furthermore, we participate in the research regarding the efficiency of new formulations of insecticidal preparations. The paper presents the chronology of activities in this area of our work.

Keywords: stored products pests, beetles, inert dusts, essential oils, biological control, Slovenia

1. Introduction

In Slovenia, the systematic research and professional work in the field of stored product pest control began in 2005, when in laboratory conditions we exposed the selected stored products beetles to entomopathogenic nematodes, whose effects were then tested on different species of insect pests. In the next 12 years, also with the help of foreign experts, we expanded the scope of stored product pest research to include other fields, primarily the fields of natural products, and physical and other techniques for stored products pest control. Bellow, we present the results of our work and the complete overview of references in this field.

2. Chronology of scientific and professional work in the field of stored products pests protection

2.1. First attempts or entomopathogenic nematodes against stored products beetles

Four entomopathogenic nematode species (*Steinernema feltiae, Steinernema carpocapsae, Heterorhabditis bacteriophora*, and *Heterorhabditis megidis*) were tested in a laboratory bioassay with the aim of studying their efficacy in control of the adults of two stored grain pests, *Sitophilus granarius* and *Oryzaephilus surinamensis*. Activity of the biological agents studied was determined at three different concentrations (500, 1000, and 2000 infective juveniles [IJs] per adult) and temperatures (15, 20, and 25°C). The granary weevil mortality rate was higher than the mortality rate of the saw-toothed

grain beetle. *Heterorhabditis megidis* proved to be the least efficient in control of both pests, while no significant differences were recorded between any of the other three nematode species. The experiment demonstrated that the entomopathogenic nematodes were most efficient in the control of *S. granarius* at 20°C (LC_{50} after 7-day exposure 803-1195 IJs/adult) and 25°C (LC_{50} 505-1175 IJs/adult). A satisfactory level in control of the pest *O. surinamensis* was reached at 20°C (LC_{50} 921-1335 IJs/adult). The concentration of the suspension used in our experiment was shown to be a less important factor affecting the biological activity of nematodes against the adults of both stored grain pests. Though the use of entomopathogenic nematodes for control of the tested pests is not possible at the present time, it may be possible to combine this approach with some other (biotechnical) methods in the future (Trdan et al., 2005; Trdan et al., 2006).

The efficacy of three new strains (B30, B49 and 3162) of the entomopathogenic nematode *Steinernema feltiae* in controlling rice weevil (*Sitophilus oryzae*) adults was tested in a laboratory bioassay. The aim of the study was to determine the activity of selected biological control agents

against one of the most important primary stored product pests to prevent the occurrence of rice weevil resistance to insecticides. The pathogenicity of biological agents was studied at four different temperatures (15, 20, 25 and 30°C) and for five concentrations of nematode suspension (125, 250, 500, 1000 and 2000 IJs per adult). Beetle mortality was determined on 4, 6 and 8 days after treatment. The results showed that all studied strains were most pathogenic (42-72% mortality) at 25°C and the highest concentration of the nematode suspension, while the lowest pathogenicity (from 6 to 11%) was found at 30°C and the lowest concentration of the nematode suspension. Besides, at higher concentrations the suspension of entomopathogenic nematodes can be an effective biological agent in controlling adult rice weevils. The lowest LC_{50} value (1165 IJs/adult after an 8-day exposure) was obtained for the Hungarian strain 3162 at 25°C, while the highest (2533 IJs/adult after an 8-day exposure) was obtained for the Slovenian strain B30 at 30°C (Laznik et al., 2010; Laznik and Trdan, 2010).

2.2. Seasonal dynamics of stored products lepidopteran pests

In the period 2004-2006 seasonal dynamics of Mediterranean flour moth (*Ephestia kuehniella*), Indianmeal moth (*Plodia interpunctella*) and Angoumois grain moth (*Sitotroga cerealella*) was studied in the mills and grain warehouses in central Slovenia. For this purpose pheromone traps were used from April until December, and the males of all three lepidopteran pests were counted in two week intervals. The three insect pests under investigation developed two peaks in capture per year that might represent two distinct generations per year. In the maize open air storage *Ephestia kuehniella* was the most numerous, while *Plodia interpunctella* was more frequent in the closed storage in mills and warehouses, *Sitotroga cerealella* was slightly less common in these latter closed warehouses (Trdan et al., 2010).

2.3. Diatomaceous earth, quartz sand, plant powders, wood ashes, and zeolites in single and combined use

Laboratory experiments were carried out to evaluate the impact of diatomaceous earth (DE) samples of different origin with their insecticidal properties to control *Sitophilus oryzae*. We tested the efficacy of three local DEs, from Serbia, Greece and Slovenia, and commercial formulation SilicoSec against the adults in stored wheat. The experiments were carried out at three temperatures (20, 25 and 30 °C) and two relative humidity (RH) levels (55 and 75 %). Mortality of pest was counted 7, 14 and 21 days after exposure (DAT) at the following DE dose rates: 100, 300, 500 and 900 ppm. The mortality of adults normally increased with increasing dose rates and DAT. In all samples the mortality of rice weevil adults (dose rate 900 ppm, 21 DAT) was above 90 %, except at Slovenian DE (at 20 °C and 55 % RH) and Greek DE (at 25 °C and 75 % RH), when the mortality was 85.3 and 67.6 %, respectively. With 100 % mortality (14 DAT and at 900 ppm) the most effective was SilicoSec. Slovenian DE was more effective at 55 % RH than at 75 % RH (7 DAT at all temperatures) (Rojht et al., 2008, 2010a; Rojht et al., 2012a).

Laboratory experiments were done to determine the effect of geochemical composition of diatomaceous earth (DE) on insecticidal activity of DE against adults of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Samples of DE were mined from DE-deposits in Slovenia, Greece, and Serbia. In addition, a commercially available DE formulation (SilicoSec®) was used in the tests and served as a positive control. The bioassays were carried out at temperatures 20, 25, and 30°C, relative humidity levels of 55 and 75%, and at application rates of 100, 300, 500, and 900 ppm. Adult mortality was recorded after 7, 14, and 21 days of exposure. Prior to bioassays with *S. oryzae*, the geochemical composition of all DEs that were used in the tests was determined by whole rock ICP geochemical analyses. Silica (in the form of SiO_2 or opal-A) was the DE ingredient that was significantly correlated with efficacy in most of the bioassays. Some weak positive correlation was observed between *S. oryzae* mortality and MnO or CaO content. All significant correlations between mortality and Al_2O_3, Fe_2O_3, K_2O, TiO_2, Cr_2O_3, P_2O_5, and MgO content were negative, while correlation between Na_2O content and mortality was generally not significant (Rojht et al., 2010b).

The efficacy of Slovenian quartz sands admixed with stored wheat was tested against rice weevils (*Sitophilus oryzae*) in laboratory conditions. Five different samples of quartz sand of different ages were tested. Samples from the location Raka-Ravno (with admixture and clean) and the location Moravče (with admixture and clean) and commercially available cleaned quartz sand from the locality of Puconci (Plantella) each were used at six concentrations: 100, 300, 500, 900, 1200, and 1500 ppm. The amount of SiO_2 in all sand samples was high and varied from 91.52 to 99.24%. For each dose rate, the treated wheat grains were placed at four temperatures (20, 25, 30 and 35°C) and at 55 and 75% relative humidity level. After 7, 14 and 21 days of exposure dead adults were counted. All samples showed only a slight insecticidal effect on adults of rice weevil and are not appropriate for wider use against rice weevil adults in stored wheat. The highest mortality (15%) of rice weevil adults was confirmed 21 days after treatment at 900 ppm, 30°C and 55% RH level for quartz sand with admixture from the Moravče location (Rojht et al., 2010c; Rojht et al., 2011).

In the search for an effective and sustainable control method against the bean weevil, *Acanthoscelides obtectus* (Say), three different powders were tested against adults under laboratory conditions. The three powders were diatomaceous earth (DE) (commercial product SilicoSec®), common lavender (*Lavandula angustifolia*) powder and field horsetail (*Equisetum arvense*) powder. The substances were tested at five temperatures (15, 20, 25, 30, and 35°C), two relative humidity levels (RH) (55 and 75%), and four concentrations (100, 300, 500, and 900 ppm). The mortality of adults was measured after the 1st, 2nd, 4th, and 7th days of exposure. The efficacy of the powders increased with the temperature, whereas in general, RH did not have a significant effect on the adults' survival. According to common practice of storing common beans, we recommend the use of DE against the pest in question, as this inert powder showed the highest efficacy at lower temperatures and concentrations. Concerning the wider use of common lavender and field horsetail powders, we suggest studying their combined use with other environmentally friendly methods with the aim of achieving the highest synergistic effect possible (Trdan and Bohinc, 2011; Bohinc et al., 2013).

In the search for an effective and sustainable control method against the maize weevil (*Sitophilus zeamais* Motschulsky), an important insect pest affecting stored grain, different inert dusts were tested under laboratory conditions. We treated wheat grains with quartz sand, zeolites, and diatomeaceous earth. Inert dusts of different origins were used, namely diatomaceous earth from Slovenia and SilicoSec, quartz sands from two locations from Slovenia, and three different zeolites (two types of natural zeolite from location in Slovenia, and synthetic zeolite Asorbio®). Untreated winter wheat grains served as control treatment. The substances were tested at three different temperatures (15, 20 and 25 °C) and two different relative humidity levels (55 and 75%). Mortality was measured 7th, 14th and 21st day after exposure. Inert dusts were applied at two different concentrations, 450 and 900 ppm. The analysis of pooled results provoked significantly the highest mortality of beetles in treatments with SilicoSec® (52.31 ± 2.07%), and in treatment with one type of Slovenian zeolite (31.48 ± 1.42%). The lowest mortality was recorded in treatments with quartz sands from both Slovenian locations, Moravče (18.84 ± 1.31%), and Raka (9.12 ± 0.66%). Mortality of *S. zeamais* was significantly the highest in treatments exposed to 25 °C (28.32 ± 1.16%), and in treatments exposed to higher concentrations (900 ppm) of inert dusts (27.30 ± 0.87%). The use of diatomaceous earth is well established in stored products pest management, however the knowledge on the efficacy of zeolites is very week and offers a lot of opportunities for future researchers (Trdan et al., 2015).

The effectiveness of three different wood ashes from black locust (*Robinia pseudoacacia*), beech (*Fagus sylvatica*), and Norway spruce (*Picea abies*) were evaluated on maize weevil (*Sitophilus zeamais*) regarding adult (2-4 weeks old) mortality. Diatomaceous earth served as positive control. We have tested wood ashes as surface treatment (10 and 20 g/m2) and as admixtures (2.5 and 5 w%). Mortality of weevils, when wood ashes were applied as surface treatment was evaluated every day till 7th day of application, and every day till 14th day of application (as delayed mortality). When wood ashes were admixed, we have evaluated mortality after 7, 14 and 21st day. Research was

performed at two different relative humidty values (55 and 75%) and at three different temperatures (15, 20 and 25 °C). Based on the results of our survey we conclude that mortality of *Sitophilus zeamais* adults was influenced by wood ash species, air temperature and relative humidity. As surface treatment, 99.69 ± 0.31% mortality was achived at treatment with Norway spruce on day 7 at 25 °C. When admixed, 100% mortality was achieved on day 14, when Norway spruce's wood ash has been applied at 25 °C. Use of wood ash as stored product protectant proved to be efficient in our survey, although additional reseach should be made (Bohinc et al., 2017a).

Laboratory experiment was carried out to evaluate the impact of zeolites of different origin on the mortality of the maize weevil (*Sitophilus zeamais* Motschulsky) adults. We have tested the efficacy of natural zeolites (Slovenian and Serbian) and synthetic zeolites ('Asorbio'). Diatomaceous earth (product SilicoSec® was used as positive control). We have applied zeolites as surface treatment (at concentrations 10 and 20 g/m2) and as admixtures (at concentrations 450 and 900 ppm). Mortality of weevils, when zeolites were applied as surface treatment was evaluated everyday till 7th day after application, and everyday till 14th day after application (as delayed mortality). When zeolites were admixed, we have evaluated mortality after 7th, 14th and 21st day. Research was performed at two different relative humidty values (55 and 75%), and at three different temperature (15, 20 and 25 °C). We conclude that mortality of maize weevil adults was influenced by higher temperature values and lower relative humidity value. When we have applied 'Zeolite Slovenia' (at 900 ppm, 15 °C, 55% Rh) as admixture we have recorded 69.69 ± 7.04% after day 21, meanwhile mortality reached 83.66 ± 3.21% after day 21, when 'Zeolite Slovenia' was applied at 25 °C. 100% mortality of maize weevil adults was recorded, when 'Zeolite Slovenia' (after day 7 at 25 °C) was applied at surface. There was no impact of zeolite's dose on mortality of maize weevils. Mortality of weevils was alike in two natural zeolites (Slovenian and Serbian), meanwhile mortality of maize weevils was the lowest in treatments with 'Asorbio'. Use of natural zeolites proved to be efficient as stored product protectant in our research, although additional surveys should be made (Bohinc et al., 2017b).

Laboratory experiment was carried out to evaluate the insecticidal efficacy of different environmentally acceptable substances on the mortality of the granary weevil (*Sitophilus granarius*) adults. We treated wheat grains with diatomaceous earth (commercial formulation SilicoSec®), quartz sand, leaf powder of neem tree (active ingredient azadirachtin, commercial formulation Neem listni prah®), and wood ash. Wheat grains were also treated with combination of diatomaceous earth and wood ash, combination of leaf powder and wood ash, quartz sand and wood ash and with a combination of four different substances (diatomaceous earth, wood ash, leaf powder and quartz sand). Substances were applied at different concentrations. Mortality of the granary weevil adults was tested at 3 different temperatures (20, 25 in 30°C) and at 2 different relative humidity levels (55 and 75%). Mortality was evaluated 7, 14 and 21 days after exposure. We have detected significant impact of different substances on the mortality of the beetles. Significantly the highest mortality of the beetles was evaluated in treatments with wood ash in single or combined use, i.e. individual use of 2,5 w% wood ash (69.73±2.52%), and combined uses of diatomaceous earth (450 ppm) and 2.5 w% wood ash (71.94±2.40%), quartz sand (450 ppm) and 2.5 w% wood ash (68.72±2.80%), and diatomaceous earth (225 ppm), wood ash (1.25w%), leaf powder (0.625 w%), and quartz sand (225 ppm) (68.76±2.75%). We established that wood ash in single or combined use can perform environmentallyacceptable alternative to synthetic insecticides in controlling granary weevil adults, however for final confirmation of this thesis we have to study the activity of the substances against the eggs and the larvae of the pest (Trdan and Bohinc, 2013, 2014; Bohinc and Trdan, 2017).

2.4. Essential oils and helbal extracts

Fumigant toxicity of essential oils from *Rosmarinus officinalis, Salvia officinalis, Laurus nobilis, Citrus bergamia*, and *Cinamomum camphora* against *Acanthoscelides obtectus* adults reared on common bean seeds was assessed. Properties of essential oils were tested at two different dose rates (245 and 980µl/l). Insecticidal efficacy was tested at five different temperatures (15, 20, 25, 30, and 35°C)

and two relative humidity (RH) levels (55 and 75%). Responses varied with type of essential oil, time of exposure, dose of essential oil, as well as with temperature and relative humidity levels. Three days after treatment over 90% adult mortality was achieved. An essential oil from rosemary gave over 94% efficacy after three days. At 75% relative humidity essential oils were significantly more effective than at 55% relative humidity level. The plant essential oils described in this paper could be useful for managing populations of *A. obtectus* in warehouses (Trdan and Bohinc, 2012; Bohinc and Trdan, 2013).

A trial was conducted to assess the fumigant toxicity of the essential oils from *Rosmarinus officinalis* L., *Salvia officinalis* L., *Lavandula angustifolia* Mill. and *Mentha balsamea* Willd. against the adults of *Sitophilus granarius* (L.). The relationships between the time after treatment (1, 2, and 3 days), temperature (20, 25, 30, 35, and 40°C), concentration of essential oils (2.4 and 7.4 ml/L air) and mortality were investigated. In the experiment, the efficacy of the essential oils at 40°C was 95%, whereas their efficacy was considerably lower at lower temperatures (from 12 to 36%). Throughout the experiment, the essential oil of rosemary proved to be the most effective fumigant, causing more than 60% mortality of the granary weevil adults. When applying the essential oil of rosemary, more than 50% mortality in the adults of granary weevil was attained at 35°C (89%) and 40°C (99%). A satisfactory efficacy of the other essential oils, common lavender (90%), peppermint (97%) and common sage (94%), was attained only at the highest temperature. The activities of the essential oils were better at higher concentrations (36%) than at lower concentrations (32%). When assessing the effect of the concentration on the adult mortality, we achieved more than 50% efficacy only with rosemary (2.4 ml/L of air, 58%; 7.4 ml/L of air, 63%). The data for the other essential oils ranged between 19% (peppermint, 2.4 ml/L of air) and 34% (common sage, 7.4 ml/L of air). The calculated values for the LC_{50} and LC_{90} showed that only rosemary produced satisfactory fumigant activity on the adult granary weevils, especially in relation to the temperature. However, the positive efficacy identified in our laboratory experiments needs to be validated under conditions similar to those of the applied conditions, that is, warehouses (Laznik et al., 2012).

Ethanol extracts of *Rosmarinus officinalis*, *Lavandula angustifolia* and *Ruta graveolens* were tested against adults of *Acanthoscelides obtectus*, an important insect pest affecting stored common beans and other legumes. Using a newly developed computer tracking system, a choice test revealed that all of the extracts have a repellent action. The highest repellent activity against the bean weevils adults showed the ethanol extract of rue. We suggested that a cocktail of volatile components in the ethanol extracts was responsible for the observed repellent action. All three of the extracts have insecticidal effects on bean weevils, reducing F1 adult emergence, with no side effects on the germination of the bean plants (Rojht et al., 2012b).

2.5. Biological control agents of stored products pests

In Slovenia, biological control agents, whose introduction, rearing and use are permitted according to Rules on biological control of plant pests (Official Gazette RS No 45/06), are classified in the List of indigenous biological control agents. Since 2006, when the first List was composed, until 2015 the number of indigenous biological control agents increased for 11 species (to present 25 species). The knowledge about occurrence and distribution of indigenous natural enemies is the key factor for their implementation in food and ornamental plants production systems (Trdan and Bohinc, 2016).

In 2012, we established the first record of parasitoid wasp *Dibrachys microgastri* (Boche, 1834) in Slovenia. The wasp was detected in the Laboratory of Entomology (Biotechnical Faculty in Ljubljana) in the rearing containers filled with wheat grains, which are used for reproduction of population of the granary weevil (*Sitophilus granarius*) (Trdan et al., 2013). During 2013 and 2014, we first recorded nine beneficial organisms in Slovenia, among them also parasitic wasp *Anisopteromalus calandrae* Howard. In our opinion this insect species has the potential for future implementation in plant production, since it is already used in some European countries in biological control of some stored products beetles like *Sitophilus* species and *Rhyzopertha dominica* (Bohinc and Trdan, 2015).

2.6. Environmentally acceptable insecticides

The efficacy of spinetoram and spinosad against 2-4 weeks old *Sitophilus* adults has been tested under laboratory conditions. Spinetoram and spinosad were applied at three different dose rates (0.5., 1 and 2 mg/kg). Experiment was performed at 25 °C and 65% rh, on four different winter wheat varieties. Mortality counts were assessed on day 7, day 24 and day 21. Our research demonstrated impact of grain type, dose of exposure, day of evaluation and *Sitophilus* species on mortality of weevils. Mortality of weevils was higher in treatments treated with spinetoram (90.19 ± 0.48%). After day 21, spinosad caused 91.64 ± 0.93% mortality, meanwhile 96.13 ±0.51% mortality was detected in spinetoram treatment after day 21. When applied spinosad as 2 mg/kg, 96.35 ± 0.44% was detected. Spinetoram caused 96.79 ± 0.38% at 2 mg/kg. Efficacy of spinosad (69.47 ± 1.87%) and spinetoram (78.23 ± 0.83%) was the lowest on variety 'Fidelius'. Spinosad caused the highest mortality in treatments with Serbian maize weevil (96.64 ± 0.31%), meawhile spinetoram prooved to be the most efficient in treatments with Serbian rice weevil (94.58 ± 0.77%) and Serbian granary weevil (94.07 ± 0.64%). Our resarch on the efficacy of spinetoram and spinosad against stored product insect pests is the first one for Slovenian agriculture. It presents good basics for further studies on implementation of tested insecticides as protectant of stored grains in Slovenia (Trdan et al., 2017).

2.11 Other types of investigation

In a study the effects of the production systems of wheat from different production systems on the mortality, progeny production and preference of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) were evalueted. The factors tested were production system (integrated [INT], organic [ORG], biodynamic [BD] and control), which differed in plant protection and fertiliser procedures during plant growth and development; exposure interval (7, 14 and 21 d); relative humidity (r.h.) (55% and 75%) and temperature (20°C, 25°C and 30° C). Mortality after 7 d increased with the temperature increase and decreased with the increase in r.h. in most of the tested combinations. The mortality of weevils was higher in ORG compared to INT-produced wheat after 7 d. Progeny production was recorded 56 d after removal of parental adults and was higher at 75% r.h. in comparison to 55% r.h. At 55% r.h. and 20°C, progeny was 60.8% higher when *S. zeamais* were exposed to ORG in comparison to INT-produced wheat. Wheat from different production systems influenced mortality rates which were higher in alternative compared to INT production systems under optimal conditions for wheat storage (low temperature and r.h.). The reverse was recorded for temperature and r.h. increase. Progeny was not affected by wheat from various production systems. Significantly more *S. zeamais* adults were found in traps containing wheat from BD and control in comparison to INT. An understanding of the agricultural processes, biotic and abiotic factors which alter the post-harvest response of storage pests could be useful for the development of efficient post-harvest strategies for ORG and BD farms and the processing industry (Turinek et al., 2016).

2.8 Organization of workshops for agricultural specialists and IOBC Conference

In 2017, we have organized the 11th Conference of the IOBC/wprs Working Group on Integrated Protection of Stored Products (Ljubljana, 3-5 July), which was attended by 136 participants from 25 countries (Trdan and Trematerra, 2017; Trematerra and Trdan, 2018). We also transfer knowledge to Slovenian agricultural specialists about the harmfulness and possible ways of controlling stored products insects pests. In 2014, we have organized a workshop on this topic ("From Technological Maturity to Storing of Cereals and Legumes") (Trdan, 2014ab), however even three years before we transfered our knowledge in the field of stored pests protection to Slovenian agricultural specialists in a siminal event (Trdan and Bohinc, 2011). In 2015, we have hosted C. Athanassiou as an invited lecturer at the 12th Slovenian Conference on Plant Protection with international participation in Ptuj (Athanassiou, 2015; Trdan, 2015ab).

2.9 Cooperation with foreign experts

In 2007, we participated as partners in the project SEE-ERA.NET "Development of a non-toxic, ecologically compatible, natural-resource based insecticide from diatomaceous earth deposits of South Eastern Europe to control stored-grain insects pests" (coordinated by C. Athanassiou), and we thus became acquainted with the research work in the field of investigation the efficacy of diatomaceous earth in controlling beetles from the *Sitophilus* genus (Athanassiou et al., 2009, 2011).

The opportunity to reduce the amount of pirimiphos-methyl applied to grain by formulating it in an electrostatic powder was investigated in a study of international research group. The insecticidal efficacy of pirimiphos-methyl in EC formulation or formulated using electrostatic powder (EP) as an inert carrier was investigated against *Sitophilus oryzae* (L.), *Oryzaephilus surinamensis* (L.), *Rhyzopertha dominica* (F.) and *Tribolium confusum* Jacquelin du Val. Furthermore, the adhesive properties of EP to rice, corn and wheat, together with the effect on bulk density and bread- and pasta-making properties, were investigated. The results showed that pirimiphos-methyl formulated with EP provided better efficacy against adultswhencompared with EC formulation for O. surinamensis and T. confusum, but there was no difference for R. dominica. Progeny production was consistently lower in grain treated with the EP formulation than in grain treated with the EC. Tests showed that EP adhered to the kernels for longer on hard wheat than on maize or rice. In most commodities, EP did not alter the bulk density. Finally, the addition of EP did not affect flour- and bread-making properties, nor the pasta-making properties. The results of the present study suggest that an EP could be used to reduce the amount of pirimiphos-methyl applied to grain for effective pest control, with no detrimental effects on grain quality (Athanassiou et al., 2016, 2017).

The study of Slovenian-Serbian research group focused on examining of spinosad and spinetoram efficacy after 21 days of *S. granarius* and *S. oryzae* adults exposure in treated wheat grain and their influence on weevils offspring production and wheat grain damage rates. Investigation was conducted under laboratory conditions at 25±1°C and 70±5% r.h. Both insecticides were applied to untreated wheat grain with 12.3±0.1% of m.c. at the rates of 0.5, 1.0 and 2.0 mg a.i./kg for both weevil species. Then, 25 adults were added to each plastic dish containing 50 g of treated wheat, in six replicates, for each insecticide/species tested. Mortality of weevils was determined after 21 days, and the effect on progeny production was determined seven weeks after parental exposure. When the offspring were counted, damage caused by the weevils were also assessed on 100 randomly selected kernels. Spinosad and spinetoram demonstrated the highest mortality (96-100%) of *S. granarius* and *S. oryzae* parents after 21 days of contact with 1-2 mg/kg and 2 mg/kg, respectively. The highest *S. granarius* offspring reduction (>90%) was found in wheat treated with 2 mg/kg spinosad and 1-2 mg/kg spinetoram, while S. oryzae offspring reduction was the greatest in wheat treated with 2 mg/kg spinetoram. In these experimental conditions, the percentage of grains damaged by *S. oryzae* was ≥50% in wheat treated with 0.5-1 mg/kg spinosad and 0.5 mg/kg spinetoram, while grain damage below 5% was found only in wheat treated with 2 mg/kg spinetoram. The results show that spinetoram was more effective than spinosad. Also, *S. granarius* was more susceptibility to both insecticides than *S. oryzae*. Under these experimental conditions, spinosad and spinetoram can be successfully used to control both weevil species at the rate of 2 mg/kg (Andrić et al., 2016, 2017).

Discussion

In 12 years of our work in the field of stored product pest control, our laboratory studies most often included three species from the genus *Sitophilus* (*S. granarius, S. oryzae* in *S. zeamais*) and the bean weevil (*A. obtectus*), i.e. harmful organisms which are in Slovenia of great economic significance. The economically harmful butterflies (E. *kuehniella, P. interpunctella* and *S. cerealella*) have been so far addressed in only one study. The main body of our research work included studying insecticidal properties of diatomaceous earth and other inert dusts, e.g. quartz sand, plant powders, wood ashes and zeolites. Diatomaceous earth was due to its efficiency often used as a positive control when studying the efficiency of other inert dusts. Considerable fumigant effects on harmful beetles were

in our laboratory experiments displayed also by some essential oils, particularly rosemary essential oil. In our professional work, in which we have been systematically sampling autochthonous natural enemies for more than 10 let years, we found two parasitoids of stored products beetles. In our view, the more important of these two is *Anisopteromalus calandrae*, which is already being systematically introduced in cereal storages in some European countries, while the Slovenian legislation in the field of biological control do not yet enable its use in practice. By organising expert meetings for the needs of the domestic experts who study the significance and control of stored product pests, we provide implementation of expert knowledge into practice, and we were delighted when the IOBC-WPRS entrusted us with the organisation of the 11th Conference of the IOBC/wprs (OILB/srop) Working Group on Integrated Protection of Stored Products (Ljubljana, Slovenia, 3-5 July 2017), which is also a result of our recognisability and our good cooperation with foreign experts. Studies in the field of monitoring and control of stored product pests will remain a part of our research-professional activities, as this group of harmful organisms will be also in future, due to the increasing world population, intense international trading in plant materials, climate changes and some other factors, one of the economically most important groups of plant pests.

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Investigations on the efficacy of Turkish diatomaceous earth comparing with SilicoSec? against the stored grain pests

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Abstract

In this research, both Turkish diatomaceous earth from Central Anatolia and SilicoSec? was evaluated against *Tribolium confusum* Jacquelin du Val (Tenebrionidae: Coleoptera), *Rhyzopertha dominica* (F.) (Bostrychidae: Coleoptera) and *Sitophilus granarius* (L.) (Curculionidae: Coleoptera) adults. Different rates (0, 250, 500, 750, 1000, 1500 ve 2000 mg/kg wheat) of diatomaceous earth from both sample mixed with wheat were evaluated for 1, 2, 3 and 4 -weeks exposure period at 55% r.h. and 25 adult/vial insect density in ten replicates. Mortalities were determined at the end of exposure, whereas F1 adult production were determined at 8 weeks after mortality

observation. Mortalities and F1 adults of *T. confusum*, *R. dominica*, and *S. granarius* was significantly changed by the rates of diatomaceous earth. In untreated wheat mortality was less than 10% for three species. Mortalities at 2000 mg/kg wheat applications at the end of 4-week of exposure against *S. granaries* were found to be 95.6% and 98% for SilocoSec and local DE, respectively. For *R. dominica* adults, mortalities were recorded as 90% and 92.4% for SilocoSec and local DE, respectively. Additionally, mortality of *T. confusum* at 2000 mg/kg wheat was 100% for both DE samples. Results showed that local DE and SilocoSec are equally effective against three major stored grain pests.

The Effectiveness of Silicosec, Diatomaceous Earth Against the Lesser Grain Borer, *Rhyzopertha dominica* (L) (Coleoptera: Bostrichidae)

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Abstract

In this research, the efficacy of SilicoSec was assessed in two sets of experiments. In the first set of experiment, the efficacy of SilicoSec at the rates of 0, 250, 500, 1000, 1500, 2000, 2500 and 3000 mg/kg wheat were evaluated against *Rhyzopertha dominica* adults at 55% r.h and 25°C. Insect counts were performed at the end of 3 week of exposure for the mortality. F1 progeny assessment were made after 8 weeks.

In the second set of experiment, the efficacy of SilicoSec against the same insect pest was evaluated at dose rates of 0, 250, 500, and 1000 mg/kg wheat for three months of exposure. In each experimental vial there were 10 adults per 250 g wheat with 10 replicates. Insect counts as dead and alive were made at the end of three months of exposure. For each vial, progeny production was determined as total insects excluding 10 adults introduced at the beginning of the experiment.

According to results, increase in dose rates increased the adult mortality, while it decreased the progeny production. As the exposure time increased, mortality rates were also increased. At the dose of 2000 mg/kg, 98.5% adult mortality and 10.55 adult progeny per vial were obtained.

For three months exposure, population development was inversely proportional to dose rates. According to results, increase in dose rates increased the adult mortality, while it decreased the progeny production. Population confinement was achieved at 1000 mg/kg dose rate of SilicoSec for three months of exposure.

Key words: Diatomaceous earth; *Rhyzopertha dominica*; exposure interval; progeny production; mortality

Host-preference and parasitic capacity of five *Trichogramma* species (Hym.: Trichogrammatidae) against some stored product moth pests

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Abstract

Most stored product insects are either beetles or moths. Moth pests are important hazards to the storage of a wide variety of products. Natural enemies are applied commercially against stored-product moths in Central Europe .So, the host-preference and parasitic capacity of four local *Trichogramma* spp.(*T. bourarachae*, *T. cordubensis*, *T. euproctidis* and *T. cacoeciae*) , towards four species of stored product moth eggs were investigated in laboratory experiments in order to select new candidate species for use in mass rearing and biological control against moths in storages. The results were compared with *T. evanescens,* the common wasp used commercially for biological control. The naturally occurring *Trichogramma* species were collected for the first time in Egypt from two representative olive growing areas in arid area (170 km south of Alexandria) and semi-arid area (60 km west Alexandria, near the coast). All these wasps were also bred from naturally parasitized host eggs during

favorable and even at unfavorable temperature conditions of June-August. The presence of warm weather wasp-strains may suggest the existence of well-adapted wasp species or strains which may be appropriate candidates for the control of stored product pests. The strains had also been collected in late winter and summer, thus demonstrating activity also during less favorable weather conditions, raising again the possibility of using these egg parasitoids as an inundative biological control agent in stored products.

Experiments were carried out by offering eggs of the Indianmeal moth *Plodia interpunctella* (Hübner), the Mediterranean flour moth *Ephestia kuehniella* Zeller, the warehouse moth *E. elutella* (Hübner), and the almond moth *Cadra cautella* (Walker) in choice and no-choice assays to a single female parasitoid. Two different choice experiments were used to certify the same conclusion in both methods. The bioassay for host-preference of *Trichogramma* spp. was carried out by offering a single female wasp the choice between equal numbers of host eggs on square cards "Petri dish tests "and /or strip cards "strip card tests". In both methods ,countingthe number of*Trichogramma* developing in the host eggs (parasitism) show the preference of the wasp for ovipositingand indicated theabilityof theparasitoid todevelopin theseeggs (i.e., host suitability).

In Petri dish tests, *E. kuehniella* was a highly accepted host species for *T. bourarachae*, *T. euproctidis*, and *T. cacoeciae* wasps while *E. elutella* and *C. cautella* eggs were more accepted by *T. evanescens* and *T. cordubensis,* respectively. In the strip card tests, *E .kuehniella* eggs were highly accepted by *T. bourarachae, T. cacociae* and *T. evanescens*. Eggs of *E. elutella* and *C. cautella* were more acceptable for *T. euproctidis* and*T. cordubensis,* respectively. Furthermore, a comparative study of the parasitic capacity of the *Trichogramma* spp. was carried out under 'no choice conditions' by exposing a freshly emerged single wasp to an unlimited number of host eggs. Significant differences were found among the parasitic capacity of the tested *Trichogramma* spp.: *T. bourarachae* showed a good parasitic potential against *S. cerealella* and *E. kuehniella; T. evanescens and T. cacoeciae* against *S. cerealella*; *T. cordubensis* against *S. cerealella* and *P. interpunctella* and *T. euproctidis* against *P. interpunctella.* However, dissection of host eggs with wasp-emergence holes showed that all tested wasps had a propensity to superparasitize the host eggs. Results of the present work suggest that the test wasps failed to discriminate parasitized hosts eggs among a large number of non-parasitized eggs, thus superparasitism occurred. Also, both of Petri dish and strip cards methods may underestimate the actual parasitization capacity due to self-superparasitism and mortality in black eggs that suffered desiccation during the early stages.

T. cordubensis, *T. euproctidis* and *T. bourarachae* showed promise for further investigation into selecting new biological control agents against some stored product lepidopterous pests.

Keywords: Stored product moths; *Trichogramma* spp.; host preference; parasitization capacity; superparasitism.

Monitoring of the Indian meal moth and its parasitoids in long-term grain storage

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Abstract

The Indian meal moth *Plodia interpunctella* became a major pest in bulk grain storage in Germany in recent years. Monitoring with adhesive pheromone-baited traps revealed a dependence of the number of generations of the moth from the temperature conditions in store, which themselves depend on insulation of the storage structure. The larval parasitoid *Habrobracon hebetor* was monitored with the help of cone traps placed in the grain. Baiting these traps with moth webbings significantly increased the number of female wasps trapped in 5 cm depth in wheat. Field trials showed both the pest and the beneficial can be monitored in stores, but more research is needed to develop a biological control strategy for *P. interpunctella*.

Keywords: stored products, bulk grain, Pyralidae, Trichogrammatidae, Braconidae

Introduction

Monitoring of pest populations is a basic prerequisite for biological control of stored-product pests (Zimmermann 2004), but difficult in large quantities of bulk grain. Within the frame of a project on the application of beneficials in long-term grain storage of grain, the phenology oft the Indian meal moth *Plodia interpunctella* (Hübner, 1813) (Lepidoptera, Pyralidae) was studied in different grain flat

stores. In German grain storage, large populations of the Indian meal moth were observed in the past six years resulting in significant damage and repeated control activities, consequently new control options were evaluated. The Indian meal moth is laying up to 400 egges close to the stored product. The emerging larvae typically develop through five instars in grain. A first signal of infestation are webbings on the grain surface produced by the larvae, produced presumably for protection. The last larval instar shows increased mobility in order to find a pupation site, and is consequently called wandering larva (Mohandass et al., 2007).

For the development of a biological control strategy a monitoring oft he beneficials is helpful. The larval parasitoid *Habrobracon hebetor* (Say, 1836) is naturally occuring in Central Europe, among its hosts are the Indian meal moth, the warehouse moth *Ephestia elutella* (Hübner, 1796) and the flour moth *E. kuehniella* Zeller, 1879 (Prozell & Schöller 2001).

In this study, the effectiveness of different monitoring techniques for parasitoids is described and the potential release of benefials within the frame of an integrated control concept discussed.

Materials and Methods

Monitoring of the Indian meal moth *P. interpunctella*: The phenology was studied in different flat grain stores. Various methods were applied, i.e. adhesive surfaces for larvae and adults, artificial pupation aid structures for larvae, and pheromone-baited traps for adults (Fig. 1).

Monitoring of the larval parasitoid *H. hebetor*: 20 kg grain, wheat or oats, respectively, were filled in a Hobbock. Per Hobbock, one cone trap was placed (Fig. 1). The cone trap has two components, a vial and a perforated lid. The lid is regularly vaulted, this is where the insect enter. The diameter of the holes in the lis is approximately 2 mm. Liquid teflon is added to the upper rim oft he vial, in order to hinder the insects to move back tot he lid. The cone trap was placed either on the grain surface, or 5 cm deep in the grain. The cone traps on the surface were carefully placed in one level with the grain surface. The cone traps were either unbaited, or baited with 5 g of webbings of the flour moth. Per Hobbock, 50 *H. hebetor* were released, i.e. 25 females and 25 males. The Hobbocks were closed and stored for 14 days at 20.8°C ± 0.95°C (mean ± SD) and 43.6% ± 5.5% RH (mean ± SD). After that period, the cone traps were removed and the traped *H. hebetor* counted. The sex ratio of *H. hebetor* was determined. Moreover, six cone traps each were placed in different grain flat stores (3000–4000 metric tons wheat) and removed ca. four weeks after parasitoid release, again the number of insects traped was counted.

The number of trapped insects were compared with the help of a t-test, in case data were not normally distributed, the Mann-Whitney Rank Sum Test was applied. Statistical analyses were performed using the software package SigmaStat 3.1.

Fig. 1 Cone trap used for monitoring of *Habrobracon hebetor* (a) and adhesive trap baited with sex pheromone (ZE-TDA) (b) for monitoring of *Plodia interpunctella* in flat grain storage.

Fig. 2 Records of adult *Plodia interpunctella* (green bars) in a non-isolated store in Northern Germany, 2015. Left axis: surface temperature [°C], right axis: no. of moths. Blue line: mean temperature, red line: maximum temperature.

Results

Monitoring *of P. interpunctella*: In a non-isolated storage building in Northern Germany, adult moths were recorded already in June, 2015 (Fig. 2), while in a well isolated storage building in Southern Germany, adult moth activity started in August (Fig. 3). At that time, a second generation of adults developed already in the non-isolated store (Fig. 2). A peak in the number of adult moths in the non-isolated store was reached in August.

While the F_2-moth progeny in the non-isolated store overwintered in the last larval instar, only one moth generation was recorded in the well-isolated grain store (Fig. 3).

Monitoring of Habrobracon hebetor:

(1) Unbaited cone traps: both in wheat (t-Test, t = 8.061, DG=4, P<0.001) and in oats (t-Test, t = 8.061, DG=4, P<0.001), significantly more wasps were traped in 0 cm compared to 5 cm depth (Fig. 4). On the surface, significantly more wasps were traped in wheat compared to oats (t-Test, t = -3.742, DG=4, P = 0.020). In 5 cm depth, no difference in recapture was detected comparing wheat and Oats (t-Test, t = -0.707, DG=4, P > 0.05).

(2) Baited cone traps: baited with webbings, cone traps on the surface, caught more wasps in oats compared to such unbaited traps on the surface (t-Test, t = -8.721, DG=4, P <0.001) (Fig. 4). In wheat, baited traps caught on the surface a larger number of wasps, however, this difference was not significant (t-Test, t = 2.286, DG=4, P = 0.084).

Baited traps placed in 5 cm depth in wheat caugth more wasps compared to unbaited traps (t-Test, t = -3.530, DG=4, P = 0.024). In oats, no difference in wasps caught in traps placed in 5 cm depth was detected whether the traps were baited with webbings or not (t-Test, t = -1.444, DG=4, P = 0.222). More female wasps were caught in total with baits (t-Test, t = -2.119, DG=22, P = 0.046), but the presence of webbings as bait did not increased the number of males traped (Mann-Whitney Rank Sum Test, T = 125.5, n=12, P = 0.163).

Fig. 3 Records of adult *Plodia interpunctella* (red bars) in a well-isolated grain store in Southern Germany in 2015. Left axis: surface temperature [°C], right axis: no. of moths. Blue line: mean temperature, green line: maximum temperature.

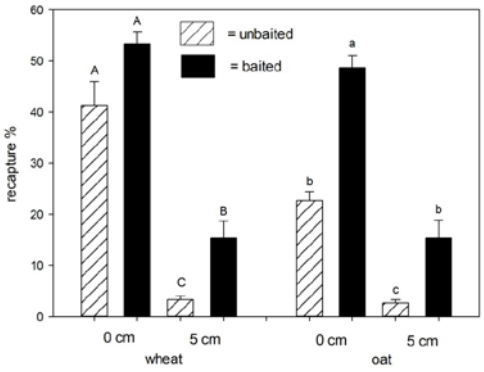

Fig. 4 Recapture of *Habrobracon hebetor* with the help of cone traps in percent, with or without 5 g webbings of *Ephestia kuehniella* as bait in wheat or oats. 50 *H. hebetor* (males and females) released. Different caps indicate significantly different recapture, major caps for wheat, small caps for oats (p<0.05).

Discussion

Under field conditions, pupation of moth larvae and the start of flight of the adult Indian meal moths in spring are mainly depending on temperature in the store. While in a non-isolated store the progeny of the second generation overwintered as larvae, in an isolated cooler store only one generation was observed anually, These observation under practical conditions is well explainable by the developmental time known from laboratory data (Mohandass et al., 2007).

The attractiveness of moth webbings in the cone traps placed in grain on the females confirms olfactometer laboratory trials showing kairomonal activity of webbings produced by different species of pyralid Lepidoptera for *H. hebetor* (Strand et al. 1989). Moreover, foraging H. hebetor were shown to enter into bulk grain in previous studies (Schöller, 2000). In the present study, female *H. hebetor* were shown to exploit signals from moth webbings in bulk grain, too. Consequently, parasitisation of Indian meal moth larvae can be expected below the grain surface, too. This behaviour of *H. hebetor* can also be used to monitor the foraging behaviour of the wasps under practical conditions of storage. In wheat, more *H. hebetor* were traped compared to oats. This might be due to the three-dimensional structure of the bulk grain.

Both male and female *H. hebetor* were caught with the cone traps. The capture of females in unbaited traps indicates this trap type is able to record passivly the movement activity oft the parasitoids. Males could potentially be attracted by already caught females, however, in our trials, a signicantly higher number of females in the baited traps did not result in a significant increase in the number of males caught.

The results on monitoring showed the possibility to record data on the phenology oft he Indian meal moth and *H. hebetor* under practical field conditions. The abiotic conditions in differnt grain stores are subject to wide variation, consequently more field trials are needed in order to develop recommendations for biological control of the Indian meal moth.

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A preliminary study of growth and development of *Cheyletus malaccensis* (Oudemans) under different humidity conditions

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Abstract

Cheyletus malaccensis (Oudemans) is a species of predatory mite, which is widely distributed in grain storage, and is a potential natural enemy of stored-product pests. Based on the typical temperatures and humidities that occur in granaries, the growth and development of *C. malaccensis* was studied at 24°C with different relative humidities (RH 65±2%, 75±2%, 85±2% and 95±2%). During this study, *C. malaccensis* was fed on *Acarus siro* (Linnaeus), a very important stored grain pest to investigate its potential to control this pest and production of this natural enemy in the laboratory. The results showed that *C. malaccensis* has five developmental stages, egg, larva, protonymph, deutonymph and adult. The deutonymph stage is absent in males. For females, the developmental time from egg to adult was shortest at 85±2 % RH and averaged 16.3 days; developmental time was longest at 65±2 % RH and averaged 18.6 days. The male mites in the 95±2% RH trials had the shortest developmental time which averaged 12.6 days; it was longest at 65±2% RH where it averaged 14.7 days. At 95±2

% RH, the male adult lived 83.5 d and its longevity from egg to adult was 95.8 d. Humidity had a significant effect on how long the adults lived and the duration of all developmental stages. At 85±2 % RH, the maximum average number of eggs per female, oviposition period and daily fecundity were 493.0, 46.2 d, and 10.3, respectively. This study provides basic biological parameters for *C. malaccensis*, a potential biological control agent for mite pests infesting stored grain.

Key words: *Cheyletus malaccensis*; development, reproduction, biological control

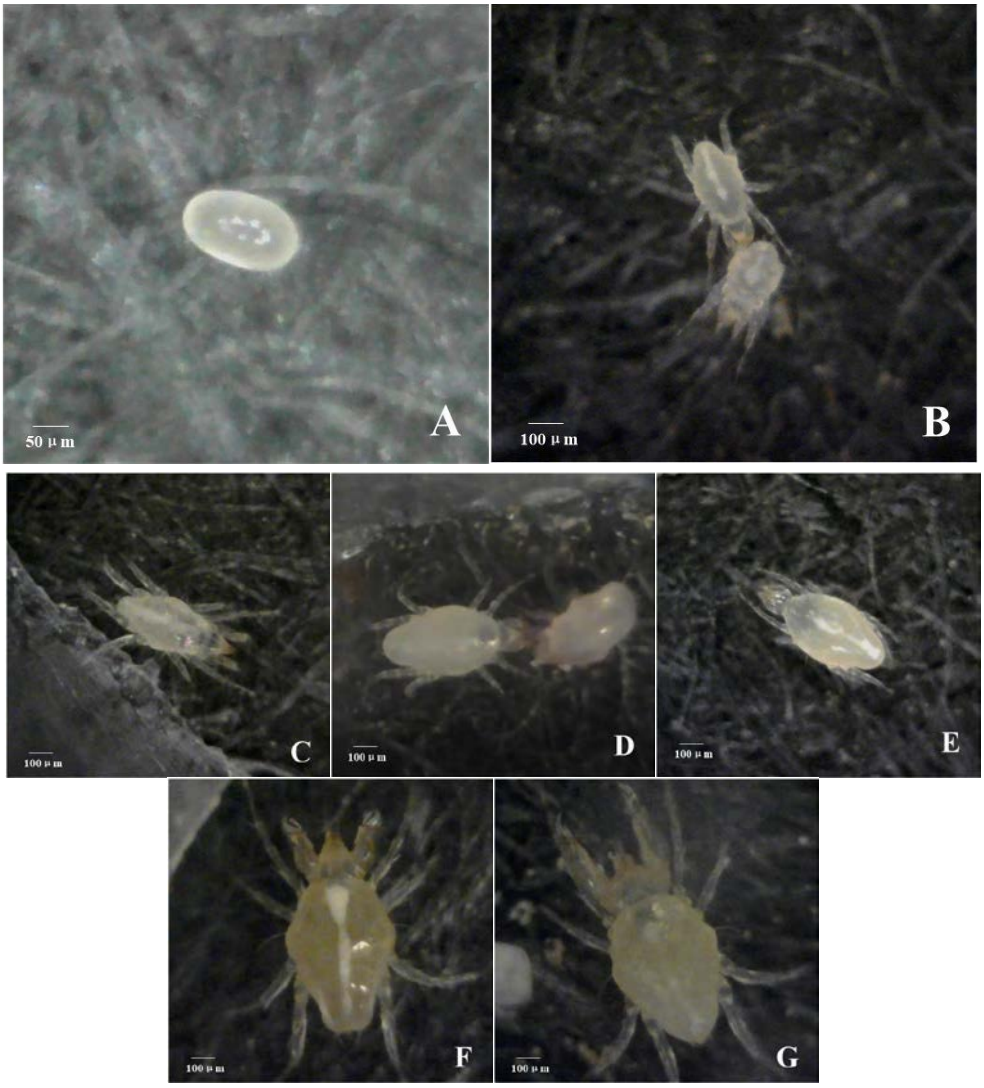

Fig.1 The development stages of *C. malaccensis* , A: Egg B: Larva C: Protonymph D: Deutonymph E: Hypopus F: Female G: Male

Table 1 The developmental duration of *C. malaccensis* **at different relative humidity conditions**

Relative humidity (%)	Egg(d)		Larva				Protonymph				Deutonymph		Life history(d)		Adult(d)		Development duration of all stages	
			Mov. (d)		Qui. (d)		Mov. (d)		Qui. (d)		Mov. (d)	Qui. (d)						
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Female	Female	Male	Female	Male	Female	Male
65±2	2.9±0.26	2.6±0.18	4.2±0.31	5.0±0.37	0.9±0.07	1.6±0.18	4.0±0.38	4.0±0.38	1.1±0.14	1.6±0.38	3.8±0.46	1.6±0.24	18.6±0.48	14.7±0.69	51.8±10.83	47.8±2.83*	70.2±10.79	61.8±2.98*

75±2	3.0±0.00	2.4±0.15	3.3±0.33	4.6±0.47	1.0±0.00	1.4±0.21	3.7±0.33	4.0±0.22	1.0±0.00	1.1±0.09	3.3±0.67	2.0±1.00	17.3±0.33	13.9±0.73	57.0±21.59	40.8±5.69*	74.3±21.26	54.7±5.50*
85±2	2.0±0.38	2.4±0.29	3.7±0.57	5.3±0.92	1.1±0.14	1.3±0.18	3.3±0.36	3.1±0.51	1.0±0.00	1.0±0.00	3.4±0.43	1.7±0.42	16.3±1.27	13.1±1.10	53.8±7.07	71.0±5.80*	70.0±6.72	83.5±6.00*
95±2	2.9±0.14	2.1±0.1	4.3±0.29	3.6±0.29	1.1±0.14	1.1±0.14	3.7±0.18	4.3±0.57	1.7±0.57	1.4±0.20	3.1±0.34	1.3±0.18	18.1±0.46	12.6±0.48	50.5±8.59	83.5±7.53*	68.7±8.39	95.8±7.61*

Note: The data in the table are means±SE. *Mean significantly different ($P < 0.05$).

Table 2 The developmental duration of stages and oviposition between *C. malaccensis* and *C. eruditus*

Prey	Predator	Temperature (℃)	Relative Humidity (%)	Sex	Egg	Larva		Protonymph		Deutonymph		Life history	No. of eggs per female	Oviposition period	Author
						Mov.	Qui.	Mov.	Qui.	Mov.	Qui.				
T. putrescentiae	*C. malaccensis*	24-25	75		4	3.5		3.5		5.5		19.5	73	6	Zhaopeng Shen
	C. malaccensis	25	80±5	Female	4.3	7.5		6.9		6.1		24.8			Palyvos 、Emmanouel
				Male Virgin	3.9	7.4		6.7				18.0	47.6±6.9	15.3±0.6	
				Fertilized	4.3	7.8		7.2				19.3	88.6±10.1	17.5±0.5	
	C. eruditus	24	80		3.34	3.85	1.66	3.32	1.71	2.79	1.65	18.32			Bin Xia
L. destructor	*C. malaccensis*	18-22			5-6	3	2	3-4	2	3-4	3		77-107	14-16	Yanxuan Zhang
	C. eruditus	25	76		3.3	3.5		4.5		4.1			132.8	25.3	Barker
A. ovatus	*C.malaccensis*	25±0.1	75±2	Female	3.3	5.2	1.2	4.5	1.4	3.5	1.6	20-23		10.1±0.3	Saleh.M
				Male	3.3	5.2	1.3	4.4	1.6			15-17			
D. gallinae	*C. malaccensis*	25	80±5		4.74	5.24		4.38		3.96		18.38			Maicon Toldi 、Faleiro
A. siro	*C. eruditus*	24	75		5.0	7.8		7.2		6.3					Peihuan He

Table 3 The oviposition of ***C. malaccensis*** parthenogenetic at different relative humidity conditions

Relative humidity（%）	65±2	75±2	85±2	95±2
No. of eggs per female	418.0±91.90	427.3±178.44	493.0±104.52	348.2±101.06
Oviposition period	45.4±10.57	44.7±18.10	46.2±8.21	34.0±7.39
No. of eggs laid by each female per day	9.5±0.33	9.4±0.99	10.3±1.20	9.0±1.70
Max. no. of eggs laid by each female per day	23.6±1.81	19.3±1.86	20.0±1.00	21.2±3.30
Pre-oviposition	3.2±1.09	2.3±1.15	3.0±1.00	1.3±1.50
Post-oviposition	3.6±2.40	2.7±2.67	7.0±2.67	7.2±1.29

Note: The data in the table are means±SE.

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Evaluation of the potential value of the F_1H and F_2H Diatomaceous earth formulations as grain protectants against *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae)

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Abstract

An insecticidal efficacy of two **newly developed** grain protectant formulations were assessed against lesser grain borer *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) on wheat and corn after 6 months period of. Tested formulations, marked as F_1H and F_2H, based on inert dust, laurel leaves, lavender essential oil, corn oil, silica gel (both F_1H and F_2H) and pyrethrin (only F_2H) were tested at six doses (from 100 ppm to 600 ppm) depending on formulation and type of grain. The appropriate weights of each formulation, were added seperately to plastic containers containing 10 kg of wheat or corn. An initial population of 200 adults of *R. dominica* were added into each container and left under natural environmental conditions for up to 6 months. A commercial diatomaceous earth (DE) insecticide, Celatom® Mn 51, was used for the comparison of the results, in addition to the untreated control. After six months, both formulations showed higher insecticidal effect than DE Mn 51 in corn and in wheat. Furthermore, the initial population of *R. dominica,* introduced in wheat was suppressed almost completely, with only 0.7%-5.3% live adults found, depending on formulations and dose. The order of efficacy was $F_1H>F_2H>$DE Mn 51. Similar suppression of the initial population was recorded in corn, where F_2H was slightly more effective than F_1H with 2.0%-10.6% and 4.1%-9.5% live adults found, respectively. At the same time, in the treatments with DE Mn 51 there were 4.7%-74.7% and 33.4%-56.1% live adults in wheat and corn, respectively.

Keywords: inert dust, botanicals, grain protectant, stored product insects, insecticidal effect

Introduction

Minimising food commodity losses, both qualitative and quantitative, during longer period of storing represents a main challenge for all economies. Stored-product insects play a significant role in postharvest losses, causing losses in grain weight, affects on baking quality and seed viability (Sánchez-Mariñez et al., 1997; Stejskal et al., 2015), which lower cereal market value. The use of synthetic insecticides is globally the most common way of controlling stored product insect pestsof the negative effects of pesticides on stored products includes: toxic residues (Fang et al., 2002), resistant strains within the insect populations (Chaudhry, 2000; Boyer et al., 2012), adverse effect on human health and environment (Fields and White, 2002). Thus there is an urgent need for alternative strategies which would be sufficiently effective against insects but less toxic for the environment.

The use of inert dusts, especially diatomaceous earth (DE), suits most of those requirements. Its main advantages are low mammalian toxicity and stability (Maceljski and Korunic, 1972; Subramanyam and Roesli, 2000) and an efficient insecticidal activity without leaving hazardous residues (Korunic, 1998; Shah and Khan, 2014; Liška et al., 2015; Korunić et al., 2017.). Despite this there are several limitations which hinder wider commercial use of DE for direct mixing with grains and are described by Korunić (2016). Diatomaceous earths, inert dusts in general, have physical mode of action and therefore act more slowly than conventional contact insecticides. Depending greatly on ambient conditions, it could take a several days to control most target insect species (Korunić et al, 2016), providing enough time for oviposition. Further, there are different sensativities of insect species to DEs, varying effects of DE on insects depending upon the commodity being treated and a negative effect on bulk density (Korunic, 2016; Korunić et al, 2017). Possible solutions for minimising or avoiding those implications include incorporating DE with other methods, such as extreme temperature (Dowdy, 1999), mixture with synthetic insecticides (Athanassiou, 2006; Korunic and Rozman, 2010), mixture with entomopathogenic fungi (Batta and Kavallieratos, 2018) or with botanicals (Korunic et al, 2014; Adarkwah et al, 2017).

Most experiments with inert dusts and their mixtures are carried out in controlled conditions, and less in the real conditions where various environmental and storage conditions could impact efficacy and subsequently, storage duration of the commodity.

The objective of this study was to test insect activity of two new developed formulations based on Croatian inert dust, bay leaves, lavandin essential oil, bait, corn oil, silica gel and pyrethrin against *R. dominica* in wheat and corn grain after six months period of storage.

Materials and Methods

Test insects

A local strain of *R. dominica*, was used in the experiments. Insects were reared on clean soft whole wheat kernels under controlled conditions (28±2°C, 65±5% RH, in dark). Two hundred, unsexed adults (7-21 days old) were used for each treatment.

Commodity

Locally available commercial corn and soft wheat were used in the treatments. Commodities were sifted prior to tests in order to segregate broken kernels and other impurities. Grain moisture content and grain temperature were measured by the GAC 2100-Agri Grain analysis computer (Dickey-john). The initial measurements were 11.1 % m. c. and 23.2°C for wheat, and 10.7 % m. c. and 23.3°C for corn. Ten kg of clean wheat and corn grain was used for each treatment.

Formulations

Two powder formulations labelled as F_1H and F_2H, were based on an inert dust of Croatian origin, dried and milled bay leaves, essential oil of lavandin (Lavender x Intermedia), bait, corn oil, silica gel

and pyrethrin (only F_2H). A commercial DE insecticide, Celatom® Mn 51, was used for the comparison of the results. It belongs to a group of DE's with medium to high efficacy against stored-product insects. Formulations and DE were tested at different doses depending on the treatment. In wheat, F_1H and DE Celatom® Mn 51 were tested at 300, 400 and 500 ppm, and F_2H at 100, 150 and 200 ppm, while in corn F_1H and DE Celatom® Mn 51 were tested at 400, 500 and 600 ppm, and F_2H at 200, 250 and 300 ppm.

Bioassay

The dose rates of the tested formulations and DE were chosen based on results of preliminary laboratory test (unpublished data). Plastic containers of 15 L volume were filled with 10 kg of clean wheat or corn respectively. The appropriate weights of the formulations or Celetom DE were added into each container and mixed thoroughly with a power drill. Containers with untreated grain served as controls. After the dust had settled, 200 unsexed, 7-21 days old adults of *R. dominica* were added into each container which were then closed with perforated plastic lids. The bioassay was conducted for six months storing the containers in a wooden structure which simulated an average floor warehouse. The air temperature during the entire period of storing varied between 18.5°C and 23.0°C and relative humidity between 55.0% and 82.0%. After the end of the bioassay trial, the entire content of the plastic containers was sieved and all insects, dead and live, were counted.

Results

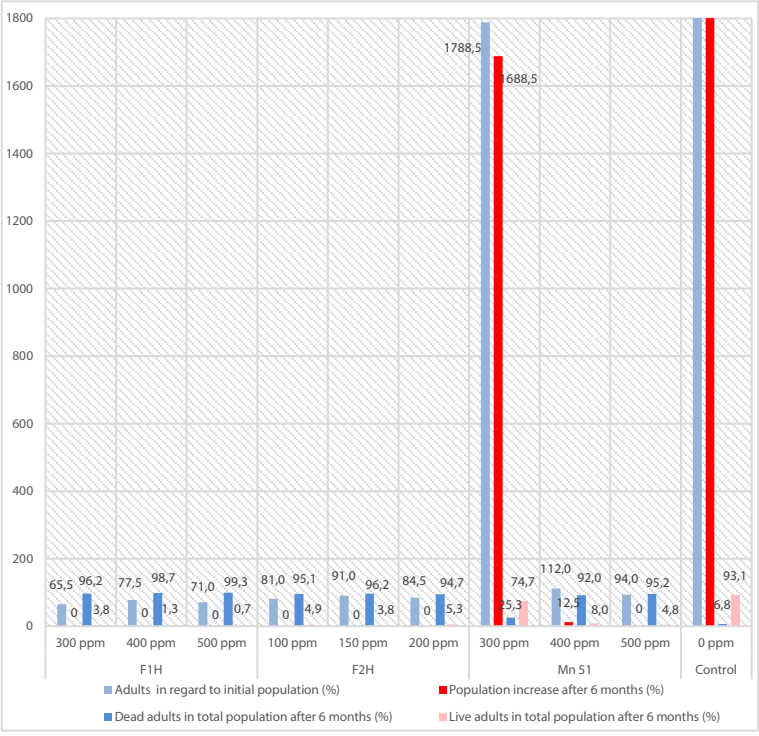
After six months of storing of treated corn and wheat, tested formulations F_1H and F_2H showed different efficacy against *R. dominica* depending on the cereal type and the applied dose.

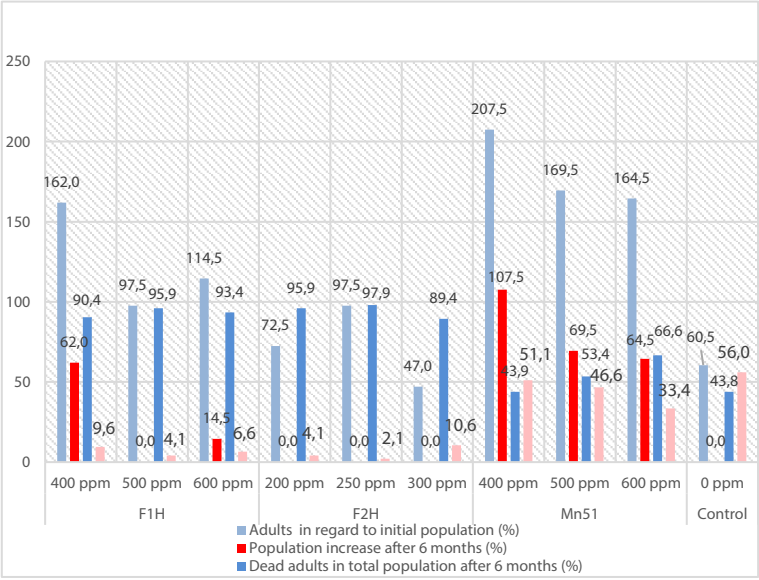
In wheat (Figure 1), both formulations successfully preserved grain quality against *R. dominica* during the period of six months. Even at the lowest dose, both formulations (300 ppm of F_1H and 100 ppm of F_2H) almost completely suppressed the initial population of *R. dominica,* therefore after six months, there was no population increase with only 0.7-3.8% (depending on dose of F_1H) and 3.8-5.3% (depending on dose of F_2H) of the original live adults found. For comparison, Celatom® Mn 51 at the lowest dose was not effective, resulting in a population increase (1688.5% higher than the initial population) within 74.7% live insects found. Population increases were also observed in the control treatment in wheat where number of insects from the initial 200 individuals increased up to 11817 within 93.13% live adults.

In corn (Figure 2), both formulations also showed better efficacy then Celatom® Mn 51. According to the number of adults found at the end of the testing period, formulation F_2H was more effective in regard to F_1H. Further, even at the lowest dose (200 ppm) no population increase was observed, while F_1H resulted with 62% and 14.5% of the population increase (at the dose of 400 ppm and 600 ppm, respectively). However, in the treatment with DE Mn 51, the highest dose (600 ppm) was not efficient enough to control *R. dominica,* and the population increase was in the range from 64.5 to 107.5% (higher than initial population) (depending on dose) within average of 45.4% live adults found. Concerning control treatments, unlike in wheat no population increase was observed in corn. Due to high temperature and high moisture content of the grain during six months fungi developed intensively and the whole stock become glued mass, so insects could not survived in those conditions.

Fig. 1 Efficacy of the formulations F_1H and F_2H against *R. dominica* after six months of wheat storing

Fig. 2 Efficacy of the formulations F_1H and F_2H against *R. dominica* after six months of corn storing





Discussion

Increased efficacy of the tested formulations against *R. dominica,* compared to DE alone was expressed because of the mixture of different active ingredients within their composition and due to their different modes of action. Besides inert dusts, the main composition of the formulations are the botanicals powdered bay leaves and lavandin essential oil. These compounds possess fast toxic activity against many coleopteran pests of stored products (Kostyukovsky et al 2002; Rajendran and Sriranjini, 2008; Nerio et al 2010; Koutsaviti et al, 2018). Comprised of volatile monoterpenoids and sesquiterpenenoids, the essential oils interfere with basic metabolic, biochemical, physiological and behavioural functions in insects possessing contact, inhalation and ingestion toxicity, antifeedant activity, developmental delay of adult emergence and fertility, different effects on oviposition and repellent activity (Obeng-Ofori 2007; Caballero-Gallardo et al, 2012; Nenaah 2014; Germinara et al, 2017). DEs and general inert dusts, with physical mode of action, are slow acting protectants (Korunic, 1998). Apparently, as a mixture of plant powders, essential oils and inert dusts, our formulations accelerate the knock down effect of the adults within the initial population of *R. dominica* which resulted in prevention of mating and reduced oviposition. Food grade bait composed within our formulation probably also accelerated insect mortality. Presumably, it attracted insects which kept them in the contact with inert dust for longer period. Consequently, insects picked up more inert dust particles on their body which led to faster desiccation (Korunić et al, 2016).

Adarkwah (2017, 2017a) reported faster activity of the mixture of DE and plant powders against three different stored product insects.While, mentioned authors conducted their trials in laboratory conditions and only during 7 days of exposure, our tested formulations secured effective control of the tested pest during the whole testing period of six months in conditions that might more realistically represent true storage conditions.

Overall two developed formulations, as a combination of inert dusts and botanicals could be promising insecticides with residual effect for protecting stored wheat and corn against insect infestation.

Acknowledgement

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based on inert dusts and botanicals to replace synthetic, conventional insecticides", www.diacromixpest.eu.

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Olfactory host location and host preference of *Holepyris sylvanidis* (Hymenoptera: Bethylidae) and *Cephalonomia waterstoni* (Bethylidae), two natural enemies of *Tribolium* and *Cryptolestes* species

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Abstract

Parasitoids can suppress populations of their host and thus play a primary role in Integrated Pest Management. In the stored product environment, stimuli deriving from plant products, damaged plant products and hosts might be important for host location by the parasitoids. We studied foraging cues in *Holepyris sylvanidis* (Hymenoptera: Bethylidae), a larval parasitoid of *Tribolium* species and *Cephalonomia waterstoni* (Bethylidae), a natural enemy of the rusty grain beetle *Cryptolestes ferrugineus* (Coleoptera: Cucujidae). Our studies in a four-chamber olfactometer revealed that the host complexes of both *Tribolium* species and different living host stages attract naive *H. sylvanidis* females, whereas no reaction was observed to uninfested substrates. The olfactory response of *C. waterstoni* was found to be strongly elicited both by chemicals emitted by the dust, adult *C. ferrugineus* and *C. ferrugineus* third and fourth instar larvae. Our findings may contribute to the development of biological control strategies of *T. castaneum*, *T. confusum* and *C. ferrugineus* with parasitoids.

Keywords: natural enemies, Bethylidae, stored product pests, biological control

Introduction

The bethylid wasp *Cephalonomia waterstoni* Gahan is an external, arrhenotokous idiobiont larval ectoparasitoid. Hosts are *Cryptolestes ferrugineus* (Stephens), *C. pusillus* (Schönherr) and *C. turcicus* (Grouvelle) (Coleoptera: Cucujidae) (Finlayson, 1950a; 1950b). *C. waterstoni* is able to find hosts by recognizing residual kairomonal cues on infested substrates, similar to other parasitoids (Howard & Flinn, 1990). Hagstrum (1987) and Reichmuth et al. (2007) reported the ability of *C. waterstoni* to maintain the population of rusty grain beetles below the economic threshold.

The parasitic wasp *Holepyris sylvanidis* (Brèthes) (Hymenoptera: Bethylidae) is a larval parasitoid of *Tribolium confusum* Jacqueline du Val and *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae), the economically most important stored product pests worldwide (Athanassiou et al., 2005; García et al., 2005). The host-searching behaviour of *H. sylvanidis* is influenced by the presence of host faeces, in which two compounds are thought to be responsible for the attraction: (*E*)-2-nonenal and 1-pentadecene (Fürstenau et al., 2016). The ability of *H. sylvanidis* to penetrate cracks and crevices makes it a promising natural enemy against stored-product pests. Pest larvae are often hidden under thin layers of substrate, in aeration ducts, in machines and in areas that are difficult to clean, but this wasp is able to access these critical environments.

Materials and Methods

Olfactory responses of *C. waterstoni*

The experiments were carried out in a static four-chamber olfactometer proposed by Steidle & Schöller (1997). The experiments evaluated the parasitoids response towards the following odour sources: (1) healthy grain (HGR), consisting of undamaged and uninfested wheat harvested in Germany in 2013; (2) grain infested by *Sitophilus granarius* (L.) (Coleoptera, Dryophthoridae) (IGR), which was obtained from the stock rearing of Hohenheim University; (3) a mixture of grain dust from the mass rearings of *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae), *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae) and *C. ferrugineus* on durum wheat (DST), which was collected from cultures kept at 30°C and 60 % relative humidity (RH), the insects and the wheat were kept in a 100 l bin for about 6 months; (4) dust which was obtained by sieving infested durum wheat after being fed on by adult S. granarius from Hohenheim's University laboratory (DSH); (5) diet without *C. ferrugineus* (DIT), consisting of big oats, small oats and wheat in the ratio 1:1:2, this diet also contained one tea-spoon of yeast and water, respectively; (6) diet plus *C. ferrugineus* (DCR), which was like the diet without *C. ferrugineus*, but in this case 50 randomly chosen adults were added, (7) male and female *C. ferrugineus* adults (ADU), (8) mixed larvae (LRM), i.e. 50 randomly chosen larvae of *C. ferrugineus*.

Olfactory responses of *H. sylvanidis*

In the experiments we studied the parasitoid females reaction towards the following odour sources: (1) *T. castaneum* host complex (CAH) consisting of 0.5 g whole grains, 0.5 g broken grains, first to second instar larvae (n = 2), fourth instar larvae (n = 2), pupae (n = 2) and adults (n = 2); (2) *T. confusum* host complex (COH) consisting of 0.5 g whole grains, 0.5 g broken grains, first to second instar larvae (n = 2), fourth instar larvae (n = 2), pupae (n = 2) and adults (n = 2); (3) whole grains (WHG) consisting of 1 g whole grains (*Triticum durum*); (4) flour (FLR) consisting of 1 g of flour (*Triticum aestivum*); (5) *T. castaneum* first to second instar larvae (CA1-2) (n = 10); (6) *T. castaneum* fourth instar larvae (CA4) (n = 10); (7) *T. confusum* first to second instar larvae (CO1-2) (n = 10); (8) *T. confusum* fourth instar larvae (CO4) (n = 10); (9) *T. castaneum* pupae (CAP) (n = 5); (10) *T. confusum* pupae (COP) (n = 5); (11) *T. castaneum* adults (CAA) (n = 5); (12) *T. confusum* adults (COA) (n = 5).

Results

Olfactory responses of *C. waterstoni*

Table 1 Substrates tested and mean walking time upon each odour source.

Substrates	Mean Time ± SE	P-value
DCR vs DIT	189.50 (±27.70) – 96.80 (±14.00)	P<0.05
DST vs EMP	202.43 (±28.10) – 94.22 (±15.70)	P<0.05
DSH vs EMP	198.16 (±28.40) – 110.70 (±17.70)	P<0.05
DST vs HGR	272.54 (±24.80) – 77.36 (±13.70)	P<0.05
DST vs IGR	214.53 (±33.03) – 71.30 (±20.50)	P<0.05
DCR vs DST	203.50 (±33.50) – 107.75 (±17.70)	P>0.05
ADU vs 3-4 L	164.16 (±31.40) – 95.39 (±21.80)	P>0.05
LRM vs ADU	121.74 (±28.10) – 102.64 (±22.80)	P>0.05
LRM vs EMP	178.29 (±15.40) – 114.43 (±10.70)	P<0.05
3-4 L vs 1-2 L	166.50 (±25.60) – 103.90 (±22.30)	P<0.05

Olfactory responses of *H. sylvanidis*

Table 2 Substrates tested and mean walking time upon each odour source

Substrates	Mean Time ± SE	P-value
CAH vs EMP	250.16 (±81.90) – 81.9 (±61.30)	P<0.05
COH vs EMP	248.22 (±93.20) – 102.74 (±46.55)	P<0.05

CAH vs COH	282.78 (±85.49) – 118.78 (±48.57)	P<0.05
WHG vs EMP	162.00 (±52.00) – 129.00 (±44.00)	P>0.05
FLR vs EMP	131.00 (±81.00) – 163.00 (±48.00)	P>0.05
WHG vs FLR	135.00 (±55.00) – 162.00 (±57.00)	P>0.05
CA1-2 vs CA4	183.36 (±70.78) – 123.54 (±42.06)	P<0.05
CO1-2 vs CO4	228.85 (±83.21) – 97.18 (±56.40)	P<0.05
CA1-2 vs CO1-2	204.77 (±74.37) – 143.92 (±62.77)	P<0.05
CA4 vs CO4	150.79 (±69.59) – 120.35 (±66.03)	P>0.05
CAP vs COP	190.25 (±64.27) – 138.26 (±69.90)	P<0.05
CAA vs EMP	208.00 (±96.00) – 136.00 (±80.00)	P<0.05
COA vs EMP	177.10 (±78.89) – 118.79 (±46.85)	P<0.05
CAA vs COA	164. 38 (±72.04) – 152. 18 (±59.85)	P<0.05

Discussion

C. waterstoni is an arrhenotokous ecto-parasitoid ables to locate hosts recognizing their kairomonal cues left on infested substrates (Amante et al., 2017 b,c; Howard & Flinn, 1990). Among the odours tested in the host habitat experiments, dust was the most attractive. The dust contains host faeces and particles from the host's feeding substrate, i.e. plant materials. Our study showed dust from infested products plus larvae and adults were most attractive to the parasitoid *C. waterstoni*. Consequently our study suggests laboratory reared *C. waterstoni* released for biological control would be arrested in areas infested by *C. ferrugineus*, but would not stay in uninfested stored products.

The present study demonstrates that *H. sylvanidis* primarily relies on volatile chemical cues from all host stages for host location. Our results demonstrate that the host complexes of at least two *Tribolium* species, *T. castaneum* and *T. confusum*, release volatile chemical signals, which are attractive for naive *H. sylvanidis* females. In our experiments designed to identify the attractive elements of the host complex, naive wasps did not react to uninfested whole grains and flour, but to most of the host stages of both *Tribolium* species. Whether the kairomonal cues identified could be used to manipulate the behaviour of these parasitoids in order to increase the effectiveness of biological control has to be investigated in future studies (Amante et al., 2017 a, b, c).

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Session 6
Fumigants, Controlled Atmospheres, and Hermetic Storage

The significance of vapor pressure in quality preservation of stored commodities under gastight conditions

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Abstract

While investigating the preservation of the aroma of various spices we compared the effects of hermetic conditions, vacuum and carbon dioxide versus aerated storage. The quality of the tested spices stored under hermetic conditions was comparable to those stored in vacuum after 120 d of storage. At a given temperature, a substance with higher vapor pressure vaporizes more readily than a substance with a lower vapor pressure. Throughout the investigation on specialty coffee, the volatility was evaluated as particularly important because coffee taste and aroma are influenced by compounds that are volatile. We hypothesize that hermetic storage reduces the rate of the volatiles to spread to the atmosphere. Dry food commodities can be stored for extended periods, provided there is no insect infestation and their water activity is low enough to prevent microbial growth. However, in aerated storages quantitative and qualitative losses still occur. If the moisture content is maintained sufficiently low, insects and quality loss remain the main concern for the quality preservation of durable agricultural commodities. Although in hermetic storage, the major emphasis is placed on the control of insect pests, for quality preservation just maintaining the vapor pressure in the sealed structure is sufficient. Quality preservation under hermetic conditions remains an aspect that deserves more attention. This characteristic of hermetic storage is the tendency to maintain within the hermetic structure the substances that have the ability to vaporize.

Key words: Hermetic storage, vapor pressure, volatile substances, aroma, quality preservation, modified atmospheres, low oxygen, vacuum, stored-product insects, stored-product microflora.

Introduction

Hermetic storage is a type of modified atmosphere (MA) that can be applied for the protection of commodities. This method takes advantage of sufficiently sealed structures that enable insects and other aerobic organisms in the commodity or the commodity itself to generate the MA by depleting the O_2 and increasing the CO_2 concentrations through respiratory metabolism (Navarro, 2006).

Dry food commodities can be stored for extended periods, provided there is no insect infestation and their water activity is low enough to prevent microbial growth. However, in aerated storages quantitative and qualitative losses still occur. Qualitative losses, for example, may consist of changes in physical appearance, in color change, loss of flavor, in nutritional degradation due to oxidation and increase in free fatty acids, the presence of insects or their fragments, or contamination by mold or the presence of mycotoxins. If the moisture content is maintained sufficiently low, insects and quality loss remain the main concern for the quality preservation of durable agricultural commodities (Navarro and Donahaye, 2005). Although in hermetic storage, the major emphasis is placed on the control of insect pests, for quality preservation just maintaining the vapor pressure in the sealed structure is sufficient.

Vapor pressure is a less investigated characteristic of hermetic storage. It is the tendency to maintain within the hermetic structure the substances that have the ability to vaporize. Such volatility is directly related to a substance's vapor pressure.

In chemistry and physics, volatility is the tendency of a substance to vaporize. Volatility is directly related to a substance's vapor pressure. At a given temperature, a substance with higher vapor pressure vaporizes more readily than a substance with a lower vapor pressure.

According to Weast et al. (1987) "vapor pressure is the pressure exerted when a solid or a liquid is in equilibrium with its own vapor. The vapor pressure is a function of the substance and of the temperature".

Vapor pressure or equilibrium vapor pressure is "the pressure exerted by a vapor in thermodynamic equilibrium with its condensed phases (solid or liquid) at a given temperature in a closed system" (Wikipedia Vapor pressure 2018). The equilibrium vapor pressure is an indication of a liquid's evaporation rate. It relates to the tendency of particles to escape from the liquid (or a solid). A substance with a high vapor pressure at normal temperatures is often referred to as volatile. The pressure exhibited by vapor present above a liquid surface is known as vapor pressure. As the temperature of a liquid increases, the kinetic energy of its molecules also increases. As the kinetic energy of the molecules increases, the number of molecules transitioning into a vapor also increases, thereby increasing the vapor pressure.

There are many examples for commodities with volatiles that make the aroma, the taste and the flavor special. For example, in spices and beverages like cocoa, coffee and spices, volatility is particularly important because taste and aroma are influenced by certain compounds that are relatively volatile. Retaining those volatiles during storage period of time has not been possible in aerated storage, but hermetic storage gave excellent results.

Quality preservation under hermetic conditions remains an aspect that deserves more attention. In the present paper, this aspect of hermetic storage in the quality preservation capacity of volatile substances that are emitted from the products is emphasized. The objective of this presentation was to explore the significance of vapor pressure in quality preservation of stored commodities under gastight conditions.

Materials and methods

A- Laboratory trials

Source of spices:

The spices were freshly imported from Bangladesh from a spices company interested in exploring the effects of vacuum and other storage methods on quality preservation of spices. The selected spices for test were: cumin seeds (Vijayanand et al., 2001), chili pods (Duman, 2010), coriander seeds (Bandoni et al., 1998) and turmeric rhizomes (Goyal and Korla, 1993).

Samples of spices:

Prior to tests, five samples from each spice were taken. Samples sizes were: 100 g of cumin seeds, 100 g of turmeric rhizomes, 100 g of coriander seeds, and 50 g of chili pods. The experimental samples were kept in a room at 27±2°C and 65±5% relative humidity (r.h.) for 120 d.

Relative Humidity:

The equilibrium relative humidity (ERH) of the spices was checked using Defensor® Novasina model ms1, Switzerland, with box sensor enMBRK-3. The equilibrium r.h. values expressed in this paper as percentages are equivalent to the decimal values in terms of water activity (a_w) which is the ratio of the water vapor pressure in the agricultural commodity to the water vapor pressure of pure water at the same temperature. Tests were carried out at 26.0-26.3°C. The ERH of the cumin seeds was 52.2%, chili pepper pods was 43.3%; coriander seeds was 44.5% and turmeric rhizomes was 75.4%.

Storage conditions of spices: The spices were stored under the following conditions:

a- *Control under continuous airflow:* burlap sack was filled with a sample of the tested spice. Each sack was then inserted into a separate 500 mL glass container (for the chili pods we used 1,000 mL

glass container). A plastic tube was then inserted from the top of the container and placed at the bottom below the burlap sack. The plastic tube was then connected to a small aquarium air pump allowing the air to flow through the sack at in airflow of 350 mL/min. The glass container remained open at the top for the entire duration of the test .

b- *Hermetic storage:* Each of the tested spices was sealed in a separate 1,000 mL glass jar. The jars were hermetically sealed for the entire duration of the test.

c- *High CO_2 (90% to 100%) atmosphere*: Each of the tested spices was sealed in a separate 750 mL glass jar. The jars were saturated with 100% CO_2 inserted through a rubber sealer at the jar lead. The CO_2 concentrations were examined periodically (once a week) and corrected when needed to adjust to CO_2 concentrations higher than 90%.

d- Vacuum: Each of the tested spices was sealed in a 500 mL vacuum glass container. The pressure in the container was initially reduced to 6.5 mmHg absolute pressure. The pressure was examined periodically (once a week) and maintained below 25 mmHg. In some samples higher pressures than 25 mm were recorded due to the difficulty in maintaining the low pressure. Those pressures were immediately rectified to the target pressure.

e- Burlap sack without aeration (also used as control): Each sack was filled with the tested spice. All burlap sacks were placed on shelve, exposed the room temperature and humidity.

Quality test: After 120 days of storage, all treated spices were removed from their experimental containers and placed in a container covered with a plastic Petri dish. The treatments were given a score from 1-5 for aroma and color 1 (dark color or other defects) represent the poorest and 5 best aroma and the best color (bright red color with no defects).. For these tests 15 individuals (6 women and 9 men) from the Department of Stored Products of the Israel Agricultural Research Organization were asked to score the sensory evaluation. Only chili pods were scored for color change and their pungency by smelling. Comparisons for all pairs were analysed using Tukey-Kramer HSD (honest significant difference) test method (SAS, 2014)

B- Field trials

The following two field trials were conducted by commercial companies that market chili pepper. Test results reported to the authors were not complete, but they were supported by available data description of the tests and pictures taken during and at the end of the trials.

Field Trial 1: One set of trial was conducted by the company HAJISONS at Kunri of District Umar Kot in the Province of Sindh area of Pakistan during the period of 7 months between December 3 - 9, 2016 and July 5, 2017. The area has foggy winter (Nov –Feb) with few western disturbances causing rain; pleasant spring (Feb –April); summer (April – June) with dust, rain storms and heat wave periods and rainy monsoon (July – August). The hottest month is June, where average highs routinely exceed 40°C. Coldest month is January with average high 19.8°C. Dry chili was stored in jute bags that served as control and compared with GrainPro hermetic bags indoors.

Field Trial 2: The second set of demonstration trial was conducted within the facilities of Olam Agro India Limited at District Guntur, Andhra Pradesh, India. The trial lasted for 6 months. The climate in Guntur is tropical. The average temperature is warm to hot year-round. The average annual temperature is 28.5°C. The trials were conducted by Mr. Madhu Nagaraj and Mr. Hari Babu of the Olam Agro India Limited. The practice was storing the whole dried RCPs (red chili pepper). However, this method requires a lot of space in a storage facility or during transport. It was recommended to test storing dried RCPs in powdered form to maximize capacity for storage and transport. Various parameters were measured every month for 6 months of storage including weights, moisture, colour, pungency and aflatoxins.

Results

Results of Laboratory Trials:

Results of blind test are shown in Table 1 which indicates that the control with airflow and in burlap sack gave the poorest results with scores below 2. For aroma tests best results obtained after 120 d under hermetic storage and vacuum. Although high CO_2 provided better preservation compared to controls, it was still inferior to hermetic and vacuum. The purpose of providing an active airflow in the control was to demonstrate that aerating to remove the aromatic volatiles would be more effective than the static control without aeration in burlap sack. The fact that airflow provided better results than the burlap sack may indicate that the jars where airflow of 350 mL/min were exposed was not sufficiently aerated compared to the volatiles that were evaporated from the burlap sacks.

Statistic test of comparisons for all pairs using Tukey-Kramer HSD showed that there was no significant difference for all spices stored under vacuum or hermetic storage. The storage under high CO_2 showed also no significant difference from stored under vacuum or hermetic storage excluding turmeric rhizomes. In both, storage in burlap sack or airflow showed the poorest performance compared with the other treatments. Vacuum storage gave best results followed by hermetic storage and high CO_2.

Table 1- Blind tests (Mean ± SE) for aroma of spices stored for 120 d at 27°C and 65% r.h.

Treatment	Cumin seeds	Chili pods*	Coriander seeds	Turmeric rhizomes
Airflow (control)	1.9 ± 0.31	1.5 ± 0.31	1.5 ± 0.40	1.4 ± 0.22
Hermetic storage	4.2 ± 0.36	3.7 ± 0.49	3.9 ± 0.36	4.1 ± 0.54
High CO_2	3.6 ± 0.40	4.0 ± 0.49	3.5 ± 0.31	2.7 ± 0.56
Vacuum	4.1 ± 0.36	4.1 ± 0.45	4.4 ± 0.45	4.6 ± 0.27
Burlap sack (control)	1.1 ± 0.13	1.2 ± 0.36	1.5 ± 0.40	1.3 ± 0.22

The Standard d Error of all means was 0.2

*For chili pods pungency tests served as criteria instead of aroma.

Table 2 shows results obtained using blind test for color difference of chili pods. Statistic test of comparisons for all pairs using Tukey-Kramer HSD showed that there was no significant difference for the color of the chili pods stored under vacuum or hermetic storage. This in spite of the fact that vacuum has better results. Storage under high CO_2 showed no significant difference from stored under hermetic storage, but was significant difference for those stored under vacuum.

In both, storage in burlap sack or airflow showed the poorest performance compared with other treatments. vacuum storage gave best results followed by hermetic storage and high CO_2.

Table 2- Blind tests (Mean ± SE) for color and pungency of chili pods stored for 120 d at 27°C and 65% r.h.

Treatment	Chili pods
Airflow (control)	1.0 ± 0.0
Hermetic storage	4.2 ± 1.2
High CO_2	3.5 ± 0.5
Vacuum	5.0 ± 0.0
Burlap sack (control)	1.5 ± 0.8

Results of Field trials

Field trial 1:

Test results of the are shown in Table 3 which gave a good indication of quality preservation capacity of dry chili peppers after 7 months of storage. In this case the quality parameters of color preservation, pungency, freshness, moisture preservation and fruit weight were well preserved in hermetic storage compared to lower quality observed in chili peppers stored in jute bags.

The weights of both whole and powdered chili peppers (RCP) remained constant throughout the 6-months storage period. For RCPs stored in Cocoon Indoor, the moisture content for whole RCP remained stable at 7.15%, while for powdered RCP, no change in moisture content was observed. For whole RCPs stored in SuperGrainBags (SGB), fluctuating moisture was observed taking into consideration the moisture analyzer that was utilized and the non-uniform moisture content of RCP

lots that were stored. Cocoon Indoor and SGBs are designed to prevent moisture ingress during storage, maintaining moisture of the stored commodity. It is a must that properly dried agricultural commodity are dried and stored in safe moisture to inhibit mold growth thus preventing aflatoxin or mycotoxin production. In the case of RCPs, a final moisture content of about 8% is ideal, as moisture content above 11% allows mold growth and below 4% causes excessive color loss.

Table 3- Results recorded at the end of storage period of dry chili peppers in GrainPro hermetic bags and jute bags at Haji Sons, Pakistan after 7 months.

	Hermetic	Jute bag
Color	No discoloration, shined red	Discolored, dull
Pungency	Same as fresh/high	Diluted
Freshness	High	Low
Moisture	No change	Increased
50 fruit weight (g)	36	24

Field trial 2:

The initial pungency of both whole and powdered RCPs measured in heat units were comparable to the heat units taken from RCPs stored for 6 months. Pungency of the spices is caused by several compounds, such as capsaicin for RCPs, which are volatiles. In a gastight system such as SGBs and Cocoon Indoor, these volatiles create equilibrium within the storage, trapping these compounds thus preserving pungency of RCPs.

Colour of whole and powdered RCPs were observed to decline during the storage period. The coloring pigment of chilies is carotenoid which is sensitive to light. Carotenoid pigments degrade when exposed to light, thus storing RCPs in the cold storage or dark room have low rate of color loss. During the trial at Olam, the set-up was placed outdoor since there was not enough space indoor. Even though RCPs were placed in woven polypropylene bags along with SGBs and Cocoon Indoor, light could still penetrate the stack leading to change in color of RCPs.

Light and oxygen contributed to the rate of colour loss. Color degradation of whole RCPs were more evident compared to powdered RCPs since it is less compact allowing more light to enter the stack.

The aflatoxins of RCPs were observed to be 0.5 ppm initially up to 6 months, except on 2nd month for powdered RCPs and 3rd month for whole RCPs in SGBs. These fluctuations in reading might be brought by limitations of the method of analysis for aflatoxin. SGBs and Cocoon Indoor were effective in preventing aflatoxin production during storage. In the previous discussion, moisture ingress is prevented when using SGBs and Cocoon Indoor thus inhibiting molds by maintaining safe moisture for dried RCPs during storage. When molds are inhibited, production of mycotoxins, including aflatoxins is also prevented.

During the trial, whole and powdered dried RCPs were stored. The capacity of SGBs was maximized when storing powdered RCPs (i.e. 40 kg/SGB) compared to storing whole RCPs (i.e. 8 kg/SGB). Several parameters were tested including moisture, colour, pungency, weight and aflatoxin. For 6 months of storage, these parameters measured from powdered and whole RCPs were comparable. Moreover, the colour was observed to be better when storing powdered RCPs compared to whole RCPs.

Discussion

Ambient humidity is an abiotic factor of the air surrounding the commodity. Within the confined storage space, the moisture of the commodities, tend to reach equilibrium with the humidity of the intergranular air. Its greatest influence is on molds, which begin to develop at intergranular air humidities above 65% (Navarro and Donahaye, 2005).

Micro-organisms are the biotic factor composed of molds, yeasts and bacteria. They are universally present on the grain, but are inactive when the equilibrium relative humidity is below 65% .

When discussing microflora activity and preservation of grain quality, it is more meaningful to consider the moisture content of the intergranular environment or the equilibrium relative humidity (ERH) corresponding to a particular commodity moisture content. This is because various grain types may have different moisture contents at the same ERH. The microfloral activity and susceptibility of grain to deterioration is correlated to the ERH. An additional term frequently used in food microbiology is "water activity". Water activity (a_w) and ERH are numerically equivalent, but ERH is expressed as a percentage and aw as a decimal of ERH, thus a_w 0.8 = 80% ERH (Lacey et al. 1980).

Favorable conditions occur when the moisture content of the grain or the relative humidity of the intergranular atmosphere rises above a certain threshold. This threshold is generally considered to be around 75% RH (termed the critical relative humidity) or the corresponding equilibrium moisture content of the grain (e.g. for wheat it is about 14%) - often termed its critical moisture content. Beyond this threshold, microflora become activated, and starts to grow, accompanied by active respiration, liberation of metabolic heat and water. At humidity or moisture conditions above this level, deterioration increases at an exponential rate .

The availability of water in the food medium is a vital factor determining both the types of bacteria or fungi capable of growth, and the rate at which they can grow. It is usually measured in term of water activity, and is a function of the moisture content of the food.

Bacteria grow best at water activities near to unity, and will not grow at a water activity less than about 0.95. Yeasts occupy an intermediate range, and they will grow at water activities as low as 0.85. Fungi are more resistant to the effect of dry conditions, although the vast majority are inhibited by water activities lower then 0.70, a very few species will show some growth at a water activity as low as 0.65 (Lacey et al. 1980).

Tests were carried out in various climate conditions to observe product preservation under hermetic conditions. Among other quality parameters such as insect infestation, milling recovery, head rice, yellow kernels, germination, and weight loss, changes in moisture content of hermetic and non-hermetic storage of paddy was reported (Navarro et al,1997). Accordingly they report paddy stacks of capacities ranging from 13.4 to 31.9 tonnes that were stored outdoors in flexible enclosures for 78 to 183 days. The quality of the paddy was compared with that of three control stacks (5.3–5.6 tonnes capacity) held under tarpaulins in the open for 78–117 days. The trials were conducted at the NAPHIRE compound, Nueva Ecija, the Philippines (Navarro et al., 1997). There was a real trend towards an increase in moisture content in the two control stacks during the wet season and toward a decrease in moisture content in the control stack stored during the dry season. No significant changes were noted, in the eight hermetic stacks and two silos. These field trials indicated that the changes in moisture content and weight of the grain changed only very slightly within the stacks and the silos due to hermetic storage.

Lane and Woloshuk (2017) studied small hermetic bags (50 and 100 kg capacities) used by smallholder farmers in several African countries as a low-cost solution for preventing storage losses due to insects. In their study they compared the effects of environmental temperature and relative humidity at two locations (Indiana and Arkansas) on dry maize (14% moisture content) in woven polypropylene bags and Purdue Improved Crop Storage (PICS) hermetic bags. The results indicated that the PICS bags prevented moisture penetration over the three-month storage period. In contrast, maize in the woven bags increased in moisture content. The work of Lane and Woloshuk (2017) is an additional indication that hermetic storage enables maintaining the water vapor as expressed as humidity in their work. They concluded that the PICS hermetic bags are effective at blocking the effects of external humidity fluctuations as well as the spread of fungi to non-infected kernels.

The more volatile the compound is the faster it will vaporize. This is why the coffee brewing temperature is so important; it allows proper and fast extraction of nonvolatile components, while preserving the volatile ones. Espresso extraction is a few degrees lower than drip

coffee. Pressure helps extract more total dissolved solids (TDS) at a lower temperature, while preserving volatile components. TDS is a measure of the combined content of all inorganic and organic substances contained in a liquid in molecular, ionized or micro-granular suspended form. Generally the operational definition is that the solids must be small enough to survive filtration through a filter with two-micrometer (nominal size, or smaller) pores (Wikipedia, TDS, 2018).

Ribeiro, et al. (2011) evaluated the physical, chemical, and sensory qualities of green coffee beans (*Coffea arabica* L.) during storage in different types of packaging. Coffee was stored in a warehouse in Brazil. The treatments consisted of two types of packaging (hermetic big bags with the injection of up to 60% CO_2 in a controlled atmosphere; similar bags but without the injection of CO_2 in a modified atmosphere). The storage of green coffee beans under these conditions was viable over a 12-month period. The coffee packed in big bags maintained its quality and exhibited an intensification of the green coloration of the grains during storage. Sensory analysis of coffee beans stored in a controlled atmosphere showed that the medium sampling position yielded the best ratings. The results of Ribeiro, et al. (2011) analysis demonstrated that the tested storage technique can potentially increase the effectiveness in preserving the sensory quality of coffee beans.

In another study Borém et al,. (2013) commercially validated the effects of an artificial atmosphere on the color, flavor and aroma of green coffee beans stored after 12 months. The coffees were evaluated by a sensory panel composed of 13 tasters who were judges certified by the Specialty Coffee Association of America and who operate commercially in various coffee-producing regions of Brazil. The evaluation consisted of hermetic big-bags with and without CO_2 injection. Two additional treatments served as controls: jute sacks and GrainPro sacks. The beans were qualitatively evaluated for their color and for their beverage quality attributes including their fragrance, sweetness, acidity, flavor, body and aftertaste. The beans packaged in hermetic big-bags with a CO_2 injection maintained a specialty coffee classification. Impermeable packaging preserved the initial color of the coffee beans. Coffee storage in hermetic packaging preserved the desirable aromas of the coffee. In these studies Borém et al,(2013) showed that undesirable flavors and aromas predominated in the coffees packaged in jute sacks. In all these studies with coffee it is most possible that the quality preservation under sealed conditions, weather under vacuum, hermetic storage or CO_2 assisted modified atmospheres, the packages were maintained sealed that most possible the escape of the volatiles from the coffee to maintain its organoleptic qualities.

We do not have a current method to assess the vapor pressure of the volatiles in the commodities. However, all these studies are in line with the basic understanding that hermetically sealed storages maintain the vapor pressure of the volatiles enabling the commodities better quality preservation. Whereas in all tested aerated storages loss of the volatiles dues to their vapor pressure that should have maintained, was accompanied with loss of quality. Therefore, the conclusions from these studies lead to the understanding for the preservation of the quality of spices and beverages the preferred method of storage should be hermetic sealed storage. Additional supporting research should be carried out on quality preservation of the hermetic storage as an added benefit to the control of insects.

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Hermetic storage technology for handling of dry agricultural commodities: Practice, challenges, opportunities, research, and prospects in Zimbabwe

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Abstract

Storage pest management practices have relied on synthetic pesticides comprising: dust powders, liquid formulations and fumigants. Reduced efficacy against targeted species, negative health-related issues and increase in consumer awareness on potentially detrimental effects of synthetic pesticides have led to a shift towards safer and environmentally-benign alternatives. Hermetic technology is a pesticide-free storage alternative currently being used in Zimbabwe and other African countries. In the current paper, we review forms and characteristics of the hermetic technology available, organisations driving the technology, research and development (R&D) initiatives, and access and uptake trends in the country. The review draws out future prospects in terms of: stakeholder partnerships and roles, up-scaling/adoption options, R&D gaps, capacity building, and funding mechanisms for effective and sustainable uptake. Critical areas identified in the review include: the need for increasing the number of hermetic plastic liner brands available to enhance access and competitive pricing, improved distribution mechanisms for hermetic storage containers for easy access in remote areas, and generation of evidence-based efficacy data on the various hermetic storage containers in preserving quality of commercial, parent and foundation seed. Future opportunities include use of hermetic containers in the disinfestation of organic horticultural products using carbon dioxide gas hermetic fumigation. However, supporting policies are necessary to ensure sustainable adoption of the hermetic technology at subsistence and commercial scales.

Keywords: synthetic pesticides, pesticide-free storage, gas hermetic fumigation, hermetic technology adoption

Introduction

Agriculture is the primary source of livelihoods in developing countries accounting for up to 15 % of the Gross Domestic Product (GDP) in sub-Saharan Africa (SSA)(OECD, 2016). The crop sector constitutes 85 % of the total production value in SSA. Efficiency along the crop value chain is important to ensure that whatever is produced reaches the end user in optimum quality and quantity. Noxious pests and unfavourable ambient conditions are among factors that are associated with bio-deterioration of agricultural products along the value chain (Kumar and Kalita, 2017; Bradford *et al.*, 2018). Insect pests are among the major loss causing agents during the storage stage (Snelson, 1987; Muatinte *et al.*, 2014) and control strategies have been centred on synthetic pesticide use (Chaudhry, 1997; Daglish, 2006). However, negative attributes have been associated with use of chemical pesticides which include development of resistance by storage insect pests (Chaudhry, 2000; Boyer *et al.*, 2012), toxicity against untargeted species (Isman, 2006; Sarwar, 2015), health hazards to users (Aktar *et al.*, 2009) and health risks posed to consumers as a result of pesticide residues in food products (Navarro *et al.*, 2012). This has led to a paradigm shift towards research on, promotion, uptake and adoption of, environmentally-benign non-chemical pest control methods.

Hermetic storage is an environmentally-benign method being used globally for postharvest handling and disinfestation of agricultural commodities (Navarro *et al.*, 1993; Jayas and Jeyamkondan, 2002; Villers *et al.*, 2010). The method allows commodities to be stored without using any chemicals but by sorely utilising airtight storage conditions which deprive storage pests (arthropods and fungi) of oxygen leading to mortality as a result of asphyxiation (Villers *et al.*, 2006). Use of hermetic storage containers in Africa is increasing especially for handling of dry agricultural commodities at smallholder farmer level (Mvumi *et al.*, 2013; Murdock and Baributsa, 2014; Baoua *et al.*, 2015) and commercial sector (Jonfia-essien, 2012). However, hermetic technology use is not widespread in Zimbabwe although it is increasingly being adopted as a result of promotional activities by development agencies in partnership with various stakeholders including government, research institutions and farmers (Mvumi *et al.*, 2013). Hermetic storage options available on the commercial market in SSA are: small-scale storage options (hermetic bags) eg Purdue Improved Crop Storage (PICS) Bags, AgroZ® and AgroZ® Plus bags, SuperGrain™ Bags (SGB), ZeroFly® bags, Ecotact bags, metal silos and plastic silos; large-scale storage options eg GrainSafes, GrainPro Cocoons™, SiloBags; and transport options eg TranSafe Liners (TSLs) and SGB Oceans. Except for metal and plastic silos, hermetic storage containers readily available on the market in SSA are flexible plastic liners that are manufactured using high density polyethylene (HDPE) and polyvinyl chloride (PVC) (Baributsa *et al.*, 2010; Villers *et al.*, 2010) (Table 1). The hermetic containers are being used for storage and/or transportation of dry agricultural commodities like coffee, cocoa, spices, cereals and pulses (Jonfia-Essien et al. 2010; Villers et al. 2010; Mvumi et al. 2013; Baoua et al. 2015; Walker et al. 2018).

Locally available hermetic storage options, use and scale

Various hermetic technology options are now available in Zimbabwe (Table 1). The hermetic technology was first promoted in the country by FAO in 2012 in eight districts focussing on metal silos and hermetic bags (Mvumi et al., 2013). The project included Government of Zimbabwe and an NGO (Practical Action) as implementing partners focussing on training artisans in the fabrication of hermetic metal silos and introducing both the silos and hermetic bags to smallholder farmers, as alternative grain protection methods. In the same year CIMMYT partnering Government of Zimbabwe, the private sector, an NGO and the University of Zimbabwe also embarked on a similar project in two districts. In 2013, GrainPro Philippines Inc, a green and not only for profit company, and one of the leaders in the manufacturing of hermetic products for handling dry agricultural commodities, partnered Farm & City, an agricultural inputs retailer as its sole distributor in Zimbabwe. This eased local availability of hermetic plastic liner products. Use of hermetic plastic liners is increasing with promotional work being carried out by more NGOs in smallholder farming

communities (Table 2). Catholic Relief Services also imported PICS bags from Malawi in 2017 to assist farmers in reducing storage losses in the arid southern parts of Zimbabwe. The total national usage of hermetic bags is around 100 000 units per season based on estimates from various project interventions. This is a small figure in relation to the approximately 1.5 million smallholder farmers in the country especially considering that 70 % of the population rely on agriculture for their livelihood. Therefore, the product has potential for wide-scale adoption by more smallholder farmers.

Initially, hermetic storage options being used were mainly for household food security purposes. However, in 2015 WFP Zimbabwe realised the opportunity of enhancing smallholder farmers' income through grain aggregation and partnered Fintrac with funding from the USAID to promote community aggregated maize grain storage (Fig 1). This initiative allowed farmers to store grain soon after harvesting and then sell later to take advantage of favourable prices that exist during the lean season. The project donated GrainPro Cocoons™ (5 MT and 10MT) to farmers in two maize producing districts. However, sustainability of such commercial-oriented initiatives is usually hampered by fluctuating harvests, lack of business acumen and management capacity by communities, and conflicting objectives among community members. Ever since the WFP project, there has been a slow but gradual increase in use of GrainPro Cocoons™ by farmers and private sector. Anectodal evidence suggest that use of GrainPro Cocoons have enabled poultry farmers to store grain meant for stockfeed production for periods exceeding eighteen months with insignificant bio-deterioration. This increases the profit margins of farmers because they are able to buy grain soon after the harvest season when prices are low and then use the grain during the lean season when prices are high. On the other hand, SGB Premium is also being used for storing coffee by small-scale coffee producers in the Eastern Highlands of Zimbabwe. The use of SGBs for coffee storage is common in Latin America (Villers et al., 2010) while cocoons were tested at large-scale in storing cocoa beans in Ghana (Jonfia-Essien et al. 2008) and maize grain in Zimbabwe (Chigoverah et al. 2016) Maize seed is being exported to Asia and West Africa in TSLs to limit the effect of fluctuating weather conditions and insect pest development, hence preserving germination in transit. Silobags are also being used in large-scale storage of bulk maize and soyabean seed.

Fig. 10 Community aggregated maize grain stored in a 5MT GrainPro Cocoon in Gokwe South district, Zimbabwe (Source: Authors).

There is need to improve the distribution network for hermetic storage options especially in remote areas where the majority of smallholder farmers are located. Although Farm and City has a nationwide branch network, they have no presence at village level which results in farmers having to travel considerable distances to access hermetic bags at town or district centres. The company can enhance its distribution network by partnering with smaller agro-dealers who operate in smallholder areas. This will increase accessibility of these products to smallholder farmers and enhance adoption (Baributsa et al., 2010).Poor road networks and long distances from district centres of some of the communities can increase the final cost of products.

Tab. 6 List of hermetic containers available in Zimbabwe and their respective capacities (Compiled by authors, 2018).

Manufacturer	Product	Use	Available capacities (based on maize)	Commodities being handled	Source
GrainPro Philippines Inc	SuperGrainBag Farm	Crop Storage	50 kg	Dry cereals and pulses	Farm & City Pvt Ltd (has countrywide distribution network)
	SuperGrainBag Premium		50 kg, 90 kg	All dry agricultural commodities (cereals, pulses, oilseeds, spices, retained seed)	
	GrainSafe		1.3 MT		
	GrainPro Cocoon		5 MT, 10 MT, 20 MT		
	TranSafe Liners	Transportation	20 Ft	Commercial seed maize	
Ministry of Lands Agriculture and Rural Resettlement	Metal Silos	Crop Storage	50 kg, 100 kg, 0.5 MT, 1 MT, 3 MT	Dry cereals and pulses	Manufacturers
Peak Trading Pvt Ltd	Metal Silos	Crop Storage	50 kg, 100 kg, 0.5 MT, 1 MT, 3 MT	Dry cereals and pulses	Manufacturers
Farmyard Investments	Metal Silos	Crop Storage	50 kg, 100 kg, 0.5 MT, 1 MT, 3 MT	Dry cereals and pulses	Manufacturers
Local artisans	Metal Silos	Crop Storage	50 kg, 100 kg, 0.5 MT, 1 MT, 3 MT	Dry cereals and pulses	Manufacturers
SiloBag International	SiloBags	Crop Storage	200 MT	Dry cereals and pulses	RadZim Pvt Ltd

Tab. 7 Promotional activities of hermetic storage options by development partners.

Product	Promoting Agency
SuperGrainBag	FAO (2013, 2018/19), CIMMYT (2013/14), Action Contre La Faim (2015/16, 2018/19), World Vision (2016-18), German Agro Action (2016), Action Aid (2017/18)
Metal Silo	FAO (2012-15), CIMMYT (2013/14), Action Contre La Faim(2015/16, 2018/19), Oxfam (2017)
GrainSafe	German Agro Action (2016)
GrainPro Cocoons	WFP (2015/16)

Availability of metal silos is also a challenge. Even though artisans have been trained in some communities, low demand has resulted in most of the artisans switching to fabricating high demand products like watering cans and cooking pots. Furthermore, metal silo manufacturing companies are few in the country and the Government of Zimbabwe through Ministry of Lands Agriculture and Rural Resettlement (MLARR) have conducted capacity building initiatives to private companies to increase the number of service providers. Despite these efforts, there are only a few manufacturers and they cannot service the sparsely distributed smallholder farming communities. This has resulted in MLARR also providing the service through its Postharvest Department which is centrally located in Harare. There is need for more players to enable farmers to easily access the product. However, both Farm & City and RadZim are strategically located to be able to fully service the commercial sector and farmers near major towns and district centres.

Hermetic plastic liners being distributed by Farm and City and RadZim are imported from the Philippines and Latin America, respectively. The products are charged import duty which increases cost which in turn is transferred to the end-users. Cost is usually a major adoption factor especially for resource-constrained African smallholder farmers who tend to opt for low-cost pest control

alternatives regardless of the efficacy of the product (Nukenine *et al.*, 2010).The issue can be addressed by enforcing tax exemption on imported hermetic plastic liners. There is only one brand of hermetic bags (SGB) currently available in the country; hence the need for more players to come on board to avoid monopoly on the market. Competition can also lower prices thereby enhancing affordability by smallholder farmers. In East Africa local manufacturing of hermetic bags has also lowered costs (Baributsa *et al.*, 2010) and enhanced perennial supply of the product on the market. There is need for local plastic manufacturers to also consider venturing into this line of business.

Evidence-based performance of hermetic technology

Research findings have reported hermetic storage products namely SGBs, metal silos (Chigoverah and Mvumi, 2016; Mlambo *et al.*, 2017; Nyanga and Ambali, 2017), and GrainPro Cocoons (Chigoverah *et al.*, 2016) to be effective in suppressing storage pest-induced bio-deterioration of maize and pulses under simulated and field conditions. Metal silos and SGBs were also reported to be more effective than conventional pesticides in suppressing storage insect pests development and consequently preserving germination of commodity maize grain over a storage period of up to one year (Chigoverah and Mvumi, 2016). These findings have led to widespread acceptance of the technology by government, farmers, private sector and development agencies. However, knowledge gaps exist on the reusability of the hermetic containers across storage seasons, maintenance of hermeticity at the Cocoon zipper, performance of plastic liners in areas with severe rodent and *P. truncatus* infestation, performance of other hermetic technology options like SiloBags, GrainSafes and TSLs under local conditions although unconfirmed reports suggest that they are effective in comparison to non-hermetic methods. Furthermore, most research has been centred on commodity grains namely maize and pulses; thus, there is need to generate evidence-based performance data on other commodities like spices, herbs, seed (commercial and foundation), dried fruits and stock feeds.

Future prospects

There is potential for increased use of hermetic technology in Zimbabwe judging by increase in sensitisation initiatives and positive feedback from end users (Nyanga and Ambali, 2017). However, supporting policies are essential to enhance participation of more industry players. Policies which include removal of, or reduction in, import duty tax on hermetic plastic liners and metal silo sheets can significantly reduce costs. Metal sheets constitute the largest proportion of the total cost of the metal silo averaging 60% (Kimenju et al., 2009). The Government of Zimbabwe included metal silos in the country's economic blueprint document (ZIMASSET) as key agricultural equipment that can be useful in enhancing household food security (ZimVac, 2014). There has been lobbying by stakeholders for the country to formulate a Postharvest Policy which if in place can also enhance promotion and uptake of hermetic storage options.

Hermetic technology presents various opportunities especially in the handling of oilseeds like groundnuts and sesame, and disinfestation of organic horticultural products (Navarro, 2010). Groundnuts are usually stored in shells (Harish *et al.*, 2014) which is inefficient in terms of space utilisation. Shelled groundnuts are less bulky but are susceptible to insect infestation and aflatoxin contamination. Hermetic storage (Gas Hermetic Fumigation) has been reported to be effective in storing shelled groundnuts and other oilseeds (Navarro and Navarro, 2014).

Financial constraints faced by local companies and farmers willing to invest in hermetic technology can negatively affect uptake and adoption. Intervention by financial institutions offering credit to both companies and farmers can catalyse availability and adoption of hermetic containers. Farmer groups can also approach retailers of hermetic bags with a payment plan which can facilitate access to the bags on credit terms for payment upon marketing of their harvested commodities. Similarly, trained artisans need access to quality equipment for manufacturing metal silos. They need to be facilitated to approach financial institutions as associations or groups and access soft loans. They also need to be trained in agribusiness so that the investment is sustainable. Given that agricultural

production is seasonal in SSA, most farmers face financial challenges at the onset of the storage season hence end up selling their crops at a lower price. Moreover, lack of appropriate storage technologies also force farmers to sell soon after harvesting to minimise risks associated with prolonged storage (Tefera and Abass, 2012). Capacitating farmers with effective storage facilities enables selling at a higher price during the lean season thereby enhancing household income security. A credit facility will enhance livelihoods and also stimulate demand for the storage products among both smallholder and larger-scale farmers.

Hermetic storage cannot be effective as a standalone postharvest loss reduction strategy but should be complemented by sound crop postharvest management practices and continuous training of both artisans and farmers. There is need to capacitate users on recommended handling practices for both commodity and hermetic storage containers. Users should be made aware that commodities to be stored for long periods of time should be adequately dried prior to loading into the storage containers. Furthermore, some hermetic storage options should be placed in clean spaces free from sharp or overhanging objects and wild animal species. This is critical for hermetic plastic liners which are susceptible to damage by sharp objects and storage pests. Followups of trained personnel followed by refresher training workshops are essential to reinforce the skills and promoting co-learning from practice. Standardised manufacturing procedure for metal silos is critical to ensure optimum performance of the product and avoid sub-standard material. There is need for MLARR to come up with standardised fabrication and testing procedures that will be used during training of artisans to minimise faulty products.

There is an increase in awareness by consumers on the benefits associated with hermetic storage and demand is likely to increase. This might lead to an increase in the manufacturers and suppliers of hermetic storage products. Henceforth, MLARR should engage Standards Association of Zimbabwe formalise hermetic standards for metal silos and hermetic plastic liners. This will enable Government of Zimbabwe to effectively monitor in future the quality of products available on the market. Inferior hermetic bags have been reported in West Africa leading to PICS bags manufacturers branding their bags for easy identification by customers (Baributsa *et al.*, 2010). Standardisation will be key to branding of the hermetic products.

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Evaluation of hermetic technologies in the control of insect infestation and mycotoxin contamination in stored maize grains

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Abstract

Grain losses due to moulds during on-farm storage increase food insecurity, result in economic losses, negatively affect farmers' livelihoods, and increase exposure to mycotoxins that can harm human and animal health. Hermetic storage technologies provide a reliable solution for maize grain that may also preserve food safety. Several studies report the effectiveness of these technologies against post-harvest insects in Africa but provide limited evidence on effectiveness against mould proliferation and mycotoxin contamination. Hermetic technologies were superior to farmer practice in reducing insect infestations and mycotoxin accumulation. Among hermetic technologies, there were no significant differences ($P>0.05$) in performance between metal silos and hermetic bags for mycotoxin accumulation and insect infestation regardless of the mode of infestation. In non-inoculated grain, fungal populations were varied but included mycotoxin-producing *Aspergillus* and *Fusarium* spp., indicating that the grain was naturally contaminated and acted as a good reservoir for these fungi. Mycotoxin levels increased with higher moisture even in non-inoculated grain. Meanwhile, aflatoxin and fumonisin levels at 4 months were not significantly different from baseline values in dry inoculated grain across all storage technologies ($P>0.05$), indicating that hermetic technologies can prevent mycotoxin contamination in dry grain for at least 4 months of storage. Aflatoxin and fumonisin were significantly higher by 1.69 ppb and 0.25 ppm respectively in non-inoculated grains at high moisture indicating the need to adequately dry grain before storage in hermetic technologies. This trend was observed collectively in all the technologies registering 2.03 ppb and 0.311 ppm respectively. In inoculated grains at high moisture, there was an increase in aflatoxin in both hermetic treatments and the control by 5.7 ppb and 12.14 ppb respectively. Therefore, a trial was conducted to compare hermetic technologies with farmer practice in their effectiveness against both insect infestation and mycotoxin contamination.

Keywords: Insect infestation, mycotoxin contamination, stored maize, hermetic storage, food security

1. Introduction

Maize (*Zea mays* L.) can conveniently be classified as the most important cereal crop owing to its nutritional value and utilization of its by-products. Grain losses due to insect pests during on-farm storage increase food insecurity, result in economic losses, negatively affect farmers' livelihoods, and increase exposure to mycotoxins that can harm human and animal health (Obeng-Ofori, 2008). Among these mycotoxins, the two commonest and highly toxic mycotoxins compound encountered in maize in the tropical and sub-tropical region of the world are aflatoxins and fumonisins (Krska *et al.,* 2008). Aflatoxins are toxic metabolites produced by fungal species during their growth under favorable conditions of temperature and moisture. The major aflatoxin producing species are *Aspergillus flavus* and *Aspergillus parasiticus*. The main cereals affected are maize, sorghum, rice and wheat and other crops like groundnuts and cassava. Aflatoxin-producing fungi have very few nutritional, environmental and reproductive requirements, and that is their strategy to survive and develop (Wu *et al.,* 2011). Fumonisins are mycotoxins produced by the grain moulds *Fusarium verticilliodes* and *Fusarium proliferatum,* which is frequently a universal inhabitant of corn. Fumonisins are categorized as, B1, B2 and B3 and are usually found to be greater than 1

ppm in the corn samples tested. However, the FDA/USDA advises less than 4 ppm in corn meant for human consumption and less than 50 ppm for cattle feed. Fumonisins are not always produced where the fungi have colonized on the kernels, but many factors contribute to the subsequent mycotoxin contamination, including host susceptibility and environmental conditions. All these factors together determine the incidence and severity of mould contamination on the grain. The conditions that favor fumonisin production are not well known; *Fusarium* moulds thrive well in hot followed by cool conditions, in wet conditions during pollination and ear development. The magnitude of the effect of mycotoxin exposure is facilitated by the level and exposure period, as well as health, age and the species of the animal.

Damages caused by insect pests represent a huge setback in the world's effort to achieve food security globally. According to Ileleji *et al*. (2007) and Nukenine, (2010) an estimated 1% to 5% of stored grain in developed countries and 20% to 50% of stored grain in developing countries are lost due to insect damage. Cracked or broken grains provide an entry point for infestation by insects and moulds during storage. Variation in temperature and humidity has been identified to support the metamorphosis of *Prostephanus truncates* (Horn) (Hodges and Meik, 1984). They lay eggs which hatch in about three days at 27 **°C** day temperature and the dust provide the nourishment to the larvae. Larva development to adult stage takes place within 27 days and is facilitated by ideal conditions of 32 °C and 80% relative humidity (Hodges, 1986). Maize weevil, *Sitophilus zeamais* (Motschulsky), is one of the cosmopolitan pests of stored cereals, especially maize (Throne, 1994). It damages stored maize and of cob maize prior to harvest. It may also infest other cereals if the moisture content is moderate or high (Longstaff, 1981). Eggs are laid at temperatures between 15 and 35 °C (with an optimum around 25 °C and at grain moisture contents over 10%). Subsequent infestations in stores result from the transfer of infested grain into store or from the pest flying into storage facilities, probably attracted by the odour of the stored grain. Dry weight loss from *S. zeamais* infestation alone averaged about 5% by weight after six months of storage. The 5% dry weight loss translates into 22% of total grains displaying damage (Holst *et al*., 2000). As a start, it should always be recognized that an intact grain is an essential item for successful storing.

Insect infestation could have significant impact on the mycotoxin contamination of maize. It is worthwhile to know that, the level of insect damage influences the extent of mycotoxins contamination. Insects act as vectors by carrying spores of mycotoxin producing fungi from plant surfaces to the interior of the stalk or kernels or create infection wounds through their feeding habits (Munkvold, 2003). Insects attack in storage could also be devastating because their level of damage influences the extent of mycotoxin production in the store. Hermetic storage technologies provide a reliable solution for maize grain that may also preserve food safety.Several studies report the effectiveness of these technologies against post-harvest insects in Africa but provide limited evidence on effectiveness against mould proliferation and mycotoxin contamination. Hermetic bags have also been known to preserve the quality of grain, appearance and aroma by reducing mould growth (Moussa *et al.,* 2014). Hermetic technology works synergistically to promote conditions of limited oxygen and high carbon dioxide levels produced by aerobic metabolism of insects, micro-organisms and grain respiration, creating a non-toxic, cost effective and environmentally friendly option over the use of chemicals in the control of insects and mycotoxin contamination in stored maize (Williams et al., 2014). Aerobic metabolism uses up oxygen and produce carbon dioxide to levels that are lethal to insects in the grain mass (Yakubu *et al.,* 2011). In the world today, concerns on the environment and food safety have increased and consumers are demanding high quality products that are free from chemical residues, aflatoxin and insect contamination (Weinberg *et al.,* 2008).

Improved storage technologies at both household and national levels which reduce losses by preventing mould growth are important component of food security. Improved storage technologies, based on hermetic sealing in high density polyethylene bag or metal and plastic silos provide affordable and more effective storage alternative for farmers, especially the vulnerable

women, that would markedly contribute to food security (Gitonga *et al.,* 2013; Obeng-Ofori, 2011; Ndegwa *et al.,* 2016; Mutambuki *et al.,* 2012).

This study is, therefore to analyze the synergy effect of hermetic storage to control mould proliferation as well as mycotoxin contamination in safe and environmentally friendly system. The generated data from this study will facilitate sustainable adoption of the hermetic technologies among smallholder farmers in Sub Saharan Africa. This study suggests the ideal storage options for the small holder farmers considering the robustness and cost of the hermetic storage that will have been identified as effective and less expensive. The study also tries to answer the question of how the use of improved storage technology impact the quantity and quality of grain stored and also the length of storage while holding other factors constant at farmers' practice level.

2. Materials and methods

The trial was conducted at CIMMYT/KARLO Kiboko Research Centre (Makuen icounty), 170 km from Nairobi in a semi-arid region in Eastern Kenya. The trial site was selected for being a trouble spot for aflatoxin outbreaks in Kenya. Two factors were used in the design of this study: 1) low (12-13%) or high (14-15%) grain moisture levels; 2) ten storage technologies. The hermetic storage technologies under study were metal and plastic silos, while the hermetic bags were: Super Grain IV-RTM, AGRO-Z with pesticides, AGRO -Z without pesticides, PICS, Elite and ZeroFly. The two controls were two farmer practices, the standard woven polypropylene bags, one with grain treated with insecticide and one without insecticide treatment. The experimental design was a 2 x 10 randomized complete block design (RCBD) with 3 replications. The duration of the experiment was 4 months with non-destructive sampling at baseline and every 120 days afterwards. Each grain sample was divided in two for insect pest testing and mycotoxin analysis.

2.1 Sample collection and preparation

About one kilogram of sample was required for the analysis. Sampling was done from five different points, about 1 inch from the walls of the storage technology using a grain sampling spear. Sampling was done carefully not to puncture the linings of the bags and the spear cleaned with cotton dampened with 75% ethanol before sampling the next storage technology to avoid cross contamination. The sampled grain was transferred into the ziplock plastic bag and sealed carefully to exclude air. Three people were involved in the sampling procedure; one person opens the storage technology, draws samples and transfers to the plastic sample bags held by another person while the other person immediately tightly seals up the bag/silo.

2.2 Materials

The grain used for this study was of H614 and H618 hybrid, purchased from farmers in Nakuru county and Naivasha sub-county. The untreated grain was cleaned by sieving to remove chaff, broken and rotten kernels. At the onset of the experiment, the grain was mixed and conditioned at the appropriate moisture content before transferring in the respective study technologies.

2.3 Grain moisture

The high moisture content (14-15%) was achieved by subjecting the grain to high relative humidity and tests were carried out progressively to determine the required moisture contents. The grain spread on plastic sheet was sprayed with potable water for 1.5 to 2 days. The water was calculated from the formula below:

$$Quantity\ of\ water\ required\ (g) = weight\ of\ grain\ x\ \frac{mcf - mc}{100 - mc}$$

Where *mcf* is the final moisture content; and *mc* the initial moisture content (Kiburi *et al*., 2014).

To achieve the moisture range of 12-13%, the grain will be sun dried in the case their moisture content was above 13%.

Insects assessment

One kilogram of the grain was analyzed for the dead and the alive of insects. This was done to investigate whether the storage technologies are able to prevent entry of insects/encourage insects' activities. The number of live and dead insects, both adult weevils and larger grain borers was counted and recorded. The grains of the subsample were sorted into undamaged, damaged and discolored fractions. The number of kernels and the weight of each fraction were recorded to investigate the extent of damage if any as follows:

$$Discolored\ grain(\%) = \frac{Number\ of\ discolred\ grain}{Total\ number\ of\ grain} \times 100$$

$$weight\ loss(\%) = \frac{[(Wu \times Nd) - (D \times Nu)]}{Wu \times (Nu + Nd)} \times 100$$

Where W_u = Weight of undamaged grain; N_u = Number of undamaged grain; W_d = Weight of damaged grain and N_d = Number of damaged grain

Grain weight loss was determined by count and weight method (Boxall 1986).

Aflatoxin and fumonisin analysis

Aflatoxin and fumonisin levels were determined in each working sample collected at cero and four months after stocking using the VICAM method (VICAM Science Technology, 1998), as describe by (Fandohan *et al.*, 2005). Three samples from each bag were taken.

Statistical analysis

Variances of insect count, (x) was stabilized by log transformation Y=log(x+1) whereas percentage data (P) was arcsine Y=sin−1$\sqrt{P}$), transformed, where Y is the result of transformation. The transformed data was then be subjected to analysis of variance (ANOVA) using Stata SE version 12 (StataCorp LP, Texas, USA). Further due to inherent limitations of ANOVA in describing difference in progression of variables over time, the analysis of covariance (ANCOVA) which combines features of both ANOVA and regression were applied to test effects of treatment and storage duration, and the interaction effects. Means were separated using Bonferroni adjustment at 95% confidence level (Ognakossan *et al.*, 2014).

Results

Aflatoxin and fumonisin

Aflatoxin contamination increased with relative humidity in both hermetic and farmer practice storages at a significance level of P<0.001, it was also observed that aflatoxin contamination increased in all the inoculated storage technologies and very high in the farmer practice (Fig. 1 and table 3). The treatment type had an effect on the level of aflatoxin contamination at the significance level of <0.001 with the mean value of 2.93, 1.31, 2.59, and 1.65 for high humidity, low humidity, inoculated and in not inoculated grains respectively. The level of fumonisin contamination increased in woven storage bags while hermetic storage technologies reduced fumonisin contamination (Table 5 and 6). There was a relationship between moisture levels, mode of inoculation and the fumonisin contamination in the storage technologies with the grand mean of 0.315 and 0.275 respectively, Fig 2. However, there was not a significant difference observed between treatment and the level of fumosin contamination (Table 1). There was no interaction between the aflatoxin and the fumonisin P>0.05 but a strong correlation between the insects and the aflatoxin contamination at P<0.05 and the number of dead insects was linked with the type of storage where hermetic bags had less insects infestation than the farmer practice. At high relative humidity, the aflatoxin, fumonisin and insects was significantly high regardless of the mode of inoculation compared with the dry grains Fig.1.

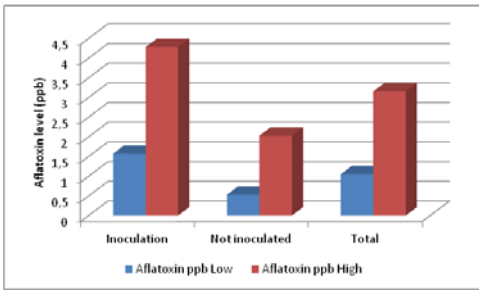

Fig. 1 Mean values of aflatoxin for both hermetic storages and the farmer practice.

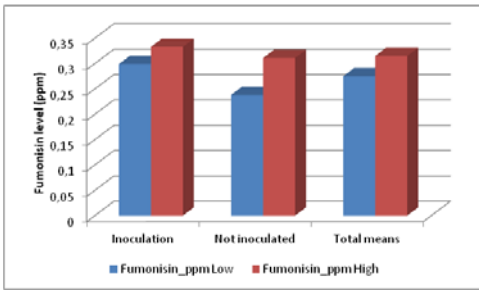

Fig. 2 Mean fumonisin levels in the storage technologies in relation to humidity and mode of inoculation

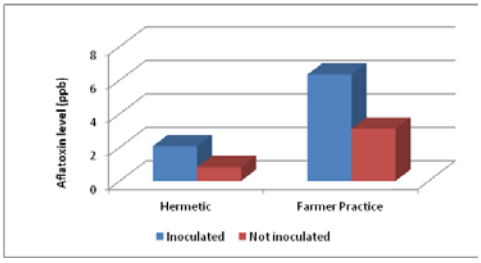

Fig. 3 Effects of technology and inoculation on aflatoxin

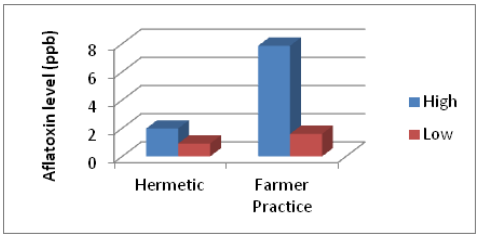

Fig. 4 Effect of technology and RH on aflatoxin

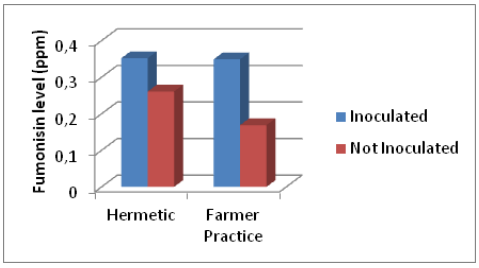

Fig. 5 Effects of technology and inoculation on fumonisin

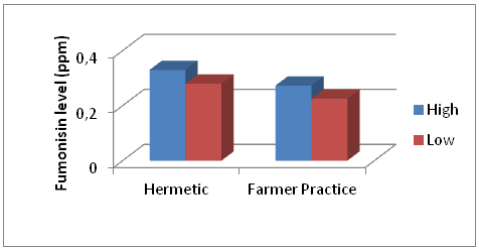

Fig. 6 Effect of technology and RH on fumonisin

Tab. 1 Interaction between aflatoxin/fumonisin and RH, inoculation and treatments

Mycotoxin		P- Value	corrected p-value		Significance
Aflatoxin					
	Relative Humidity	<.001	1.711		Sig.
	Treatment	<.001	0.904		Sig.
	Inoculation	<.001	1.032		Sig.
Fumonisn					
	Relative Humidity	0.555	0.059		n.s
	Treatment	0.092	0.169	Sig.	
	Inoculation	0.413	0.069		n.s

Insect infestation in different sets of treatment

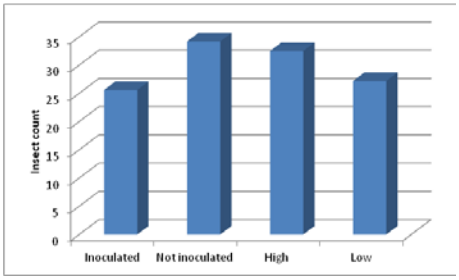

Fig. 7 Insect infestation comparing RH and mode of inoculation.

There was a significant correlation between the total insects infestation and the type of storage technology (treatment), at P= 0.109. Mode of inoculation and RH also did have any effect on the insect infestation in the four months storage period (Fig 7).

Tab. 2 Effects of treatment and insect infestation

Insects				Significance
		P- Value	corrected p-value	
	Inoculation	0.196	0.169	n.s.
	Relative	0.492	0.048	n.s.
	treatment	0.109	0.26	Sig

Discussion

Hermetic storage technologies can be an effective solution to reduce insect infestation and mycotoxin contamination during on-farm storage, thereby reducing potential human and animal exposure to mycotoxins. However, if farmers do not adequately dry grain, even hermetic storage technologies may not be effective in the control of mycotoxin contamination, and contamination will be even greater underconventional storage systems. This observation is in agreement with Cotty (2007), who described water activity as one of the conditions that encourage aflatoxin development high levels of fumonism in woven bags could be attributed to large open spaces that allow for free flow of air hence contamination. Hermetic storage technologies restrict gaseous exchange and act as a barrier hence reduced contamination. There was a correlation between inoculation and insect infestation where insect infestation was higher in the maize that was not inoculated. This is because maize already infested with aflatoxin and fumonism may have reduced in the nutritional components and palatability desired by the insects. This is also agreeable with the findings that mycotoxins development increases with the insects activities in the grain (Munkvold, 2003). This work supports the promotion of both hermetic storage technologies and improved drying practices. Currently the analysis of samples after eight months of storage is ongoing and will also be presented.

Acknowledgement

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Postharvest treatment research at USDA-ARS: stored product fumigation

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Abstract

The overall goal of this USDA-ARS research is to ensure the protection and quality of stored product foodstuffs. The results of this research directly enhance production, distribution, and safety of foodstuffs, promote and retain access of United States-grown crops to domestic and foreign markets, and protect the United States and trading partners from the agricultural, ecological and economic threat posed by quarantine and invasive pests. In general, USDA-ARS research related to the fumigation of stored products focuses on the development of techniques to rapidly disinfest raw products of field pests, control pests in processed products amenable to re-infestation and microbial infection, and reduce reliance on fumigation as a stand-alone measure for postharvest disinfestations and disinfections. Specific research objectives include: comparative evaluation of alternative fumigants to methyl bromide in postharvest applications, development of novel technologies to reduce and eliminate atmospheric emissions from chambers used in postharvest fumigation, and design production strategies that allow for a more strategic postharvest use of methyl bromide and alternative fumigants. Recent research findings will be presented and discussed, including: exposure requirements of phosphine on key stored product pests (as related to resistance management), the establishment of efficacy and experimental criterion for quarantine applications, and the development of models to quantitatively understand the underpinnings of fumigations and related phytosanitary treatments.

Keywords: food security, food safety, quarantine treatments, postharvest methyl bromide

1. Introduction

The use of postharvest phosphine fumigation as a quarantine phytosanitary requirement is increasing coincident with the globalization of agriculture. However, operational and regulatory framework for implementing and certifying efficacious treatments have not bee firmly established. In this work we describe a postharvest fumigation with phosphine to control Warehouse beetle, *Trogoderma variable* (Ballion) (Coleoptera, Dermestidae), a pest of concern to certain countries that import Dried Distillers Grains (DDGs) from USA. A series of laboratory-scale exploratory fumigations with phosphine at 10.0 ± 0.3 ºC ($\bar{x} \pm 2s$) were conducted to evaluate the postharvest control of

eggs as well as diapausing larvae of *T. variable*, the most phosphine-tolerant life stages of this pest. Models of the duration-mortality response predicted >99% mortality when headspace concentrations of phosphine, [PH3], are maintained at levels ≥ 0.8 mgL^{-1} (500 ppmv) and ≤ 1.5 mgL^{-1} (1000 ppmv) for ≥ 120 h, as estimated by the lower boundary limit of the 95% confidence interval. A fumigation schedule is proposed based on the results of this research and the seminal studies of Vincent and Lindgren (1975) as well as Banks and Cavanaugh (1985). Data is presented and discussed in the context of controlling *Trogoderma variable* following commercial fumigations for export of DDGs.

2. Materials and Methods

Insects and egg collection

Specimens were cultured in the insectary at the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), San Joaquin Valley Agricultural Sciences Center, Parlier, CA (USDA, 2012). Cultures were housed in an 15.2-m^3 rearing unit maintained at 27 ± 1°C ($\bar{X} \pm s$) and 60 ± 5% RH ($\bar{X} \pm s$) with a photoperiod of 16:8 (L:D) h, unless otherwise noted. Rearing procedures and diets, briefly mentioned below, were as reported in the Crop Protection and Quality Research Unit electronic rearing manual (USDA, 2012). Methods for collection of eggs and diapausing larvae are detailed below.

Warehouse beetle (WHB), *Trogoderma variable* (Ballion) (Coleoptera, Dermestidae), was originally collected in 1967 from whereabouts unknown in Fresno County, California USA. WHB adults (200 to 300) were transferred to a 946-mL glass jar filled with 20 to 25 g of a dried dog food substrate. The jar was sealed with filter paper (Whatman® #1, 90-mm diameter) followed by a wire screen (U.S. #40 mesh, 90-mm diameter) and both were secured a top the jar with a threaded metal ring. The jar was transferred to the rearing unit for a 72-h ovipositional period, after which, eggs were separated from the adults and flour using a stack of sieves (Seedburo Equipment Company, Des Plaines, IL). The contents of the jar were poured into the top sieve (U.S. #25, 0.71 mm^2 openings) and shaken vigorously for a few minutes. The eggs were retrieved from the underlying sieve (U.S. #60, 0.25 mm^2 openings) by decanting them into a glass Petri dish (100 mm diameter x 15 mm h). Counting eggs required for treatments was performed under the microscope by transferring small amount of eggs into a trough made out of black construction paper. Using a small, horsehair brush, ca. 100 eggs were transferred onto black velour paper that lined the inside of a 35-mm diameter Petri dish (Falcon, Oxnard, CA). Wheat bran diet (5 g), prepared as described in the rearing manual (USDA, 2007), was placed in each of several 10-cm diameter plastic Petri-dish cages. The diet was spread concentrically to the outer edge of each cage bottom and a single 35-mm Petri dish, containing the < 72-h old eggs, was placed in the center of the void. After fumigation treatment, or concomitant use as non-treated control specimens, the Petri-dish cages were lidded.

As described in Banks and Cavanaugh (1985), larvae known to be in diapause were obtained by isolating single larvae taken from stock cultures. Each larvae was introduced, along with 0.5 g of diet, 7-dram clear plastic "snap cap" cages modified with 8-mm diameter stainless-steel 100 wire mesh gas-portals on the bottom, snap cap, and side. The cages were incubated at 30 ± 1°C ($\bar{X} \pm s$) and 60 ± 5% RH ($\bar{X} \pm s$) with a photoperiod of 16:8 (L:D). Larvae that had not metamorphosed after 6 wk were considered to be in induced diapause, as described by Burges (1961, 1965).

Chemical Analysis and calibration of standards

Cytec Canada, Inc. (Niagara Falls, Ontario, Canada) provided the 300-lb cylinders of 1.6 % (v/v) phosphine balanced with nitrogen. A source cylinder (300-lb) of breathing air was obtained from Airgas (Fresno, CA, USA). The 1.6% PH3 mixture was used as the source for gas chromatography calibrations and the exploratory fumigations. [PH3] and steady-state concentrations thereof, $[PH3]_{ss}$, were measured using gas chromatography (GC); retention time (PH3, t_r = 3.2 ± 0.2 min, $\bar{X}$

$\pm s$, n = 10) was used for chemical verification and the integral of peak area, referenced relative to liner least-squares analysis of a 5-point concentration – detector response curve, was used to determine concentration. Detector response was determined by diluting known volumes of gases into volumetric gas vessels. A response curve was generated respective to each sampling interval with each sample referenced to the response. [PH3] levels were reported as average (±) standard deviation ($\overline{X} \pm s$) from duplicate measurements (vide infra). Analyses were with a Varian 3800 and splitless injection (140 °C) using a gas sampling port with a 10 μL-sample loop, a Teflon column (L = 2 m, OD = 2 mm) packed with Porpak N (80/100 mesh) held at 130 °C for 10 min, and a pulsed flame photometric detector (PFPD) detector (13 mL/min H_2, 20 mL/min air, and 10.0 mL/min N_2 make-up) at 250 °C that received only 10% of the 15 ml He/min column flow.

Exploratory fumigations

Laboratory-scale exploratory fumigations were conducted in a matching set of 24 Labonco® 28.32-L vacuum chambers housed in a walk-in environmental room with programmable temperature and humidity (USDA, 2010). Temperature and humidity set-points were 10.0 °C and 80% RH, respectively. A series of exploratory experiments was used to determine the treatment duration, ranging from 24 to 168 h, required to control larvae with applied doses, and subsequently, steady-state concentrations of phosphine in chamber headspace (i.e, $[PH3]_{ss}$) of ca. 250 (0.4), 500 (0.8), 1000 (1.5), or 2500 ppmv (μLL^{-1}) (3.7 mgL^{-1}) phosphine, respectively. Each of five chambers was loaded with an egg dish; four of the chambers were, respectively, subject to the phosphine treatments above and the fifth was not fumigated to yield non-treated control specimens. In addition, the control chamber and the chamber treated with 1000 ppmv (μLL^{-1}) (1.5 mgL^{-1}) $[PH3]_{ss}$ were loaded with 30 caged larvae. Each "block" of five chambers was subject to treatment durations of 24, 48, 72, 96, 120, 144, and 168 h. Each "block" was conducted in triplicate, which yielded a total of ca. 300 eggs at each $[PH3]_{ss}$ and each treatment duration, as well as 90 larval specimens treated with 1000 ppmv (μLL^{-1}) (1.5 mgL^{-1}) $[PH3]_{ss}$ at each treatment duration. This design corresponded to a total of 630 and ca. 2100 control larvae and eggs, respectively.

Loaded chambers, 300-lb source cylinders of breathing air (Airgas, Fresno, CA, USA) as well as 1.6 % (v/v) phosphine balanced with nitrogen, and gas-tight syringes were acclimated to fumigation temperature (i.e., tempered) within the walk-in environmental room for at least 24 h prior to treatment. Air temperature in the walk-in room was confirmed prior to fumigation by a HOBO data logger (HOBOware version 2.7). Chamber lids were then clamp-sealed in preparation for treatment. A slight vacuum of approximately 76-127 mmHg was established in each chamber. Gas-tight super-syringes (Hamilton ® 500, 1000, or 1500 mL) were filled with a volume of phosphine from the 300-lb source cylinder of 1.6 % (v/v) phosphine to achieve the requisite dose as predetermined in preliminary calibration studies. The syringe was fitted to a LuerLok ® sampling valve, which was subsequently opened so that fumigant was steadily drawn into the chamber. The syringe was then removed and normal atmospheric pressure (NAP) was reestablished; this marked the beginning of the exposure period.

Flow from 300-lb source cylinders of breathing air (Airgas, Fresno, CA, USA) and 1.6 % (v/v) phosphine were metered, respectively, into each of four gas blending manifolds (Aalborg Model G gas proportioner meter) that allowed for tunable $[PH3]_{ss}$ to exit the manifold, and ultimately enter a respective chamber. Exit flow from the manifold, which totaled 25 $mLmin^{-1}$ regardless of $[PH3]_{ss}$ (i.e. breathing air was the make-up gas), was directed to the input port/valve on the chamber; ¼'-diameter Teflon tubing was used for all plumbing and all connections were with standard stainless-steel Swedgelock fittings, unless otherwise noted. Flow exiting the chambers was directed through a LuerLok® sampling port into a centralized ventilation system (USDA, 2010). $[PH3]_{ss}$ and air inputs were tuned to the desired level in preliminary calibration studies, prior to the introduction of any test specimens into the chamber.

A gas sample of the chamber headspace was acquired using the LuerLok® sampling valve, which accessed the effluent of the respective chambers. A B-D® 100-mL gas-tight syringe was allowed to slowly fill to ~ 40 mL with the chamber effluent. Contents of the syringe were quantitatively analyzed with gas chromatography (GC) as described below. In the exploratory fumigations, the standard sampling interval for measurement of $[PH3]_{ss}$ was at 0.12 h (initial) and every 12 h thereafter through the duration of the treatment. Carbon dioxide and oxygen concentration were measured with a gas sampling pump connected in series between a port accessing chamber effluent and an atmospheric gas analyzer (GFC-7000E, Teledyne Instruments, City of Industry, CA), which recorded at standard temporal intervals over the duration of treatment.

After the final sampling of $[PH3]_{ss}$, cylinder valve-stems were shut, thereafter inputs of breathing air and phosphine ceased, chamber valves were opened to atmosphere, and a 30-min aeration period was initiated. Chamber lids were then opened and the treated as well as non-treated specimens were retrieved and transferred to an incubator at 27.0 ± 1.0 °C and 80 ± 2% RH ($\overline{X} \pm s$) in prelude to mortality evaluation (vide infra).

Mortality evaluation

Mortality of diapausing larvae was diagnosed visually by discoloration, while survivability was diagnosed by locomotion or by prodding-induced motion 14 to 21 d post treatment. Ultimately, however, evidence of pupation served as diagnostic of survival. Egg mortality assessments were conducted using a dissecting microscope (8 to 10 x magnification) 14 d after treatment, as the 5 to 7 days typically required for hatching was delayed due to physiological suppression at the 10°C treatment temperature.

Mortality was calculated as a percentage of the response per treatment. Mortality of control specimens was assumed to be equal to that in fumigation trials, per the method of Abbott (1925), and was included as a natural response in modeling the efficacy results from exploratory trials. The total number of specimens that were treated for each exploratory-trial was estimated by summing the numbers treated, while the total number of specimens treated (*n*) across exploratory-trials was estimated by summing the numbers from each respective trial. Mortality was analyzed via probit analysis of Finney (1944 & 1977) at the 95% confidence level, as further derived in Couey and Chew (1986) as well as Liquido and Griffin (2010).

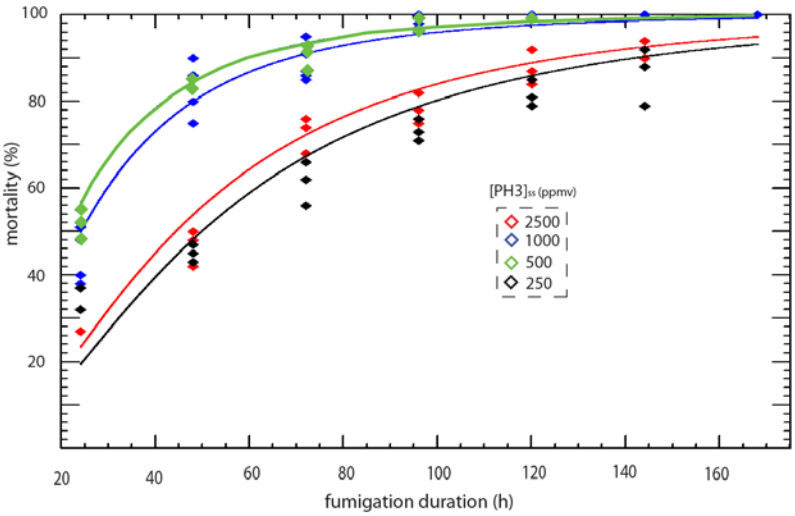
3. Results

Exploratory fumigations

The average air temperature ($\overline{X}$), 10 °C, was calculated across all trials. Deviation in temperature was assumed to follow a normal distribution with the estimated margin of error reported as ± 2*s*, 0.3 °C, the 95% confidence interval (Quinn, 1983). Of the 630 untreated diapausing larvae, only 24 expired with no more than 3 deaths per 30-specimen control grouping. As for mortality in 2,106 untreated eggs, 45 expired with no more than 5% mortality in each control grouping of ca. 100. While the control mortality of the diapausing larvae was consistent with previous reports (Banks and Cavanaugh, 1985), control mortality of eggs was ~15 % less than observed by Vincent and Lindgren (1975).

Respective duration-mortality regressions for (applied doses and) $[PH3]_{ss}$ of 250 (0.4), 500 (0.8), 1000 (1.5), or 2500 ppmv (μLL^{-1}) (3.7 mgL^{-1}) were modeled using Polo Plus (LeOra Software, 2002-2007) with the mortality of control specimens included as a natural response. The number of egg specimens treated (250 ppmv: 2105 subjects; 500 ppmv: 2112 subjects; 1000 ppmv: 2109 subjects, 2500 ppmv: 2093 subjects), the regression heterogeneity (H), the projected durations to cause 50, 95, and 99% mortality in the treated population (respectively LT_{50}, LT_{90}, and LT_{99}), and the bounds (upper (UL) and lower (LL) limits) at the 95 % confidence level (CL) are shown in Figure 1. Likelihood ratio-based hypothesis testing of equality was rejected ($P < 0.05$, $\chi^2 = 621$, $df = 6$), indicating that the

slopes as well as the intercepts of the regressions respective to $[PH3]_{ss}$ were significantly different. Likelihood ratio-based hypothesis testing of parallelism was rejected ($P < 0.05$, $\chi^2 = 44.7$, $df = 3$), indicating that the slopes of the regressions respective to $[PH3]_{ss}$ were significantly different.



Probit Regression Parameters

	250 ppmv			500 ppmv			1000 ppmv			2500 ppmv		
	"duration"	95% CL LL	UL	"duration"	95% CL LL	UL	"duration"	95% CL LL	UL	"duration"	95% CL LL	UL
LT_{50}	46.8	38.7	54.4	24.4	21.6	26.9	28.2	24.5	31.6	43.9	38.3	49.0
LT_{95}	241.6	189.8	342.6	73.9	67.4	82.7	83.5	74.4	96.4	174.0	149.3	212.5
LT_{99}	475.0	335.6	802.8	117.2	102.5	138.6	130.9	111.3	161.9	308.0	247.1	414.1
Slope	2.3 (+/-) 0.2			3.4 (+/-) 0.2			3.85 (+/-) 0.2			2.7 (+/-) 0.1		
Heterogeneity	3.95			1.30			2.29			2.64		
Treated	2105			2112			2109			2093		

Fig. 1 Mortality of warehouse beetle, *Trogoderma variable* (Ballion), eggs following phosphine fumigation at 10.0 ± 0.3 ºC ($\bar{x} \pm 2s$) and probit regression analyses (Polo Plus, LeOra Software, 2002-2007) of the duration-mortality response respective to applied doses and steady state headspace concentrations, $[PH3]_{ss}$ of ca. 250 (0.4), 500 (0.8), 1000(1.5), or 2500 ppmv (μLL^{-1}) (3.7 mgL^{-1}), showing the number of specimens treated, the regression heterogeneity (H), the projected durations to cause 50, 95, and 99% mortality in the treated population (respectively LT_{50}, LT_{90}, and LT_{99}), and the corresponding estimates of the bounds (upper (UL) and lower (LL) limits) at the 95 % confidence level (CL).

Lethal time ratios (LTRs) were calculated with (+/-) 95 % confidence intervals (CI) across the durations projected to cause 10 to 99% mortality in the treated population. Figure 2 shows that fumigation with $[PH3]_{ss}$ of 250 or 2500 ppmv required longer treatment durations, relative to 1000 ppmv, to yield the same egg mortality response as noted by respective LTRs < 1 (unity) (Fig. 2). On the other hand, the LTRs for $[PH3]_{ss}$ of 500 ppmv overlapped or superseded a value of 1 (unity) respective to all projected durations > LT_{10}, indicating that time required for a particular percentage of egg control is equivalent when $[PH3]_{ss}$ is 500 or 1000 ppmv.

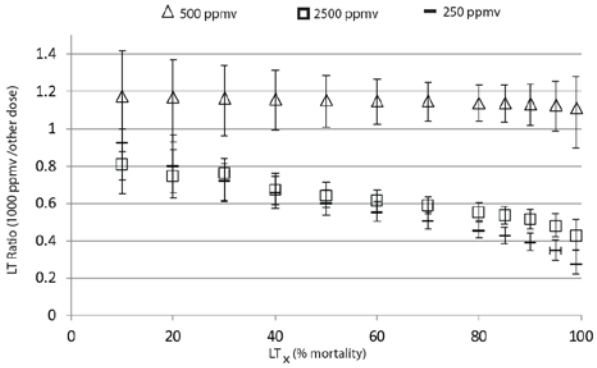


Fig. 2 Lethal time ratios (LTRs) associated with steady-state headspace concentrations, $[PH3]_{ss}$, of ca. 250 (0.4), 500 (0.8), 1000 (1.5), or 2500 ppmv (μLL^{-1}) (3.7 mgL^{-1}) were calculated ± 95% confidence intervals across the treatment durations projected to cause 10 to 99% mortality in the treated population of warehouse beetle, *Trogoderma variable* (Ballion), eggs. LTRs respective to durations predicted to yield >10% mortality overlapped a value of 1 (unity) for 500 ppmv, indicating that maintaining $[PH3]_{ss}$ at 500 ppmv was no more efficacious than maintaining $[PH3]_{ss}$ at 1000 ppmv levels. However, LTRs were less than a value of 1 (unity) for 250 and 2500 ppmv, indicating that these treatments required significantly longer durations to evoke an equivalent response in the treated populations, relative to treatments with $500 \leq [PH3]_{ss} \leq 1000$ ppmv.

Additionally for a $[PH3]_{ss}$ of 1000 ppmv, LTRs were calculated ± 95% confidence intervals across the treatment durations projected to cause 10 to 99% mortality in the treated population of eggs and diapausing larvae. LTRs respective to durations predicted to yield >10% mortality overlapped a value of 1 (unity), indicating that equivalent treatment durations resulted in equivalent response of eggs relative to diapausing larvae (Fig. 3). This finding is consistent with that of Banks and Cavanaugh (1985) in that neither study establishes diapausing larvae as being more phosphine-tolerant than eggs, which are clearly more phosphine tolerant than all other life stages of *T. variable* (Vincent and Lindgren, 1975).

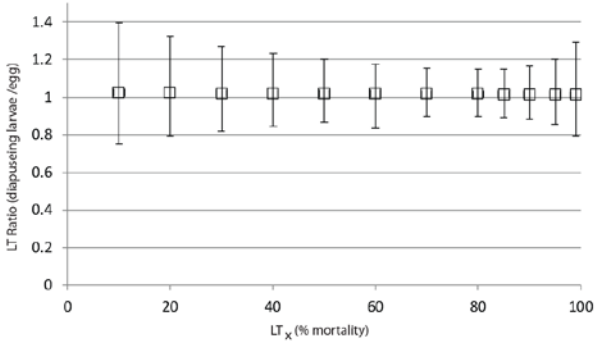


Fig. 3 Lethal time ratios (LTRs) associated with steady-state headspace concentrations, $[PH3]_{ss}$, of 1000 ppmv (μLL^{-1}) (1.5 mgL^{-1}) were calculated ± 95% confidence intervals across the treatment durations projected to cause 10 to 99% mortality in the treated population of warehouse beetle, *Trogoderma variable* (Ballion), eggs and diapausing larvae. LTRs respective to durations predicted to yield >10% mortality overlapped a value of 1 (unity), indicating that equivalent treatment durations resulted in equivalent response of eggs relative to diapausing larvae.

Figure 4 shows the projected durations to cause 99% mortality in the treated population (LT_{99}) of eggs varies as a function of $[PH3]_{ss}$. To rationalize this result, note the seminal work of Winks on phosphine (1984, 1985, 1986, 1994) as related to Haber's Rule ($C^z t = \omega$), which forms the basis for relating concentration (C) and time (t) to toxicological efficacy (ω), at least with respect to fumigation science (Bliss, 1940; Miller et al., 2000). For phosphine, z, the response evoked by a

specific toxicant in a particular organism, changes with *C*. When considering data on mortality collected at "fixed" concentrations over varying times, such as was done in the exploratory fumigations, the applied dose correlative to the onset of deviation (i.e., change in *n*) is termed the "narcosis threshold", the concentration above which further change in z results in the narcotic effect of phosphine and an increased tolerance. Recently, the work of Walse et al. (2013, 2016, 2017) has expanded on the concept of the "narcosis threshold" in the context of quarantine treatments as well as mitigation strategies for phosphine resistance. The results from the exploratory studies indicates the "narcosis threshold" of *T. variable* eggs spans the range 500 ≤ $[PH3]_{ss}$ ≤ 1000 ppmv and is centered at 750 ppmv.

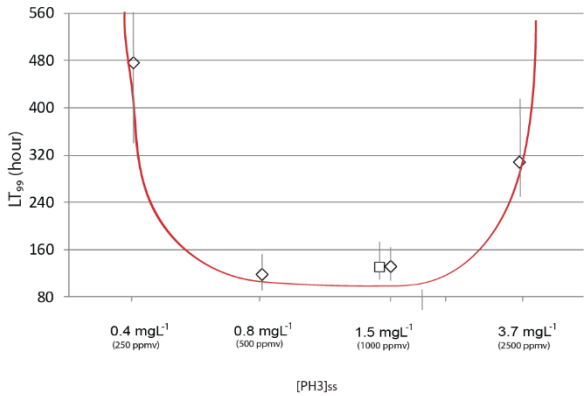


Fig. 4 The projected durations to cause 99% mortality in the treated population (LT_{99}) of warehouse beetle, *Trogoderma variable* (Ballion), eggs (◇) varied as a function of steady-state headspace concentrations, $[PH3]_{ss}$, over the range 250 to 2500 ppmv. However, an equivalent mortality response was observed when at $[PH3]_{ss}$ of 500 and 1000 ppmv, indicating that variability in $[PH3]_{ss}$ within the range 500 ≤ $[PH3]_{ss}$ ≤ 1000 ppmv, will not change the efficacy of fumigation. It is critical to note that the predicted duration required to control 99% of a treated population of diapausing larvae (□) is equivalent to that required for eggs. Error bars are the estimates of the upper (UL) and lower limits (LL) at the 95% confidence interval (see Fig. 1). The "narcosis threshold" for *T. variable* (Ballion) eggs spans the range 500 ≤ $[PH3]_{ss}$ ≤ 1000 ppmv as indicated by horizontal portion of the red trace.

The LL (95% CL) of the durations predicted to cause 99% mortality in the treated population (LT_{99}) of eggs and diapausing larvae were ca. 120 h. Moreover, none of the specimens (1,815 eggs & 270 diapausing larvae) survived fumigation with 500 ≤ $[PH3]_{ss}$ ≤ 1000 ppmv for a duration ≥ 120 h, results that suggest fumigation at ≥ 10.0 ˚C will control *T. variable* infestations if [PH3] is maintained at ≥ 500 and ≤ 1000 ppmv for a duration ≥ 120 h. In general, an increase in treatment temperature is commensurate with an increase of insect metabolism and increase in the efficacy of a fumigant (Monro, 1969). The work of Vincent and Lindgren (1975) supports this conclusion with respect to *T. variable*, as fumigation at 21.1 ºC with 500 or 750 ppmv for 72 h resulted in complete mortality of 1- to 6-d old eggs. Collectively, results provide the technical framework of a fumigation schedule:

Phosphine concentration maintained at 750 ppmv (μLL^{-1}) (1.1 mgL^{-1}) or higher for 72 h or greater at commodity temperature of 20.6 ºC or greater

Phosphine concentration maintained at 750 ppmv (μLL^{-1}) (1.1 mgL^{-1}) or higher for 96 h or greater at commodity temperature of 15.0 ºC but less than 20.6 ºC

Phosphine concentration maintained at 750 ppmv (μLL^{-1}) (1.1 mgL^{-1}) or higher for 120 h or greater at commodity temperature of 10.0 ºC but less than 15.0 ºC

4. Discussion

The use of postharvest phosphine fumigation as a quarantine phytosanitary requirement will only increase in years to come. Here we provided operational and regulatory framework for

implementing and certifying efficacious treatments. Although ISPM 27 ultimately leaves efficacy acceptance criterion to the discretion of the importer, interantional scientific consensus helps guide such reguialtory decisions.

Acknowledgement

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Quantifying grain storage structure leakage by testing effects of environmental conditions on pressure loss

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Abstract

A major concern in grain storage and management facilities is the effective control of insects and pests that reside in stored grain. Currently, the best studied method of subduing these insects is fumigating the grain bins with phosphine. However, many grain storage insects have developed a resistance to phosphine due to its misuse over the years, partially due to bin leakage, leading to minimum pest control in grain and increased product damage. The first step in managing the issue of fumigant leakage is by identifying environmental conditions that may impact the bins' total air loss, and ultimately, fumigant loss. One way to quantify the leakage potential of a structure is to perform pressure tests. Data collected from these tests statistically quantify the significance of atmospheric conditions on bin leakage, as well as quantify leakage area in the bin. These tests were performed on a 500 bushel grain bin filled with canola seed, sealed with plastic sheeting and Gorilla duct tape. A PVC pipe "arm" and shop vacuum (Shop-Vac® 5-gallon 6-Peak HP) contraption was designed for pressure application. Constant pressure testing methods were performed to collect data for calculations of leakage area. Tests were repeated in varying environmental conditions. Data analysis included performing single sample t-tests to determine significance of environmental conditions, as well as using previously established relationships to quantify predicted leakage area in each scenario. It was concluded that atmospheric conditions significantly affect gas leakage from structures ($p < 0.001$), so pressure test conditions should match fumigation conditions for an accurate initial fumigant dosage. Constant pressure tests accurately predict equivalent leakage area of bin, with areas demonstrating a variance of 3.4×10^{-5}. Future tests to improve fumigating processes could include relationships between phosphine concentration and the leakiness of the bin, as well as automated constant pressure testing devices.

Keywords: Phosphine resistance, fumigation, grain storage, leakage area, pressure testing.

1. Introduction

With populations across the world continuing to grow at an exponential rate and increasing life expectancies, it is inevitable that food production and storage will need to be continuously innovated to meet this growing need. One method that has been used for centuries is storing agricultural products, such as grain, as long as possible to ensure they are available when needed after harvesting season. Grain is known to last for long periods of time in storage facilities after drying due to lower moisture content. Nevertheless, it is still susceptible to damage from pests. The most effective way of controlling these pests is by utilizing fumigants and chemicals to eradicate their populations before the grain incurs too much damage. Over the years, the public has developed a growing concern regarding their foods, especially unfamiliar chemicals. Despite adverse public response, some chemicals, such as fumigants (i.e., phosphine, methyl bromide, etc.), are necessary to keep food products safe from disease-carry pests, specifically in stored products such as grain. These pests are not just detrimental to the fate of stored grain, but they have also proven extremely difficult to eradicate from the storage bin due to their resistance to most toxic chemicals. Grain pests include: *Rhyzopertha dominica* (F.), commonly known as the lesser grain borer; *Sitophilus oryzae* (L.), the rice weevil; *Tribolium castaneum* (Herbst.), also known as the red flour beetle; and *Oryzaephilus surinamensis* (L.), the saw-toothed grain beetle. While there are many other stored product insect pests, the aforementioned are four of the more commonly studied species. Not only do the insects damage the grain, they also leave waste product in the grain, which quickly diminishes the quality of grain. If left untreated, these insects have the ability to cause detrimental damage on the grain they are infesting.

One of the most common and cost effect methods of eliminating insects from grain is through fumigation. Attempts to eradicate pests through fumigation involve introducing a chemical toxic to the pests, such as phosphine or methyl bromide. However, due to its ozone-depleting properties,

methyl bromide has been banned as a fumigant in developed countries. Sulfuryl fluoride is a fumigant that is gaining popularity, but phosphine is still the most commonly used fumigant worldwide due to its low cost and minimal residue on treated grain. Moreover, due to incomplete fumigations using phosphine, grain storage pests have developed resistance (Daglish, 2004; Lorini et al., 2007; Price, 1984). A cause of incomplete fumigation is leakiness in grain storage structures. One way to predict the leakage area of grain storage structures prior to fumigation is by performing pressure tests, such as half loss time or constant pressure tests. Half loss pressure tests, also known as half loss time (HLT) have been used extensively in many studies (Chayaprasert et al., 2012), but it has been proven to not be the most reliable method of predicting the estimated leakage area from the bin (Mann et al., 1999). In this study, the constant pressure testing method is utilized since it has potential to be a more reliable method of pressure testing. It is also known that there is an expected correlation between atmospheric conditions, such as wind speed, temperature, and relative humidity, but previous studies have not used field trial data to statistically quantify this correlation (Chayaprasert et al., 2009). Therefore, this study will focus on using constant pressure testing methods to determine whether atmospheric conditions significantly affect gas leakage from grain storage structures, as well as estimating the leakage area using constant pressure data to predict a more accurate phosphine dose to reduce development of insect resistance.

2. Materials and Methods

Initial bin modifications

A selected grain storage bin at Oklahoma State University's Stored Product Research and Education Center (SPREC) was modified prior to testing. Modifications included drilling a hole and developing an extension arm that allowed for a shop vacuum hose to connect to the headspace of the bin (Fig. 1). The selected grain bin was a 500 bushel (12.5 metric tonnes approximately) steel bin bolted to a concrete slab and filled with canola seed. External valves correlated with various depths of the bin were already installed, and were used in the experiments to measure gauge pressure. To create an opening for an airflow source to perform pressure testing, a 1 ½ inch hole was drilled into the headspace of the bin. A 1 ½ inch PVC pipe fitting was then sealed into the hole using silicone caulking. To keep out moisture and other atmospheric conditions between testing, a cap was placed over the fitting. In order to create a path for air to flow from a pressurization source (shop vacuum) to the bin, a 90° elbow, which connected to the fitting in the headspace, was attached to one end of a three foot section of 1 ½ inch PVC pipe. A 60° PVC elbow was attached to the other end to connect a seven foot section, which went down the outside wall of the bin. To control airflow going into the bin (for the constant pressure/variable flow testing), a PVC ball valve fitting was attached to the other end of the seven foot PVC section. All PVC connections were made using PVC primer and cement. The arm was utilized to pressurize the bin by sealing the end of the shop vacuum hose to the ball valve using Gorilla duct tape.

Fig. 1 Extension arm created from PVC pipe to extend down side of bin for pressurization using shop vacuum

Fig. 2 HOBO Wind Speed Smart Sensor attached to top of pole to measure wind speed free from obstruction

Constant pressure tests with varying weather conditions

In order to correlate grain bin leakage with weather conditions, added instrumentation was installed to measure relative humidity (r.h.) and temperature inside and outside of the bin, and wind speed outside of the bin. To measure wind speed, an Onset HOBO® Wind Speed Smart Sensor was attached to a 15 foot pole (Fig. 2) near the grain bins and above obstructions. An Onset HOBO® micro station data logger was connected to the wind speed sensor to record wind speed data. Temperature and r.h. were measured using Onset HOBO® Pro v2 temp/RH sensors. One of the sensors was placed outside next to the bin according to manufacturer's directions. The second sensor was taped to the inside entry latter of the bin with Gorilla duct tape. All sensors were launched using HOBOware v. 3.7.8 and were programmed to take measurements once every 30 seconds starting at the time of each test.

There were four varying weather conditions in which the constant pressure tests were performed: hot and windy, hot and still, cold and windy, and cold and still. Conditions for each test can be seen in Table 1. Constant pressure tests were conducted by first resealing the aeration fan intake and the hatch entrance with tape and double-layered plastic sheets. Other leakage-prone areas that were previously sealed with tape were re-sealed prior to testing. Next, the extension arm was inserted into the headspace of the bin, and the shop vacuum hose was securely sealed to the ball valve. A Pitot tube was inserted upstream of the ball valve in the extension arm, and worked in conjunction with a Fluke 922 Airflow Meter to measure the air velocity going into the bin. Testing began by turning the shop vacuum on with the ball valve 100% open, and monitoring the U-tube manometer until a constant pressure was reached. As soon as the pressure stabilized, a timer was started and the air velocity was measured and recorded. For each varying weather condition constant pressure test, five trials were performed at each ball valve position, where air velocity and pressure data were collected once every thirty seconds for 15 minutes. To create control data, additional testing was performed on a day with an average temperature of 75°F and average wind speed greater than 8 mph and less than 10 miles per hour. Five trials were performed by adjusting the ball valve position to vary the air velocity. Once the pressure readings from the inside of the bin stabilized, the pressure value was recorded as well as the average air velocity at that pressure. Ten data points representing different input air velocities and stabilized internal bin pressures were collected for each test. A total of five tests were performed to create a standard curve for predicted pressure retention at given input air velocity into the bin.

Statistical analysis of effects of weather conditions on air leakage

Single sample t-tests with a confidence interval of 0.005 were performed in order to statistically quantify the effect of varying weather conditions on gas leakage rate from bins during pressure testing and fumigation. A standard model to predict pressure retention in the bin at a given input air velocity was developed using data from the neutral conditions (average temperature of 75°F and wind speed of no less than 8 mph, and no greater than 10 mph). This model was developed by plotting the data in a scatter plot, then matching a curve to the data. The resulting equation can be useful for this particular bin to decide if the bin is sealed to a standard level according to its pressure retention with a given input velocity. However, in this case, it was used to determine if weather conditions significantly affect gas leakage rate from bins. Average velocity for each stabilized pressure was calculated in order to perform t-tests on the data collected from the varying weather conditions. T-tests compared the average velocities needed to maintain the stabilized pressures for each weather condition with the predicted pressure that should be maintained at the given velocity, taken from the developed standard model.

Determining estimated leakage area (ELA) from constant pressure tests

In a previous study by Lawrence et al. (2012), ELA was calculated using constant pressure tests on a flour mill using Equation 1:

$$A_L = 10{,}000 Q_r \frac{\sqrt{\rho / 2 p_r}}{C_D}$$

[Eq. 1]

Where A_L is the predicted leakage area (cm^2), Q_r is the airflow rate (m^3/s), ρ is the air density (1.15 kg/m^3), p_r is the reference pressure (inch H_2O, $P_{gauge} - P_{atm}$ in this case), and C_D is the discharge coefficient (0.61 in this case, taken from Mann et al., 1999). This same equation was used to determine the ELA of the tested grain bin from constant pressure test data. Air flowrate was calculated by multiplying the input velocity by the diameter of the drilled hole in the headspace of the bin (0.0312 m^2). Air velocity values for Q_r that were used for this model were the average flow rates for each weather condition, and p_r was the correlated constant pressure with the air flowrate.

3. Results

Results from single sample t-tests

Weather conditions were tested on days with the conditions seen in Table 1:

Tab. 1 Weather conditions (temperature and wind speed ranges) for constant pressure test to ensure varied weather data to correlate with gas leakage rate.

Weather Condition	Avg. Wind Speed Range	Avg. Outside Temperature Range
Hot & Windy	> 10 mph	> 80°F
Hot & Still	< 8 mph	> 80°F
Cold & Windy	> 10 mph	< 70°F
Cold & Still	< 8 mph	< 70°F

After collecting data from constant pressure tests for all varying weather conditions and control data, the control data was plotted to develop a model to predict the ideal relationship between input air velocity and the maintained constant pressure at that velocity:

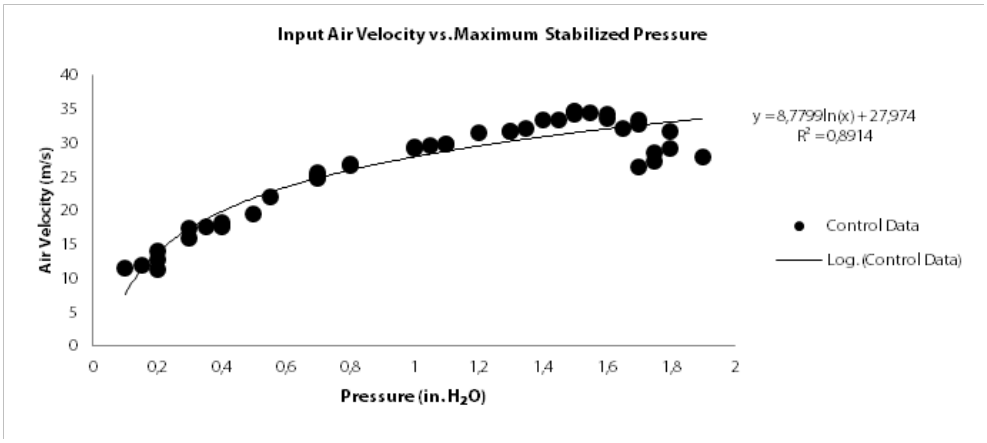

Fig. 3 Model of control data for constant pressure test.

The logarithmic equation developed from this model was used to predict the air velocity at the given constant pressure. Constant pressure test data from varying weather conditions were then compared to these predicted values. Single sample t-tests compared the predicted pressure for the average measured input air velocity with the actual maximum stabilized constant pressure maintained during the testing period. T-tests were performed for each constant pressure observed for each varying weather condition. The resulting p-values indicated if the weather factors had a significant impact on air leakage from the bin based on the maximum pressure that can be achieved in ideal weather conditions compared with maximum pressure maintained at the same input air velocity/flowrate in non-ideal conditions.

Tab. 2 Results from t-tests for constant pressure test in varying weather conditions, comparing theoretical constant pressures with actual constant pressures maintained in non-ideal weather conditions.

Weather Condition	Air Velocity (m/s)	Pressure (in.H_2O)	p-value
Hot and Windy	32.887	1.75	<0.0001
Hot and Windy	31.534	1.5	<0.0001
Hot and Windy	26.015	0.8	<0.0001
Hot and Windy	13.843	0.2	<0.0001
Cold and Windy	32.101	1.6	<0.0001
Cold and Windy	31.297	1.46	<0.0001
Cold and Windy	27.431	0.94	<0.0001
Cold and Windy	13.843	0.2	<0.0001
Hot and Still	23.489	0.6	<0.0001
Cold and Still	26.443	0.84	<0.0001

Predicted equivalent leakage areas (ELA) were also calculated using these same averages from the constant pressure test data in varying weather conditions to validate that constant pressure testing is an accurate method of estimating the ELA. Using Eq. 1, the ELA was calculated for each average input air velocity measured from the varying weather condition data.

Tab. 3 Estimated ELA for the same bin under varying weather conditions.

Q_r (m^3/s)	P_{gauge} (Pa)	A_L (m^2)
1.03	435	0.06
0.98	373	0.06
0.81	199	0.07
0.43	50	0.08
1	398	0.06
0.98	363	0.06
0.86	234	0.07
0.43	50	0.08
0.73	149	0.07
0.83	209	0.07

Average predicted ELA from this data is 0.07 m^2, with a standard deviation of 0.006 and variance of 3.4×10^{-5}.

4. Discussion

From the calculated p-values, it is evident that weather conditions significantly impact the air leakage rate from grain storage structures. This indicates not only that a higher dosage of phosphine (or other fumigant) may be needed in non-ideal conditions, but also that the atmospheric conditions at which the pressure testing takes place should match the conditions during the fumigation period for a more effective fumigation. Constant pressure testing used as a method of predicting leakage area from a grain storage structure is further validated by using the varying weather data to calculate the estimated ELA. The resulting ELA demonstrated low variance (3.4×10^{-5}), even though the air leakage rate from the same bin varied significantly with weather conditions. For future fumigations, constant pressure testing should be considered as not only a viable but more accurate method of estimating ELA than pressure HLT testing. Fumigations that utilize data from constant pressure testing to calculate the initial phosphine dose are likely to be more effective, and thus reduce the development of insect resistance to phosphine in grain storage. Future studies will automate the constant pressure test, as well as correlate initial phosphine dosage with leakage area predicted from constant pressure tests.

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Three and Half Decades of Research on Controlled Atmosphere Storage of Grains under Nitrogen and Recent Utilization of the Technology in Nigeria

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Abstract

A major breakthrough of Nigerian Stored Products Research Institute (NSPRI) is in the development of Inert Atmosphere Metal Silo (IAMS) in bulk grain storage for suitability of the climate, adoption and utilization in Nigeria. This technology uses nitrogen gas to achieve a controlled atmosphere (N2-CA) or environment for the control of stored products pests infestation and damage. Achieved 100% mortality of all life adults and immature stages of stored products insect pests; inhibited mould growth, maintained biochemical composition of stored grain and germinability (85% -91%) recorded at 12 months of storage. The system has been used to effectively store white maize, groundnut, Ife brown cowpea, wheat, paddy rice and yellow sorghum for periods of 12 – 48 months. The only system that has ability to store cowpea which cannot be stored in conventional silos. Research activities commenced from laboratory trials to pilot scale and later to medium and commercial levels. Shading the IAMS top against direct sun effect with palm fronds or hood for insulation prevented moisture migration and condensation, and maintained temperature below 30 °C in stored grain. A return per unit on investment of 0.44 was recorded when utilized for storage of wheat for a period of 48 months. IAMS has economic advantages over conventional silos which require frequency of pesticides application, turning of grains to prevent caking, food poisoning and high cost of labour. The recent utilization of this technology is due to increased awareness and demands for availability of grains for food safety, quality and nutrition. IAMS is being taken up by some entrepreneurs, marketers and Landmark University, Omu-Aru, Kwara state for grain storage in Nigeria. This technology is available for use at smallholder, medium, commercial and strategic grain reserve levels. Three and half decades of application of IAMS technology in grain storage in Nigeria is discussed.

Keywords: Inert atmosphere metal silo (IAMS), Nitrogen, grain storage and quality, control of pests, utilization.

1. Introduction

The Nigerian Stored Products Research Institute (NSPRI) is one of the National Agricultural Research Institutes (NARIs) in Nigeria, being supervised by Agricultural Research Council of Nigeria (ARCN)

and a parastatal under Federal Ministry of Agriculture and Rural Development (FMARD). NSPRI has been conducting research in technology development suitable for postharvest handling, storage and preservation of grains for food safety and quality for over fifty years. Nigeria is the world largest producer of cowpea. Other cereal and pulses grains are produced in large quantities and consumption of safe and quality food for good nutrition and health is imperative. Research into use of phosphine and other conventional insecticides shoed that several insect pests have developed resistance ([1]). In late 1970s Nigerian Stored Products Research Institute (NSPRI) has developed improved warehouse, improved ventilated maize cribs and hermetic grains storage bags/containers ([2,3]). These technologies are not only effective in reduction of postharvest loss in cereals and pulses but reduce the impact of aflatoxin contaminations ([4]). However, handlers and operators are exposed to health hazards, poisoning and resistance to pesticides by stored products pests are developed ([5,6]). In 1980 the need for alternate pest control became imperative and the Institute in line with best global practices developed a non-chemical technique for storage of agricultural produce. Researches started with laboratory trials on susceptibility and insect infestations in mini IAMS of 0.65m^3 and 4-ton volumetric capacity; to pilot scale of 45 ton volumetric capacity in Ibadan for storage of maize ([7,8,9,10,11,12]). Due to 2006 bean scare incident due to reckless misuse and abuse of pesticides for control of stored products pests by the farmers and grain aggregators in Nigeria, which resulted in accidental poisoning and deaths, NAFDAC one of the regulatory agencies banned importation and use of pesticides in stored products food ([13]). NSPRI seized the opportunity and stored cowpea under nitrogen in a 45-ton volumetric capacity Inert Atmosphere Metal Silo (IAMS) at NSPRI Ibadan. The release of the stored cowpea in 2008 attracted Federal government intervention in construction of other battery of 2 units of 50-ton volumetric capacity IAMS in Ilorin and Kano in 2010 and 2013 respectively. In 2015, some of Nigeria agricultural produce were rejected and cowpea was banned by the European Union countries due to misuse and abuse of pesticides on grains ([14]). By 2013 utilization of IAMS was introduced as there was increasing awareness on food safety and quality. NSPRI being the only Federal Government agency mandated by its Act of establishment to conduct research among other issues into the postharvest activities of agricultural crops in the country became part of the problem solving mechanism. By 2015 entrepreneurs, marketers, industrialists, university and other relevant stakeholders became interested in the utilization of IAMS due to increased awareness on grain availability for food safety and quality and the successful application of nitrogen controlled atmosphere in grain storage. Researches have proved use of N2-CA as safe alternative to synthetic pesticides for protection of grains from attack of stored products insect pests ([14,15]). Low temperature and controlled atmospheres (CA) are internationally standard recognized grain storage technologies. The two principal types of atmosphere that could be used for controlled atmosphere (CA) storage and disinfestations of grains are: low concentration of Oxygen (O_2) and high concentration of Carbon Dioxide (CO_2)([14]). The principles and operation of Nitrogen Controlled Atmosphere (N_2-CA) in grain storage involve maintenance of food safety and grain quality by control of storage pests, inhibiting storage fungi, prevention of re-infestation of storage insects, maintenance of viability and germinability of stored seeds and delaying deterioration of grain quality ([15]). The use of N_2 gas as a medium of modifying atmosphere is most preferable and suitable for grain storage in Nigeria. IAMS is therefore a promising alternative for appropriate storage of grains under nitrogen. This technology does not require use of any synthetic pesticides for control of stored products pests, no residues in stored grains and no food poisoning involved. Also resistance to pesticides and hazards to operators are eliminated ([5]).

The research in IAMS technology has moved from laboratory trials to pilot scale, to medium scale and finally to commercial and industrial levels. It is necessary to appraise the research efforts of this technology to further sensitize the public on the importance, relevance and benefit of the technology, as well as open up more research opportunities and collaboration for improvement studies. The research work carried out and development of IAMS technology for grain for the smallholder, medium, commercial and strategic reserve levels for storage in Nigeria are reviewed subsequently.

2. Materials and Methods:

Construction of Inert Atmosphere Metal Silo (IAMS)

In the laboratory trials, an airtight mini-silo of a battery of 2 units volumetric capacity 0.65m3, were constructed in NSPRI Ibadan and Kano respectively. The IAMS was installed under shade ([9]). The mini silo was provided with three sampling points located at the bottom, middle and top positions of the metal silo. A dial bi-metallic thermometer probe was fitted midway of the silo with sensing probe penetrating to the central axis. Nitrogen was supplied to the metal silo through a nitrogen distribution system consisting of a pressure cylinder and a gas flow instrument panel from the silo downward through the stored grain. A pressure relief valve was fitted at the base of the mini silo ([9]).

In the pilot trials, an airtight mini metal silo of a battery of 4 units volumetric capacity 4-ton were erected at the premises of NSPRI Ibadan supported by point load bearer concrete columns and structural bars, in such a way that they were fully exposed to varied effects of the sun, wind and rain throughout the experimental period. The mini silos were all painted white so as to increase the reflectivity of the surface and thus cause a cooler environment inside. The whole body and top of silo was insulated with 5 cm glass wool and galvanized sheets but not shaded; or top of silo was insulated with 5 cm glass wool but not shaded; or no part of silo was insulated or shaded; or silo not insulated but shaded on the top with palm fronds ([16]).

In the medium level, an airtight Inert Atmosphere Metal Silo of a battery of 2 units volumetric capacity 45-tons were constructed at the premises of NSPRI Ibadan in such a way that they were fully exposed to varied effects of the sun, wind and rain throughout the experimental period. The IAMS was made up of bin, plinth, gallery, gas supply network, monitoring devices and handling equipment. The bin is a cylindrical structure with conical top constructed of food grade coated steel plate. It has three outlets for loading, discharging and accessibility. The plinth is constructed of reinforced concrete and the basement supports the bin and the gallery. The gallery comprised of ladder and walkway designed for the silo accessibility. The gas supply system of the silo is responsible for nitrogen supply to the bin. The components of the system included gas cylinder, gas line, gas control valve and gas regulators. The gas line is supported with gas line tray and vertical support. The monitoring devices included pressure gauge and temperature probes. The pressure gauge is used to measure the pressure level in the silo especially during purging while the temperature probes are installed at different gradients in the silo to monitor the temperature at different levels of the grain mass in the silo. The handling equipment are accessories for operation of the silo include grain bunker, grain sampling probe, loading and unloading auger conveyors. The silo is also provided with a generator powered augur for mechanized loading with grains. The top of the silo shaded with palm fronds ([14,16,17]).

In the commercial level an airtight IAMS of a battery of 2 units of 50-tons capacity each were constructed at the premises of NSPRI Ilorin and Kano. Similar procedure as in medium levels was followed. While 4 units of 5-ton capacity was installed at Dawanu grain market in Kano.

At the Industrial level, NSPRI was commissioned by a private university, Landmark University, Omu-Aru, Kwara State, Nigeria, where it constructed and installed 2 units of 250-ton capacity IAMS.

Principles and operation of IAMS

IAMS is an airtight system with facilities to purge out the air content of the enclosure and replace it with nitrogen gas (N_2), thereby making the system inert and inhabitable for stored products insect pests.N_2 released into the silo eliminated O_2 in the process and created inert condition within the bin that could not support the growth or survival of any organism irrespective of its developmental stages. Operation of IAMS and maintenance of grains in the structure entail three major operations; loading of grain, purging of silo and unloading of grain from the bin.

Loading of grain: Dried grain stored at safe moisture content (≤ 13 % depending on the grain) determined with in-situ moisture test before the commencement of the loading operation were

used. The grain bunker (for receiving the grains) and the bins were properly cleaned, dried and made free from dust and other extraneous materials. Thereafter, the air tightness was guaranteed to prevent gas leakage. All openings were properly sealed using bolts and nuts except the loading spout. Setting up of the loading conveyor preceded the conveyance of the grains into bin. After loading, the loading spout was properly sealed, and provision was made for appropriate insulation on the bins to reduce direct impact of the sun ([16,17]).

Purging of bin: This is a process of replacing the air content of the silo with N_2 gas. After loading the silo with grain, the interstitial atmosphere within the bulk was purged by introducing nitrogen at the bottom of the silo at a conveniently high rate. All valves that aided the supply of gas through the gas line were adequately opened to supply N_2 to the bin. The gas was allowed to fill the bin for some time after which the gas release valve was opened. The waiting time is a function of the size of the silo. It was 1h 30 mins for the 45-ton silo. The oxygen gas content of the bin was measured with the aid of oxygen analyser and less than 5%. Immediately this condition was achieved, the gas supply line was then switched off after the water in the purging container has bubbled for 5 minutes in case of silos of less than 2 tonnes and for 30 minutes for 50 tonnes capacity silo and for 1 - 2 hours for 250 tonnes silo and above. The O^2 concentration within the silo was checked once every week, and after every purge which followed sampling for analysis ([14,16,17]).

Sampling of grains from IAMS and quality assessment: Initial sample of different grains used for studies were drawn by opening IAMS top tight lid to sample at intervals of 6 month storage from the top, middle and bottom levels of the mini silo, 45-ton or 50-ton by means of silo sampling dip tool. The samples from the three different points were bulked and quartered for triplicate sampling 100 g each for assessment and quality parameters. Oxygen concentration within the silo was checked with the Taylor Servomex oxygen analyzer, type OA 272 forth nightly, and after every purge following grain sampling. The samples were subjected to the following tests: insect infestation count, insect damage kernel, mould count, proximate composition, viability and germinability, organoleptic evaluation according to standard methods ([8,9,11,14,16,17,18]).

Temperature fluctuations and relative humidity inside the silo: Each mini 4-ton silo was equipped with 5 temperature probes each containing 5 thermistors. Two of these were located axially while the other 3 were located radially round the silos so that temperatures can be measured axially and radially at the surface of the bins and at various depths in the stored grain. The temperature probes were led into a programmable electronic temperature recorder system, making it possible to record the temperatures at pre-set time intervals. Similar procedure for 45-ton and 50-ton IAMS ([16,19]).

Discharging of stored grains: After cleaning of the conveyor, the unloading spout of the bin was opened for grain discharge with aid of the unloading conveyor. The collected grains were later packaged in bags.

Research works carried out on IAMS by NSPRI for three and half decades

A battery of two units of 45 tons (in Ibadan) and a battery of two units of 50 tons capacity (in Ilorin and Kano) air tight metal silos were constructed at NSPRI offices located in different ecological zones in the country for bulk storage of grains. Apart from these capacities, there are laboratory and pilot scale capacities for experimental purposes only. Prominent among grains that have been successfully stored in the silos under nitrogen at the three locations were white maize, yellow maize, brown cowpea, wheat var. *Atilla gans atilla* and *Cetia*, paddy rice 'Faro 2' and yellow sorghum. The storage was carried out at different intervals spanning a total of 36 years. Series of research activities have been carried out on the aforementioned crops since inception of the technology.

Storage of maize: Experiments on storage of maize in inert atmosphere metal silo were carried out at five different levels and time. Six experiments were conducted in Ibadan in 1980, 1982, 1983 and 1984. The first experiment was on susceptibility of the life stages of *Sitophilus zeamais* and *Trogoderma granarium* larvae to nitrogen atmosphere in mini silos. This was carried out to determine how effective the technology was to control stored products insects attack in maize

storage. The mini silos with capacity of 5 tons each were used for the experiment. The second was application of artificial controlled atmosphere in grain storage in Nigeria. The third experiment on maize storage was a laboratory examination of yellow maize stored under nitrogen in Nigeria. The fourth research was on microbiological studies on yellow maize stored in sealed mini-silo filled with nitrogen in two metal silos of 0.65m^3. The fifth research on maize was on the effect of shading and insulation on maize stored under nitrogen in 4 mini-metal silos of 4-ton capacity each. The sixth storage of maize was in 45-ton silo at commercial level for 24 months.

Storage of rice and cocoa: The study was carried out in two airtight, stainless steel mini-silos labelled A and B of 0.65m^3 capacity each erected under shade at the premises of NSPRI Ibadan in 1984 to determine the effects of nitrogen atmosphere on the adults and immature stages of some stored products insects: (eggs, larvae, pupae and adults of *Dinoderus porcellus* Lense, *Lasioderma serricorne* (F), *Callosobruchus maculatus* (F.), *Rhyzopertha dominica* (F.), *Dermestes maculatus* (DeGeer) and *Necrobia rufipes* Degeer) when used for storage of rice and cocoa.

Brown cowpea: Experiments on cowpea storage were carried out at Kano (North West) and Ibadan (South West). The Kano experiment which lasted 30 months was at the laboratory level to test the efficacy of the technology in controlling of insect pests in stored cowpea as well as its ability to maintain the quality of cowpea stored under the condition. Another study carried out on cowpea at the laboratory level was to establish the effect of insect infestation of cowpea stored under N_2 on biological evaluation of protein quality. The second stage of the experiment was at the commercial level. One of the 45-ton metal silos in Ibadan was used for storage of cowpea. A 45-ton metal silo was loaded with Ife brown cowpea variety for storage under nitrogen in NSPRI Ibadan. The IAMS was refurbished and purged with nitrogen to 0.05% level to remove O_2 and continuously maintained at regular intervals throughout the storage period. Sampling was done on monthly basis for evaluation of moisture content, microbial count, insect infestation, seed damage, aflatoxin level, germinability and organoleptic assessment which was carried out at the end of 24 months storage period.

Wheat seed: Two different wheat seeds (*Atila gan atila and Cetia*) were stored in silo facility located at the headquarters of NSPRI in Ilorin (North Central of Nigeria). An aspect of the study was to monitor temperature fluctuation in the silos, while the other two focused on maintenance of wheat seed germinability and nutritional quality. Another important area of the study was effect of the technology on mortality of insects at different developmental stages. The wheat was stored for 48 months (2010 to 2014). The germinability test was terminated after 12 months of storage, while the other parameters continued.

Paddy rice: The storage of paddy rice research was conducted in Ibadan at commercial level. The study was carried out to establish the potential of IAMS in preservation of the germinability and nutritional qualities of the paddy rice. The research work lasted for 24 months (2010 to 2012).

Yellow sorghum: This experiment was conducted in Kano for 30 months (2014 to 2016). The storage was at commercial level and was carried out to ascertain the effect of the storage conditions on nutritional qualities of sorghum.

Economic appraisal: The economic appraisal of the technology was conducted to establish how cost effective it is, and the expected return on investment. Different economic tools were jointly adopted to carry out the exercise. Such tools included budgetary analysis and profitability analysis in 1987 and 2015 ([20,21]).

Promotion and utilization: Series of steps have been taken to aid the adoption and utilization of the technology. The techniques adopted for this include training of grain stakeholders on importance and principles of operation of the silo and installation of smaller capacity at grain markets as government intervention in postharvest management of grains. The utilization component covers rentage of the facility to interested individual for storage of grains and installation for individual or corporate ownership. The Institute was commissioned by Landmark University, Omu-Aru, Kwara State, and a battery of 2 units of 250 tons IAMS were constructed on their Research Farm. In late

February 2018 loading of white and yellow maize harvested from their farm is ongoing. The loading is being carried out and supervised by Staff of NSPRI Postharvest Engineering Research Department.

3. Results

Construction of IAMS

Silo at NSPRI Headquarters is used as case study for all others. All the materials required for construction and installation of the functional IAMS were readily and locally available in the country. The silo consisted of two units of 50-ton inert atmosphere metal silo located at Headquarters, Ilorin. The geometry of the silo is shown below: Diameter and cylinder of cone = 4 m; Height of the cylinder = 5.4 m; Total height of silo = 6.4 m; Based on the geometry of the silo structure, the dimension of the cellular raft foundation base is as follows: Total width = 12 m; Total breadth = 6 m with top slab thickness = 300 mm; Width of the beam – 600 mm; Height of the beam = 1000 mm; Bottom raft thickness = 400 mm (Fig. 1) ([19]).

Impact of IAMS on insect control in stored grains

Results obtained by the authors showed that exposure time required to achieve 100% mortality at 28±2°C in inert atmosphere for adults and of larvae which developed internally varied from that required for the eggs stage. Adults of internally developing species such as *Sitophilus zeamais* Motsch, *Trogoderma granarium Dinoderus porcllus* Lense, *Callosobruchus maculatus* Fab..and *Rhyzopertha dominica* F. were more tolerant to N_2 gas than those of *Dermestes maculatus* DeGeer, *Lasioderma serricorne* F which develop externally. Susceptibility of these insects to the atmosphere varied both between species and the various stages of development within each insect species ([8,12]). White maize, brown cowpea, wheat, paddy rice and yellow sorghum under nitrogen in IAMS 45-ton or 50-ton capacity stored for 48, 24, 48, 24 and 30 months respectively and 100% mortality of all life stages of each insect species was recorded, inhibited zero mould growth and no re-infestation.

During the commercial utilization of the technology for cowpea storage, the quantity of N_2 gas used was 101.25m^3 for the 24 month period. It was observed that 40.7% of N_2 gas was used during the first month of storage. A greater quantity of N_2 is needed at the initial stage to establish complete purging of the silo, which in turn is required to maintain the IAMS. This decreased with length of storage period. The moisture content increased form initial 9% to 10.13% at the end of 24 months storage within safe moisture content limit.

Microbial load at the top of the silo ranged from 400-9000 colony (cfu/ml after 6 months), while at the bottom of the silo the value was below 300-4000 cfu/ml which are below safe limits ([23]).

Physical observation showed that the cowpea grains stored in IAMS were clean and without any mould growth. There was no insect infestation or seed damage. Percentage cowpea seed damage decreased from initial 13.64% to 12% after 24 months.

Nutritional quality of grains stored under N_2

Results of analyses carried out on grains stored under N_2 showed that the technology is not just efficient in insects control but also in maintenance of nutritional quality of grains. The outcome of the study carried out on brown cowpea showed that free fatty acid (FFA) contents increased from 2.60% to 6.51% under nitrogen, but increased rapidly to 58.60% in the control cowpea ([14]). Results of previous researchers carried out using the technology showed that crude protein content increase observed in grains stored as controls was not applicable to grains stored under nitrogen, this was attributed to excretory products of the insects that infested the control grains ([10]). Organoleptic tests carried out stored cowpea under N_2 after 24 months to assess the palatability of the stored cowpea and fresh cowpea by processing the cowpea into wet paste and made into bean cake showed no difference in taste and appearance in the fried “akara” balls.

Effects of IAMS on germinability of seeds

Seed germinability and grain quality were also maintained. Germinability of the stored paddy rice was maintained (≥85%) for 3 months in 45-ton IAMS at NSPRI Ibadan ([12]).The germinability of cowpea seeds stored in the inert atmosphere silo in 0.65m^3 at NSPRI Kano was maintained above 85% at 12 months of storage, which shows that the technology is effective for seeds storage ([14]). Germinability of Ife brown cowpea stored under N_2 in a 45-ton capacity IAMS at NSPRI Ibadan was maintained from initial 94% to 80% at 24 months. Also, the result of wheat seed variety *Atlila gans Atilla* stored under N_2 in a battery of 2 units 50-ton at NSPRI Ilorin showed that the technology was able to maintain the germinability 91 % at 12 month of storage.

Moisture migration and condensation in IAMS

Shading with palm fronds or hood to provide insulation for the silos prevented moisture migration and condensation that are peculiar to the conventional metal silos ([10, 11, 16]).The technology was able to maintain the temperature of the grain stored in the silo below 30 °C even when the ambient was as high as 36 °C during the hot season in Ilorin. The mean temperatures at the top, middle and the bottom of the inert atmosphere silos when used to store wheat in Ilorin were approximately 29.35 °C, 28.19 °C and 26.51 °C respectively. This depicts a temperature decrease from the top of grain bulk towards the floor of the inert atmosphere silos ([19]).The recorded temperatures in the silo used for storage of brown cowpea in NSPRI Kano, ranged from 21 °C (in the night) to 35 °C (in the day) with an average value of 28.5 °C; while the ambient temperature was in the range of 14 °C to 43 °C and an average temperature of 33.2 °C ([14]).

Cost benefit of IAMS

These economic advantages of IAMS over conventional silos were observed. The conventional silos require frequency of application of pesticides, turning of grains to prevent caking and high cost of labour. A cylinder of 50 kg N_2 gas used in storage of Ife brown cowpea in a 45-ton capacity IAMS at commercial level in NSPRI Ibadan, Oyo State cost N7014 and was used between 8 and 15 weeks. All indices of economic analysis adopted showed that the technology is economically viable for storage of grains with a return per unit of investment of 0.44 when utilized for storage of wheat for a period of 48 months in NSPRI Headquarters, Ilorin, Kwara State ([20,21,23]).The cost effectiveness has revealed that any investment in the facility is capable of huge economic return within short period of the investment.

Utilization of IAMS

The benefits of the technology coupled with the economic return on the storage structure have prompted the adoption of the technology. The beauty of the technology is that it could be used for storage at all levels (ranging from domestic storage to commercial/industrial grain storage). These are some of the factors that aided the decision of the management of Landmark University Farm to put up a battery of two units of 250 tonnes capacity of the structure for safe keeping of grains for production of feeds for the livestock arm of its commercial farm located in Omu-Aran, Kwara State. Aside from this, a number of individual grain farmers/handlers in the Northern part of the country have committed themselves to the adoption of the technology because of the huge benefits attached to it. In fact, this was referred to as a sustainable and reliable investment opportunity for retirees ([22]). The government of Nigeria through NSPRI was able to construct and install some units of 5 tonnes capacity IAMS for the benefit of stakeholders in the grain sector of agriculture as intervention and a means of popularizing the technology among the grain farmers and marketers. Four units of the silos were installed at Dawanu grain market (the largest grain market in West Africa) in Kano state of Nigeria.

One of the major factors responsible for the low adoption of the technology despite the enormous benefits attached to it, is the initial cost of construction and installation. Awareness has commenced

on the rentage of the facilities for storage of grains for benefit of those that have interest and confidence in it but lack the initial capital require to put up such facility. Another means been adopted to propagate the technology is through training. IAMS was the main storage structure recommended aside from PICS bag for storage of sorghum in the training organised and facilitated by International Crop Research for the Semi-Arid Tropics (ICRISAT) and NSPRI respectively in six (6) states of the federation. Another capacity building workshop was organized by NSPRI on principles and operations of IAMS for grain merchants and industrialists across Nigeria.

4. Discussion

The practical application of N2-CA in stored grain showed that the effect of N2-CA control pests and closely related to the nitrogen concentration and processing time. The efficacy of nitrogen controlled atmosphere is closely related to the pest species, nitrogen concentration, grain temperature and exposure time. Only pure nitrogen eliminated those fungi, preserved grain and oil quality ([15]). Construction of new IAMS will engage fabricators and thereby creating more job opportunities and wealth for the people in the sector. The previous researches carried out by different researchers on different grains at different time and different locations within Nigeria have shown that the principle of operation of the silo is key to its functionality and efficiency. Irrespective of the capacity of the silo, once the principle is strictly adhered to, the efficiency and effectiveness of the structure in handling grains is guaranteed. Capacities of the silo range from 100 kg to thousands of tons. Construction of new IAMS will engage fabricators and thereby creating more job opportunities and wealth for the people in the sector.

Essentially, the adoption of any storage structure for grains is to protect the grains against insect pests attack. IAMS has proved its efficiency in dealing with stored products insects. The technology has potential to attack insect at its every developmental stages. The cost effectiveness has revealed that any investment in the facility is capable of huge economic return within short period of the investment.

One of the major factors responsible for the low adoption of the technology despite the enormous benefits attached to it, is the initial cost of construction and installation. Awareness has commenced on the rentage of the facilities for storage of grains for benefit of those that have interest and confidence in it but lack the initial capital required to put up such facility. Another means being adopted to promote the technology is through capacity building training. IAMS was the main storage structure recommended aside from PICS bag for storage of sorghum in the training organised and facilitated by International Crop Research for the Semi-Arid Tropics (ICRISAT) and NSPRI respectively in six (6) states of the federation. Another capacity building workshop was organized by NSPRI on principles and operations of IAMS for grain merchants and industrialists across Nigeria in October, 2017.

In conclusion, the recent achievement of the technology is that its utilization has been taken up by entrepreneurs, marketers and universities for grain storage in Nigeria due to the success of application, operating cost and increased awareness for availability of grains for food safety, grain quality and nutrition. Investment in this technology has economic benefits to the stakeholders in particular and the country. The technology is an advanced environmental friendly, and construction and operating cost and application is feasible all the agro-ecological zones of Nigeria. Apart from providing a green environment for the preservation of grains, it has the capacity to create jobs for those that will be involved in the management of the structure. IAMS application will encourage farmers to produce more grains as there is a technology that is effective and efficient for grain storage. The cumulative success recorded on different grains stored in IAMS is an evidence research input by NSPRI to ensure food safety and quality thereby meeting MDGs in food security. Finally, there is opportunity for collaborative research work to further strengthen the present achievement.

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Toxic effects of ozone on selected stored product insects and germ quality of germinating seeds

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Abstract

The merchant grain beetle (MGB), *Oryzaephilus mercator* (Fauvel), the cigarette beetle (CB) *Lasioderma serricorne* (F.) and the rice weevil, *Sitophilus oryzae* (L.) cause significant damage to stored grain, grain-based products, and other durable commodities. Ozone, a highly oxidative toxic gas, has the potent to kill insects, meantime degrades rapidly to oxygen, making it a potential alternative to phosphine, a fumigant to which insects are developing resistance. The adults of MGB and CB were exposed to ozone concentrations of 100 - 400 ppm at 50 ppm increments for one hour and at 100 ppm for 1-6 h. Adults of rice weevil buried at 5, 15 or 25 cm depths within a wheat mass placed in 10 cm diameter 30 cm high PVC pipes were exposed to ozone concentration of 200 ppm for six hours and then at 12-h increments up to 60 h. Adult survival was recorded at 0, 24, and 48 h post-treatment. Significantly fewer MGB or CB adults survived when exposed to higher ozone concentrations or when exposed to ozone in the absence of food. RW adult mortality at 5 cm depth were significantly higher than that of 15 or 25 cm depths. This paper further discusses about mortality of MGB, CB and RW adults at different exposure periods at various ozone concentrations and effect of ozone on wheat germination.

Keywords: Fumigants, germination, ozone, stored product insects, wheat

Introduction

The ban of methyl bromide, the most effective fumigant for the control of many stored product insect pests, has necessitated the search for other potential alternative management methods. One of the potential alternatives is ozone (O_3), a highly oxidative, environmentally safe gas that degrade into molecular oxygen (O_2) within 20-50 minutes. Ozone is formed by the excitation of molecular oxygen, into atomic oxygen (O), and then combination of three atomic oxygen to form ozone. The use of ozone against stored product insect pests has gained tremendous attention over the past decade (Mahroof el al., 2018).

There have been few studies (Hasan et al., 2012) on the effect of ozone on the merchant grain beetle, *Oryzaephilus mercator* (Fauvel), the cigarette beetle, *Lasioderma serricorne* (F.) and the rice weevil, *Sitophilus oryzae* (L.). Published studies did not investigate the effect of ozone when externally feeding insects are treated in the presence or absence of food. Therefore, the study described here was conducted using adults of *O. mercator*, *L. serricorne* and *S. oryzae* exposed to different concentrations of ozone for different durations. The objectives of this study were to evaluate the relative susceptibility of adults to different ozone concentrations, determining concentration-mortality and time-mortality relationships and to determine germination quality of wheat treated using ozone.

Materials and Methods

A bench-top ozone generating equipment that produces ozone in the range of 0-8000 ppm was used in the experiment. Detailed descriptions of the Ozone generator were provided in Mahroof et al. (2018).

Exposure of *O. mercator* and *L. serricorne* to different concentrations of ozone

Adults of *O. mercator* and *L. serricorne* were exposed to ozone concentrations beginning 100 ppm and then at 50 ppm increments up to 400 ppm for one hour. Ten jars with 20 adults were used for

ozone treatment and 10 jars served as control. Five of the either treatment or control jars had 10 g diet mix and the other five were without food. Insects were treated either in the presence or absence of diet to determine the effect of ozone, if any. For each concentration tested, each treatment was replicated five times. Each experiment for any particular concentration was repeated for a total of three times. Adults were observed for mortality soon after treatment (0). Then, adults were placed in an incubator at 28 °C and 65% RH and observed again at 1 and 2 days after treatment (DAT).

Exposure of *S. oryzae* to 200 ppm ozone concentration for different durations

The effect of 200 ppm ozone concentration on adult *S. oryzae* adults at different depths within a column of wheat mass was tested using PVC pipes. A nylon pouch containing 20 adults was placed at the 5, 15 and 25 cm depths from the top of the PVC pipe filled with wheat. Three of the pipes were exposed to ozone by placing them in the ozone chamber set at 200 ppm for 12, 24, or 36 h. The remaining three pipes served as control. Adults were observed for mortality immediately after ozone exposure (0 DAT). Then, adults were provided with wheat kernels and placed in an incubator at 28 °C and 65% RH and observed again at 1 and 2 DAT.

Germination test

Wheat kernels exposed at 200-ppm of ozone for 12-60 h with an increment of 12 h were germinated in a Petri dish and germination percentage was compared with untreated wheat kernels. Seeds layered on a wet paper towel placed in the Petri dish were maintained in an environmental growth chamber at 28°C, 65% RH and 18:6 D: L photoperiodism for 10 days to record germination.

Data analyses

For experiments exposing the adults of *O. mercator* and *L. serricorne* to different concentrations of ozone, the corrected mortality data were fitted to a complementary log-log (CLL) regression model to estimate the concentration required to kill 50% (LC_{50}) and 99% (LC_{99}) of insects exposed to ozone (SAS Institute 2013). In the CLL model, the concentration was transformed to $\log_{10}$ scale. The goodness-of-fit of the model to the data was compared using a χ^2 statistic (SAS Institute 2013). For *S. oryzae*, we determined the differences between treated and corresponding control for each depth. The means were separated using Tukey's Honest Significant Difference (HSD). ANOVA was considered significant if $P < 0.05$ (PROC GLM, SAS Institute, 2013). Results from germination tests were summarized in a graph and represented as mean % germination at 5, 15 and 25 cm depths.

Results and discussion

Exposure of *O. mercator* and *L. serricorne* to different concentrations of ozone

The Probit estimates from the mortality response of adult *O. mercator* exposed to different ozone concentrations are summarized in Tab. 1. The results showed that susceptibility for adults varied to ozone when they were treated with or without diet. When adults were treated with diet, they were more tolerant to ozone than without diet. In *L. serricorne*, when diet were provided, similar to *O. mercator* adults were more tolerant than when diet was absent (Tab. 1). The χ^2 values were significant for the concentration-mortality regression models, an indication that the responses to ozone by the *O. mercator* and *L. serricorne* adults were heterogeneous whether they were treated with or without food. In height of heterogeneous response, the CLL model estimated a very high LC_{50} and LC_{99} values that did not yield any fiducial limits for O. Mercator adults, those were treated with food.

Tab. 1: Relative toxicity of ozone to adults of *O. mercator* and *L. serricorne* determined through concentration-mortality bioassays

Stage	Treatment type	Total # of insects	Intercept ± SE	Slope ± SE	LC_{50} (95% FL) (ppm) [a]	LC_{99} (95% FL) (ppm) [a]	χ^2 (df) [b]
***O. mercator* Adult**	With food	2100	-4.1 ± 1.5	0.7 ± 0.6	338432 [c]	212033356 [c]	157.5 (40) *
	Without food	2100	-13.5 ± 1.9	5.5 ± 0.8	239.0 (211.3–261.0)	525.7 (454.6 – 669.8)	2,207.7 (69) *
							739.0 (103) *
***L. serricorne* Adult**	With food	2100	-9.3 ± 1.4	2.8 ± 0.6	1442 (879–4620)	6692 (2628–62082)	
	Without food	2100	-9.0 ± 1.2	2.9 ± 0.5	854 (630–1549)	3769 (1931–14532)	955.0 (103) *

[a] FL, Fiducial Limits

[b] χ^2 values for goodness-of-fit of the CLL regression model to the observed mortality data

[c] Fiducial Limits were not calculated

Significant ($P < 0.05$)

Means within treatments followed by different letters are significantly different (Tukey's HSD test, $P < 0.05$). Upper case letters are for comparisons within 15 cm depth

* indicates a significant difference between a treatment and its corresponding control for a given day.

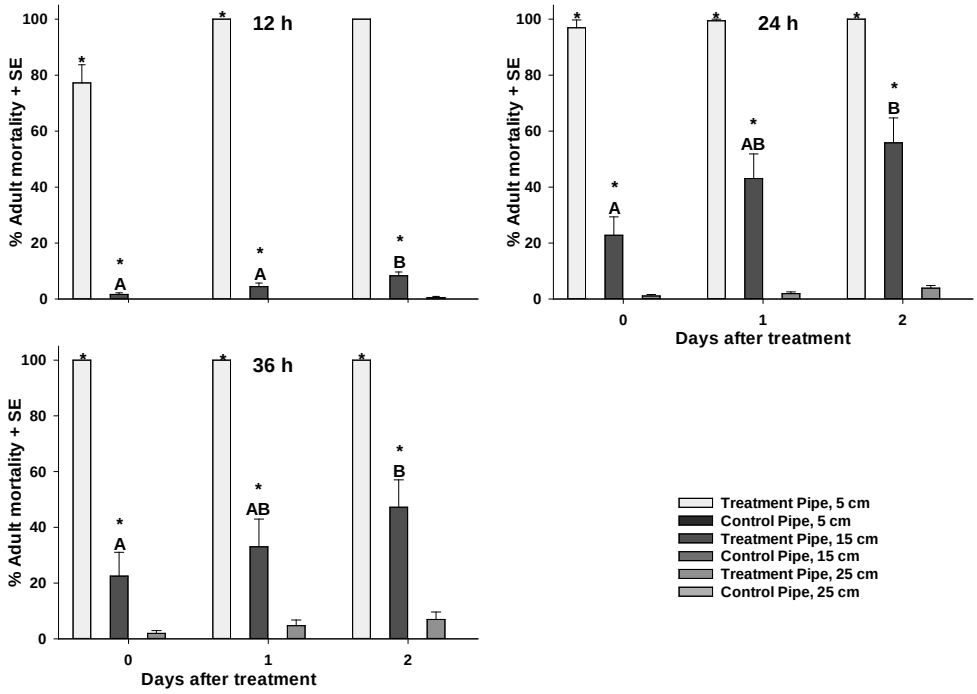

Fig. 1: Mean % mortality ± SE of adult *S. oryzae* exposed to 200 ppm of ozone for 12, 24 and 36 hours at 5, 15 and 25 cm depths in a wheat column.

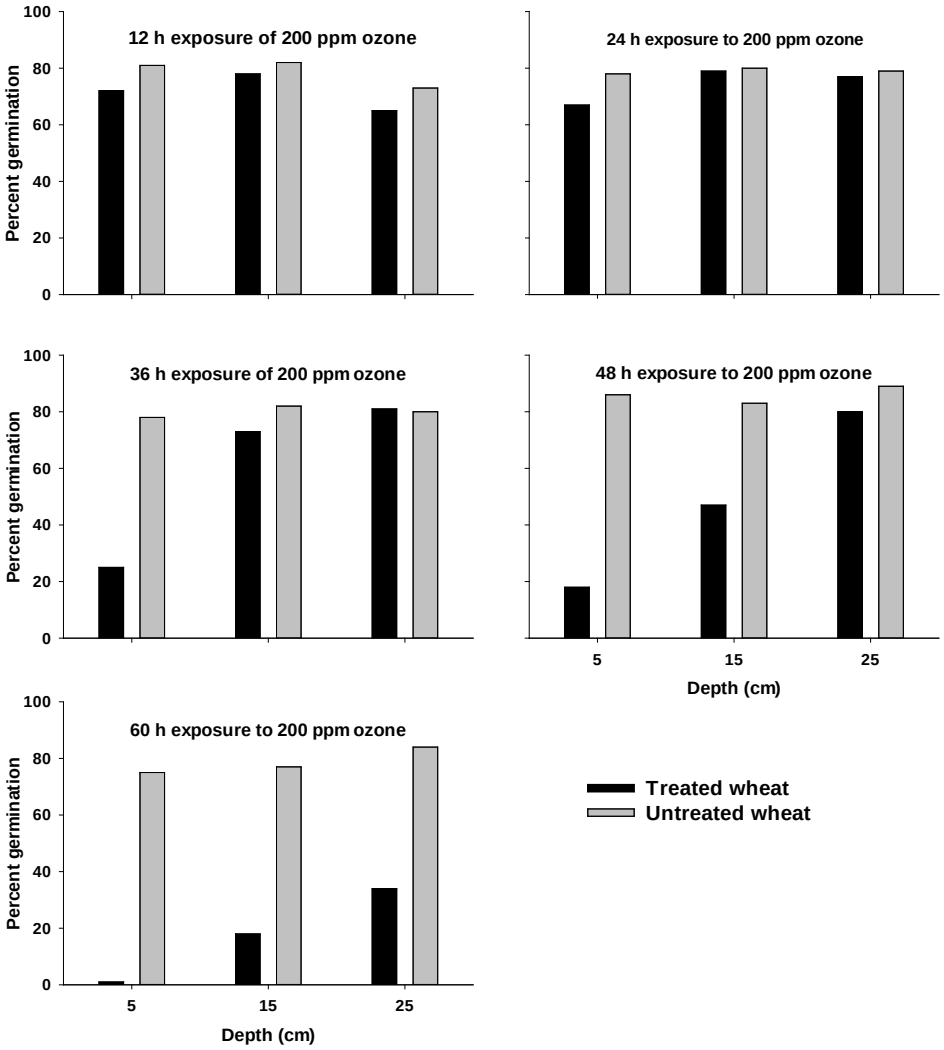

Fig. 2: Percentage germination of wheat seeds exposed to 200 ppm of ozone for 12-60 h at an increment of 12 h. Seeds were collected from 5, 15 and 25 cm depths in a wheat column.

Exposure of *S. oryzae* to 200 ppm ozone concentration for different durations

The percentage mortality of *S. oryzae* adults when exposed to 200 ppm for different durations, is presented in Fig. 1. Higher mortality was recorded at the 5 cm depths for each of the durations investigated. The longer the exposure period, the higher the mortality recorded. Exposure durations of 12 h or higher resulted in 100% mortality at the 5 cm depths by 2 DAT. At 24 h exposure duration, there were significant higher adult mortality at the 15 cm depth compared to control, soon after treatment, or at 1 and 2 DAT ($P < 0.001$). However, at the deepest depth, at 25 cm, there was no significant difference among days and between a treatment and its corresponding control for three durations tested ($P > 0.05$).

Based on our data, the highest concentration of ozone tested, 400 ppm, could not result in 100% mortality for both *O. mercator* and *L. serricorne*. Complete mortality in adult *L. serricorne* was achieved with 24 ppm after 48 h exposure, and shorter exposure periods required significantly

higher ozone concentrations to elicit similar effects (Hassan et al., 2012). Longer exposure durations and or higher concentrations will also be required to be able to control *S. oryzae* adults placed at the 25 cm depth of the PVC pipes. Ozone adsorption in grain layer depends on factors such as the ozone concentration supplied to the layer, ozone degradation rate, and the exposure duration to ozone (Tojanowska, 1991). Hence, longer exposure periods and or higher ozone concentrations may be required to control *S. oryzae* adults placed in deeper depths of a wheat column.

Germinations tests

Wheat germination was not adversely affected when seeds were exposed to 200 ppm up to 24 hours (Fig. 2). However, percentage germination was reduced, when compared to control germination at 5 cm depth, beyond the 36 hour-exposure to ozone at 200 ppm. Germination of wheat seeds considerably decreased when wheat was exposed to 60 hours at depths of 15 or 25 cm. Seeds collected and germinated from the 5 cm depth at 60 hours of exposure had germination closer to 0%. To our knowledge this is the first experiment to report effect of ozone on germination of wheat seeds in relation to insect mortality. It is clear with this study, that prolonged exposure of grain to ozone may adversely affect the germ quality. If grain is stored for seeding and cultivation purposes, or extracting germ for commercial uses, care must be taken if ozone treatment is an option for stored insect management.

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Update on ProFume® gas fumigant (sulfuryl fluoride) use for post-harvest pest control

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Keywords: Sulfuryl fluoride, ProFume, *Cryptolestes pusillus*, *Sitophilus zeamais*

ProFume® gas fumigant (99.8% sulfuryl fluoride), first registered in 2003, is a broad spectrum, non-ozone depleting fumigant for the control of rodent, insect and other invertebrate pests. It is used to treat a wide range of stored products and structures which transport, store, and process commodities and is currently registered in 22 countries. Sulfuryl fluoride is not cross-resistant with phosphine and has been documented to effectively control quarantine pests, including the pinewood nematode and brown marmorated stinkbug.

Continued reduction of methyl bromide availability for non-quarantine fumigations coupled with the emergence of phosphine tolerant or resistant populations has led to increased interest in registration of sulfuryl fluoride in several tropical and sub-tropical countries. As part of the registration effort additional efficacy data has been developed to support the use in these countries. hile found in temperate zones, both Flat grain beetle (*Cryptolestes pusillus* (Schönherr)) and Maize Weevil (*Sitophilus zeamais* (Motschulsky)) are more problematic in tropical environments. As

sulfuryl fluoride was developed mainly for use in temperate zone countries, limited efficacy data has been developed for these pests. In 2018, laboratory studies were conducted in California by the Dried Fruit and Tree Nut Association (DFA, Fresno, CA, USA) to determine the dosage required for control of all life stages of these pests. The results of this study coupled with earlier work may allow for inclusion in the Fumiguide® program.

The Fumiguide program is required for use with ProFume to calculate dosage and dose requirements. The program allows users to tailor applications based on job specific parameters to best meet customer needs for cost and time. The new Fumiguide includes improvements in the underlying algorithms, additional functionality for fumigators and the ability to easily add new pests.

Since the purchase of sulfuryl fluoride from The Dow Chemical Company in 2015, Douglas Products has continued to expand product use through new country registrations, expanded efficacy data and development of an updated Fumiguide program. This presentation provides updates on registrations for ProFume, details efficacy work for two insect species of interest in tropical regions, and reviews the updated Fumiguide program, a required tool for dose and dosage determination.

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Nitric oxide as a new fumigant for postharvest pest control

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Abstract

Nitric oxide (NO) is a new fumigant for postharvest pest control. It is effective against all pests tested to date, including external and internal pests of fresh and stored product insects, and mites. Efficacious treatment time ranges from 2 h to 72 h, and NO concentrations range from 0.1% to 5%, depending on species and life stages of the pests.

Nitric oxide fumigation must be conducted under ultralow oxygen conditions because NO reacts with O_2 spontaneously to produce nitrogen dioxide (NO_2), which is toxic to perishable fresh products. Fresh product fumigation must, therefore, also be terminated by flushing with N_2 to dilute NO at the end of fumigation to avoid damage to delicate products by NO_2. Nitric oxide fumigation was safe in small-scale tests to postharvest quality of all fresh commodities when terminated with N_2 flush. In addition, NO fumigation resulted in better postharvest quality of strawberries and apples as compared with controls, indicating its beneficial effects on postharvest quality of fresh products.

Twenty fresh fruit and vegetables and 10 stored products were fumigated with NO to determine residue levels of nitrate and nitrite. When terminated properly with N2 flush, NO fumigation does not increase nitrate or nitrite levels in fumigated products. NO fumigation was demonstrated to be effective against all pests, safe to fresh products, and has no toxic residues and, therefore, has the potential to be a practical alternative to methyl bromide fumigation for postharvest pest control on both fresh and stored products.

Keywords: Nitric oxide, fumigation, quarantine treatment, residue, postharvest quality.

Introduction

There is a severe lack of safe and effective alternative treatments for postharvest pest and disease management after phasing out of methyl bromide. The current main alternatives, including phosphine and sulfuryl fluoride, have difficulties in meeting the need for postharvest pest control on stored products or fresh commodities. Phosphine fumigation typically has long treatment time and is not effective against some pests due to tolerance or resistance (Hole *et al*., 1976; Benhalima *et al*., 2004). Sulfuryl fluoride is not effective against insect eggs (Bell *et al*., 1998) and therefore has limited efficacy in addition to its phytotoxicity to fresh products (Aung *et al*., 2001). Nitric oxide (NO) is a newly discovered fumigant for postharvest pest control and has high efficacy against insects

and mites and no toxic residues (Liu, 2013, 2015). It was also demonstrated to be safe to fresh commodities and enhance postharvest quality of fresh commodities (Liu, 2015, 2016, Liu *et al*., 2016). Therefore, NO may have potential to be a practical alternative fumigant for postharvest pest control on both fresh and stored products.

Nitric oxide is a chemical produced naturally in fossil fuel combustion and lightning and commercially as an intermediate in fertilizer production. Since the discovery of NO as a cell messenger molecule in 1980s, NO has been studied extensively and was found to play diverse roles in physiological and biochemical processes in organisms (Lamattina *et al*., 2003). Nitric oxide has also been used in medical fields to treat certain respiratory and cardio vascular conditions (Roberts *et al*., 1993; Ricciardolo *et al*., 2004) and was also found to be an inhibitor of ethylene biosynthesis in plants and can be used to enhance postharvest quality and prolongs shelf-life of fresh fruit and vegetables (Wills *et al*., 2000; Soegiarto and Wills, 2004; Manjunatha *et al*., 2012; Saadatian *et al*., 2012).

As a new fumigant, NO is effective against a wide variety of pests (Liu, 2013, 2015, 2017; Liu and Yang, 2016; Liu *et al*., 2016). Nitric oxide fumigation is also safe to fresh products (Liu, 2016, 2017, Liu et al. 2016). In fact, NO fumigated strawberries and apples have better postharvest quality as compared with untreated controls (Liu, 2016; Liu *et al*., 2016). Nitric oxide fumigation also has no toxic residues on fumigated fresh products (Yang and Liu, 2017). In this paper, NO fumigation past research was reviewed and discussed, and new data on efficacy and residues were also presented and discussed.

Procedures of nitric oxide fumigation

Nitric oxide fumigation must be conducted under ultralow oxygen (ULO) conditions. This is due to the nature of spontaneous reaction between NO and oxygen (O_2) to produce nitrogen dioxide (NO_2). The reaction not only consumes NO, but also produces NO_2 which can cause injuries to sensitive fresh products at high concentrations. Therefore, ULO needs to be established in a fumigation chamber by flushing with nitrogen (N_2) to reduce O_2 concentration to a minimum level. The fumigation chamber also needs to be sealed airtight to prevent O_2 leaking into the chamber. For stored product fumigation, carbon dioxide (CO_2) can also be used to flush the fumigation chamber as CO_2 is unlikely to affect stored products. At the end of fumigation, especially for fresh products, the fumigation chamber also needs to be flushed with N_2 to dilute NO before opening the chamber to prevent the reaction between NO and O_2 and production of NO_2.

Nitric oxide fumigation procedures have been published in a video article (Liu *et al*., 2017) and also described previously (Liu, 2013, 2015; Liu and Yang, 2016; Liu, 2017). Nitric oxide fumigation starts by establishing ULO conditions in an airtight fumigation chamber with a N_2 flush. Greasing with petroleum jelly often is required to achieve an airtight seal of a fumigation chamber. Tubing with low permeability or non-permeable to O_2, such as nylon tubing, should be used. Oxygen analyzers with zirconia sensors are recommended for their high sensitivities and longevity. To have high efficiency in establishing ULO conditions, the fumigation chamber can be flushed with N_2 at a high flow rate at the beginning and then reduce the flow rate when O_2 is close to a desired level. The ULO levels for NO fumigation can vary depending on NO concentrations and products. Higher ULO levels will consume NO and therefore reduce effective NO levels for pest control. Some fresh products are also sensitive to high NO_2 levels. We used $\leq$30 ppm O_2 in all of our small scale NO fumigation tests.

The length of time to achieve a desired ULO level may vary greatly depending on the type of products to be fumigated and the quantities of the products. Large fruit such as apples can take long time to establish ULO conditions as they contain large volume of air inside. Many fresh products are packed in perforated plastic bags or wraps with very limited air exchanges capabilities and therefore limit efficiency of N_2 flush in establishing ULO conditions. Vacuum may also be used to increase the efficiency in establishing ULO conditions.

Nitric oxide concentration, fumigation time, and temperature will depend on pest species and products. For packaged fresh products, it is recommended to use lower NO concentration but longer treatment time, because N_2 flush is needed to dilute NO at the end of fumigation and lower NO concentrations are easier to be diluted than higher concentrations. The level of dilution may also change depending on sensitivity of fresh products to NO_2. For fumigations of leafy vegetables and delicate fruits, it is preferably that NO be diluted to 100 ppm or lower before opening the chamber to ensure safety to products.

Currently there is no suitable analyzer to monitor NO concentrations in NO fumigations. The high concentration NO sensors in commercial flue gas monitors typically have a maximum concentration limit of 5000 ppm. Because of its reactive nature with O_2, NO cannot be quantified using a gas chromatograph. Therefore, NO concentrations in small chamber fumigations were calculated based on NO gas volumes used and the chamber volumes. For large fumigations, a flue gas monitor with a high concentration NO sensor can be used in conjunction with a dilution device to monitor NO concentrations in NO fumigations. The dilution device we made and used consisted of four equal length micro-tubes with one tube for sample gas and the other three for nitrogen. Under the condition of equal air pressure in the fumigation chamber and nitrogen in a foil bag, the air sample can be diluted four times and, thereby, a fumigation with 2% NO can be monitored using the monitor with a 5000 ppm NO sensor. However, a custom built NO analyzer with suitable maximum NO level for NO fumigation is possible from certain vendors.

Efficacy of nitric oxide fumigation

Over 10 species of insects and mites have been tested with NO fumigation. Nitric oxide fumigation is effective against all pest species tested to date at different life stages (Table 1) (Liu, 2013, 2015; Liu and Yang, 2016). However, there are considerable variations among species and life stages in susceptibility to NO fumigation. Nitric oxide fumigation is particularly effective against small external soft-body insects on fresh products. Western flower thrips (*Frankliniella occidentalis* (Pergande)), lettuce aphid (*Nasonovia ribisnigri* (Mosley)), and longtailed mealybug (*Pseudococcus longispinus* (Targioni Tozzetti) can be controlled in a few hours with 1-2% NO at a low temperature of 2°C (Liu, 2013).

Nitric oxide fumigation is also effective against internal feeding insects. Spotted wing drosophila (*Drosophila suzukii* (Matsumura)) larvae in infested cherries were controlled in 8 h with 2.5% NO fumigation. For codling moth (*Cydia pomonella* (L.)) larvae in infested apples, NO fumigation treatments of 24 h at 5% concentration at 2°C resulted in 100% larval mortality (Liu *et al*., 2016). Nitric oxide fumigation at 1-2% concentrations takes 24 h to 72 h at 15-25°C to control stored product insects such as Indianmeal moth (*Plodia interpunctella* (Hubner)), confused flour beetle (*Tribolium confusum* (Jacquelin du Val)), and rice weevil (*Sitophilus oryzae* (Linnaeus)). The treatment time is shorter for mobile stages than for pupa and egg stages (Liu, 2013, 2015; Liu and Yang, 2016). Bulb mites (*Rhizoglyphus spp*.) on infested peanuts were also controlled with 2% NO in 24 h at 20°C (Liu, 2017) (Table 1).

The efficacy of NO fumigation increases with concentration, time, and temperature. Concentration x Time (C×T) products correlate well with mortality and can be used to determine NO fumigation treatments. Effect of temperature on efficacy of NO fumigation is lower as compared with concentration and time (Liu, 2013).

Safety of nitric oxide fumigation to product quality

Safety of NO fumigation to product quality includes possible injuries to fresh products and likely residues in fumigated products. In small scale tests, NO fumigation is safe to all fresh products tested to date including lettuce, broccoli, cucumber, pepper, tomato, strawberries, apple, pear, orange, and lemon when terminated with N_2 flush as there are not significant differences between the treatment and the control (Table 2) (Liu, 2016). When NO fumigation is terminated by directly

opening the fumigation chamber to ambient air without flushing with N_2, NO reacts with O_2 to produce NO_2 in the fumigation chamber and results in stains on delicate fresh products including leafy vegetables, broccoli, squash, and peach. Stains also occur to some apples (Liu, 2016).

Tab. 1 Summary of nitric oxide fumigation treatments that had 100% control of different pest species at specified life stages*

Species	Life stage	NO (%)	Time (h)	Temp (°C)	Note
Western flower thrips	larva, adult	0.2	8	2	on lettuce leaves
		2	2	2	
Lettuce aphid	nymph, adult	0.2	12	2	on lettuce leaves
		0.5	9	2	
		1	3	2	
Long-tailed mealybug	nymph, adult	2	2	2	on grape leaves
Confused flour beetle	larva, pupa	0.5	24	20	on flour diet
	adult	0.5	8	20	
	egg	2	24	10	
Rice weevil	adult	1	24	25	on pearled barley
	egg	1	48	25	
Indian meal moth	egg	1	24	20	
Light brown apple moth	larva, pupa	2	8	2	on artificial diet
	egg	3	12	2	
		5	6	2	
Codling moth	egg, larva, pupa	2	48	2	on artificial diet
	larva	5	24	2	in apples
Spotted wing drosophila	egg, larva	3	8	2	in sweet cherries
Bulb mites	larva, adult	2	24	20	on peanuts

* Reprint from Liu and Yang (2016).

For some fresh products, properly conducted NO fumigation not only is safe to product quality but also help to extend storage/shelf-life. Nitric oxide fumigations for control of western flower thrips results in better postharvest quality of strawberries with significantly firmer and brighter, richer color as compared with the control one week after treatment (Liu, 2016). Nitric oxide fumigation for control of codling moth larvae in apples also results in better apple quality as compared control four weeks after fumigation (Liu *et al*., 2016).

Residues of nitric oxide fumigation

Nitric oxide fumigation can result in nitrate (NO^{3-}) and nitrite (NO^{2-}) as residues as NO reacts with O_2 to produce NO_2 which can be further converted to nitrate and nitrite. However, both nitrate and nitrite occur in varying quantities in fresh and stored agricultural products. Twenty fresh products and 10 stored products have tested for residues. For most fresh products, NO fumigation does not lead to significantly higher nitrate or nitrite if the treatment is terminated properly with N_2 flush (Yang and Liu, 2017). For the 10 stored products, there were also no significant increases in nitrate or nitrite in fumigated stored products as compared with the controls (Yang and Liu, unpublished). When NO fumigation is terminated without N2 flush, there are significant increases in nitrate and sometime also nitrite levels in fumigated fresh and stored products (Yang and Liu, 2017, unpublished).

Nitrogen dioxide release rates and nitrate and nitrite levels were evaluated for different treatments from five selected fresh products and five selected stored products at 24 h after NO fumigation. This study showed considerable differences between fresh and stored products and among different fresh products, as well as among different stored products (Tab 3). Apples from both NO-N_2 (NO fumigation terminated with N_2 flush) and NO-Air (NO fumigation terminated without N_2 flush) treatments had similar significantly higher NO_2 release rates as compared with the control. Lettuce from the NO-Air treatment, however, had a NO_2 release rate which was about 1000 times as those of the NO-N_2 treatment and the control. For other fresh products: asparagus, broccoli and strawberries, NO-Air treatments had significantly higher NO_2 release rates as compared with NO-N_2

and controls. Higher retention of NO_2 on the fresh products led to corresponding higher levels of nitrate and nitrite in the products (Yang and Liu, 2017).

Tab. 2 Effects of nitric oxide fumigation treatments on postharvest quality of fresh fruit and vegetables after 14 days post-treatment storage at 2°C*

Product	Treatment	N	Quality score (Mean±SE)	ANOVA	
Lettuce	Control	7	6.4±0.9a	df = 2, 18	F = 15.754
	NO-N_2	7	4.9±0.6a		P = 0.0001
	NO-Air	7	1.4±0.2b		
Broccoli	Control	7	8.0±0.3a	df = 2, 17	F = 9.193
	NO-N_2	7	7.9±0.5a		P = 0.0020
	NO-Air	6	5.2±0.7b		
Pepper	Control	15	8.1±0.2a	df = 2, 42	F = 9.026
	NO-N_2	15	7.3±0.3a		P = 0.0005
	NO-Air	15	6.0±0.5b		
Squash	Control	7	7.1±0.3a	df = 2, 18	F = 9.546
	NO-N_2	7	6.6±0.2a		P = 0.002
	NO-Air	7	4.1±0.8b		
Tomato	Control	9	8.3±0.3a	df = 2, 24	F = 2.886
	NO-N_2	9	7.6±0.4a		P = 0.075
	NO-Air	9	6.9±0.5a		
Apple	Control	15	7.9±0.2a	df = 2, 42	F = 11.667
	NO-N_2	15	8.1±0.2a		P < 0.0001
	NO-Air	15	6.3±0.3b		
Lemon	Control	7	8.4±0.3a	df = 2, 18	F = 0.214
	NO-N_2	7	8.3±0.3a		P = 0.809
	NO-Air	7	8.1±0.3a		
Orange	Control	7	8.4±0.2a	df = 2, 18	F = 0.079
	NO-N_2	7	8.4±0.2a		P = 0.924
	NO-Air	7	8.3±0.4a		
Peach	Control	7	8.4±0.2a	df = 2, 18	F = 6.584
	NO-N_2	7	7.4±0.3ab		P = 0.007
	NO-Air	7	5.1±1.1b		
Pear	Control	9	8.1±0.3a	df = 2, 24	F = 5.375
	NO-N_2	9	8.3±0.2a		P = 0.012
	NO-Air	9	6.8±0.5b		

* Reprint from Liu (2017). All products from the treatments and the control were stored at 2°C for 14 days before being scored for postharvest quality. The visual quality was scored for marketability using the 1 (extremely poor) to 9 (excellent) scale for lettuce (Kader *et al*., 1973) with 3, 5, and 7 representing poor, fair with major defects, and good with minor defects (Liu, 2016).

For stored products, NO_2 release rates were much lower as compared with the fresh products. The variations among the three treatments were also much smaller as compared with the three treatments for the fresh products (Table 3). However, for the most products, NO-Air treatment still had significantly higher NO_2 release rate as compared with NO-N_2 treatment and the control. Nitrate and nitrite levels also varied among the three treatments in consistence with the NO_2 release rate variations. Higher NO_2 release rates corresponded with higher nitrate and nitrite levels for all of the stored products (Table 3).

Discussions

Nitric oxide fumigation is effective against all pests tested to date, is safe to fresh product quality and leaves no toxic residues in fumigated products when terminated properly. Over 10 tested pest species have been effectively controlled and they represent different taxonomical groups, both external and internal feeders, both fresh and stored product pests, and different life stages. The efficacy data suggest that NO fumigation is likely effective against all insect pests and mites. Because of large variation in susceptibility to NO fumigation among different species and life stages, different pests will likely need different combinations of NO concentration and treatment time at certain temperatures to achieve effective control. CxT products can be used to determine appropriate NO fumigation treatments because they correspond well with mortality for individual species.

Nitric oxide has advantages in efficacy in comparison with other methyl bromide alternatives, including phosphine, sulfuryl fluoride, and ethyl formate. Phosphine is the major methyl bromide alternative fumigant for both fresh and stored product pests. However, phosphine fumigation is not effective against some pests due to tolerance or resistance. In general, phosphine fumigation

also has long treatment times which may extend over 10 days to achieve effective control of some pests (Hole *et al*., 1976). Although recently developed oxygenated phosphine fumigation has significantly increased efficacy of phosphine fumigation against phosphine tolerant insects (Liu, 2011; Liu *et al*., 2013), the prospect of commercial application is still unclear. Sulfuryl fluoride is not effective against insect eggs (Bell *et al*., 1998) and is also phytotoxic to fresh products (Aung *et al*., 2001). Therefore, it has limited effectiveness against postharvest pests. Ethyl formate has high absorbing rates in fresh products and also has phytotoxicity on fresh products (Stewart and Mon, 1984; Zoffoli *et al*., 2013). In contrast, NO is effective against all pests and all life stages and has high efficacy against small external pests on fresh products with short treatment times and very low absorbance in fresh products.

Tab. 3 Nitrogen dioxide (NO_2) release rate and nitrate (NO^{3-}) and nitrite (NO^{2-}) contents in selected fresh and stored products at 24 h after nitric oxide fumigation*

Product	Treatment	NO_2 (mg kg^{-1} h^{-1})	NO^{3-} (mg/kg)	NO^{2-} (mg/kg)
Fresh products				
Apple	NO-Air	58.721±8.114a	15.96±1.20a	4.95±1.57a
	NO-N_2	45.613±7.442a	13.64±1.33ab	0.30±0.14b
	Control	0.019±0.005b	7.61±2.80b	0b
Asparagus	NO-Air	3.050±0.704a	21.85±1.32a	0.75±0.42a
	NO-N_2	0.387±0.052b	7.00±0.25b	0a
	Control	0.184±0.073b	8.36±0.74b	0a
Broccoli	NO-Air	0.499±0.165a	186.86±37.54a	1.70±0.63a
	NO-N_2	0.183±0.018ab	185.12±34.16a	0b
	Control	0.081±0.031b	122.58±23.07a	0b
Lettuce	NO-Air	1643.704±395.573a	1128.49±201.70a	79.87±20.15a
	NO-N_2	13.452±5.189b	389.66±58.69b	0.98±0.79b
	Control	14.677±13.652b	406.41±108.06b	0b
Strawberry	NO-Air	3.322±1.147a	60.14±6.20a	0
	NO-N_2	0.334±0.055b	52.99±7.65a	0
	Control	0.079±0.018b	61.62±10.61a	0
Stored products				
Almond	NO-Air	0.034±0.008a	16.86±1.10a	4.22±0.37a
	NO-N_2	0.024±0.008ab	12.21±1.83ab	1.91±0.89b
	Control	0.020±0.008b	11.34±0.79b	0b
Barley	NO-Air	0.037±0.010a	26.36±0.50a	6.23±0.35a
	NO-N_2	0.031±0.008b	8.29±1.10b	2.04±0.36b
	Control	0.018±0.005b	8.48±0.56b	0c
Pinto beans	NO-Air	0.017±0.005a	39.58±3.53a	9.54±1.47a
	NO-N_2	0.013±0.001b	33.62±9.0b	1.12±0.16b
	Control	0.001±0.001c	28.37±5.84b	0b
Rice	NO-Air	0.042±0.009a	14.41±2.02a	3.44±0.28a
	NO-N_2	0.033±0.008a	8.53±1.60ab	1.69±0.13b
	Control	0.034±0.009a	7.76±0.71b	0c
Walnut	NO-Air	0.023±0.008a	19.04±3.61a	3.20±0.07a
	NO-N_2	0.015±0.007b	11.73±2.12a	0.82±0.47b
	Control	0.016±0.007b	13.84±0.22a	0b

*Fresh products: apple, asparagus, broccoli, lettuce, and strawberries were fumigated with 5, 3, 3, 2, and 2.5% NO respectively for 16h at 2°C. Stored products were fumigated with 3% NO for 24 h at 20°C. Treatments NO-Air and NO-N_2 refer to nitric oxide fumigation that was terminated by flush with air and N_2 respectively. For each product, the values in each column followed by different letters were significantly different based on Tuckey HSD multiple range tests at $P \leqslant 0.05$ (SAS Institute, 2012). Data on fresh products are from a previous article (Liu and Yang, 2016).

In small scale fumigation tests, NO fumigation is safe to fresh products if it is terminated properly with N_2 flush to dilute NO prior to open the fumigation chamber to ambient air. These results are encouraging and need to be demonstrated in large scale trials. Commercial fresh products often are sealed in plastic packing materials such as perforated wraps and bags with very limited air exchange ability and then packed in cartons and crated on pallets. All of these restrict air exchange and

increase difficulty in establishing ULO conditions for NO fumigation and diluting NO at the end of fumigation.

For delicate fresh fruits and vegetables, the additional benefits of NO fumigation for pest control on postharvest quality can have significant economic impact as it increases shelf-life and enable wider distribution of the products. Fumigation of flower bulbs for controlling bulb mites with or without N_2 flush to dilute NO also did not have any effects on their germination or growth, indicating NO fumigation was safe to propagating plant materials (Liu, 2017).

Some harvested fresh products are treated with chemical agents to maintain proper storage life. For example, diphenylamine (DPA), a plant growth regulator, is used to control storage scald of apples in USA. Nitric oxide, however, is an inhibitor of ethylene biosynthesis (Manjunatha *et al*., 2010) and can also help to maintain postharvest storage life (Wills *et al*., 2000; Soegiarto and Wills, 2004; Manjunatha *et al*., 2012; Saadatian *et al*., 2012; Liu *et al.,* 2016). It is possible that NO fumigation for postharvest pest control can also reduce or replace the usage of chemical agents such as DPA for postharvest storage of fresh fruit. This potentially bring additional benefits of NO fumigation and enhance food safety.

Nitric oxide fumigation does not leave toxic residues on fumigated products. When NO fumigation is not terminated properly by directly opening the fumigation chamber to ambient air without prior N_2 flush, the fumigation will likely cause significant increases in nitrate levels and sometimes also nitrite levels in fresh products. However, nitrate and nitrite are nutrients and they exist in both fresh and stored products at various levels (Santamaria, 2006; Hord *et al*., 2009) and the increases after NO fumigation are also well within their normal ranges in prospective products.

Nitrogen dioxide has a boiling point of about 21°C and high solubility in water. This is likely the main reason for higher NO_2 release rate 24 h after fumigation on fresh products than on stored products. Fresh products were fumigated at 2°C and stored products were fumigated at 20°C. At the end of fumigation, NO_2 from oxidation of NO will more likely to stay on fresh products because of the low ambient temperature and high relative humidity than on stored products. Retaining of NO_2 not only cause increases in nitrate and nitrite contents, also affect management of fumigated products due to increased health risks related to workers' exposures to released NO_2 from fumigated products. So, even the increases in nitrate and nitrite from NO fumigation are acceptable, it is preferably to terminate NO fumigation with N_2 flush to avoid prolonged emission of NO_2 from fumigated products, especially for fresh products since NO_2 at high levels can also cause injuries to delicate fresh products (Liu, 2016).

Due to the reactive nature of NO with O_2, NO fumigation, however, must be conducted under ULO conditions in airtight fumigation chambers. Therefore, NO fumigation requires complex and strict procedures and efforts are needed to develop protocols for commercial applications of NO fumigation. The complex fumigation procedures also add costs to NO fumigation. These costs include initial capital expenses on N_2 generation equipment and fumigation chambers and operation related costs including electricity, equipment maintenance, and NO supply. Nitrogen generation equipment is widely available commercially. Electricity cost varies depending on locations. Nitric oxide gas is also available commercially. Previous analysis suggests that NO fumigation is technically feasible and cost effective (Liu, 2015).

Nitric oxide has been studied extensively over past three decades since it was found to be a ubiquitous cell messenger. However, as a newly discovered fumigant, the mode of action of NO for pest control is still unknown. In addition, NO is known to be toxic to humans. Therefore, even NO is produced naturally by almost all organisms, it still needs to be registered as a chemical pesticide in USA in order to be used for postharvest pest control. Nitric oxide will also need regulatory approval in other countries in order to be used commercially for postharvest pest control. Participation of industry will be critical for eventual registration and commercial use of NO for postharvest pest control.

There have been extensive efforts to find alternative treatments for postharvest pest control since the start of global phase out of methyl bromide production. However, progresses are very limited and there is a severe lack of safe and effective alternative fumigants to meet the demand for postharvest pest management. Nitric oxide fumigation has high efficacy against a wide variety of pests, no toxic residues on fumigated products, and can be used on both fresh and stored products. In addition, NO fumigation has potential to extend storage life of fresh products. All these advantages of NO should far offset the disadvantages of complex and strict fumigation procedures and associated costs on acquisition and operation of N_2 generation equipment. More efforts are needed in several fronts in order to speed up the commercial applications of NO fumigation. They include research to develop effective and safe treatments for various pests on a variety of products, developmental efforts for suitable and reliable systems and protocols for commercial scale NO fumigation, including techniques to reduce emission of NO into atmosphere and registration efforts from industries to attain regulatory approval from respective countries for commercial applications.

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Bluefume (HCN) and EDN® as fumigation alternatives to methy bromide for control of primary stored product pests

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Abstract

The presented paper provides preliminary results on the fumigation potential of two preparations: Bluefume (HCN - hydrogen cyanide) and EDN®. (Ethane-dinitrile). Their biological efficacy was tested on Granary weevil (*Sitophilus granarius*; Curculionidae; Coleoptera) as a primary stored product pest in the Czech Republic. In fumigation chamber, we tested temporal survival of various *S. granarius* strains following exposure of a dose of 9 $g.m^{-3}$ HCN (Bluefume). We compared differential sensitivity of one laboratory (i.e. sensitive) CRI-strain and 9 field strains collected from the Czech stores and mills. The HCN Ct products required to kill the tested *S. granarius* strains ranged from CTp= 30.5 $g.m^{-3}.h^{-1}$ to CTp= 51.7 g.m3.h^{-1}. The efficacy of EDN (30 $g.m^{-3}$) on various developmental stages *S. granarius* was tested in a fumigation chamber. No live individual of *S. granarius* belonging to any life stage was recorded following 18 hours of EDN exposure.

Keywords: gas, ethane dinitrile, hydrogen cyanide, Granary weevil, Sitophilus granarius,

Introduction

Fumigation of stored product pests has become a real challenge for both farmers and pest control professionals (PCOs) in the last two decades. The reason is that broad-spectrum pesticide methyl bromide is no longer available and pest resistance to the remaining major fumigant phosphine is on the rapid increase (Nayak, et al., 2017). Therefore, the alternatives to methyl bromide or "resistance phosphine breakers" (e.g., Nayak et al., 2016) are urgently needed. However, there are only few candidate active ingredients available even at the worldwide scale (Ducom, 2006). Currently two of them (EDN and HCN) are produced in the Czech Republic (Lucebni zavody Draslovka Kolin a.s.).

HCN (Bluefume)

Various formulations of hydrogen cyanide (HCN) has previously been used for pesticide/biocide fumigation in several countries, including USA, South Korea, France, Germany, Czech Republic, and Switzerland (Rambeau et al., 2001). HCN as an active ingredient shows quick and high efficacy on structural pests infesting mills (Bond 1984, Rambeau et al. 2001, Aulicky et al., 2015a) and ships (Monro, et al., 1952). Aulicky et al., (2015a) demonstrated a higher activity of HCN on *Tribolium confusum* eggs than the one documented for phoshine during the commercial mill fumigations in Czechia (Aulicky et al., 2015b). HCN has been historically used for the fumigation of many dry

foodstuffs, grains, tobacco and seeds (Bond 1984, Emekci, 2010, Stejskal, et. al., 2014b). HCN also shows promising level of biocidal activity on package and structural wood infesting pests such as *Hylotrupes bajulus*, *Anoplophora glabripennis* and pine wood nematode, *Bursaphelenchus xylophilus* (Stejskal et al., 2014a, Douda et al., 2015). Recent works Zouhar et al., (2016) reported high nematicide potential of hydrogen cyanide against *Ditylenchus dipsaci* nematode present inside garlic seedlings.

EDN®

Ethane-dinitrile (EDN) is an ozone-friendly alternative to methyl bromide. Its advantages are good penetration characteristics, high efficacy and short application time (Ryan et al., 2006). The EDN® main use is aimed at limiting the risks of pests and disease spreading, within the agricultural and timber industry. It can be used to sterilize soil and control insects, diseases, nematodes, weeds and other parasites, before planting. It can also be used to fumigate harvested timber and logs. Its excellent penetration characteristics and high efficacy make EDN® a great solution for eliminating wood-boring insects in timber as well as pathogens and nematodes which present a direct biosecurity risk to many importing countries.

As apparent from the previous paragraphs, most of the recently published studies on both fumigants dealt mainly with wooden, soil or structural pests. However, there are only limited (Hooper et al., 2003) and/or outdated (Monro, et al. 1952, Lindgren, et al. 1954, Lindgren, Vincent 1965) information documenting the efficacy of ECN or EDN on stored product pests.

Therefore in the present work we evaluated the potential of two fumigantion preparations (Bluefume -HCN- hydrogen cyanide and EDN® -Ethane-dinitrile) regarding their biological efficacy on the primary stored product pest granary weevil (*Sitophilus granarius*).

Materials and Methods

Pest species

Granary weevil (*Sitophilus granarius*, Curculionidae; Coleoptera) was selected as a model species, since it is a major primary pests in the Czech Republic stores (Stejskal et al., 2014b; Stejskal et al. 2015). Both field (for HCN testing) and laboratory (for EDN or HCN testing) strains of *S. granarius* were included in the study.

HCN (Bluefume)

The efficacy of HCN on various strains of *S. granarius* was tested. In the tests, the efficacy of BLUEFUME (with the active substance HCN) at the initial dose of 9 g. m^{-3} (0.75%) of HCN on *S. granarius* adults was estimated. Testing was carried out in a small fumigation chamber of 650 litres (for detailed description of methods see Stejskal et al., 2014a). In total, 10 strains (one laboratory and 9 field strains) were included in the experiments. The following HCN exposure times were used: 15; 45; 60; 90; 180; 240; 300; 360; 420; 480; 540; 600 and 660 minutes. The reason for using short exposure times and a reduced dose of HCN was to divide the mortality of the tested individuals over a wider time span.

EDN®

The efficacy of EDN on various stages of *S. granarius* was tested. The tested EDN dose was 30 $g.m^{-3}$ and the exposure time was 18 hours. The tests were carried out in a 650-liter fumigation chamber (Stejskal et al., 2014a). The individual stages of development of the *S. granarius* were separately placed (in grain mass of winter wheat) in plastic containers with a diameter of 50 mm and a height of 70 mm. A hole of 40 mm diameter was formed in the lid and bottom of the plastic container, which was overlaid with a breathable fabric (miralon). Biological samples with grain containing

different stages of *S. granarius* were exposed in separate plastic containers. Adults aged 7-14 days were inserted into grain mass in the test vial one day before trial.

Eggs of *S. granarius* were ovtained by exposing adult females to grain mass for 3-4 days before trial. For the testing of pupae, the infested grains were used 42-48 days after exposure to *S. granarius* females. The biological efficacy of EDN on various stages was evaluated after removal and ventilation of samples in the laboratory. In adults, the efficacy was assesed visually, directly on the basis of their knockdown and mortality. In pupae, larvae, and eggs, however, the EDN efficacy was evaluated indirectly: according to adult emergence from the exposed grain-infested samples.

Results and Discussion

HCN (Bluefume)

Figure 1 shows comparison of the HCN sensitivity of one laboratory strain with 9 field strains of *S. granarius* (collected from the Czech stores and mills). The maximum time required to kill all of the tested 10 *S. granarius* strains was 660 minutes. The Ct products - required to kill the tested 10 *S. granarius* strains - ranged from CTp= 30.5 $g.m^{-3}. h^{-1}$ to CTp= 51,7 $g.m^{-3}. h^{-1}$.

Monro, et al. (1952), were the first who published a comparison of the efficacy of HCN and methyl bromide as fumigants for the treatment of stored product pests in empty ships. Later Lindgren, et al. (1954) compared the laboratory efficiency of 10 fumigants to selected types of food and warehouse pests. Their work showed the high efficiency of HCN in 8 tested species. Grain weevil (*S.granarius*) and Rice weevil (*S. oryzae*) were less tolerant to HCN than other pests including *Tribolium confusum, Acathoscelides obtectus, Oryzaephilus surinamensis* and *Rhizopertha dominica*. However, Ct products were not established in their work. The first study, which documented the HCN Ct products required to kill *S. granarius*, was the work of the French researchers Rambeau et. al. (2001). They claimed that *S. granarius* was more tolerant to HCN than to methylbromide (CH_3Br). The Ct product and mortality of *S. granarius* for HCN was 36 $g.m^{-3}$ for LD_{90}, and 15.7 $g.m^{-3}$ for CH_3Br. Rambeau et al. (2001) did not use HCN formulations in the form of liquid or liquid soaked in porous matter - a formulation that uses BLUEFUME but worked with HCN released from cyanide salts.

EDN®

Table 1 shows that the exposure of *S. granarius* by EDN fumigant at a dose of 30 $g.m^{-3}$ led to 100% mortality in all of the tested pest stages. According to Ducom (2006) EDN was much more toxic than methyl bromide and killed most of the pests very quickly. However, S*itophilus* sp. was an exeception, since a very long exposure of 5 days was required to achieve complete mortality of the egg stage. However, our laboratory experiments suggested that 100% mortality can be reached after 18 hours in all stages for the EDN dose of 30 $g.m^{-3}$. This preliminary result show good potential of EDN to be used to combat infestations from storage pests and it is therefore, desirable to continue with further validation tests against other pest species.

Tab. 1 The efficacy of EDN on various stages of *Sitophilus granarius* (dose: 30 g/m3, exposure time: 18 hours) in a fumigation chamber.

	Mortality (%)		Larval emergence (ks)	
Developmental stage	Treated	Control	Treated	Control
Adults	100	3,6	n	n
Larvae	n	n	0	483,4
Pupae	n	n	0	651,6
Eggs	n	n	0	2,8

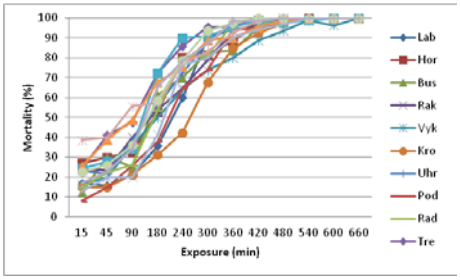

Fig. 1 Mortality of 10 strains (9 field and 1 laboratory) of *Sitophilus granarius* after HCN exposure in a dose of 9 $g.m^{-3}$ in a fumigation chamber.

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Improved Analysis of Propylene Oxide, Propylene Chlorohydrin and Propylene Bromohydrin

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Abstract

The benefits and deficiencies of several methods of analysis for PPO and PXH, including the aqueous extraction used in ASTA method 23.1 and the MTBE extraction method previously reported by the authors, will be discussed. Novel methods utilizing dynamic headspace extraction and solid phase microextraction (SPME) will also be reported with particular emphasis on preventing artefactual effects. Preliminary experiments have found that dynamic headspace sampling can lower detection limits by up to 3 orders of magnitude.

Keywords: Propylene Oxide, Fumigation, Sterilent, Headspace-SPME, Pesticide Degradants.

Introduction

The importance of propylene oxide (PPO) treatments for stored product protection has only increased in recent years, especially as the implementation of FSMA in the US puts pressure on tree nut producers to pasteurize their product. In the search for post-harvest methyl bromide replacements; PPO/SF blends, with PPO overcoming the ovicidal deficiencies of sulfuryl fluoride, have been shown to be effective against several stored product pests.

With the increasing variety in PPO treatments across commodity types and a "deharmonized" global MRLs comes an increasing need for the quick and accurate quantification of PPO residues. Analysis is complicated by the ease with which PPO will undergo nucleophilic reaction with water to form propylene glycol, or with chloride and bromide to form propylene chloro and bromo- hydrin, which can artificially lower the detected PPO residue. Avoiding the formation of these halohadrins (PXH) is of particular importance as they face regulatory scrutiny as carcinogens.

Materials and Methods

Jimenez et. al. Method:

Almonds or walnuts are added to an explosion proof blender along with chilled, deionized water and MTBE and homogenized. 45mL of the homogenate is centrifuged and a 1 mL aliquot of the MTBE supernatant is transferred to a 2 mL amber glass vial for analysis. A 10x concentration (10 mL to 1 mL) of the MTBE supernatant could be performed to increase detection of PBH-1 and PBH-2. Analysis was performed via cool on-column injections in an Agilent 6890 gas chromatograph (GC) equipped with a 5973N mass spectrometer (MS).

Dynamic Headspace Extraction Method:

Three almonds or walnuts are chopped roughly, transferred into a 20 mL headspace vial and sealed. The vial is then incubated at 80C for 42 min in a Perkin-Elmer Turbomatrix dynamic headspace autosampler, and three cycles of pressurizing the vial to 15 psi and allowing it to vent through an adsorbent trap are performed prior to ballistically heating the trap and directing the sample flow into a Perkin-Elmer Clarus SQ8 GCMS.

SPME-Headspace Method:

An approx. 50g sample of almond or walnuts is cryogenically milled under liquid N2 and a 2g subsample is transferred to a 20 mL headspace vial and sealed. SPME extraction is performed with

a Carboxen / DVB / PDMS fiber at room temperature and 30min sorbtion time. Analysis is performed on an Agilent 7890B GC with a LECO Pegasus BT TOF-MS.

For each methods, % recovery and LODs were determined by spiking known amounts of each analyte onto the surface of a walnut or almond and extracting. The amount of side reaction (amount of PCH or PBH formed during extractioin and analysis) was determined by spiking PPO-treated nuts with d6-PPO and measuring the amount of deuterated PCH (PCD) and PBH (PBD) formed. The reaction kinetics between PPO and chloride / bromide will be examined by spiking PPO onto the surface of nuts or nut grounds and measuring the amount of PCH and PBH formed at varying reaction times and temperatures.

Results

Negative chemical ionization MS (NCIMS) was not found to improve analyte sensitivity for the target analytes when compared to electron impact MS (EIMS). GC-ECD (electron capture detection) demonstrated improved sensitivity for PBH-1 and PBH-2 in non-concentrated MTBE extracts (approx. 0.7 mg/kg compared to 50 mg/kg for EIMS). Ten-fold concentration of the MTBE extract yielded a 10x improvement in detection limits for PBH, but recoveries for PPO and PCH, respectively, dropped below 50% and ranged from 50 to 72%.

The use of dynamic headspace extraction demonstrated a great improvement in the simplicity, speed and sensitivity of analysis compared to MTBE extraction, with detection limits for PCH and PBH around 10 ng/g (ppb). The incubation temperature required for the sensitive detection of PBH, however, was shown to also cause further reaction of PPO into PCH and PBH. Results from the the use of a saturated KI solution to preemptively react with PPO will be reported.

Preliminary experiements show that SPME-Headspace extraction has been shown to have excellent sensitivity for each target analyte, able to detect as little as 1.5 ng of material, dissolved in 5 µL H_2O, and spiked into an empty 20 mL HS vial. Spiking approx. 20 µL of PPO into almond grounds and analyzing with SPME-headspace analysis has shown that PBH and PCH begin to form in as little as 20 min at room temperature.

Tab. 1 Limits of detection for each target analyte for the MTBE extract and dynamic headspace methods.

Compound	Matrix	LOQ - Solvent Extract	LOD - Head Space Trap
PPO	Almond	0.85 ug/g	0.54 ug/g
	Walnut	0.81 ug/g	0.08 ug/g
PCH-1	Almond	2.10 ug/g	10.0 ng/g
	Walnut	2.31 ug/g	12.1 ng/g
PCH-2	Almond	2.22 ug/g	N/A
	Walnut	1.95 ug/g	N/A
PBH-1	Almond	75.1 ng/g	6.01 ng/g
	Walnut	74.8 ng/g	19.0 ng/g
PBH-2	Almond	75.3 ng/g	4.92 ng/g
	Walnut	77.3 ng/g	N/A

Discussion

Preliminary experiments have demonstrated that while headspace sampling methods can significantly improve sensitivity for PPO, PCH and PBH, great care must be taken to avoid aretfactually raising PCH and PBH levels. The use of autosamplers (either dynamic headspace or L-PAL3 with SPME attachment) can greatly reduce injection to injection variability and reduce the number of person-hours required for analysis, but to fit walnuts or almonds into headspace vials they must be chopped or ground exposing further chloride or bromide to react with PPO. Future experiments using manual SPME sampling will allow the use of glassware that can accommodate whole nuts. The use of iodide, or other nucleophiles, to compete with chloride and bromide for the reaction with PPO will also be examined.

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Monitoring of post-harvest fumigation with Gasmet Multikomponent FTIR gas detection systems

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Keywords: Post-Harvest, Fumigation, Fourier Transform Infrared (FTIR), Fumigant, Library Search Tool

Fumigation business has changed dramatically after the Montreal protocol came into effect on January 1st 1989. Methyl Bromide had to be replaced in all its widespread application. A lot of fumigators having experience with Methyl Bromide are still mourning in regards of its outstanding fumigation performance. Today, almost 30 years after, we are having a big variety of different alternatives to Methyl bromide, developed by research institutes around the world.

Focusing on new gaseous alternatives to Methyl Bromide, FTIR technology is an extremely versatile detection principle, offering a widespread use in the fumigation industry.

Fourier transform infrared (FTIR) is a powerful gas measurement technology that offers true multicomponent capability. This technology that was originally used for challenging research applications has since proven to be very reliable and versatile and has become the industry standard in many challenging emissions monitoring applications.

Most gases absorb infrared light at some wavelengths in the infrared spectrum. The position and intensity of the absorptions are determined by the molecular structure of the gas and this means that each gas will have a unique absorption pattern. This unique pattern can be used like a fingerprint to identify and measure each gas in the sample.

An FTIR analyzer works by simultaneously scanning the entire infrared spectrum and then calculating the concentrations of each gas in the sample based on their characteristic absorptions. The fact that the entire infrared spectrum is scanned at once means that all the gases in the sample can be measured simultaneously. This allows for very quick multicomponent measurements and for compensation for any cross-interference.

As all gases are measured by scanning the same infrared spectrum, adding new compounds can be done easily in the software without requiring any changes to the hardware. The recorded spectra are also unaltered by the analysis performed on them and can therefore, always be re-analyzed at a later point. This allows for traceable data and facilitates for instance retrospectively checking the measurements for new gases.

All this makes FTIR the ideal solution for a variety of applications where multiple gases need to be measured quickly, accurately and reliably.

Working on approving, registering, developing or applying new fumigation procedures has become much more demanding than what experienced for Methyl Bromide. The need for an ideal gas detection device is enormous.

FTIR technology brings some outstanding advantages for the fumigation industry as listed below:

1. Detection of several different fumigants with the same instrument
2. No changes of sensors required for change in gases
3. Extremely easy and low cost calibration
4. Detection of complex gas mixtures

5. Due to the evidential detection of the target gas cross sensitivities are reduced to a minimum and can be evaluated and analysed even after the measurement.
6. Generating of ct-diagrams is a crucial part of the whole FTIR measurement.
7. Ready to measure new fumigants
8. High concentrations of fumigants during fumigations and low concentrations for clearance /entry permits can be measured with one instrument

A lot of gases can be detected, qualitative and quantitative

	Gas	Fumigation procedure	Clearance/ entry permit	LDL in N2
1.	Methyl Bromide	X		0,4 ppm
2.	Phosphine	X		0,2 ppm
3.	Sulfuryl Fluoride (Vikane®, Profume®)	X	X	0,03 ppm
4.	HCN	X	X	0,35 ppm
5.	EDN	X	X	0,9 ppm
6.	COS	X	X	0,004 ppm
7.	Ethyl Formate	X	X	0,1 ppm
8	Propylene Oxide	X	X	0,1 ppm
9.	Methyl Iodide	X	X	0,1 ppm
10.	Chloropricrin	X	X	0,08 ppm
11.	Formaldehyde	X		0,09 ppm
12.	Ethylene Oxide	X	X	0,2 ppm

A real big advantage is to detect several gases (up to 50) parallel. By using the entire spectrum between 850 and 4200 waves/cm.

Examples for parallel evaluations are:

1. HCN and CN2
2. SO2F2 and Chloropricrin

This options enables the user to check for interactions with the fumigated material, metabolisms and other tasks where more than one gas has to be evaluated.

Determination of safe storage moisture content of commercial maize (*Zea mays*) seeds during hermetic storage

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Abstract

Germination declines during storage and meeting official standards (90% limit) can be challenging for the seed industry. Hermetic storage, through the establishment of self-modified atmospheres has shown to preserve germination in high-moisture maize seeds, but in the range of the low-moisture contents (m.c.) used by the seed industry, the relationship between hermetic storage and seed quality has not been fully studied. The aim of this work was to determine the safe storage m.c. of commercial maize seeds during hermetic storage considering both germination and microbiological aspects. Maize seeds with 95% initial germination were conditioned to m.c.s. between 11.5 and 14.5% and stored hermetically at 25ºC for 6 months. Germination, % oxygen, % infected grains, and colony forming units (CFU) were evaluated. Germination declined with increasing m.c.s, dropping to 50% at 14.5% m.c. Microflora respiration started to be detected at 13.5% m.c. and an anaerobic self-modified atmosphere was reached at 14.5% m.c. Despite the higher relative humidity, % infected grains and CFU count at 14.5% m.c. were lower than at 13.5%, probably due to the suppressive effect of the anaerobic atmosphere. In conclusion, 11.5% was a safe storage m.c. as it preserved germination above marketing requirements without microbiological risk. Hermetic storage was useful to generate self-modified atmospheres for m.c.s above 13.5%, but these self-modified atmospheres were not effective to protect germination. Further research on the effects

of controlled and self-modified atmospheres on the quality of different maize genotypes is needed to evaluate the benefit of hermetic storage of commercial seeds.

Keywords: seed germination, microbial activity, modified atmosphere, controlled atmosphere, respiration.

Introduction

Relative humidity and temperature are two major factors that must be controlled during seed storage to preserve germination (Krishnan et al., 2004; Kong et al., 2015; Mansouri-far et al., 2015). At an equilibrium relative humidity (e.r.h) above 75%, the seed associated microflora will develop rapidly during storage affecting seed quality (Pixton, 1967; Navarro and Donahaye, 2005). For most grains and oilseeds, a threshold of 70% e.r.h is used to determine the microbiological safe storage moisture content (Pixton, 1967; Giner and Gely, 2005). Also, degenerative reactions associated with seed ageing are hastened above 70% e.r.h. (Bewley et al., 2013). Because seed deterioration increases with temperature (Barzali et al., 2005), commercial maize seeds must be stored in refrigerated (5-10ºC) and controlled e.r.h chambers at the expense of high energetic costs (Robertson et al. 1984; Chiu et al. 2003; Sun et al. 2007; Abreu et al. 2013).

Besides e.r.h. and temperature, low oxygen concentrations during storage have shown to benefit germination in many species (Chiu et al., 2003). Low-oxygen concentrations limit the development of various aerobic genera of seed fungi (Hocking, 1990; Weinberg et al., 2008; Taniwaki et al., 2009; Marcos Valle, 2015) and also impair oxidative aging reactions (Chiu et al., 2003; Yeh et al., 2005; Groot et al., 2015). Exploring the potential of low-oxygen modified atmospheres as an alternative technology to refrigeration is of great interest for the seed industry, as it could help to save energy and reduce storage costs.

Low-oxygen atmospheres can be easily obtained storing seeds hermetically. During hermetic storage, the respiration of the seed associated microflora will consume the oxygen and release carbon dioxide originating a self-modified atmosphere (Navarro and Donahaye, 2005). Seed respiration starts to be detected above 90% relative humidity (Bewley et al. 2013), and thus it is not expected to occur in the range of low-moisture contents used in the seed industry.

Hermetic storage of high-moisture maize (above 14%) has proved to benefit germination compared to open-air storage (Moreno et al. 1988; Cardoso et al. 2016). However, in the range of the low-moisture contents used by the seed industry, the relationship between hermetic storage and maize seed quality has been less studied. The aim of the present work was to determine the safe storage moisture content (s.s.m.c.) of commercial maize seeds under hermetic storage considering not only microbiological risk but also germination decay. Therefore, the s.s.m.c. was defined as the maximum moisture content (m.c.) that preserves germination above 90% (official requirement in Argentina for maize seed marketing, SAGyP (1993) with no evidence of microbial activity after six months of storage at 25ºC.

Materials and methods

Maize samples preparation and experimental procedure

Maize seeds harvested in February 2016 (GLStack 4500, KWS) were divided into four batches and put into plastic bags for moisture conditioning to four target m.c.s. (11.5, 12.5, 13.5, and 14.5%). Calculated amounts of distilled water were poured into the bags and seeds were thoroughly mixed after wetting. The four bags were stored for 7 days at 4ºC with daily mixing of the seeds for moisture stabilization.

The maize of each batch was divided into three and placed in glass jars, each with a rubber septum on the lid for gas sampling. Maize samples were collected from the jars before closing the lid for initial measurements of germination, colony forming units (CFU), and infected grains. The sealed jars were stored at 25ºC for 6 months.

Analytical procedure

The germination percentages were determined following ISTA guidelines (ISTA, 2015). Briefly, three replicates of 50 seeds per jar were placed on extended wet paper towels, rolled, and introduced into plastic bags to be incubated at 25°C for 7 days. Results were expressed as percentages of normal seedlings.

The m.c. of the maize samples was determined by forced-air oven drying at 130ºC for 72 h according to ASAE (2003). Equilibrium relative humidity (e.r.h.) of the headspace was determined at 25ºC using relative humidity sensors (I-button, Hygrochrom, EEUU). Oxygen and carbon dioxide concentrations in the headspace were determined using a gas analyzer (CheckPoint, DanSensor, Denmark).

Microbiological analysis

Colony forming units (CFU) were determined by homogenizing 10g of maize seeds in 90 ml of peptone water, serial diluting 1ml in 9ml of the same diluent, and spreading 0.1ml aliquots on potato dextrose agar (PDA) plates. Plates were incubated at 28ºC for 5-7 days, when colonies were counted (Castro et al., 2002). Results were expressed as logCFU/g of maize.

Infected grains were determined by the direct plating technique. Fifty kernels were randomly sampled from each jar, and they were surface-disinfected in a 1% sodium hypochlorite solution for 2 min. The samples were plated on potato dextrose agar (PDA) plates (10 seeds by plate) under a sterile hood, and incubated at 28ºC for 5-7 days, when the percentage of fungi contaminated seeds was determined for each sample (Berardo et al., 2005).

Statistical analysis

Linear or Generalized Linear Models were fitted to the data after checking model´s assumptions by means of residual plots. The statistical analysis included analysis of variance and Tukey´s multiple comparisons test. The packages nlme (Pinheiro et al., 2017), lattice (Sarkar, 2008), and emmeans (Lenth, 2018) of the statistics software R version 3.4.3 were used.

Results

At the beginning of the hermetic storage period, the m.c.s. of the maize in the 11.5%, 12.5%, 13.5%, and 14.5% levels were (11.64±0.01)%, (12.68±0.06)%, (13.70±0.05)%, and (14.70±0.10)%, respectively. The corresponding initial e.r.h.s. were (63.0±1.6)%, (70.5±2.5)%, (75.2±2.7)%, and (77.2±4.2)%, respectively. M.c.s. showed a slight increment during the storage period, because of respiration activity. The final m.c.s. were (12.2±0.4)%, (13.7±0.2)%, (14.8±0.1)%, and (14.9±0.8)% and the corresponding final e.r.h.s. were (63.2±1.1)%, (72.3±0.1)%, (77.1±0.5)%, and (80.7±0.8)%.

Table 1 summarizes the germination percentages of the maize seeds at the beginning and after 6 months of hermetic storage. The initial germination percentage was higher than 95% in all the four seed lots conditioned to the different m.c.s. Final germination remained unchanged at 11.5% m.c. and started to decrease at 12.5% m.c., dropping by half at 14.5% m.c. However, due to variability in the data, no significant differences were found between final germination for 11.5, 12.5, and 13.5% m.c.s.

Tab. 1. Estimated marginal means ± model standard error of germination percentage under hermetic storage.

Time (months)	Moisture content (%)			
	11.5	12.5	13.5	14.5
0	95.7±2.9 Aa	97.7±2.9 Aa	97.0±2.9 Aa	99.0±2.9 Aa
6	94.0±2.9 Aa	83.7±2.9 Aa	81.3±2.9 Ba	48.0±2.9 Bb

Within each column, means followed by different upper case letters are significantly different; within each row means followed by different lower case letters are significantly different ($P<0.05$).

As a Linear Model was fitted to the germination data, the model standard error is the same for all groups.

Table 2 shows the concentration of oxygen within the sealed containers of maize seeds after 3 and 6 months of storage. At 11.5% m.c. oxygen remained constant during the whole storage period and similar to the normal atmospheric concentration, indicating there was no respiration at this m.c. At 12.5% m.c., a slight self-modification of the atmosphere occurred by 3 months, indicating the onset of respiration at this m.c. At 13.5% and 14.5% m.c.s., oxygen dropped markedly by 3 months revealing a more intense respiration. An anaerobic self-modified atmosphere was reached only at 14.5% m.c. The carbon dioxide concentration always remained below 21% (data not shown), suggesting that aerobic respiration prevailed in the range of m.c.s. studied.

Tab. 2. Estimated marginal means ± model standard error of oxygen concentration during hermetic storage of maize seeds. Normal atmospheric oxygen content: 20.9%

Time (months)	Moisture content (%)			
	11.5	12.5	13.5	14.5
3	20.0 ±0.2 Ac	19.4 ±0.4 Ab	1.3±1.1 Aa	0.0±0.1 Aa
6	20.1 ±0.2 Ac	18.3±0.4 Ab	2.6±1.1 Aa	0.5±0.1 Ba

Within each column, means followed by different upper case letters are significantly different; within each row means followed by different lower case letters are significantly different ($P<0.05$)

Tab. **3** shows the results of the microbiological analyses before and after 6 months of hermetic storage. The initial percentages of infected seeds were similar for the different m.c.s. After the storage period, the percentages of infected seeds were similar at 11.5% and 12.5% m.c.s., and remained on average below 40%. The final percentage of infected seeds increased abruptly at 13.5% m.c., where practically all the seeds were infected by molds. The percentage of infected seeds at 14.5% was near 60%, and was lower than at 13.5% m.c. despite the higher e.r.h. However, due to variability in the data, the differences were not significant.

The initial CFU counts were similar between the four m.c. level seeds (Tab. **3**). Only the seeds stored at 13.5% m.c. showed a significant increment in the CFU counts after the hermetic storage. There were no differences between the final CFU counts at 11.5%, 12.5%, and 14.5% m.c.s., which remained similar to the initial counts.

Tab. 3. Estimated marginal means ± model standard error of percentage of infected seeds (Inf) and CFU counts (expressed as log10CFU/g) in maize seeds under hermetic storage.

Time (months)	Moisture content (%)							
	11.5		12.5		13.5		14.5	
	Inf	CFU	Inf	CFU	Inf	CFU	Inf	CFU
0	20±8.4 Aa	3.5±1.1 Aa	22.7± 2.3 Aa	2.7±0.8 Aa	14.7± 3.6 Aa	3.5±0.2 Aa	16.5± 13.1 Aa	2.0±1.1 Aa
6	39.5±8.4 Aa	2.6±1.1 Aa	26± 2.3 Aa	2.3±0.8 Aa	98.7± 3.6 Bb	5.2±0.2 Bb	62.8± 13.1 Aa,b	1.8±1.1 Aa

Within each column, means followed by different upper case letters are significantly different; within each row means followed by different lower case letters are significantly different ($P<0.05$)

Discussion

Low-oxygen atmospheres are a promising alternative to refrigeration for seed storage. This technology could help to reduce costs and is friendly with the environment (Weinberg et al., 2008). The availability of new plastic liners with oxygen barrier (Cardoso et al., 2016) and of portable gas analyzers that provide quick results makes it feasible to implement modified atmospheres in the commercial scale. Hermetic storage may serve as a practical, simple way for obtaining low-oxygen atmospheres.

The results of this work show that, under hermetic storage, maize seeds with up to 11.5% m.c. can be stored safely for six months at 25ºC (Tab.4). At this m.c., oxygen concentration and microbial indicators remained unchanged (Tab. **2.** Estimated marginal means ± model standard error of oxygen concentration during hermetic storage of maize seeds. Normal atmospheric oxygen content: 20.9%

Time (months)	Moisture content (%)			
	11.5	12.5	13.5	14.5
3	20.0 ±0.2 Ac	19.4 ±0.4 Ab	1.3±1.1 Aa	0.0±0.1 Aa
6	20.1 ±0.2 Ac	18.3±0.4 Ab	2.6±1.1 Aa	0.5±0.1 Ba

Within each column, means followed by different upper case letters are significantly different; within each row means followed by different lower case letters are significantly different ($P<0.05$)

Tab. **3** shows the results of the microbiological analyses before and after 6 months of hermetic storage. The initial percentages of infected seeds were similar for the different m.c.s. After the storage period, the percentages of infected seeds were similar at 11.5% and 12.5% m.c.s., and remained on average below 40%. The final percentage of infected seeds increased abruptly at 13.5% m.c., where practically all the seeds were infected by molds. The percentage of infected seeds at 14.5% was near 60%, and was lower than at 13.5% m.c. despite the higher e.r.h. However, due to variability in the data, the differences were not significant. andTab. **3**) indicating that there was no microbial activity. This result was expected due to the low e.r.h. (63.1% e.r.h. on average for the whole storage period). Final germination was 94%, higher than the official requirements for maize seed marketing (90%, Tab. **1**) and therefore 11.5% m.c. resulted a safe storage moisture content. Additionally, since the absence of microbiological activity was related to the low m.c. (oxygen was not a limiting factor), storing 11.5% m.c. corn seeds in non-hermetic conditions would have the same results.

At 12.5% m.c., the microbial activity was also undetectable what can be attributed to the still low e.r.h. (71.4% on average for the storage period). However, germination dropped to 84%. Hence, other mechanisms rather than microbial damage must have been involved in the germination loss observed at 12.5% m.c., i.e. intrinsic aging of the seed. Because it failed to meet the germination criterion, 12.5% m.c. was not a safe storage moisture content at least for the quite challenging temperature used in this work (25ºC).

Because no appreciable modification of the atmosphere occurred at 11.5 or 12.5% m.c., it is not expected to find differences in germination or microbial charge between hermetic and open-air storage at these m.c.s. In contrast, removing the oxygen from the beginning of storage at 12.5% m.c. by means of a controlled atmosphere could have an impact on final germination that remains to be studied.

At 13.5% m.c., final germination dropped to 81%. The higher e.r.h. (76.2% on average through the whole storage period) favored fungal growth. This was reflected in the oxygen depletion and the significant increase of infected grains and CFU counts. Because it failed to meet both the germination and microbiological criteria, 13.5% is not a safe storage moisture content (Tab.4).

It is noteworthy that, despite the higher e.r.h. and microbial charge, the final germination at 13.5% m.c. was similar to the observed at 12.5% m.c. The low-oxygen atmosphere at the higher m.c. might have prevented a larger germination loss. Indeed, the higher respiration rate consumed the available oxygen (less than 2% of oxygen by 3 months of storage, Tab. **2.** Estimated marginal means ± model standard error of oxygen concentration during hermetic storage of maize seeds. Normal atmospheric oxygen content: 20.9%

Time (months)	Moisture content (%)			
	11.5	12.5	13.5	14.5
3	20.0 ±0.2 Ac	19.4 ±0.4 Ab	1.3±1.1 Aa	0.0±0.1 Aa
6	20.1 ±0.2 Ac	18.3±0.4 Ab	2.6±1.1 Aa	0.5±0.1 Ba

Within each column, means followed by different upper case letters are significantly different; within each row means followed by different lower case letters are significantly different ($P<0.05$)

Tab. **3** shows the results of the microbiological analyses before and after 6 months of hermetic storage. The initial percentages of infected seeds were similar for the different m.c.s. After the storage period, the percentages of infected seeds were similar at 11.5% and 12.5% m.c.s., and remained on average below 40%. The final percentage of infected seeds increased abruptly at 13.5% m.c., where practically all the seeds were infected by molds. The percentage of infected seeds at 14.5% was near 60%, and was lower than at 13.5% m.c. despite the higher e.r.h. However, due to variability in the data, the differences were not significant.), which became limiting for further fungal development. The results of this experiment agree with the findings of various authors who reported reduction of microbial activity in low-oxygen environments (Hocking 1990; Weinberg et al. 2008; Taniwaki et al. 2009; Marcos Valle 2015). The high percentage of infected grains and CFU counts at the end of storage at 13.5% m.c. could be, in effect, a picture of what happened before oxygen became limiting for the microflora. Intermediate measurements of oxygen and microbial indicators are needed to characterize the fungal development more precisely. The low-oxygen atmosphere might have also contributed to limit other intrinsic degenerative reactions in the seed, what remains to be evaluated. Despite final germination was similar at 13.5% and 12.5% m.c., the former is a more risky condition from the microbiological point of view. Any change in the storage conditions could rapidly impact germination.

14.5% m.c. resulted a high-risk condition for hermetic storage at 25ºC (Tab.4). Final germination dropped markedly to 50%. The high e.r.h. (79% on average through the whole storage period) enabled an intense respiration that generated an anaerobic atmosphere by 3 months of storage. Microbial indicators, however, were lower at 14.5% than at 13.5% m.c., probably because oxygen was consumed earlier at 14.5% m.c. and rapidly became limiting for microbial growth. At 14.5% m.c., indeed, the oxygen concentration reached 0%. Some authors report that fungal growth is completely inhibited only below 0.5-1% of oxygen (Taniwaki et al., 2009; Marcos Valle, 2015). The anaerobic atmosphere, nevertheless, was not effective to protect germination. Open-air versus hermetic storage experiments are needed at 13.5 and 14.5% m.c.s. to explore the benefits of storing the seeds in a low-oxygen atmosphere.

Finally, this research was carried out using a single corn hybrid. Since there is evidence that seed storability is affected by genotype (Friday et al., 1989; Marks and Stroshine, 1995), additional research including different hybrids should be conducted before drawing general conclusions.

Tab.4. Summary of safe storage moisture contents for maize seeds under hermetic storage.

	Moisture content (%)			
	11.5	12.5	13.5	14.5
Final germination >90%	Yes	No	No	No
Absence of microbial activity	Yes	Yes	No	No
Safe storage moisture content	Yes	No	No	No

Conclusions

The results of this work show that 11.5% m.c. is a safe m.c. for storing maize seeds hermetically for 6 months at 25ºC, because germination remains above 90% (official standard for maize seed) and the seed microflora is inactive. At 12.5% m.c. the microflora is still inactive but germination falls below the official standard. Hence, seeds should only be stored at 12.5% m.c. if they are not intended for marketing. Moisture contents of 13.5% m.c. and above are not compatible with safe seed storage because of microbiological activity and damage to germination.

At 11.5 and 12.5% m.c.s., the hermetic storage was not useful to generate low-oxygen self-modified atmospheres. In contrast, self-modified atmospheres were observed for m.c.s. of 13.5% and above (e.r.h. higher than 75%). The self-modified atmospheres, however, were not effective to protect germination.

Further research is needed on the potential of low-oxygen modified atmospheres for seed storage. In the future it would be important to study the effect of anaerobic environments on germination and microbial activity from the beginning of storage, by means of controlled atmospheres. It would also be important to expand the temperature range, to include seed aging indicators for a better understanding of the mechanisms of germination loss, and to extend the studies to other maize genotypes.

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Application of $ECO_2FUME^®$ Phosphine Fumigant for the Complete Control of Major Stored Product Insect Pests in Milled Rice in Thailand

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Abstract

$ECO_2FUME^®$ phosphine fumigant was used to fumigate milled rice in a commercial plastic bag (5 kg) and milled rice in a jumbo bag (1,000 kg) under gas-proof sheets to assess its performance against a mixed-age culture of *Sitophilus zeamais, Tribolium castaneum* and *Oryzaephilus surinamensis*. The trials were divided into 2 groups: 1) milled rice in a commercial plastic bag (packed rice) treated with a 50 g/m^3 $ECO_2FUME^®$ application rate (700 ppm phosphine) for 2 days with two bag stacks of 46 m^3 and 55 m^3 and for 3 days with two bag stacks of 50 m^3 each; and 2) milled rice in a jumbo bag (raw material rice) with stack size of 314 m^3 treated with a 35 g/m^3 $ECO_2FUME^®$ application rate (500 ppm phosphine) for 3 days and a stack size of 435 m^3 treated with 50 g/m^3 $ECO_2FUME^®$ application rate for 2 days. Gas sampling lines were installed in the stack to measure the phosphine concentrations during the fumigation period. The results of the fumigation trials showed that mixed-age cultures of the three insect species in packed rice stacks were completely controlled at 2 and 3 days when applied with an $ECO_2FUME^®$ application rate of 50 g/m^3, whereas most insects in untreated control cages remained alive. $ECO_2FUME^®$ was also 100% effective in raw material rice stacks with complete control of mixed-age cultures of the three insect species using 35 g/m^3 of $ECO_2FUME^®$ for 3 days and 50 g/m^3 of $ECO_2FUME^®$ for 2 days. Commercial tarp fumigation of milled rice with $ECO_2FUME^®$ can be fumigated successfully without "top up" with good sealing procedures. Gas monitoring at regular intervals throughout the whole fumigation period is part of best fumigation practice to ensure that the minimum recommended phosphine concentration is maintained for complete control of all stages of target insect pests.

Keywords: $ECO_2FUME^®$ phosphine fumigant, fumigation, stored-product insect pests, milled rice, commercial plastic bag (5 kg), jumbo bag (1,000 kg).

1. Introduction

One of the largest rice-producing countries in the world, Thailand has an estimated 1.06 million hectares of cultivated land. In 2016, Thailand exported 9.88 million tons of rice with a total value of 154,434 million Baht (Office of Agricultural Economics, 2016).

Milled rice is prone to damage due to insect infestation. When the grain has no protection, the insect population will build up rapidly. Therefore, the losses and damage to stored milled rice due to insect

pests are related to the storage duration. The percentage of loss is very difficult to determine as the figure varies from 1% to as high as 25%. In 1977, FAO reported that the loss of rice ranged from 8 to 14% (Champ and Dyte, 1967) within the post-harvest system in Thailand. Suprakarn (1985) reported that 70 species of beetles and moths had been recorded in association with grains and other agricultural products in Thailand. Fortunately, only a handful of these insects have caused major economic damages. The major insect pests adversely affecting stored rice in Thailand are *Sitophilus zeamais*, *Tribolium castaneum*, and *Oryzaephilus surinamensis,* among others. Considered as an individual factor, the number of insect pests is not significant to the health of the harvested rice; however, preventing contamination is imperative as these pests can impact the quality and weight losses of stored rice. If contamination via insects occurs in the exported product, the rice may be rejected and returned to the country of origin, negatively affecting the reputation and revenue of the grain producer and exporter. Future exporting issues can occur as a result.

$ECO_2FUME^®$ fumigant gas is a cylinderized formulation of 2% PH_3 and 98% CO_2 by weight. It is packaged in high pressure aluminum or steel cylinders with net content of 31 kg of PH_3/CO_2 mixture and an equivalent phosphine amount of 620 g (Tumambing et al., 2012). $ECO_2FUME^®$ was first registered and commercialized in Australia during the 1990s. It was then registered in the USA in 2000, and approved for a shorter fumigation time of 24 hours for 1,000 ppm phosphine concentration at 27 °C or higher temperature for susceptible insect species (Cavasin et al., 2001).

There are several advantages when applying $ECO_2FUME^®$. The dose of $ECO_2FUME^®$ phosphine fumigant applied to the commodity is rapid delivery, easy maintenance of the required dose during the fumigation period, shorter exposures and ease of application. $ECO_2FUME^®$ does not require the applicator to enter the fumigation space. The ready-to-use cylinders can be dispensed from outside of silos or structures being fumigated. This eliminates the need for entry into confined spaces to apply fumigants and solid waste disposal (Bonjour, 1998; Phillips, 1998).

The controlled application of fumigant gas resulting in less fumigant was introduced in stored product instead of the traditional solid formulation. It relies on the generation of a high initial phosphine concentration followed by a slow deterioration to ensure that the phosphine concentration - time product (CT) - will result in an effective fumigation. With $ECO_2FUME^®$ fumigant gas, the concentration can be easily controlled by the applicator to maintain an efficacious concentration and can be precisely measured by adding the required amount of gas when needed. Disposal of solid waste products from tablets is becoming more difficult every day. $ECO_2FUME^®$ fumigant gas eliminates the concern associated with deactivating unspent metal phosphide residue and disposal of the waste product (Cavasin et al., 2001).

The objective of this research is to study the application of $ECO_2FUME^®$ phosphine fumigant on milled rice in a packaged plastic bag and as a raw material in a jumbo bag for the control of major stored-product insect pests under commercial fumigation conditions of selected Thai rice companies.

2. Materials and Methods

Test insects and preparation of mixed-age cultures

Strains of *Sitophilus zeamais*, *Tribolium castaneum* and *Oryzaephilus surinamensis* were investigated. All insects used in this study were from the stored-product insect colonies maintained at the Post-harvest and Processing Research and Development Division Lab of the Thai Department of Agriculture (DOA). The mixed-age population was prepared by adding 50 young adults (2-3 week olds) of each strain of each of *S. zeamais*, *T. castaneum* and *O. surinamensis* into the cage of a glass bottle containing 200 g of culture medium and kept for 3 weeks. The culture medium was different for each species: brown rice for *S. zeamais*, rice bran for *T. castaneum* and grinded rice for *O. surinamensis*. Afterwards, all adults were removed and kept in the laboratory for 4 weeks at a temperature of 30 ± 2°C and relative humidity of 65 ± 5% before fumigation. All of the life stages were examined for their presence in the mixed-age culture glass bottle prior to fumigation. Before the

experiment was carried out, the test insects were transferred from glass bottles to the spun-bonded bags.

Fumigant

$ECO_2FUME^®$ phosphine fumigant is manufactured at Solvay's Niagara Falls, Canada, facility (known also by its associated legal entity name, Cytec Canada).

Fumigation of mixed-age culture

The experiment was conducted in a concrete warehouse of Patum Rice Mill and Granary Public Company Limited Group of Companies, located at Sikhiu district, Nakhon Ratchasima province, Thailand, in 2015. The trials were divided into 2 groups, as outlined below.

1Milled rice in a commercial plastic bag of 5 kg capacity (packed rice) was treated with a 50 g/m³ $ECO_2FUME^®$ application rate (700 ppm phosphine) for 2 days with 2 bag stacks of 46 m³ and 55 m³, and for 3 days with 2 bag stacks of 50 m³ each (Figure 1).

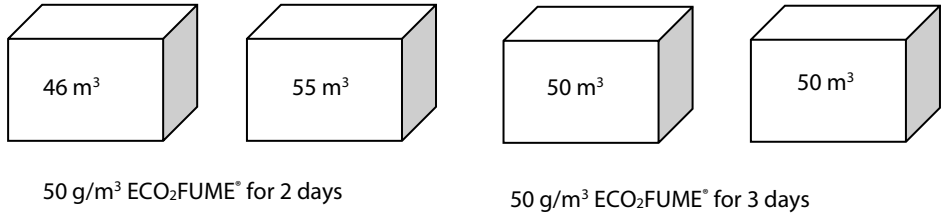


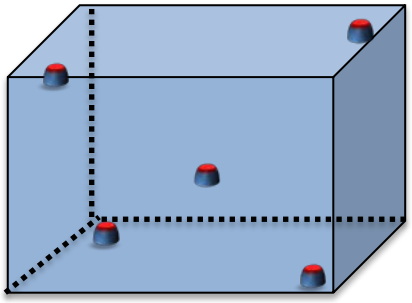
Fig. 1 Bag stack arrangement of milled rice in commercial 5-kg plastic bags

2) Milled rice in a jumbo bag of 1,000 kg capacity (raw material rice) and a stack size of 314 m³ was treated with a 35 g/m³ $ECO_2FUME^®$ application rate (500 ppm phosphine) for 3 days, and a stack size of 435 m³ was treated with 50 g/m³ $ECO_2FUME^®$ application rate (700 ppm phosphine) for 2 days (Figure 2).

Fig. 2 Bag stack arrangement of milled rice in 1,000 kg - jumbo bags

The raw material rice stacks and packed rice stacks were piled on the floor. Afterwards, 15 spun-bonded bags of test insects in a culture medium were placed in the stacks at 5 different locations: top (left and right), middle and bottom (left and right). Each spun-bonded bag of test insects contained a mixed-age culture of each insect species; *S. zeamais, T. castaneum* and *O. surinamensis*.

For the fumigation of both packed rice and raw material rice, the spun-bonded bags of mixed-age test insects in the culture medium were placed inside the bag (Figures 3 and 4).



Test insect locations in the jumbo bag stack

Fig. 3 Placement of test insects inside the jumbo bag stack

Fig. 4. Test insect placement inside the commercial 5-kg plastic bag.

The commercial 5-kg plastic bag of milled rice in the market of Thailand was punched 4 holes with diameter 1.0 mm. On the front and back of the bag, 2 holes were punched for each side. These holes allowed the air transfer which the bag could be stacked on the shelf during the distribution. Additionally, phosphine gas would penetrate into the plastic bag through these holes.

Two holes on the front of the plastic bag

Two holes on the back of the plastic bag

Fig. 5 Position of holes on the plastic bag

Gas sampling lines were installed at 3 locations in the rice stack (top, middle and bottom). Gas sampling lines were also installed inside the packed rice bag and the raw material bag to measure the phosphine concentration during the fumigation period.

Each milled rice stack with test insects was then covered with a clear polyvinyl chloride (PVC) sheet (0.2 mm thickness) as a fumigation enclosure to keep the phosphine gas inside the stack. The sheets were sealed to the concrete floor with sand snakes and masking tape.

The equivalent amount of phosphine from $ECO_2FUME^{®}$ was injected inside the tarp using a stainless steel quick-dispensing hose and gas injector with a gas flow rate of 6.8 kg/min. The exact amount of dispensed $ECO_2FUME^{®}$ was determined by the weight change of the cylinder on the top of a digital weighing scale accurate to 0.01 kg or 10 g.

Monitoring of gas concentration

Phosphine concentration was monitored at each of the following time intervals: 1) 1, 18, 24 and 42 hours for 2-day exposure time; 2) same as item 1 plus 48 and 66 hours for 3-day exposure time. The two phosphine target concentrations of 500 ppm and 700 ppm were maintained during the whole fumigation period. When the phosphine concentration fell below the target concentration, ECO2FUME® dosing was topped up to bring the concentration back to or above the target level. Phosphine concentration was monitored with calibrated SILOCHEK phosphine monitor (0 - 2000 ppm).

Fumigation was terminated at 2 and 3 day exposure times followed by aeration of the slightly opened enclosure until the phosphine concentration reached the threshold limit value (TLV) of 0.3 ppm or lower. The plastic cover sheets were completely removed afterwards.

Assessment of insect mortality

Effectiveness of $ECO_2FUME^®$ against the test insect was determined by mortality of the mixed-age culture. After fumigation, the spun-bonded bags that contained the test insects were retrieved from each stack. The mortality of adults from each experiment was recorded after the removal of the fumigation sheet cover. Dead and alive insects were separated and culture media were returned to the bottles and then kept in the laboratory at 30 ± 2°C and 65 ± 5% for 6 weeks and were observed weekly for any newly emerged adults. This period was sufficient for emergence of all insects in the treatment as well as the control. The occasional death from the control was corrected by Abbott's formula (Abbott, 1925).

Monitoring of temperature, relative humidity and moisture content

The temperature and relative humidity in the warehouse were monitored by a thermo-recorder every day during the fumigation period. The moisture contents of maize were measured before and after treatment by applying the samples to an SB 900 Steinlite moisture meter.

3. Results

The effectiveness of $ECO_2FUME^®$

The results of the fumigation on packed rice stacks shown in Table 1 demonstrated that mixed-age *Sitophilus zeamais, Tribolium castaneum* and *Oryzaephilus surinamensis* were completely controlled at 2 and 3 days when applied with an $ECO_2FUME^®$ application rate of 50 g/m^3 (700 ppm phosphine). No live insects were observed immediately after fumigation or 6 weeks later. Most insects in non-fumigated control cages remained alive. $ECO_2FUME^®$ was also 100% effective in raw material rice stacks, completely controlling the mixed-age cultures of the three insect species with concentrations of 35 g/m^3 (500 ppm phosphine) for 3 days and 50 g/m^3 (700 ppm phosphine) for 2 days (Table 2).

The concentration of phosphine

Fumigation on packed rice in stacks at 50 g/m^3 $ECO_2FUME^®$ (700 ppm) phosphine concentration was achieved at 2 and 3 days of exposure period times, as shown in Table 3. One hour after gas injection, the phosphine concentrations in the rice stacks were higher than 2,000 ppm, yet phosphine gas did not penetrate the plastic bag of packed rice. After fumigation for 18 hours, the phosphine concentration decreased but was still higher than the target concentration of 700 ppm in all rice stack fumigations. Phosphine concentration was monitored throughout the fumigation period. There were small reductions in phosphine concentrations, but all were still over the target concentration. The phosphine concentrations inside the plastic bag were nearly equal to the phosphine concentrations inside the fumigated stacks. This indicated that phosphine penetrated well into the plastic bag.

Phosphine concentrations on raw material rice fumigated with 35 g/m^3 $ECO_2FUME^®$ for 3 days and 50 g/m^3 $ECO_2FUME^®$ for 2 days from the rice stacks were shown in Table 3. The phosphine concentration of the fumigated raw material rice was quite similar to the fumigated packed rice stacks. After fumigation for an hour, the phosphine concentrations in raw material rice stacks were higher than 2,000 ppm. However, phosphine concentration could not be found inside the jumbo bag, indicating that there was no gas penetration yet.

After fumigation for 18 hours, the concentrations of phosphine decreased but were still well above the target concentration in the rice stacks. All phosphine concentrations were above the target concentrations of 500 and 700 ppm throughout the fumigation period. These results show that phosphine concentrations inside the jumbo bags were close to the concentrations of the fumigated stacks, indicating that phosphine could also penetrate well into the jumbo bag.

Monitoring of temperature, relative humidity and moisture content

The moisture content of maize was 11.8-13.5%. Temperature and relative humidity in the warehouse were 29.0-30.3ºC and 66.9-68.2%, respectively.

4. Discussion

The commercial fumigation trials of milled rice in 5 kg plastic bags and 1,000 kg jumbo bags using $ECO_2FUME^®$ showed complete control of all stages of the target major grain insects at selected rice mills in Thailand. Results were achieved using phosphine fumigation protocols of 35 g/m^3 $ECO_2FUME^®$ (500 ppm phosphine) for 3 days, and 50 g/m^3 $ECO_2FUME^®$ (700 ppm) for 2 days at a daily temperature fluctuation of 26 - 33°C.

The results showed that the amount of phosphine gas absorbed by milled rice is minimal. The good sealing of the stack also minimized the gas loss due to gas leaks, and the higher phosphine concentration achieved at the start of fumigation remained higher than the target phosphine concentration throughout the entire fumigation period. In this study, top up of $ECO_2FUME^®$ was unnecessary to maintain the target concentration. Hence, the commercial fumigation of milled rice during storage of both raw material rice and packed rice with $ECO_2FUME^®$ before distribution could be completed successfully without top up.

Despite the fact that the target phosphine concentration was maintained throughout the duration of the fumigation period, it is still necessary to monitor phosphine concentrations at regular intervals. If the phosphine concentration is not regularly monitored during the entire exposure period, there could be a risk of greater gas loss due to gas leak and gas sorption, which can render the fumigation ineffective.

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I wish to express my sincere gratitude and appreciation first to Patum Rice Mill and Granary Public Company Limited Group of Companies and Ms. Jurairat Wongnam, IPM AGRO CO., Ltd. for supporting staff, place and commodities for the experiment. Finally, I would like to express my appreciation and gratitude to my colleagues for the invaluable support in the completion of my experiment.

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Tab. 1 Mortality of 3 insect species after fumigation on packed rice stacks with $ECO_2FUME^®$ fumigate at different exposure times.

Dosages (g/m^3)	Time (Days)	Stack size (m^3)	Location	%Mortality of insect species					
				Sitophilus zeamais		Tribolium castaneum		Oryzaephilus surinamensis	
				Immediately after fumigation	6 weeks after fumigation	Immediately after fumigation	6 weeks after fumigation	Immediately after fumigation	6 weeks after fumigation
50 g/m^3 (700 ppm)	2	55	Top-left	100	100	100	100	100	100
			Top-right	100	100	100	100	100	100
			Middle	100	100	100	100	100	100
			Bottom-left	100	100	100	100	100	100
			Bottom-right	100	100	100	100	100	100
		46	Top-left	100	100	100	100	100	100
			Top-right	100	100	100	100	100	100
			Middle	100	100	100	100	100	100
			Bottom-left	100	100	100	100	100	100
			Bottom-right	100	100	100	100	100	100
	3	50	Top-left	100	100	100	100	100	100
			Top-right	100	100	100	100	100	100
			Middle	100	100	100	100	100	100
			Bottom-left	100	100	100	100	100	100
			Bottom-right	100	100	100	100	100	100
		50	Top-left	100	100	100	100	100	100
			Top-right	100	100	100	100	100	100
			Middle	100	100	100	100	100	100
			Bottom-left	100	100	100	100	100	100
			Bottom-right	100	100	100	100	100	100
Control (Unfumigated)	3			0[1/]	0	0	0	0	0

[1/] Mean of 3 replications

Tab. 2 Mortality of 3 insect species after fumigation on raw material rice stacks with $ECO_2FUME^®$ fumigate at different dosages and exposure times.

Dosages (g/m^3)	Time (Days)	Stack size (m^3)	Location	%Mortality of insect species					
				Sitophilus zeamais		Tribolium castaneum		Oryzaephilus surinamensis	
				Immediately after fumigation	6 weeks after fumigation	Immediately after fumigation	6 weeks after fumigation	Immediately after fumigation	6 weeks after fumigation
35 g/m^3 (500 ppm)	3	314	Top-left	100	100	100	100	100	100
			Top-right	100	100	100	100	100	100
			Middle	100	100	100	100	100	100
			Bottom-left	100	100	100	100	100	100
			Bottom-right	100	100	100	100	100	100

50 g/m³ (700 ppm)	2	435	Top-left	100	100	100	100	100	100	
			Top-right	100	100	100	100	100	100	
			Middle	100	100	100	100	100	100	
			Bottom-left	100	100	100	100	100	100	
			Bottom-right	100	100	100	100	100	100	
Control (Unfumigated)	3			0[1/]	0	0	0	0	0	

[1/] Mean of 3 replications

Tab. 3 Phosphine concentrations inside the stacks during milled rice fumigations with $ECO_2FUME^®$ fumigation at different dosages and exposure times.

Application	Dose	Time	Stack	Volume of	Location	Phosphine concentration (ppm) Hours					
						1	18	24	42	48	66
5-kg in plastic bag	50 g/m³ (700 ppm)	2	55	2.73	Top	>2000	1,325	1,289	1,213	1,195	
					Middle	>2000	1,293	1,295	1,225	1,197	
					Bottom	>2000	1,284	1,297	1,255	1,199	
			46	2.32	Top	>2000	853	860	829	819	
					Middle	>2000	864	849	837	826	
					Bottom	>2000	867	855	845	830	
					Inside plastic bag	-	805	875	812	811	
			50	2.48	Top	>2000	1,365	1,369	1,283	1,144	1,060
					Middle	>2000	1,360	1,361	1,281	1,147	1,065
					Bottom	>2000	1,359	1,380	1,279	1,148	1,069
		3	50	2.50	Top	>2000	1,145	1,125	1,070	1,069	1,075
					Middle	>2000	1,163	1,112	1,085	1,065	1,078
					Bottom	>2000	1,173	1,120	1,095	1,075	1,085
					Inside plastic bag	-	999	1,001	1,104	1,101	1,127
1,000-kg jumbo bag	35	3	314	11.22	Top	>2000	1,239	1,225	1,230	1,171	910
					Middle	>2000	1,227	1,231	1,242	1,189	926
					Bottom	>2000	1,244	1,237	1,259	1,236	940
	50	2	435	21.77	Top	>2000	1,492	1,494	1,150	986	
					Middle	>2000	1,505	1,510	1,146	1,087	
					Bottom	>2000	1,512	1,514	1,192	1,098	
					Inside jumbo bag	-	1,490	1,534	1,483	1,493	

Residual behaviour of phosphine in different commodities

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Abstract

Phosphine is one of the most common active substances used in storage protection worldwide. As it is very efficient amongst a broad range of living organisms, it has become the favoured product after phasing out methyl bromide in 2010, as it can be used in many commodities.

In 2005, the regulation 396/2005 was enacted and came into force in 2008. With this, the European commission started to evaluate residues arising from the use of a pesticide and to set maximum residue levels (MRLs) for safe and regulated food trade.

To proof residue levels are below MRL and therefore far below concerning concentrations of phosphine in food or feed, residue studies are permanently conducted. In addition to support MRL settings, the intention of these trials is to determine withholding periods needed in storage protection, corresponding to PHI (pre harvest interval) for field and glasshouse treatments.

Results of those studies show different levels and differences in decrease of residues after defined time periods. Thus, withholding periods for various commodities can differ. Residue trials with repeated exposure were conducted as well to determine possible additive effects.

Keywords: Phosphine, maximum residue level (MRL), withholding period

Regulatory background

To ensure safe and fair trading conditions among the EU member states and non-European countries, regulation 396/2005 came into force on 1st of September 2008. The major aim of this regulation was to set maximum residue levels (MRL) for pesticide residues in and on cereals, foodstuff of animal origin, and products of plant origin in one regulation only. This became necessary due to formerly separate regulations, inhibiting harmonization and complicating registration processes during plant protection authorization. Besides, the setting aims to avoid unacceptable risks posed by residues in treated commodities to humans and in animals feeding. MRLs are being reviewed and updated on a regular basis.

To evaluate a potential risk arising from treating goods with plant protection products (but also biocides), manufacturer of active contents and products composing those actives destined for plant protection uses are obliged to characterize the residual behavior of the product inside each targeted commodity. One important basis to set an MRL for a commodity or a group of commodities is provided by residue trials.

Behaviour of phosphine

There are many forms of phosphine (PH_3) emitting products, such as compacts, bags and plates. The reaction is based on the very simple reaction of the metal phosphide with humidity and air.

During fumigation with metal phosphide based formulations, the small molecule phosphine is being formed as a reactive intermediate. As it has many advantageous properties, the possibilities of use are various. One of these is the excellent capability of the gas to penetrate inside the commodity, facilitating all stages of stored product pests to be effectively eliminated.

As the commodities vary highly in nutrient composition, structure, and size, the phosphine molecules show very different binding behaviours and elimination times inside the goods.

It has become clear that these characteristics are essential during evaluating MRLs and play a crucial role in setting withholding periods after fumigation.

Materials and Methods

To represent a possible worst-case scenarios, different storage goods from commodity groups defined as in COMMISSION REGULATION (EU) 2016/1785 (2016) are chosen to be fumigated with different metal phosphide formulations containing magnesium or aluminium phosphide. All trials are conducted following GLP principles set be OECD.

In the present study, 20 kg of different storage goods were placed in 4 different airtight containers. After exposing the test items to 5 g PH_3/m^3 (Degesch Magtoxin) and 5.5 g PH_3/m^3 (Degesch Plate) for 5 days, and to 10 g PH_3/m^3 (Phostoxin Tablet and Detia Gas-Ex-B) for 14 days, samples were taken in intervals to determine residues. The longest withholding period was 28 day after aeration. To ensure negative control levels, residue analysis were conducted prior to fumigation.

The dosage and exposure time represented the highest registered doses and exposure times in the EU.

Commodities tested were:

cereals

tobacco

flour (wheat)

coffee beans

cocao beans

dried fruit (apricot)

dried vegetable (leak)

legumes (lentils)

expeller (oilseed rape press cake)

oilseed (flaxseed)

hay

herbs (dried, marjoram)

malt (dried)

nuts (hazelnut)

pistachios

coriander

medical plants (blossoms, chamomille)

liquorice

spices (leaf, laurel leaves)

black tea

potato starch

The treated commodities were sampled and stored deep frozen until analysis, which was conducted within 24 hours after sampling.

Results

No residues were detected in any of the control samples prior to fumigation.

For all tested commodities, the residue analysis directly after the end of aeration showed values below 0.05 mg/kg, except for hazelnuts and pistachios. For the two products, Degesch Plate and Detia Gas-Ex-B, residues after right aeration were increased in hazelnuts and pistachios. For Degesch Plate treatments, the residues in hazelnuts and pistachios were below 0.05 mg/kg after 14 days, while for Detia Gas-Ex-B, pistachios achieved values below 0.05 mg/kg after 7 days and hazelnuts after 21 days.

Discussion

The trials show a different behavior of storage goods regarding elimination of phosphine. Especially, high fat containing representatives of the group of tree nuts show a slower removal of the gas. Therefore, the withholding periods after aeration are increased.

As analytical methods have become significantly more accurate, residue trials were and are necessary to determine MRLs. Withholding periods are therefore a necessary and important indication for food and feed industry to be regarded on behalf of safe and fair trading or processing of the goods after fumigation.

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Phosphine Resistance Status in Lesser Grain Borer *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae) Strains Originating from the Tropical Countries

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Abstract

Stored product beetles that are resistant to the fumigant phosphine (hydrogen phosphide) have been reported for more than 50 years in many places worldwide. The high levels of phosphine resistance in lesser grain borer *Rhyzopertha dominica* (F.) have been noted from several countries including Bangladesh. This study was designed to evaluate the status of resistance to phosphine in Bangladeshi *R. dominica* and to verify the possible comparison among other phosphine resistant strains from tropical countries *viz.* Burkina Faso and Malaysia. The data reported and summarized here showed varied levels of resistance compared to the laboratory phosphine susceptible strain (*RDLAB*). *Rhyzopertha dominica* strains originating from Bangladesh (*RDBGD*) and Burkina Faso (*RDBKF*) exhibited higher levels of resistance to phosphine compared to the Malaysian strains (*RDMAL*). Analysis of dose–response data indicated that the *RDBGD* and *RDBKF* strains were the most resistant to phosphine under different exposure periods. At LC_{50}, these two strains were more than 80-fold more resistant at all exposures compared to the susceptible strain. Results also revealed that *RDBGD* and *RDBKF* strains required a relatively high concentration of 334.94 and 240.081 mg L^{-1} for 99% mortality. The mean survival time (MST) for the phosphine resistant and susceptible also varied significantly. The maximum MST was recorded for *RDBGD* and *RDBKF* strains. The present findings further confirmed that the Bangladeshi originated *R. dominica* strain contained higher resistance to phosphine compared to strains from other countries. This study could be useful in developing management strategies to prevent stored grain from being infested by resistant strains of *R. dominica* in tropical countries.

Keywords: phosphine, resistance, grain borer, tropical countries

Introduction

Tropical environments provide most favourable conditions for insect growth and multiplication in the ideal medium of food stored by humans. Some insects begin infestation in the field and continue attack in storage. In tropical developing countries, especially in humid zones, pest infestation in stored food is inevitable due to lack of proper management. Fumigation with phosphine (hydrogen phosphide, PH3) is the preferred means of controlling pests in many countries of the tropics.

The first global survey of phosphine resistance was conducted during the 1970s by Champ and Dyte (1976) who used a discriminating dose bioassay on adult insects (FAO 1975) and documented the occurrence of phosphine resistance in several key stored grain pest species across many countries. However, further studies indicate a substantial increase in phosphine resistance in stored product pests worldwide including Australia (Nayak et al. 2013), India (Kaur et al. 2015), Brazil (Lorini et al. 2007), and Malaysia (Rahim et al. 2004).

Phosphine resistance has been detected in most of the major species of stored product insects including *Rhyzopertha dominica*, *Tribolium spp., Sitophilus spp.*, *Oryzaephilus surinamensis* and *Cryptolestes* spp. (Taylor, 1986). This appears to be due to the increased selection pressure in some countries (Halliday *et al,* 1983; Friendship *et al.,* 1986). The distribution of phosphine resistance is not known in detail but it is likely that resistance to phosphine is not an unusual phenomenon in most countries in which the fumigant has been used. The reported incidence of phosphine resistance is particularly high in the lesser grain borer *R.dominica* (F.) and two species of *Tribolium* (Champ and Dyte, 1976; Taylor, 1986). It has also been noticed that some of the strains of *R. dominica* collected from the field showed very high levels of resistance to phosphine (Mills, 1983). However,

fumigation failures have been reported from Bangladesh that were caused, in part, by high levels of phosphine resistance in *R.dominica* (Mills, 1983, Tylor *et al.* 1983). Potential replacement fumigants lack the versatility possessed by phosphine. The indiscriminate uses of phosphine fumigation may lead to the increase of risk in control failures. The aim of the present investigation was to examine the relative phosphine toxicity relationship between concentration and time exposure to phosphine resistant and susceptible strains of *R.dominica* originating from the tropical countries of Bangladesh, Malaysia and Burkina Faso.

Materials and Methods

Insects

Phosphine susceptible laboratory strain RDLAB and phosphine resistant strains RDBGD, RDBCF and RDMAL of the lesser grain borer *Rhyzopertha dominica*, originated from a population sampled in Bangladesh, Burkina Faso and Malaysia respectively, were used in this study. The frequency of phosphine-resistant adults in samples from *R. dominica* field populations was determined by earlier using the Food and Agriculture Organization Method No. 16 (FAO, 1975) (Reichmuth, 1983; Mills 1983; Rahim and Sulaiman, 1999). All these strains had been cultured for many decades at the Federal Research Centre for Cultivated Plants – Julius Kühn-Institut, Berlin, Germany (Table 1). They were reared on whole wheat and dried cassava in a controlled temperature room set at 25°C, 16L: 8D h photoperiod and 75% RH.

Fumigation Procedure

Production of phosphine

Pure (100%) phosphine was generated from magnesium phosphide granules (*Degesch Co*., Frankfurt am Main) reacting to water ($Mg_3P_2 + 6H_2O = 3Mg(OH)_2 + 2PH_3$) (Hasan and Reichmuth, 2004). The method provides a convenient source of phosphine for dosing purposes over a period of time, depending on the rate of removal.

Table 1: Culturing details and specification of reference and phosphine-resistant strains of *R.dominica*.

Strains	Origin of strains	Collection & Test year	# adults to seed culture	Adult wt. (mg) Mean ± SE*	Original Culture maintained
RDLAB (S)	Germany	1978	150	1.45 ± 0.02	BBA Inst., Berlin
RDBGD(R)	Bangladesh	1982	150	1.42 ± 0.02	CSL,UK
RDBCF (R)	Burkina Faso	1993	150	1.25 ± 0.02	BBA Inst., Berlin
RDMAL (R)	Malaysia	1993	150	1.31 ± 0.01	BBA Inst., Berlin

S- Susceptible; R- Resistant; * mean of four replicates each having 50 insects.

Fumigation Test

Newly emerged (8-10 day-old) mixed-sex adults of *R. dominica* were selected for phosphine fumigation. The fumigation was carried out in cylindrical steel gauze cages (5.0 x 1.5 cm) containing a small quantity of diet (0.5 g). Treatments of phosphine consisted of three replicates containing 25 insects each. An untreated reference sample was kept for each type.

Glass Dressel flasks (2.5 l) were used as fumigation chambers. The flasks were connected to each other with a gas-tight PVC tube. A small electric pump was set up to recirculate the gas evenly throughout the apparatus. A silicon rubber septum fitted in the narrow tube, protruding from the gas reservoir, was used to inject and withdraw a phosphine sample with a gas-tight Hamilton syringe. An equal volume of air was withdrawn from the flask before injecting the respective concentration of phosphine. The sealed Dressel flasks contained saturated sodium chloride solution to achieve about 75% RH by the end of exposure period. The time of injection was recorded and the pump was operated to distribute the gas evenly. The concentration of phosphine in each of the flasks was assessed at the beginning and end of the fumigation period using quantitative gas chromatography (Hasan and Reichmuth, 2004). The glass flasks containing the experimental insects

were then disconnected without losing any gas and immediately transferred to the CT-room conditioned at 25°C, where they were kept throughout the exposure periods.

Post-fumigation

The adults *R. dominica* were fumigated at 25°C at a range concentrations of phosphine, 0 (control), 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mg/l and exposure periods of 4.0, 5.0, 6.0 7.0 and 8h. The Exposure periods for each test was designed to encompass the dose-response range ensuring, where possible, that a 100% kill was achieved at the maximum dose. At the end of desired fumigation periods, the insect cages were removed from the fumigation chambers and kept in small petri dishes containing food media for assessment of mortality. The mortality of adult *R. dominica* was recorded each day after exposure to phosphine until complete mortality occurred.

Data processing

The LCs and LTs were estimated from a Probit regression analysis using PROC PROBIT from SAS version 9 (SAS 2002). Comparison among populations for differences in their level of resistance were made after computing resistant ratios based on the LC_{50} value for the population of interest divided by the LC_{50} of the laboratory-susceptible strain, referred to as the RR_{50}.

Mean Survival Times (MST): The mean survival time (MST) for adult *R. dominica* strains exposed to phosphine was calculated following the model developed by Cheng and Ducoff (1989):

$MST = 1/n \Sigma(t \times Yt)$

Where, n = number of beetles in the group, t = hour t, and Yt = number of beetles that died at hour t.

Results

The effect of phosphine on the mortality of resistant adult *R. dominica* originating from tropical countries varied significantly ($P < 0.001$). Results of dose–mortality studies with three phosphine resistant populations of *R. dominica* and susceptible laboratory strain are reported in Tables 2 and 3. The results indicated clearly that the adults *R. dominica* originated from Burkino Faso and Bangladesh required higher concentration of phosphine at the mortality level of LC_{50} when compared with resistant RDMAL strain from Malaysia. The strain RDBCF was 152-fold more resistant than the susceptible strain RDLAB when exposed for 5 h to phosphine (Fig. 5). However, the strains RDBCF and RDBGD showed more or less similar resistance as the RDLAB strain when exposed to 6, 7 and 8 h exposures at LC_{50} level (Table 2 & Fig. 5). The RR_{50} for the RDMAL strain gradually decreased as the exposure levels increased (Fig. 5). This pattern of relative resistance was also reflected in the LC_{99} values (Table 2). At the LT_{50}, adult *R. dominica* exhibited several fold resistance to phosphine compared to the susceptible strain particularly at lower exposure levels (Table 2). The strains RDBGD and RDBCF showed more resistance to phosphine compared to RDMAL strain at the exposure up to 2h exposure at LT_{50} level, but they exhibited more or less similar trends of resistance at higher exposures levels of 6 to 8 h (Table 2). However, this trend did not follow at the mortality level of LT_{99} at which the RDBCF and RDBGD strains showed higher resistance to phosphine compared to strain RDMAL. Table 3 shows, the higher level of resistance in RDBCF and RDBGD at lower concentration of 0.25 and 0.50 mg/l. Moreover, the strain RDBGD required 193.32 h for 99% mortality at concentration 3 mg/l. The present findings show that the estimated fiducial limits for LT_{50} and LT_{99} values were narrow and overlapping for all the phosphine resistant strains tested, indicating a good fit of the data in the linear regression model (Table3).

The mean survival time (MST) for the phosphine resistant and susceptible strains of *R. dominica* varied significantly ($P < 0.001$). The $F_{(df\ 7,39;\ F_{crit\ 2.36})}$ values for phosphine concentration for the strains RDBGD, RDBCF, RDMAL and RDLAB were 43.72, 40.91, 10.17 and 5.12 respectively. The maximum MST was recorded for *RDBGD* and *RDBKF* strains in all concentrations (Figs. 1 & 2) while the minimum was in susceptible strains RDLAB (Fig. 4). Figure 4 shows that the MST for RDLAB strains was below

to the range of 3 day in all concentrations and exposures except 0.50 mg/l at 4 h exposure. The strains RDBCF and RDBGD exhibited higher fold of resistance in terms lethal time at lower phosphine concentrations ranging from 0.25 to 2.0 mg/l compared to strain RDMAL (Fig. 6). However, the RDMAL showed higher fold of resistance compared to RDBGD strain at concentration ranging from 4.0 to 6.0 mg/l.

Table 2: Probit analysis for the mortality responses of phosphine susceptible and resistant adult *R. dominica* after different exposure periods to varying concentrations of phosphine at 25°C.

Expo -sure (hr)	Strains	*n*	LC_{50} mg/l (95% FL*)	LC_{99} mg/l (95% FL*)	Slope ± SE	Intercept±S E	χ^2 values (df) P
4	RDLAB	600	0.03 (0.01-0.10)	0.04 (0.01-0.10)	0.15±0.05	-0.45 ± 1.02	62.28 (22) P<.0.001
	RDBGD	600	3.54 (2.47-5.89)	452.23 (107.18 - 894.00)	0.28±0.04	0.02 ± 0.43	49.47 (22) P< 0.001
	RDBCF	600	5.97 (4.32-9.62)	1704.00 (411.32-2453.00)	0.38 ±0.04	-0.29 ± 0.38	23.14 (22) P< 0.393
	RDMAL	600	3.25 (1.67-12.85)	1837.00 (120.08-22463.0)	0.16 ±0.05	0.90 ± 0.61	105.70(22) P<0.001
5	RDLAB	578	0.03 (0.01-0.09)	0.03 (0.01-0.09)	0.13 ±0.05	-0.43 ± 1.11	25.22 (22) P> 0.286
	RDBGD	259	2.57 (1.75- 4.01)	90.02 (29.89-109.50)	0.19±0.04	0.57 ± 0.46	98.16 (22) P <0.001
	RDBCF	237	4.09 (2.62- 8.62)	3754.00 (400.59-4609.00)	0.29 ±0.05	-0.15 ± 0.54	38.35 (22) P<0.017
	RDMAL	375	1.34 (0.68- 2.24)	601.81 (88.28-1178.23)	0.22±0.04	-0.19 ± 0.59	67.60 (22) P <0.001
6	RDLAB	568	0.02 (0.01-0.07)	0.03 (0.01-0.08)	0.13±0.05	-0.39 ± 1.11	35.37 (22) P<0.035
	RDBGD	299	1.82 (1.23 - 2.65)	69.97 (25.98- 524.45)	0.22±0.02	-0.07 ± 0.35	86.53 (22) P <0.001
	RDBCF	307	1.67 (1.18- 2.31)	312.29 (91.52- 430.13)	0.25±0.03	-0.46 ± 0.51	35.74 (22) P< 0.032
	RDMAL	381	0.75 (0.42-1.09)	143.49 (44.65- 284.24)	0.23± 0.04	-0.87 ± 0.57	42.97 (22) P<0.002
7	RDLAB	593	0.02 (0.001-0.08)	0.02 (0.01-0.07)	0.12±0.05	-0.11 ± 1.16	21.60 (22) P> 0.484
	RDBGD	312	1.65 (1.08-2.42)	93.97 (31.66- 910.81)	0.20±0.04	0.08 ± 0.49	77.41 (22) P<0.001
	RDBCF	318	1.48 (0.97- 2.13)	308.83 (79.77- 474.68)	0.24±0.04	-0.45± 0.53	44.69 (22) P <0.003
	RDMAL	408	0.61 (0.33- 0.90)	65.96 (25.10- 412.12)	0.21±0.04	-0.79 ± 0.61	48.00 (22) P<0.001
8	RDLAB	599	0.01 (0.002-0.06)	0.02 (0.01 – 0.08)	0.11±0.05	-0.10 ± 1.16	11.02 (22) P> 0.974
	RDBGD	338	1.17 (0.71- 1.71)	334.94 (79.68 – 682.00)	0.24±0.04	-0.54± 0.58	45.27 (22) P< 0.002
	RDBCF	334	1.25 (0.85-1.72)	240.08 (73.91- 421.03)	0.25±0.04	-0.61± 0.53	35.98 (22) P< 0.031
	RDMAL	437	0.32 (0.10 - 0.57)	133.11 (35.31- 262.26)	0.20±0.04	-0.81±0.74	44.58 (22) P<0.003

*FL-fiducial limits

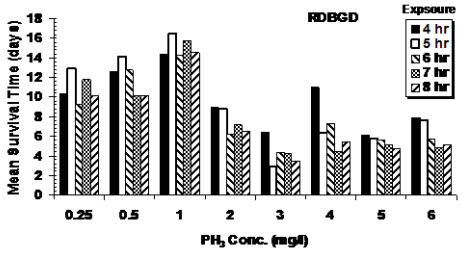

Fig 1: Mean survival time of phosphine resistant Bangladeshi strain of adult *R. dominica* fumigated at different concentration of phosphine and exposure periods.

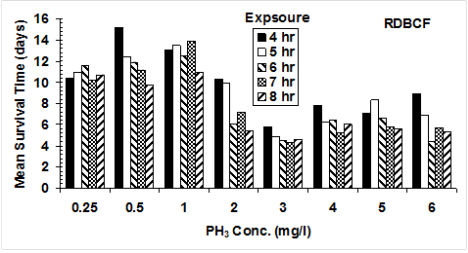

Fig 2: Mean survival time of phosphine resistant Burkina Faso strain of adult *R. dominica* fumigated at different concentration of phosphine and exposure periods.

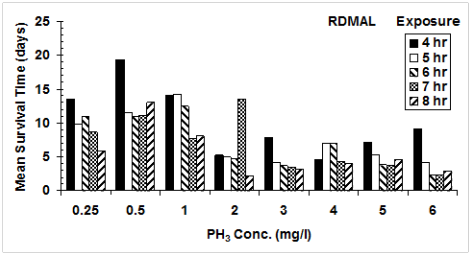

Fig 3: Mean survival time of phosphine resistant Malaysian strain of adult *R. dominica* fumigated at different concentration of phosphine and exposure periods.

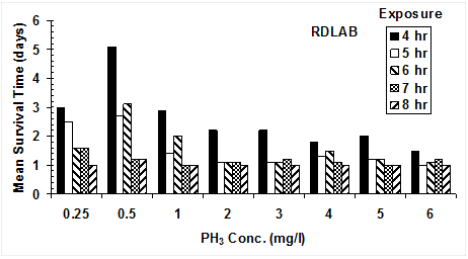

Fig 4: Mean survival time of phosphine susceptible laboratory strain of adult *R. dominica* fumigated at different concentration of phosphine and exposure periods.

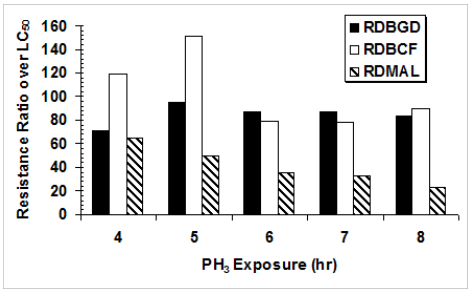

Fig 5: Resistance ratio in different strains of adult *R. dominica* over 50% lethal concentration fumigated at different exposures of phosphine.

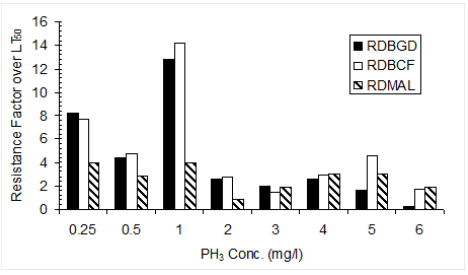

Fig 6: Resistance ratio in different strains of adult *R. dominica* over 50% lethal time fumigated at different concentrations of phosphine.

Table 3: Probit analysis for the mortality responses of phosphine susceptible and resistant adult *R. dominica* after five days exposures to phosphine at different concentrations at 25°C.

PH_3 Conc mg/l	Strains	*n*	LT_{50} (hr) (95% FL)	LT_{99} (hr) (95% FL)	Slope ± SE	Intercept± SE	χ^2 values (df) P
	RDLAB	357	1.89 (0.36- 2.80)	8.16 (6.59- 18.21)	0.27 ±0.03	0.35±0.69	13.79 (13) P< 0.388
	RDBGD	76	15.59 (9.43-37.70)	230.19(37.44-317.02)	0.59 ±0.14	2.44±0.75	21.37 (13) P<0.065
0.25	RDBCF	92	14.56 (9.66-65.84)	303.13(195.82-491.01)	0.78± 0.10	1.03±0.60	7.19 (13) P<0.891
	RDMAL	138	7.55 (6.55- 10.81)	40.76 (20.27- 77.47)	0.45±0.06	1.57±0.58	20.37 (13) P<0.086
	RDLAB	326	3.47 (2.29- 4.05)	8.52 (6.99-14.70)	0.28±0.03	-0.12±0.52	28.24 (13) P< 0.008
	RDBGD	375	15.29(10.27-118.21)	200.248(47.94-416.29)	0.74±0.15	1.90±0.73	12.59 (13) P< 0.479
0.50	RDBCF	84	16.43(10.26-72.61)	374.15(59.41- 543.16)	0.93± 0.09	0.64±0.48	4.05 (13) P< 0.990
	RDMAL	115	9.88 (7.98- 20.04)	104.58 (36.65-289.61)	0.62± 0.08	1.05±0.57	8.87 (13) P< 0.782
	RDLAB	359	1.64 (0.093- 2.68)	8.24 (6.52- 27.65)	0.26±0.03	0.31±0.71	14.30 (13) P<0.352
	RDBGD	375	21.10(17.26-63.19)	344.55(116.92-517.23)	0.59±0.19	3.13 ±0.77	24.10 (13) P<0.03
1.0	RDBCF	95	23.31(14.9-39.14)	125.10(68.15-201.73)	0.84±0.11	0.56±0.63	4.97 (13) P<0.975
	RDMAL	165	6.53 (5.96- 7.41)	33.69 (20.68- 99.42)	0.43±0.05	1.07±0.49	14.17 (13) P<0.36
	RDLAB	344	2.09 (0.98- 5.21)	8.61 (4.61-13.28)	0.87±0.02	0.09±0.04	23.91 (13) P<0.020
	RDBGD	375	5.47 (4.79- 6.07)	34.96 (20.35-131.67)	0.39±0.04	0.69±0.55	18.53 (13) P< 0.138
2.0	RDBCF	188	5.83 (5.43 - 6.27)	19.56 (14.82- 32.17)	0.36±0.04	1.26±0.49	16.87 (13) P< 0.205
	RDMAL	301	1.65 (0.03-2.99)	49.41 (17.77-120.87)	0.31±0.03	-0.10±0.63	11.65 (13) P< 0.559
	RDLAB	362	2.37 (0.14 - 3.31)	6.40 (5.30-22.65)	0.26±0.03	-0.29±0.70	22.55 (13) P< 0.047
	RDBGD	302	4.73 (2.29-8.98)	193.32(95.17-319.16)	0.28±0.04	0.32±0.80	31.82 (13) P< 0.002
3.0	RDBCF	264	3.32 (1.60- 4.15)	35.93 (18.36- 83.97)	0.34±0.03	0.01±0.54	12.75 (13) P< 0.466
	RDMAL	270	4.38 (3.46-4.94)	11.756 (9.14- 22.22)	0.27±0.03	0.86±0.53	33.23 (13) P< 0.002
	RDLAB	357	1.49 (0.04- 2.59)	9.17 (6.96- 49.90)	-0.33±0.70	0.27±0.03	14.58 (13) P< 0.331
	RDBGD	253	3.84 (0.04-4.98)	30.32 (13.39–79.12)	0.76±0.69	0.30±0.04	39.02 (13) P<0.001
4.0	RDBCF	237	4.34 (3.29-4.94)	30.87 (18.10-126.35)	-0.05±0.36	0.38±0.02	5.27 (13) P<0.968
	RDMAL	246	4.55 (3.97-4.97)	17.07 (12.97-28.78)	0.43±0.42	0.33±0.03	13.67 (13) P<0.397
	RDLAB	364	2.26 (0.67- 3.04)	6.21 (5.36-10.07)	-0.34±0.70	0.26±0.03	6.13 (13) P<0.941
	RDBGD	234	1.89 (0.15 – 4.26)	72.36 (31.65–97.25)	0.22±0.78	0.37±0.05	12.51 (13) P< 0.485
5.0	RDBCF	196	5.45 (4.29- 6.39)	81.25 (29.73-150.17)	0.36±0.54	0.42±0.04	11.93 (13) P< 0.533
	RDMAL	261	3.55 (0.27- 4.64)	31.07 (14.08- 102.51)	0.39±0.60	0.32±0.03	28.54 (13) P<0.007
	RDLAB	371	1.194 (0.21 – 2.43)	5.32 (3.92 - 7.89)	0.25±0.03	0.22±0.78	13.67 (13) P< 0.39
	RDBGD	247	4.50 (2.17 -6.83)	51.97 (30.51–74.98)	0.32±0.05	0.56±0.75	27.57 (13) P< 0.010
6.0	RDBCF	239	3.98 (2.39-4.75)	45.97 (21.57-70.88)	0.37±0.03	0.02±0.48	9.44 (13) P<0.738
	RDMAL	276	4.29 (3.17- 4.92)	11.42 (8.76-24.44)	0.86±0.55	0.27±0.03	42.67 (13) P<0.001

Discussion

Our results revealed that the response of the adults of phosphine resistant and susceptible strains of *R. dominica* to phosphine fumigation varied significantly (P<0.001). All the phosphine resistant strains exhibited high levels of resistance compared to the susceptible strain. The strain RDBCF showed more resistance to phosphine compared to other resistant strains tested. Results also indicate that for resistant as well as susceptible strains, time of exposure to phosphine was more critical a factor for effective fumigation than concentration (Tables 1 & 2). The MST in all the resistance strains varied substantially. The MST values were found to be phosphine concentration dependent (Figs. 1-4) and it decreased as the concentration increased. The values of resistance ratio indicate that RDBGD and RDBCF strains were several folds higher resistant compared to the RDMAL

strain (Fig. 5). Phosphine resistance in lesser grain borer *R. domiica* has been reported from a number of countries and it is to be expected that this number will increase (Champ and Dyte, 1976; Mills, 1983; Taylor, 1986; Reichmuth, 1986; Price and Mills, 1988 Opit *et al.* 2012, Afful *et al,* 2018). Moreover, phosphine resistance has been detected in most of the other major species of stored product insects including *Tribolium* spp., *Sitophilus* spp., *Oryzaephilus surinamensis* and *Cryptolestes* spp. (Taylor, 1986). This appears to be due to the increased selection pressure in some countries (Halliday *et al*, 1983; Friendship *et al*., 1986). Our results confirm the findings of others who reported the geographic variation in phosphine resistance in *R. dominica* (Collins *et al*. 2000, Benhalima *et al.* 2004, Lorini *et al*. 2007, Cato *et al*. 2017 Auful *et al*, 2018).

The distribution of phosphine resistance is not known in details but it is likely that resistance to phosphine is not an unusual phenomenon in most countries in which the fumigant has been used. The reported incidence of phosphine resistance is particularly high in the lesser grain borer *R.dominica* (F.) and two species of *Tribolium* (Champ and Dyte, 1976; Taylor, 1986). It has also been noticed that some of the strains of *R. dominica* collected from the field showed very high levels of resistance to phosphine (Mills, 1983; Afful et al., 2018). Fumigation failures have been reported from Bangladesh that were likely caused by high levels of phosphine resistance in those populations of *R.dominica* (Tylor *et al.* 1983). Mills (1983) also reported that the susceptible individuals of *R.dominica* could be killed at 0.03 mg/l phosphine dose at 20 h exposure while a field strain from Bangladesh could survive as high as 1.45 mg/l of phosphine for 20 h, and 72 hr exposure to this dose was require to produce a complete kill of these insects. Rahim et al. (1999) reported the presence of phosphine rresistance in stored grain insects including *R. dominica* from nine of the 13 states in Malaysia. The molecular mechanisms of phosphine resistance in *R. dominica* as well as other stored product pests are multifaceted and it is still under investigations (Chaudhry, 2000, Jagadeesan *et al.* 2012, Schlipalius *et al*. 2012, Chen *et al.* 2015).

Phosphine fumigation has the advantages of needing relatively low dosages when compared with other fumigants, being cost-effective, and least effects on the quality of fumigated grains. Consequently, despite the drawbacks in the existing storage and fumigation practices in the tropical countries, phosphine fumigant has helped preservation of food grains economically and at the same time meeting consumer demands with regard to the quality of the food grain. It is possible that these resistant strains could be controlled in tropical countries using phosphine fumigation, if conducted properly. This study also suggests that proper resistance assessment techniques can help to determine occurrence of phosphine resistance in populations of *R. dominica* in the field level of tropical as well as developing countries.

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Phosphine resistance in Saw-toothed Grain Beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) in the United States

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Abstract

Sub-lethal dose application of phosphine (PH_3) that is mostly caused by leakage during fumigation has resulted in resistance in *Tribolium castaneum*, *Rhyzopertha dominica*, *Cryptolestes ferrugineus*, *Sitophilus oryzae*, and other stored-product insect pest species worldwide. However, PH_3 resistance in the saw-toothed grain beetle, *Oryzaephilus surinamensis,* has not been reported in any country. Additionally, the discriminating dose of PH_3 for eggs of *O. surinamensis* has not been estimated. In this study, the discriminating dose for eggs of the susceptible strain of *O. surinamensis* was estimated as 28.4 ppm applied for 3 d. Adults from 4 out of 14 field-collected populations showed detectable resistance to PH_3 whereas eggs in 9 out of 14 populations had detectable resistance. Resistance frequencies in both adults and eggs in Box BF, Box BR and OKWat populations were > 90%. Levels of resistance (LC_{99}) in these three populations were estimated using probit analysis. LC_{99} values for adults of Box BF, Box BR, and OKWat populations were 320.5, 290.7 and 263 ppm, respectively, whereas those in eggs from the same populations were 1055.9, 1030.7, and 564.5 ppm, respectively, over 3-d fumigation. Resistance levels of adults and eggs of the most resistant population, Box BF, were 24.3- and 43.6-fold, respectively, higher than those of the lab-susceptible strain. The resistance levels in eggs from these three populations were > 3-fold higher than that in adults and this shows eggs of *O. surinamensis* are more tolerant to PH_3 than adults. These results indicate that it may not be practical to use PH_3 to control Box BF and Box BR populations. Therefore, it is important to develop alternative pest management strategies for controlling highly PH_3-resistant populations of stored-product insect pests.

Keywords: almond storage, resistance management, fumigation, resistance level

1. Introduction

The Central Valley of California produces ~ 840,000 metric tons/year of almonds valued at ~ $6.5 billion, and this accounts for nearly all almond production in the United States (National Agricultural Statistics Service [NASS], 2017). Such almond production levels are associated with a high level of risk from stored product insect pest infestation. Postharvest fumigation using phosphine is usually the method of choice for disinfestation of most stored agricultural commodities such as almonds (Johnson et al., 2012).

Phosphine or hydrogen phosphide (PH_3) is the most widely used fumigant for stored product insect pest control in the world because it is relatively inexpensive, easy to apply, and nearly no residue is left in the treated commodity. Sub-lethal dose application of PH_3 that is mostly caused by leakage during fumigation has resulted in resistance in *Tribolium castaneum* (Herbst) (Coleoptera: Tenerionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and other stored-product pest species worldwide (Zettler and Cuperus, 1990; Zettler and Keever, 1994; Rajendran, 1999; Collins et al., 2001; Cao et al., 2003; Benhalima et al., 2004; Pimental et al., 2010; Lorini et al., 2007; Opit et al., 2012; Ahmad et al., 2013; Nayak et al., 2013; Jittanun and Chongrattanameteekul, 2014; Chen et al., 2015; Koçak et al., 2015; Sağlam et al., 2015; Gautam et al., 2016; Afful et al., 2017; Cato et al., 2017; Konemann et al., 2017).

There are currently no published studies on PH_3 resistance in saw-toothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) in the United States and other countries worldwide despite PH_3 resistance having been reported in several species of storage pests including *T. castaneum*, *R. dominica*, *C. ferrugineus*, *S. oryzae* and other stored-product pest species globally. Therefore, the objectives of this study were to estimate the discriminating dose for eggs of a laboratory-susceptible strain of *O. surinamensis,* given that this information is lacking in literature, and to estimate the resistance frequencies of both adults and eggs of 14 *O. surinamensis* populations collected from almond storage and processing facilities in California and wheat storage facilities in Oklahoma. An additional objective was to estimate PH_3 resistance levels (LC_{99}) in adults and eggs of those populations found to have resistance frequencies ≥ 40%.

2. Materials and methods

Insects

A PH_3-susceptible laboratory strain (Susceptible *STGB*) of *O. surinamensis* maintained in culture since 1972 was obtained from the Center for Grain and Animal Health Research (CGAHR) of the USDA Agricultural Research Service, Manhattan, KS. Fourteen field-collected populations of *O. surinamensis* were used. Eleven out of 14 populations were collected from almond storage and processing facilities in the Central Valley of California during the period 2013–2016 whereas 3 populations were collected using probe traps from wheat storage bins in Oklahoma in 2015 and 2016. Each sample received from California was transferred to a glass jar, labeled, and kept in an incubator at 28 ± 1°C and 65 ± 5% RH for 2–3 weeks to allow for the immature stages to develop. Adult *O. surinamensis* were then transferred to laboratory diet comprising 95% oats and 5% Brewer's yeast (wt: wt) and kept in an incubator at the same conditions described above. Code names were given to different populations, for example "Box A *STGB*" representing an *O. surinamensis* population from facility "A"; the purpose of code names was to conceal identities of the facilities insects were obtained. Voucher specimens of 20 adult insects of each population preserved in 95% ethanol that were used in this study were deposited at K.C. Emerson Entomology Museum at Oklahoma State University under lot numbers: 163 (Box A *STGB*), 164 (Box BF *STGB*), 165 (Box BR *STGB*), 166 (Box Q *STGB*), 167 (Box S *STGB*), 168 (Box U3 *STGB*), 169 (Box W *STGB*), 170 (Box X *STGB*), 171 (Susceptible *STGB*), 192 (OKBur *STGB*). 193 (OKSti *STGB*), 194 (OKWat *STGB*), 195 (Box 16A), 196 (Box 16B) and 197 (Box 16C).

In the estimation of discriminating dose for eggs of the lab susceptible strain, resistance frequencies of both adults and eggs of 14 field-collected populations, resistance levels of both adults and eggs of three populations with resistance frequencies ≥ 40% (resistance frequencies were > 80%), procedures in FAO Method No. 16 (FAO, 1975), Opit et al. (2012), Gautum et al. (2016), and Konemann et al. (2017) were used and are briefly described below**.**

Estimation of discriminating dose

Experimental procedures to estimate the discriminating dose for eggs of *O. surinamensis* laboratory strain were similar to those described by Gautam et al. (2016) and Konemann et al. (2017). To obtain eggs for fumigation, 200–300 *O. surinamensis* adults were transferred from lab culture into a glass jar containing ~ 20-g mixture of oats and wheat flour. Three jars were set up. After 3 d, *O. surinamensis* eggs were harvested by sifting contents of jars using U.S. Standard #40 and #70 (0.42- and 0.297-mm openings, respectively) pair of sieves (Seedburo Equipment Company, Des Plaines, IL). Fifty 0- to 3-d-old eggs were placed on a transparent piece of double-sided sticky tape that was attached to a piece of black filter paper. Each sticky tape with eggs was then transferred to a glass vial. The glass vials with eggs were then placed in fumigation jars as described by Gautam et al. (2016). Based on preliminary experiments, concentrations of PH_3 required ranged from 2.5–38.2 ppm with seven dose points over a 72-h (3-day) fumigation period at 25°C. After the fumigation, vials were removed from jars and kept in an incubator maintained at 28 ± 1°C and 65 ± 5% RH. Eggs that hatched were counted 10 d post fumigation.

Resistance frequencies

Discriminating doses of 37.5 ppm of PH_3 for 20 h for adults and 28.4 ppm of PH_3 for 3 d for eggs, respectively, at 25°C were applied to estimate PH_3 resistance frequencies in these life stages of 14 field-collected populations of *O. surinamensis*. Preparation of egg samples from 14 field-collected populations was conducted as described above. For *O. surinamensis* adults, for the laboratory susceptible strain and each of the 14 field-collected populations, 50 adult insects of each population were selected randomly and placed in individual glass vials that contained 0.5 g of oats diet. Vials containing insects were then placed in each of three fumigation jars. Insects were also placed in three additional fumigation jars and prepared as previously described but fumigant was not added

to these jars which served as the controls. Mortality assessments for eggs and adults were conducted 10 d and 14 d post fumigation, respectively.

Levels of resistance

The susceptible *O. surinamensis* (Susceptible *STGB*) and three field-collected populations, Box BF, Box BR and OKWat were tested in dose-response assays to estimate their levels of resistance for both adults and eggs. Populations Box BF, Box BR and OKWat had resistance frequencies > 40%. Concentrations of PH_3 used for dose-response tests for the Susceptible *STGB* strain have previously been described above whereas those for adults of Box BF, Box BR and OKWat were 24.4–354.1, 24.4–354.1 and 30.0–366.1 ppm, respectively. For eggs, these concentrations were 28.4–449.2, 28.4–449.2 and 42.4–420.0 ppm, respectively. Experimental set-up and fumigation procedures were similar to those described in Opit et al. (2012), Gautam et al. (2016) and Konemann et al. (2017). Fumigation period was 3 d and mortality assessments for adults and eggs were conducted 5 d and 10 d post-fumigation, respectively.

PH_3 concentration analysis

The concentration of PH_3 gas in each fumigation jar was measured at the beginning and at the end of the respective exposure periods using a gas chromatograph coupled with a flame photometric detector (GC-FPD) (Model 8610C, SRI Instruments, Torrance, CA). The concentrations were established using a standard curve based on 50, 40, 30, 20, and 10 µl of 200 ppm PH_3. The areas under the peak in microvolts (µV) were recorded along with the volume of PH_3 injected. PH_3 volumes were regressed against measured peak areas to generate a linear regression equation that had a coefficient of determination (r^2) value between 0.96 and 0.99 in all cases. Thirty microliters gas samples from each fumigation jar were analyzed using the GC-FPD and quantified using the regression equation generated from the standard curve.

Data analysis

The experimental designs for determining discriminating doses were completely randomized designs with three replications. LC_{50} and LC_{99} values and their 95% confidence intervals (CIs) of both adults and eggs were estimated by probit analysis using PoloPlus (LeOra Software, Petaluma, CA) (LeOra Software, 2005). The discriminating dose of eggs was the upper limit of the 95% CI of the LC_{99} value at a given exposure period at 25°C. A ratio test to compare LCs was also conducted for eggs and adults of *O. surinamensis* (Robertson et al., 2007) to estimate the degree by which the field populations were more resistant to PH_3 than the susceptible laboratory strain. In order to ascertain that the value of the mean is within the limit at 95% probability, we calculated G-factor using the equation, $t^2\ V(b)/b^2$, where t = student's *t* test with error degrees of freedom, V(b) is the slope variance estimate given in the variance-covariance matrix, and b is the slope estimate. If a G-value is less than 0.5, it suggests that the value of the mean is within the limit at 95% probability.

3. Results and Discussion

Estimation of discriminating dose for eggs of *Oryzaephilus surinamensis*

Given that there was no previously published phosphine discriminating dose for eggs of *O. surinamensis*, we first estimated it in using a dose-response experiment and a laboratory susceptible strain of this species. PH_3 discriminating dose for eggs of *O. surinamensis* was estimated as 28.4 ppm over a 3-d fumigation period at 25°C (Table 1).

Tab. 1 Estimation of PH_3 discriminating dose for eggs of the laboratory susceptible strain of saw-toothed grain beetle, *Oryzaephilus surinamensis* (Susceptible *STGB*) based on 3-d fumigation at 25°C.

Population/Strain	N	Slope ± SE	LC_{50} (95% CI)	LC_{99} (95% CI)	X^2 (df) [H*]	G-factor
Susceptible *STGB*	1200	9.8 ± 0.6	14.0 (13.3 – 14.7)	24.2 (21.8 – 28.4)	59.4 (17) [3.5]	0.016

*Heterogeneity factor, chi-square value/ degrees of freedom (chi-square is significant < 0.05).

Resistance frequencies

Adults from 4 out of 14 populations showed detectable resistance to phosphine whereas eggs in 9 out of 14 populations had detectable resistance to phosphine. The resistance frequencies in both adults and eggs in "Box BF", "Box BR" and OKWat *STGB* populations were > 80% (Table 2). These results suggest that in some almond storage and processing facilities in California, populations of *O. surinamensis* with strong phosphine resistance co-exist with populations of *T. castaneum* with strong phosphine resistance (Gautam et al. 2016).

Tab. 2 Survival of adults from a laboratory susceptible strain (Susceptible STGB) and 14 field-collected populations of *Oryzaephilus surinamensis*. Data for adults are based on 20-hour exposure to a PH_3 discriminating dose of 37.5 ppm at 25°C and for eggs, a 3-day exposure to a discriminating dose of 28.4 ppm.

Life Stage	Population	Percentage survival			
		Rep. 1	Rep. 2	Rep. 3	Mean ± SE
Adults	Box BF *STGB*	100	94	80	91.3 ± 4.7
	Box BR *STGB*	98	98	100	98.7 ± 0.5
	Box A *STGB*	4	0	2	2.0 ± 0.9
	Box Q *STGB*	0	0	0	0.0 ± 0.0
	Box U3 *STGB*	0	0	0	0.0 ± 0.0
	Box S *STGB*	0	0	0	0.0 ± 0.0
	Box X *STGB*	0	0	0	0.0 ± 0.0
	Box W *STGB*	0	0	0	0.0 ± 0.0
	Box 16A *STGB*	0	0	0	0.0 ± 0.0
	Box 16B *STGB*	0	0	0	0.0 ± 0.0
	Box 16C *STGB*	0	0	0	0.0 ± 0.0
	OKBur *STGB*	0	0	0	0.0 ± 0.0
	OkSti *STGB*	0	0	0	0.0 ± 0.0
	OKWat *STGB*	100	98	98	98.7 ± 0.5
	Susceptible *STGB*	0	0	0	0.0 ± 0.0
Eggs	Box BF *STGB*	100	100	98	99.3 ± 0.5
	Box BR *STGB*	98	98	100	94.7 ± 1.4
	Box A *STGB*	2	0	0	0.7 ± 0.5
	Box Q *STGB*	4	6	2	4.0 ± 0.9
	Box U3 *STGB*	2	10	2	4.7 ± 2.1
	Box S *STGB*	2	8	0	3.3 ± 1.9
	Box X *STGB*	0	8	2	3.3 ± 1.9
	Box W *STGB*	0	4	0	1.3 ± 1.1
	Box 16A *STGB*	0	0	0	0.0 ± 0.0
	Box 16B *STGB*	0	0	0	0.0 ± 0.0
	Box 16C *STGB*	0	0	0	0.0 ± 0.0
	OKBur *STGB*	0	0	0	0.0 ± 0.0
	OkSti *STGB*	0	0	0	0.0 ± 0.0
	OKWat *STGB*	100	97	98	98.3 ± 0.7
	Susceptible *STGB*	0	0	0	0.0 ± 0.0

Levels of resistance

LC_{99} values for adults of Box BF", "Box BR" and OKWat *STGB* populations were 320.5, 290.7 and 264.1ppm over 3-d fumigation, respectively, whereas those in eggs of the same populations were

1055.9, 1030.7 and 564.5ppm, respectively (Tables 3 and 5). The resistance levels of adults and eggs in the population with the highest resistance, Box BF were 24.3 and 43.6-fold, respectively, higher than that in the lab-susceptible strain (Table 4 and 6). The resistance levels in eggs of these two populations were >3-fold higher than that in adults. These data show that eggs of *O. surinamensis* have much higher levels of resistance than adults; similar results were reported by Gautam et al. (2016) for *T. castaneum*, also collected from almond storage and processing facilities.

Tab. 3 Probit analyses of dose-response data for the susceptible and three phosphine-resistant populations of *Oryzaephilus surinamensis* adults. LC values are lethal concentrations of phosphine (ppm) over 3 d fumigation period at 25°C.

Population/ Strain	N	Slope ± SE	LC_{50} (95% CI)	LC_{99} (95% CI)	X^2 (df) [H*]	G-factor
Susceptible	1447	5.4 ± 0.3	4.8 (4.6 – 5.2)	13.2 (11.5 – 15.9)	49.6 (20) [2.5]	0.013
Box BF	1155	6.0 ± 0.3	118.7 (107.6 – 129.7)	290.8 (249.1 – 362.8)	55.8 (19) [2.9]	0.014
Box BR	1321	3.0 ± 0.2	52.8 (44.3 – 60.9)	320.5 (249.9 – 456.9)	49.2 (19) [2.6]	0.014
OKWat	1000	3.5 ± 0.2	56.6 (52.0 – 61.5)	264.1 (215.1 – 344.3)	15.7(15) [1.1]	0.019

*Heterogeneity factor, chi-square value/ degrees of freedom (chi-square is significant <0.05).

Tab. 4 Comparison of lethal concentrations of phosphine (ppm) required to kill 50, 95, and 99% of insects in samples from three phosphine-resistant populations of *Oryzaephilus surinamensis* and those required to kill similar percentage from the lab susceptible population.

Samples compared	Lethal concentration ratios		
	LC_{50} (95% CI)	LC_{95} (95% CI)	LC_{99} (95% CI)
Box BF vs Susceptible	24.4 (22.9 – 25.9)	22.7 (20.5 – 25.2)	22.1 (19.2 – 25.2)
Box BR vs Susceptible	10.9 (9.8 – 12.0)	19.2 (16.7 – 22.1)	24.3 (19.9 – 29.7)
OKWat vs Susceptible	12.0 (10.6 – 12.6)	17.9 (15.9 – 18.8)	19.9 (18.2 – 22.6)

Tab. 5 Probit analyses of dose-response data for three phosphine-resistant populations of *Oryzaephilus surinamensis* eggs. LC values are lethal concentrations of phosphine (ppm) over a 3-d fumigation period at 25°C.

Population	N	Slope ± SE	LC_{50} (95% CI)	LC_{99} (95% CI)	X^2 (df) [H*]	G-factor
Box BF	1050	2.5 ± 0.2	122.2 (105.4 – 139.5)	1055.9 (755.6 – 1706.0)	44.0 (19) [2.3]	0.015
Box BR	1050	2.3 ± 0.1	101.7 (85.2 – 118.5)	1030.7 (714.9 – 1762.5)	50.0 (19) [2.6]	0.016
OKWat	1000	3.1 ± 0.2	98.0 (89.0 – 106.8)	564.5 (473.3 – 704.7)	19.6(18) 1.1	0.014

*Heterogeneity factor, chi-square value/ degrees of freedom (chi-square is significant <0.05).

Tab. 6 Comparison of lethal concentrations of phosphine (ppm) required to kill 50, 95, and 99% of insects in samples from three phosphine-resistant populations of *Oryzaephilus surinamensis* eggs and those required to kill similar percentage from the lab susceptible population.

Samples compared	Lethal concentration ratios		
	LC_{50} (95% CI)	LC_{95} (95% CI)	LC_{99} (95% CI)
Box BF vs Susceptible	8.7 (7.9 – 9.6)	27.2 (23.1 – 34.1)	43.6 (34.7 – 60.1)
Box BR vs Susceptible	7.3 (6.4 – 8.1)	25.4 (21.1 – 32.9)	42.6 (32.8 – 62.1)
OKWat vs Susceptible	7.0 (6.7 – 7.3)	16.4 (15.7 – 17.0)	23.3 (21.7 – 24.8)

4. Conclusions

Resistance frequencies in adults and eggs of 14 field-collected populations of *O. surinamensis* ranged between 0–100 for both stages but were highest in three populations, namely "Box BF", "Box BR" and OKWat *STGB*, where frequencies were > 80%. Resistance levels (based on LC_{99}) in adults of these 3 most resistant populations were 22.1-, 24.3- and 19.9-fold, respectively, higher than in the susceptible strain, whereas those in eggs were 43.6-, 42.6- and 23.3-fold higher than in the

susceptible strain. These results show that phosphine-resistant populations of *O. surinamensis* are found in both almond storage and processing facilities in California and in wheat storage facilities in Oklahoma. For the control of stored product insect pests, currently the almond industry in California recommends a dose is 500–1,000 ppm of PH_3 for a minimum of 3 d, but 5–7 d are highly recommended, at 20–30°C. Therefore, it may not be possible to use PH_3 to effectively control Box BF and Box BR populations given that the LC_{99} values of eggs of these populations are 1055.9 and 1030.7 ppm, respectively. These data highlight the importance of developing alternative pest management strategies for controlling highly PH_3-resistant populations of stored product insect pests.

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Molecular mechanisms of metabolic resistance in booklice (Psocoptera: Liposcelididae)

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Abstract

The psocids from the genus *Liposcelis* are also named booklice, which are stored-product insect pests. Recently, apparent insecticide resistances have been observed in booklice. Here, we mainly focus on mechanisms of metabolic resistance associated with the three major enzymes, Cytochrome P450 monooxygenases (P450s), Estrases (ESTs), and Gluthione-S-transferases (GSTs) in booklice. We developed four comprehensive transcriptomic databases for four booklice, and a large number of detoxification genes potentially involved in insecticide resistance were identified. Totally, 49, 68, 94 and 82 P450 genes, 31, 37, 35 and 23 GST genes, 21, 19, 34 and 19 EST genes were identified for *L. bostrychophila*, *L. entomophila*, *L. tricolor* and *L. decolor*, respectively. The large number of P450s and GSTs implied that Liposcelis species could potentially develop high level of insecticide resistance. The mRNA expression levels of detoxification genes showed that these genes expressed at all tested stages, but exhibited stage-specific patterns, with the higher expression in adults and elder nymphs. Additionally, mRNA abundances of P450 genes were relatively more abundant in adult females than in adult males. The research on different strains showed that the resistance strain of both *L. bostrychophila* and *L. entomophila* had significantly higher mRNA expression and enzyme activity of the detoxification enzymes than the sensitive strain. The above data indicated that detoxification genes might be associated with metabolism insecticides in psocids.

"Remote Sensing, Predictable Storage of Agricultural Commodities and Advances in Hermetic Storage"

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Abstract

Modified atmosphere hermetic storage, now used in over 115 countries for fumigant-free storage of dry commodities from coffee to rice and maize, has been available for almost three decades. This paper describes the progress in the field use of hermetic postharvest storage systems and of recent innovations in this technology which include the introduction of remote monitoring of temperature, humidity and O_2 or CO_2 levels in large, sealed hermetic containers. Also, introduced in 2018, is the Cocoon Lite™, a 2nd generation multi-tonne container with major improvements in the permeability, weight and cost of high performance, large hermetic storage systems. Early examples of the uses of these innovations and data obtained from their study is expected by year end. The GrainPro EcoWiSe™ is a remote sensing system that enables monitoring of temperature, moisture and oxygen/carbon dioxide levels, thus providing real-time data on the conditions of the stored commodity involved without manual intervention. One (or more) low-cost, remote, wireless sensors/transmitters placed inside sealed. Postharvest hermetic storage units can be read remotely on laptops or cellphones. Data collected and accumulated over time enables development of an "algorithm" for a stored commodity to define an alarm, where the user can be notified immediately of any unsafe humidity or oxygen storage conditions. A substantial advance in large hermetic storage containers known as Cocoons™ is the new Cocoon Lite, a 500:1 improvement in permeability to oxygen as well as a unit weight only 28% of existing Cocoons with the same capacity and a significantly lower cost. The paper also discusses prevention of the public health hazard of exponential growth of aflatoxin levels in conventional postharvest storage such as in rice, maize, and groundnuts; field data is provided on the control achieved through hermetic storage.

Keywords: hermetic, grain storage, aflatoxins, remote sensors, safe storage, seed storage

1. Brief overview of current hermetic storage use for postharvest storage

Over 25-years ago, we saw the first commercial introduction of fumigant-free, hermetic (airtight) postharvest storage for many types of grains and seeds. Where used, this resulted in drastically reduced, multi-month storage postharvest losses of the stored grains and other dry commodities. Hermetic storage to prevent both qualitative and quantitative postharvest losses for periods of one-year (or more) is now used in over 115 countries and takes many forms. Hermetic storage has the further proven benefit of arresting the exponential growth of aflatoxins, a major public health hazard for key commodities, especially in hot, humid climates.

In 2018, a new large multi-tonne storage unit, the Cocoon Lite was introduced to supplement the existing 5 to 1,000 tonne capacity Cocoon for indoor or outdoor use. The Cocoon Lite described further is much lighter, has 500 times lower O_2 permeability and is significantly less expensive than the standard Cocoon.

Various manual means have been used to verify the continued integrity of hermetically sealed postharvest storage of commodities, from sampling to taking measurements with an oxygen analyzer. With the 2018 introduction of remote sensing, continuous monitoring of the container environment is now possible. The capacities of currently available hermetic storage units in current commercial use, typically range from 15kg to as much as 1,000 tonnes.

A 2016 Fintrac/USAID study in Kenya concluded: "Hermetic technologies offer small-scale farming families effective, cost-efficient, insecticide-free methods for on-farm storage." (Fintrac 2016).

2. The principle of hermetic storage and its requirements

The goal of hermetic storage is to create a container sufficiently airtight to allow the total insect respiration rate of infesting insects plus, where applicable, that of the stored commodity itself, is to be greater than the rate of residual infiltration of oxygen through the ultra-low permeability hermetic container surface. To successfully accomplish this goal in small size, man-portable storage containers, a special, coextruded PE plus barrier layer with oxygen permeability of available models of between 1 to 50 cc/ m^2/day is used. In large containers of 1-tonne (or more) capacity, use of a PVC with oxygen permeability of less than 500 cc/m^2/day is typical. This approach, which uses 0.8mm thick modern plastic materials, also requires proper sealing (and unsealing) mechanisms to permit the loading and discharge of commodities.

Because of the larger ratio of volume to surface area, small containers such as man-portable bags need much lower permeability than large containers to reach the same equilibrium between respiration and infiltration of air, and therefore to achieve the same degree of oxygen depletion in the container.

Thus, the principle of successful use of hermetic storage is that insect respiration alone, plus any respiration of the commodity itself as well as microflora activity, can create a modified atmosphere and either approach (or reach) the unbreathable atmosphere levels of 3% oxygen. Somewhat higher oxygen levels can be successful in killing insects when synergistic carbon dioxide levels rise to 12 - 15% or above, primarily, through insects exhaling of CO_2.

In those few instances where insect respiration needs to be supplemented, or where rapid disinfestation is desired (or required), the natural insect respiration process can be supplemented by an adjuvant to reduce the time required to reach LT99. The typical adjuvant is carbon dioxide, or for smaller containers, a smaller commercial oxygen-absorbing sachet. In most instances, an initial dose of 80-90% CO_2 alone will kill the total insect population in approximately 10 days, provided that the final concentration of CO_2 remains > 35% (Navarro, et all, 2012).

3. Introduction of remote sensing

GrainPro EcoWiSe™ was introduced in the first half of 2018 as an alternative to manual measurement or observation of the content of a sealed, hermetic container. The remote sensing technology enables monitoring of temperature, moisture, and oxygen/carbon dioxide levels. Measurements are made wirelessly and information is displayed on a laptop or desktop computer capable of receiving wireless signals. Real-time data on the conditions of the stored commodity are produced without manual intervention.

Data collected and accumulated over time enables development of an "algorithm" for a stored commodity using an alarm setting, where the user and others, can be notified immediately of any preset unsafe humidity or oxygen storage conditions. If any of the preset values being monitored are exceeded, the alarm indicates that an out of control condition exists and requires immediate attention. The algorithm helps as well in predicting the "storability" of the commodity.

One (or more) low-cost, remote, wireless sensors/transmitters placed inside sealed, postharvest hermetic storage units can be read without opening the container and at distances up to 500 meters (and more with a repeater), the small electronic module (Figure 1) placed inside the sealed storage container broadcasts information through a nearby computer to the "cloud". A single, remote receiver communicates with one or more sensors/transmitters placed inside. The sensors weigh a fraction of a kilogram and are powered by a 5-year active battery, A single receiver can handle up to one hundred sensors.

Fig. 1 Wireless sensor/transmitter

Fig. 2 Cocoon Lite™ capacity 5-tonne, PE-based, weight 7.75 kg (Courtesy, GrainPro, 2017)

4. A new class of lightweight, multi-tonne hermetic container

2018 also marked the introduction of the Cocoon Lite, an innovating addition to the widely used, PVC-based hermetic Cocoon. Cocoon Lite is lighter and far more airtight than the Cocoon.

The Cocoon Lite (Figure 2) is composed of an improved, 205µ thick composite material consisting of a special formulation of polyethylene, a compound barrier layer, plus a white, opaque barrier layer, which also adds UV-resistance and strength against penetration by insects. It is designed for both indoor and outdoor use.

Although the 5-year rated life of the Cocoon Lite is significantly shorter than the 15-year life of the existing PVC-based Cocoon, advantages include significantly lower cost and a weight of only 20% of the equivalent size PVC Cocoon. Its permeability to O2 improved to <1 cc/m^2/day instead of the conventional Cocoon permeability of <500/cc/m^2/day. This shortens the time needed to reach an unbreathable atmosphere and low oxygen level.

Because it is much lighter, the Cocoon Lite is easier both the transport and install, and it better protects against the entry of outside humidity, with permeability to water vapor of <2g/m^2/day versus <8g/m^2/day for the PVC-based Cocoon.

First deliveries of the Cocoon Lite took place in the first half of 2018.

5. Hermetic storage for control of exponential aflatoxin growth in storage

The health consequences of high levels of aflatoxins (produced by *Aspergillus flavus* and *Aspergillus parasiticus*) are widely recognized as major health problems, particularly in hot, humid climates. The international community and many individual countries have set strict limits on acceptable levels of aflatoxin – most commonly 10-20 parts per billion (ppb), or even 5 ppb for direct human consumption. In practice these limits are often greatly exceeded or not being monitored at all, causing serious health effects by depressing the human immune system (Williams, et. al., 2004).

In humans, high aflatoxin levels contribute to many health problems ranging from cancer and susceptibility to HIV, to stunting growth among children. In African countries, Dr. Williams cites a sampling survey of several local markets which showed that 40% of the commodities tested had food aflatoxin levels exceeding the international standard of 10 to 20 ppb, putting an estimated 4.5 billion people in developing African countries at risk. (Williams, 2011).

A cross-sectional study conducted in Ghana (Dr. Williams, 2011) showed that the immune systems of recently HIV-infected people had above-median levels of aflatoxins and that "people with a high aflatoxin biomarker status in Gambia and Ghana were more likely to have active malaria." Small holder farmers were particularly affected: "A major area of neglect and opportunity is foods stored by small farmers for their own consumption. A very common consequence of quality control in markets is for farmers to retain, for their own use, grains that would reduce the price offered in the market place." Further Williams writes "studies of groundnuts in local storage facilities show a steady increase in (aflatoxin) contamination levels and these differences are observed in the cyclical variation in the biomarkers of rural African people." (Williams, 2011).

"Hermetic (i.e., airtight) storage devices arrest aflatoxin growth nearly entirely", according to a 2017 ACDI/VOCA brief, sponsored by the Bill and Melinda Gates Foundation. The report states "This has been substantiated through research in both real-world and controlled settings. Hermetic storage offers tangible hope in mitigating aflatoxin's blight." (AflaSTOP, 2017).

Figure 3 shows the relation between the growth of aflatoxin-producing molds and humidity. 80% relative humidity and above is common in many parts of Africa, South America and Asia.

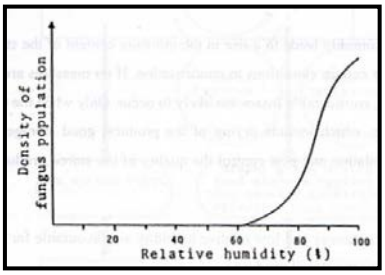

Fig. 3 Effect of relative humidity on mold/fungus density (Harrington, 1972)

Field study results from ICRISAT in Mali (Table 1), show how exponential growth of aflatoxins takes place within months. (Waliyar, et. al., 2013).

Tab. 1 Increased aflatoxin levels in groundnuts during conventional storage in farmers' fields in Mali.

Village In Mali	Aflatoxin content (ppb)		
	At harvest	1 month later in storage	2 months later in storage
Bamba	101.3	168.9	275.5
Gouak	61.4	118.0	174.7
Kolokani	119.2	352.6	400.0
Sido	53.7	93.6	166.2

Another 2013 study from Ruhira, Uganda, by Millennium Villages shows the growth of aflatoxins in conventional storage versus hermetic storage methods and illustrates the major suppression of aflatoxin growth when using hermetic storage versus alternative, conventional storage. (Figure 4), (private communication, 2013).

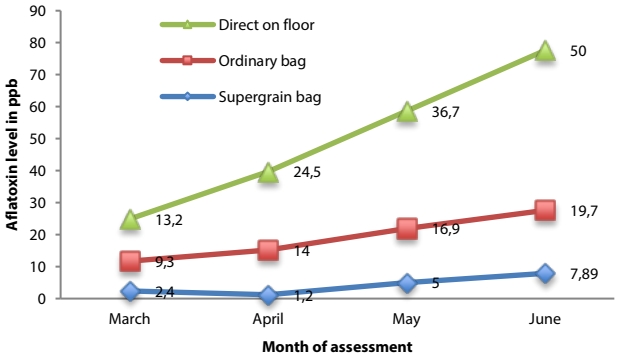

Fig. 4. Comparing aflatoxin concentration over one season in maize stored in conventional and hermetic methods. Millenium Village study in Ruhiira, Uganda (2013).

6. Hermetic storage performance in storing major commodities

Postharvest hermetic storage systems, in addition to contributing to public health through inhibiting aflatoxin growth, dramatically reduce quantitative postharvest losses from insects, rodents and molds. In grains, such as maize conventionally stored for many months, total postharvest losses including storage can (and often) exceed 25%. (Zorya, et. al., 2011).

Field performance of postharvest, hermetic storage systems and the large-scale applicability of Ultra Hermetic storage to preserve dry grains for up to a year, have been studied for several commodities that include: maize, wheat, rice, seeds, coffee, cocoa and ground nuts.

These studies generally have documented reduction of losses using hermetic storage in hot, humid climates to less than 1% per year for up to 1-year. For some commodities, such as coffee and cocoa, the primary goal is preserving premium quality for up to a year without use of refrigeration, pesticide, or fumigants. Examples of results for a few of these key commodities are shown in the following sections, including ground nuts where the injection of CO_2 or oxygen absorbers as an accelerant has been found important.

6.1 Hermetic maize storage

Babban Gona, an integrated contract farming model project, involving 20,000 small farmers in Northern Nigeria, provide farmers with seeds, fertilizer, technical support, financing and take-out in aggregation centers catering especially to quality buyers for high quality/low aflatoxin uses including baby food. Babban Gona features their low aflatoxin levels in its maize. (Figure 6). They report a doubling of farmer yields for maize.

Fig. 5 Storing maize in 150 MT Cocoon at Babban Gona Aggregation Center in northern Nigeria, emphasizing low aflatoxin in their maize (courtesy, Babban Gona, 2017).

According to Babban Gona's records in Oct 2017, the maize losses out of 26,000 tons of the 2016 harvest were an average of 0.003% (about 7 bags out of 250,000 bags stored in 170 Cocoons with 1,500 bags each). (Private communication, Donna Etiebet, Babban Gona, 2018)

A report by Kukom Edoh Ognakossan in Togo compared maize storage in woven polypropylene versus hermetic storage for 150-days with populations of Prostephanus truncates and Sitophilus zeamais. Losses from Sitophilus zeamais were less than 0.5% in hermetic storage versus 19.2% in woven polypropylene. For P. truncates losses in hermetic storage were 6% in hermetic storage versus 27.1% in woven polypropylene. (Ognakossan, I.E., et al, 2013).

6.2 Hermetic rice & rice seed studies

Extensive postharvest studies of hermetic storage of rice and rice seeds (paddy) at IRRI (International Rice Research Institute, Los Banos, Philippines) (Villers and Gummert, 2009), and at PhilRice (Philippines) (Sabio, et. al., 2006)) have shown that rice seeds stored hermetically without fumigants, can be stored for up to a year with negligible loss rates, while maintaining germination rates and vigor. Results are comparable to refrigerated or air conditioned storage. Rice seed, as see in Table 2, can be stored hermetically for six months with germination levels equivalent to air-conditioning or cold room storage without the energy cost or capital investment (Villers, et al., 2006)

In a field study by IRRI, they reported that "in Cambodia, the germination for hermetically stored seeds was 90% after 6 months and 63% after 12 months. In comparison, seed stored in traditional systems had germination of 51% and 8%, respectively" (Villers and Gummert, 2009)

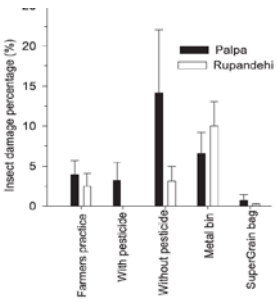

Fig. 7 Insect damage after 6 months in wheat seed versus storage method, Nepal (Devkota et. al., 2017)

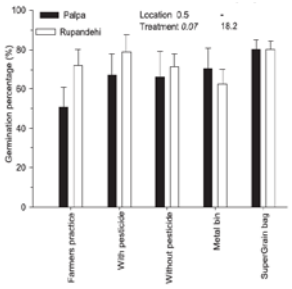

Fig. 8 Comparison of germination rates, wheat seeds in Nepal (Devkota et. al. 2017)

6.3 Hermetic wheat seed storage

An article published in the Journal of Stored Product Research (Devkota, et al, 2017) describes a joint 2017 IRRI and CIMMYT study conducted in Nepal of wheat seed storage measuring quantitative germination performance, insect damage and seed vigor. (Devkota, M., 2017)

The study demonstrated that wheat seed stored in hermetic SuperGrainbags (SGBs®) maintained a lower moisture content, and produced higher seed germination and seed vigor with less insect damage than other storage methods evaluated in the study (Figure 7). It also demonstrated that maintaining wheat seed storage quality is more challenging using traditional storage methods in the Nepalese hills, which have higher rainfall and lower temperature than in the Terai Plains. According to the authors, "the higher germination percentage and lower insect infestation under SGB storage was attributed to maintaining low seed moisture during storage. It is known that every 1% increase in seed moisture content reduces the seed shelf-life by half (Harrington, 1972).

Shown in Figure 7 and Figure 8 from their study showing data on insect damage and germination %, respectively from their study.

Tab. 2 Mean % germination rate of Mestizo 1 hybrid paddy (unmilled) seeds stored using different storage technologies. (Sabio, G.C., et al, 2006)

	Storage time after harvest (months)			
Storage method	**0**	**3**	**6**	**9**
Hermetic	96.2	96.5	93.3	86.2
Cold Room	96.8	97.6	93.0	89.6
Air-conditioned	94.3	94.8	88.1	85.8
Control (unprotected)	92.9	92.9	76.4	74.7

Tab. 3 Mean percent adult insect density1 per kg sample of Mestizo 1 hybrid paddy seeds stored under different storage technologies and durations. (Sabio, G.C., et al, 2006)

Months	Open Storage	Air-Conditioning	Cold Room	Hermetic (5 tons)
0	3.2	8.4	8.4	8.8
3	135	1.6	0	0
6	114	3.0	0	0.4
9	54	3.4	0	0.4
12	27	9.0	0	2.2

6.4 Green coffee storage

In the case of coffee, hermetic bags typically sized 15kg to 69 kg capacity, have become the defacto standard worldwide for the specialty coffee sector.

In 2012, Dr. Flavio Borem's Brazil study concluded "The coffee beans stored in the hermetically sealed packaging predominantly had desirable flavors such as chocolate, vanilla, citrus and red fruits. Conversely, the coffee stored in the jute sacks had predominately undesirable odors such as papery and jute." Also, "The lowest losses were observed when coffee was stored with artificial atmosphere. After 12-months, no differences were observed between vacuum and GrainPro bags." (Borem et. al., 2013)

Variations on hermetic storage of green coffee beans have been studied, including another study in Brazil by Dr. Borem that found a small, but not statistically significant, improvement can be obtained by injecting CO_2 during hermetic storage. (See Table 4). As to vacuum storage, in a different study, Dr. Flavio Borem writes: "This result confirm the thesis that it is possible to maintain coffee quality equal to the quality of vacuum-packed coffee, up to now considered by most coffee importers as the best storage system for specialty coffees". (Borem, 2016).

Tab. 4 Quantitative Value for Coffee Quality after 12-months of storage.
Mean values of the overall score of the coffee beans after 12-months of storage.

Big-bag (one-tonne hermetic)	Position	Score
With CO2	Upper	80.00a
With CO2	Middle	80.80a
Without CO2	Upper	78.09a
Without CO2	Middle	78.06a
Other treatments:		
GrainPro (SuperGrainbag, no CO2)	GrainPro	78.98a
Jute sack alone	Jute sack	73.03b

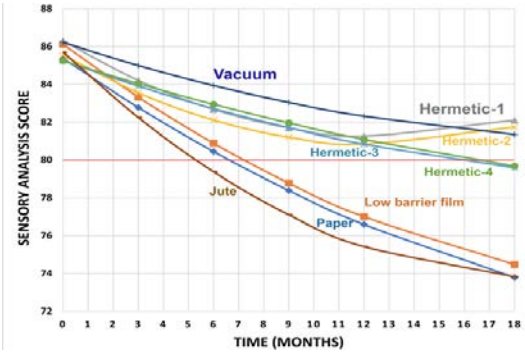

Fig. 9 Adapted from "Evaluation of Packages and Storage Methods for Specialty Coffees", Courtesy of Prof. Flavio Borem, UFLA, Brazil, 2016.

6.5 Hermetic cocoa bean protection

According to a Ghana Cocoa Board report "Experiments were conducted in the year 2008 in Tema, at the research department of the Ghana Cocoa Board with 150 tonne GrainPro Cocoons (for stacked bags of cocoa beans), SuperGrainbags (for 69 kg bags of cocoa), and TranSafeliners™ (gas-tight liner for shipping containers), all showing that the low oxygen/high carbon dioxide atmosphere was able to eliminate the insect population completely in less than two weeks…In conclusion, storing cocoa beans in hermetically-sealed structures inhibits activity of insect pest and mold development; consequently, the FFA deriving from microflora development was inhibited. Most possible that the toxicogenic mycotoxins are also inhibited…" (Jonfia-Essien et al, 2010)

6.6 Special requirements for safe storage of groundnuts

CO_2 injection is currently used in a number of countries for expelling air in order to achieve a faster suppression of mold growth than achieved through respiration alone, which, in the special case of groundnuts, can take 30 days or more to reach 3% oxygen. See table 5.

Tab. 5 Shows the effect of interventions on aflatoxin levels (ppb) in groundnuts for Drobonso Village, Ashanti Region, Ghana, 2014/2015 major season (Appaw, 2016).

	Field (Harvesting Stage)	Storage Stage + (Poly sac vs Hermetic Bag)	
Practice	**Aflatoxin level**	**Aflatoxin level**	**% Reduction**
Farmer (conventional)	Not detected	6.61 – 438.79 (133.22 average)	
Improved (hermetic)	Not detected	0.88 – 31.36 (10.89 average)	86 – 99 (95% average)

7. Comparative cost effectiveness data on hermetic storage

Three different examples of cost analysis each for a different commodity, are shown in Table 6, Figure 10, and Figure 11.

The cost effectiveness of utilizing hermetic storage of rice seed versus alternatives was studied at PhilRice in the Philippines, with results as shown below in Table 6. (Sabio et. al., 2006)

Tab. 6. Cost comparison (Philippines) using four storage methods for preserving Mestizo 1 hybrid paddy seeds (all values in US dollars; $1 = 50 Philippine pesos).

	3 months' storage				6 months' storage			
Costs	**Control**	**Hermetic**	**Cold room**	**Air-conditioned**	**Control**	**Hermetic**	**Cold room**	**Air-conditioned**
Investment	82,250	1,744	12,820	16,230	82,250	1,744	12,820	16,230
Operating expenses	24,991	504	3,548	3,820	31,086	504	4,196	3,950
Per bag	2.50	2.52	3.55	2.55	3.11	2.52	4.2	2.63

Comparative cost for green coffee storage in figure 10 and comparative cost of storing wheat seed in figure 11.

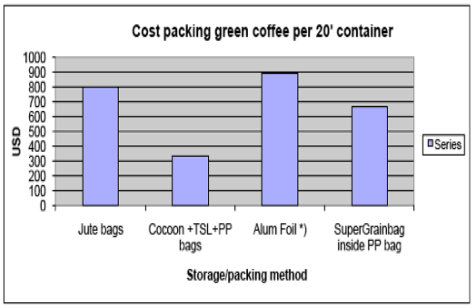

Fig. 10 Comparative Cost Calculations Dorman (VolCafe Kenya) (in USD), (De Bruin et. al., 2012)

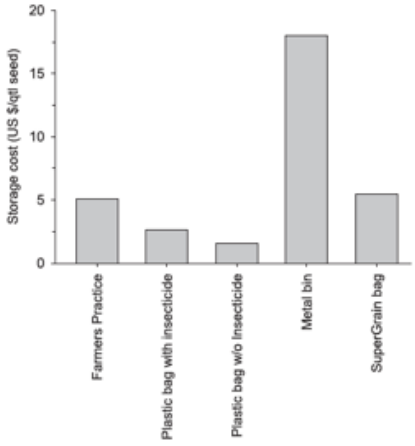

Fig. 11 Comparing storage costs of wheat seeds. Devkota, et al, 2017

8. Range of Hermetic Systems currently in use

The need for hermetic storage varies, in size and form, and in loading/unloading requirements, and thus a variety of such systems are marketed. These range from the traditional Cocoon™, the new Cocoon Lite™ and 1-tonne GrainSafes™ to the light, man-portable 15kg to 90kg SuperGrainbags® (SGBs).

The same material is used in the TranSafeliner™ (TSL), also in Figure 12, which applies the hermetic principle to lining for standard 20- or 40-foot shipping containers. TSLs are used in the coffee, cocoa and spice businesses to prevent deterioration of their high value commodities in intercontinental shipments.

At the other end of the range, 69kg, man-portable storage containers such as the 50 kg cost SuperGrainbag Farm (0.078mm), made of PE with a thinner barrier layer and a higher permeability up to 50cc/m2/day, demonstrate satisfactory performance for storage of non-premium commodities, such as maize or rice crops.

Fig. 12 Examples of different sizes of hermetic storage on-farm and coop storage, ranging from SuperGrainbag liners of 15 – 69 kg and GrainSafes of 1-tonne and Cocoons of 5- to 300-tonnes. (Courtesy of GrainPro, Inc.)

9. Conclusion

More than 25-years ago, the hermetic, postharvest storage once used by the ancients in very large airtight jars sealed with beeswax, was revived in a modern form and became available commercially. Hermetic storage from 15 kg to 1,000 ton capacity is now used within 115 countries worldwide, generally with no more than 1% quantitative losses per year, and highly effective maintenance of quality, including prevention of exponential growth of aflatoxins. Recently, the introduction of remote sensing can largely eliminate the danger of unobserved accidental damage to a hermetic container that leaves a stored commodity unprotected. Also, recently introduced, is the 2nd generation large hermetic container, which has a 500:1 improvement on oxygen permeability and a weight reduction of 75%. The hermetic storage industry continues to grow and provides a variety of products and performance characteristics as specialized needs are addressed.

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Establishing the value of modern seed storage methods for wheat in diverse production ecologies in Nepal

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Abstract

In the developing-country context of Nepal, farmers often incur in seed losses of 15-30% due to improper storage. To evaluate the efficacy and costs of modern storage alternatives, experimental trials were set up among ten farmers each in two contrasting ecologies, i.e. Palpa (hills) and Rupandehi (terai plains) districts of Nepal in 2013. Several wheat seed storage options were contrasted including farmer practices (FP) such as reused fertilizer bags, polythene bags, household metal containers, and mud bins. Modern storage methods that were evaluated included plastic bags (with and without pesticide), metal bins, and hermetic 'SuperGrainbag' (SGB). Seed quality and losses were assessed after six months of storage (May-October) with parameters such as grain moisture content, insect damage, seed germination, and seedling vigor. The overall quality of seed with FPs was lower in the hills than in the terai plains. Among the treatments, SGBs were more effective in maintaining acceptable seed moisture levels, controlling insect damage (<1%), preserving germination (>90% lab, >65% field), and promoting seedling vigor. Metal bins and plastic bags without pesticide had higher insect damage (7-15%) compared to FP and plastic bags with pesticide (2-5%). In terms of storage costs, SGBs were comparable with the farmers' storage methods ($5-6 per 100 kg seed storage). Our findings demonstrate that SGBs are better at maintaining seed quality and more economical than not only FP but also the other modern storage methods evaluated in this study across different production ecological regions in Nepal.

Keywords: SuperGrain bag, seed quality, germination, insect infestation, seed moisture.

Introduction

As a versatile crop, wheat is an essential part of the diet and food trade in many parts of the world (Uthayakumaran and Wrigley, 2010). In Nepal, wheat is the third most important cereal after rice and maize in terms of area and production. Moreover, it is widely adopted across the country with cultivation ranging from 50 to 4000 m in elevation. It shares 16% of the total calorie and 20% of the total protein supplied from plant products in diets of both the hills and plains in Nepal (CBS, 2015). The plains share 55% of the wheat area and contribute 62% to the total production, compared to 45% and 38%, respectively, by the hills (NARC, 2017).

Good quality seed is considered as the most basic and cheapest, yet most critical input for enhancing productivity (Rana, 1997). However, in Nepal, the seed replacement rate for wheat is only 13% (GoN, 2013). Only 15-20% of the total quantity of wheat seed required for planting is supplied by seed producing agencies that have proper storage structures (warehouses) with moderate cooling and periodic drying facilities. The majority of the seed is exchanged among farmers and stored at room temperature in various kinds of storage materials such as plastic or fertilizer bags, and small to medium sized metal bins, with or without pesticides (FGD, 2013). Seeds, being hygroscopic in nature, are prone to changes in moisture content in response to weather, which

ultimately affects their quality during storage (Ellis and Roberts, 1980). High temperature, seed moisture content and relative humidity during storage as well as poor on-farm storage facilities are the key reasons that lead to insect and mold infestation. Insect and pest damage are effectively responsible for most of the decline in quantity, quality, and germination potential of stored seed (Olakojo and Akinlosotu, 2004). In Nepal, grain storage losses due to insect-pests, rodents and mold range from 15-30% annually (K.C., 1992). Thus, knowledge on proper seed and grain storage methods is important to minimize these storage losses (Kibar, 2015).

The use of hermetically sealed bags such as SuperGrain bags (SGB) (GrainPro, 2017) and Purdue Improved Crop Storage (PICS) bags have been reported as alternative storage options to maintain quality of stored seeds and grain for many crops in Africa and South Asia (Afzal et al., 2017; Baoua et al., 2012; De Groote et al., 2013; Murdock et al., 2012; Mutungi et al., 2014; Vales et al., 2014). Hermetic storage refers to a modified atmosphere of low oxygen and high carbon dioxide (CO_2) content created by respiration of living organisms such as insects. It is designed to protect stored agricultural commodities such as seeds, cereal grain, pulses, and coffee (Baributsa et al., 2014; Chigoverah and Mvumi, 2016; Navarro, 2006; Villers et al., 2010).

In Nepal, the storage season for wheat seed (May-October) is wet and humid, with >90% of the annual rainfall occurring during this period. Thus, preventing post-harvest losses while maintaining seed quality is a major challenge for small holder farmers in both the hills and plains. There is a need for economically feasible, less labor intensive, safe (no use of chemicals) and convenient (easy to transport) storage technology that would benefit farmers and reduce losses. The present study was therefore conducted to evaluate the performance of alternative storage devices in maintaining seed quality of wheat as well as being economically competitive with farmers' traditional storage practices in climatically and geographically distinct areas.

Materials and methods

Experimental sites

The experiments were conducted at two sites, Palpa (Madiphat) and Rupandehi (Basantapur and Dhagdahi), selected based on different ecology and climatic conditions. Madiphat lies in the mid-hills of Palpa at an altitude of 800 m, and has a cool but humid climate with annual rainfall of 1513 mm and temperature of 20.5°C on average. Rupandehi lies in central terai plains at an altitude of 99 m, and has a hot and humid climate with annual rainfall of 1762 mm and temperature of 25°C on average. In both areas, over 85% of the total annual rainfall occurs in the four months of June-September (Fig.1) (MoSTE, 2014).

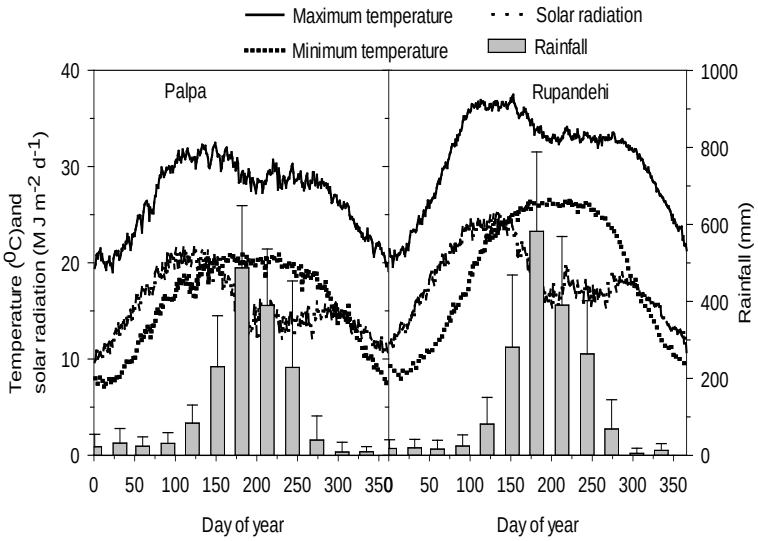

Fig. 1 Daily maximum and minimum temperature, solar radiation and the monthly total rainfall in Madiphat, Palpa and in Rupandehi. Data from long term average from 1987-2013 in Palpa and from 1977-2013 in Rupandehi. The vertical bars are the standard deviation. Source: (MoSTE 2014).

Experimental details and treatments

A participatory experiment was established in ten different wheat cultivating farmers' storage houses each in Rupandehi (terai plains) and Palpa (hills) in 2013. Five types of storage materials were evaluated at each site, i.e. traditional farmers' practice (FP), plastic bag with pesticide (celphus: a common pesticide used for storage pests), plastic bag without pesticide, metal bin and SuperGrain bag (GrainPro, 2017). Individual farmers were considered as replications; hence, each treatment was replicated 10 times in both sites.

Wheat variety NL-297 was used for storage in all the treatments. Prior to storage, the seeds were dried down to at least 12% moisture and then cleaned by removing all the broken seeds and other inert material. The storage duration was six months (May-October), i.e., after wheat harvesting (April) to before wheat seeding (end of October). Twenty kg of seed was stored in each storage treatment except FP, where samples were taken from the farmer's storage method.

The metal bins were fabricated from gauge 24 galvanized metal sheets (0.51 mm) by locally trained tinsmiths and had a seed holding capacity of 30 kg. Plastic bags with dimensions of 80 cm (height) by 50 cm (width) and seed capacity of 50 kg were bought from the local market. Celphus was applied only once to the selected plastic bags at the beginning of the experiment. The SGBs were purchased from Mero Agro Pvt. Ltd. (Kathmandu, Nepal), a local product distributor of GrainPro, Inc. (Zambales, Philippines). These bags are manufactured using high density polyethylene that essentially reduces gas exchange from the stored product. After filling the bag with seed, the empty portion of the bag was squeezed to remove excess air. The opening was then closed by tightly twisting the free portion and sealing it with a special strap fastener provided with the bag. For air-tightness, the top of the bag was twisted twice, folded back and sealed with another fastener. As per recommendations, the SGBs were used as liner bags inside the polypropylene bags, which provide more support and ease in handling. The top of the outer bag was also fastened and sealed in the same manner.

Seed sampling and data collection

After six months of storage, all the treatments were taken out to an open space and the seed inside each was thoroughly mixed. From each storage treatment, two seed samples of ~500 g and ~200 g

were taken to inspect for insect damage and for evaluation of seed germination and vigor, respectively. The samples were kept in clean, labeled plastic bags and transported to the laboratory.

Seed moisture content: It was measured immediately after sampling using a grain moisture meter (GMK-303CF. GrainPro, Inc., Zambales, Philippines).

In the laboratory, a sub-sample of ~200 g was taken to count for insect damage, and their number and weight were recorded. Seeds with holes and cracks in them were considered infested by insects. The count and weigh method, which is the most common method to measure loss by insects and pests in storage (Adams and Schulten, 1978), was used for insect damage assessment.

Determination of seed germination and vigor

One hundred seeds were randomly drawn per household from each treatment for the lab germination test which was conducted at the National Seed Testing Laboratory in Bhairahawa. The seed samples were placed evenly in germination paper and rolled around in it; then, they were wetted with distilled water and placed in an incubator at 25° C for seven days. Moisture was maintained by misting with distilled water as needed.

For the field germination test, 100 randomly drawn seeds from each treatment were sown in line (one treatment per line) in a well-prepared field with sufficient soil moisture at the National Wheat Research Program (NWRP), Bhairahawa. Germination, expressed as a percentage, was indicated by appearance of sprouts for lab seeds and seedlings coming out from the soil for field seeds. The seed vigor represents a more sensitive parameter than the germination test and aims to classify seed with higher probability to perform well after sowing and/or during storage (Marcos Filho, 2015). It was assessed based on germination percentage and seedling length, as suggested by Abdul-Baki and Anderson (1973).

Cost estimation for storage treatments.

The total cost involved in each storage treatment was calculated for storing one quintal of wheat seed. It included the cost of storage material and labor used for drying and storage for each treatment. Further, to understand the farmers' current practice for wheat storage, focus group discussions (FGDs) with 15 farmers in each group were carried out in both Rupandehi (3 FGDs) and Palpa (2 FDGs).

Statistical analysis

Data collected for different parameters were analyzed for analysis of variance (ANOVA) of completely randomized block design, considering farmers as replications using GenStat Version 18. Most variables were not normally distributed (except seed moisture content). Prior to analysis, the variables were transformed to normality using the Johnson Transformation Function of Minitab. Differences in locations and individual treatment effect were analyzed using Fisher's Protected Least Significant Difference (LSD) and treatment differences were considered statistically significant at $p<0.05$. Graphical representations were made in Sigma Plot version.

Results

Current farmers practice of wheat seed storage

From the FGDs, it was found that 70-75 % farmers stored wheat seed in fertilizer bags with 2-3 times sun drying during storage, while about 15-20% farmers' stored seed in metal bin with pesticide in both Rupandehi and Palpa districts. Ten percent of farmers used other storage structures, for example, mud bin, plastic bin, or plastic containers. In FGD, it was reported that 10-90% insect damage (10-80 % in Rupandehi, 15-90% in Palpa) occurred under the farmers' current method of storage in both districts.

Seed moisture content

After six months of storage, seed moisture content increased significantly ($p < 0.001$) from the initial level (~12%) in all treatments, with greater increment in Palpa (12.9-14.8 %) than in Rupandehi (9.3-13.1 %). In Palpa, a significant increase in seed moisture content was observed in plastic bags without pesticide (by 3%), followed by plastic bags with pesticide, metal bins and farmers' practice (by 2%), while that in SGB was <1%. In Rupandehi, the seed moisture content in SGBs and plastic bags with pesticide was unchanged, while it increased by 1% under metal bins and plastic bags with pesticide. Conversely, the moisture content dropped down to 9% under the farmers' storage practice, which could be attributed to frequent sun-drying, higher air temperature, and comparatively low rainfall (Fig. 6; Fig. 1).

Insect damage

Significant treatment effect (p=0.01) was observed in the insect damage levels in both the study sites. In Palpa, insect infestation was the highest in plastic bags without pesticide (14±3%), followed by metal bin storage (9±2%), plastic with pesticide (3.2%), FP (2.4%) and SGB (< 1%) at the end of the storage period. In Rupandehi, seed stored in metal bins had the highest level of damage (10±2%) followed by plastic bags without pesticide and farmers' method (3-4%), while plastic bags with pesticide and SGBs were found to have negligible damage. In both the locations, seed stored in SGBs showed negligible insect infestation (Fig. 2).

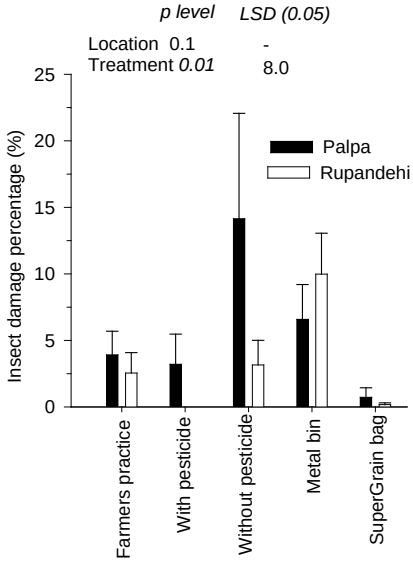

Fig 2. Variation in percent insect damage after six months storage under different treatments in Palpa and Rupandehi.

Seed germination and vigor

Seed obtained from all storage treatments in Palpa showed significantly lower (p=0.02) germination percentage than in Rupandehi, which is a similar trend to that of seed moisture content (Fig. 3A). A significant treatment effect was observed in both lab and field-tested germination percentage in both locations. In Palpa, seed from plastic bags without pesticide showed the lowest germination percentage (63% lab, 43% field), followed by metal bin storage (66.3% lab, 56% field), FP (69.3% lab, 53% field), plastic bags with pesticide (75.2% lab, 51.5% field) and SGBs (90% lab, 68% field) (Fig.3). In Rupandehi, the seed in metal bins showed the lowest germination percentage (67% lab, 50% field), followed by plastic bags without pesticide (81% lab, 54% field), FP (90% lab, 56% field) and

plastic bags with pesticide (89% lab, 65.4% field). The highest germination percentage was observed in SGB storage (92.8% lab, 69% field) as seen in Figure 3.

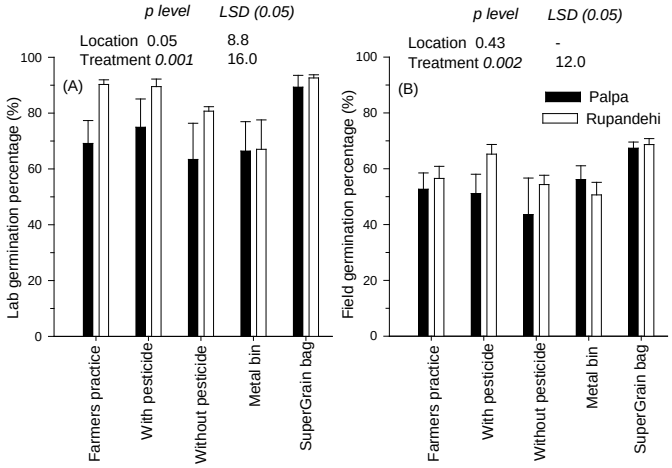

Fig. 3. Variation in laboratory and field germination percentage under different storage treatments in Palpa and Rupandehi.

Similarly, the vigor test resulted is comparable results as the lab germination test for all the storage treatments in the two locations. SGBs showed higher vigor percentage (~80%) than other treatments in both the locations. The seed from FP treatment in Palpa resulted in low vigor (~50%) while that for the other methods ranged from 60 to 70% (Fig. 4).

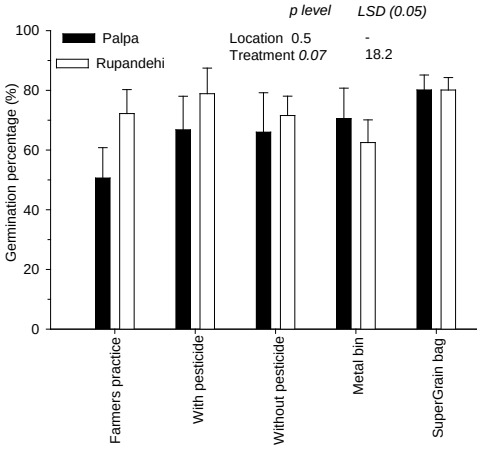

Fig. 4. Variation in seed vigor percentage under different storage method tested in Palpa and Rupandehi.

Cost involved with different storage structures

The total cost to store per quintal of wheat seed under different methods involves the cost of material and labor used for sun-drying, and storage and fabrication (in case of metal bins) of the storage structure. In terms of individual costs, the initial investment with metal bins was significantly higher ($18 per 100 kg) than the other storage structures tested in this study (Fig. 5). The cost of SGBs and FP storage method was $6 and $5 per 100 kg, respectively, which is one-third of the cost of metal bins. Moreover, the plastic bags with and without pesticide cost less than half of the SGBs and farmers' traditional practice (Fig. 5).

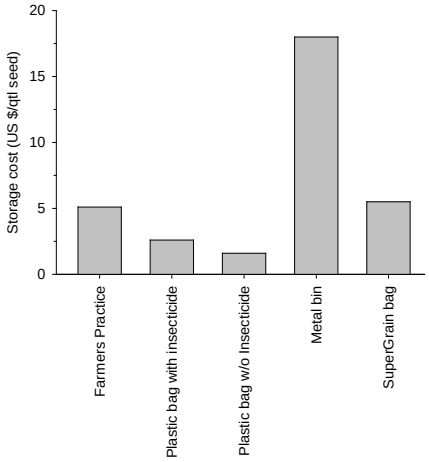

Fig. 5. Variation in cost involved ($/qtl. seed stored) for storing per quantal seed under different storage methods.

Discussion

In the six-month storage period, seed moisture content of all treatments increased. The increment was greater in hills compared to terai plains, which could be related to higher rainfall (Palpa: 2375 mm vs. Rupandehi: 1797 mm), more rainy days (Palpa: 85 days vs Rup: 70 days) and lower average temperature (Palpa: 23° C vs. Rup: 29° C) during storage period (Fig. 6). Interestingly, lower moisture and insect damage, and better germination percentage were observed under the farmers' method in terai plains. It suggests that farmers can maintain seed quality under ordinary storage structures to some extent in high temperature and low rainfall areas if they frequently check and dry their seed and grain during storage.

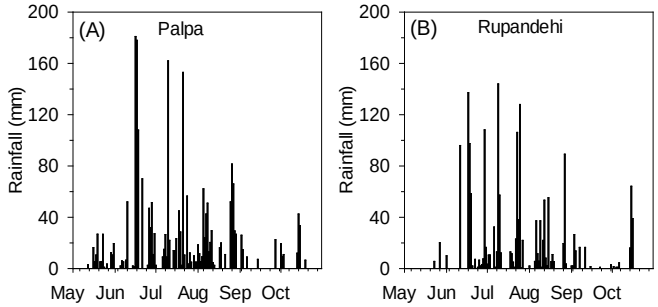

Fig. 6. Daily rainfall in Palpa (A) and in Rupandehi (B) during wheat seed storage period (1 of May to 31 October, 2013).

Seed stored in the SGBs maintained low moisture content with higher seed germination, seed vigor (Fig. 4) and less insect damage (Fig. 2) compared to other storage methods evaluated in both ecologies. It shows that maintaining wheat seed storage quality is more challenging under the ordinary storage method in the hills with higher rainfall and lower temperature than in the terai plains. Further, the higher germination percentage and lower insect infestation under SGB storage was mainly due to maintaining low seed moisture during storage. It is known that every 1 % increase in seed moisture content reduces the seed shelf-life by half (Harrington, 1972). This shows that the hermetic SGBs were mostly successful in preventing moisture migration during storage in both

ecologies, which was also observed in findings from previous work (Edoh Ognakossan et al., 2013; Navarro et al., 2002). In Kenya, Ndegwa et al. (2016) observed that after four months of storage, grain damage was 14% in farmers' storage methods and only 4% in hermetic bags. Baribusta et al. (2014) also found that airtightness of hermetic bags resulted in low oxygen and water vapor permeability, ultimately leading to low grain moisture variation during storage. However, some fluctuation in moisture content was observed in the SGBs, which can be attributed to the loss of air-tightness in storage containers as observed by Chigoverah and Mvumi (2016).

Increased seed moisture content in plastic bags without pesticide in Palpa can be directly linked to higher insect damage (Fig.2), since insects and pests thrive at high moisture conditions. Moreover, the germination percentage for the same was lower than other treatments which can also be ascribed to the higher insect damage. Rodent and insect damage to polypropylene bags has been found to be common in on-farm storage (Ndegwa et al., 2016). The use of a pesticide (celphus) in plastic bags was somewhat effective in reducing insect infestation and maintaining higher germination percentage compared to seed stored in plastic bags without the pesticide. Moreover, the effectiveness of pesticides in controlling insect infestation is well documented (Dales and Golob, 1997; De Groote et al., 2013; Golob et al., 1985). However, use of chemical pesticides in storage may be hazardous if the farmers do not take proper precautions in choosing pesticides and handling them. Again, pesticides may degrade rapidly in tropical climates because of the high temperatures and humidity (Vales et al., 2014).

Metal bins were not able to control insect infestation despite the large investment. The insect damage in the bins was higher compared to other treatments, even the farmers' method (Fig 2), which further led to lower germination (Fig. 3) and vigor of the stored seed (Fig. 4). One of the possible explanations is that the bins were not sufficiently air-tight and the hot and humid climate caused an increase in moisture and in the stress levels of the seed. Another likely reason is believed to be the fact that the bins were only filled up to ~15 kg when the total capacity was 20 kg, which might have left room for enough oxygen for insects to thrive. Hence, it is strongly advised that standardized procedures such as filling grain or seed up to the brim are followed, and artisan trainings are provided during metal bin fabrication to ensure airtightness of the containers. Chigoverah and Mvumi (2016) found that non-standardization of metal bin fabrication procedure, subjective air-tightness testing, and varying artisan experience may result in variable field performance of the technology. They observed loss of airtightness especially at joints near the inlet.

The cost involved for storing seed in SGB was comparable with the farmer's method (Fig. 5) and cheaper than the metal bin, which shows that it can be economical and potentially affordable to the small to medium farmers. For large-scale adoption of hermetic bags, farmers must know or be made aware of the technology, which leads to them expressing their demand for it. It would help if the farmers are in easy geographical reach to make the bags affordable in a sustainable way. Moreover, further communication, awareness programs, and trainings are recommended to inform extension workers and farmers on the importance of drying their seed and grain prior to storage as well as the benefits of hermetic storage. Ndegwa et al. (2016) reported that under basic price, hermetic bags become potentially profitable if they last for four seasons or more, when used for at least four months per season. Among the several hermetic storage bags available in the market, it is important to evaluate their efficiency and durability to maintain seed/grain quality and economics for storing different crops.

Acknowledgements

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Hermetic storage - an ecofriendly safe storage method for long term storage of black gram

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Abstract

India is the primary origin of the black gram that is majorly cultivated in the southern part of Asian countries. About 70% of world black gram production comes from India. Black gram is more prone to insect infestation and microorganisms resulting in deterioration of grain quality. These losses can be controlled by following appropriate storage method at farmer's level. Eco-friendly, safe storage methods are demanded by the customers due to food safety, quality and environmental issues. Hermetic storage is a safe storage method, suitable for long term storage without usage of chemical pesticides. It creates an air tight environment to rapidly exterminate insect development and suppresses micro floral activity. A study was conducted to identify the suitable, cost effective storage method for safe storage of black gram at the farm level. Hermetic bags were made by using different combinations of gunny, polypropylene & storezo bags for the safe storage of black gram. The properties of packaging materials viz., thickness, and water vapour transmission rate significantly affected the quality parameters of the black gram stored in various bags. Moisture content, thousand grain mass, bulk density, insect emergence, and germination percentage of black gram stored in various bags were studied over a storage period of 12 months. Black gram stored in polypropylene and gunny bags was infested with pulse beetle by the third month of the storage period. But black gram stored in bags with hermetic bag as inner layer was not infested up to 12 months and could retain the grain quality.

1. Introduction

India is the leading producer and consumer of pulses. The total pulse production in India significantly increased to 22.14 million tons during 2016-17 which is 2.89 million tons higher than the previous production of 19.25 million tons achieved during the year 2013-14 (Anonymous, 2018). About 70% of the total production is stored by the farmers. Due to insufficient and poor storage facilities, lack of knowledge in post harvest pulse management and storage, the risk of damage due to post harvest losses are huge up to 25-50% (Lal and Verma, 2007). During storage, pulse beetle (*Callosobruchus chinensis Linn*) attacks the pulse seeds and causes about 5-10% losses. The most important factors that cause grain deterioration are the interaction of temperature and moisture, which are the determining factors in accelerating or delaying the complex degradation reactions. In India, grains are generally stored in gunny bags which are inexpensive, reusable with good inherent toughness but high permeability and low resistance to insect and rodent attack results in frequent application of pesticides to prevent infestation (Maina et al., 2016). Consumer resistance against the use of chemical fumigants in stored products and international trade treaties increase the focus to find green and residue free technology. The main objective of the study is to develop an alternative ecofriendly safe storage method for controlling bruchid infestation in stored pulses. As there are no standard methods available for storing black gram at farmer's level, the present study was carried out to monitor the quality changes of black gram when stored under hermetic condition. Hermetic bags create modified atmospheres by increasing carbon dioxide concentration and decreasing oxygen due to the respiration metabolism of insects and aerobic microorganisms present in grains. Hermetic bags are less permeable, made up of a blend of polymers with LDPE (Low-density polyethylene) as inner liner which prevents the growth of microorganism and insect attack during long term storage. Hence, hermetic, gunny and polypropylene (PP) bags were used for the storage of black gram. Moisture content, thousand grain mass, bulk density, insect emergence, and germination percentage of black gram stored in various bags were studied over a storage period of 12 months.

2. Materials and Methods

Experiment was conducted at IIFPT, Thanjavur (Tamil Nadu) for the safe storage of black gram. The black gram variety ADT-5 was procured from National Pulse Research Centre, Vamban (Tamil Nadu) with an initial moisture content of 11.5% wet basis (w.b). The initial moisture content of the black gram was determined by hot air oven method at 135°C for 2 h. The aim of the study is to evaluate the performance of hermetic bags for the safe storage of black gram. Gunny, polypropylene, hermetic storezo bags (50 kg capacity) and their combinations were used for the storage of black gram (Table 1). Here, gunny bag was considered as control for the experimentation. Each treatment was replicated thrice and the experiment was conducted for a period of 12 months. Changes in grain quality parameters were analyzed at the beginning and at the end of the storage period. The following combination of bags was used for the study as given in Table 1.

Tab. 1 Treatment of bags chosen for experiment

Treatments
T_1 - Storezo+ Polypropylene
T_2 - Storezo+ Gunny
T_3 - Storezo+ Polypropylene+ Gunny
T_4 - Polypropylene+ Gunny
T_5 – Storezo
T_6 – Polypropylene
T_7 – Gunny

Properties of packaging materials

The thickness of the different packaging materials was determined using screw gauge. The water vapour transmission rate of the gunny, polypropylene and storezo hermetic bags was determined by the method followed by Othman et al. (2017) with slight modifications. A small cup was used to determine the water vapour transmission rate of the packaging material. The packaging material was initially made into circular shape that was slightly larger than the inner diameter of the cup. Then, the cup was placed on a horizontal platform. Known amount of anhydrous Calcium Chloride as a desiccant was placed inside the cup. Subsequently, the packaging materials were placed on top of the permeability cup (Talja et al. 2007). The cup was then covered and sealed with paraffin and weighed in order to calculate the initial weight. The weight of the cup was recorded every 24 hours for 3 days. The water vapour transmission rate (WVTR) was determined using equation 1:

$$WVTR = \frac{\Delta m}{\Delta t A} \quad \text{..... (1)}$$

Where Δm/Δt is the moisture gain weight per unit time (g/day), A is the exposed surface area of the film (m^2)

Moisture content

The moisture content of black gram was determined by Oven-drying method (AOAC, 2005; no.930.15); ground black gram was dried at 135°C for 2 h.

Thousand grain mass (M_{1000})

The thousand grain mass was measured by weighing 1000 whole grains in a properly calibrated electronic balance.

Bulk density

It is the ratio of mass of black gram to its total volume. A 250 ml graduated cylinder filled with black gram was tapped for the seeds to consolidate. The weight and volume occupied by the black gram were recorded and the bulk density was calculated using equation 2:

$$\rho_b = \frac{W_s}{V_s} \quad \text{..... (2)}$$

Where ρ_b is the Bulk density (kg m^{-3}), W_s is the Weight of the sample (kg), V_s is the Volume occupied by the black gram (m^3).

True density

The true density was determined using the liquid displacement method. Here, toluene was used as a displacement liquid instead of water to prevent absorption. 10 g of black gram were immersed in 50 ml of toluene in a 100 ml graduated cylinder. The amount of toluene displaced was recorded from the graduated scale of the cylinder. The ratio of the grain weight to the true volume of displaced toluene gives the true density of the black gram.

Porosity

The porosity of the bulk grain was determined from the true and bulk density of the grains using equation 3:

$$\varepsilon = \frac{\rho_t - \rho_b}{\rho_t} X\ 100 \quad \text{..... (3)}$$

Where ε is the porosity (%), ρ_b is the bulk density (kg m^{-3}) and ρ_t is the true density in (kg m^{-3}).

Germination percentage

Germination is a key index to test the seed viability of stored grains. One hundred seeds of black gram were soaked in distilled water for 24 h, put on a germination paper placed inside a Petri dish, and incubated at 25°C. The filter paper was moistened every day using fresh water to facilitate germination (Gupta et al. 2010). The ratio of the number of seeds germinated after seven days of incubation to the total number of seeds kept for germination were recorded as germination percentage.

3. Results

Properties of packaging materials

The thickness of the packaging materials is expressed in terms of microns as shown in the Table 2. The water vapour transmission rate (WVTR) is the rate of water vapour permeating through the packaging material. The WVTR is determined from the slope of the regression line of the sample weight versus time and the slope is then divided by the area of the film being exposed to the transmission (Equation 1). Table 3 shows that the least WVTR was observed in Storezo hermetic bags with 1.57 g/m^2 day, followed by polypropylene and gunny bags with 6.69 and 25.85 g/m^2 day, respectively.

Tab. 2 Packaging material properties

Properties	Storezo bags	PP bags	Gunny bags
Thickness (microns)	75.21±0.95	80.41±1.91	1346.67±27.22
Water vapor transmission rate (g/m^2 day)	1.57±0.56	6.69±1.65	25.85±2.12

Grain moisture content

Black gram moisture content when stored in different bags was determined and the variations in moisture content of black gram during storage are given in the Table 3. In T_7 (gunny) and T_6 (polypropylene) bags the moisture content was increased from the initial moisture content of 11.5% to 12.57% and 12.26%, respectively, because environmental factors such as temperature and relative humidity significantly affect the black gram stored in gunny and PP bags due to its higher permeability. There was no significant difference observed in the black gram moisture content

when stored in treatment T_3. Thus, the change in moisture content of stored black gram depends on the permeability of the bags used for the storage. From the study conducted in laboratory, it has been confirmed that the water vapor transmission rate of gunny bag was higher followed by polypropylene and storezo hermetic bags. Hence, less permeability in hermetic bags helps in the retention of the grain moisture content.

Thousand grain weight

The thousand grain weight of black gram stored in different bags varied from 43.22 to 41.93g. Black gram moisture content and infestation significantly affect the thousand grain mass because *Callosobruchus chinensis* feed on the endosperm portion of the black gram during infestation which results in a decrease in grain weight. The infested grains were sieved to separate grains from insects and grains dust. From the separated grains thousand grain mass was determined.

Bulk and true density

The bulk (ρ_b) and true (ρ_t) density of black gram stored in different bags are given in Table 3. The initial bulk density of black gram was 729 kg m^{-3}. After 12 months of storage period, it has been observed that maximum ρ_b was observed in the treatment T_3 with 697.42 kg m^{-3} followed by T_1 and T_5 with 688.69 and 687.54 kg m^{-3} respectively. The least bulk density was found in T_7 with 601.76 kg m^{-3}. The decrease in bulk density is due to emergence of insects from the stored black gram which results in weight loss. Thus, change in grain mass is directly proportional to bulk density.

The true density of initial black gram was recorded as1230.00 kg m^{-3}. At the end of storage period, true density decreased with the decrease in black gram moisture content. Similarly, in the infested treatments T_4, T_6 and T_7, the true density decreased to 1190.25, 1115.56 and 1108.00 kg m^{-3} respectively.

Porosity

The initial porosity of black gram was 40.73%. The porosity is inversely proportional to change in bulk density and true density of black gram. From Table 3, it has been observed that the maximum porosity was observed in the treatment T_7 with 45.69% followed by T_6 and T_4 with 44.86 and 43.80%, respectively. Hence, it is confirmed that the change in grain moisture content and insect infestation significantly affect the porosity.

Germination percentage

The initial germination percentage of black gram was 94.67%. There was significant difference ($P < 0.05$) between initial and final germination percentage of the black gram in all the treatments. After eight months of storage, the germination rate decreased in treatment T_7 followed by T_6 and T_4 with 77.67, 80.00 and 84.00 %, respectively. Maximum germination percentage was found in treatments T_3 and T_2 with 87.67% and 84.67%, respectively. Germination of black gram decreased with the increase in storage period. These changes may be due to variation in moisture content, moisture loss, and emergence of insect during storage. The germination percentage of black gram stored in PP (T_6) and gunny bags (T_7) decreased more due to emergence of insect from the stored commodity.

Insect emergence

Insect emergence from the black gram stored in various treatments was monitored regularly. *Callosobruchus chinensis* is a predominant internal feeder pest that infects pulses and affects the germination ability and nutritive value of the black gram seed during storage. No infestation was observed in the black gram stored in different treatments where hermetic bag is used as an inner layer, while black gram stored in gunny bag alone (T_7) starts to get infested from the third month of the storage period with 25.46% infestation at the end of the storage. Similarly, black gram stored with PP (T_6) and PP and gunny (T_4) got infested in the fourth and tenth month, with 19.87% and

5.16% of infestation respectively. Hence, black gram moisture content and packaging material permeability significantly affect the quality of the stored commodity.

Tab.3. Effect of different treatments of bag storage on black gram properties.

Treatments	Moisture content (w.b.%)	Thousand grains Mass (g)	Bulk density (kg m^{-3})	True density (kg m^{-3})	Porosity (%)	Germination (%)	Insect emergence (%)
Initial	11.50	43.22	729.00	1230.00	40.73	94.67	Nil
T_1	10.29	42.56	688.69	1205.00	42.85	83.67	Nil
T_2	10.55	42.83	685.12	1211.00	43.43	84.67	Nil
T_3	10.79	43.09	697.42	1224.00	43.02	87.67	Nil
T_4	11.62	42.26	668.89	1190.25	43.80	84.00	5.16
T_5	10.18	42.44	687.54	1194.48	42.44	82.33	Nil
T_6	12.26	41.97	620.66	1125.56	44.86	80.00	19.87
T_7	12.57	41.63	601.76	1108.00	45.69	77.67	25.46

4. Discussion

The *Callosobruchus chinensis* is one of the most serious pests infesting stored pulses in India. Saleem (1982) reported that *C. chinensis* causes serious damage to the pulses majorly in Bangladesh, India and many countries of the world. It causes greater damage to the pulses during storage which leads to heavy economic losses. The results coincide with the results of Mutungi et al. (2014), who report that grains stored in hermetic bags will prevent change in grain moisture content. Murdock et al. (2012) confirmed that triple layer hermetic bags are more effective in protecting grains from insect infestation. Munde (1999) reported that the increase in thousand grain mass is due to increase in moisture content for black gram. Theertha et al. (2014) found that the bulk density of black gram decreases with increase in moisture content. According to Pandiselvam et al. (2014), it is confirmed that there is a proportional relationship between grain moisture content and porosity. This might be due to increase in shape and size with respect to increase in moisture content.

Farmers in India use primitive gunny bags as a grain storage material. But within 3 months of storage period the quality of the grains gets deteriorated due to improper storage condition, which leads to insect infestation and fungal attack. To overcome these issues, the study was conducted by using storezo hermetic bag as an inner layer in multilayer bag for storage practice in godowns and at farmer's level. From the results it is evident that grain quality can be retained and insect infestation can be prevented by adopting hermetic storezo bag as an inner liner in multilayered bag storage.

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Hermetic storage of dry soybean (Glycine max): creating an effective modified atmosphere using soaked grain as O_2 depletor

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Abstract

Hermetic storage of grains and oilseed has been proposed as a solution for reducing food losses in developing countries. However, to obtain full benefit of the hermetic storage it is required to achieve a low O_2 concentration (below 2%) or high CO_2 concentration (above 20%). The gas concentration inside the hermetic container is the result of the balance between the respiration and gas exchange rates with the outside (permeability and leakage). When the grain is dry, an insufficient modification in the internal atmosphere is achieved (exchange rate higher than respiration rate), allowing insect development and, hence, grain losses. This study focuses in creating an effective modified atmosphere during the hermetic storage of dry soybean using soaked grain as O_2 depletor. Three big bags with internal polyethylene liners of 70 µm thickness were filled with 590 kg of soybean (Glycine max, with 12.5% m.c.) and sealed. Gas concentration evolution was measured during 15 days (basal condition). Later, four plastic perforated bottles filled with of 4.3 kg of soaked soybean (44% m.c.) were inserted in each bag. The bags were re-sealed and gas concentration was measured during 45 days. Results indicated that the soaked soybean acted as an O_2 depletor, reducing the gas concentration to 1% in only 8 days, and maintained below 1% during 45 days. This research indicated that a small portion of soaked grain (0.4% dry matter (d.m.)) can be used to generate an effective modified atmosphere to prevent biological activity in the entire grain mass. This is a simple and inexpensive approach to reduce food losses under low cost hermetic storage.

Keywords: food losses; pest control; controlled atmosphere; respiration; gas leakage

Introduction

Hermetic storage of grains and oilseed has been proposed as a solution for reducing food losses in developing countries. However, to obtain full benefit of the hermetic storage it is required to achieve a low O_2 concentration (below 2%) or high CO_2 concentration (above 20%) (Navarro et al., 2012). The gas concentration inside the hermetic container is the result of the balance between the respiration and gas exchange rates with the outside (permeability and leakage). When the grain is dry, an insufficient modification in the internal atmosphere is achieved (exchange rate higher than respiration rate) (Abalone et al., 2011a, 2011b), allowing insect development and, hence, grain losses.

The use of liners with gas barrier is an alternative to improve the effect of hermetic storage systems, since the O_2 entrance and CO_2 losses are strongly reduced. This creates an internal atmosphere with a higher modification level than standard liners, with potential conservation benefits (Cardoso et al., 2016). However, liners with gas barrier are expensive and it might not be affordable for family storage systems. Additionally, small perforations in the liner (as small as 1 mm) could eliminate the

benefit of the barrier (Abalone et al., 2011a), preventing the conformation of an effective modified atmosphere.

Injecting CO_2 or N_2 to create an effective atmosphere from the beginning of storage is a widely used technique, usually known as controlled atmosphere (CA) (Navarro, 2012). Carpaneto et al. (2016) implemented CA treatments injecting CO_2 in flexible liners storage systems (silo bags), and concluded that airtightness level is critical to maintain a lethal gas concentration. The lethal atmosphere can be lost in a few days after injection due to gas leaks through small perforations and would fail to achieve insect mortality. A survey made in the field reported that the airtightness level of silo bags is quite variable, due to perforations in the liners from wild animal activity or problems in the sealing (Cardoso et al., 2012). Thus, placing storage systems made of flexible liners in the field might result in perforations that could compromise the effect of the CA treatment.

One alternative is to incorporate an O_2 depletor in a storage system made of a standard liner to speed up the conformation of a lethal atmosphere, even storing dry grains, and also consume the O_2 that is entering by permeability through the linear or through the small perforations. Thus, this study focuses in creating an effective modified atmosphere during the hermetic storage of dry soybean in a big bag using soaked grain as O_2 depletor.

Methodology

1800 kg of healthy and fresh soybean with 12.5% moisture content (m.c.) was bought from a local grain elevator in August 2017. Soaked soybean was obtained by sinking dry soybean seeds in distilled water for 60 minutes. After removing the superficial water with towel paper, the m.c. of the soaked soybean was determined by the oven method (15 g of soybean at 104°C during 72 hs) (ASAE, 2007).

Respiration soaked soybean was characterized. Samples of 50 g of soaked soybean were placed in glass jars of 225 ml, sealed were incubated in a temperature control chamber at 21.8 °C during 7 days. Jars were opened, ventilated for one hour, re-sealed and stored again in the temperature chamber. CO_2 and O_2 concentrations were measured every 1.5 hours until 6 hours with a portable gas analyzer (Checkpoint, Dansensor, Denmarck). Respiration rate in terms of O_2 consumption and CO_2 generation was computed according to the procedure described in Ochandio et al. (2017) (the respiration rate of dry soybean reported in this publication was used as reference).

Three big bags with internal polyethylene liners of 70 micrometers thickness and dimensions of 1.0 m x 1.0 m x 1.8 m were filled with 590 kg of 12.5% m.c. soybean, with a total exchange area for gas permeability of 4.07 m2. After filling, the big bags were thermo-sealed with portable sealing equipment, and airtightness was tested by a pressure decay test (PDT). The PDT consisted of generating a negative pressure inside the big bag of 1200 Pa with a vacuum pump (Dosivac, DV 95, Argentina) and measuring the time at which the pressure dropped to half the initial value (Navarro, 1998). Following this, hermeticity was restored and CO_2 was injected until an average concentration of 60% CO_2 was achieved. CO_2 and O_2 concentrations were measured once a day for 10 days and permeability of CO_2 and O_2 was calculated. Additionally, samples of plastic liners were sent to the Science and Technology Polymers Laboratory (PLAPIQUI, CONICET-UNS, Bahía Blanca, Argentina) for O_2 standard permeability analysis.

Later, the big bags were opened and internal atmosphere was allowed to equilibrate with ambient atmosphere, and big bags were sealed again. Gas concentration evolution was measured once a day for 15 days to obtain the basal O_2 and CO_2 concentration for dry soybeans. After this characterization, four plastic perforated bottles filled with 4.3 kg of soaked soybean (44% m.c.) were inserted in each bag (2.4 kg dry matter) (Figure 1). The soaked soybeans were placed inside plastic bottles to prevent the contact (and spoilage) with the dry soybean. Additionally, the bottle was perforated to allow the free gas exchange between the inside and outside. The bags were re-sealed and gas concentration measured during several days of storage with a portable gas analyzer.

Figure 1. Plastic bottle perforated and filled with soaked soybean.

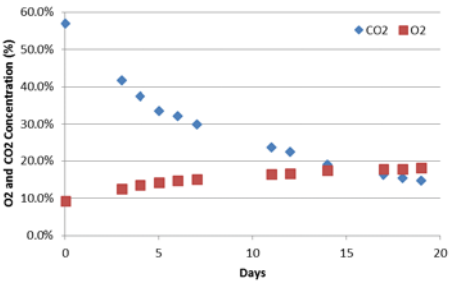

Figure 2. O_2 and CO_2 evolution inside a big-bag injected with CO_2 until 60% of concentration.

Results

Soybean respiration

The respiration rate of soaked soybean in comparison to dry soybean was about five thousand times greater in terms of O_2 consumption and eleven thousand times greater in terms of CO_2 generation (Table 1).

Table 1. Respiration rates of dry and soaked soybean.

Soybean moisture content (w.b.)	Respiration rate (mg/(kg d.m. d))		Source
	O_2	CO_2	
Dry (12.5 %)	1.465	0.617	Ochandio et al. (2017)
Soaked (44 %)	7418	6947	This study

The total O_2 consumption and CO_2 generation of the 590 kg of soybean with 12.5% m.c. (516 kg d.m.), were 1321 cc/day and 202 cc/day, respectively; and for the 4.3 kg of soaked soybean with 44% m.c. (2.4 kg d.m.) were 26870 cc/day and 9148 cc/day, respectively. This implies that the contribution to O_2 consumption of the soaked soybean was 20 times greater than the contribution of the dry soybean, while the contribution to CO_2 generation of the soaked soybean was 45 times greater than the contribution of the dry soybean (Table 2).

Table 2. Daily O_2 consumption and CO_2 generation of the dry (516 kg d.m.) and soaked (2.4 kg d.m.) soybeans (cc/day).

Gas	Dry soybean (12.5% m.c.)		Soaked soybean (44% m.c.)		Total
	(cc/day)	% of total	(cc/day)	% of total	(cc/day)
O_2 consumption	1321	4.7	26810	95.3	28131
CO_2 generation	202	2.1	9148	97.9	9350

Pressure decay test and permeability of the big bags

Results of the PDT for the three big bags were higher than 5 minutes. According to Navarro (1998), an hermetic structure 95% full should have a PDT of 3 minutes to be suitable for CA treatments and 5 minutes for modified atmosphere storage. However, Carpaneto et al. (2016) speculated that the threshold set by Navarro (1998) might be too strict for flexible grain storage systems, since there is no head space and, theoretically, no leakage of gas occurs as result of pressure release. Nevertheless, the results of the PDT indicated that there were some small leakage, which should be considered to estimate the gas exchange between the inside and the outside of the big bag, besides the permeability through the plastic liner.

The effective permeability of the plastic liner (taking into consideration the permeability through the plastic liner plus the gas exchange occurred through small openings) was calculated. Figure 2 shows the O_2 and CO_2 evolution after injecting CO_2 gas in the big bag up to a concentration of 60% of CO_2 and 10% of O_2. The rate loss (cc/(m^2 d Δ[%])) was derived from this figure, and linear models

for O_2 and CO_2 effective permeability were obtained (Figure 3). The permeability to both gasses was similar, 6941.3 and 7545.2 cc /(m^2 day atm) for CO_2 and O_2, respectively. The result of the standard permeability test of the liner for O_2 (without perforations) was 1434 cc /(m^2 day atm), indicating the effect of small perforations on the effective permeability of the system, which could quintuplicate the permeability of the liner. Abalone et al. (2011a) studied the effect of perforation in the effective permeability of flexible plastic liners (silo bags) and concluded that perforations provide non-selective permeation of gases, d.m. and also caused a significant modification in the internal atmosphere (although the effective permeability value of liners with perforation was not reported).

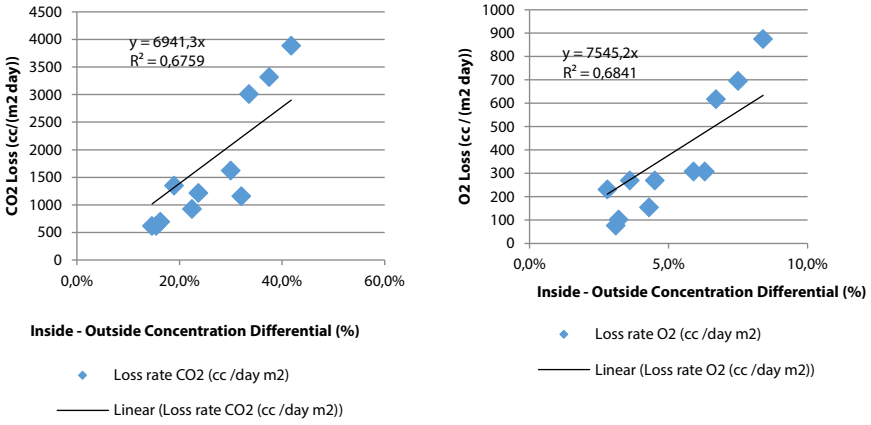

Figure 3. O_2 and CO_2 permeability rate (cc/(m^2 day). Note: the intercept of the linear model was set to cero.

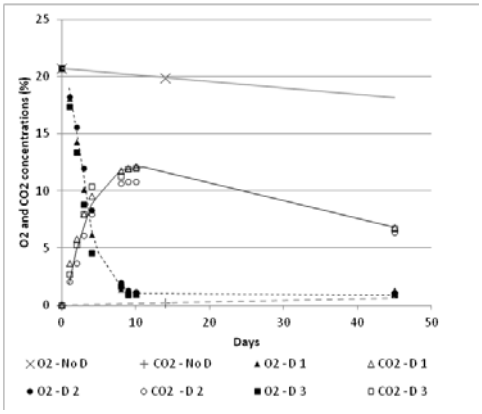

Figure 4. O_2 and CO_2 concentration inside the big bags with (D 1; D 2; D 3) and without (No D) O_2 depletor.

Figure 5. Plastic bottle with soaked and spoiled soybean after 45 days of storage.

Gas concentration inside the big bag with and without O2 depletor

Gasses concentration when dry soybean was stored in big bags did not change during 14 days of storage, being the O_2 and CO_2 concentrations of 19.9% and 0.2% respectively. This is because the respiration rate of dry soybean was low in comparison with the permeability of the big bag (Table 1). However, when the soaked grain was inserted inside the big bag, the respiration rate of the grain was high enough to create a substantial modification of the internal atmosphere (O_2 consumption and CO_2 generation increased from 20 to 45 times, respectively (Table 2)). The O_2 concentration dropped at a rate of 2.5 percentage points per day, reaching a concentration of 1% after 8 days and

maintaining such a low O_2 level until 45 days of storage, while the CO_2 concentration increased up to 12% at 8 days of storage and slowly decreased to 7% at 45 days (Figure 4).

After 45 days of storage the big bags were opened and the plastic bottles with the soaked and spoiled grain were removed. The grain inside the plastic bottles was completely spoiled, while the rest of the grain was without any visible damage (Figure 5). However, a strong smell to spoiled grain was detected and it was necessary to ventilate the grain to partially remove the odors.

Discussion

The key point to achieve an effective hermetic storage is to create an internal atmosphere with low O_2 concentration (below 2%) to prevent insect development and reduce microbial activity. For most insect species, 15 days of exposure to O_2 concentration below 2% would be enough to achieve 100% mortality.

Hermetic storage of dry products has some limitations due to the low respiration rate. In most cases, the respiration rate is about at the same magnitude order (or lower) than the permeability of the liners (or rigid hermetic structure), thus the system reach an equilibrium at a O_2 and CO_2 concentrations that is not effective for preventing insect or mold activity. Only if the insect or mold activity increases substantially, respiration rate surpass the permeability rate and an effective internal atmosphere is achieved. However, when this occurs, uncontrolled quality deterioration in the stored product is observed.

One possible solution for this problem is to incorporate liners with O_2 barrier, which extremely reduce the permeability to O_2 and allow achieving an effective atmosphere even when the respiration rate of the dry product is low. However, liners with O_2 barrier are not always available and also are more expensive, which prevent its use in low cost family storage systems. Additionally, regardless the permeability of the liner, there is gas exchange through small perforations or micro-failures in the sealing (most plastic liners are fragile and, during storage, wild and domestic animals can cause damage) substantially increasing the effective permeability of the liner. Thus, it is difficult to guarantee a low effective permeability during storage, even if O_2 barrier liners are used.

Other alternative to achieve an effective internal atmosphere is to incorporate O_2 depletor to consume the O_2 that is entering into the storage system. In this study, 2.4 kg (d.m.) of soaked soybean (44% m.c.) in 516 kg (d.m.) of dry soybean (12.5% m.c.) reduced the O_2 concentration to 1% in less than 10 days, and after 45 days of storage the O_2 concentration did not change. Such a low O_2 concentration is effective to control insects (Navarro et al., 2012) and would also reduce microbiological activity (Ochandio et al., 2017). The results of this study indicated that with a controlled loss of 0.46% of d.m. a safe storage condition can be achieved. However, even though the modification in the internal atmosphere was satisfactory, a strong smell to spoiled grain was detected after 45 days of storage inside the big bag. Using a chemical compound as O_2 depletor could be a better solution. In this case the chemical compound should react with the O_2 without generating toxic bi-products neither unpleasant odor. Taking as an example a big bag full with 590 kg of soybean, it would be required to consume 66080 cc of O_2 (86.45 g) to reach 0% concentration. Additionally, to maintain 0% concentration during 15 days it would be necessary to consume 6030 cc/day (7.9 g) extra of O_2 per day to compensate the permeability of the big bag. Thus, the O_2 depletor must be able to capture a total of 156530 cc (205 g) of CO_2 to reach and maintain 0% of O_2 during 15 days. If such chemical product is identified, effective hermetic storage of dry grains in big bags could be implemented using standard polyethylene liners.

Conclusions

The incorporation of 2.4 kg (d.m.) of soaked soybean in a big bag containing 590 kg of dry soybean (12.5% m.c.) reduced the O_2 concentration to 1% in less than 8 days. Additionally, the O_2 that permeated into the system was consumed by the soaked soybean at the same rate, maintaining the O_2 concentration below 1% after 45 days of storage.

A small portion of soaked grain (0.4% d.m.) could be used as O_2 depletor to create an effective modified atmosphere during storage of dry products in hermetic systems made of liners without O_2 barriers or with small perforations.

This is a simple and inexpensive approach to reduce food losses under low cost hermetic storage systems.

O_2 depletors made of chemical compounds could be investigated to obtain the same results as using soaked grain, but without generating unpleasant smell.

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Biocidal efficacy of nitrogen (anoxic atmosphere) applied in operational condition to stored hazelnuts against pest insects at different stages of development.

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Abstract

Recently, a test was conducted in Italy for the evaluation of the biocidal efficacy of Nitrogen saturation (anoxic conditions). One application was carried out in a controlled atmosphere cell of a logistic center specialized in receiving, storing and shipping foodstuffs. The cell, circa 3682 m^3 volume, with capacity of 752 big bags of fresh shelled hazelnuts on 4 height levels was saturated with Nitrogen (99,9%) and maintained at 15-18°C for 21 days. Five test species of insects *Plodia interpunctella*, *Cadra cautella*, *Corcyra cephalonica*, *Tribolium confusum*, *Oryzaephilus surinamensis* were observed at different development stages (egg, larva, adult). The target species were sorted in special biotest and inserted in the big bags to simulate an infestation. At the end of the exposure period the biotests were collected and analyzed. The treatment resulted sufficient to achieve a total control on eggs of Lepidoptera test species only. This result confirmed and integrates the available information in literature that showed the need of a longer minimum exposure period for total control of common stored pest insects.

Keywords: stored pest insects, nitrogen, anoxic atmosphere, entomological biotest.

Introduction

As a result of the ban to use of the methyl bromide and the necessity to use control techniques with minimum impact on men and the environment, the attention was increased in treatments that do not involve the use of biocidal molecules (Navarro, 2006; Fields, *et al.,* 2002; Fleurat-lessard, 1990). In recent years new technologies have been developed to increase the efficiency and effectiveness of physical control methods such as modified atmospheres (Conyers and Bell, 2004). Modified or controlled anoxic atmospheres, including nitrogen, are among the most promising non-toxic alternatives for control of stored product insects and mites in many types of dry stored products (Aulicky *et al.,* 2016). The same authors also reported that ten days of exposure to a concentration of 99% N_2 led to 100% mortality of all adults of *Tribolium castaneum* (Herbst) and *Sitophilus granarius* (L.) at two different level in a metal silo bin. A study in grain at less than 12% moisture, 23°C with 98-100% N_2 concentration, showed that 28 days were needed to kill all the insect pests; while to reach the same insecticidal effect at 18°C the treatment lasted 105 days (Jian *et al.,* 2016).

The present work is aimed to providing data support to avoid phosphine in the process of stocking fresh hazelnuts, verifing that the biocidal effect of the exposure to 99.9% concentration of N_2 for 21 days at 15-18°C temperature is sufficient to ensure total control on the common pests of stored food, in particular of shelled hazelnuts, by evaluation on alive insects at different stages of development immersed within special probes, here named biotest, in 58 big bags that were homogeneously sorted in the cell space.

Materials and Methods

Insects

The insects used as test species were provided by Agroblu Laboratory of Applied Entomology (LEAA), where they were raised at 26 ± 2 °C, 70% RH and photoperiod light-darkness 16:8.

The test organisms used were typical insects infesting hazelnuts such as Plodia interpunctella (Hübner), Cadra cautella (Walker), Corcyra cephalonica (Stainton), Tribolium confusum (Jaqcquelin du Val) and Oryzaephilus surinamensis (Linnaeus) at different stages of development (egg, larva, adult).

Per each of the 58 test unit (big bag), one group of 7 Biotest was prepared and provided (table 1).

Tab. 1 Species, stages and substrates used for the test.

Insect	Stage	Quantity	Substrate
P. interpunctella	Eggs	50	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran
P. interpunctella	Larvae	10	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran
C. cautella	Eggs	50	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran
C. cautella	Larvae	10	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran
C. cephalonica	Eggs	50	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran
T. confusum	Mix population	20 eggs 5 larvae 5 adult	Semolino, brewer's yeast, bran
O. surinamensis	Mix population	20 eggs 5 larvae 5 adult	Honey, glycerin, white flour, semolino, yellow flour, oatmeal, sesame, bran

Substrates

Biotests containing adult insects were prepared with non-infested substrate normally used for breeding. In order to ensure the presence of all insects stages at the same time in biotest containing *Coleoptera,* 10 adults were transferred from the breeding to 10 grams of uninfested substrate 21 days before introduction in the big bags.

Insects were prepared in containers suitable for being inserted into big bags. At the same time, control biotests were prepared and transported with all the others but were kept away from the treatment in order to verify possible mortality during transport. An equal number of control biotest were left at LEAA at 26 ± 2 ° C; 70% HR; L: B = 16: 8.

Test site data

The site selected for the test was a logistic center with climatic cell. The cell has a controlled atmosphere permanent plant, capable of extracting oxygen and pumping nitrogen to reach 99.9% saturation of nitrogen at a chosen range of temperature (15-18°C). Such range of temperature was chosen to achieve a minimum pest development condition. This cell has 752 pallets capacity, sorted in 9 lines of 8 units, each replicated by 4 vertical levels (Fig. 1). The total dimension of the cell is 27.8 m length, 15.14 m width and 8.60 m height.

Fig. 1 The cell structure.

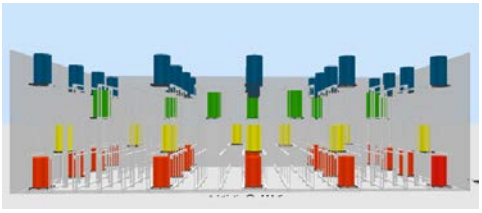

Fig. 2 The 58 big bags sorted in all cell levels from side 1

Test System and application

The test system is characterized by 58 fresh shelled hazelnuts big bags. Each big bag was considered as test unit and was sorted in a specific position in the cell to ensure homogeneus distribution in the cell at all 4 levels. (Fig. 2 and 3).

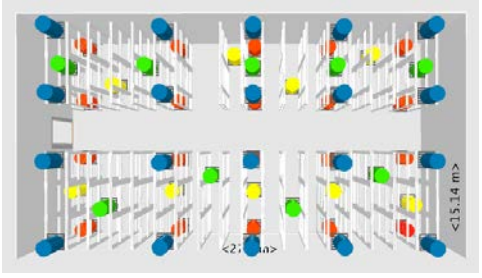

Fig. 3 The 58 big bags sorted in all cell levels from top

Fig. 4 Average temperature registered during the test in the treatment chamber

The test started when the N_2 saturation reached 99.9%. After 21 days of complete and constant N_2 saturation, the desaturation process started.

At the end of de desaturation process LEAA staff could remove all the biotests from the 58 big bags by pulling the relevant twines, then packing and transporting the biotests back to LEAA in the same day.

Table 2 shows the cronological details of the test.

Tab. 2 Test phases

Activities	Progress
Introduction of biotests	Day 1
Saturation process	Day 2
Complete saturation	Day 5
Desaturation process	Day 21
Complete oxygenation	Day 24
Biotest evaluation	Day 25-30

Evaluation method

All the evaluations of the biotests were performed at LEAA by the laboratory staff, through visual assessment, count and record of alive and dead individuals, within 5 days after the extraction.

The assessment was based on the observation of alive individuals. The assessment was recorded as "positive" at the first alive individual observed.

Climatic data and atmosphere monitoring

The internal temperature of the big bags during the trial were collected at least every 60 minutes by digital data loggers provided by the laboratory and immersed together with the biotests in 10 of the 58 test units. The graph (Fig. 4) below represents the mean temperature during the application period (average of ten big bags).

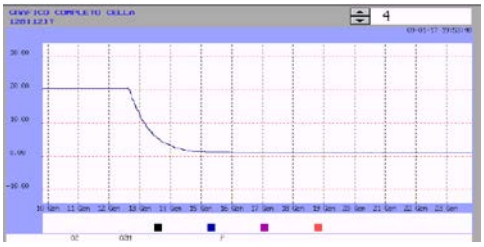

Fig. 5 Percentage of oxygen during the process

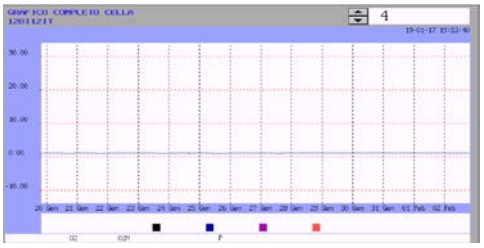

Fig. 6 Percentage of oxygen during the process

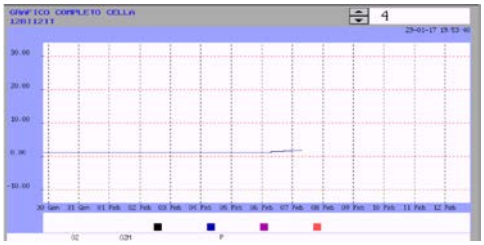

Fig. 7 Percentage of oxygen during the process

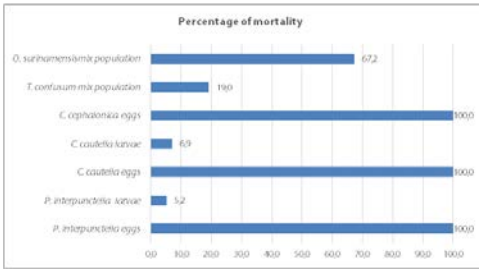

Fig. 8 Percentage of mortality of the different insect species and stages

During the application, the oxygen % in the cell was constantly monitored by a built-in oxygen meter. The data wasprovided by the storage company and reported in the figures 5, 6 and 7.

Results

The results elaborated with Abbott's formula showed that the high effect of the treatment was reached on the eggs of the lepidoptera species (100% mortality). On the Coleoptera species the mortality observed was 19.0% and 67.2% respectively for *T. confusum* and *O. surinamensis* (Fig. 8).

Tables 3 and 4 highlights the percentage of mortality and the number of biotest containing alive individuals in the treatment and the control experiment, respectively, corrected with the Abbott's folmula.

Tab. 3 Counts of positive biotest (with live insects) and mortality percentage in TREATED treatment for the different species and stages considered

Insect	Stage	Positive biotests (alive)	Mortality (%)
P. interpunctella	Eggs	0	100,0
P. interpunctella	Larvae	55	5,2
C. cautella	Eggs	0	100,0
C. cautella	Larvae	54	6,9
C. cephalonica	Eggs	0	100,0
T. confusum	Mix population	47	19,0
O. surinamensis	Mix population	19	67,2

Tab. 4 Counts of positive biotest (with live insects) and mortality percentage in UNTREATED treatment for the different species and stages considered

		TNT		LNT	
Insect	**Stage**	**Positive (alive) biotests**	**Mortality (%)**	**Positive (alive) biotests**	**Mortality (%)**
P. interpunctella	Eggs	2	0	2	0
P. interpunctella	Larvae	2	0	2	0
C. cautella	Eggs	2	0	2	0
C. cautella	Larvae	2	0	2	0
C. cephalonica	Eggs	2	0	2	0
T. confusum	Mix population	2	0	2	0
O. surinamensis	Mix population	2	0	2	0

*TNT=transfer untreated treatment - *LNT= Laboratory untreated treatment

Discussion

The test highlighted that an exposure to N_2 saturation at temperatures 15-18 °C for 21 days was not sufficient for a total control on mobile stages of all pests, while a total control of the *Lepidoptera* eggs, was observed. In fact no silk webs, feces or newborn larvae, even dead, were present in the biotests.

The population of *O. surinamensis* was the most susceptible to the treatment with 67.2% efficacy. The larvae of *P. interpunctella* were the least susceptible, 5.2% efficacy, similarly, the population of *C. cautella* with 6.7% efficacy. The treatment was barely effective on the population of *T. confusum*, with 19% efficacy.

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Effect of modified atmosphere on larval and pupal stages of *Rhyzopertha dominica* in stored chickpeas

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Abstract

The lesser grain borer, *Rhyzopertha dominica* (Fabricius), is a pest of stored chickpeas in Mexico. The control of this pest is based largely on the application of pesticides, but this strategy has important limitations: there are few active compounds available, there is a high risk of development of resistance to them and the residues left in the chickpeas have harmful effects on consumer health and on the environment. For this reason, an alternative strategy to pesticides for the conservation of stored chickpea was evaluated with the use of modified atmospheres (MA). The effect of three different MAs (50%, 70% and 90% CO_2, in air) on the larval and pupal stages of *R. dominica* were evaluated. To obtain larvae and pupae of *R. dominica*, eggs were incubated for a variable period of time until reaching the desired stage: 9-15 days for 1st and 2nd larval instar and 35-39 days for pupae. Tests were carried out by placing chickpeas containing a total of 15 larvae or pupae plus 50 g of healthy chickpea in small ventilated boxes. These ventilated boxes were individually placed inside of plastic bags (30 x 21 cm, Cryovac BB4L µm). Bags were filled with desired MA before sealing, which were previously prepared in a gas mixer (Witt Km 100-3M/MEM). A control treatment without MA was also included. To verify the CO_2 and O_2 content inside the plastic bags a gas analyzer (OXYBABY®) was used and the gas levels were determined at the beginning and at the end of the treatment. Plastic bags were opened at different periods of exposure (larvae up to 5 days; pupae up to 10 days) and ventilated boxes were kept until adult emergence to assess mortality. Results show that increasing the concentration and exposure time of CO_2 increases the mortality rate of larvae and pupae of *R. dominica* (Fig. 1). The most resistant developmental stage was the pupae, with an LD_{90} of 241 h (50% CO_2) compared to the larval stage with an LD_{90} of 22 h (90% CO_2). The tolerance of the MA is greater in the pupal stage due to the reduction of respiration in this stage.

Keywords: *R. dominica*, pest, chickpea, alternative strategy, modified atmosphere.

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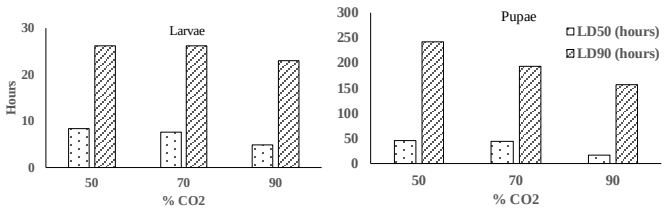

Fig 1. LD_{50} and LD_{90} of larval (left) and pupal (right) stage of *R. dominica* exposure at MA with 50, 70 and 90% of CO_2 on air.

CARVEX – Pressurized Pest Disinfection with CARBO Carbon Dioxide

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Abstract

Keywords: CARVEX, Carbon Dioxide, Pressure Fumigation, CARBO, Pest control.

Introduction

Pest control with insecticides and toxic gases have polluting effects on persons and the environment. However, CO_2 pressurized pest control is a real revolution. It does not present any hazard either to nature or to persons. Benefit from an innovative, efficient and future-capable process for optimized hygiene results!

The entrainment of pests right to the stage of final packaging is an enormous source of danger for manufacturers. If the reputation of a brand is damaged by infected products, confidence can be restored with difficulty only. Politicians and consumers demand appetizing and hygienic foodstuffs which are equally uncompromising. CARVEX assists in assuring compliance with strict legal general conditions and provides for brand confidence and consumer protection.

The employment of insecticides or toxic gases, such as methyl bromide, phosphine or hydrocyanic acid, is still usual. However, they leave residues and are problematic, also from the viewpoint of environmental protection and occupational safety. This is being increasingly criticized publicly by consumers and associations. The solutions from CARVEX are now convincing in many ways, where they provide for "clean" products while avoiding harmful residues. In this way, the justified demands of consumers are optimally fulfilled - as well as possible damage to image avoided as early as in the initial stages.

CARVEX offers you perfect solutions so that your products reach end customers in an optimum condition hygienically. The charged nature of the issue is highlighted for example in that almost all harvest herbs, as well as tea products, are infected by harmful insects as well as by their larvae or eggs. In this case also, as with all other possible areas of application, the processes from CARVEX enable the best utilization capability and value-added utility of your products.

Materials and Methods

Optimum pressurized pest control with carbo carbon dioxide

CARBO carbon dioxide has a side to it which is to a large extent unknown: Consumers hold natural products in high esteem where they provide a refreshing "sparkle" in mineral water, lemonade or beer. However, carbon dioxide because of its bacteriostatic and bactericide effects, is also increasingly used in the packaging of foodstuffs in inert gas. This tendency is increasing. The advantages are obvious: Complete disclaimer on the use of toxic gases. This follows the present Hazardous Material Ordinance (GefStoffV) which gives first priority to the health protection of persons.

Top hygiene without any losses in quality

With the proper selection of process parameters, the effects of CO_2 pressure processing on feedstock can be excluded. For example, a detailed study related to the CO_2 pressure processing of medication and herb teas indicates impressively that the amount of the material contents remains unchanged after pest control using the CARVEX process. Likewise, no negative impairments could be verified in the product quality of grain with the same processing method. As well as this, neither

the baking quality nor the germination characteristics were influenced in any way disadvantageously.

Three effects in perfect Interaction

In case of pressurized pest control, the exceptional effect of CO_2 results through the interaction of just three effects: Firstly, a forced dissolving of carbon dioxide in the bodily fluids of insects leads to an over-acidification of the cell fluid and hemoglobin, through which carbon dioxide is formed. In addition to this is the oxygen withdrawal effect and, a most important factor, the so-called pressure effect, which is also known to deep-sea divers as the bends.

The effect of pressure is particularly significant. Only the combination of CO2 and pressure allows the treatment to work well in a short period of exposure. For example, in the case of the dried fruit moth, treatment time increased from a few minutes at a pressure of 37 bars to 64 hours at a pressure of 3 bar

Neither a high nitrogen pressure, comparable with the pressurized CO2 process, nor high CO2 concentrations at atmospheric pressure, achieve the desired effect. To kill weevils using pressureless CO2 treatment an exposure time of around 28 days is required

Technical description

CARVEX systems are designed especially flexibly, where they are characterized by their quality as well as ease of operation and can also be re-equipped or retrofitted afterwards. In case of a large volume demand, the chamber number can be increased to three chambers, where the performance and cost-effectiveness are increased. Below on picture 1, you can see an example for a realized double chamber system.

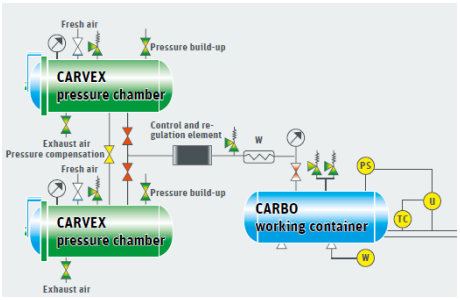
In addition, CARVEX offer different system lengths and diameters according to the area of application. With every system type, the number of pest control operations per shift is regulated by increasing pressure, which further increases throughput. Regardless of which solution you select, you can always choose whether the loading should be done manually, by lifting trolley or by roller conveyor.

The CO2 process for pressurized pest control works at ambient temperature above 15°C and pressures up to 30 bars. The material to be processed is inserted in bags in specially constructed pest disinfection chambers. After closure of the chamber, gaseous CO2 flows into the system until the required pressure has been reached. After the contact period, the pressure in the chamber is released and the material can be taken out.

The necessary CO2 is stored and refilled in a CARBO working container. The CO2 will be evaporated and tempered before entering in the chamber by heating. The pressure will be increased up to 30 bars by heating or by liquid pumping before heating.

The duration of the pressure treatment is varying depending on the chosen pressure (1-16 h), the product and the insect to be treated.

Picture 1: CARVEX Double Pressure Chamber



Picture 2: PID of a CARVEX Double Chamber

Results

CARVEX pressurized pest control with harmless CARBO carbon dioxide is based on experience which has been acquired over decades in inventory protection and pest control. Thus, not only are harmful insects, such as the grain weevil, the cigarette beetle, the rice-meal beetle, the meal beetle or the flour moth, rendered harmless within the shortest time, but also their larvae and eggs. Currently there has been positive experience with more than 30 different pests from different areas of inventory protection. The CARBO carbon dioxide is approved as plant production product in Germany and Austria.

Following effects have influence on the CARVEX process.

Packaging and product density

The type of packaging, as well as the existing product density, represent important factors in determining the contact period. For example, compressed goods such as bales, flour sacks or sealed bulk materials in big bags significantly increase the respective contact period. The reason is that it requires some time before the necessary concentration is reached in the package middle. A further influence factor on the contact period is the pest type to be expected, as well as their stage of development. For example, eggs and pupae are generally the most resistant.

The role of temperature

Increased temperatures effectively decrease the contact periods. For example, with pressure-free CO2 processing, the contact period of 28 days at 10°C for the death of the grain weevil is shortened to only a few hours at 40°C. Of course, the temperatures
cannot be increased at will because this would lead to a decrease in the product qualities. On the other hand, the influence of the temperature is reduced with increasing pressure, which is why it is always recommended to implement the processing based on optimum parameters.

Optimum co2 concentration

The CARVEX process achieves optimum effectiveness with an atmosphere which includes at least 90% CO2. The CO2 content is of particular relevance in case of pressure-free application.

The pressure-effect

As a result of the interaction of CO2 and pressure, the CARVEX systems achieve an exceptional effect while at the same time providing for short processing times. Thus, in comparison with a system which works pressure-free, the processing time of the Indian meal moth at an adjusted pressure of approx. 30 bars is decreased from approx. 240 hours to only ½ an hour. The advantage of the pressurized pest control can be demonstrated even more efficiently in the example of the grain weevil. While conventional, pressure-free gas flushing takes approx. 28 days, only a few hours are required in a CARVEX system in order to achieve the same result.

Discussion

Acknowledgement

I wish to thank Herbert Saal for his assistance and his treasure trove of experience.

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Fumigant toxicity of essential oils and their combinations on population buildup of three stored product coleoptera in stored wheat and effect on quality of wheat

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Abstract

Experiments carried out to find the fumigant toxicity of three essential oils and their combinations from *Murraya koenigii*, *Citrus reticulata*, *Curcuma longa* on population buildup of *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum* in stored wheat at different days of infestation. The oil of *M. koenigii*, *C. reticulata* at 0.2% and *M. koenigii*+*C. reticulata*, *M. koenigii*+*C. longa* at 0.1% each were found highly effective against *S. oryzae* fumigated after 5, 10, 15 and 20 days. The oil of *M. koenigii* and *C. reticulata* at 0.2% *M. koenigii*+*C. reticulata*, *M. koenigii*+*C. longa*, *C. reticulata*+*C. longa* at 0.1% each and *M. koenigii*+*C. reticulata*+*C. longa* at 0.07% each were found highly effective against *R. dominica* fumigated after 5, 10, 15 and 20 days. Only *M. koenigii* at 0.2% was found highly effective against *T. castaneum* fumigated after 5, 10, 15 and 20 day. The fumigation of grain with *M. koenigii* at 0.2% completely suppress the infestation and weight loss when it was fumigated after 5, 10, 15 and 20 days while very low infestation and weight loss was observed in grain treated with *M. koenigii* +*C. reticulata* at 0.1% each and not affect the organoleptic properties and germination of wheat.

Key words: Fumigant toxicity, essential oils, Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum

Introduction

Stored commodities are infested by more than 600 species of beetles, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses at different storage level (Rajendran and Srirajinia, 2008). Stored product insects cause 10 percent postharvest losses in developing countries, but they also contaminate the food products by presence of live insects, insect products such as chemical excretions or silk, dead insects and insect body fragments. In India, only aluminum phosphide and methyl bromide are available for fumigation of stored commodities. The use of aluminum phosphide is restricted while methyl bromide has been banned and their injudicious use during many years induced resistance development in stored grain insects.

Essential oils from more than seventy five plant species belonging to different families, such as Anacardiaceae, Apiaceae, Araceae, Asteraceae, Brassicaceae, Chenopodiaceae, Cupressaceae, Graminaceae, Lamiaceae, Lauraceae, Liliaceae, Myrtaceae, Pinaceae, Rutaceae and Zingiberaceae have been studied for fumigant toxicity against several insect pests of stored grain (Rajendran and Srirajinia, 2008). In several storage structures, fumigants are the most economical and convenient tools for managing stored grain insects due to their easy penetration in stored commodities and avaibility at cheaper rates (Azelmat *et al.,* 2006). Recently research has been focusing on the utilization of essential oils and their bioactive constituents as possible alternative to traditional fumigants (Negahban *et al.,* 2007, Ogendo *et al.,* 2008). *Sitophilus oryzae* (L), *Rhyzopertha dominica* (F), and *Tribolium castaneum* (Herbst) are one of the important stored product beetles and cause serious loses to stored commodities worldwide (Kumar, 2016). *Murraya koenigii* is an annual herb growing as medicinal plants and their leaves are used as some culinary and treatment of human disease. *Curcuma longa* is used as spice while peel of *Citrus reticulata* is wastage materials. The attempt has been made to study the fumigant toxicity of essential oils and their combinations on

population buildup of three stored product coleoptera in stored wheat and their effect on quality of wheat**.**

Material and Methods

Culture of the insects

Pure culture of test insects were developed in the BOD incubator maintained at 27°C±1 temperature and 70±5% relative humidity. Plastic jars of 1000 ml capacity were used for rearing of insects. At the center of the lid a hole of 1.8 cm diameter was made and covered with 30 mesh copper wire net to facilitate aeration in the jar. Adults of *R. dominica* (F) (Coleoptera: Bostrichidae) and *S. oryzae* (L) (Coleoptera: Cuculionidae) were reared on the wheat variety PBW-343 while *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae) was cultured on its flour fortified with 5 per cent yeast powder. Before use, wheat was disinfested in the oven at 60^{oC} for 12 hrs. After disinfestation the moisture content of the wheat was measured and raised to 13.5 per cent by mixing water in the grain. The quantity of water required to raise the moisture content was calculated by using the formula described by Pixton (1967).

Extraction of essential oils

Oils selected for the study were extracted from the locally available plants by steam distillation at Medicinal and Aromatic Plants Research and Development Centre, Pantnagar, and by Clevenger Apparatus in Post Harvest Entomology Laboratory Pantnagar.

Preparation of grain

All fumigation experiments on *R. dominica, S. oryzae* and *T. castaneum,* were conducted on untreated seed grade wheat of variety PBW-343. The experiment was conducted on *R. dominica, S. Oryzae* and *T. castaneum* to confirm the fumigant toxicity of essential oils and their combination and experiment was conducted same as rearing conditions.

Effect of essential oils on population build up of *R. dominica, S. oryzae*, and *T. castaneum*

The experiment was conducted in 2000 ml capacity air tight plastic jar to study the effect of essential oil and their combination on population build up of *R. dominica, S. oryzae* and *T. castaneum*. Wheat variety PBW- 343, (1500g) was filled in each plastic jar. Four sets comprising eight treatments with three replications were prepared. Twenty newly emerged adult (0-4 days old) of each specie were released in each jar after filling grains. The treatment applied after 5, 10, 15 and 20 days after artificial infestation, essential oil was poured on the absorbing mats and then mats were inserted inside the plastic jars. Screw cap of jars tightly closed and made completely airtight by sealing with parafilm wax strip. After one year of storage each jar was analyzed to count the number of adults emerged to calculate percent inhibition and the number of healthy and infested grain to calculate per cent infestation, per cent weight loss and germination atributes.

Organoleptic properties of *chapattis* made from grains treated with essential oils

Organoleptic test was performed on *chapattis* made from wheat grains treated with essential oils and their combination without washing and after washing thoroughly with water. 1000 g of grains was drawn out from each treatment and sieved thoroughly to remove unwanted impurities. After sieving 500 g wheat was used as such for preparation of flour while rest 500 g of the grain was washed thoroughly with water. Both washed and unwashed grains were sun dried for ten days, 8 hrs per day. The wheat grain was then ground in local market of Pantnagar to obtain wheat flour. The flour was then passed through a fine sieve to get a flour of the required fineness. Equal amount of flour was weighed and kneaded to dough using equal quantity of water and dough balls of same size were prepared. These balls were rolled out as thin as possible into a chapatti and were cooked on both sides on a hot iron plate without any fat or oil.

Various characteristics of chapatti i.e. colour, flavour, texture, taste, appearance and overall acceptability were tested as described by Amerine *et al.* (1965). For the evaluation of chapatti ten evaluators were invited randomly from the different departments of the university. Each member of the panel evaluated the characteristics of chapatti on a grading scale as 1-2 Very poor, 3-4 Poor, 5-6 Fair, 7-8 Good, 9-10 Very good.

Effect of essential oil on germination of wheat

Samples were drawn from experiment to record the effect of different essential oils and their combinations on germination of wheat. Germination test was done as per protocol of Chalam *et al.* (1967). Seedling vigour (vigour index) was computed as per Abdul-Baki and Anderson (1973).

Statistical analysis

Data were analyzed in Completely Randomized Design after suitable transformation with log (X+ 1).

Results

The efficacy of essential oils and their combinations was classified in different categories on the basis of first progeny production.The more weightage was given to suppression of first progeny, with this assumption, products inhibiting more than 90 percent of first progeny were classified as highly effective while inhibition of 80 to 89 and 70 to 79 percent were ranked as moderately and less effective respectively. The products showing less than 70 percent of first progeny suppression were ranked as least effective for the control of test insects.

Effect of essential oils and their combinations on population build up of *S. oryzae*

The essential oils and their combinations on population build up of *S. oryzae* is presented in Table 1. The table indicates that *M. koenigii* oil at 0.2 % and its combination with *C. reticulata* at 0.1 % each completely suppressed the feeding and breeding of the *S. oryzae* in all the treatments. The combination of *M. koenigii* + *C. longa* at 0.1 percent each was also highly effective against *S. oryzae* as caused 100, 99.81, 100 and 100 percent inhibition in grain fumigated after 5, 10, 15 and 20 days respectively. The oil of *C. reticulata* caused 100, 97.92, 100 and 93.84 % inhibition at 0.2 % in grain fumigated after 5, 10, 15 and 20 days, respectively. The combination containing *M. koenigii* + *C. reticulata* + *C. longa* at 0.07 percent each caused 95.69, 91.97, 100 and 77.44 percent inhibition in grain fumigated after 5, 10, 15 and 20 days respectively. The combination of *M. koenigii* + *C. longa*, *C. reticulata* + *C. longa* at 0.1 percent each and *M. koenigii* + *C. reticulata* + *C. longa* at 0.07 each percent each was highly effective against *S. oryzae* in the grain fumigated after 5, 10, 15 and 20 days of artificial infestation.

Effect of essential oils and their combinations on Population build up of *R. dominica*

The effect of essential oils on population build up of *R. dominica* is given in Table 2 which indicates that *M. koenigii* at 0.2 percent concentration and its combination with *C. reticulata* at 0.1 percent each were highly effective as it completely suppressed feeding and breeding of the *R. dominica* after one year of stored grain fumigated after 5, 10, 15 and 20 days. The combination containing *M. koenigii* + *C. longa* at 0.1 percent each was also highly effective against *R. dominica* as it caused 100, 99.34, 100 and 100 percent inhibition in grain fumigated after 5, 10, 15 and 20 days respectively. The oil of *M. koenigii* + *C. reticulata* + *C. longa* at 0.07 percent each concentration caused 100, 97.88, 100 and 100 percent inhibition in grain fumigated after 5, 10, 15 and 20 days respectively. The oil of *C. reticulata* at 0.2 percent caused 100, 95.70, 100 and 97.32 percent inhibition at 0.2 percent in grain fumigated after 5, 10, 15 and 20 days respectively, while its combination with *C. longa* at 0.1 percent each caused 100, 90.60, 100 and 100 percent inhibition in grain fumigated after 5, 10, 15 and 20 days, respectively.

Effect of essential oils and their combinations on Population build up of *T. castaneum*

The effect of essential oils on population build up of *T. castaneum* after one year of storage is presented in Table 3, which indicates that only *M. koenigii* at 0.2 percent completely suppressed feeding and breeding of the *T. castaneum* in grain fumigated after 5, 10, 15 and 20 days. The oil of *C. reticulata* at 0.2 % was found effective at 15 days of artificial infestation, while its combination with *C. longa* at 0.1 percent each was effective at 20 days after artificial infestation.

Tab. 1 Effect of essential oils and their combinations on population buildup of *Sitophilus oryzae* after one year of storage.

Essential Oils	% Conc.	5 days		10 days		15 days		20 days	
		Adult emerged	% inhib.	Adult emerged	% inhib.	Adult emerged	% inhib.	Adult emerged	% inhib.
M. koenigii	0.2	0.0 (0.0)	100.00	0.0 (0.0)	100.00	0.0 (0.0)	100.00	0.0(0.0)	100.00
C. reticulata	0.2	0.0 (0.0)	100.00	29.3(1.4)	97.92	0.0 (0.0)	100.00	26.7 (1.4)	93.84
C. longa	0.2	875 (6.7)	38.22	1109(6.9)	21.71	13.3(1.2)	90.61	163.7(5.0)	62.20
M.koenigii+C. reticulata	0.2	0.0 (0.0)	100.00	0.0 (0.0)	100.00	0.0 (0.0)	100.00	0.0 (0.0)	100.00
M.koenigii+C. longa	0.2	0.0 (0.0)	100.00	2.7 (0.7)	99.81	0.0 (0.0)	100.00	0.0 (0.0)	100.00
C.reticulata+C. longa	0.2	235 (5.3)	83.42	414 (5.9)	70.77	0.0 (0.0)	100.00	401.7(5.9)	70.23
M.koenigii+C. reticulata+C. longa	0.2	61 (3.1)	95.69	113.7(4.6)	91.97	0.0 (0.0)	100.00	97.7 (3.3)	77.44
Untreated		1416.3(7.2)	0.0	1416.7(7.2)	0.0	142 (4.8)	0.0	433 (6.0)	0.0
S. Em.±		(0.55)		(0.61)		(0.45)		(0.78)	
CD at 5%		(1.67)		(1.84)		(1.37)		(2.36)	

Data in parenthesis indicate log (X+1) transformed value.

Tab. 2 Effect of essential oils and their combinations on population buildup of *Rhyzopertha dominica* after one year of storage.

Essential Oils	% Conc	5 days		10 days		15 days		20 days	
		Adult emerged	% inhib.	Adult emerged	% inhib.	Adult emerged	% inhib.	Adult emerged	% inhib.
M. koenigii	0.2	0.0(0.0)	100.00	0.0(0.0)	100.00	0.0 (0.0)	100.00	0.0(0.0)	100.00
C. reticulata	0.2	0.0(0.0)	100.00	19.7(1.3)	95.70	0.0 (0.0)	100.00	15.3 (2.1)	97.32
C. longa	0.2	0.7(0.3)	99.76	130.7(4.8)	71.45	0.0 (0.0)	100.00	119.3(4.5)	79.19
M.koenigii+C. reticulata	0.2	0.0(0.0)	100.00	0.0 (0.0)	100.00	0.0 (0.0)	100.00	0.0 (0.0)	100.00
M. koenigii+C. longa	0.2	0.0(0.0)	100.00	3.0 (0.7)	99.34	0.0 (0.0)	100.00	0.0(0.0)	100.00
C.reticulata+C. longa	0.2	0.0(0.0)	100.00	43 (2.7)	90.60	0.0 (0.0)	100.00	0.0 (0.0)	100.00
M.koenigii+C. reticulata+C. longa	0.2	0.0(0.0)	100.00	9.7 (1.1)	97.88	0.0 (0.0)	100.00	0.0 (0.0)	100.00
Untreated		276.3 (5.6)	0.0	457.7(6.1)	0.0	393.7(5.9)	0.0	573.7(6.3)	0.0
S. Em.±		(0.13)		(0.84)		(0.29)		(0.42)	
CD at 5%		(0.39)		(2.53)		(0.88)		(1.28)	

Data in parenthesis indicate log (X+1) transformed value

Tab. 3 Effect of essential oils and their combinations on population buildup of *Tribolium castaneum* after one year of storage.

Essential Oils	% Conc	5 days		10 days		15 days		20 days	
		Adult emerged	% inhib.	Adult emerged	% inhib.	Adult emerged	% inhib.	Adult emerged	% inhib.
M. koenigii	0.2	0.0 (0.0)	100.00	0.0 (0.0)	100.00	0.0 (0.0)	100.00	0.0 (0.0)	100.00
C. reticulata	0.2	0.0 (0.0)	100.00	44.7 (2.7)	89.75	29.7 (3.3)	88.33	262 (5.5)	31.17
C. longa	0.2	167.3 (5.0)	52.24	254.3 (5.5)	41.66	0.0 (0.0)	100.00	346.3 (5.8)	9.01
M.koenigii+C. reticulata	0.2	350.7 (5.8)	76.19	343 (5.8)	71.33	33 (2.5)	87.02	263.3 (5.5)	30.82
M. koenigii+C. longa	0.2	555.3 (6.3)	58.06	605.3 (6.4)	78.76	0.0 (0.0)	100.00	380.7 (5.9)	90.00
C.reticulata+C. longa	0.2	7.3 (1.0)	97.91	21.7 (2.3)	95.03	38.7 (2.6)	84.79	206.7 (5.3)	45.71
M.koenigii+C. reticulata+C. longa	0.2	331.7 (5.7)	75.59	187 (4.8)	57.11	60.3 (3.5)	76.27	196.3 (5.2)	48.42
Untreated		351.3 (5.8)	0.0	436 (6.0)	0.0	254.3(5.4)	0.0	380.7 (5.9)	0.0
S. Em.±		(0.38)		(0.68)		(0.74)		(0.11)	
CD at 5%		(1.16)		(2.04)		(2.19)		(0.35)	

Data in parenthesis indicate log (X+1) transformed value.

Effect of essential oils and their combinations on infestation and weight loss

The effect of essential oils on infestation and weight loss due to *S. oryzae, R. dominica* and *T. castaneum* after one year of storage is presented in Table 4, which indicates that fumigation of grain with *M. koenigii* + *C. reticulata* or *C. longa* at 0.1% each or *C. longa* at 0.2 % observed 2.85, 2.42 and 2.61 percent infestation, respectively, which was very low as compared to untreated control which recorded 11.76 percent infestation. Fumigation of grain with *M. koenigii* + *C. reticulata* + *C. longa* at 0.07 percent each, *C. reticulata* + *C. longa* at 0.1% each and *C. longa* at 0.2% concentration permitted 0.69, 0.62 and 0.49% weight loss, respectively while 6.0 percent weight loss observed after one year of storage in untreated control. The fumigation after 10 days only *M. koenigii* at 0.2 percent completely suppressed the progeny development of *S. oryzae, R. dominica* and *T. castaneum,* while *M. koenigii* + *C. reticulata* at 0.1% each permitted very low infestation and weight loss. The combination of *C. reticulata* + *C. longa* oil at 0.1 percent each, *M. koenigii* + *C. reticulata* + *C. longa* at 0.07 percent each, *C. longa*, *C. reticulata* at 0.2 percent and *M. koenigii* + *C. longa* at 0.1 percent each 5.56, 5.35, 4.73, 3.88 and 3.66% infestation, respectively, while in untreated control 17.07 percent infestation was found. The percent weight loss in grain treated with *M. koenigii* + *C. reticulata* + *C. longa* at 0.07 percent each, *C. reticulata* + *C. longa*, *M. koenigii* + *C. longa* at 0.1 percent each, *C. longa* and *C. reticulata* at 0. 2 percent each concentration were 2.36, 1.63, 1.19, 1.11 and 0.39 as compare to untreated control in which 7.96 percent weight loss found after one year of storage. No infestation or weight loss was recorded in grain treated with *M. koenigii* or *C. reticulata* oil at 0.2 percent and its combination at 0.1 percent each in grain fumigated after 15 days. Very less infestation and weight loss was observed in grain treated with a combination of *M. koenigii* + *C. reticulata* + *C. longa* at 0.07 percent each, and *M. koenigii* + *C. reticulata* at 0.1 percent each concentration. The weight loss amounting to 0.67 and 0.38 percent were recorded in grain treated with *C. longa* at 0.2 percent and *C. reticulata* + *C. longa* at 0.1% each, respectively, while in untreated control 5.54 percent weight loss found, The grain was fumigated after 20 days, *M. koenigii* at 0.2 percent and *M. koenigii* + *C. reticulata* at 0.1 percent each concentration were completely suppressed the progeny of *S. oryzae, R. dominica, and T. castaneum* after one year of storage.

Tab. 4 Effect of essential oils and their combinations on infestation and weight loss due to *Sitophilus oryzae, Rhyzopertha dominica and Tribolium castaneum* after one year of storage.

Essential Oils	% Conc	5 days		10 days		15 days		20 days	
		% Infestation	% Weight loss	% Infestation	% Weight loss	% Infestation	% Weight loss	% Infestation	% Weight loss
M. koenigii	0.2	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
C. reticulata	0.2	0.0 (0.0)	0.0 (0.0)	3.88 (1.5)	0.39(0.3)	0.0 (0.0)	0.0 (0.0)	0.54 (0.4)	0.04 (0.3)
C. longa	0.2	2.61 (1.2)	0.49(0.3)	4.73 (1.7)	1.11(0.5)	2.19 (0.8)	0.67 (0.3)	6.77 (2.0)	2.86 (1.3)
M.koenigii+C. reticulata	0.2	0.0 (0.0)	0.0 (0.0)	0.51 (0.4)	0.32(0.2)	0.32 (0.2)	0.05 (0.4)	0.0 (0.0)	0.0 (0.0)
M. koenigii+C. longa	0.2	0.12 (0.1)	0.04(0.4)	3.66 (1.3)	1.19(0.6)	0.0 (0.0)	0.0 (0.0)	0.74 (0.3)	0.11 (0.1)
C.reticulata+C. longa	0.2	2.42 (1.2)	0.62(0.7)	5.56 (1.8)	1.63(0.8)	3.2 (1.4)	0.38 (0.2)	4.4 (1.6)	1.3 (0.5)
M.koenigii+C. reticulata+C. longa	0.2	2.85 (1.3)	0.69(0.5)	5.35 (1.8)	2.36(1.0)	0.96 (0.6)	0.12 (0.1)	2.38 (0.9)	0.21 (0.1)
Untreated		11.76 (2.5)	6.0 (1.6)	17.07 (2.8)	7.96(2.1)	14.05 (2.6)	5.54 (1.8)	16.33(2.8)	5.81 (1.9)
S. Em.±		(0.1)	(0.48)	(0.18)	(0.29)	(0.21)	(0.13)	(0.24)	(0.18)
CD at 5%		(0.32)	(1.4)	(0.56)	(0.87)	(0.62)	(0.41)	(0.73)	(0.56)

Data in parenthesis indicate log (X+1) transformed value

Tab. 5 Percent germination, vigour index and significance of viability of grain treated with essential oils.

Essential Oils	% Conc	Germination parameter			Organoleptic Properties						
		% Germination	Vigour Index	Significance of viability	Condition	Colour	Flavour	Texture	Taste	Appearance	Overall Acceptability
M. koenigii	0.2	100.00	15590.67	1.1	Unwashed	9.5	9.0	9.0	9.1	9.5	9.5
					Washed	10.0	9.7	10.0	10.0	10.0	10.0
C. reticulata	0.2	96.66	17271.33	1.1	Unwashed	9.4	9.0	9.7	9.6	9.5	9.5
					Washed	9.7	9.7	10.0	10.0	10.0	10.0
C. longa	0.2	95.33	16554.00	1.1	Unwashed	8.3	4.1	6.2	6.1	4.3	4.3
					Washed	9.0	7.1	8.2	6.5	6.8	7.3
M.koenigii+ C. reticulata	0.2	96.66	17813.33	1.1	Unwashed	9.1	8.7	8.2	7.2	9.7	9.7
					Washed	9.1	6.3	7.8	8.1	10.0	7.5
M. koenigii+C. longa	0.2	97.33	17838.00	1.1	Unwashed	9.0	8.6	6.3	7.8	5.3	5.3
					Washed	9.0	6.2	7.1	6.9	9.5	7.0
C. reticulata+C. longa	0.2	94.66	17866.67	1.1	Unwashed	9.0	6.3	7.8	7.1	6.2	6.2
					Washed	9.0	6.8	7.4	6.1	9.5	7.1
M.koenigii+ C. reticulata+C. longa	0.2	96.66	18041.33	1.1	Unwashed	9.3	7.9	7.1	7.2	6.1	6.1
					Washed	9.0	6.1	7.1	8.0	9.3	7.2
Untreated		86.00	10882.70		Unwashed	9.1	8.7	8.1	7.9	7.2	7.2
					Washed	9.5	8.7	7.5	8.0	9.5	8.1
S.Em.±		1.58	489.9								
CD at 5%		4.73	1468.88								

Effect of essential oils and their combinations on germination of wheat

Percent germinations is presented in table 5 which indicates that the 100% germination was recorded in grain treated with *M. koenigii* while 94.66 to 97.33% germination was recorded in grain treated with *C. reticulata, C. longa* and their combination with *M. koenigii*. Only 86.00% germination was recorded in untreated control. As compared to untreated control very high vigour index was recorded in seed treated with essential oils. The significance of viability was 1:1 in all treated grain after one year of storage.

Organoleptic properties of chapattis made from grain treated with essential oils

The organoleptic properties of chapattis made from wheat grain treated with essential oils is presented in table 5 which indicates that colour, flavor, texture, taste, appearance and overall acceptability of chapattis prepared from treated grain were supirior as compare to untreated grain. The overall acceptability rating of chapattis prepared from unwashed grain treated with *M. koenigii, C. reticulata* at 0.2% or at 0.1% each were very good as compare to untreated grain in which rating was good. Similarly the chapattis made from washed grain of different treatments showed very good overall acceptability in grain treated with *M. koenigii* and *C. reticulata* while remaining showed good overall acceptability as compare to untreated grain.

Discussion

Seventeen essential oils were evaluated against S. oryzae, R. dominica, T. castaneum and C. chinensis in wheat and checkpea, ane the ones extracted from Allium sativum, Artimisia annua, Callistemon citrinus, Chenopodium botrys, Cinnamomum zylanicum, Citrus reticulata, Cuminum cyminum, Foeniculum vulgare, Murraya koenigii, Pinus roxburghii and Piper nigrum were found highly effective at 0.2% (Kumar and Tiwari, 2017). The essential oils of Murraya koenigii, Citrus reticulata and Curcuma longa were evaluated against S. oryzae, R. dominica, T. castaneum in stored wheat to protect the grains for one year in metal bins and did not affect the organoleptic propertis of chapatti made from treated grains and germination of wheat (Kumar and Tiwari, 2017). The essential oils of Murraya koenigii, Citrus reticulata, Curcuma longa and Calistimone citrinus at 0.2% caused 100 percent mortality in R. dominica and T. castaneum after twenty four hours of treatment (Kumar et al., 2018). Essential oil of Sweet Annie, Artemisia annua evaluated by Tripathi et al.(2002) against T. castaneum and C. maculatus at 1% v/v proved to be adult repellent and revealed negative correlation between larval survival, pupal survival and adult emergence of T. castaneum. Bio-efficacy of leaf oil of Murraya koenigii was evaluated against C. chinensis, oil attracted insects at 25 mg dose and repelled at 300 mg dose in the dual choice repellency test. In both contact (0.125 mg/cm^2) and fumigant (22.5 mg/ml) toxicity test 100% mortality was observed with LC₅₀ value of 0.08 mg/cm^2 and 22.5 mg/ml, respectivily (Paranagama et al., 2002). Pathak et al. (1997) investigated toxicity and repellent activity of Murraya koenigii against C. chinensis in stored green gram, chickpea at 340 ppm and oil showed toxic effect and ovicidal properties. They concluded that the oil can be used for small level protection of legumes. Liu et. al. (2007) evaluated 40 species of Chinese medicinal herbs from 32 different botanical families for their contact, fumigant and feeding deterent activities against two stored grain coleopterans S. zeamais and T. castaneum. Thirty Chinese medicinal herbs exhibited insecticidal or feeding deterent activities against test insects. The oil of Artemisia argyi, Dictamnus dasycarpus, Evodia rutaecarpa, Lietsea cubeba, Narcissus tazetta var chinensis, Polygonum aviculare, Rhododendrum molle, Sophora flavescens, Stemona sessilifolia, Tripteryginni wilfordii, and Torreya grandis were most effective against both tested insects.

Resuls concluded that the exploration of fumigational potential of essential oils may leads to development of non-synthetic, economical, ecological safe and easily degradable alternative for sustainable stored grain insect pest management. Essential oils of *M. koenigii*, *C. reticulata* at 0.2% and *M. koenigii*+*C. reticulata*, *M. koenigii*+*C. longa* at 0.1% each were found highly effective against *S. oryzae* fumigated after 5, 10, 15 and 20 days. The oil of *M. koenigii* and *C. reticulata* at 0.2% *M.*

koenigii+C. reticulata, *M. koenigii+C. longa*, *C. reticulata+C. longa* at 0.1% each and *M. koenigii+C. reticulata+C. longa* at 0.07% each were found highly effective against *R. dominica* fumigated after 5, 10, 15 and 20 days. Only *M. koenigii* at 0.2% was found highly effective against *T. castaneum* fumigated after 5, 10, 15 and 20 day. The fumigation of grain with *M. koenigii* at 0.2% completely suppress the infestation and weight loss when it was fumigated after 5, 10, 15 and 20 days while very low infestation and weight loss was observed in grain treated with *M. koenigii +C. reticulata* at 0.1% each. Essential oils did not affect the organoleptic properties and germination of wheat.

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Fumigant toxicity of *Haplophyllum tuberculatum* (Rutaceae) and *Nepeta crispa* (Lamiaceae) on the Indian meal moth

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Abstract

The insecticidal activity of essential oil vapors of *Haplophyllum tuberculatum* (Sapindales: Rutaceae) and *Nepeta crispa* (Lamiales: Lamiaceae) were evaluated on third instar larvae of *Plodia interpunctella* (Lepidoptera: Pyralidae) as one of the major insect pests of stored products. Essential oils of the plants were obtained using Clevenger-type water distillation. GC-MS analyses of the oils demonstrated that the main compounds of *H. tuberculatum* were ρ-menth-2-en-1-ol-cis (20.15 %), p-menth-2-en-1-ol-trans (16.92 %), trans-Piperitol (13.23 %), Piperitone (7.34 %) and cis-Piperitol (6.72 %). It is an important plant that has many medicinal properties. 1,8-Cineole (32.98 %), β-Pinene (8.70 %), 4aα,7α,7aα-Nepetalactone (8.08 %) and 4aβ,7α,7aβ-Nepetalactone (6.1 %) were detected as the predominant component in *N. crispa*. The aerial parts of that are used in traditional medicine. The LC_{50} values were estimated after 24 hours for *H. tuberculatum* and *N. crispa* as 4.301 and 5.579 µl L^{-1} air, respectively. LC_{50} values were projected using probit analysis. Results on *H. tuberculatum* showed more toxicity against the Indian meal moth compared to the *N. crispa*. In conclusion, the essential oil of the two plants could have potential for application to stored grain and agricultural commodities to control of stored crop pests in IPM programs.

Key Words: Essential oils, insecticidal toxicity, Plodia interpunctella, Haplophyllum tuberculatum, Nepeta crispa.

Introduction

Plodia interpunctella Hubner (Lepidoptera: Pyralidae), the Indian meal moth, is a common domestic pest, feeding on stored food products including nuts, beans, processed foods and dried fruits (Simmons and Nelson 1975). The larvae spoil stored food by spinning a silky web inside and on top of the food surface and feed in it (Phillips et al., 2000). According to earlier experiences, fumigants are commonly utilized for control of stored-products pests (Cox et al., 1984) Chemical insecticides led to various problems to our environment and health (Taylor, 1989). Plant-derived products have proved to be excellent source of a variety of volatiles with the potential for development as alternatives to conventional insecticides (Atta-ur-Rahman et al., 1999; Tripathi et al., 2000; Lee et al. 2004; Ebadollahi et al., 2010; Wu et al., 2015; Plata-Rueda et al., 2017).

The genus *Haplophyllum*, belongs to the Rutacea family, comprises about 70 species represented in Mediterranean, Saharo-Arabian, Irano-Turanian, and Sudano-Zambezian regions (Willis, 1980; Takhtajan, 1986). *Haplophyllum* species are generally used in traditional medicine as a remedy for headaches and arthritis, skin discoloration, wart removal, and against parasitic diseases and other infections. *Haplophyllum tuberculatum* Forssk is considered to protect livestock from biting insects and flies (Miller et al., 1988). Insecticidal activity of the aerial parts of *H. tuberculatum* against *Culex quinquefasciatus* has been proved (Zohair et al., 1989).

The genus *Nepeta* (Lamiaceae) contains almost 280 species endemic to Europe, Asia and a few parts of Africa (Rechinger, 1982). Some of the species of *Nepeta* are grown as a garden herb and some of the species used as medicinal herb for cancer, toothache, colds, anemia, headache, diarrhea, indigestion, tuberculosis, and other various ailments (Duke and Ayensu, 1985; Duke, 2002). This genus, with 67 species in Iran, also is reported to be used as phytotherapy in Iranian traditional medicine (Amin, 1991). Essential oils of some species of *Nepeta* have been described to possess insecticidal activity (Zhu et al., 2006; Ali et al., 2016).

The review of the literature revealed the scarcity of information over the toxicity of *H. tuberculatum* and *N. crispa* Willd essential oils. Hence at the present study, the potential of fumigant toxicity of the essential oils from the two aromatic plant species examined against the third instar larvae of the Indian meal moth, *P. interpunctella*, in order to contribute for the development of new approaches for controlling this insect pest.

Materials and methods

Rearing of insects

P. interpunctella was collected from the Department of Plant Protection, Faculty of Agriculture of Urmia University, Iran. It was reared using the method of Adler (2010), with some modifications, on a diet containing 400 g of wheat bran, 15 g of broken almonds, 48 g of glucose, 80 g of dried yeast,

80 ml of glycerin and 20 ml of water. Stock cultures were maintained in a temperature-controlled chamber with 25±5 °C and 65±5 % R.H. and photoperiod of 14:8 (L:D) h.

Plant material

Aerial parts of *Nepeta crispa* and *Haplophyllum tuberculatum* were collected at the flowering stages in the middle of August (from the mountain areas of West Azerbaijan province (Northwestern Iran)) and November (from the fields of Kerman province (Southern Iran)) 2016, respectively. Samples were dried at room temperature and chopped into small pieces. The essential oils were extracted from the samples using a Clevenger-type apparatus for 4 h. The obtained essential oils were kept in the refrigerator at 4 °C for subsequent experiments.

Analysis of the essential oil

Essential oils were analyzed using GC–MS. For GC–MS analysis an Agilent 7890. A gas chromatograph coupled to a 5975A mass spectrometer using a HP-5 MS capillary column (5% Phenyl Methylpolysiloxane, 30 m length, 0.25 mm i.d., 0.25 µm film thickness) was used. The oven temperature was programmed as follows: 3 min at 80 °C, subsequently 8 °C min^{-1} to 180 °C, held for 10 min at 180°C. Helium was used as carrier gas at a flow rate of 1 mL min^{-1} and Electronimpact (EI) was 70 eV. The injector was set in a split mode (split ratio of 1:500) and mass range acquisition was from 40 to 500 m/z. Essential oil constituents were identified by using the calculated linear retention indices (Wiley 2007; NIST 2005) and mass spectra with those reported in the NIST 05 and Wily 07.

Bioassays

Fumigant toxicity of essential oils from *H. tuberculatum* and *N. crispa* investigated against *P. interpunctella*. Fumigation experiments carried out using the method of Ziaee et al. (2013) with some modifications. Ten third instar larvae of the insect were presented to the 100 ml glass jars as experiment units. The jars were covered with muslin cloth for inhibition of larvae contact to filter paper. Different concentrations of the essential oils (2, 2.91, 4.24, 6.18 and 9 µl L^{-1} air for *H. tuberculatum*, 15, and 2, 3.31, 5.48, 9.06 and 15 µl L^{-1} air for *N. crispa*) were pipetted onto a filter paper (Whatman No.1) and appended to the under surface of the jar's lid. After 24 h, the data were recorded in terms of the number of dead larvae and percentage mortality determined for each insect. Each treatment was replicated six times. The bioassay was conducted at 25±5 °C and 65±5 % R.H. and photoperiod of 14:8 (L:D) h. The control groups were treated except that no essential oils were employed.

Data analysis

Analysis of variances, using SPSS 21.0 (followed by Tukey's test to compare differences among various treatments at α= 0.05 level), were carried out to determine the significance of differences between mortality of larvae of each species at different concentrations of essential oils. Probit analysis was used to estimate LC_{50} and LC_{95} values (Abbott, 1925).

Results

Chemical composition of essential oil

Thirty-one and 27 chemical constituents were identified which represented 93.64 and 91.62 % of the total oils of *H. tuberculatum and N. crispa*. The major components of *H. tuberculatum* oil were ρ-menth-2-en-1-ol-cis (20.15 %), p-menth-2-en-1-ol-trans (16.92 %), trans-Piperitol (13.23 %), Piperitone (7.34 %) and cis-Piperitol (6.72 %). Chemical components analysis of the essential oils revealed that the predominant composition of *N. crispa* oil was 1,8-Cineole (32.98 %) followed by β-Pinene (8.70 %), 4aα,7α,7aα-Nepetalactone (8.08 %) and 4aβ,7α,7aβ-Nepetalactone (6.1 %) (Tables 1 and 2).

Table 1. Chemical constituents of *Haplophyllum tuberculatum* essential oil.

No.	Component	RI	%
1	Alpha-Pinene	931	0.45
2	Sabinene	960	0.53
3	α-phellandrene	1001	0.68
4	3-Carene	1011	0.90
5	*p*-Cymene	1026	1.16
6	β-phellandrene	1030	2.78
7	Linalool	1099	1.14
8	p-menth-2-en-1-ol-cis	1125	20.15
9	p-menth-2-en-1-ol-trans	1142	16.92
10	Isoborneol	1164	0.55
11	Terpinine-4-ol	1180	1.92
12	p-Cymen-8-ol	1186	0.60
13	Alpha-Terpineol	1193	0.80
14	cis-Piperitol	1198	6.72
15	trans-Piperitol	1210	13.23
16	Carvone	1246	1.22
17	Piperitone	1258	7.34
18	Bornyl acetate	1288	0.49
19	Thymol	1290	0.68
20	Carvacrol	1300	1.38
21	Piperitenone	1344	0.56
22	Alpha-Copaene	1380	0.43
23	Beta. Bourbonene	1389	0.75
24	trans-Caryophyllene	1425	2.39
25	Alpha.-curcumen	1484	1.13
26	Germacrene D	1486	4.70
27	7-epi-.alpha.-selinene	1524	0.47
28	Germacrene B	1563	1.07
29	Caryophyllene oxide	1590	0.99
30	beta.-Gurjunene	1593	1.02
31	(-)-Spathulenol	1633	0.49
	total		93.64

* RI= Retention indices

Insecticidal activity of essential oils against *P. interpunctella* larvae

The essential oils of *H. tuberculatum* and *N. crispa* possessed fumigant toxicity on third instar larvae of *P. interpunctella* with the LC_{50} and LC_{95} values ($P < 0.0001$) of 4.301, 13.538 and 5.579, 24.808 µl L^{-1} air, respectively (Table 3).

The results of one-way analysis of variances represented that effect of concentrations of the essential oils of *H. tuberculatum* (F= 76.054; df= 4, 25; $P < 0.001$) and *N. crispa* (F= 81.709; df= 4, 25; $P < 0.001$) on the mortality of third instar larvae of *P. interpunctella* were significant. The mortalities of the larvae of *P. interpunctella* were 86.7 and 88.3 % after 24 h exposure to the highest concentrations of *H. tuberculatum* and *N. crispa* essential oils, respectively. The increasing concentration of essential oils created significant increase in mortality. (Table 4).

Table 2. Chemical constituents of *Nepeta crispa* essential oil.

No.	Component	RI	%
1	α-Thujene	928	0.78
2	α-Pinene	936	2.89
3	Sabinene	971	2.66
4	β-Pinene	978	8.70
5	Myrcene	983	0.80
6	Dehydro-1,8-cineol	989	0.51
7	α-Terpinene	1014	1.86
8	ρ-Cymene	1017	0.18
9	1,8-Cineole	1033	32.98
10	γ-Terpinene	1054	1.18
11	trans-Sabinene hydrate	1061	1.92
12	α-Terpinolene	1086	0.34
13	cis-Sabinene hydrate	1089	2.52
14	Linalool	1094	1.94
15	α-Campholenal	1113	0.23
16	trans-Pinocarveol	1124	0.39
17	trans-Verbenol	1132	0.04
18	Sabinol	1137	0.68
19	δ-Terpineol	1154	2.69
20	4-Terpineol	1167	3.38
21	α-Terpineol	1179	4.26
22	4aα,7α,7aα-Nepetalactone	1337	8.08
23	4aβ,7α,7aβ-Nepetalactone	1348	6.10
24	4aβ,7α,7aβ-Nepetalactone	1368	5.78
25	trans-β-Farnesene	1444	0.46
26	α-Farnesene	1492	0.17
27	Germacrene-B	1510	0.10
	total		91.62

* RI= Retention indices

Table 3. Probit analysis data for *H. tuberculatum* and *N. crispa* essential oils against third instar larvae of *P. interpunctella*

Essential oil	n	LC_{50} (µl L^{-1})	*CI (µl L^{-1})	LC_{95} (µl L^{-1})	*CI (µl L^{-1})	Slope ± SE	**χ^2
H. tuberculatum	10	4.301	3.847–4.814	13.538	10.725–19.278	3.029±0.374	0.641
N. crispa	10	5.579	4.822–6.464	24.808	18.369–39.007	3.53± 0.284	0.637

* CI: confidential interval; ** χ^2: chi-squared value

Table 4. Fumigant activity of *N. crispa, S. hortensis* and *A. graveolens* essential oils against third instar larvae of *P. interpunctella*

Pest species	Concentration (µl L^{-1} air)	Mortality (%) mean±SE
H. tuberculatum	2	15.0 ± 3.34 a*
	2.91	26.7 ± 3.33 a
	4.24	50.0 ± 3.65 b
	6.19	68.3 ± 3.07 c
	9	86.7 ± 3.33 d
N. crispa	2	13.3 ± 3.33 a
	3.31	28.3 ± 3.07 b
	5.48	50.0 ± 3.65 c
	9.06	66.7 ± 3.2 d
	15	88.3 ± 3.07 e

* Means within column with the same letter(s) are not significantly different ($P > 0.05$) according to Tukey's test.

Discussion

Fumigation is one of the most effective methods of rapidly controlling insects infesting stored foods. A new approach for the control strategies that are environmentally sustainable and avoids the use of conventional pesticides is of paramount important. The essential oils of plants are assumed as viable alternative of controlling many insect pests (Mauchline et al., 2005; Lopez et al., 2008; Razavi 2012). Some reports have been stated that the most promising botanical insect-control agents belong to the families of Annonaceae, Asteraceae, Canellaceae, Apiaceae, Lamiaceae, Meliaceae and Rutaceae (Jacobson, 1989; Kim and Ahn, 2001; Chaubey, 2006, 2007; Taghizadeh-Saroukolai et al., 2010).

Chemical composition of essential oil

The insecticidal compositions and essential oils obtained of many plants are monoterpenoids (Ahn et al. 1998; Ayvaz et al., 2008). Monoterpenoids may inhibit the nervous system of insects (Tong, 2010).

In the current study, the principal constituents of *H. tuberculatum* essential oil were ρ-menth-2-en-1-ol-cis (20.15 %), p-menth-2-en-1-ol-trans (16.92 %), trans-Piperitol (13.23 %), Piperitone (7.34 %) and cis-Piperitol (6.72 %) that the toxic impacts of the oil could be attributed to the major constituents. Previous studies on the essential oil of this species demonstrated variable chemical components (Raissi et al., 2016). The main composition of the essential oil of *H. tuberculatum* were characterized as β-phellandrene (23.3%), limonene (12.6%), (Z)-β-ocimene (12.3%), β-caryophyllene (11.6%), myrcene (11.3%), and α-phellandrene (10.9%) (Al-Burtamani et al., 2005). Giles and Bisits (2007) isolated the chemical compounds of *H. tuberculatum* essential oil and found cis-p-menth-2-en-1-ol and trans-p-menth-2-en-1-ol (22.9 and 16.1 %, respectively) as the main constituents in the oil. *H. tuberculatum* essentials oils were constituted by oxygenated monoterpenes (71.0 % of the whole oil). In another investigation in Larestan, Iran, main constitute of *H. tuberculatum* was borneol (25.73%) followed by α-Pinene (14%), Bornyl acetate (18.07%) and β-caryophyllene (7.43%) (Vahdania et al., 2011). According the current review we revealed that all samples comprise mainly of monoterpenes, and the differences could be due to differences in time of harvest, plant parts applied, agroclimatic and geographic conditions.

The dominant compounds obtained from *N. crispa* essential oil contained 1,8-Cineole (32.98 %) followed by β-Pinene (8.70 %), 4aα,7α,7aα-Nepetalactone (8.08 %) and 4aβ,7α,7aβ-Nepetalactone (6.1 %). The major composition of *N. crispa* (71 %) and *N. menthoides* (41.1 %) oils were identified 1,8-cineole (Mojab et al., 2009). Ali et al (2016) stated 1,8-cineole was the main component of *Nepeta racemosa* and *N. faassenii* essential oils. While *N. sibirica* and *N. subsessilis* essential oils mainly possessed of sesquiterpenes: (Z)-β-farnesene, β-bisabolene, δ-cadinene or β-caryophyllene, and caryophyllene oxide. Previous investigations represented that the essential oils of *Nepeta* species consist mainly of 1,8-cineole (Sonboli et al., 2004; Sefidkon et al., 2006). It has been found that the main constitutes of *N. racemosa* essential oil, collected from a wild source in western Iran, were 1,8-cineole (37%) and nepetalactone (2.3%) (Daryasari et al., 2012). 1,8-cineole has been indicated as a toxic volatile against insect pests (Obeng-Ofori et al., 1997; Lee et al., 2004; Kordali et al., 2006; Stamopoulos et al., 2007; Rozman et al., 2007).

Insecticidal activity of essential oils against *P. interpunctella* larvae

Some previous studies have described that many of the plant's essential oils possess an insecticidal and antifeedant activity on stored-product pests (Negahban et al. 2007; Rajendran and Sriranjini 2008; Karabörklü et al. 2010, 2011; Saeidi and Yousefi, 2013; Wu et al., 2015; Plata-Rueda et al., 2017).

Current study is the first investigation on the insecticidal activity of the essential oils of *H. tuberculatum* and *N. crispa* against stored product pests. Based on the LC_{50} values (Table 3) the examined essential oils were strongly toxic against *P. interpunctella*. Mortality rate of 86.7 and 88.3 % observed in third instar larvae of *P. interpunctella* exposed to different concentrations of *H.*

tuberculatum and *N. crispa* oils, respectively. In both oils, mortality of larvae increased as concentration of the essential oils increased. Similar findings were obtained by El-Khyat et al. (2017) who reported insecticidal activity of *Matricaria chamomilla* L., *Origanum majorana* L. and *Citrus aurantium* L. on *Ephestia Cautella* and stated that the insecticidal activity increased with the increase of concentration. Rafiei-Karahroodi et al. (2011) investigated characterization of essential oil of different essential plant oils on *Plodia interpunctella* and detected that the oils have toxicant effect on first instar larvae of Indian meal moth. These reports are confirmed by Ebadollahi et al. (2010), Moazeni et al. (2013), and Pandir and Baş (2016) who indicated that essential plant oils were very toxic on insects due to their active volatiles. Ahmad et al. (2016) stated that crude extracts and fractions from *N. leavigata* and *N. kurramensis* indicated insecticidal activity against *Tribolium castaneum* which supports the traditional anti insect value (mosquito replant) of the Nepeta species (Baser et al., 2000). Insecticidal properties of *N. recomena* on *Sitophilus granaries*, *E. kuehniella* and *Lasioderma serricorne* has been reported (Aslan et al., 2005). The essential oil of *S. hortensis* has been reported to possess high toxic effect against *E. kuehniella* and *P. interpunctella* (Mollaei et al., 2011). Insecticidal activity of the essential oils varies depending on the experimental method, exposure time, origin of the essential oil, concentration of the oil, insect stage and species (Chiasson et al., 2001; Choi et al., 2003; Sedy and Koschier, 2003; Negahban et al. 2007; Ayvaz et al., 2010; Mollaei et al., 2011).

In conclusion, findings of this investigation revealed the essential oils from *H. tuberculatum* and *N. crispa* showed potent fumigant activities on *P. interpunctella*. Nevertheless further studies, including evaluation of the residues of the oils in food, flavor quality of food and persistence experiments, are required to clarify the competency of *H. tuberculatum* and *N. crispa* to reduce stored-products insect populations in IPM programs.

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Efficiency of ozone gas treatment against *Plodia interpunctella* (Hübner) (Lepidoptera:Pyralidae) (Indianmeal Moth) in hazelnut

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Abstract

In this study, ozone gas at different concentrations (16.7, 33.3 and 66.6 mg/L) were exposed to all biological stages (egg, larva, pupa and adult) placed at top and bottom of the hazelnut for various exposure periods (2, 4 and 6 hours). In biological tests conducted in presence of hazelnuts, 100% mortalities of all biological stages of *P. interpunctella* placed at top of the commodity were obtained at tested ozone concentrations and exposure periods while it was easier to kill the adult and pupa stages that the larva and egg stages. While it was possible to kill 100% of the adults and pupae placed at bottom of the commodity at tested ozone concentrations and exposure periods, 100% mortality of the larvae and eggs were not obtained at any of the ozone treatments. Generally, the mortalities of all life stages of *P. interpunctella* placed at bottom of the commodity for ozone treatments were lower than those placed at top of the commodity. It was easy to kill the pupae and adults of *P. interpunctella* placed at bottom of the commodity while the ozone treatments resulted in low mortalities of the egg and larvae placed at bottom of the commodity. Just as 100% mortalities of the larva and adult stages were not obtained even at the highest ozone concentration for the longest exposure period. In conclusion, in this study, it was observed that ozone gas only at high concentrations can control all biological stages of *P. interpunctella* in hazelnut and therefore could have an alternative potential for methyl bromide in quarantine applications in short application period.

Keywords: *Plodia interpunctella*, ozone gas, hazelnut, fumigation

Introduction

Hazelnut production is an important agricultural activity in Turkey. Hazelnut cultivation is mainly performed on steeplands in Black Sea region of Turkey and it is being an important source of income for a large number of family farms (Dikmen, 1999). Turkey produces 73% of world production and exports 84% of its production, which accounts for around 20% of total agricultural exports from Turkey (Fiskobirlik, 2003). Storage pests infesting hazelnut especially during drying and storage period may cause significant problems in hazelnut sector. The Indianmeal moth (*Plodia intepunctella* (Hubner)) reduces fruit quality by feeding and damaging the fruit and contaminating by leaving its excretions and other residues as silky net weaves (Damarlı et al., 1997). Large populations can develop before being detected and severe damage may occur rapidly (Jarratt, 2001). Moreover, from a phytosanitary point of view, during export, the presence of insects, or their fragments, has cost inestimable losses due to cargo returns.

Ozone is a triatomic form of oxygen (O_3) and is referred to as activated oxygen, allotropic oxygen or pure air. It is an unstable gas and its life span lasts about 20 minute, depending on the temperature. Electrical generation of ozone eliminates the handling, storage, and disposal problems of conventionally used post-harvest pesticides. Attractive aspect of ozone is that it decomposes rapidly (half-life of 20-50 min) to molecular oxygen without leaving a residue. These attributes make ozone an attractive candidate for controlling insects and fungi in stored products. Ozone in its gaseous form has been also considered to have potential to kill insect pests in commodities and was subjected of several research studies (Erdman, 1980; Mason et al., 1997; Kells et al., 2001). High mortality was achieved for adults of the maize weevil, *Sitophilus zeamais* (Mostsch.), and the red flour beetle *Tribolium confusum* (Jacqueline de Val), and the larval stage of the Indian meal moth, *Plodia interpunctella* (Hübner) exposed to lower ozone concentrations ranging from to 5 to 45 ppm (Erdman, 1980; Kells et al., 2001).

Methyl bromide has frequently used as a fumigant for disinfestations of other stored agricultural commodities such as nuts, cereals and fruits since it kills the insects rapidly, has a wide spectrum of activity and relatively low-cost (Fields and White, 2002). However it had been banned in developed countries since 2005 and scheduled for worldwide withdrawal from routine use as a fumigant in 2015 under the directive of the Montreal Protocol on Substances that Deplete Ozone Layer (Schneider et al., 2003) except quarantine, laboratory and pre-shipment purposes. As a consequence, there is a critical need to develop new fumigants for quarantine purposes, where rapid insect mortality is required (exposure time less than 1 day). Thus, the objective of present study was to determine toxicity of ozone at high concentrations and short exposure time against all life stages of *Plodia interpunctella* (Hübner) in hazelnut under laboratory conditions.

Material and Methods

Commodity

In-shell hazelnuts with m.c. of 10.5%±0.5 were used in the tests. In order to minimize the reaction of microbial loads in the commodity with ozone the hazelnuts used in the tests were sterilized under high pressurized stem.

Fumigation chambers

Test chambers consisted of 3 liter glass jar, each capped with a metal stopper equipped with entry and exit tubing. Two pieces of rubber tubing, 5 cm long, 6.2 mm ID, were attached to the tubing and sealed with pinch-clamps. The desiccators were sealed with silicone vacuum grease.

Test insects

Tests were carried out on all life stages (adult, larva, pupa and egg) of *P. interpunctella*. All life stages of *P. interpunctella* were obtained from cultures reared at 26 ± 1°C and 65 ± 5% r.h. on a diet of a 10:2:1 mixture of wheat flour: wheat germ: dried Brewers yeast using standard culture techniques. Eggs aged 1-2 days in 9 cm Petri dishes were placed in 3 litter jars and then were exposed to the treatments. Larvae were removed from culture jars and exposed to the treatments 21 days after oviposition. Two days old pupae were obtained by daily separation from culture jars and were exposed to the treatments. Newly emerged, aged 0-1 day, adults were placed in culture jars and then were exposed to the treatments.

Ozone fumigation procedures

Ozone generator in laboratory scale was provided from the company Ozomax Inc., Ozone gas was generated using a laboratory corona discharge ozone generator (Model OZO-1VTT) from purified extra dry oxygen feed gas. Ozone was introduced as gaseous into the exposure jars using an ozone generator. Pressure in each jar was measured using a 0 to 800 mm Hg vacuum digital gauge. The 100 mm Hg measure referred to herein is absolute pressure, with 760 mm Hg considered as atmospheric pressure. Prior to each test, twenty larvae, pupae or adults were confined, separately, inside 3 cm diameter by 8 cm long wire-mesh cages. For eggs mortality evaluation, fifty eggs placed in opened Petri dishes were used per fumigation.

For intermittently repeated ozone treatment in presence of commodity, each desiccators was loaded separately with 1.3 kg of in-shell hazelnut, and then 50 eggs, 25 pupae, adults and larvae were confined inside the wire-mesh cages and inserted into top and bottom position of the commodity, and the desiccators were briefly evacuated to 760 mm Hg. Afterwards, ozone gaseous at concentrations of 16.7, 33.3 and 66.6 mg/L was flushed into exposure jar until reaching atmospheric pressure and it was repeated every half an hour for 2, 4 and 6 hours. Untreated control insects were exposed to atmospheric conditions. Each test was replicated at three times. For all ozone fumigations, r.h. and temperature were maintained at 65±5% at atmospheric pressure and 26±1°C, respectively.

Data processing and analysis

After each treatment, larvae, pupae, and adults were transferred to 250-mL jars containing standard diets and were held at 26 ± 1°C and 65 ± 5% r.h. until examined for mortality. The eggs in their Perspex slides were held under the same conditions until the oviposition sites were examined for egg hatch. Mortality data was subjected to Arcsin transformation and then, were analyzed using one-way analysis of variance (ANOVA). The means were separated using the LSD method at 5% level.

Results and Discussion

Toxicity data for empty space ozone treatments indicated a remarkable difference in susceptibility between the life stages of *P. interpunctella*. Adult and pupae stages mortality rates were found to be high at low ozone concentrations (8.4 and 16.7 mg/L) and short application periods (30 at 60 minute), whereas egg and larval mortality rates were very low. High mortality rates were obtained from the larvae and egg stages with the increase in the application period, but 100% mortality of the larvae and egg was achieved at the highest exposure times (240 and 360 minutes) and ozone gas concentration (66.6 mg/L). In empty space fumigation of ozone, susceptibility of the adult and pupae stages was similar to ozone gas while they were sensitive to ozone gas than the larvae and egg. As a result, it was easy to kill the adults and pupas at low ozone concentrations for short exposure times while higher ozone concentrations and longer exposure periods were required to obtain complete mortality of the larvae and eggs.

In biological tests conducted in presence of hazelnuts, 100% mortalities of all biological stages of *P. interpunctella* placed at top of the commodity were obtained at tested ozone concentrations and exposure periods while it was easier to kill the adult and pupae that the larvae and egg stages. While it was possible to kill 100% of the adults and pupas placed at bottom of the commodity at tested ozone concentrations and exposure periods, 100% mortality of the larvae and egg were not obtained. Similarly to the results obtained from empty space ozone fumigation, there was significant difference in susceptibility of biological stages of *P. interpunctella* to ozone treatments. The mortalities of the adults and pupas was higher than those of the larvae and eggs. Generally, the mortalities of all life stages of *P. interpunctella* placed at bottom of the commodity for ozone treatments were lower than those placed at top of the commodity. It was easy to kill the pupas and adults of *P. interpunctella* placed at bottom of the commodity, while the ozone treatments resulted in low mortalities of the egg and larvae placed at bottom of the commodity. Complete (100%) mortalities of the larvae and adult stages were not obtained, even with the combination of the highest ozone concentration and the longest exposure period.

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Ethyl formate application trials for in-transit fumigation of shipping containers

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Two ethyl formate/nitrogen in-transit trials have been conducted on containerized goods in shipping containers under winter and summer temperature ranges to provide a representative dataset of year round temperature conditions in Western Australia. The winter trial was conducted in September 2017 and the summer trial in December 2017. Overall, the trials demonstrated that ethyl formate/nitrogen fumigation exposure periods could be successfully completed in-transit, with zero risk to the public or workers from exposure to ethyl formate throughout the two-day journey. The results show that more than half of the applied ethyl formate/nitrogen concentration was maintained over the six-hour exposure period. This is consistent with the fumigant decay of a shipping container undergoing a stationary fumigation exposure period. These results also show that the environmental influence on a moving container under fumigation was negligible in reducing the efficacy of the treatment. Environmental gas concentration measurements taken throughout the journey indicated nil presence of ethyl formate in the immediate surroundings of the containers up to 15 metres downwind, as well as inside the cab of the truck. These results further suggest that there would be zero risk to workers if the containers were vented at the end of the two-day journey. Continuing the in-transit period would eliminate the requirement for ventilation screens to be installed whilst undergoing ventilation.

Safe and cost-effective method for application of liquid ethyl formate (Fumate™) as a methyl bromide alternative for perishable commodities

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Abstract

The cylinderized liquid ethyl formate (EF) formulated with CO_2 is one of the great potential fumigants to replace methyl bromide (MeBr) for fresh fruit. However, it is too expensive to adapt commercial practices, and also involves work place safety issue including handling of heavy cylinders as well as restrict emission of CO_2, particularly for use in large scale commercial fumigationw. Therefore, it is urgently needed to develop environmental friendly, safe for workers and cost-effective alternative method for application of liquid ethyl formate as a MeBr alternative for perishable commodities. Recently, the environmentally friendly, cost-effective and practically safe use of liquid EF (Fumate™, registered name) with nitrogen gas has been developed and commercialized in Republic of Korea and Australia. The new technology for application of liquid EF is 100 times safer than MeBr in terms of threshold values (EF, TLV = 100 ppm). Ethyl formate is known as food additive and naturally occurred substances as well as a non-ozone depletion chemical. In this report, we demonstrate the liquid EF application technology that offers a clean environment (no ozone depletions and CO_2 emissions), safe to fumigators and related workers and practically cost-effective technology to fumigation industry.

Keywords: Quarantine fumigation, Ethyl formate, Fumate™, perishable commodities.

1. Introduction

Methyl bromide (MeBr) had being planned for phasing out and recommended to reduce its usage by the International Plant Protection Convention (IPPC) since the 1990s. Therefore, from point of view of protecting ozone policy and conducting safe fumigation by operators and related workers, MeBr must be urgently replaced. Intially ethyl formate (EF) with carbon dioxide formulation in cylinder offered replacment of MeBr for fumigation of fresh fruit (Ryan et al., 2013). However, cyliderized EF formulation has some hudles for its broad commerical use. This is because it is too expensive to use as fumigant, especially at a high dose rate (70g m^{-3}), such as fumigation of citrus. It also frequently involved worker safety issue for handling of heavy cylinders. In order to sucessfully achieve cost-effective and practically safe use of liquid EF with nitrogen gas, long-term and continousely cooperative research and developement has been conducted between Murdoch University and Animal and Plant Quarantine Agency (APQA). Here, we report various commerical demonstration trials with new liquid EF application technology on perishable commodities such as orange, banana, lemon and grapefruit and vertify the current quarntine protocols in Korea.

2. Materials and Methods

2.1 Fumigants and fumigation

Ethyl formate (Fumate™, 99%) was supplied from Safefume Inc. Korea. Ethyl formate was vaporized with heated nitrogen gas through the nitrogen heater, which fitted in vaporizer (SFM-1) and discharged into the fumigation chamber.

2-2. Commercial scale fumigation tests

Commercial scale trials were performed at Busan port, South Korea. A 40ft shipping container and PVC-tarpaulin fumigation chambers (81-424 m^3) were used. Ethyl formate was applied with SFM-1 (EF vaporizer) at dose rate of 70 g m^{-3} for citrus (orange, lemon and grapefruit) and 35 g m^{-3} for banana; exposed at > 5°C (citrus) and >13°C (bananas) for 4 hours. After 4 hours of fumigation, the container and chamber were opened and ventilated for > 1 hours. The phytotoxicity of ethyl formate on commodity was investigated after 2 weeks of fumigation held at room temperature conditions.

2-3.Measurement of fumigant concentration

Ethyl formate was drawn with an electric pump at timed interval and stored in Tedlar's gas sampling bags (1L, SKC Inc.). The concentration of EF was measured by a portable EF analyzer (iBRD MX, Industrial Scientific) and some random gas samples were analysed with a GC-FID (Agilent Technology 7890N) at laboratory to compare accuracy of portable EF analyzer.

3. Results and Discussion

The cumulative Ct products of ethyl formate in inside and outside bag of banana were calculated at the range of 26.1 to 42.0 g h m^{-3} and 35.5 to 78.8 g h m^{-3}, respectivly. In case of citrus fumigation, the Ct products were 102.6 to 133.7 g h m^{-3}. However, the same commodites even treated with same calulated dose of fumigant, the Ct products can be significantly different which depend on the conditons of loading ratio, types of application facilities (period of application) etc. The commercial trials with liquid EF applied with SFM-1 (EF vaporizer) have obtained different Ct products at different treatment conditions. However, liquid EF fumigation with nitrogen has met the current quarantine protocols on fruit fumigaiton in Korea. The efficacy of fumigation with EF achieved complete control of target pests such as citrus mealy bugs (*Planococcus citri*), California red scale (*Aonidiella aurantii*), Foller'rose weevil (Naupactus godmanni) etc. on imported Citrus and *Aspidiotus excisus* on banana. The results from phytotoxic acessment demonstrated privously by Kim et al., (2017) and Yang et al., (2016, 2017) and indicate that this new technology to apply liquid EF suits

the commercial fumigation practice and regulatory point of view for replacement of MeBr and conducting of good fumigation practice to ensure worker and envorimental safety.

4. Acknowlodgements

We thank for Donga Fumigator Co. in Busan, Korea for providing and supporting all fumigation facilities, Fumate™ and operation for the tirals.

Table 1. Cumulative CTP (Concentation X Time product, g h m^{-3}) of 4 hr Ethyl formate fumigation on imported fruits depending on different conditions in commercial trials

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Safe and high efficient method for application of liquid ethyl formate (Fumate™) to replace methyl bromide for treatment of imported nursery plants

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Abstract

There have been significantly increased reports of finding invasive quarantine pests with increasing import plants into Korea. Moreover, the efficacy and work safety issues have been reported regarding use of methyl bromide (MeBr) for fumigation of imported nursery plants. For replacement of MeBr use on imported plants, a new technology of using liquid ethyl formate has been registered in South Korea as Fumate™. The technology involved to mix ethyl format with nitrogen gas to form non-flamable ethyl format formulation. It has been evaluated on various imported plants. The Fumate™ is recently developed and commercialized in Republic of Korea and Australia for quarantine treatments on fresh fruits, grains etc. Fumigation with Fumate™ offers environmental-friendly and practically safe use of liquid ethyl formate. We have extended the use of liquid EF application technology to quarantine treatment of imported nursery plants.

Keywords: Quarantine fumigation, Ethyl formate, FumateTM, Nursery plantsIntroduction

1. Introduction

Methyl bromide (MeBr) had long been planned for its phasing out and recommended for reduction of its usage by the International Plant Protection Convention (IPPC). Therefore, for protecting the ozone policy and work safe applications, MeBr needs to be urgently replaced. Ethyl formate (EF) offers great potential to replace MeBr for fresh fruit fumigation. The Animal and Plant Quarantine Agency (APQA) of the Korean Government, is under progress for developing new alternative fumigants and fumigation technology to replace MeBr. However, intially, the cylinderized liquid EF with carbon dioxide formulation faced with challenges from higher costs, work safety issues and phytotoxicity of furits and vegetables (Ryan et al., 2003; Simpson et al., 2004). Recently, the cost-effective and practically safe use of liquid EF with nitrogen gas has been developed by cooperative research between Murdoch University in Australia and APQA in Korea. An innovative application of EF (Fumate™) with nitrogen is replacing cylinderized formulation because new EF application technology benefits to reduce operation costs of fumigation, provide safety for workers, handling heavy cylinders as well as reduction of green house gas (Yang et al., 2016). Here, we report the phytotoxicity of EF on imported nursery plants.

2. Materials and Methods

2-1. Preparation of samples

In this preliminary test, 12 different variety of imported nursery plants were used as listed below:

Alocasia amazonica, Cactaceae spp, Sansevieria trifasciata, Codiaeum variegatum, Peperomia obtusifolia, Peperomia puteolata, Hoya carnosa, Viburnum odoratissimum, Spathiphyllum wallisii, Rhapis excalsa, Hedera helix, Fatsia japonica and Sansevieria stuckyi.

2-2. Fumigant

Fumate™ (Ethyl formate, 99%) was supplied from Safefume Inc., Korea. EF was vaporized with heated nitrogen gas through a nitrogen heater, which was fitted in vaporizer (SFM-1) and discharged into the fumigation chamber.

2-3. Preliminary test of phytotoxicity to nursery plants

Phytotoxic tests were performed at Gyeongsang National University (GNU) site. A fumigation chamber (0.55m x 0.50m x 1m) was used and the loading ratio of plants was 5 to 15%. After loading the nursery plants, we calculated the required dosage of EF (70 g m^{-3}) that was injected into the chamber for fumigation for 4 hours. The air temperature in fumigation room was controlled with an air conditioner at 20±2°C. After fumigation, the chamber was ventilated for more than 1 hour, and phytotoxicity on nursery plant was investigated after 1st and 7th day after treatment. Assessment for recovery of post-fumigated plants was undertaken after 4 weeks after treatment.

2-4. Measurement of fumigant concentration

For monitoring concentration of EF in the chamber, gas samples were drawn with an electric pump at timed intervals and stored in Tedlar gas sampling bags (1 liter, SKC Inc.) before analysis. The concentration of EF was determined by using an Agilent Technology 7890N gas chromatography (GC) equipped with a flame ionization detector (FID) after isothermal separation on a 30 m × 0.32 mm I.D. HP-5 (0.25 μm film)-fused silica capillary column (Restek Co. Ltd.). The GC oven, injector and detector temperature were 150, 200 and 200°C, respectively. Helium was used as the carrier gas at a rate of 2 mL/min. The peak areas were calibrated periodically using a standard (inject the known volume of ethanedinitrile in 1 L Tedlar gas sampling bags).

2-5. Assessmenet of post-fumigation phytotoxicity

The market qualities on nursery plants post-fumigation was assessed by three different ways. The external damage was measured by researchers and numbered as index ranged from 0 (none) to 4 (severe). Chrolophyll contents were measured with chrolophyll meter. Color changes of leaf was measured with colorimeter. Unfumigated nursery plants were used as controls for these assessments.

3. Results

Phytotoxicity of EF to nursery plants

The cumulative concentation X time products (Ct) of EF were 74.9 to 143.4 g h m^{-3}. The Ct products decreased with the increase of loading ratio of plants. Even though there were small damages in terms of reducion of chlorphyll and changes in colors in post-fumigated nursery plants within the first week, there were no serious damages found in terms of recoveries at 4 weeks post-fumigation. This is the time (4 weeks), at which, usually post-quarantine plants are distributed in markets in Korea. However, there was no recoveries in *Spathiphyllum wallisii* and *Peperomia puteolata* plants (Table 1)

Tab. 1 One week end point damage of nursery plants after 4 hr EF fumigation (20°C)

Nursery plants	Filling ratio (%)	CT value (mg h/L)	Damage index[a] (Mean ± SE)		Chlorophyll			Hue[b]			recovery
			Control	Treatment	Control	Treatment (Damaged leaf)	Treatment (Undamaged leaf)	Control	Treatment (Damaged leaf)	Treatment (Undamaged leaf)	
Alocasia amazonica	5	141.7	0.0 ± 0.0	3.0 ± 0.0	37.9 ± 0.6	-	-	8.3 ± 4.2	-	-	O
Cactaceae spp	10	143.4	0.0 ± 0.0	0.0 ± 0.0	-	-	-	-	-	-	-
Sansevieria trifasciata	15	110.6	0.0 ± 0.0	1.0 ± 0.0	56.4 ± 2.7	-	35.8 ± 5.2	17.3 ± 8.6	-	10.3 ± 5.2	-
Codiaeum variegatum	5	141.7	0.0 ± 0.0	1.7 ± 0.3	78.3 ± 1.4	32.5 ± 2.7	75.1 ± 0.0	6.2 ± 3.1	-	11.8 ± 6.2	O
Peperomia obtusifolia	8	104.1	0.0 ± 0.0	2.7 ± 0.3	41.6 ± 1.5	19.4 ± 2.7	34.6 ± 1.0	6.9 ± 3.5	19.4 ± 2.7	13.2 ± 0.6	O
Peperomia puteolata	15	80.1	0.0 ± 0.0	1.3 ± 0.3	41.9 ± 0.9	-	39.6 ± 0.3	8.6 ± 4.3	-	18.2 ± 0.6	X
Hoya camosa	8	104.1	0.0 ± 0.0	2.7 ± 0.3	58.5 ± 0.8	25.6 ± 5.8	65.8 ± 3.0	6.5 ± 3.2	18.2 ± 2.1	9.3 ± 0.7	O
Vibumum odoratissimum	5	141.7	0.0 ± 0.0	3.0 ± 0.0	59.0 ± 0.5	33.4 ± 5.7	22.5 ± 4.6	7.0 ± 3.5	16.4 ± 2.6	17.0 ± 0.6	O
Spathiphyllum wallisii	5	141.7	0.0 ± 0.0	4.0 ± 0.0	55.4 ± 0.2	36.4 ± 2.6	58.3 ± 6.6	7.3 ± 3.7	11.1 ± 0.2	15.5 ± 1.4	X
Rhapis excalsa	8	104.1	0.0 ± 0.0	4.0 ± 0.0	-	-	-	-	-	-	O
Hedera helix	8	104.1	0.0 ± 0.0	4.0 ± 0.0	47.7 ± 0.4	18.9 ± 2.6	57.8 ± 1.2	8.2 ± 4.1	19.2 ± 1.9	11.6 ± 0.2	O
Fatsia japonica	8	104.1	0.0 ± 0.0	4.0 ± 0.0	52.4 ± 0.4	41.9 ± 1.0	-	5.8 ± 2.8	8.5 ± 4.2	-	O
Sansevieria stuckyi	10	74.9	0.0 ± 0.0	0.0 ± 0.0	-	-	-	-	-	-	-

[a]: Damage score : 0 (0%), 1 (<5% affected shoot), 2 (5-25% affected shoot), 3 (25-50% affected shoot), 4 (>50% affected shoot)
[b]: [Color L x 2+ Color a x 2+ Color b x 2]/2
- : Impossible to check

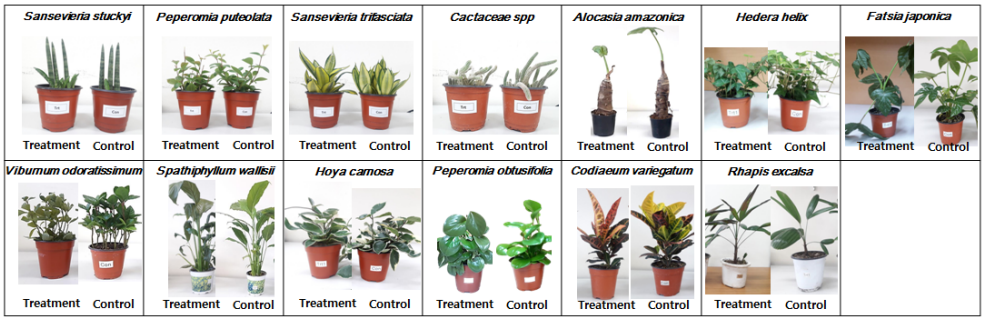

Fig. 1 Comparison of untreated and EF treated nursery plants

4. Discussion

Although EF fumigation caused decrease of chrolophyll contents on leaf and withering from some imported plants after one week of fumigation, this research showed that ethyl formate (EF) fumigation could be potentially used on imported nursery plants in terms of recoveries, 2 weeks post-fumigation, which is more than the period required in Korea to distribute the plants to consumers. The imported nursery plants in Korea might be grown in remote nusery sites for several months depending on plants for satisfication of market values and to certify disease-free, and during this period the damaged plant can be recoverred. The degree of phytotoxic damages with EF fumigation could be various depending on species, ages and physical conditions of plants when imported in Korea. It is known that MeBr fumigation can cause serious and unrecoverable damage on imported nursery plants as well as chronic inhalation damages to fumigator and related workers in post-fumgation process (Kim et al., 2016). Ethyl formate fumigation of nursery plants could be provide better options than current MeBr application in tems of work safety and less phytotoxic to commodities as well as classification of definatly non-ozone depletion chemical.

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A new concept for controlling tiny-scale insect pest in green house – noval technology to apply liquid ethyl formate

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Abstract

As increased agricultural insecticide uses and trends in insecticide resistance, increased labor cost to apply insecticide and limited its application to fertility season in green house. There is a need of a safe, labor-saving and confined space application concept to manage control tiny-scale insect pest such as thrips and whitefly. Fumigation with ethyl formate (EF), which is considered as effective to various insect pest and safely use in quarantine treatment, was evaluated in the confined space (glass house) and semi-confined space (vinyl house). The new application technology for application of liquid EF could be the one of the key options for control of tiny flying insects in greenhouses that would save labor and operation costs. It could be connected to smart-farm technologies in the near future.

Keywords: Ethyl formate, Inert gas application, green houses, future smart-farm technology

1. Introduction

Fumigants, like methyl bromide (MeBr) and phosphine, are widely used for quarantine and pre-shipment (QPS) fumigations and restriced use in preplant soil disinfestation because physically fumigants have less residues than solid/liquid active ingredients. Its use has advantages for better efficacies because it can penetrate into deep and easy in application even in tiny small space without addtional labor work. This means that fumigants have a potential to replace liquid/solid pesticides in plant cultivation in case they are grown in sealed or semi-sealed enviroments. Even though we know the benefits, usage of fumigant in agricultural purpose especially in sealed system is limited because most fumigants are expensive to apply and normally higher toxic to mammals and human being in terms of acute inhalation toxicity. Ethyl formate (EF), a MeBr alternative which was re-evaluated and commercialized in recent years, is less toxic than other fumigants and has less risk on environments (Muthu et al, 1984). Use of EF on plant cultivation could solve the issue like emerging and increasing pesticide resistant insects (PSI) and residues on harvest. Moreover, in case of application in a sealed system, workers do not need to be exposed to pesticide solution directly which is hazardous and there will be decreased labor-cost. EF fumigation in greenhouse was considered as higher operation costs than pesticide and concerns of leaked out environments. In recent report, liquid EF (Fumate™) with inert gas application was cost-effective and safer protocols than formulated in gas cylinder to apply various imported and exported fruits (Kim et al, 2017, Yang et al, 2016, 2017a, 2017b). In the preliminary studies, efficacies of EF on tiny small insect pest in cucumber green house was reported by Kim et al (2016). In this research, we reported liquid EF with nitrogen applied in the confined space (glass house) and semi-confined space (vinyl house) model and was evaluated for the accumulative Ct product in two system and monitoring of ethyl formate level in spaces after fumigations and aerations for accessment of worker safety.

2. Materials and Methods

2.1. Green houses

Two types of green houses were used, one was vinyl house (3.5m x 20.0m x 5.0m scales) and the other was glass house (8.0m x 5.0m x 24.0m), located at KNU site in Jinju, Korea.

2.2. Fumigation, gas sample collection and aeration

Ethyl formate (FumateTM, 99%) was supplied from Safefume Inc., Korea. Ethyl formate was vaporized with SFM-1 vaporizer system which fitted with an internal gas heater to heat inert gas (nitrogen or carbon dioxide) through the liquid ethyl formate and purge mixture into the greenhouse. For analyzing of EF concentration inside greenhouse, gas sampling lines were placed in green houses (6 locations in vinyl house (VH) and 12 locations in glass house (GH)). The gas samples in greenhouse were taken at timed interval by withdrawing the gas through an air pump into gas bag (SKC Tedlar bag, 1L). Prior to fumigation, the green houses were sealed and calculated dosages of ethyl formate (10 g m-3 for VH trials and 5 g m-3 for GH trials) were applied. For assessment of work safety in work environment, ambient air samples were collected from 4 locations (W, E, S and N) with jumbo syringe (1L, SGE); during during application (0-30 min), fumigation holding period (4-12 hours) and aeration (2 hours). The gas samples were stored in Tedlar gas bags until analysis.

2.3. A nalysis of collected gas sample.

The concentration of EF was determined using a Agilent Technology 7890N gas chromatography (GC) equipped with a flame ionization detector (FID) after isothermal separation on a 30 m × 0.32 mm I.D. HP-5 (0.25 μm film) fused silica capillary column (Restek Co. Ltd.). The temperatures of the GC oven, injector and detector were 150, 200 and 200°C, respectively. Helium was used as the carrier gas at a rate of 2 mL/min. The peak areas were calibrated periodically using a standard.

3. Results

3.1. Concentration of EF inside green house during fumigation

The concentration of EF was continuously decreased at both vinyl house (VH) and glass house (GH). However, the aspects of decrease in two trials were different. Concentration decreased rapidly at overnight in VH and at day time in GH. EF concentration in VH was higher than GH at both 4 and 12 hr trials. Although there were little difference in concentration of EF depending on location of samples, there was no signicant difference in tems of cumulative CT products. The cumulative CT products of EF were 17.53 and 22.67 g h m^{-3} for overnight (12hr) and day time (4hr) application, respectively, in VH application. In GH trials, CT products of EF were lower than VH's, 4.53 and 2.62 g h m^{-3} for overnight (12hr) and day time (4hr) application, respectively. (Fig. 1-Fig.4). Even though 2 times dose applied in VH, concentration of EF in VH is lower than expected. It could be different depending on sealing conditions, temperature when applied etc.

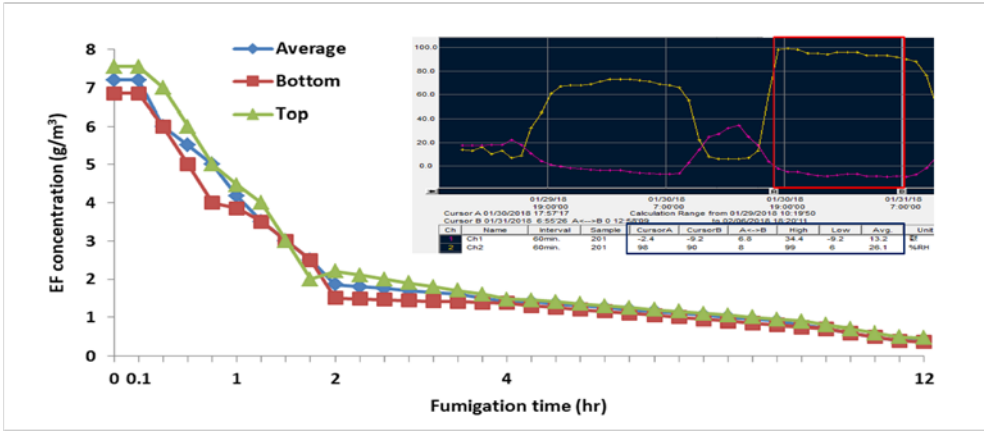

Fig. 1 Concentration of EF (g m^{-3}) inside vinyl house for 12 hr fumigation.

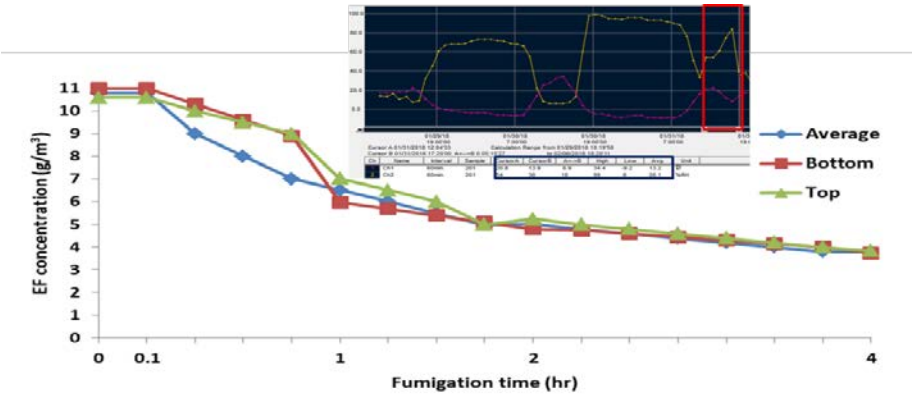

Fig. 2 Concentration of EF (g m^{-3}) inside vinyl house 4 hr fumigation.

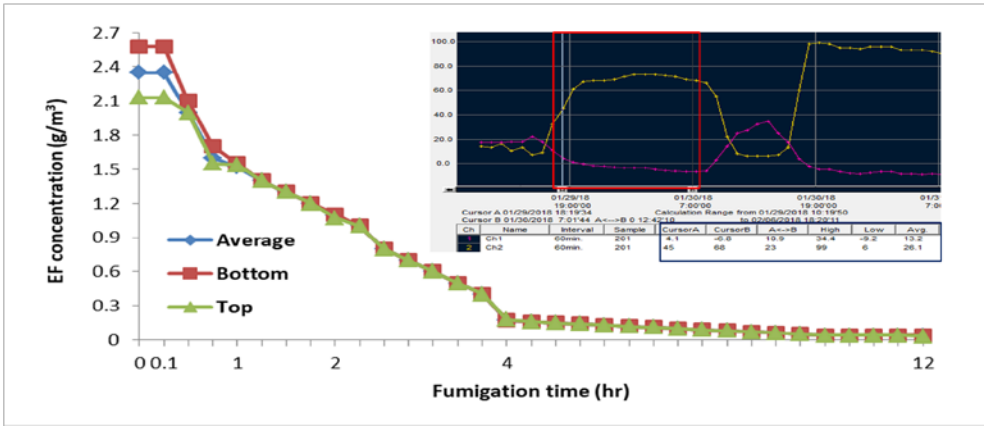

Fig. 3 Concentration of EF (g m^{-3}) inside glass house for 12 hr fumigation.

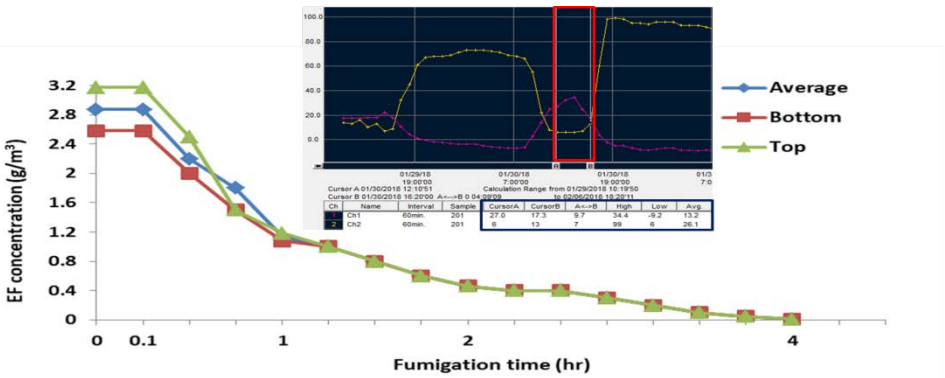

Fig. 4 Concentration of EF (g m^{-3}) inside glass house for 4 hr fumigation.

3.2. Ethyl formate levels in the air samples outside green house during the EF application.

Ethyl formate levels from all ambient air samples were < 50 ppm in all trials during the 30 min application of EF (Tab. 1, 2).

Tab. 1. Ethyl formate levels surrounding vinyl house after 0 - 30 min of injection in 4 and 12 hr fumigation experiment.

Sampling time (min)	12 hr fumigation EF concentration (ppm)				4 hr fumigation EF concentration (ppm)			
	East	West	South	North	East	West	South	North
0	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
10	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
20	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
30	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50

Tab. 2. Ethyl formate levels surrounding glass house after 0 - 30 min of injection in 4 and 12 hr fumigation experiment.

Sampling time (min)	12 hr fumigation EF concentration (ppm)				4 hr fumigation EF concentration (ppm)			
	East	West	South	North	East	West	South	North
0	< 5	< 5	< 5	< 5	20	20	20	20
10	< 5	< 5	< 5	< 5	20	40	20	< 5
20	< 5	< 5	< 5	< 5	7	< 5	< 5	< 5
30	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5

3.3. EFhyl formate levels inside green house during 2 hours of ventilation.

EF levels in the green house (GH) during 2 hours of ventialion were shown in Tab 3. As expected, EF levels in green house were continuously decreasing. After 30 min-ventilation, EF levels in GH was decreased to <10 ppm at both trials.

3.4. Carbon dioxide levels inside green house during the fumigation.

In case of 12hr-EF application in green house fumigation, liquid EF applied with inert gas (carbon doxoide) as carrier gas, it could be expect to help growing plants some crops depending on variety, concentration and exposure time of CO_2 etc. In this experiment, carbon doxoide (CO_2) levels inside green house (GH) for 12hr fumigation was also monitored in Fig. 5 and 6. The levels of CO_2 was initially increased up to 700-800 ppm and decreased to 600-700 ppm at the end of fumigation.

Tab. 3 Ethyl formate levels in the vinyl house during 2 hours of ventialations after completion 4 and 12 hr fumigation experiment.

	12 hr fumigation EF concentration (ppm)		4 hr fumigation EF concentration (ppm)	
	Entrance	Exit	Entrance	Exit
0 min	< 10	< 10	1560	923
5 min	< 10	< 10	966	873
10 min	< 10	< 10	226	236
15 min	< 10	< 10	130	122
20 min	< 10	< 10	50	26
30 min	< 10	< 10	< 10	< 10
60 min	< 10	< 10	< 10	< 10
120 min	< 10	< 10	< 10	< 10

Tab. 4 Ethyl formate levels in the glass house during 2 hours of ventialations after completion 4 and 12 hr fumigation experiment.

	12 hr fumigation EF concentration (ppm)		4 hr fumigation EF concentration (ppm)	
	Entrance	Exit	Entrance	Exit
0 min	< 10	< 10	90	90
5 min	< 10	< 10	15	18
10 min	< 10	< 10	15	< 5
15 min	< 10	< 10	< 5	< 5
20 min	< 10	< 10	< 5	< 5
30 min	< 10	< 10	< 5	< 5
60 min	< 10	< 10	< 5	< 5
120 min	< 10	< 10	< 5	< 5

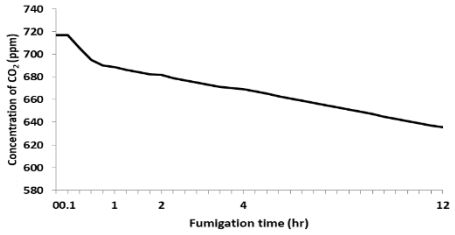

Fig. 5 CO_2 level (ppm) in vinyl house during 12 hr fumigation (Mean CO_2 level in the atmosphere is 400 ppm).

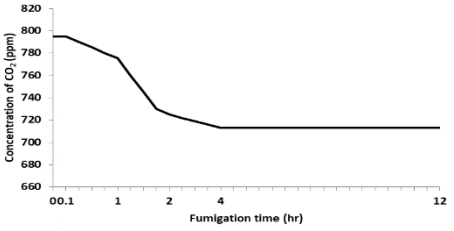

Fig. 6 CO_2 (ppm) in glass house during 12 hr fumigation (Mean CO_2 level in the atmosphere is 400 ppm).

4. Discussion

In this preliminary study on vinyl house, we found that liquid EF with inert gas (nitrogen or carbon dioxide) application, was cost-effective and labor-saving, which showed the promise for further reseaches. Because vinyl house application showed better sealed sytem than glass house system and EF levels during the application and ventilation after completion of fumigation was relatively safe in terms of current workplace safety guideline of EF (TLV-TWA, 100 ppm). The cumulative Ct products (>10 g h m^{-3}) calculated in vinyl house both for 4 and 24 hr applications could be expected to be enough to control especially flying insect such as whitefly and thirps, which are hard to control with insecticides in green house system. However, for commercial use of EF in green house system, there is more researche required in terms of phytotoxic damages of different plants and their growing stages as well as fumigation conditions such as temperature and humidity in detail. Nevertheless, this newly emering liquid EF technology could provide a new concept to partly replace intergrated insecticide approach, which leads to build-up of resistance to insecticide and restricts application frequencies when crops are ready for markets.

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Supporting quarantine and health & safety monitoring of fumigants and industrial chemicals in offshore transport containers with Gasmet Multicomponent FTIR gas detection technology

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Keywords: container, fumigation, Fourier Transform Infrared (FTIR), fumigant, library search tool

Abstract

Cargo containers and wooden packing materials are fumigated to control the spread of pests and micro-organisms. However, fumigant gases are toxic and present a danger to human health even at low concentrations. Additionally, products shipped in containers may release VOCs from the solvents, coatings and glues used in manufacturing processes, and the concentrations of these vapors may be significant in the confined space of the container. Gas measurements are required in order to protect the health of any workers involved in opening these containers.

As potentially hazardous gases originate from a variety of different sources, the amount of gases that need to be monitored, in order to ensure a safe working environment, is very large. The Fourier Transform Infra-Red (FTIR) measurement principle allows simultaneous measurement of a large amount of inorganic and organic substances, regardless of their molecular weight. The portable Gasmet™ DX4040 Multicomponent FTIR Gas Analyzer records infrared spectra at 10 scans/second and is capable of sub-ppm detection. When used with a laptop computer and the pro version of Calcmet™ software, the DX4040 is capable of analyzing up to 50 components simultaneously with compensation for cross-interference effects.

A standard application has been developed for container measurements. The application consists of a gas library of 50 gases that has been configured to include all of the most important fumigants and other hazardous gases found in containers, along with a number of other commonly found gases for correction of cross-interference effects. A built-in QA/QC routine ensures reliable results and alerts the user of the possibility of unknown gases in the sample.

If the presence of unknown gases in the sample is suspected, these can be identified using the library search function available in the CalcmetTM software. Identification is undertaken by automatically finding matching spectra in the library of hundreds of different reference spectra measured by Gasmet for different compounds. Once the unknown compound has been identified, it can be added to the analysis for quantification. The measured sample spectra are not altered by the analysis and are saved so they can be re-analyzed at a later time if needed.

The Gasmet™ DX4040 is battery powered, backpack sized designed for use in field conditions. The analyzer is portable, so there is no need for separate sampling and the sample can be collected and analyzed directly on site. Quick and easy sampling, coupled with fast, simultaneous analysis of all compounds makes for an

exceptionally quick measurement procedure per container. The DX4040 requires no span gas calibrations and uses no consumables for sampling or analysis. Only a short zero calibration with nitrogen is required once per day. This means that containers can be measured quickly and with a negligible cost per measurement.

The Gasmet DX4040 provides a powerful and cost effective solution to the challenge of measuring gases inside cargo containers. The use of FTIR technology enables the simultaneous measurement of an unparalleled amount of gases for a portable device, which leads to improved safety of workers. The DX4040 is also durable, requires no calibration gases (other than N2 for zero measurement) and requires no consumables for sampling. This means that the cost of ownership for this solution is also exceptionally low.

Efficiency of phosphine and modified atmospheres against five different stored products insects

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Abstract

There has been a notorious resistance to phosphine over the last decade, and a wide variety of factors can be associated with this rise to tolerance in stored products in the northwest of México, which can be due to bad exposition times and application of phosphine, and others causes; investigations were conducted in a warehouse place comparing the efficacy of phosphine with the use of mixtures gases in order to create the modified atmosphere against five diferrent adults: *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Hbst.), *Rhyzopertha dominica* (Fabricius.), *Oryzaephilus surinamensis* (L.), and *Prostephanus truncatus* (Horn.). An application of 1.4 ± .21 gr/m^3 of phosphine for 72 ± 1 h exposure time could achieve 100% mortality to four species just like: of *Tribolium castaneum*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis*, and *Prostephanus truncatus*. While for *C. ferrugineus* the 100% mortality could be achieved after 4.2 ± 63 gr/m^3 of phosphine for 120h exposure time.

Modeling the distribution of phosphine in cylindrical grain silos with CFD methods for precision fumigation

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Abstract

In the present study, the distribution of phosphine gas in a cylindrical silo was modeled and compared with available sensor data. The cylindrical silo was filled with wheat and a recirculation system was used to enhance the diffusion of phosphine throughout the grain volume. A Computational Fluid Dynamics (CFD) model was developed with OpenFoam software, which accounted for gas transport in porous media and sorption effects of phosphine into the grain. A time-dependent source was used to model the phosphine release from Aluminum Phosphide bags. Furthermore, simulation results were obtained for insect mortality as a function of their exposure to phosphine gas. The phosphine concentration measurements were available from calibrated wireless sensors provided by Centaur Analytics, placed near the silo walls at various heights. As the agreement of phosphine measured data with the simulation results was satisfying, it led to considering that the proposed CFD model (equations, boundary conditions, grain properties, recirculation system approach, etc.) was accurate. Utilizing the capabilities of fumigation modeling, the phosphine concentration could then be determined for every location inside the storage volume and at any given time, thus a prediction method for fumigation

duration and success could be enabled. Additionally, as the CFD model correlates phosphine exposure with insect mortality, a methodology for planning precision fumigations can now be established.

Keywords: phosphine, modeling, fumigation, cylindrical silo.

Introduction

Phosphine (PH_3) is the single most relied-upon fumigant to control grain pests, due to its inexpensiveness, ease of application and universal acceptance as a residue-free treatment. Since the use of Methyl Bromide was phased out due to its significant contribution to the destruction of the earth's stratospheric ozone layer, phosphine has emerged as a viable replacement. However, there are several factors that occasionally prevent phosphine fumigations to be successful (e.g. phosphine sorption, leaky storage structures, poor monitoring procedures). Improper use leaves the treated commodity susceptible to insects, increasing the possibility of spoilage, but is also known to lead to tolerant strains among key stored product insects throughout the world (Athanassiou et al., 2016).

In view of the above, it is important to bolster the effectiveness of phosphine fumigation processes and ensure the ecosystem can continue to rely on this important fumigant. To achieve this, an in-depth knowledge and understanding of fumigant behavior are crucial. An efficient method for tackling this is through the combination of field experiments and computer simulation. To the authors' knowledge, there are a limited number of studies in the literature adopting this approach. Except for Lawrence et al. (2013), none of them combine field experiments with detailed numerical simulations. Lawrence et al. (2013) presented a 3D transient heat, mass, momentum, and species transfer model for the stored grain ecosystem which was developed using the finite element method. However, they validate their model against average phosphine concentration measurements which are not indicative of treatment effectiveness. Other relevant publications concerning phosphine simulations are the ones by Boac et al. (2014), Isa et al. (2016), but both are lacking validation with experimental data. Specifically, Boac et al. (2014) studied phosphine distributions in bulk storage structures (bunkers) including the effect of wind phenomena, whereas Isa et al. (2016) made predictions of phosphine flow during grain fumigation in leaky cylindrical silos. Mills et al. (2001) studied a positive pressure system for combating dilution during phosphine fumigations of bulk grain. Nonetheless, their CFD model is not extensively documented. Chayaprasert et al. (2006) used CFD to develop 3D computer models for structural fumigations upon datasets collected at a fumigation treatment in a commercial flour mill. The fumigation models were divided into two parts: internal and external flow models.

In this work, a detailed description of the fumigation treatment inside a cylindrical silo is presented, presenting – for the first time – correlations of numerical (CFD) analysis with wireless gas sensor readings based on a rich sample of phosphine distribution during the entire duration of treatment. Numerical results are employed to provide a map of insect mortality rates, thus binding the analysis with the end objective of pest treatment. Additionally, information about the PH_3 sensing devices as well as the simulation approach is given and a methodology for planning and implementing precision fumigations is outlined.

Materials and Methods

Silo description

The silo under consideration (Fig. **11**) was located in the area of Volos, Greece and the fumigation treatment took place during December. The steel silo diameter was D=15 (m) and its height was H=12 (m). A recirculation system was installed and used during the process. Stored grain (whole wheat) temperature was 12 (°C).

Measurement of phosphine concentration

Data collection of phosphine concentration inside the silo was made with sensor devices provided by Centaur Analytics, Inc. The devices are based on electrochemical sensors thus providing high

accuracy, and are equipped with wireless connectivity with the ability to transmit data frequently (e.g. every 2 hours) from inside stored grain. The data were transmitted in real time to Centaur's cloud platform, from which they were downloaded and further processed. Fig. **11** shows the position of the 4 sensors inside the silo, whereas Fig. **12** shows how one of the sensors is installed inside the silo.

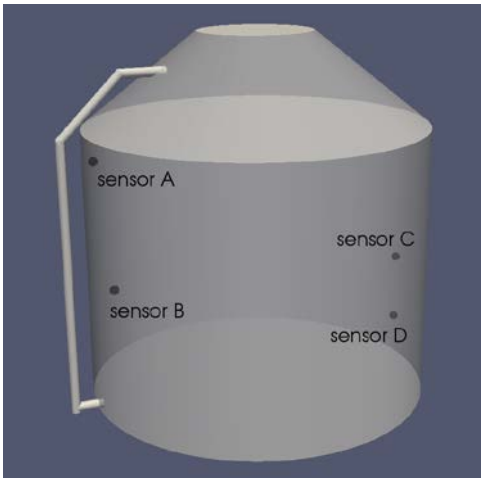


Fig. 11 The three-dimensional model of the cylindrical silo considered in this work

Fig. 12 Installation of sensor B inside the silo

Fumigation parameters

Phosphine gas was generated using Aluminum Phosphide bags. Approximately 10 gr of AlP per tonne of stored product was used, which is equivalent to 2.53 gr of phosphine gas per m^3. The degassing evolution of phosphine is presented in Fig. **13**.

Simulation technique

In phosphine fumigations, it is important to ensure that phosphine concentration exceeds the predefined ppm levels in the entire storage space, to eliminate all insects (for 99.9% mortality). In order to increase the spatial resolution of sensor data, Computational Fluid Dynamics (CFD) models are used. CFD is a branch of fluid mechanics that uses numerical analysis and data structures to solve and analyze problems that involve fluid flows. Computers (typically on the cloud) are used to perform the calculations required to simulate the interaction of liquids and gases with surfaces defined by boundary conditions.

Governing equations

The CFD solver is implemented using OpenFoam v.3.0.1 (OpenFOAM Foundation, Ltd.) in order to solve the following transport equations for incompressible fluid flow, heat, and mass transfer, accounting for porous media effects:

$$\nabla u = 0 \quad (1a)$$

$$\frac{\partial u}{\partial t} + \frac{1}{\phi} u \nabla u = -\phi \nabla p + \nu \nabla^2 u - \phi \frac{\nu}{K} u - \phi \frac{F_e}{\sqrt{K}} |u| u + \phi g \beta (T - T_{ref}) + \phi g \beta_c (C - C_{ref}) \quad (1b)$$

$$\frac{\partial T}{\partial t} + \phi \frac{(\rho C_p)_f}{(\rho C_p)_{eff}} u\nabla T = \frac{k_{eff}}{(\rho C_p)_{eff}} \nabla^2 T \qquad (1c)$$

$$\phi \frac{\partial C}{\partial t} + \phi u\nabla C = \phi\nabla^2 (\frac{D_m}{\tau} C) - \phi B_1 C + B_2 q \qquad (1d)$$

$$\frac{\partial q}{\partial t} = -B_3 q + \phi B_4 C \qquad (1e)$$

In the above equations (1a-1e), u is the velocity vector, and p, T, and C are the pressure, temperature, and phosphine concentration in air, respectively. D_m is the binary diffusion coefficient ($m^2\ s^{-1}$). Buoyancy forces created by both temperature and concentration gradients are considered in the momentum equations using the Boussinesq approximation. Under the Boussinesq approximation, the variation of density ρ with temperature T is linear, according to $\rho = \rho_{eff} - \rho_{eff}\beta(T - T_{ref})$. The volumetric coefficient of thermal expansion β and the species expansion coefficient β_c for ideal gases are given by Eqs. 2a and 2b respectively:

$$\beta = -\frac{1}{\rho}(\frac{\partial \rho}{\partial T})_p = \frac{1}{T} \quad (2a) \qquad \text{and} \qquad \beta_c = -\frac{1}{\rho}(\frac{\partial \rho}{\partial C})_p = \frac{1}{\rho_{air}}(\frac{MW_{air}}{MW_{gas}} - 1) \quad (2b)$$

where MW_{air} and MW_{gas} are the molecular weights of air and phosphine gas respectively.

According to Shen et al. (2007) in order to represent the role of porosity on ordinary molecular diffusion, the diffusion coefficient must be scaled with tortuosity. Specifically, an effective diffusivity coefficient could be set as:

$$D_{eff} = \frac{D_m}{\tau} \qquad (3)$$

Neethirajan et al. (2008) calculated τ = 2.4 for wheat.

Porous media approach

In order to account the effect of grains on the gas flow, the grains were assumed to be a porous medium. Flow in porous layers is described by the Darcy-Brinkman formulation. The geometric function Fe and the permeability K of the porous medium are related to the porosity φ based on Ergun's experimental investigations:

$$F_e = \frac{1.75}{\sqrt{150\ \phi^3}} \qquad (4a) \qquad \text{and} \qquad K = \frac{\phi^3\ d_p^2}{150(1-\phi)^2} \qquad (4b)$$

The effective properties $(\rho\ C_p)_{eff}$ and k_{eff} are calculated as a function of the fluid and porous material:

$$(\rho\ C_p)_{eff} = (1-\varphi)(\rho\ C_p)_{solid} + \varphi(\rho\ C_p)_f \qquad (5a)$$

$$k_{eff} = (1-\varphi)\ k_{solid} + \varphi\ k_f \qquad (5b)$$

Sorption effects

Phosphine is adsorbed by grain at differing rates depending on the grain type. Sorption can reduce the concentrations of fumigation doses to sublethal levels before grain has been disinfected. A model to predict fumigant losses due to sorption is considered necessary. Researchers (Darby, 2008) have suggested that the relationship between the fumigant concentration in the interstices between the grain, C, and the average concentration of fumigant within the grain kernel q, is modelled by Eqs 1d and 1e which assert that phosphine is absorbed into the grain and at the same time also degrades in air. The coefficients B_1, B_2, B_3, and B_4, are independent of C and q.

$$B_1 = \frac{S_{sorp}\, k_f}{B_{fill}} \quad (6a), \qquad B_2 = \frac{S_{sorp}\, k_f}{B_{fill} F} \quad (6b)$$

$$B_3 = \frac{S_{sorp}\, k_f}{(1-\phi)F} + k_{bind} \quad (6c), \qquad B_4 = \frac{S_{sorp}\, k_f}{(1-\phi)} \quad (6d)$$

$$B_{fill} = \varphi + \frac{1-R_{fill}}{R_{fill}} \quad (6e)$$

where: S_{sorp} is the specific adsorption surface area, k_f is a linear mass transfer coefficient, F is the partition relation coefficient, k_{bind} is the coefficient for irreversible reaction/binding of the adsorbed fumigant in the grain kernel. For wheat, the above parameters have the following values: $S_{sorp}\, k_f$ =0.0125, F=0.3, and k_{bind}=0.0569.

Boundary conditions

In order to evaluate accurately the storage (computational domain) interaction with its surroundings, the following convective boundary conditions were used for phosphine concentration and heat transfer (Barreto et al., 2013), respectively:

$$-D_m \frac{\partial C}{\partial x}|_{x=0} = h_m (C - C_{amb}) \quad (7a)$$

$$-k \frac{\partial T}{\partial x}|_{x=0} = h_c (T - T_{amb}) - \alpha_h G + \epsilon \sigma (T^4 - T_{sky}^4) \quad (7b)$$

Coefficients h_m, h are a function of silo geometry (cylinder, orthogonal), fluid medium (air, water) and fluid velocity (e.g. wind velocity). In Eq. 7b, the second term on the right-hand side is the heat gain due to solar radiation and the third term is the net radiation heat loss rate for a hot object which is radiating energy to its cooler surroundings (Adelard et al., 1998):

$$T_{sky} = 0.0552\, T_{amb} \sqrt{T_{amb}} \quad (8a)$$

$$h_c = 10.45 - U_{wind} + 10 \sqrt{U_{wind}} \quad (8b)$$

For the purposes of the present study, the time series of ambient temperature, wind velocity, and solar radiation that are used as inputs are presented in **Fig. 14**.

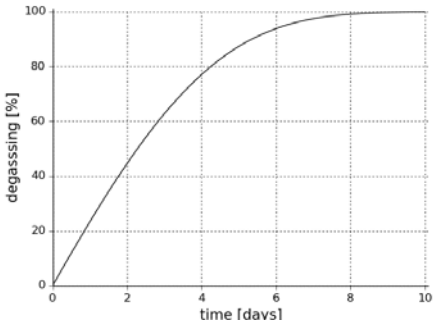


Fig. 13 Degassing evolution of phosphine gas from AlP bags during fumigation (data provided by Detia Degesch)

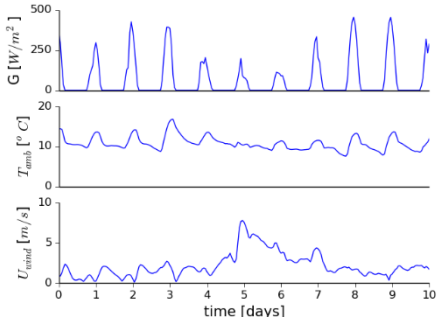


Fig. 14 Time variation of ambient conditions: solar radiation, temperature and wind velocity

Domain discretization

Meshing is the discrete representation of the geometry that is involved in the problem. Essentially, it partitions space into cells over which the equations can be approximate. In the present study, the computational grid used (Fig. **15**) was structured, thus ensuring greater accuracy, and all cells

(approximately 85000) were hexahedra. Furthermore, grid-clustering was employed near the side to properly capture large gradients.

Insect mortality

It is known that the effect of phosphine on the mortality of grain insects is due to both the level of the phosphine concentration and the time of exposure (Collins et al., 2005; Isa et al., 2016). An insect mortality indicator function IM(x, t) could be defined as:

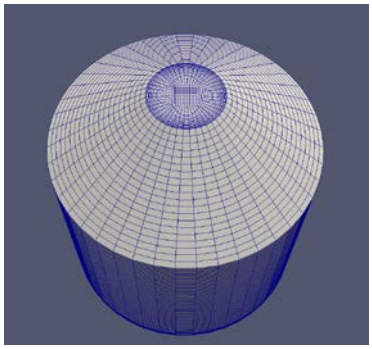
$$IM(x,t) = \frac{1}{K_1}\int_0^t C(x,t)^{K_2}\, dt \qquad (9)$$

The constants K_1 and K_2 are empirical and depend on the species and strain of insect. IM(x,t) account for the period of exposure to phosphine that an insect has encountered. For a given point in the grain, when IM(x,t)<1 there are some insects in the grain still alive. When IM(x,t)>1 at least 99.9% of the insect population have been killed. For the present simulations K_1=4.04 and K_2=0.6105 which accounts for the *Rhyzopertha dominica*.

Results

The simulation model yielded, among other results, the development of phosphine concentration for the entire duration of the fumigation treatment (9 days). In **Fig. 16**, the time evolution of phosphine at the 4 locations is presented. Specifically, sensor data are compared against model predictions. The best correlation occurs for A and B positions which are located on the silo side where the recirculation system was installed. Their maximum concentration is reached at the end of the 4th day, followed by a decrease due to diffusion, losses, and sorption by the stored product. Concerning, locations C and D, sensor data reveal lower concentration values as the model also predicts. A small discrepancy is observed on the time that the maximum value is reached. According to sensor data, phosphine concentration has an upward trend until the end of the 7th day, whereas the CFD model underestimates to the end of the 5th day. Minor fluctuations, with hourly timescales, occur due to natural convection currents which are the result of temperature differences imposed by the unsteadiness of ambient conditions. The currents create upward and downward air movements that transport phosphine along.

The overall performance of the CFD model is considered satisfactory ensuring the validity of the phosphine concentration predictions for the entire silo space as the ones presented in **Fig. 17**. Particularly, **Fig. 17** shows the spatial distribution of phosphine at four time instances. The advantages of using a recirculation system can be clearly seen since at the second day, phosphine has reached every position inside the silo. Until the 6th day, higher concentration values are observed on the top regions of the silo, near the aluminum phosphide bags but as their degassification completes a more uniform phosphine distribution is reached (**Fig. 17**, 8th day). A video showing the model predictions for the entire fumigation process could be found here: https://youtu.be/iISBS7eoWb8



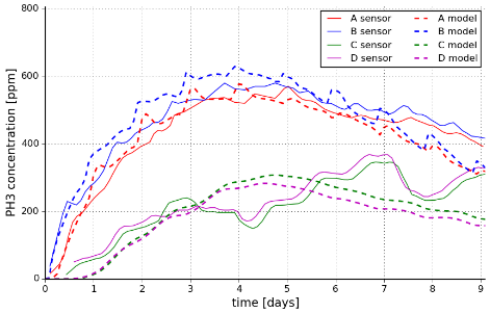


Fig. 15 The computational mesh used for the silo simulation

Fig. 16 Phosphine concentration (ppm) comparison of sensor data (solid lines) vs. simulation predictions (dashed lines) at 4 locations inside the storage.

A useful augmentation of the phosphine concentration profiles is the prediction of the insect extinction. **Fig. 18** shows the areas (red color) in which the Rhyzopertha Dominica species could not survive the fumigation process. As expected, areas near the Aluminum Phosphide bags and at the piping outlet are the first ones that reach lethal levels. According to the simulation, at the end of the 7th day there are still some areas that insects could be still alive. A video showing the insect extinction predictions for the entire fumigation process could be found here: https://youtu.be/54uJ1ZJlkrk

Discussion

A 3D heat, momentum, and species transfer model for stored grain ecosystems was developed in this work, able to predict phosphine concentration changes. As the agreement of phosphine measured data with the simulation results was satisfying, it is safe to assume that the proposed CFD model (equations, boundary conditions, grain properties, recirculation system approach, etc.) is accurate for the purpose. Utilizing the capabilities of fumigation modeling, the phosphine concentration was determined for every location inside the storage at any given time, thus a prediction of fumigation duration and pest elimination success could be provided. The main benefit of the CFD approach is its wide applicability on any type of commodity, storage or phosphine formulation. As the CFD model correlates phosphine exposure with insect mortality, a methodology for planning precision fumigations can be established.

In general, the results presented here illustrate that gas distribution is uneven during the entire treatment period, suggesting that there are large areas within the treated area that are exposed to low concentrations. This may result in increased survival of the exposed insects in these areas, and, to some extent, lead to tolerance development. Circulation of phosphine (through J-system) may be a solution to this implication, but additional experimental work is needed to estimate the relative benefits. In light of the present findings, it is evident that distribution is uneven right after the start of the application, and is likely to exhibit "diurnal circles", as has been previously reported for other trials (Athanassiou et al., 2016). By the use of sensors, however, monitoring of these variations may provide the inferences necessary for designing a strategy to overcome this phenomenon, under the premise of a 'precision fumigation' approach. This was definitely not possible, at least not at an acceptable accuracy level, with the 'traditional' phosphine concentration measurement techniques.

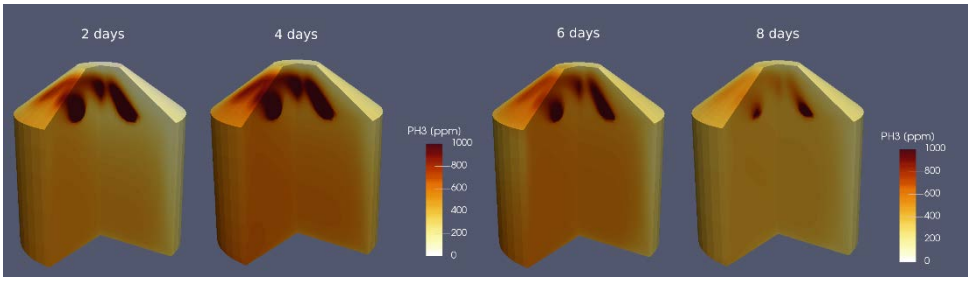


Fig. 17 Phosphine concentration profiles at 4 time instances

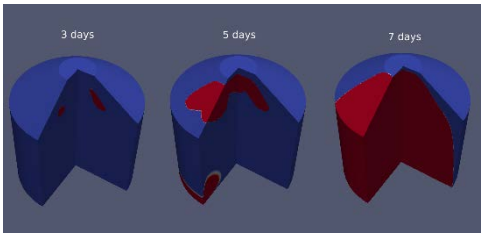


Fig. 18 Extinction of insects at 3 time instances. Red color indicates zones with 99.9% insect mortality.

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Phosphine distribution during fumigation of wheat in steel bins: extended abstract

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Abstract

Phosphine is a widely used fumigant for controlling insects in stored grain, but fumigation effectiveness is often compromised by suboptimal distribution of the gas. Leaks in the grain bin wall and roof, foreign material in the grain, and phosphine placement contribute to regions of insufficient concentration of fumigant, resulting in insect survival and leading to phosphine-resistant insect populations. Phosphine distribution was studied during field tests in temporarily sealed bins to compare distribution from conventional probed tablets to the distribution using a closed-loop recirculation system. The results showed uneven distribution patterns and leakage over time with conventional probed tablets, which resulted in some areas in the lower half of the grain

mass receiving no phosphine and some other locations remaining below the target phosphine concentration for the entire period of fumigation. The closed-loop fumigations with the same phosphine dosage yielded much more uniform phosphine concentrations, but suffered from equal or greater phosphine leakage losses.

Keywords: grain storage, phosphine resistance, stored product insects, closed-loop fumigation.

Introduction

The fumigant phosphine is extensively used for stored grain insect control and is considered one of the most effective insect control measures when properly applied (Philips et al., 2012). With this widespread use and high expectations, the effectiveness of phosphine fumigations is a fundamental concern for all users; however, there is little or no control of where gas may go during conventional fumigation. Improper application or leakage from the storage structure can result in insufficiently treated areas in the bin that will harbor surviving insects and likely select for resistant insects in the survivors. Thus, ineffective fumigations increase grain losses and contribute to the development of pesticide resistance in stored grain insects.

Conventional phosphine fumigation methods include probe and tarp, automatic dispenser, and gravity fumigation (Kenkel et al., 1993; Noyes et al., 1995). Phosphine is usually applied to grain as aluminum or magnesium phosphide in pellet or tablet form. The pellets or tablets react with water vapor in the air to produce phosphine gas. In gravity fumigation diffusion is used to distribute phosphine gas throughout the grain mass. There is little or no control of where gas may go during conventional fumigations. Each of these conventional methods offers increased risk of exposure during insertion of fumigant into the grain and the distribution of phosphine is often suboptimal. Leaks in the grain storage bin and foreign material in the grain can lead to regions of insufficient concentration of fumigant. Phosphine is also available in gaseous form mixed with carbon dioxide which can be directly injected into a grain storage bin. In the probe and tarp method, Noyes et al. (1995) recommended using a probe to place about three-quarters of the fumigant dosage 0.3 to 1.5 m below the surface of the grain mass and placing the remaining fumigant in aeration ducts in the base of the structure. Tarps can then be applied to partially filled bins to limit the fumigated volume and minimize leakage. In probe and tarp fumigation, workers must enter the grain bin and are exposed to entrapment hazards and fumigants during the tarping process.

A concentration of 200 ppm for 100 hours is the guideline to kill common stored wheat pests in Oklahoma (Noyes and Phillips, 2004) and in Kansas. It is virtually impossible to completely seal existing grain storage bins so that some phosphine does not leak out over the course of the fumigation. When sufficient levels of phosphine are not maintained for the duration required to eradicate all life stages of insects, the surviving life stages can continue to infest the grain. Furthermore, the surviving insects are likely to be the most resistant members of the population. Incomplete fumigations are a significant cause of development of phosphine resistance, which has been reported in stored grain pests (Benhalima et al., 2004; Lorini et al., 2007). Resistance to phosphine is a critical concern for grain storage managers because of the widespread use pattern.

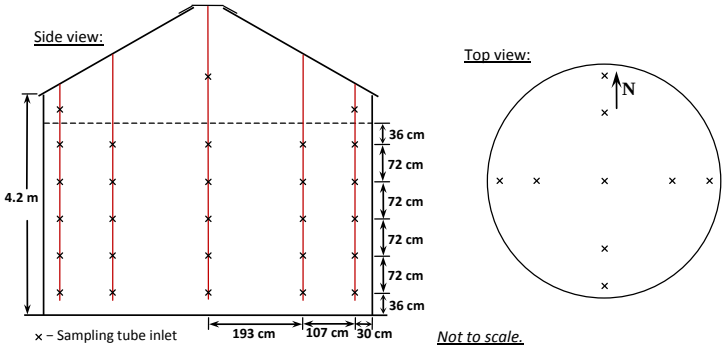
A safer and more effective alternative to traditional fumigation practices is the use of closed-loop fumigation (CLF) systems in grain handling and storage facilities. The typical CLF system uses a small fan and duct system to recirculate fumigant in the grain storage bin by drawing it out of the headspace and injecting it back into the bottom of the grain storage bin. The fumigant rises up through the grain until it enters the headspace where the cycle repeats. After several cycles through the grain storage bin the fumigant is evenly distributed. Recommended CLF flow rates of 0.0016 to 0.008 m^3/min per m^3 grain (0.002 to 0.010 cfm/bu) provide several air changes through the grain storage bin per day to provide sufficient mixing in the usual time that phosphine pellets/tablets react (Noyes et al., 2002). CLF systems that distribute fumigant evenly throughout a grain storage bin can allow the use of less phosphine in a fumigation because the manufacturer's recommended application rates are elevated to allow for unequal fumigant distributions in typical grain storage bin (Kenkel et al., 1993; Noyes and Kenkel, 1994; Noyes et al., 1995; Hardin et al., 2009).

Phosphine is chemically stable at the normal conditions inside a grain storage bin and diffusion through the envelope of the structure is generally negligible, while the major loss of phosphine is through leakage from the structure through cracks and other openings. Pressure from wind and thermal buoyancy are the primary forces that drive the exchange of fumigant with the air outside the structure (Cryer, 2008). Wind flowing around a grain storage bin induces areas of high and low pressure. Wind velocity, direction, and the presence of other structures all affect the pressure distribution on the grain storage bin, and in turn, influence leakage (Mulhearn et al., 1976; Banks et al., 1983; Bibby and Conyers, 1998). CLF systems produce a pressure differential across the grain mass that can significantly contribute to leakage in a grain bin that is not sufficiently sealed.

The objective of this study was to evaluate the distribution of phosphine in temporarily sealed grain storage bins during conventional fumigation with probed tablets and compare to distribution during closed-loop fumigation of the same bins.

Materials and Methods

The fumigation experiments were conducted in two corrugated steel bins each containing 95 metric ton of hard red winter wheat. Bins were 6.6 m in diameter with 4.2 m eave height and 6.0 m peak height. The wheat was center-loaded in the bin and leveled at 3.6 m deep. Plastic sampling tubes (3 mm inside diameter) were attached to support cables, which were installed with two in each cardinal direction plus one in the center with five sampling tubes on each support cable (Fig. 1). This provided nine sampling tube inlets at each of five depths in the grain mass plus three in the headspace, giving 45 sampling points distributed through the grain mass out of 48 total sampling points in the bin. The tubes ran outside the bins through an opening designed for that purpose with the ends arranged in a grid on a board for easy access. The bins were temporarily sealed using 4 mil plastic sheets covering all opening using contact adhesive. The sidewall to eave joint had been



previously sealed with caulk.

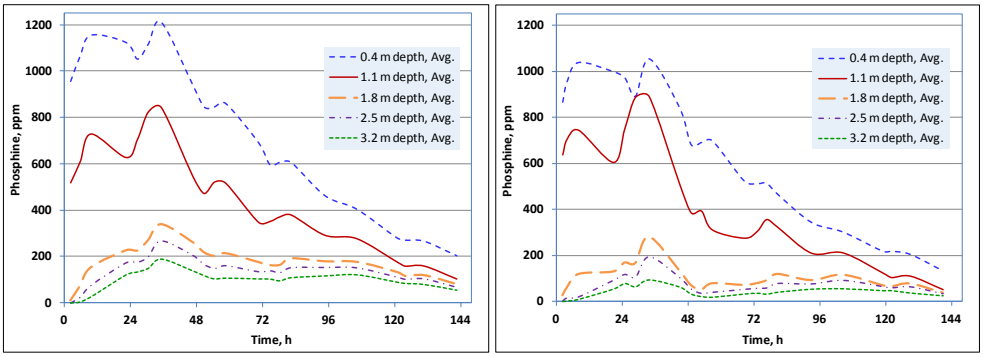
Fig. 1 Experimental bin showing sampling tube locations.

Each bin was fumigated at the minimum label rate of 90 tablets per 27 metric ton of grain for both conventional and CLF fumigations. In the conventional fumigations the tablets were evenly dispersed among three depths of 1.2, 0.6, and 0.3 m from the surface at nine locations near the nine support cables. In the CLF fumigations the tablets were dispersed across the top surface of the grain and circulation fans were run for 45 minutes every six hours throughout the fumigation. After phosphine application, the concentrations of phosphine gas at various depths were measured manually with a Dräger X-am 5000 (Drägerwerk AG & Co., Lübeck, Germany) personal monitoring instrument using a Dräger X-am 1/2/5000 pump to draw the gas from the grain mass through the sampling tube and the lines of the gas sensor. The readings were collected approximately at 4-8 h intervals for 5 to 6 days. Phosphine concentrations were averaged for all nine sampling points at each depth in the grain and for the three sampling points in the headspace and the resulting means

graphed versus time. The full data from all 45 sampling points in the grain were analyzed to determine statistics such as mean, standard deviation, minima, and maxima for each sampling time.

Results

Figure 2 shows phosphine concentrations during a six-day conventional fumigation in the two bins. The two monitored depths with the highest readings (0.36 m and 1.1 m) had average doses above 200 ppm for most of the first five days, with only minor differences in the trends between the two bins. These two depths with the highest readings were the two nearest the top surface and fell within the range of depths where the pellets were introduced. The three lower depths monitored (1.8, 2.5, and 3.2 m), all below the depths where the pellets were placed, received average doses at each level less than 200 ppm with a few exceptions between 20 and 60 h. The peak readings at those three lower depths occurred at 34 to 35 h in both bins and one of the six average readings at those depths (from three readings each in two bins) reached 339 ppm at that peak (Fig. 2), while the rest were all below 300 ppm at the peak. The readings at the upper two depths also peaked at 34 to 35 h indicating the aluminum phosphide tablets were spent shortly after that time and the subsequent declining phosphine readings resulted from continued leakage out of the bins. In general, bin 2 had slightly lower average phosphine readings at all depths and at all times than did bin 1. These lower readings were likely due to bin 2 having slightly greater overall leakage around the upper portions of the bin, where maximum concentrations occurred, than did bin 1. The upper two depths in these bins (0.4 m and 1.1 m) had average concentrations above 200 ppm for the recommended 100 h (Jones et al, 2008), but lower three depths did not in either bin.

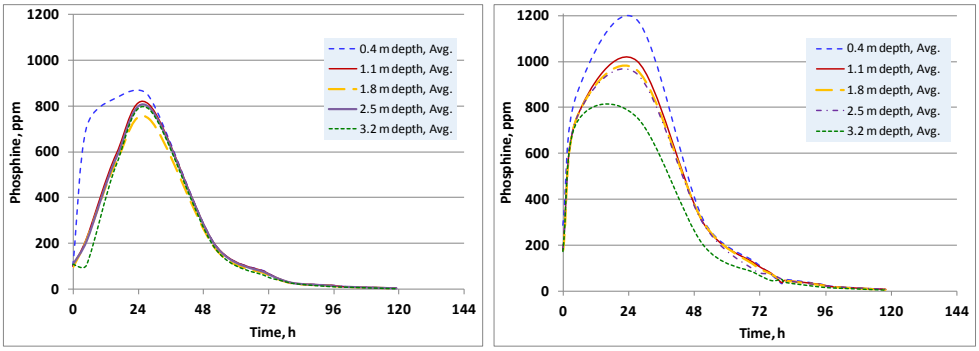


(a) (b)

Fig. 2 Phosphine concentrations during conventional, probed-tablet fumigation in (a) bin 1 and (b) bin 2, each containing 95 metric ton of wheat at 24°C.

Some of the individual sampling points at the lower three depths had zero readings for much of, and occasionally all of, the six-day conventional fumigation. The maximum number of sample points with a zero phosphine reading was 26 and 24 for bin 1 and 2, respectively, which occurred at the first reading (2.3 h). The minimum number of sample points with a zero phosphine reading was six and ten for bin 1 and 2, respectively, which occurred at 104 h. After 104 h the number of sample points with a zero reading began to increase again in both bins. It was also observed that 14 of 45 monitored locations in both grain masses received no phosphine (all readings during the fumigation were zero) or nearly no phosphine (majority of readings during the fumigation were zero). Both bins had similar patterns of phosphine average dosage for all depths, but there was slightly more variation observed between the two bins for the highest monitored depths (Fig. 2 and 3). However, the greater variability at the upper two depths could be due to the overall higher phosphine readings for those depths having proportionally higher deviations than in the lower three depths with the lower readings.

Figure 3 shows phosphine concentrations during a five-day CLF fumigation of the two bins. All peak readings, which occurred during the first 48 hours of fumigation, were in the same range as the highest peak readings in the conventional fumigations, 800 to 1200 ppm, with no depths having very low peaks (below 300 ppm) as seen at many of the lower depths in the conventional fumigations.



(a) (b)

Fig. 3 Phosphine concentrations during CLF fumigation in (a) bin 1 and (b) bin 2, each containing 95 metric ton of wheat at 24°C.

In bin 1, the distribution of average readings at each depth showed very little variation with only one data point early in the fumigation, at 5 h, deviating from the uniform trends. The peak readings at 28 h in bin 1 were all very close to each other at approximately 750 to 850 ppm. In bin 2, the peaks at 27 h varied from 800 ppm to 1200 ppm, but these were much more uniform than the peaks in the conventional fumigations. For both bins, average concentrations remained above 200 ppm for all five heights for 48 h, but no depths stayed above 200 pm for more than 60 h. In general, bin 1 had slightly lower average phosphine readings at all depths and at all times than did bin 2. These lower readings may have been due to bin 1 having slightly greater overall leakage around the lower portions of the bin, which received the maximum pressurization from the circulation fan, than did bin 2.

The variation between readings at different depths in both bins for both conventional and CLF fumigations was evaluated by calculating the coefficient of variation from the mean and standard deviation of the 45 concentration values in each bin (Fig. 4). The conventional fumigations showed much larger values of coefficient of variation because of the large deviation between readings at different locations, especially between different depths (Fig. 2), within the grain mass. The CLF fumigations almost always had coefficients of variation under 30% except one data point in one test early before the recirculating airflow had produced uniform distribution.

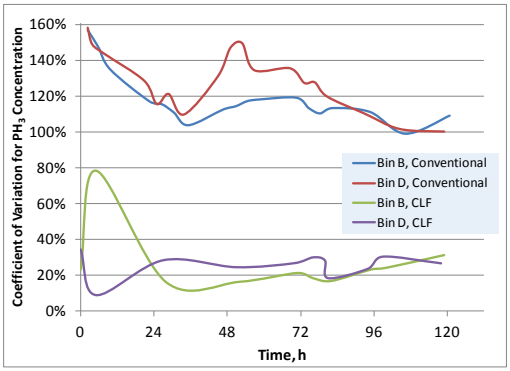


Fig. 4 Coefficient of variation over time for the average of the phosphine concentrations at 45 locations in the four bins during fumigations.

Discussion

Flinn and Reed (2008) found similar results to ours in tall concrete bins when fumigating with pellets. In the absence of wind or chimney effects the phosphine gas did not move far from the pellets so that locations in the bins without pellets did not receive lethal concentrations of gas for fumigation. When there were significant chimney effects in those tall bins due to temperature differences, the phosphine gas moved to other locations and moved out of the bins through leakage from openings in the top and bottom of the bins. Cook (2016) measured phosphine gas concentrations during CLF fumigation of small (45 to 50 metric ton), well-sealed metal bins. Gas was circulated using a thermosiphon system (Boland, 1984). The CLF systems of Cook also maintained relatively uniform, but higher, phosphine gas concentrations during fumigations similar to our CLF fumigations. With the well-sealed bins in that study, phosphine gas concentrations always remained above 100 ppm for 125 h. The longer maintenance of gas concentrations was clearly a result of more effective sealing on those bins compared to the temporary sealing of our bins.

Fumigation treatments reported by Jones at al. (2008) demonstrated some similarities and some differences in comparison to our results. In their tests, pellets were distributed uniformly in tall concrete bins while turning the grain in the conventional application and the resulting gas concentrations were compared to those in identical bins under CLF fumigation. In the CLF bins, the same number of pellets were distributed on the top surface of the grain with phosphine gas then distributed with intermittent running of a recirculation fan. In the conventional bins the three monitored locations, top, middle, and bottom never reached 200 ppm of phosphine gas at any time during 72 h of monitoring, which is like the locations in our conventional fumigation bins that were not in close proximity to the tablets. CLF bins in their tests maintained an average phosphine gas concentration above 1000 ppm for approximately the last 60 h of the same test period, which indicates there was much less leakage from those concrete CLF bins than from our steel CLF bins.

Our measurements showed uneven phosphine distribution patterns and leakage over time when fumigating with conventional and CLF techniques. With the conventional probed tablet fumigation, the uneven distribution of phosphine at the minimum label rate resulted in effective doses in only some portions of the bin. The distribution of phosphine gas was much more uniform when using the CLF fumigation method. Both types of fumigations exhibited wind-driven leakage that was often excessive, while the CLF bins also exhibited continual high leakage due to the fan pressures in the bin and ductwork during the intermittent fan operation. Leakage driven by wind effects and recirculation fan pressure in these temporarily sealed bins prevented lethal phosphine gas dosages for the recommended length of time in all or part of the bin in all tests.

Acknowledgement

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Fumigation of Apples and Sunflower Seeds with Phosphine – Desorption Behavior and Aroma Profiles

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Since many decades, fumigations of stored products are an accepted and worldwide used method to control pest organisms. Infested stored goods can be treated with anoxia and chemical fumigants to eradicate pests very effectively and without any movement of the products. Stored-product insects present a serious problem causing economic loss and contamination of food destined for animal or human consumption as well as a direct physical damage of materials and objects.

Therefore, fumigations are an effective option in manufacturing, storage and shipment. Since the International Standard for Phytosanitary Measures No. 15 (ISPM 15) is approved, container fumigations against quarantine pest have become important and customary in international trade.

We investigated several subjects related to fumigation such as occupational safety, modification of flavor profiles in fumigated crops and the development of resistance against fumigants.

Fumigation of goods for protection against pests is common practice in the context of global trading. One of the most commonly used fumigants for this purpose is phosphine (PH3). Apples are fumigated prior to export to control eggs of pest insects like the codling moth (*Cydia pomonella*). In this study we addressed the question whether phosphine fumigation affects the aroma profile of apples (*Malus domestica* 'Royal Gala'). For this purpose, a headspace solid-phase micro-extraction (HS-SPME) technique was developed and coupled to subsequent gas chromatography-mass spectrometry (GC-MS).

Previously we looked into the desorption behavior of phosphine after the fumigation of apples and sunflower seeds. Furthermore, the effects of fumigation on the overall volatile profiles were studied. Alterations of the volatile profiles were observed for apples and sunflower seeds.

A second question addressed concerns the adsorption and desorption behavior of phosphine from apples and sunflower seeds under different conditions as well as the chemical residues. The impact of the initial fumigation concentration and of the storage temperature was analyzed. The phosphine concentration was thereby monitored using GC-MS instrumentation.

Dates fumigation with phosphine

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Abstract

Stored dates are usually infested by sap beetles and moths. For years, the common practice for dates disinfestation was fumigation with methyl bromide (MB). After MB phase-out, heat treatment and modified atmosphere are used. However, there are several limitations of these methods. In search for alternatives for dates disinfestation, fumigation by phosphine was evaluated.

Commercial fumigations of Medjool dates variety using phosphine were conducted in a standard 20 ft. shipping container. Two formulations of phosphine were used: Magtoxin® Plates 56% (Detia Freyberg GmbH, Germany), and Phostoxin® Tablets 56% (Detia Freyberg GmbH, Germany). The phosphine dosage range was 1-4 g/m^3. The exposure time range was 24-72 hrs. Several fumigations were carried out by an innovative phosphine generator model OMT 501 developed by Universal Probes. Most fumigations carried out demonstrated total dates disinfestation. The application of Magtoxin plates, especially using the OMT 501 demonstrates significant advantages versus Phostoxin tablets; the advantages were in quicker gas development, and achieving much higher maximum and pre-ventilation phosphine concentration levels. Upon fumigation using the OMT 501, plates are easily collected and disposed, no residual dust left on the dates, which avoided their contamination. No phosphine residues were found in the fumigated dates, neither changes in organoleptic properties were noted. Phosphine fumigation using the phosphine generator model OMT 501 provides safer, quicker, more efficient dates disinfestation.

Keywords: fumigation, phosphine, dates

Introduction

Stored dates are usually infested by sap beetles and moths. For years, the common practice for dates disinfestation was fumigation with methyl bromide (MB). After MB phase-out, heat treatment and modified atmosphere are in use (Navarro, 2006; Navarro and Navarro, 2015; Rafaeli et al., 2006). However, there are several limitations of these methods. Today, phosphine is the main fumigant for postharvest treatment in stored products, such as grain and dry food. To improve the phosphine

fumigation some innovative technologies were suggested (Kostyukovsky and Shaaya, 2012; Kostyukovsky et al., 2010; 2013). In search for alternatives for dates disinfestation, fumigation by phosphine was evaluated.

Materials and Methods

Two standard 20 Ft shipping container were used for the fumigations. One container was used as it is without special sealing. The other one was sealed especially for the treatments.

Two formulations of phosphine were used: Magtoxin Plates, 56% (Detia-Degesh, Germany), or Phostoxin Tablets, 55% (Detia-Degesch, Germany). The range of phosphine concentrations used was 1-4 g/m^3. The range of exposure time was 24-72 hrs (1-3 days). The treatments were done with or without the phosphine generator model OMT 501 (Universal Probes, Israel). The list with all fumigation treatments is shown in table 1.

Tab. 1 Fumigation treatments

Trial #	Phosphine formulation	OMT 501	Dosage g/m^3	Quantity	Exposure time
1	**Magtoxin Plates**	V	4	4 Plates	1 day
2	**Magtoxin Plates**	V	2	2 Plates	1 day
3	**Magtoxin Plates**	V	2	2 Plates	2 days
4	**Magtoxin Plates**	-	2	2 Plates	3 days
5	**Phostoxin Tablets**	-	2	66 Tablets	5 days
6	**Phostoxin Tablets**	-	1	33 Tablets	1 day
7	**Phostoxin Tablets**	-	2	66 Tablets	1 day
8	**Magtoxin Plates**	-	2	2 Plates	1 day
9	**Magtoxin Plates**	V	1	1 Plates	1 day
10	**Phostoxin Tablets**	-	3	99 Tablets	2 days

Before starting the fumigation treatments, a sample of non-treated dates of 1 kg each, was taken as a control for efficacy evaluation. After the fumigation treatments, three dates samples 0.5 kg each were taken from different locations in the container for efficacy evaluation and phosphine residues analysis.

During the entire fumigation period, the phosphine concentrations levels inside the containers were monitored every two hours.

Results

The results showed that concentrations of phosphine in the non-special-sealed containers were very low (table 2, trials 4, 5). In contrast, in the container that passed special sealing before fumigation, a much higher concentration of phosphine were recorded (table 2, trials 1-3, 6-10). The highest concentrations were reached using Magtoxin plates, 4 g/m3 X 24 hrs with the phosphine generator model OMT 501 (table 2, trial 1). However, also at lower dosage of 2 g/m3 X 24 hrs and 2 g/m3 X 48 hrs using the OMT 501, high phosphine concentrations were achieved (table 2, trials 2, 3). The exposure time of 48 hrs does not have a significant advantage compared with 24 hrs (trials 2, 3). When the Magtoxin plates were used without the OMT 501, the highest concentrations were recorded much later compared with the trials with the OMT 501 (Table 2, trials 2, 9). Using Phostoxin tablets, only at the dosage of 3 g/m3 X 48 hrs the concentrations of phosphine were satisfactory (table 2, trial 10), but were reached much later compared with the Magtoxin plates and especially when using the OMT 501.

Tab. 2 The Phosphine concentrations in the field trials

Trial #	Phosphine formulation	OMT 501	Dosage g/m^3	Exposure time h	Concentration ppm after 4 h	max	final	Time (h) for max. conc.
1	**Magtoxin Plates**	V	4	24	1000	2100	900	8
2	**Magtoxin Plates**	V	2	24	650	1200	500	9
3	**Magtoxin Plates**	V	2	48	600	1050	350	10
4*	**Magtoxin Plates**	-	2	48	20	160	16	24
5*	**Phostoxin Tablets**	-	2	72	21	240	0	12

6	**Phostoxin Tablets**	-	1	24	60	300	300	24
7	**Phostoxin Tablets**	-	2	24	60	580	460	21
8	**Magtoxin Plates**	-	2	24	175	900	750	21
9	**Magtoxin Plates**	V	1	24	270	400	280	10
10	**Phostoxin Tablets**	-	3	48	13	770	730	35

* - non-special-sealed containers

The range of the dates infestation in the control was 3% to 30%. The dates were infested with alive adults of sap beetles (Coleoptera: Nitidulidae) and the larva of moths. Post fumigations no live insects were found. The dates infestation by dead insects in Magtoxin plates using the OMT 501 was 0-1%, in plates without the OMT 501 2%, in the trials with the tablets 2-18% (table 3).

Tab. 3 The efficacy of Phosphine fumigation in dates disinfestation in the field trials

Trial #	Phosphine formulation	OMT 501	Dosage g/m³	Exposure time h	Date infestation % control		treatment	
					alive	dead	alive	dead
1	**Magtoxin Plates**	V	4	24	6	0	0	0
2	**Magtoxin Plates**	V	2	24	9	0	0	3
3	**Magtoxin Plates**	V	2	48	6	0	0	2
6	**Phostoxin Tablets**	-	1	24	3	0	0	2
7	**Phostoxin Tablets**	-	2	24	6	0	0	18
8	**Magtoxin Plates**	-	2	24	12	0	0	2
9	**Magtoxin Plates**	V	1	24	22	7	0	5
10	**Phostoxin Tablets**	-	3	48	3	3	0	8

No phosphine residues were found in any of the fumigated dates.

Discussion

The best results were achieved in the trials with Magtoxin plates using the OMT 501. The plates have significant advantages versus tablets by achieving the highest levels of phosphine concentrations much faster, resulting in dates disinfestation. In addition, plates have obvious safety advantages versus tablets. Standard (common) containers without special sealing are not suitable for fumigation.

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Determination of phosphine concentration for *Cryptolestes ferrugineus* (S.) control in wheat in Sonora, Mexico

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The rusty grain beetle (*Cryptolestes ferrugineus*) is one of the most common insect pest of stored products. Phosphine (PH_3) is a major fumigant used for treating various food commodities, and the wrong aplication has led to resistance to phosphine. The development of high levels of resistance to phosphine has been reported in México. For that reason, different doses and exposure times were used to control of *C. ferrugineus* in two stages, larvae, and adult. In a warehouse using a Grainbag (GrainPro®) with 50 kg of wheat (*Triticum aestivum* L.) as support. Three doses of phosphine were used, 1.4 gr/m^3, 2.8 gr/m^3 and 4.2 gr/m^3 and 3, 5 and 7 days to determine the proper dose and exposure time for control *C. ferrugineus*. An application of 2.8 gr/m^3 with 5 days could achieve 100% mortality in both stages.

Efficacy Studies on $ECO_2FUME^®$ Phosphine Fumigant for Complete control of *Sitophilus zeamais* and *Tribolium castaneum* in stored maize in Thailand

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Abstract

$ECO_2FUME^®$ fumigation of maize bag stacks under gas proof sheets was conducted to establish the optimal dosages (application rate) and exposure times (fumigation period) against mixed-age cultures of *Sitophilus zeamais* and *Tribolium castaneum*. The Complete Randomized Design (CRD) experimental design was employed, with 3 replications and 4 treatments. The experiments were divided into three groups: 1) treatment with a 25 g/m^3 $ECO_2FUME^®$ application rate (350 ppm phosphine) for 3, 4, and 5 days and a control treatment; 2) treatment with an $ECO_2FUME^®$ application rate of 50 g/m^3 (700 ppm phosphine) for 2, 3, and 4 days and a control treatment; and 3) treatment with a 70 g/m^3 $ECO_2FUME^®$ application rate (1,000 ppm phosphine) for 1, 2, and 3 days and a control treatment. The three target phosphine concentrations of 350 ppm, 700 ppm and 1,000 ppm were maintained during the whole fumigation period. Results of the studies showed that no insect was alive at all dosages and exposure times. The studies also indicated that fumigation with $ECO_2FUME^®$ could reduce the fumigation period by increasing the phosphine concentration. The effective fumigation protocols on maize against mixed-age cultures of *S. zeamais* and *T. castaneum* were ECO_2FUME application rates of 25 g/m^3 for 3 days, 50 g/m^3 for 2 days and 70 g/m^3 for 1 day. The target phosphine concentration must be maintained throughout the fumigation period to achieve 100% mortality of all stages of insects.

Keywords: $ECO_2FUME^®$ phosphine fumigant, stored-product insects, *Sitophilus zeamais*, *Tribolium castaneum,* fumigation protocols, stored maize

1. Introduction

Maize is a primary ingredient of animal feed. Thailand's maize demand in 2016 was 5.85 million tons, which increased by 2.77% from 5.72 million tons in 2015. Because of the expansion of the livestock industry, demand for maize for animal feed increased. In 2016, Thailand exported 0.58 million tons of maize with total value of 4,855.34 million baht, a significant increase from 0.08 million tons with total value of 716.79 million baht in 2015. Maize production and value increased 7.25 and 6.77 times, respectively, because maize was increasingly exported to ASEAN markets including The Philippines, Indonesia and Vietnam (Office of Agricultural Economics, 2016).

For use as animal feed, maize must be stored for several months to sustain the continuous supply to the feed processors. When maize is stored at the production sites for a period longer than 3 months, insect infestation becomes a common and serious problem. The major insect pests that negatively affect the quality and quantity of stored maize in Thailand are *Sitophilus zeamais*, *Tribolium castaneum* and *Crytolestes ferrugineus* among others.

In Thailand, phosphine has been used as an effective fumigant to control several stored grain insect pests for more than 50 years (Sukprakarn et al., 1996). Formulations of phosphine available in Thailand are tablets or blankets of the metallic salts aluminium phosphide and magnesium phosphide that emit phosphine gas (PH_3) when exposed to air and moisture. Following application of tablets, concentration of PH_3 increases over several hours or days until all of the material is reacted. The increase rate of concentration depends on temperature and relative humidity. It is highly important to establish and maintain the most appropriate concentrations and exposure times in each particular situation. Precise dosing of PH_3 and the assurance of appropriate exposure times are difficult to achieve because of the dynamic release characteristics of the gas from tablet formulations and inherent structural leaks. There is the relative ease and safety in applying tablets to grain mass, but the influence of efficacy due to leaky structures may be a disadvantage (Banks, 1994; Bonjour, 1998).

$ECO_2FUME^®$ fumigant gas is a cylinderised formulation of PH_3 dissolved in liquid carbon dioxide at approximately 2% PH_3 and 98% CO_2 by weight. It is packaged in a high-pressure aluminium steel cylinder, with a net content of 31 kg of PH_3/CO_2 mixture and an equivalent phosphine amount of 620 g (Tumambing et al., 2012). $ECO_2FUME^®$ was established and approved in the U.S.A., which allows a shorter fumigation time of 24 hours for a 500 - 1,000 ppm phosphine concentration at 27°C or higher temperatures. The mixture of PH_3 and CO_2 is not flammable, which eliminates all safety concerns with the dispensing rate or dilution rate. Traditional solid formulations can generate PH_3 concentration above the lower flammability limit for PH_3 thereby creating a hazard (Cavasin et al., 2001).

There are several advantages when applying $ECO_2FUME^®$. The dose of $ECO_2FUME^®$ phosphine fumigant applied to the commodity is rapid delivery, easy maintenance of the required dose during the fumigation period, shorter exposures and ease of application. $ECO_2FUME^®$ does not require the applicator to enter the fumigation space. The ready-to-use cylinders can be dispensed from outside of silos or structures being fumigated. This eliminates the need for entry into confined spaces to apply fumigants and solid waste disposal (Bonjour, 1998; Phillips, 1998).

The controlled application of fumigant gas resulting in less fumigant was introduced in stored product instead of the traditional solid formulation. It relies on the generation of a high initial phosphine concentration followed by a slow deterioration to ensure that the phosphine concentration - time product (CT) - will result in an effective fumigation. With $ECO_2FUME^®$ fumigant gas, the concentration can be easily controlled by the applicator to maintain an efficacious concentration and can be precisely measured by adding the required amount of gas when needed. Disposal of solid waste products from tablets is becoming more difficult every day. $ECO_2FUME^®$ fumigant gas eliminates the concern associated with deactivating unspent metal phosphide residue and disposal of the waste product (Cavasin et al., 2001).

$ECO_2FUME^®$ is currently being considered for registration as an alternative to methyl bromide in Thailand by Genera Asia Co. Ltd. in 2011. Because $ECO_2FUME^®$ has never been used in Thailand therefore the study on this fumigant was needed.

The objective of this study was to establish the optimal dosages (application rate) and exposure times (fumigation period) of $ECO_2FUME^®$ phosphine gas for killing mixed-age of the major stored product insects in stored maize (*S. zeamais* and *T. castaneum*) in Thailand.

2. Materials and Methods

Test insects and preparation of mixed-age cultures

All insects of *S. zeamais* and *T. castaneum* used in this study were obtained from the stored-product insect colonies maintained at the Post-harvest and Processing Research and Development Division of the Thailand Department of Agriculture. The mixed-age cultures were prepared by adding 50 young adults (2-3 week olds) of each species (*S. zeamais* and *T. castaneum*) into a glass bottle

containing 200 g of a culture medium which was different for each species; brown rice for *S. zeamais*, rice bran for *T. castaneum* and covered with filter paper for 3 weeks. Afterwards, all adults were removed and kept in the laboratory for 4 weeks at 30±2°C temperature and 65±5% relative humidity before fumigation. All life stages were examined for their presence in the mixed-age culture glass bottle prior to fumigation.

Fumigant

$ECO_2FUME^®$ phosphine fumigant is a product of Solvay's Niagara Falls, Canada, facility (known by its legal entity name, Cytec Canada).

Fumigation of mixed-age cultures

The experiment was conducted in a concrete warehouse of Bangkok Food Products Co., Ltd. (CPF), located at the Phra Bhuttabat district, Saraburi province, Thailand, in 2014. Maize used for the trials was packaged in jumbo bags (1,000 kg capacity). The concrete floor was thoroughly cleaned, and black polyethylene floor sheets (0.5 mm thickness) were then laid out on the ground as an under-layer sheet. For construction of the stack, 8 jumbo bags of maize were piled on the floor sheet. Stack sizes were between 6.7 to 7.5 m^3. A cage of mixed-age test insects in a culture medium was placed into the maize jumbo bag on top of the stack. Each maize stack with test insects was then covered with a clear polyvinyl chloride (PVC) sheet (0.2 mm thickness) to construct fumigation enclosures. The fumigation sheet was then sealed to the floor with sand snakes (cotton bags of 100 cm x15 cm filled to 80% with sand). Considerable attention was given to the sealing operation to ensure that the fumigation sheet was neatly folded, without wrinkles, that it extended at least 1 meter away from the stack edges, and that overlapping sand snakes were laid in double rows at the corners. This type of setup was taken to ensure minimum gas leakage.

Efficacy trials were designed in Complete Randomized Design (CRD) with 3 replications and 4 treatments. The experiments were divided into 3 groups:

1) Treatment with a 25 g/m^3 $ECO_2FUME^®$ application rate (350 ppm phosphine equivalent) for 3, 4, and 5 days, and a control treatment (untreated).

2) Treatment with a 50 g/m^3 $ECO_2FUME^®$ application rate (700 ppm phosphine equivalent) for 2, 3, and 4 days, and a control treatment.

3) Treatment with a 70 g/m^3 $ECO_2FUME^®$ application rate (1,000 ppm phosphine equivalent) for 1, 2, and 3 days, and a control treatment.

The required amount of phosphine from $ECO_2FUME^®$ was injected inside the trap using a stainless steel hose and a gas injector with a gas flow rate of 6.85 kg/min. The exact amount of dispensed $ECO_2FUME^®$ was determined by the weight change of the cylinder on the top of a 100-kg digital scale with an accuracy of 0.01 kg or 10 g.

Monitoring of gas concentration

Phosphine concentration was monitored at each of the following intervals: 1) 1 and 18 hours for a 1-day exposure time; 2) same as item 1 plus 24 and 42 hours for a 2-day exposure time; 3) same as item 2 plus 48 and 66 hours for a 3-day exposure time; 4) same as item 3 plus 72 and 90 hours for a 4-day exposure time; and same as item 4 plus 96 and 114 hours for a 5-day exposure time. Three target phosphine concentrations of 350 ppm, 700 ppm and 1,000 ppm were maintained during the whole fumigation period. When the phosphine concentration fell below the target concentration, $ECO_2FUME^®$ was topped up to bring back the concentration at or above the target concentration. Phosphine concentration was monitored with calibrated SILOCHEK phosphine monitor (0 - 2000 ppm).

Fumigation was terminated at 1, 2, 3, 4 and 5 days of exposure time, followed by aeration of the slightly opened enclosure until the phosphine concentration reached the threshold limit value (TLV)

of 0.3 ppm or lower. The plastic cover sheet at each of the treatment stacks was completely removed afterwards.

Assessment of insect mortality

Effectiveness of $ECO_2FUME^®$ against the test insect was determined by mortality of mixed-age cultures. After fumigation, the glass bottles with the test insects were retrieved from each stack and the mortality of adults from each experiment was recorded. Dead and alive insects were separated, and each culture medium was returned to the bottles and kept in the laboratory at 30±2°C and 65±5% for 6 weeks. The bottles were observed weekly to determine if any newly emerged adults surfaced from hatched eggs. This period was sufficient for emergence of all insects in the treatment as well as the control. The occasional dead insect from the control treatment was corrected by Abbott's formula (Abbott, 1925).

Monitoring of temperature, relative humidity and moisture content

The temperature and relative humidity in the warehouse were monitored by a thermo-recorder every day during the fumigation period. The moisture contents of maize were measured before and after the treatment by applying the samples to a SB 900 Steinlite moisture meter.

3. Results

The effectiveness of $ECO_2FUME^®$

The effectiveness of $ECO_2FUME^®$ fumigation at different concentrations and exposure periods against mixed-age cultures of *S. zeamais* and *T. castaneum* are shown in Table 1. Results indicated that both insect species were completely controlled (100% mortality), and no live insects were observed immediately after fumigation or throughout the 6 weeks of monitoring. The test insects in the non-fumigated control samples continued to develop and emerged normally.

All of the application rates (25, 50 and 70 g/m³ $ECO_2FUME^®$) at any exposure time in each experiment were equally effective against mixed-age cultures of the two insect species in maize fumigation.

Tab. 1 The survival of insects inside the maize stacks during $ECO_2FUME^®$ fumigation with dosage 25, 50 and 70g/m³ at different exposure times.

Dosages (g/m³)	Times)Days(	The number of insects survival)insect(			
		Sitophilus zeamais		*Tribolium castaneum*	
		Immediately after fumigation	6 weeks after fumigation	Immediately after fumigation	6 weeks after fumigation
25 g/m³)350 ppm)	3	0[1/]	0	0	0
	4	0	0	0	0
	5	0	0	0	0
Unfumigated)control)	5	1,443	1,094	2,336	1,531
50 g/m³)700 ppm)	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
Unfumigated)control)	4	1,358	997	1,853	1,192
70 g/m³)1,000 ppm)	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
Unfumigated)control)	3	1,268	983	1,838	1,015

[1/] Mean of 3 replications

The concentration of phosphine

The phosphine concentration profile on the maize stack fumigated with 25g/m³ $ECO_2FUME^®$ at 3, 4 and 5 days of exposure time is shown in Figure 1. One hour after gas injection, the phosphine

concentration at 3, 4 and 5 days inside the maize stacks were 677, 682 and 527 ppm, respectively. The higher phosphine concentration compared to the target concentration was due to the phosphine gas initially occupying only the free space inside the stack. As the calculation of $ECO_2FUME^®$ dose is based on total empty space volume, the free space volume becomes smaller due to volume occupied by the maize stacks. Phosphine gas will then penetrate through the whole maize stack until equilibrium concentration is reached. After fumigation for 18 hours, the concentrations decreased and dropped down to 302, 318 and 282 ppm, respectively, indicating the distribution of phosphine gas in the entire maize stack. There was high fluctuation in the phosphine concentration inside the maize stack due to the addition of $ECO_2FUME^®$ to maintain the target phosphine concentration.

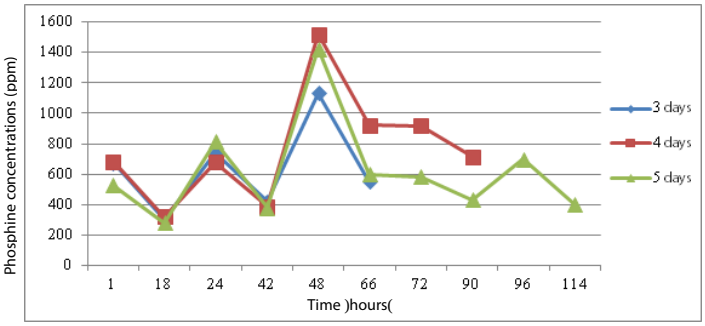

Fig. 1 Average phosphine concentrations inside the maize stacks during fumigation at the different times with $ECO_2FUME^®$ dosage 25 g/m³ (350 ppm).

The phosphine concentration curves of maize stack treated with 50 g/m³ $ECO_2FUME^®$ at 2, 3 and 4 days of exposure periods, as shown in Figure 2, display similar variations of phosphine concentrations with the treatment at 25 g/m³ $ECO_2FUME^®$. The phosphine concentration was below the target concentration after fumigation for 18 hours.

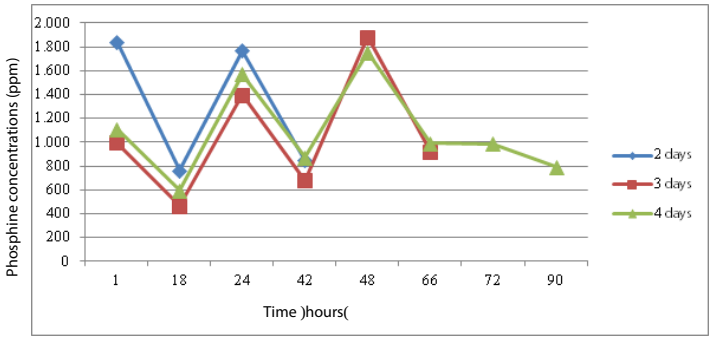

Fig. 2 Average phosphine concentrations inside the maize stacks during fumigation at the different times with $ECO_2FUME^®$ dosage 50 g/m³ (700 ppm).

The phosphine concentration profile of maize stacks fumigated with 70 g/m³ $ECO_2FUME^®$ at 1, 2 and 3 days of exposure periods is shown in Figure 3. The variation of phosphine concentration of this treatment was quite similar to the treatment with 25 g/m³ $ECO_2FUME^®$ in that the phosphine concentration reduction from an initial concentration of 2000 ppm almost hit the target concentration of 1000 ppm after a fumigation period of 18 hours.

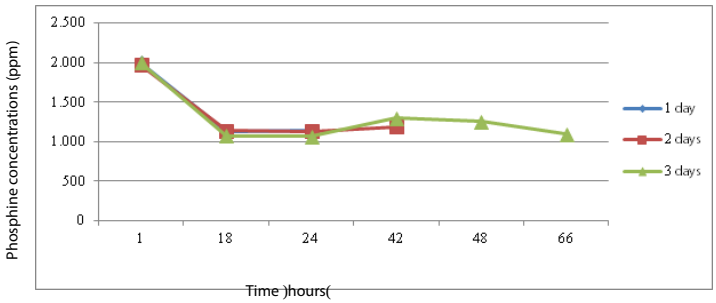

Fig. 3 Average phosphine concentrations inside the maize stacks during fumigation at the different times with $ECO_2FUME^®$ dosage 70 g/m³ (1,000 ppm).

Maintenance of target concentration

As shown in Table 2, there was addition of $ECO_2FUME^®$ conducted at 18 hours and 42 hours for the 3 days and 4 days of fumigation, and at 18, hours, 42 hours and 92 hours for the 5 days of fumigation as top up procedure. This was to maintain the target phosphine concentration at 350 ppm. Every time top up was done, the phosphine concentration was much higher than the target concentration due to the initial occupancy of free space by the gas before equilibrium distribution is reached.

In case of the treatment with 50 g/m³ $ECO_2FUME^®$ (700 ppm phosphine), Table 3 shows that top up was required at 18 hours for the 2 day fumigation, and at 18 hours and 42 hours for 3 and 4 day fumigation. For the treatment with 70 g/m³ $ECO_2FUME^®$ (1000 ppm phosphine), Table 4 shows that there was no top up needed for one day fumigation, and top up was required at 18 hours for the 2 and 3 day fumigation but there was an inconvenient in this experiment, therefore, the top up was done after fumigation for 24 hours instead of 18 hours.

The need for top up meant that maize used in the experiment absorbed some of the phosphine gas inside the stack, which caused the reduction of the phosphine concentration during the fumigation period. In this study, maize could absorb up to 40% of the initial phosphine concentration. As the gas absorption continues during fumigation, the amount increases as the fumigation period is increased. When the phosphine concentration fell below the target concentration, top up of $ECO_2FUME^®$ dosing was conducted to bring back concentration at or above the target concentration. With the ability to safely top up the stacks with $ECO_2FUME^®$, the desired concentration was maintained.

Tab. 2 The top up volumes of ECO_2FUME inside the maize stacks at the different times of fumigation with $ECO_2FUME^®$ dosage 25 g/m³ (350 ppm).

Time)Days(	Top up volume of ECO_2FUME)g)									
	Hours									
	1	18	24	42	48	66	72	90	96	114
3	-	13.44[1/]	-	5.75	-	-				
4	-	12.87	-	7.60	-	-	-	-		
5	-	15.64	-	8.87	-	-	-	4.24	-	-

[1/]Mean of 3 replications

Tab. 3 The top up volume of ECO_2FUME inside the maize stacks at the different times of fumigation with $ECO_2FUME^®$ dosage 50 g/m³ (700 ppm).

Time)Days(	Top up volume of ECO_2FUME)g)							
	Hours							
	1	18	24	42	48	66	72	90
2	-	14.22[1/]	-					
3	-	35.95	-	23.28	-	-		

4	-	27.00	-	9.47	-	-	-	-

1/Mean of 3 replications

Tab. 4 The top up volume of ECO_2FUME inside the maize stacks at the different times of fumigation with ECO_2FUME® dosage 70 g/m³ (1,000 ppm).

Time)Days(	Top up volume of ECO_2FUME)g)					
	Hours					
	1	18	24	42	48	66
1	-	-				
2	-	-	16.44 1/	-		
3	-	-	23.11	-	-	-

1/Mean of 3 replications

Monitoring of temperature, relative humidity and moisture content

The moisture content of maize was 13.2-13.8%. Temperature and relative humidity ranges inside the stacks were 29 - 35°C and 43 - 66%, respectively.

4. Discussion

Fumigation with ECO_2FUME® could reduce the fumigation period by increasing the phosphine concentration. The effective ECO_2FUME® fumigation protocols on maize against mixed-age cultures of *S. zeamais* and *T. castaneum* were 25 g/m³ (350 ppm phosphine) for 3 days, 50 g/m³ (700 ppm phosphine) for 2 days, and 70 g/m³ (1,000 ppm phosphine) for one day.

The phosphine target concentration must be maintained throughout the fumigation period to achieve 100% mortality of all stages of insects. It is necessary to monitor phosphine concentrations during fumigation. If the concentration of phosphine is not regularly monitored during the entire exposure period, the fumigation will be ineffective. Therefore, it is best fumigation practice to monitor the concentration of fumigant inside the sealed stack regularly to keep the phosphine concentration at the recommended minimum concentration.

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Application of Phosphine Fumigant for Controlling Rice Storage Insect Pests in Foundation Seeds

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Abstract

The development of phosphine resistance in storage insect pests is now problematic, so the increase of rate and frequency of phosphine fumigation in a storage room is needed. However, an adverse effect on seed germination, and human hazard needs to be tested. This experiment was aimed to find the most suitable methods used in combination with phosphine fumigation to reduce the risk of phosphine exposure. The treatments were (1) phosphine fumigation for 7 days and then open a plastic cloth (2) phosphine fumigation for 7 days and continue to cover a plastic cloth (3) phosphine fumigation for 7 days and spray pirimiphos methyl on sack (4) phosphine fumigation for 7 days and use a light trap (5) treat seeds with sweet flag powder before phosphine fumigation for 7 days (6) no phosphine fumigation with plastic cloth opening and (7) no phosphine fumigation with plastic cloth covering. In each treatment, seeds were sampled every month for 12 months to determine seed quality and insect populations. The results showed that seeds treated with sweet flag powder and fumed with phosphine for 7 days can significantly control storage rice insect pests in the first and the second year of experiments. The seed moisture content in each treatment changed in a similar pattern throughout 12 months storage in both years (13.4 – 13.7%). The seed germination showed similar results (more than 80% after 6 months storage), except the treatment of 7 days phosphine fumigation with plastic cloth covering which resulted in a slowly decline in germination. Seed weight losses and numbers of insect pests in the treatment with sweet flag powder were significantly less than the others.

Keywords: rice seed, Leb Nok Pattani, storage rice insect pests, phosphine, sweet flag powder

Introduction

Storage rice insect pests are the main cause of rice seed damage and deterioration by eating of seeds by both larvae and adults (Kaewnango et al., 2016). It also makes the amount of innert matter increases causing deterioration of seed quality and interferes in the standardization of grain types (Visarathanon et al., 2005). Particularly, the damage of the foundation seed from insect pests can affect the production of registered seed and certified seed. Phosphine fumigation is the most popular method for preventing insect pests damage to rice seeds after harvesting because it can kill insects at all stages of growth, no toxic residue and easy to operate. Phosphine fumigation under a sealed canvas for 7 days can kill insects at all stages of growth but if not used properly, it can cause insect resistance (Bullen, 2007). Increasing the frequency of use and rate of phosphine is a way to solve the problem of insect resistance; however, this method is not only harmful to the users, but also can impact on seed germination. So finding a safe way to undertake phosphine fumigation for control storage rice insect pests is probably a good solution to this problem. In addition to reducing the cost of seed storage, it also protects farmers from the toxic residues of phosphine in rice seeds.

Materials and Methods

A two - year experiment was conducted at Pattani Rice Research Center, Rice Department, Thailand, with Randomized Complete Block Design (RCBD) of 4 replications. The details of the operation are as follows.

Foundation seeds of Leb Nok Pattani variety packed in 60 kilograms of jute sacks was used as test specimens.

Testing of insect pests control in rice seeds, which was prepared with 7 treatments include:

1. Phosphine fumigation of 7 days and open plastic cloth
2. Phosphine fumigation of 7 days and cover plastic cloth

3. Phosphine fumigation of 7 days and spray pirimiphos methyl on sack
4. Phosphine fumihation of 7 days and use light trap
5. Treat seed with sweet flag powder and fumigant phosphine for 7 days
6. No fumigation with open plastic cloth (control)
7. No fumigation with cover plastic cloth (control)

Placing of rice seeds that was tested with each treatment in the storage shed for 12 months. Each month, about 1 kilogram of rice seeds was sampled for the following purposes:

- 500 grams for identification of insect pest species and recording their numbers
- 250 grams for moisture content measurement
- 50 grams for counting and weighing of normal seeds and broken seeds to calculate percentage of weight losses by insect damage from the formula of Adams (1976)
- 400 seeds for germination test

Statistical analysis and Duncan's Multiple Range Test (DMRT) comparison.

The experiment of the first year was conducted during May 2014 to April 2015 and May 2015 to April 2016 in the second year.

Results

The effect of using phosphine fumigant with other methods for control storage rice insect pests

The number of storage rice insect pests found in rice seeds from some treatments was highly significantly ($p < 0.01$) in both years. The best treatment for insect pests control was found to be treating seeds with sweet flag powder and fumigation with phosphine for 7 days, as supported by the results of the lowest number of insect pests recorded in seed samples: 602.3 and 625.5 insects/500 grams seeds, in the first and second year, respectively. The second most successful treatment of seed was phosphine fumigantion for 7 days and sprayed with pirimiphos methyl on sack and phosphine fumigantion for 7 days and covered plastic cloth, respectively. While phosphine fumigantion for 7 days and opened plastic cloth was effective in the first year only. We found that phosphine fumigantion for 7 days and use of light trap was not able to control insect pests in both years (Tab. 1)

Rice seed quality

The seed moisture content in each treatment changed similarly throughout 12 months storage in both years **(**13.4 **-** 13.7%). The seed germination showed similar results **(**more than 80% after 6 months storage**)** except the treatment of 7 days phosphine fumigation with plastic covering which result in slowly declined germination**.** Seed weight losses and number of insect pests in the treatment with sweet flag powder were significantly less than the others**.**

Discussion

From the results which showed that seeds was treated with sweet flag powder and fumed with phosphine for 7 days was the best treatment for control storage rice insect pests because essential oils (acalamol aldehyde) in the rhizomes of sweet flag which is toxic to the nervous system of insects. It also has the effect of repelling, inhibit eating and inhibit the reproduction of insects. (Supawan, 2014) Whereas Paneru *et al*. (1997) studied on wheat seeds treated with sweet flag powder for control storage rice insect pests which found that wheat seeds was treated with 2% w/w of sweet flag powder could control 100% of adults of rice weevil (*Sitophilus oryzae* Linnaeus) and grain weevil (*Sitophilus granaries*) within 7 days. In addition, Shuka *et al*. (2009) also found that chick peas seeds that was treated with 0.3 - 0.4 mg/g of sweet flag powder could decrease oviposition and egg hatchability of southern cowpea weevil (*Callosobruchus sinensis* Linnaeus) and seed germination remained 100% after 6 months.

Tab. 1 Total number of storage rice insect pests found in Leb Nok Pattani foundation seeds which applied phosphine fumigant with many methods at Pattani Rice Research Center during May 2014 - April 2015 and May 2015 - April 2016. (Fig.1)

Treatment	No. of insects/500 g of rice sample	
	1st year	2nd year
Phosphine + open plastic cloth	716.8 ab	1,321.3 c
phosphine + cover plastic cloth	702.0 ab	726.0 a
phosphine + pirimiphos methyl	725.3 ab	686.8 a
phosphine + sweet flag powder	602.3 a	625.5 a
phosphine + light trap	858.0 bc	1,083.5 b
no fumigation + open plastic cloth (control)	1,009.5 c	1,853.5 d
no fumigation + cover plastic cloth (control)	-	1,059.3 b
CV (%)	18.4	12.6

[1/]Average on 4 replications
[2/]Means in the same column followed by a common letter are not significantly different at 5% level by DMRT
(-) No treatment test

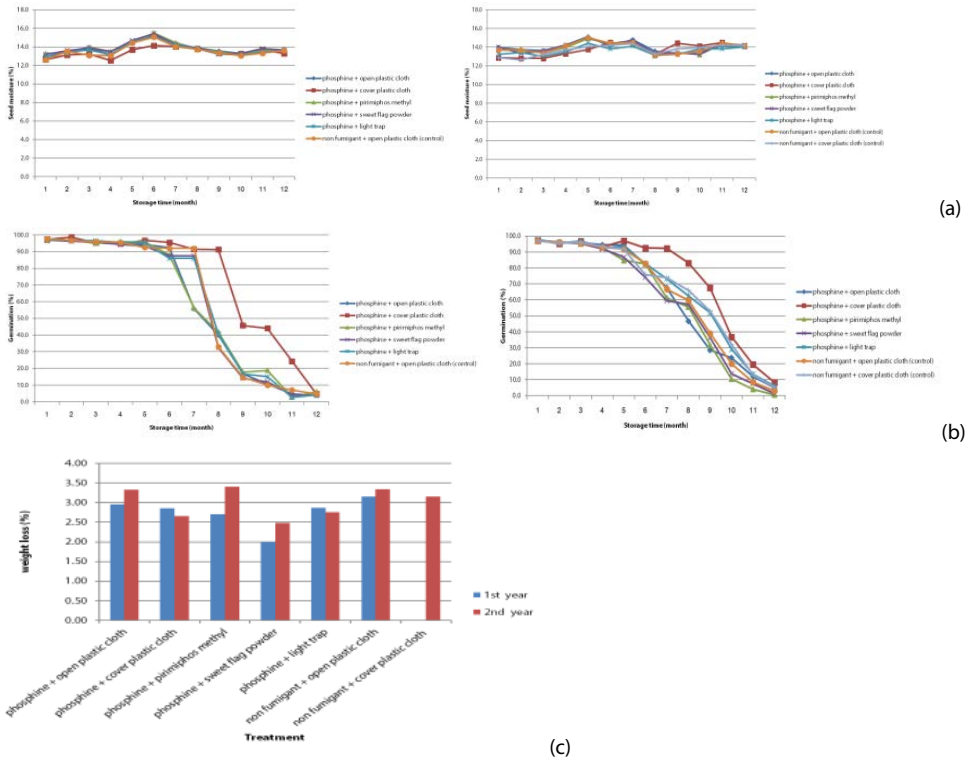

Fig. 1 Line graph showing moisture in first and second year (a) germination in first and second year (b) and weight loss in first and second year (c) of Leb Nok Pattani foundation seeds which applied phosphine fumigant with many methods at Pattani Rice Research Center during April 2014 - May 2015 and April 2015 - May 2016.

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Session 7
Contact Pesticides, Residual Products, and Plant Extracts

Laboratory Evaluation of Turkish Diatomaceous Earths as Potential Stored Grain Protectants

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In this study, efficacy of local diatomaceous earths (DE) collected from different regions of Turkey against stored grain insects, *Sitophilus oryzae* (L.), *Tribolium confusum* du Val. and *Rhyzopertha dominica* (F.), was investigated. For this purpose, biological tests were carried out at concentrations of 500 and 1000 ppm (mg DE / kg wheat) of 9 local diatomaceous earths and one commercial diatomaceous earth, namely Silicosec® as positive control at 25 ± 1 ° C temperature and 65 ± 5% relative humidity in wheat. In addition, the studies on some of the chemical and physical analysis of the tested diatomaceous earths (silicon dioxide (SiO_2) ratio, particle size and adhesion rate on commodity) were also conducted. In biological tests conducted at 500 ppm concentration for 14 days of exposure in wheat the highest mortality rates (97 to 98%) of *S. oryzae* adults were recorded in CB2N-1, AGN-1 and BGN-1 diatomaceous earths, while the highest mortality rates of *T. onfusum* adults were obtained from only AGN-1 and BGN-1 diatomaceous earths. In the case of *R. dominica*, the highest mortality rate (64.4%) was recorded only in CB2N-1 diatomaceous earth. At concentration of 1000 ppm for 14 days of exposure in wheat, 100% mortality of *S. oryzae* adults was observed in all tested local diatomaceous earths except FB2N-1 and Silicosec® while mortality rates of *T. confusum* adults ranging from % 95 to %100 were obtained in all tested local diatomaceous earths except FB2N-1, FBN-1 and Silicosec®. In the case of *R. dominica* adults, mortality rates ranging from 80% to 93% were recorded in CB2N-1, CCN-1 and AG2N-1 diatomaceous earths. In conclusion, laboratory bioassays indicated that CB2N-1 and BGN-1 local diatomaceous earths had high efficacy against *S. oryzae*, *T. confusum* and *R. dominica* adults and thus could be potential to be successfully used for controlling stored grain insect pests as a grain protectant.

Key Words: Turkish diatomaceous earth, wheat, Sitophilus oryzae, Tribolium confusum, Rhyzopertha dominica

Introduction

Stored products and especially grains and their by-products are the most important durable food category for human nutrition. During storage, these commodities are attacked by a numerous pests, particularly insect species, known as stored product pests, which cause very serious quantitative losses and qualitative degradations. Apart from the direct infestation *per se*, the presence of these pests and the substances that they produce may seriously endanger human health. In these commodities, which are also known as durable stored products, residual contact insecticides and fumigants are currently used as the main way to avoid insect infestation. Nevertheless, most of these pesticides are very toxic to mammals, and some of them leave dangerous residues on the product which may accumulate in the human body through the food chain. Moreover, most major insect species are now resistant to many of these pesticides, while some of these substances, such as methyl bromide, are extremely dangerous for both human health and the environment. Methyl bromide was thus banned by 2005 in industrialized countries according to the Montreal protocol, and it is expected to be completely withdrawn from the developing countries until 2015 (Bell 2000). Therefore, there is an urgent need to evaluate alternatives to traditional pesticides, which will have low mammalian toxicity, will be cost-effective and will be environmentally-compatible.

One of the most promising alternatives over the use of traditional pesticides in durable stored products is the use of diatomaceous earths (DEs). DEs are composed by the fossil skeletons of phytoplanktons, also known as diatoms, which occur in fresh and salt water since the Eocene period

and produce a soft sedimentary rock, which is composed mainly by amorphous silica ($SiO_2 + H_2O$). The DEs currently mined vary remarkably in their insecticidal activity, depending upon species composition, geological and geographical origin as well as certain chemical characteristics, such as SiO_2 content, pH and tapped density (Korunic 1997). DEs are probably the most efficacious natural resource-based dry materials that can be used as insecticides (Korunic 1998). DEs act in the insects' exoskeleton (cuticle) causing rapid desiccation resulting in death through water loss. They are non-toxic to mammals (rat oral LD_{50}>5000 mg/kg of body weight), leave no toxic residues on the product and according to the US EPA they are classified in the category of GRAS (Generally Recognized As Safe) since they are used as food or feed additives (FDA 1995). Moreover, they are used as insulating materials against both heat and sound, as explosive additive, as well as in filters for beverages such as beer or fruit juice and abrasives in tooth paste (Korunic 1998). Finally, DEs are completely compatible with organic food production (Subramanyam and Roesli 2000). Regarding their insecticidal use, DEs can be applied with the same application technology with traditional grain protectants, which means that no specialized equipment is required (Athanassiou et al. 2005). Moreover, since they are inert (silicaceous) materials, no interaction with the environment occurs. Thus, DEs persist in the treated substrate, providing a long-term protection against pests, which is currently a ''red flag'' for the use of conventional pesticides.

Several DEs, based on natural deposits, are now commercially available, and have proved very effective against stored grain pests (Subramanyam and Roesli 2000, Athanassiou et al. 2011). However, the investigation for newer, naturally-occurring DEs that are more effective in insect control is still in progress, especially in areas rich to silicaceous rocks. Korunic (1997, 1998) in an extensive screening of DEs from several parts of the world, found that local DEs from the Former Yugoslavia were very effective, and could be used with success against stored-grain pests. Similar results have been reported by Indic et al. (1998) for certain DEs from Serbia. An extensive screening by Athanassiou et al. (2011) also illustrated the presence of several DE deposits that have certain insecticidal properties from Croatia, Serbia and Slovenia. The DEs of this area have been used in the past for several applications, including their use as insecticides, and some of them are now the main ingredients in commercially available formulations. For instance, an amorphous silica DE from the Former Yugoslavia Republic of Macedonia (FUROM) is the main ingredient for the DE formulation Protect-It (Hedley Technologies, Canada), which is one of the most commonly used DE-based insecticides worldwide (Fields and Korunic 2000).

Based on the first evidence and preliminary samplings, it seems that Turkey is considered to have rich natural DE deposits, and there is clear evidence for the existence of large DE deposits at some areas in Turkey (Özbey and Atamer 1987, Mete 1988, Sıvacı and Dere, 2006, Çetin and Taş 2012). The European continent has the richest reserves in terms of diatomite reserve in the world and America has been following it. Diatomite reserve of Turkey is about 125 million tons. Hırka (Kayseri) known in Turkey has the largest diatomite reserve (106 million tons) (Çetin and Taş 2012). However, there is no information on the efficiency of local DEs from these areas in Turkey against stored grain insects. The objective of this study was to determine the physiochemical properties of the local DE deposits in Turkey and their efficacy against several stored grain insects.

Materials and methods

Test insects:

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), adults used in the bioassays were taken from a culture that was kept in the laboratory on whole wheat at 26 ± 1ºC, 65 ± 5 % relative humidity (RH) whereas the lesser grain borer*, Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), adults were taken from a culture kept on whole wheat 29 ± 1ºC, 65 ± 5 % RH. The confused flour beetle*, Tribolium confusum* du Val (Coleoptera: Tenebrionidae), adults were obtained from cultures reared in 1 l glass jars at 25 ± 1°C and 65± 5 % relative humidity (RH) on a diet of wheat flour mixed

with dry brewer's yeast (17:1, wt:wt) using standard culture techniques. All individuals used in the tests were < 2 week old.

Wheat variety

Untreated, clean, with very little dockage (0.8%) and infestation-free wheat (*Triticum aestivum* L., variety of Elbistan Yazlığı) was used for experimentation. One gram of wheat variety tested corresponds to 21.3 seeds. The moisture content of the three products, as determined by a Dickey-John moisture meter (Dickey-John Multigrain CACII, DICKEY-John Co., Lawrence, KS), ranged between 11.0 and 11.4%.

Sampling, collection and preparation of local diatomaceous earths

In biological tests, diatomite earths with code of CCNA-1, CB2N-1 (Çankırı province), AGN-1, ACN-1, AG2N-1 (Ankara province), BGN-1, BHN-1 (Kayseri province), FB2N- 1 and FBN-1 (Aydın province) collected from different regions of Turkey and commercial DE, namely Silicosec® (Biofa Company-Germany) were used in the biological tests. In each DE reserve, 10 DE samples with 500 gr were randomly taken from different points of DE reserve. At least totally 5 kg DE sample was taken from each DE reserve. The DE samples were cut into small pieces with the aid of a hammer and placed in metal trays and then dried at a temperature of 100 ± 10ºC for 24 hours in a ventilated oven (MEMMERT UN75, Germany) to a moisture content of 3-5%. After drying, small pieces of DE samples were ground in a laboratory mill at full speed for 10 sec. All samples were then shifted through a standard sieve of 149 µm. After these DE process, final DE product called as natural DE deposit was used in the biological tests.

Physical and chemical determination of local diatomaceous earths:

The adherence percentage of DEs on wheat kernels was determined. The cleaned 500 g sample of wheat grain was then mixed by hand (by shaking) with 1000 ppm of DE (1 g/kg, or 0.5 g/500 g) in a tightly closed glass jar for 1 min. Treated grain was then sieved thoroughly using laboratory sieve No. 10 (2 mm openings) with a lid and bottom, for 1 min by hand to separate dust from the grain. The sieve, with closed lid and bottom, was left undisturbed after sieving for at least 1 min. The dust collected in the bottom of the sieve was then measured with a precision balance (mg). The weight was subtracted from 500 mg, and the value was expressed as a percentage of adherence of DE on the wheat kernel. Particle size distribution of the diatom earths were determined using the Laser Diffraction Method Silicon dioxide (SiO_2) ratio was measured by dissolution in acid and Atomic Absorption Spectroscopy (AAS) method. These analyzes were carried out in the accredited Analysis Laboratory of the General Directorate of Mineral Research and Exploration (MTA).

Bioassays procedures:

The tested insect species were the adults of three major stored grain pests, *S. oryzae*, *R. dominica* and *T. confusum*. The bioassay protocol was 3 X 3 replicates (each series of tests was repeated three times, by preparing new lots each time). In this series of tests, the DEs was applied in 1-kg lots of soft bread wheat (*Triticum aestivum* L., variety of Elbistan Yazlığı) with 11% moisture content (m.c) at the DE dose rates of 500 and 1000 ppm (one lot per dose). The grain was left previously for 7 d at the appropriate conditions to equilibrate with the desired relative humidity levels. Based on the standard procedure, the lots were placed in glass jars, and shaken manually for approx. 3 min., to achieve equal distribution of the DE dust to the entire grain mass. There was an additional series of lots with untreated grain which serve as a control. Then, 3 samples, of 50 g each, were taken from each lot, and these samples were placed in small glass vials, which were closed, apart from a 1.5 cm hole at the top, covered with fine mesh for ventilation. After this procedure, 30 mixed sex adults, <15 d old were placed in each lot. The lots were placed in incubators set at 25±1 °C temperature and 65±5% RH. The mortality was assessed after 7 and 14 d of exposure in the treated substrate.

Data processing and analysis:

For tested three species, the control mortality was very low, but where it was considered necessary the mortality counts were corrected by using the formula of Abbot (1925). The data were analyzed, separately for each species, by using the GLM Procedure of SAS (SAS Institute 1995), with insect mortality as the response variable and type of DE formulation and dose rate, as the main effects. Means were separated by using the Least Significant Difference (LSD) test at $P<0.05$.

Results and Discussion

Laboratory analysis results showed that there were significant differences in the SiO_2 ratio of the local diatomaceous earths collected from different regions of Turkey. The highest SiO_2 ratio was obtained from the local diatomaceous earths collected from Aydın (FB2N-1) and Kayseri (BHN-1) province. The particle size of all local diatomaceous earths except FBN-1 diatomaceous earths ranged from 12.31 to 20.05 µm, while the particle size of the commercial formulation of Silicosec® was 12.51 µm. Adherence rates of FB2N-1, ACN-1, BHN-1, CCN-1 and Silicosec® diatomaceous earths on wheat kernel were found to be 89% or <89% while adherence rates of AGCN-1 and FBN-1 diatomaceous earth ranged from 80% to 89%. In the adherence test no diatomaceous earths had an adherence rate below 75%.

In biological tests conducted at 500 ppm concentration for 7 days of exposure in wheat the highest mortality rates of *S. oryzae* and *R. dominica* adults were obtained from CB2N-1, AGN-1 and CCN-1 diatomaceous earths while the highest mortality rates of *T. confusum* were recorded in CB2N-1, AGN-1, ACN-1 and CCN-1 diatomaceous earths. After 14 days of exposure at 500 ppm concentration, the highest mortality rates (97 to 98%) of *S. oryzae* adults were recorded in CB2N-1, AGN-1 and BGN-1 diatomaceous earths, while the highest mortality rates of *T. onfusum* adults were obtained from only AGN-1 and BGN-1 diatomaceous earths. In the case of *R. dominica*, the highest mortality rate (64.4%) was recorded only in CB2N-1 diatomaceous earth. At concentration of 1000 ppm for 7 days of exposure in wheat, mortality rates of *S. oryzae* adults ranging from 92% to 100% were found in CB2N-1, AGN-1, CCN-1, ACN-1, AG2N-1 and BGN-1 diatomaceous earths while mortality rates of *T. confusum* adults ranging from 86% to 98% were detected in AGN-1, CCN-1 and AG2N-1 diatomaceous earths. After 14 days of exposure at 1000 ppm concentration, 100% mortality of *S. oryzae* adults was observed in all tested local diatomaceous earths except FB2N-1 and Silicosec while mortality rates of *T. confusum* adults ranging from % 95 to %100 were obtained in all tested local diatomaceous earths except FB2N-1, FBN-1 and Silicosec. In the case of *R. dominica* adults, mortality rates ranging from 80% to 93% were recorded in CB2N-1, CCN-1 and AG2N-1 diatomaceous earths. In this study, it was determined that *S. oryzae* adults were more susceptible to tested diatomaceous earths than *T. confusum* and *R. dominica* adults. Whereas the susceptibility of *T. confusum* and *R. dominica* to tested diatomaceous earths was found to be mostly similar.

Conclusion

Laboratory bioassays indicated that CB2N-1 and BGN-1 local diatomaceous earths had high efficacy against *S. oryzae*, *T. confusum* and *R. dominica* adults and thus could be potential to be successfully used for controlling stored grain insect pests as a grain protectant.

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Lethal Effect of Turkısh Dıatomaceous Earth (Bgn-1) agaınst Adults of German Cockroaches (*Blatella Germanıca* L.*)*

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In this study, mortality effects of BGN-1 which is local diatomaceous earths, were investigated against adults of German cockroach (*Blatella gemanica* (L.)) on concrete, ceramic floor tile and laminate flooring. On these three different surfaces, *B. germanica* adults were exposed to BGN-1 diatomaceous earth at the doses of 2.5, 5, 10, 20 g/m^2 along 6 days. In all surface applications of BGN-1 diatomaceous earth, exposure time and dose caused significant effect on mortality rates of *B. germanica* adults. It was determined that BGN-1 coded Turkish diatomaceous earth has the lowest mortality effect on all application surfaces at the dose of 2.5 g/m^2. 2.5 g/m^2 BGN-1 caused 100 % mortality after 6 days concrete surface and caused 100 % mortality at the end of the fourth day on ceramic floor tile and laminate flooring. On the other hand, doses of 5 and 10 g/m^2 of BGN-1 caused 100% *B. germanica* mortality on all surfaces at the end of the second day, while the highest dose of 20 g /m^2 of BGN-1 reached 100% *B. germanica* mortality at the end of the first day on all application surfaces. In general, the mortality activity of BGN-1 diatomites against *B. germanica* adults was found to be similar on all three surfaces. At the end of this study, local diatomaceous earth coded BGN-1 was found to be good alternatives for controlling *B. germanica* which is a medical pest insect.

Keywords: Turkish diatomaceous earth, *Blatella germanica*, surface application.

Introduction

The German cockroach is commonly found living area with people and scattered all over the world. It is also a major carriers of pathogens and main source of allergens. Therefore it is an important primary medical and economical insect pest. The cockroach is mainly controlled by synthetic insecticides (Rust et al., 1993). However, this cockroach widely developed resistance to these

insecticides (Jialin et al., 2007). Hence, the development of new types of selective cockroach-control alternatives are needed.

Diatomaceous earth (DE) is a component of organic origin and is a precipitate formed from the fossilized siliceous shells of algae that have lived in all aquatic ecosystems. The cell walls of the algae are amorphous cystite (SiO2 + H2O). Recent studies have shown that DE has a significant impact on warehouse pests (Waksh and Shabbir, 2005; Athanassiou et al., 2007). In addition to the insecticidal effect of DE, it can be used as filtration, absorbant, filling material in the industry, silicon reinforcing in humans, and as a moisture retainer in the packaging of nutrients (Durmuşkaya, 2009). Diatomaceous earth is probably the most effective of the natural powders that can be used as insecticides. The insecticidal effect of diatomaceous earth is regarded as a method of physical struggle because it does not have a chemical effect on insects. This physical struggle; DE has an effect on the insect cuticle and results in death of the insect's water loss (Ebeling, 1971). DE, besides its water absorber feature, can also abrade the oil quite well. For this reason it is also very effective on the protective waxy layer present on the insect cuticle. As a result, death in insects occurs due to water loss and drying (Cloarec et al., 1992).

Furthermore, the killing effect of insects may vary significantly depending on the test conditions used, the diathermy (marine or freshwater diatoms), the geographical area taken, the formulation process, the oil absorption capacity and the chemical / mechanical modification of the diatomaceous earths (Faulde ve ark., 2006). Therefore, in this study, mortality effects of BGN-1 which is local diatomaceous earths, were investigated against adults of German cockroach (*Blatella gemanica* (L.)) on concrete, ceramic floor tile and laminate flooring.

Materials and Methods

Insect

Colonies of *B. germanica* were reared in plastic containers (60 liter) and maintained at room temperature. The cockroaches were provided with water in glass tubes with cotton stoppers and dry dog food. Each container was provided with paper egg cartons as shelter. The adult cockroaches (5-10 days old) were tested for each bioassays at 25 (± 2) ºC and 50 (±5) % relatıve humidity.

Local diatoms used in biological tests:

In this study, BGN-1 coded local diatoms from Kayseri/Turkey province were used. At least 5 kg samples were taken from the diatom reserve. The samples were mixed in the gutters and brought to the laboratory. The diatom specimen brought in rock form is prepared in natural form. For natural (natural) preparation of the diatom specimen, it was dried at 100 ± 10 ° C for 2 hours, until it had a 3-5% moisture content in a controllable ventilated oven. After drying, the small pieces were grinded in a laboratory mill for 10 seconds at the highest speed. All samples were then sieved through a 100 mesh (149 µm) standard sieve and the damp, soft small pieces left under the sieve were dried in a ventilated oven at 40 ° C for 24 hours. Thus, natural powdered diatomaceous earth of a particle size of 149 microns or less is obtained.

Surfaces used in biological tests

During the test, concrete, ceramic, parquet surfaces were prepared in plastic boxes (100x100x60 mm) and ventilation holes were opened with the help of a needle to the cover parts of plastic boxes in order to provide air in and out during the established tests.

Concrete Surface: The mortar obtained by using 200 g + 50 ml water was poured into plastic boxes (100x100x60 mm) and the mortar was obtained by drying.

Ceramic Substrate: The ceramic surfaces used during this work were produced from a mixture of clay, kaolin, quartz, feldspar and limestone in sizes of 150x150x5.5 mm according to TS202 standards. The ceramic surfaces produced in TS202 standards are reduced in dimensions of 100x100 mm and working dimensions are obtained.

Parquet Substrate: Laminated parcels which are manufactured according to the standards of High Density Fiberboard (HDF) and 717 E-1 are 8x195x1200 mm in size and 100x100 mm in size and working dimensions are obtained.

Biological tests and test method

Biological tests were carried out in the climate chamber with 25 ± 1 ° C and 65 ± 5% relative humidity. Water and feed were not given to insects during the experiment and insects were exposed to 2.5, 5, 10, 20 g / m2 doses of diatomaceous earth. Diatomaceous earth weighed with the aid of precision scales for dosing experiments is placed on concrete, ceramic and parquet surfaces. After the diatomaceous earth was distributed on the surface, ten newborn individuals from *B. germanica*, which we cultured in the laboratory environment, were left. The experiments were carried out in four replicates, with 10 individuals each time. The control unit was also set up in four replications and no diathermy was applied, such as water and feed. Dose experiments were taken into the climate chamber as soon as the establishment was established and dead-live insects were counted for six days. For the time trials, 6, 9, 12, 18, 24 hour experiments were set up as separate treatments, keeping the highest dose rate constant during the dose trials, thus setting up separate control units for each exposure period. The time experiments were carried out again with four repetitions and 10 individuals each time.

Conclusion

In all surface applications of BGN-1 diatomaceous earth, exposure time and dose caused significant effect on mortality rates of *B. germanica* adults. It was determined that BGN-1 coded Turkish diatomaceous earth has the lowest mortality effect on all application surfaces at the dose of 2.5 g/m^2. 2.5 g/m^2 BGN-1 caused 100 % mortality after 6 days concrete surface and caused 100 % mortality at the end of the fourth day on ceramic floor tile and laminate flooring. On the other hand, doses of 5 and 10 g/m^2 of BGN-1 caused 100% *B. germanica* mortality on all surfaces at the end of the second day, while the highest dose of 20 g /m^2 of BGN-1 reached 100% *B. germanica* mortality at the end of the first day on all application surfaces. The dose of 10 and 20 g / m^2 on the concrete surface of the native diatomaceous earth of BGN-1, which had been used in the experiment, had statistically similar effect on the concrete surface after 24 hours and was more effective than 2.5 and 5 g / m^2 doses. The BGN-1-coded native diatomaceous earth used in the experiment had the highest activity as a 20 g / m^2 dose statistically 24 h later on the parquet surface while it was found to have the lowest activity as 2.5 g / m^2 statistically. The dose of 10 g / m^2 was statistically found to have similar efficacy with both 5 g / m^2 and 20 g / m^2. The BGN-1-coded native diatomaceous earth used in the experiment had the highest activity as a 20 g / m ^{2} dose statistically 24 h on the ceramic surface and the lowest activity as 2.5 g / m^2 statistically. The dose of 10 g / m^2 was statistically found to have similar efficacy with both 5 g / m^2 and 20 g / m^2. In general, the mortality activity of BGN-1 diatomites against *B. germanica* adults was found to be similar on all three surfaces. The mortality rates obtained from *B. germanica* adults exposed to BGN-1-coded diatomaceous earth for a period of 6, 9, 12 hours for a dose of 20 g / m^2 are statistically similar. However, at the end of 18th hour mortality rate was the highest for concrete surface and reached to 100%, whereas parquet and ceramic surfaces were statistically similar. Mortality rates following 24-hour exposure were 100% for all surfaces. The mortality rate increases as the exposure time for all surfaces increases. In addition, exposure of the BGN-1 coded native diatomaceous earth to 20 g / m^2 for 18 hours resulted in 100% mortality for the concrete surface, while the other two surfaces reached 100% mortality after 24 hours.

All these results demonstrate that BGN-1 encoded native diatomaceous earth has potential for use in the B. germanica adult struggle and may be an alternative to synthetic instecticides with a broad-spectrum spectrum used in the struggle for this bug. However, diatom earth species should be demonstrated in a comprehensive study of the applicability of the German cockroach under natural

habitat conditions and the determination of its interaction with other living factors outside cockroaches when applied in natural conditions.

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Efficacy of seven Turkish diatomaceous earths against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchninae) on stored chickpea

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Abstract

In this study, insecticidal efficacy of seven different local diatomaceous earths (DE) obtained from different deposits in Turkey together with two commercial DEs, Silicosec® (Biofa AG- Germany) and Desect® (Ep Naturals-America) against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchninae) an important pest of stored chickpea at five different concentrations (100, 300, 500, 1000 and 1500 ppm) was evaluated. The local DEs were coded as BGN, BHN, AG2N, AC2N, CB2N, CCN, FB2N. Mortality of the adults was assessed after 1, 3, 5 and 7 days of exposure, and consequently progeny (F1) production on treated chickpeas was recorded 42 days later. The tests were carried out under laboratory conditions of 25±1 °C, 55±5 % R.H. in a dark place. The most effective DEs after 1 day of exposure were CCN, AG2N and BHN causing 75%, 59%, 58% mortalities, respectively at 1500 ppm concentration. Silicosec®, Desect®, BGN, AC2N, applied at 1500 ppm concentration achieved 98-100% mortality of *C.maculatus* after 7 days of exposure, showing similar high insecticidal efficacy. The CCN, BHN, AG2N and CB2N caused 97-99% reduction in progeny (F1) production. Generally, increasing concentration significantly reduced the progeny production. In conclusion, this study has shown that three Turkish DEs, namelyCCN, AG2N and BHN highly toxic to *C. maculatus* after 3 days of exposurein comparison with commercial DEs Silicosec® and Desect®. These local DEs could be used in the management of pests of stored chickpea.

Keywords: Turkish diatomaceous earths, Silicosec®, Desect®, *Callosobruchus maculatus*, chickpea.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the rare plants that have been cultivated since thousands of years and the Turkey's southeast region is known as the homeland of the crop. Globally, it is known that cowpea beetle, *Callosobruchus maculatus* (F.) is the most important pest of stored cowpea and other legumes (Taylor, 1981) and its origin is West Africa (Decelle, 1981). This insect can damage 100% of stored products causing weight losses of up to 60% (Tanzubil, 1991). Fumigants and contact insecticides have several problems such as development of resistance, chemical residues in food, as well as harmful effects on the environment and human health. Researchers are therefore searching for alternative methods of stored product protection. Diatomaceous Earths (DE) have less

resistance problems and leave no residue in the stored product., and less harm to the environment and mammals (Fields 1998). There are a lot of commercial DE formulations which have been applied against a wide range of insect species (Fields 2000, Athanassiou et. al., 2004). The efficacy of DE products depends on several factors such as diatom morphology, DE physical properties, type of grains, temperature and relative humidity (Korunic, 1998).

Turkey is considered to have rich natural DE deposits, and there is clear evidence for the existence of large DE deposits in some regions of Turkey (Özbey and Atamer, 1987; Mete, 1988; Sıvacı and Dere, 2006; Çetin and Taş, 2012). The efficacy of DEs to control stored product insects have been studied by several researchers for almost 20 years. However, there are a few studies using DEs against *C. maculatus* in Turkey. The purpose of this study was to determine the efficacy of two commercially available DEs and seven Turkish local diatomaceous earths formulations against *C. maculatus* on chickpea.

2. Materials and Methods

2.1. Test İnsects

The Test insects, *C. maculatus* were obtained from laboratory cultures maintained on chickpea seeds at the Toxicology Laboratory of the Department of Plant Protection, University of Namik Kemal, Turkey. For this study, new cultures were set up on the chickpea seeds at 25±1°C and 55 % r.h. Adults emerging from the chickpea seeds, aged 48 h old were used in the experiments.

2.2. DE formulations

Seven local diatomaceous earth samples (coded as BGN, BHN, AG2N, AC2N, CB2N, CCN, FB2N) were mostly collected from DE reserves located at middle Anatolia of Turkey, commercial DEs were purchased from the agricultural market.

2.3. Chickpea

The seeds of the chickpea cultivar Koçbaşı (*Cicer arietinum* L.) were obtained from a supermarket. Chickpeas were sterilized at -20°C for 3-5 days before they were used in the experiments

2.4. Experimental procedure

About 500 g of chickpeas used in the experimentsput into 3-liter volume glass jars. Each diatomaceous earth was placed on the products in the glass jar, weighing 0,050, 0,150, 0,250, 0,500 and 0,750 g (100, 300, 500, 1000 and 1500 ppm) on the precision scale. Then the lids of these jars were tightly closed and shaken manually for 3 minutes to homogenize the diatomaceous earth over the chickpea. Each lot was divided into five parts of 100g and in each vial, 20 *C. maculatus* adults of mixed-sex were added with the help of a fine brush .The vials were tightly closed with a tulle which provided air inlet and outlet. One lot was kept as the untreated control. The vials were stored in incubators maintained at 25±1°C temperature and 55±5% relative humidity. Adult mortality was recorded after 1, 3, 5 and 7d. Live and dead adults were counted and recorded. After the 7th day counting, all insects have been taken out from the vials. The chickpeas were seperated according to their doses and placed in plastic containers which were drilled with the help of a pin. They were kept in the dark 80 l volume plastic container for 42 days at a temperature of 26±1°C and 65±5 % relative humidity. Sodium Bromide (94.32 g NaBr / 100 ml water) solution was used to keep the ambient air humidity constant. After 42 d, the jars were opened and the total number of F1 adults were recorded.

2.5. Statistical analysis

Mortality rates obtained from control counts were corrected using Abbot's correction formula (Abbott 1925). After applying the Arcsin transformation to the corrected mortality rates, two-way

variance analysis (ANOVA) was performed in the SPSS 15.0 Evaluation Version statistical program. F1 emergence adults were subjected to direct variance analysis (ANOVA) without any corrections. Using the Duncan test at the 5% significance level, the differences between the mean of mortality rates and the number of new generation adults were determined.

Table 1 Mean percentage mortality (±SE) of *Callosobruchus maculatus* adults exposed to 5 different concentrations of 9 DEs after 72h

Table 2 Mean (±SE) number of *Callosobruchus maculatus* F1 progeny produced after 42 d on chickpea treated with eight DE formulations at five dose rates.
*Two-way variance analysis (ANOVA) was applied to the data and the differences between the averages were based on the 5% significance level. The different uppercase letters in the same column and the different lowercase letters in the same line are statistically different.

3. Results

Toxicity of DEs against *C. maculatus*

DE dose rate (ppm)	DE Formulation / Silicosec®	Desect®	BGN	BHN	AG2N	AC2N	CB2N	CCN	FB2N		
1500	82.9±2.9Acd*	71.7±2.9Ade	82.3±4.8Acd	96.3±1.6Aab	100±0Aa	61.1±8.9Ae	90.6±1.1Abc	98±1.1Aa	78.7±6.2A	15.200	0.0001
1000	62.2±5.2Bb	70.4±3.7Ab	65.3±6.3Bb	92.1±2.6Aa	94.1±1.7Ba	40.3±7.8Bc	66.1±4Bb	96±1.8Aa	62.7±4.8Bb	17.017	0.0001
500	48.3±3.2Cb	25.2±5.8Bd	9.5±1.2Ccd	31.4±2.3Bcd	36±2.8Cc	2.9±2.2Cf	39.7±2.6Cbc	72.9±3Ba	23.9±2Cd	46.989	0.0001
300	20.4±2.6Db	8.7±2.9Cb	5.5±0.9Cb	6.8±1.1Cb	21.6±2.6Da	1±1Cc	22.5±0.9Da	23.8±4.3Ba	20.6±4.1Ca	51.558	0.0001
100	0±0Ed	0.7±1.06Dcd	4.2±2.6Ccd	4.9±1.6Cc	11.3±1.8Eab	3.5±2.4Cd	10.6±1.1Eab	6.4±3.4Cc	17.7±2.8Ca	7.441	0.0001
Control	0±0	0.5±0.5	2±1.2	0±0	0±0	2±1.2	0±0	0±0	1±1	-	-
F	150.222	67.512	68.741	131.617	247.357	28.780	204.950	76.216	35.671	-	-
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	-	-

Percent mortality of *C. maculatus* at the end of the third day of treatment of chick peas with 5 different concentrations of each diatomaceous earth are given in Table 1.

DE dose rate (ppm)	DE Formulation / Silicosec®	Desect®	BGN	BHN	AG2N	AC2N	CB2N	CCN	FB2N	F	P
1500	27.8±3.7Cb*	18.4±3.4Dc	27.8±5.8Cb	1.4±0.5Cd	2.4±0.5Dd	37.8±2.9Ca	2.8±0.8Dd	0.4±0.2Cd	20.4±3.1Cbc	22.343	0.0001
1000	29±12.1Ca	27.8±4.8Da	35.8±6.7Ca	1.2±0.5Cb	6.2±1.1Db	41.6±7.1Ca	22±5.1Dab	5.2±2Cb	36±10.2Ca	5.058	0.0001
500	39.8±5Cd	60.8±10.4Ccd	84.4±6.2Bab	3.6±0.6Ce	57.8±4.5Ccd	97.6±7.3Ba	47.6±7.6Cd	56.6±9Bcd	71.2±6.8Bbc	15.103	0,0001
300	67.6±12.1BCc	97.4±5.9Bab	97±4.9Bab	18.6±1.2Cd	61±8Cc	102.4±5.7ABa	62.4±6.8Cc	109.6±6.7Aa	78.6±6.1ABbc	15.749	0.0001
100	103.4±16.6ABa	107.6±10.5Ba	105±7ABa	77.4±18.9Ba	96.8±12.3Ba	105.2±6ABa	99.2±10.9Ba	111.6±4.9Aa	80.4±12.3ABa	1.002	0.451
Control	138.2±20Aa	93.3±17.1Aa	129.8±17.7Aa	98.8±13.3Aa	98.8±13.3Aa	129.8±17.7Aa	98.8±13.3Aa	138.2±20.2Aa	102.4±9.2Aa	0.497	0.850
F	11.863	28.117	19.149	33.924	38.762	16.841	31.905	36.477	12.919	-	-
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	-	-

Mortality of *C. maculatus* adults after 3 d of exposure to DE treated chickpea increased with increase in DE dose rate.Two-way ANOVAs showed statistically significant effects on the mortality rates of the DEs ($F_{8,180}$ = 73.866, P <0.0001) and concentrations ($F_{4,180}$ = 659.275, P <0.0001) and the interaction between these two factors $F_{32,180}$ = 9.179, P <0.0001) were found to be statistically significant. The CCN and AG2N coded DEswere found to be similar in efficacy at a concentration of 1500 ppm. Within this exposure interval, the highest (100%) mortality were recorded on AG2N coded DE at 1500 ppm. CCN-coded DE showed 96 and 98% mortality rates at 1000 and 1500 ppm

concentrations, respectively. Silicosec® and Desect® commercial DEs showed 82.9% and 71.7% mortality of the beetles, respectively. At the lowest dose rate of each of the DE formulations the efficacy was very low.

F1 progeny production

The main effects of both DE formulation and dose rate as well as their interaction were significant ($P \leq 0.0001$) for number of progeny produced by *C. maculatus*. The mean number of progeny in the untreated control was significantly higher than the numbers that developed on treated chickpeas. Progeny production was reduced by increasing DE dose rate. On treated chickpea seeds, the lowest number of progeny was 0.4 and 1.4, respectively. At 1500 ppm concentration of CCN and BHN coded Des, very few new generation adults emerged. İn all DE treatments, the number of new generation adults that emerged from the control treatments ranged from 93 to 138 and was statistically similar. Generally, as the concentration Des was increased, there was a decrease in the emergence of new generation adults.

Discussion

A few researchers have studied the toxicity of various diatomaceous earth formulations on *C. maculatus.* Most researchers have applied diatomaceous earths of different origins against adult insects at different temperature, humidity and time (Prasantha et al., 2002, Stathers et al., 2004, Islam et al., 2010, Wakil et al., 2010, Shams et al., 2011, Parsaeyan et al., 2012, Tofel et al., 2012, Badii et al., 2013, Chelav et al., 2013, Ofuya et al., 2015, Doğanay et al., 2017). The results presented in this study indicate that local Turkish DE's are effective in controlling *C. maculatus* on chickpea. However, among them, CCN, AG2N, BHN and CB2N coded DEs were found to be more effective after one day of exposure. Our results and those of previous studies suggest that as the DE dose and exposure time increased, the adult mortality rates also increased (Vayias and Athanassiou 2004, Athanassiou and Kavallieratos 2005, Korunic and Fields 2006, Vayias and Stephan 2009, Baytekin and Sağlam 2017). İn the present study at the end of the third day, only AG2N coded DE showed a 100% mortality at 1500 ppm concentration. Stathers et al., (2004) found that 0.1 g of Dryacid® commercial DE killed 100% of adults of *C. maculatus* after 3 days' exposure. Similar results were obtained with the AG2N encoded DE in this current study. When we applied at 1500 ppm concentration of Siicosec® for 3 days 83% mortality was recorded. Shams et al. (2011) conducted studies on Silicosec® on *C. maculatus* at 500 ppm concentration for 2 days and obtained a 95% mortality rate. Tofel et al. (2012) achieved a 100% mortality rate when they applied Silicosec® 2000 ppm concentration for 4 days. Islam et. al (2010) reported that after 3 days of exposure Silicosec® at 1000 ppm concentration the mortality of *C. maculatus* was 90%. İn the present study Silicosec® had a 99% mortality rate on the 5th day, but a 100% mortality rate was reached at the 7th day at 1500 ppm concentration. It is thought that the differences between the studies may be due to differences between the experimental conditions, product type and the insect populations. İn this study, it was concluded that local Des were effective in controlling *C. maculatus* adults on chickpea, but among them, CCN, AG2N, BHN and CB2N coded Des were found to be most effective. Diatomaceous earth is a potential alternative method that can be used in an integrated pest management of stored product pests. Progeny production was also significantly reduced by the application of the local DEs.Field studies on the practicial application of local DEs for the protection of grains against insect pest infestation n Turkey are necessary.

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Residual efficacy of spinosad-treated surfaces on *Rhyzopertha dominica* and *Tribolium castaneum* adults

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Abstract

Rhyzopertha dominica and *Tribolium castaneum* cause massive losses in stored food. These insects are effectively controlled by synthetic insecticides and fumigants but they accompany many demerits on biotic and abiotic environment. Spinosad is a bacterial formulation and a reduced-risk insecticide which is registered for stored grain protection in many countries. Despite many avenues of research on spinosad, its residual efficacy on certain insect species remains undiscovered. The objective of this research was to evaluate the residual efficacy of spinosad-treated surfaces on the survival of *R. dominica* and *T. castaneum* adults. The label rate of spinosad (25 ppm) was sprayed on polypropylene, jute, polythene, metal and filter paper. One-month-old twenty adults of *R. dominica* or *T. castaneum* were introduced on to the surfaces treated with spinosad and maintained at ambient environmental conditions. The mortality was counted at 2 and 6 days following introduction of adults. The mortality differed with the surface, insect species and duration of exposure. The current study highlights the possibility of controlling *R. dominica* or *T. castaneum* by spinosad sprayed on different surfaces.

Keywords: *Rhyzopertha dominica*, *Tribolium castaneum*, Spinosad, Surfaces, Residual efficacy

Introduction

Rhyzopertha dominica and *Tribolium castaneum* are serious pests of stored products. The synthetic neurotoxic insecticides are the common control methods for these insects (Ghimire *et al*., 2016; Wijayaratne *et al*, 2018) but they have many disadvantages such as negative impacts on human, animals and environment (Arthur, 1996). Reduced-risk insecticides (Arthur, 2007) are better options to overcome the above problems (Phillips and Throne, 2010). Spinosad is a bacterial formulation derived from *Saccharopolyspora spinosa* (Bacteria: Actinobacteridae) (Mertz and Yao, 1990). Spinosad negatively affects the insect nervous system (Salgado and Sparks, 2005). It has been tested against several stored-product insects (Boina *et al*., 2012; Subramanyam *et al*., 2016; Wijayaratne and Rajapakse, 2018) but information on its residual effect on different surfaces is lacking. Therefore, the objective of this research was to evaluate the residual effect of spinosad applied on different surfaces on the survival of *R. dominica* and *T. castaneum* adults.

Material and methods

The experiments were conducted at ambient environmental conditions according to completely randomized design. There were four replicates. Commercially-available spinosad was sprayed on polypropylene, gunny bag (jute), filter paper, polythene and metal at the label rate. Twenty adults of *R. dominica* or *T. castaneum* were introduced onto each surface. Survival of insects was observed at 2 and 6 days following introduction.

Results and discussion

Higher residual efficacy of spinosad was observed on polypropylene, gunny bag (jute) and filter paper than metal and polythene. Increased exposure to spinosad recorded higher mortality in both *T. castaneum* and *R. dominica*.

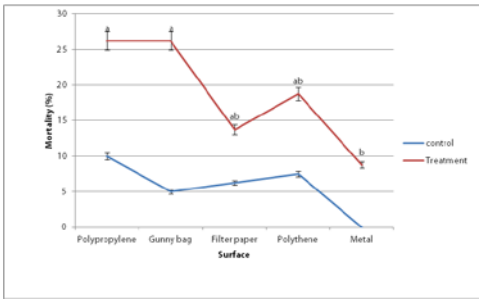

Figure 1. Mortality of *Tribolium castaneum* adults exposed to spinosad-treated surfaces for 2 days.

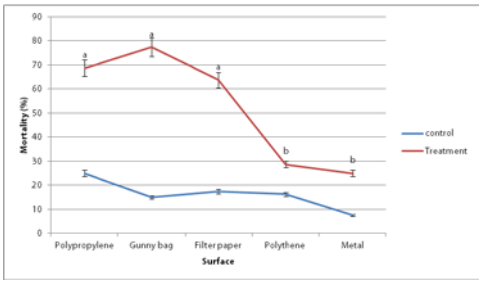

Figure 2. Mortality of *Tribolium castaneum* adults exposed to spinosad-treated surfaces for 6 days.

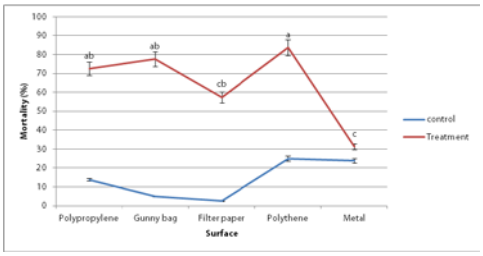

Figure 3. Mortality of *Rhyzopertha dominica* adults exposed to spinosad-treated surfaces for 2 days.

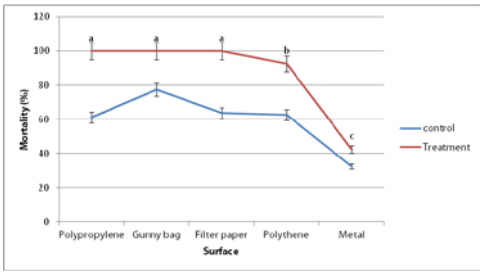

Figure 4. Mortality of *Rhyzopertha dominica* adults exposed to spinosad-treated surfaces for 6 days.

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Effectiveness of spinosad and spinetoram against five stored-product beetle pests under high relative humidity conditions

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The objective of this study was to evaluate spinosad and spinetoram effectiveness against *S. granarius, S. oryzae*, *T. confusum*, *T. castaneum* and *R. dominica* in wheat grain under high relative humidity (75%). The insecticides were applied at the rates of 0.5, 1 and 2 mg a.i./kg. Mortality was assessed after 2, 7, 14 and 21 days, and progeny reduction and grain damage caused by the insects were also assessed.

All rates of both insecticides caused 98-100% mortality of *R. dominica* after 7 days, and 100% mortality after 14 and 21 days of exposure. Both insecticides achieved high mortality (97-100%) after 21 days of contact of *S. granarius* with 1 and 2 mg/kg, and *S. oryzae* with 2 mg/kg rate. The highest mortality of *T. confusum* and *T. castaneum* was recorded after 21 days of contact with 2 mg/kg of both insecticides, 54-55% and 25-31%, respectively. All rates of both insecticides caused high progeny reduction of >99% of *R. dominica*, >90% of *T. confusum* and 94% of *T. castaneum* (only with 2 mg/kg). The highest *S. granarius* progeny reduction (>90%) was found in wheat treated with 2 mg/kg spinosad and 1-2 mg/kg spinetoram, while the greatest progeny reduction of *S. oryzae* was observed in wheat treated with 2 mg/kg spinetoram. Wheat grain damage caused by *R. dominica* was very low, i.e. up to 0.2% in wheat treated with all rates of spinosad and spinetoram, while *S. granarius* and *S. oryzae* caused up to 5% damage only in wheat treated with 2 mg/kg of spinetoram.

Keywords: stored-product beetle pests; high relative humidity; spinosad; spinetoram; effectiveness

1. Introduction

Traditional grain protecting organophosphates (OP) and pyrethroids (PY) still constitute the most important segment in the concept of IPM programs and the first option for control of stored insects in storages in which fumigation is not possible (Arthur 2012; Arthur and Subramanyam, 2012). Further use of these insecticides is limited by resistance that populations of stored-product insects have developed to traditional grain protectants and especially by an increasing consumer demand for products that are free of insects and insecticide residues and their negative impact on the environment (Phillips and Throne, 2010; Boyer et al., 2012). Dealing with the challenge and improvement of anti-resistance tactics and control programs for stored-product insects becomes possible with new insecticides that have different mechanisms of activity and good toxicological and ecotoxicological profiles (Phillips and Throne, 2010; Hertlein et al., 2011). Over the past 20 years, only spinosad and diatomaceous earths have proved to be good alternative to OPs and PYs and have been registered as grain protectants in many countries (Arthur and Subramanyam, 2012; Kljajić et al., 2014). Spinosad is a broad-spectrum insecticide of low mammalian toxicity, a mixture of spinosyn A and spinosyn D, secondary metabolites of the soil actinomycete *Saccaropolyspora spinosa* Mertz and Yao (Hertlein et al., 2011). Spinetoram is a new member of the spinosyn group, and a mixture of two synthetically modified spinosyns (spinosyn J and spinosyn L), which are also metabolites of *S. spinosa*. In the latest research, spinetoram has shown similar or higher effectiveness than spinosad against stored-product insects, and may therefore be expected to play an important role in future control of stored-product insects (Vassilakos et al., 2012; Vassilakos and Athanassiou, 2013; Athanassiou and Kavallieratos, 2014; Vassilakos et al., 2015; Rumbos et al., 2018). Unlike traditional grain protectants, such as OPs that achieve their high efficacy after exposure periods of 2-7 days (Kljajić and Perić 2009; Rumbos et al., 2013) spinosad and spinetoram show slower activity, reaching peak efficacy against most stored-product insects after 14-21 days (Fang et al., 2002; Nayak et al., 2005; Athanassiou et al., 2008; Vayias et al., 2009; Andrić et al., 2011; Vassilakos et al., 2012; Vassilakos and Athanassiou, 2013; Vassilakos et al., 2015; Rumbos et al., 2018). As a result, environmental conditions, such as temperature and relative humidity, may significantly affect the efficacy of spinosad and spinetoram. While higher temperature usually increases efficacy, high humidity mostly leads to efficacy reduction (Athanassiou et al., 2008; Vassilakos and Athanassiou, 2013). Besides reducing insecticide efficacy, high relative humidity most often has a positive effect on progeny production, insect distribution and abundance (Hagstrum et al., 1996). High humidity, especially when it extends over a longer period of time, also leads to greater grain moisture, so that wheat grains become softer and more prone to insect infestation (Gaines et al., 1996).

To our knowledge, the efficacy of spinetoram against the granary weevil *Sitophilus granarius* (L.) and red flour beetle *Tribolium castaneum* (Herbst) under high relative humidity conditions has not been

tested so far. The intention of this study was to examine and compare the efficacies of spinosad and spinetoram against *S. granarius*, rice weevil *Sitophilus oryzae* (L.), lesser grain borer *Rhyzopertha dominica (F.)*, confused flour beetle *Tribolium confusum* (Du Val) and *T. castaneum* under high relative humidity (75%), as well as their effects on progeny production/reduction in F_1 generation, and grain damage.

2. Materials and Methods

Test Insects and insecticides used

Laboratory populations of *S. granarius, S. oryzae*, *T. confusum*, *T. castaneum* and *R. dominica*, reared in an insectary, were used in the testing, and procedures described by Harein and Soderstrom (1966), and Davis and Bry (1985) were employed. *S. granarius* and *S. oryzae* were reared in 2.5 L glass jars containing whole-grain soft wheat with moisture content below 12%, while coarse ground wheat was added for *R. dominica* and *T. confusum,* and *T. castaneum* was reared on white wheat flour with 5% yeast. Air temperature in the insectary was 25±1°C, and relative humidity 60±5 %. Unsexed 2-4 week old adults of all tested species were used in the experiment.

The following commercial products were used in the experiment: Laser 240 SC containing 240 g/L spinosad, and Delegate 250 WP containing 250 g/kg spinetoram (Dow AgroSciences, Austria).

Bioassays

Investigation was conducted in the laboratory under high relative humidity conditions of 75±5% and 25±1°C temperature (both parameters were measured by a data logger Kestrel 4000, USA). Moisture content in wheat grain, variety 'Simonida', was 12.3±0.1 % and it was measured by a Dickey–John Mini GAC (Dickey–John Co., USA) device before the experiment.

Two standard solutions were prepared for both insecticide and diluted into dose series of 0.5, 1.0 and 2.0 mg a.i./kg, so that each insecticide dose was used for two treatments of 500 g lots. Each 1000 mL glass jar was filled with 500 g of wheat grain and treated with 5 mL of water solution of one of the insecticides, or 5 mL of water for control grain. After hand shaking the treated wheat for 30 s, each jar was placed on a mechanical roller for 15 minutes. For each tested species, six 50 g samples (three per treatment), representing each dose and insecticide, were placed into 200 mL plastic vessels. The next day, 25 adults of each tested species were released into each vessel, which was then covered with cotton cloth and fixed with rubber band. Adult mortality of the tested species was determined 2, 7, 14 and 21 days after the beginning of their exposure to treated wheat grain.

After the last assessment, dead and living adults were removed and the vessels were retrieved to the laboratory (25±1°C and 75±5% r.h.) for additional periods of 5 weeks for *Sitophilus* species, 7 weeks for *R. dominica* and 9 weeks for *Tribolium* species. Progeny emergence/suppression was determined by counting adults (for *Tribolium* species the total number included larvae, pupae and adults).

When the progeny were counted, damage caused by the weevils and *R. dominica* were also assessed on 100 randomly selected kernels per vessel.

Data analysis

Before analysis, percentage mortality was transformed using *arcsine* and progeny counts were transformed by *log(x+1)*. All data were submitted to a one-way ANOVA and the means were separated by Fisher's LSD test at P=0.05. Progeny reduction (IR%) in wheat grain was determined using a formula recommended by Tapondjou et al. (2002).

3. Results

All application rates of spinosad and spinetoram caused low mortality (0-16%) of all tested species after 2 days of exposure, except of *R. dominica*, whose mortality was 18.0-62.7% and 32.7-53.3%,

respectively. Seven to 21 days exposure to both insecticides at all application rates caused high mortality of *R. dominica*, 98-100%. The 7 days exposure period to all rates of spinosad and spinetoram caused *S. granarius* mortality that ranged from 18.0-81.3% and from 62.0-86.0%, considerably lower mortality of *S. oryzae* (8.7-72.0% and 24.0-87.3%), and the least (<12%) in *T. confusum* and *T. castaneum*. After 14 and 21 days, spinosad and spinetoram caused high mortality (92-100%) of *S. granarius* and *S. oryzae* in contact with 1.0 and 2.0 mg/kg, and 2.0 mg/kg of both insecticides, respectively, while the highest mortality of *T. confusum* and *T. castaneum* was recorded after 21 days of contact with 2.0 mg/kg of both insecticides, 54-55.3% and 25.3-31.3%, respectively (Table 1).

All application rates of both insecticides caused high progeny reduction: 98.6-100% of *R. dominica*, 90-100% of *T. confusum* and 94.5% of *T. castaneum* (only with 2.0 mg/kg). The highest *S. granarius* progeny reduction (90.2-95.3%) was found in wheat treated with 2 mg/kg spinosad and 1-2 mg/kg spinetoram, while *S. oryzae* progeny reduction was the greatest (94.5%) in wheat treated with 2 mg/kg spinetoram (Table 2).

No grain damage caused by *R. dominica* was detected in wheat treated with any rate of spinosad or spinetoram other than grain treated with the lowest doses of the insecticides, and even that dose caused only a very small damage of up to 0.2%. After *S. granarius* and *S. oryzae* progeny were counted in all treated wheat, grain damage was detected, peaking with 42.2-77.2% in wheat treated with 0.5 mg/kg spinosad, while the lowest was 3.0-5.2%, found in wheat treated with 2 mg/kg spinetoram (Table 2).

4. Discussion

The results in our present study show that the efficacy of spinosad and spinetoram depend on the rate, exposure interval and target species, which is consistent with previous results (Fang et al., 2002; Nayak et al., 2005; Subramanyam et al., 2007; Athanassiou et al., 2008; Vassilakos et al., 2012; Vassilakos and Athanassiou, 2013). For example, both insecticides were highly effective at the rate of 0.5 mg/kg against *R. dominica* after 7 days, at the rate of 1-2 mg/kg against *S. granarius*, and 2 mg/kg rate against *S. oryzae* after 14 days, while the 2 mg/kg rate achieved its highest efficacy against *T. confusum* and *T. castaneum* after 21 days.

In a recent study Athanassiou and Kavallieratos (2014) concluded that spinetoram was equally and in some cases even more effective than spinosad against stored-product beetle species. Based on these results, we can similarly conclude that no significant difference emerged in our experiment between spinosad and spinetoram effectiveness against the most susceptible *R. dominica* and least susceptible *Tribolium* species.

Regarding *Sitophilus* species, however, spinetoram was significantly more effective than spinosad. For example, spinetoram applied at the rates of 0.5 and 1 mg/kg was significantly more effective than spinosad after 7-21 days of exposure of *S. granarius* and *S. oryzae*, as well as considering the average progeny counts and percentage of damaged grain, which was significantly lower for both species in wheat treated with spinetoram.

A number of previous studies (Fang et al., 2002; Nayak et al., 2005; Subramanyam et al., 2007; Athanassiou et al., 2008; Vayias et al., 2009, 2010; Vassilakos et al., 2012, 2015; Athanassiou and Kavallieratos, 2014; Rumbos et al., 2018) agreed that *R. dominica* was the most susceptible of stored-product beetle species, while *Sitophilus* species were significantly less susceptible, and *Tribolium* species the least susceptible to spinosad and spinetoram, which was further confirmed in our present research under high relative humidity conditions. Furthermore, our results clearly show that *S. granarius* is significantly more susceptible than *S. oryzae* to both insecticides, while *T. confusum* is significantly more susceptible than *T. castaneum*. Differences between *Sitophilus* species were greatest when 0.5 mg/kg rate was applied, and after exposure periods of 7 and 14 days, as well as between *Tribolium* species after the application of 2 mg/kg spinosad and spinetoram and exposure for 21 days.

Tab. 1 Mean (% ± SE) mortality of *S. granarius*, *S. oryzae*, *R. dominica*, *T. confusum* and *T. castaneum* adults exposed for 2, 7, 14 and 21 days to wheat treated with spinosad or spinetoram (for each species/exposure separately, means within columns marked by the same letter are not significantly, Fisher's LSD test at $P>0.05$)

Insecticide	Rate mg/kg	Mean (% ± SE) mortality after exposure			
		2 days	7 days	14 days	21 days
S. granarius					
Spinosad	**2.0**	0.7±0.2 a	81.3±0.8 a	99.3±0.2 a	99.3±0.2 a
	1.0	0.0±0.0 a	68.0±1.3 b	98.0±0.3 a	100±0.0 a
	0.5	0.0±0.0 a	18.0±1.0 c	48.7±1.8 c	71.0±1.1 c
Spinetoram	**2.0**	0.0±0.0 a	86.0±0.6 a	100±0.0 a	100±0.0 a
	1.0	0.0±0.0 a	80.0±0.6 a	100±0.0 a	100±0.0 a
	0.5	0.0±0.0 a	62.0±1.2 b	88.0±05 b	94.0±0.7 b
S. oryzae					
Spinosad	**2.0**	4.0±0.4 b	72.0±1.1 b	92.0±0.8 ab	97.3±0.3 a
	1.0	3.3±0.3 bc	42.0±1.1 c	60.0±1.0 c	71.0±0.8 b
	0.5	0.0±0.0 c	8.7±0.8 d	18.7±1.2 d	21.3±12 d
Spinetoram	**2.0**	16.0±0.4 a	87.3±0.7 a	98.0±0.5 a	98.7±03 a
	1.0	3.3±0.3 bc	52.0±0.9 c	87.3±1.4 b	96.0±0.4 a
	0.5	0.0±0.0 c	24.0±1.4 d	36.7±1.2 d	44.7±1.3 c
R. dominica					
Spinosad	**2.0**	62.7±0.4 a	100±0.0 a	100±0.0 a	100±0.0 a
	1.0	42.7±0.6 c	100±0.0 a	100±0.0 a	100±0.0 a
	0.5	18.0±0.8 e	98.0±0.2 b	100±0.0 a	100±0.0 a
Spinetoram	**2.0**	53.3±0.5 b	100±0.0 a	100±0.0 a	100±0.0 a
	1.0	48.7±1.1bc	100±0.0 a	100±0.0 a	100±0.0 a
	0.5	32.7±0.6 d	99.3±0.2 a	100±0.0 a	100±0.0 a
T. confusum					
Spinosad	**2.0**	0.0±0.0 a	12.0±1.0 a	34.7±2.2 a	54.0±2.2 a
	1.0	0.0±0.0 a	1.3±0.2 b	2.7±0.3 b	6.0±0.6 b
	0.5	0.0±0.0 a	0.0±0.0 b	3.3±0.3 b	5.3±0.3 b
Spinetoram	**2.0**	0.0±0.0 a	10.0±0.4 a	32.7±0.9 a	55.3±1.1 a
	1.0	0.0±0.0 a	2.0±0.2 b	6.7±0.3 b	16.0±0.4 b
	0.5	0.0±0.0 a	0.0±0.0 b	6.7±0.4 b	14.7±0.8 b
T. castaneum					
Spinosad	**2.0**	0.0±0.0 a	9.3±0.6 ab	22.0±1.8 a	25.3±2.3 a
	1.0	0.0±0.0 a	4.7±0.4 bc	9.3±0.5 b	11.3±0.7 b
	0.5	0.0±0.0 a	0.0±0.0 c	4.0±0.2 b	7.3±0.5 b
Spinetoram	**2.0**	0.0±0.0 a	12.0±0.6 a	24.0±0.9 a	31.3±1.0 a
	1.0	0.0±0.0 a	2.0±0.2 c	8.0±0.4 b	10.0±0.4 b
	0.5	0.0±0.0 a	0.0±0.0 c	7.3±0.5 b	10.0±0.6 b

Vassilakos et al. (2012) reported a 1.5 times lower efficacy of 0.5 mg/kg rate of spinetoram in wheat against *S. oryzae* than *S. granarius*. Our study showed even greater differences, so that spinetoram rate of 0.5 mg/kg after 7 days and spinosad rate of 0.5 mg/kg after 14 days were 2.6 times less effective against *S. oryzae* than against *S. granarius*. After 21 days of contact with 2 mg/kg rate of spinosad and spinetoram, efficacy was 1.8 and 2.1 times lower against *T. castaneum* than against *T. confusum*.

Data from some earlier studies show that increasing relative humidity mostly leads to lower efficacy of spinosad and spinetoram (Athanassiou et al., 2008; Vassilakos and Athanassiou, 2013). Comparing efficacy data for spinosad under high humidity of 75% in the present study and our earlier findings (Andrić et al., 2011) in experiments conducted under 60% r.h., similar conclusions were drawn. For example, spinosad applied in our present study at 0.5, 1 and 2 mg/kg rates resulted in *S. oryzae* mortality of 18, 60 and 92% after 14 days, while the respective data from earlier experiments were 59, 77 and 100%. Similarly, progeny reduction of *S. oryzae* in wheat treated with 0.5, 1 and 2 mg/kg spinosad was 2.5, 34.1 and 82.2% at 75% r.h., which is significantly less than progeny reduction at 60% r.h, which was 42.2, 80,8 and 91%.

Tab. 2 Progeny emergence (adults/vessel ± SE), progeny reduction (%) and kernel damage (mean % ± SE) of *S. granarius*, *S. oryzae*, *R. dominica*, *T. confusum* and *T. castaneum* in wheat treated with spinosad or spinetoram (for each species separately, means within columns followed by the same letter are not significantly different, Fisher's LSD test at *P*>0.05)

Insecticide	Rate mg/kg	Progeny emergence (adults/vial ± SE)	Progeny reduction (%)	Kernel damage (mean % ± SE)
S. granarius				
Spinosad	**2.0**	54.3±18.0 e	90.2	13.0±2.8 de
	1.0	87.3±6.7 d	84.4	18.2±2.4 d
	0.5	377.2±13.4 b	33.1	42.2±2.7 b
Spinetoram	**2.0**	25.7±2.0 f	95.3	3.0±0.7 f
	1.0	43.3±7.2 e	92.2	8.5±1.2 ef
	0.5	169.8±19.0 c	69.8	26.8±2.7 c
	0	564.2±33.0 a	-	58.7±3.8 a
S. oryzae				
Spinosad	**2.0**	134.5±35.2 d	82.2	14.7±3.3 c
	1.0	499.2±22.1 b	34.1	51.0±3.1 b
	0.5	738.5±16.9 ab	2.5	77.2±3.5 a
Spinetoram	**2.0**	41.8±.4.2 e	94.5	5.2±0.7 d
	1.0	196.2±14.0 c	74.1	21.8±2.3 c
	0.5	511.0±41.6 b	32.5	51.2±4.0 b
	0	757.5±34.0 a	-	76.0±1.3 a
R. dominica				
Spinosad	**2.0**	0.0±0.0 b	100	0.0±0.0 b
	1.0	0.0±0.0 b	100	0.0±0.0 b
	0.5	2.2±0.4 b	98.6	0.2±0.2 b
Spinetoram	**2.0**	0.0±0.0 b	100	0.0±0.0 b
	1.0	0.0±0.0 b	100	0.0±0.0 b
	0.5	0.5±0.3 b	99.3	0.2±0.2 b
	0	238.5±42.5 a	-	29.0±0.03 a
T. confusum				
Spinosad	**2.0**	0.0±0.0 b	100	/
	1.0	0.0±0.0 b	100	/
	0.5	0.0±00 b	100	/
Spinetoram	**2.0**	0.0±00 b	100	/
	1.0	0.0±00 b	100	/
	0.5	0.2±0.2 b	90.0	/
	0	1.7±0.9 a	-	/
T. castaneum				
Spinosad	**2.0**	1.2±0.3 e	94.5	/
	1.0	3.5±0.6 d	84.7	/
	0.5	11.3±2.4 b	51.8	/
Spinetoram	**2.0**	1.2±0.6 e	94.5	/
	1.0	5.3±1.2 cd	77.0	/
	0.5	9.0±17 bc	61.6	/
	0	23.7±3.2 a	-	/

As data from tests of the effectiveness of insecticides as grain protectants may vary, it is very important to determine their effects on progeny production of storage insects, as well as on grain damage (Subramanyam and Roesli 2000; Subramanyam et al., 2007). In the present study, all spinosad and spinetoram doses caused high progeny reduction (98.6-100%) only for *R. dominica*, accompanied by almost no grain damage at all (≤0.2%), as well as high progeny reduction (90-100%) for *T. confusum*, which is consistent with earlier reports (Fang et al., 2002; Vayias et al., 2009; Subramanyam et al., 2007; Athanassiou and Kavallieratos, 2014). Regarding both *Sitophilus* species, however, only the 2 mg/kg rate of spinosad and spinetoram caused high progeny reduction of 82.2-90.2% and 94.5-95.3%, respectively, while the percentage of grain damage ranged 3-14.7% and 3-5.2%, respectively. These results are inconsistent with several earlier studies (Vayias et al., 2009; Subramanyam et al., 2007; Athanassiou and Kavallieratos, 2014) in which 1 mg/kg rate of spinosad or spinetoram applied to various types of grain resulted in high or maximum reduction of progeny of maize weevil *Sitophilus zeamais* (Motsch.), *S. granarius* and *S. oryzae*, and no maize grain damage was caused by *S. zeamais* and *S. oryzae*. Besides the lower effiacy that was observed in this study and

population origin, the observed differences may also be attributed to the high relative humidity that made wheat grain softer in our experiment (Gaines et al., 1996). It enabled progeny production of *Sitophilus* species, and consequent grain damage, as well as progeny production of *T. castaneum* even after wheat grain treatment with 2 mg/kg spinosad or spinetoram. Supporting these findings are data on grain damage caused by *S. granrius* and *S. oryzae* in control of 58.7 and 76.0%, respectively.

Based on all results in this study, we concluded that the minimum effective dose of spinosad and spinetoram for *R. dominica* and *S. granarius* control in wheat grain under high humidity conditions (75% r.h.) is 0.5 and 2.0 mg/kg, respectively, and only 2 mg/kg of spinetoram for *S. oryzae* control, while successful control of *T. confusum* requires 0.5 mg/kg of spinosad or spinetoram, and *T. castaneum* 2.0 mg/kg of either spinosad or spinetoram.

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Spinosad-induced stress on the maize weevil *Sitophilus zeamais*

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Abstract

Although seldom considered, sublethal insecticide exposure may lead to harmful, neutral, or even beneficial responses that may affect (or not) the behavior and fitness of the exposed insects. Intriguingly, little is known about such effects on stored product insect pests and even less is available regarding the bioinsecticide, spinosad. Thus, we assessed the sublethal effects of spinosad on walking, feeding, drinking and mating behaviors of maize weevils (*Sitophilus zeamais*), also assessing their survival, reproductive output, and grain loss compared with maize weevils exposed to the pyrethroid deltamethrin (as positive control), and water only (negative control). Both spinosad and deltamethrin were able to effectively control the insects, although the latter caused a faster mortality than the former. Behavioral pattern changes were caused by both insecticides, especially deltamethrin, triggering irritability (i.e., avoidance after contact). Different feeding and drinking responses were also detected with significant avoidance to deltamethrin, but not to spinosad. Maize weevil couples sublethally exposed to deltamethrin and spinosad exhibited altered reproductive behavior, a likely consequence of their altered activity, but deltamethrin caused greater behavioral changes. Curiously, higher progeny emergence and grain loss were observed in deltamethrin-exposed insects, suggesting that this pyrethroid insecticide elicits hormesis in maize weevils that may compromise control efficacy by this compound. In contrast, such effect was not detected with spinosad, which did not elicit avoidance allowing the intended weevil exposure and control.

Keywords: biopesticide, hormesis, insecticide avoidance, sublethal exposure, progeny production

Introduction

Insecticides are a familiar class of pest control agents, understandable due to their broad use since the 1940's across many sectors, including stored product protection. Although insecticides are technically defined as "*any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any insect pest*" (e.g., US Federal Insecticide, Fungicide, and Rodenticide Act), these compounds are popularly defined as substances that kills insects. The blame probably lays with the old Romans and the Latin origin of the suffix *cide* (from *cīda*; = a killer of), which is rather popular and frequent in several nouns of different languages. Regardless, the emphasis of the popular definition of insecticide is on the *killing* of insects, not managing or controlling them, as

advocated by the technical definition. Such fact leads to an important bias on dealing with insecticides – the emphasis and reliance on their mortality effect, while largely neglecting their sublethal consequences (Hardin et al., 1995; Guedes et al., 2016).

Lethal effects of insecticides are certainly important, and the primary intent of most users is indeed to quickly kill the pest species. Importantly, however, sublethal exposures can cause population suppression without necessarily causing death; reproductive impairment, for instance, can be as effective, or even more effective, for pest control. It is critical to understand the issues that contribute to a sublethal exposure. Initial insecticide deposits degrade over time, lowering residue levels, eventually to the sublethal range for a length of time that is a complex function of toxicological and environmental factors. Another contributor is that the lethal concentration applied to target a given pest is potentially sublethal to other species, particularly co-occurring species.

Sublethal insecticide exposure is known to affect arthropod development, longevity, reproduction, and even the genetic make-up of the population (Lee, 2000; Guedes et al., 2016, 2017). Sublethal exposures may lead to shifts in species prevalence (or dominance) (Cordeiro et al., 2014) creating unforeseen pest outbreaks, as well as inadvertent selection(s) for insecticide resistance in non-targeted pest species (Haddi et al., 2015; Guedes et al., 2017). Behavioral changes are also among the potential consequences of sublethal insecticide exposure. These changes may involve general activity, mobility, feeding, mating and egg-laying, among others, all potentially affecting the maintenance and growth of the arthropod pest population (Haynes, 1988; Lee, 2000; Guedes et al., 2016). Therefore, a range of responses may accrue from sublethal insecticide exposure. In fact, novel insecticidal chemistries have increasingly relied on secondary and behavioral effects (Casida and Durkin, 2013; Guedes et al., 2016). Curiously, sublethal insecticide exposure is rarely scrutinized in stored product scenarios, where it is common for pest species co-occur with finite resource (Guedes et al., 2011, 2014).

Another conceptual bias that commonly plagues the general public perception of insecticides, and also influences pest research and management, is the deeply-rooted notion that natural compounds are safer than synthetic ones. While this may be valid in certain cases, the notion in based on the false premise that origin is a determinant of toxicity, and safety (Coats, 1994; Isman and Grieneisen, 2014; Guedes et al., 2016). The selection of a "natural" insecticide, including biopesticides, reduced-risk pesticides, biorational compounds, insecticidal proteins, and an increasing variety of neologism, pleonasms, and/or misnomers should be considered based not on semantic qualification, but on the chemistry that dictates toxicity and safety.

Spinosad is an insecticide of natural origin, or bioinsectide, with recent use in stored product protection, not yet subjected to comprehensive sublethal studies. The technical active ingredient (a.i.) is a mixture of spinosyns A and D, fermentation products of the soil actynomycete *Saccharopolyspora spinose* Mertz and Yao (Thompson et al., 2000; Sparks et al., 2001). Earlier launched for field crop use, its market was more recently extended to stored products as a grain protectant efficient against a range of pest species (Toews and Subramanyam, 2003; Huang and Subramanyam, 2007; Athanassiou et al., 2008; Athaniassiou and Kavallieratus, 2014), including the grain weevils (Coleoptera: Curculionidae) (Athaniassiou and Kavallieratus, 2014). In warmer climates, particularly Neotropical America, the maize weevil *Sitophilus zeamais* Motsch is a key pest, and virtually nothing is known about (sublethal) effects of spinosad, despite its potential usefulness for conventional and organic production and storage systems.

We assessed the sublethal effects of spinosad on the overall activity, walking, feeding, drinking and mating behaviors of maize weevils (*Sitophilus zeamais*), and evaluated results relative to conspecifics exposed to the pyrethroid deltamethrin (as positive control), and water only (negative control). Differences in the sublethal effects of the two insecticides were suspected, as the mechanism and modes of neurotoxic action are distinct; deltamethrin is a Na-channel modulator of the axon of neurons (i.e., nerve cells) with quick activity, in contrast with spinosad, which is a disruptor of

nicotinic acetylcholine receptors (nAChRs) in synapses of the insect nervous system with potentially slower activity (Sparks et al., 2001; Casida and Durkin, 2013).

1. Material and Methods

1.1. Insects and insecticides

The maize weevil population used in the study was originally collected in Sete Lagoas county (State of Minas Gerais, Brazil). This population is used as a susceptible standard population in studies of insecticide resistance and has been maintained on maize grains free of insecticide residues under controlled conditions of 27 ± 2°C, 70 ± 10% r.h., and 12:12 h photoperiod (L:D).

The insecticides were used in their respective commercial formulations available in Brazil for stored product protection and at the recommended label rates, as follows: deltamethrin (K-Obiol 25 CE; emulsifiable concentrate at 25 g a.i./L; Bayer, São Paulo, SP, Brazil), and spinosad (Tracer 480 SC; suspension concentrate at 480 g a.i./L, Dow, Mogi-Mirim, SP, Brazil). The insecticides were diluted in distilled and deionized water at the concentrations of 0.25 and 0.50 mf a.i./L of deltamethrin and spinosad, respectively. The insecticide solutions were sprayed in batches of 400 g of maize grains at 0.5 and 1.0 mg a.i./kg grain using an artist air brush (Saguma SW440A, Yamar, São, SP, Brazil) connected to an air compressor (model 131 type 2VC, Primatec, Itu, SP, Brazil) at 3 bar pressure. The maize grains were sprayed within a stainless-steel container coupled to a revolving rotor to homogenize the grain coverage until the residues dried. The air brush and revolving container were cleaned with acetone; distilled and deionized water was used as negative control treatment.

1.2. Survival bioassay

Time-mortality bioassays were conducted using 3- to 7-days old adults (unsexed). Individual insects were placed inside 30-mL glass vials containing 10 g of treated maize (i.e., deltamethrin, spinosad, or water (control)). Twenty insects were used in each insecticidal treatment and their respective survival was monitored at 30 min (for first two hours) and 1 h intervals for deltamethrin, and at 1 h (first 8 hs) and 6 h-interval afterwards for spinosad and water. The insects were considered as dead if unable to respond when prodded with a fine hair brush.

1.3. Overall group activity

Adult weevils subjected to either 30 min (deltamethrin) or a 10-h exposure (spinosad and water), the duration corresponding to non-observed effect levels as determined in the survival bioassays, were clustered in groups of 10 individuals within Petri dish arenas (9-cm diameter) lined at the bottom with filter paper and coated with Teflon® to prevent insect escape (Guedes et al., 2009b). The overall group activity within each arena was recorded for 15 min and digitally transferred to a computer using an automated video-tracking system equipped with a CCD camera (ViewPoint LifeSciences, Montreal, Canada). Overall activity was digitally recognized as changes in pixels in two successive frames taken every 10^{-2} s from each other representing any change of position and posture of the insects. The bioassays were carried out under the same conditions as previously described and during daytime.

1.4. Walking bioassays

Walking bioassays in half-treated arenas were performed to assess insecticide behavioral avoidance by means of irritability (i.e., with contact with insecticide) and repellence (i.e., without direct contact with insecticide). Unexposed insects were released alone in individual Petri dish arenas lined with filter paper half-treated with either deltamethrin or spinosad and their movement was digitally recorded for 15 min with the tracking system described above (Cordeiro et al., 2010; Morales et al., 2013). Again, 20 replicates were used for each dichotomous bioassay with either deltamethrin or spinosad vs water (control).

1.5. Feeding and drinking preference

A free-choice test modified from Guedes et al. (2009a) was performed using white plastic trays (30 x 18 x 6 cm) with 200 g of water- and insecticide-sprayed grains placed in opposite sides. The inner walls of the trays were covered with Teflon® to prevent insect escape and 25 unsexed adult weevils (1-2 weeks old) were released in the center of the arena. Insect preference was recorded after one hour and the bioassays were replicated five times.

A dichotomous bioassay of water drinking preference was carried out as described by Guedes et al. (2014) providing a choice between 50-µL droplets of water uncontaminated ,or insecticide-contaminated (deltamethrin or spinosad), after maintaining the insects under 30-40% relative humidity for 24 hs. The insecticide contaminations were used at the same rates from previous bioassays, and each droplet was stained with either artificial blue or red dye (Mix Industries, São Bernardo do Campo, SP, Brazil). The choice of water droplets was provided in 9-cm Petri dish arenas and the insects were observed for 5 min. Water intake was confirmed by dissecting the insects and examining evidence of the dye coloration in the insect gut diverticula, what was performed under stereomicroscope (Stemi 2000; Zeiss, Göttingen, Germany).

1.6. Female mate-searching

Virgin weevil females (< one week old) were treated with insecticides (except in the control) as previously described, and transferred to 9-cm Petri dish arenas containing a male weevil caged in its center (Guedes et al. 2017; Cordeiro et al. 2017). The searching activity of the females was recorded again using the ViewPoint tracking system recording search time and velocity for up to 2 hours under the same conditions of the previous experiments.

1.7. Progeny emergence and grain consumption

Three groups of 35 virgin weevil couples (one week old) were treated with insecticide as previously described and subsequently released in 140-mL jars containing 50 g maize free of insecticide residues. The insects were removed after 30 days and progeny production and grain loss were recorded. Progeny production was daily assessed until emergence of the last adult, and grain loss was determined in sequence with eventual correction for humidity change, if necessary.

1.8. Statistical analyses

Time-mortality data was subjected to survival analyses using Kaplan-Meyer estimators allowing determination of the respective median survival times (LT_{50})(PROC LIFETEST; SAS, SAS Institute, Cary, NC, USA). The curves were compared using Bonferroni's method. Individual and overall group activity, irritability, repellence, and feeding and drinking preferences were subjected to general linear model and contrasted by χ^2 test (PROC GENMOD; SAS). Female searching time, progeny production and grain loss were subjected to analyses of variance and Tukey's HSD test ($P < 0.05$), when appropriate (PROC GLM; SAS).

2. Results

3.1. Survival time

The survival curves of weevils exposed to either insecticide, deltamethrin or spinosad, and the control were significantly different ($\chi^2 = 409.37$, df = 2, $P < 0.001$). Natural (i.e., control) mortality was negligible for up to 15 days, in contrast with insecticide-exposed weevils (Fig. 1). Deltamethrin led to quick mortality among the exposed adult weevils with median survival time of 3.5 hs, while median mortality by spinosad took significantly longer (i.e., 76.5 hs) (Fig. 1).

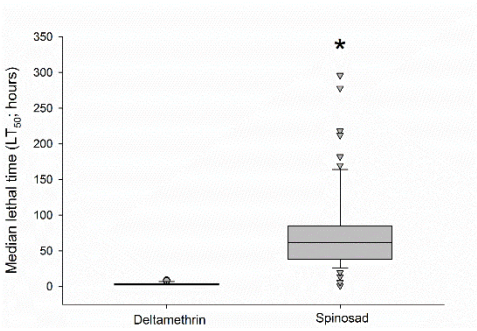

Fig. 1. Median lethal times (TL_{50}) of insecticide-exposed adult maize weevils (*Sitophilus zeamais*). The box plots indicate the median and dispersion (lower and upper quartiles, and outliers) of the median lethal times. The asterisk indicate significant difference between insects exposed to the insecticides using Bonferroni's method ($P < 0.05$).

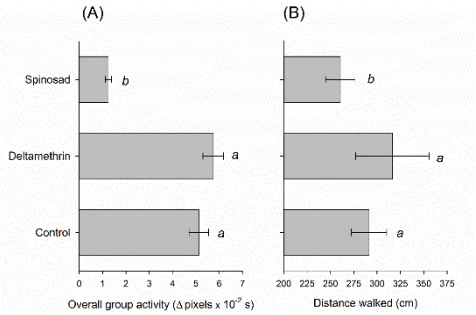

Fig. 2. Overall group activity (A) and distance walked by individual weevils (B) (± SE) subjected to insecticide exposure (except control with unexposed insects). Different low case letters in the bars indicate significant differences by χ^2 test ($P < 0.05$).

3.2. Activity

The overall group activity also significantly differed among treatments with spinosad-exposed adult weevils exhibiting significant less activity than unexposed and deltamethrin-exposed weevils ($\chi^2 = 181.45$, df = 2, $P < 0.001$) (Fig. 2A). When distance walked by individual insects was considered as a proxy of individual activity, a similar trend was observed. Again spinosad compromised activity and led to the lowest distance walked compared with deltamethrin and unexposed insects, which provided similar results ($\chi^2 = 55.68$, df = 2, $P < 0.001$) (Fig. 2B).

3.3. Feeding and drinking preference

Behavioral avoidance among insecticide-exposed weevils indicated significant irritability ($\chi^2 = 4.73$, df = 2, $P = 0.03$), or avoidance after contact with contaminated surface, but no repellence (i.e., avoidance without contact with contaminated surface). Most insects did not respond to spinosad though, in contrast to deltamethrin against which 40% of the insects exhibited avoidance by irritability (Fig. 3A).

Feeding preference also differed between insecticides when given a choice between uncontaminated and contaminated maize grains. Weevils did not exhibit feeding preference when offered uncontaminated and spinosad-contaminated grains, but the insects significantly avoided deltamethrin-contaminated grains in favor of uncontaminated maize kernels ($\chi^2 = 25.53$, df = 1, $P = 0.0004$) (Fig. 3B). Such a trend was also observed when water was provided for drinking with weevils avoiding deltamethrin-contaminated water ($\chi^2 = 39.32$, df = 1, $P < 0.001$), but no avoidance was detected with spinosad-contaminated water ($\chi^2 = 2.91$, df = 1, $P = 0.10$) (Fig. 3C).

3.4. Female-mate searching, progeny production and grain loss

The female searching for suitable male partner was significantly affected by insecticide exposure ($F_{2,57} = 39.63$, $P < 0.001$). Unexposed females were able to find their mates relatively quicker, while spinosad and particularly deltamethrin significantly extended such searching time (Fig. 4A). Nonetheless, the differences in mate searching time did not significantly affect the total progeny produced by each female weevil ($F_{2,102} = 0.35$, $P = 0.70$) (Fig. 4B), but deltamethrin-exposed weevil led to higher grain loss than those unexposed or exposed to spinosad ($F_{2,102} = 13.93$, $P < 0.001$) (Fig. 4C).

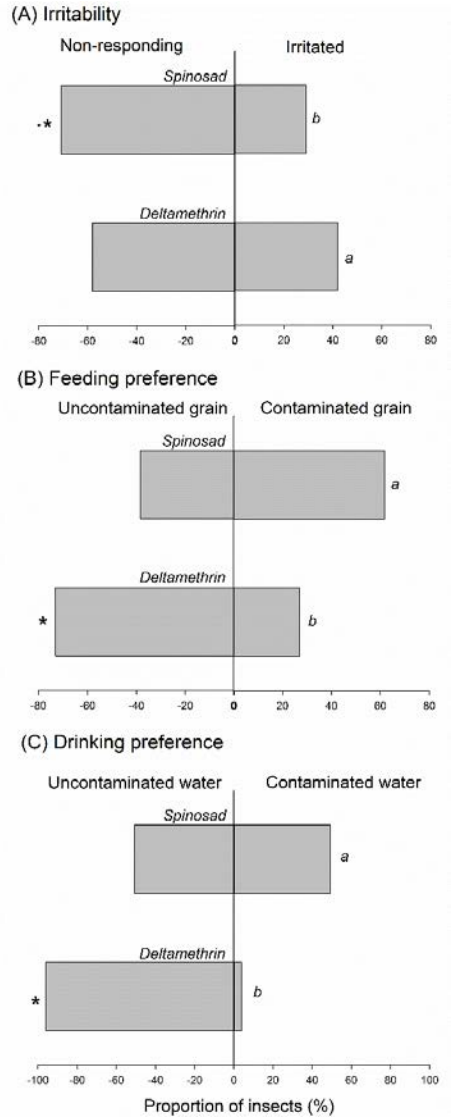

Fig. 3. Proportion of adult weevils showing irritability to insecticide-contaminated surfaces (A), and exhibiting feeding (B) and drinking preferences (C) with choice of uncontaminated and insecticide-contaminated grains and water. Different letters in each bar indicate significant differences between treatments and asterisk indicates significant difference between proportion of uncontaminated and insecticide-contaminated material (i.e., surface, grain, or water). All differences were detected with χ^2 test ($P < 0.05$).

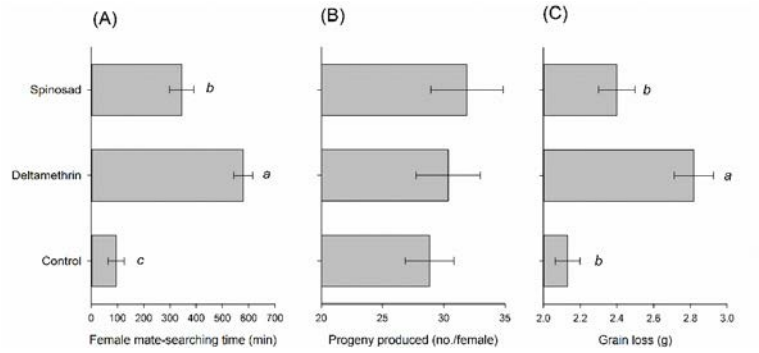

Fig. 4. Female mate-searching time (A), total progeny produced per female (B), and total grain loss (C) (± SE) by weevils subjected to insecticide exposure (except control with unexposed insects). Different lower case letters in the bars indicate significant differences by χ^2 test ($P < 0.05$).

3. Discussion

The lack of information regarding the sublethal effects of the bioinsecticide spinosad led us to evaluate its impact on walking, feeding, drinking and mating behaviors of maize weevils (*Sitophilus zeamais*), also assessing their survival and reproductive output compared with maize weevils exposed to the pyrethroid deltamethrin (as positive control), and water only (negative control). Interference of both neurotoxic compounds with weevil activity were expected with potential consequences for mating, reproduction and grain (weight) loss. Indeed behavioral changes were induced by both insecticides although the effects of deltamethrin were stronger, but unexpectedly enhancing grain loss instead of reducing it.

Both deltamethrin and spinosad were effective against adult maize weevils exposed to contaminated grains at their respective recommended label rates as grain protectants, although deltamethrin exhibits faster activity, as previously been shown (Athanassiou et al., 2008; Athaniassiou and Kavallieratus, 2014). Nonetheless, the impact of both compounds go beyond mortality. Sublethal effects of deltamethrin and spinosad have been reported in other species (Elliott et al., 1978; Huang and Subramanyam, 2007; Amakware et al., 2014; Velki et al., 2014). Overall group activity and walking activity were both significantly reduced by spinosad, a likely consequence of its mode of action modulating nicotinic acetylcholine receptors (nAChR) and interfering with receptors of γ-aminobutiric acid (GABA) at synapses of the nervous system (Sparks et al., 2001; Casida and Durkin, 2013).

Among the two different types of insecticide avoidance behavior reported among insects, repellence (i.e., avoidance with little or no contact) and irritability (i.e., avoidance after contact), only the latter was observed in weevils and sole with deltamethrin, not with spinosad. Weevils exhibited significant irritability to deltamethrin and avoided feeding on deltamethrin-contaminated grains and water, which may potentially reduce exposure to, and targeted-efficacy of this compound. Irritability and associated behavioral responses toward pyrethroids, such as deltamethrin, were already reported among other arthropod species (Quisenberry et al., 1984; Vatandoost, 2001; Pekar and Hadda, 2005; Guedes et al., 2009ab, 2014). In contrast, spinosad avoidance was reported only in a couple of species of predatory stink bugs (Castro et al., 2013).

Insecticides may also interfere with insect communication (Guedes et al., 2016, 2017), which may potentially disrupt mating and reproduction (Lürling and Scheffer, 2007; Guedes et al., 2016). Indeed, spinosad and deltamethrin extended the mate searching time in exposed female weevils, but without significant effect on progeny production. In fact, the trend was of increased progeny production with insecticide exposure, which was reinforced by the higher grain loss obtained, particularly for deltamethrin. With sublethal insecticide exposure, we expected a decrease in progeny production and an extension of the time spent searching for a mate. However, the opposite

was observed in our study, suggesting that the longer searching time may have favored the selection of better quality male partners, potentially leading to higher (and/or better quality) progeny production and more feeding leading to heavier grain losses. The lack of significant difference in the total progeny produced is a likely reflex of not accounting for the time of 1st reproduction in our assessment, and not assessing progeny quality. The former condition shortens generation time, leading to higher population growth and progeny numbers with time, conditions that were beyond the scope fo this investigation. The consequence of higher population growth is more feeding, and ultimately higher grain loss, as was observed to a greater extent for deltamethrin, relative to spinosad.

Higher grain losses resulting from sublethal insecticide exposure is a counter-intuitive outcome. However, this outcome takes place when insecticide-induced hormesis is present. Hormesis is a biphasic dose-response phenomenon that takes place when a stimulatory effect is observed from the low dose of a compound demonstrated to be toxic at higher doses (Guedes and Cutler, 2014; Guedes et al., 2016, 2017). Deltamethrin-induced hormesis has already been reported in the maize weevil (Guedes et al., 2010), and the same likely occurred in our study. The pyrethroid effective against the exposed parental population may have induced higher reproductive output of the better-quality (surviving) parents, leading to higher and/or better-quality progeny. Although the final progeny population was not significantly higher with deltamethrin, the grain loss observed provides at least partial support for this contention. Hormesis, in this case, was probably the result of a trade-off where energy resources for the parent self-maintenance are diverted to offspring production (Guedes and Cutler, 2014), which are of better quality and/or in higher numbers leading to higher grain loss.

Insecticide behavioral avoidance and hormesis are two management concerns for pest species in general, and the maize weevil in particular. The former potentially minimizes exposure to the insecticide, while the latter favors population growth with exposure. Evidence for both phenomena was observed in our study with the maize weevil, but only with the insecticide deltamethrin, not spinosad. Therefore, deltamethrin use deserves particular attention and spinosad, although not as quick in leading to adult mortality, is also a very effective insecticide against the maize weevil and without apparent risk of minimizing exposure or leading to hormesis, at least at the label rate conditions used in our study. Thus, spinosad is an attractive alternative for weevil management, not due to its natural origin, but due to its insecticidal activity. The natural origin of spinosad however, makes it an enticing alternative for organic production and storage systems, a condition in which the origin, rather than chemistry, receives emphasis.

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Effects of *Hemizygia welwitschii* leaf extract fractions on postharvest infestation of maize by *Sitophilus zeamais* Motsculsky (Coleoptera: Curculionidae)

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Abstract

As part of on-going efforts to use eco-friendly alternatives to chemical pesticides, leaf powder of *Hemizygia welwitschii* was sequentially extracted in hexane, acetone and methanol. Bioassays were carried out to establish the most active fraction(s) against *Sitophilus zeamais* in maize. Maize grains (50 g) were treated with concentrations within the range 2, 4, 6, and 10 g/kg of extract and *Azadirachta indica* seed oil (positive control) in the laboratory. The total number of progeny emerging from grains infested separately with *S. zeamais* eggs, larvae and pupae were recorded. Adult mortality counts were carried out 1, 3, 7 and 14 d post-exposure. Acetone extract was more toxic to the eggs, larvae and pupae than the other extracts, inhibiting progeny production by 90.90%, 88.10% and 100%, respectively, at the concentration 10 g/kg. For the same concentration, *A. indica* seed oil reduced progeny production by 100% for eggs, 96.08% for larvae and 70.93% for pupae. Hexane extract was more potent to the adult weevil than the other extracts, recording 100% mortality for the concentration 10 g/kg within 14 d. LC_{50} values were 0.78 (Hexane), 5.52 (acetone) and 1.69 g/kg (methanol). Extracts of *H. welwitschii* leaves had sufficient efficacy to be a component of storage pest management package for *S. zeamais*.

Key words: Leaf powder, Mortality, Grain damage, Pest management

1. Introduction

Maize (Zea mays L.) is a staple food for a large proportion of the world with significant economic importance. It is currently the third most-cultivated and traded cereal after wheat and rice (FAO, 2006). The highest amounts of maize consumed as food are found in Southern Africa at 85 kg/capita/year as compared to 27% in East Africa and 25% in West and Central Africa (Smale *et al.,* 2011). The crop is characterized by the diversity of its consumption forms: fresh, boiled, roasted, and "foufou" (Ndjouenkeu *et al.,* 2010). A world challenge is to increase the global maize production to feed nine billion people by 2050 (Godfray *et al.,* 2010).

The production and storage of maize have faced many constraints throughout developing countries such as scarcity of rain, diseases and lack of inputs (Brisibe *et al.,* 2011), and most important constraint being the field-to- store infestations of maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) (Akob and Ewete, 2007). This insect inflicts severe damages leading to weight loss and reduction of the economic value, grain viability and nutritive value of maize (Akunne *et al.,* 2013). According to Obeng-Ofori and Amiteye, (2005) and Yuya *et al.,* (2009), about 20 to 40% of

maize grain is lost due to the attack of *S. zeamais*. The control of insect pests of stored products in general, relies mainly on the use of residual synthetic chemical insecticides. This practice, although effective in the fight against insects, is toxic to consumers, pollutes the environment and induces resistance in pests (Arnaud *et al.,* 2001). The use of increasing amounts of pesticides represents a real danger since it leads to the stage where the insecticide is completely ineffective against the pest. Also, obsolete synthetic chemicals are found in our local markets, which cause serious health hazards (Bambara and Tiemtoré, 2008).

Alternative solutions to the application of synthetic chemicals is the use of phytochemicals (reduced-risk insecticides of plant origin) which is presently being encouraged in stored grain protection because there are more biodegradable, and thus may pose less environmental hazards. *Hemizygia welwitschii* Rolfe Ashby (syn. *Orthosiphon welwitschii* Rolfe) (Lamiaceae) is a bushy aromatic perennial herb, widely available in the Adamawa region of Cameroon, and used in folk medicine for the treatment of skin diseases (Ngassoum *et al.,* 1999). The essential oil is known to possess antibacterial properties and repellence activity against mosquitoes (Oyedele *et al.,* 1992, 2000). The chemical composition of the essential oil from the powdered leaf of *H. welwitschii* harvested in Ngaoundere, Cameroon, were mainly 1-octen-3-01 (14.1%), 3-octanol (4.5%) and linalool (2.6%) (Ngassoum *et al.,* 1999). To date, no scientific publication has reported the efficiency of *H. welwitschii* leaf extracts on stored product insect pests. The study was therefore aimed at determining the most active fraction of *H. welwitschii* against the eggs, larvae, pupae and adults of *S. zeamais*.

2. Materials and Methods

2.1. Collection and extraction of *Hemizygia welwitschii*

Fresh leaves of *H. welwitschii* were collected from the surroundings of University of Ngaoundere in the Vina Division, Adamawa region, Cameroon between August and November 2016 and shade-dried naturally at the room temperature for five days where they became crisp dry. The identity of the plant was confirmed at the Cameroon National Herbarium in Yaounde, where a voucher specimen (Serial number: 6910/SRFK) was deposited. The dried leaves were crushed in a mortar until the powder passed through a 0.4 mm mesh sieve. The powder was stored in a deep-freezer at the temperature of -18°C until needed for bioassay.

Two thousand three hundred grams of *H. welwitschii* powder were mixed with 7.5 L of hexane and stirred for 30 min and allowed to stand for 24 hours in the laboratory of the Institute of Medical and Medicinal Plant Research (IMPM), Yaounde, Cameroon, and then re-stirred again. After 48 hours the mixture was then filtered with a filter paper (Whatman no. 1). The residue obtained after filtration was put through the process above again and the filtrate was admixed with the one obtained initially. After the hexane extraction was done, the paste left was dried for 10 h at room temperature in the laboratory and then used for Acetone extraction and followed by methanol extraction. The filtrates obtained with hexane, acetone and methanol were then separately concentrated in a Rotavapor at 70°C, 60°C and 65°C, respectively at 120 rpm. Extracts were stored in a refrigerator at 4 °C until needed for bioassay. *Azadirachta indica* seed oil from the study of Tofel *et al.,* (2016), which was stored in a deep freezer at -18°C, was used as a positive control.

2.2. Insects

The parent adults of *S. zeamais* for the adult toxicity test were obtained from colonies maintained at the Applied Zoology laboratory of the University of Ngaoundere while those for the immature stages toxicity were taken from colonies maintained at JKI, Institute for Ecological Chemistry, Plant Analysis and Stored Products Protection, Berlin since 2007 and 1968, respectively. In Ngaoundere, *S. zeamais* were reared on Shaba maize variety while in Berlin they were reared on Ricardino variety.

2.3. Bioassays

For the adult mortality bioassay, four different masses of 0.1, 0.2, 0.3 and 0.5 g each of the hexane, acetone and methanol extracts were separately mixed with 50 g grains in glass jars, which corresponded to the concentrations of 2, 4, 6 and 10 g/kg, respectively. The negative controls consisted of grains with solvent alone. The content of each jar was hand-shaken properly to ensure complete coating of the grains with the extracts. The grains were then air-dried for 2 hours to evaporate the solvent. Groups of 20 *S. zeamais* were added to glass jars containing treated or untreated maize. Glass jars were securely covered with muslin cloth and were tightly held in place with rubber bands to ensure adequate ventilation. All treatments were arranged in a completely randomized design on shelves under fluctuating laboratory condition in Ngaoundere, Cameroon and each treatment had four replications. Mortality was recorded 1, 3, 7 and 14 days after treatment. Insects were considered dead when no movement was observed after touching them with forceps twice within two or three minutes.

Concerning the toxicity test on immature stages, individual lots of 50 g maize grains in 250 ml glass jars, containing eggs, different larval stages or pupae of *S. zeamais* were coated with *A. indica* seed oil (positive control) or the hexane, acetone and methanol extracts at the rates 1, 4 and 10 g/kg, with the aid of a rotatory shaker. The jars were closed with perforated metal lids. The negative controls consisted of grains with solvent alone. The grains were then dried for 10 min in a ventilated fume chamber to evaporate the solvent. The treated and untreated grains, which were replicated four times were keep in a controlled environment at 25 ± 1°C and 65 – 70% r.h. in a complete randomized design in Berlin, Germany for F_1 progeny emergence. All the F_1 progeny were counted.

2.4. Data analysis

Data on % cumulative corrected mortality and % reduction in F_1 progeny, were arcsine [(square root(x/100)] transformed to homogenise the variance. The transformed data were subjected to the ANOVA procedure using the Statistical Analysis System (version 9.2). Tukey (HSD) test (P = 0.05) was applied for mean separation**.** Probit analysis (Finney, 1971) was applied to determine lethal concentrations causing 50% (LC_{50}) mortality of *S. zeamais* at 1 and 7 days, after treatment application. Abbott's formula (Abbott, 1925) were used to correct for control mortality before probit analysis and ANOVA.

3. Results

3.1. Toxicity to adult

All the *H. welwitschii* extract fractions generally caused significant mortality to adult *S. zeamais* compared to the control. Mortality increased with ascending content levels and time exposure, irrespective of extract fractions. Overall, significant difference was observed among the fractions. Hexane extract was more potent to the adult weevil than the other extracts, recording the maximum 100% mortality for the concentration 10 g/kg within 14 days post infestation. Within the same time and at the same dosage, acetone and methanol extracts recorded respectively 64.87 and 75.98% adult mortality. Within 1day after infestation the methanol extract caused higher mortality of 16.25% at the dose of 10 g/kg. The lowest tested dose (2 g/kg) achieved 63.32, 51.91 and 83.14% *S. zeamais* adult mortality respectively for the methanol, acetone and hexane fractions within14 days post-exposure.

The results of the evaluation of toxicity of the different extract fractions of *H. welwitschii* s are shown in Table 1. All the extracts proved to be toxic to adult *S. zeamais* although the acetone fraction was less effective with the LC_{50} of 410.18 g/kg one day after treatment. Adult S. *zeamais* was more susceptible to the hexane fraction with LC_{50} of 0.78 g/kg (7 day) followed by the methanol fraction with LC_{50} of 1.69 g/kg (7 day). At day one, the slope of the hexane extract (4.55 ± 0.74) was steeper than that of methanol (1.89± 0.24) and acetone (0.83 ± 0.21) extracts while they seemed similar at seven

days. In general, the coefficients of determination (R^2) of the extracts were between 0.60 and 0.94. The values of chi-square (Chi^2) were not significant for all the extracts except the methanolic fraction after day one exposure.

Tab. 1 Toxicity of leaf extracts from *Hemizygia welwitschii* to adult *Sitophilus zeamais* in maize grains at different exposure periods

Extract	Slope ± S.E.	R^2	LC_{50} (g/kg)	Chi^2
Day 1				
Hexane	4.55 ± 0.74	0.70	19.04	17.31ns
Acetone	0.83 ± 0.21	0.72	410.18	15.27ns
Methanol	1.89± 0.24	0.84	34.70	30.79***
Day 7				
Hexane	0.96 ± 0.14	0.60	0.78	6.47ns
Acetone	1.15 ± 0.13	0.83	5.52	4.90ns
Methanol	1.02 ± 0.13	0.69	1.69	12.26ns

Ns $P > 0.05$, ***$P < 0.001$

3.2. Inhibition of offspring production from eggs and immature stages

Table 2 shows the result of ability of *H. welwitschii* extract to inhibit the emergence of progeny in grains containing eggs, larvae and pupae of *S. zeamais*. All the extracts significantly influenced the production of the weevil ($P < 0.05$). Overall, the bioefficacy of these extracts on the eggs and immature stages was dose-dependent. *A. indica* seed oil was more efficient in inhibiting the development of eggs at all doses (100% inhibition). Acetone extract was more toxic to the eggs, larvae and pupae than the other extracts, inhibiting progeny production by 90.90%, 88.10% and 100%, respectively, at the concentration 10 g/kg. Contrariwise, hexane fraction had less affects the eggs, larvae and pupae development, reducing them by only 25%, 33.75% and 52%, respectively, at the concentration 10 g/kg. Methanol extract was most effective on pupal stage (73.50% inhibition) at its lowest dose (1 g/kg) than at its highest content of 10 g/kg with 58.50 % emergence reduction.

Tab. 2 Inhibition of adult emergence in grains containing eggs and immature stages of *Sitophilus zeamais* and treated with extracts from the leaves of *Hemizygia welwitschii*

Product	Dose (g/kg)	Inhibition of offspring production from three life stages (%)		
		Egg	Larva	Pupa
Neem seed oil	1	100 ± 0.00 ^{a}	43.50 ± 6.95	70.25 ± 8.33 ^{b}
Hexane	1	8.50 ± 5.06 ^{c}	37.25 ± 11.95	49.75 ± 6.12 ^{b}
Acetone	1	61.00 ± 16,47 ^{b}	32.00 ± 4.56	95.50 ± 2.63 ^{a}
Methanol	1	83.50 ± 6.06 ^{ab}	30.75 ± 4.61	73.50 ± 4.87 ^{b}
$F_{3, 12}$		15.50***	0.45ns	17.09***
Neem seed oil	4	100 ± 0.00 ^{a}	82.25 ± 3.92 ^{a}	89.00 ± 3.00 ^{b}
Hexane	4	8.25 ± 8.25 ^{b}	15.00 ± 9.00 ^{b}	38.75 ± 8.34 ^{c}
Acetone	4	88.75 ± 6.57 ^{a}	37.50 ± 6.59 ^{b}	100 ± 0.00 ^{a}
Methanol	4	39.00 ± 18.73 ^{b}	16.75 ± 11.31 ^{b}	63.50 ± 8.51 ^{c}
$F_{3, 12}$		15.85***	14.86***	34.90***
Neem seed oil	10	100 ± 0.00^{a}	96.25 ± 1.65 ^{a}	72.25 ± 5.50 ^{b}
Hexane	10	25.00 ± 10.16^{b}	33.75 ± 14.30 ^{b}	52.00 ± 1.15 ^{c}
Acetone	10	93.75 ± 6.25 ^{a}	91.00 ± 4.12 ^{a}	100 ± 0.00 ^{a}
Methanol	10	79.50 ± 7.35 ^{a}	25.50 ± 6.33 ^{b}	58.50 ± 1.70 ^{c}
$F_{3, 12}$		23.74***	20.19***	109.79***

ns < P > 0.05, ***$P < 0.001$

Means ± S.E. followed by the same letter in a column do not differ significantly at $P < 0.05$ (Tukey's test). Each datum represents the mean of four replicates.

4. Discussion

Plant products and their secondary metabolites are receiving increasing attention in stored product management (Zettler and Arther, 2000). Several researchers have evaluated the insecticidal, repellent or antifeedant and development inhibiting effects of various plant parts and plant extracts on *S. zeamais* with varying degrees of success (Arannilewa et *al.,* 2006). Boulogne *et al.,* (2012) mentioned that, 656 plant species worldwide, distributed into 110 families, were identified to have a significant insecticidal activity. The most cited family is the Lamiaceae in which *H. welwitschii* belongs, with 181 species distributed into 48 genera, counting for 28 % of the plant families with an insecticidal activity. In the present study, the different extracts of *H. welwitschii* which caused significant mortality to *S. zeamais* suggests that they contain insecticidal compounds. Compounds like linalool known for insecticidal activity and present in the leaves as reported by Ngassoum *et al.* (1999) may have played a significant role. The hexane extract was the most effective against adult *S. zeamais*. This could be speculated that the fraction may possess compound with high insecticidal potency. Kosini *et al.* (2015) reported similar results with *Ocimum canun,* plant of the same family. They mentioned that the high mortality caused by hexane extract may be partially related to the gummy aspect of the hexane extract, which acetone and methanol extracts lacked. The extract may be glued to the insect's wing cover and legs and hindered mobility. The insect thus lost vigor by trying to get loose and when combined with the toxic effects of compounds in the leaf extracts, may lead to the death of *S. zeamais*. The essential oil of the studied plant was repellent against mosquitoes preventing them from feeding (Oyedele *et al.,* 2000). This repellency property may have averted the maize weevils in the present study from food intake and death occurred by starvation since mortality was time dependent. More studies are needed to elucidate the active ingredients in each fraction and to understand the mode of action of these against insect pests of stored products. Also, microscopic observations will help to clarify how and where the glue of the extract fixes on the insect.

One of the basic characteristics of an effective phytochemical is its ability to reduce progeny emergence in treated grains (Khoshnoud et al., 2008). Results of inhibition of progeny production showed that extract fractions from *H. welwitschii* inhibited adult emergence of *S. zeamais,* showing their ability to control the development of the insect. The fractions might have acted physically by asphyxiation or chemically on eggs or immature stages, depending on the compounds present in each extract. The coating of the grains with extracts might have prevented the eggs from adhering unto the grains. Afful *et al.,* (2012) indicated that the methanol extract of root of *Securidaca longipendonculata* inhibited the development of eggs and larvae of the maize weevil. This reduction in emergence is an indication of the presence of ovicidal and larvacidal compounds which need to be determined.

The results of the present investigation based on the laboratory experiments, revealed that the extracts of *H. welwitschii* leaves had sufficient efficacy to be a component of storage pest management package for *S. zeamais,* especially for low income farmers since the plant species are cheap and widely available.

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Chemical properties and efficacy of Sweet orange essential oil nanoemulsion applied as cold aerosol against two stored product beetles

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Abstract

Common control strategies to manage stored product pests are mainly based on the use of synthetic insecticides and fumigants. Consumer's demand for pesticide-free food, and the increasing resistance of pests to traditional insecticides, dictate the need to evaluate alternative control methods. For this purpose, many sustainable techniques have been tested for the control of stored product pests. Among them, Citrus essential oils can represent a valid alternative to synthetic insecticides. The effects of Sweet Orange essential oil (EO) nanoemulsion applied as cold aerosol were evaluated against adults of *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) and *Cryptolestes ferrugineus* Stephens (Coleoptera: Cucujidae). Both chemical and physical characterization of the EO-based formulation was carried out. The developed formulation had an average size belonging to the nanometer scale and a low polydispersity index. The relatively high zeta potential value confirms the stability over time of the developed formulation. The efficacy of the tested formulation showed a dose-dependent response and the cumulated mortality of the exposed insects increaseduntil 24h of exposure for *C. ferrugineus* and until 120h for *T. confusum*. The tested formulation was more effective against *T. confusum* adults (LD50= 86.30 ppm) than *C. ferrugineus* ones (LD50= 36.79 ppm). The results of this study coupled with the large availability at reasonable costs of Sweet orange EO, are promising for the potential development of new tools against stored product pests.

Keywords: Citrus, essential oil, Tribolium confusum, Cryptolestes ferrugineus, control, fumigation

Introduction

Although their high toxicity and non-biodegradable nature have already been acknowledged worldwide, the use of synthetic pesticides is still increasing (Koul et al. 2008). However, the environmental consequences, the negative impact on non-target species and the development of resistance have stimulated the interest in alternative control strategies, such as the use of naturally derived chemical compounds (biopesticides), which have selective toxicity and are easily biodegradable (Kordali et al. 2006; Regnault-Roger et al. 2012).

Among botanicals, essential oils (EOs) are effective biopesticides, due to their promising laboratory results in term of toxicity against insect pests, bacteria and other pathogens (Romeo et al. 2008; Ali et al. 2012; Russo et al. 2013; Campolo et al. 2014).; Essential oils are volatile natural compounds, synthesized by many species of plants as secondary metabolites (Bakkali et al. 2008), which may act against insects as larvicidal, antifeedant, growth inhibitor, adulticidal, fertility reducer, oviposition deterrent and repellent (Cardiet et al. 2012; Ibrahim et al. 2001; Werdin-Gonzáles et al. 2011; Licciardello et al. 2013). Furthermore, EO toxic activity against mammalians is quite reduced (rat oral LD50 = 2–5 g × kg-1) (Regnault-Roger et al. 2012), guaranteeing product specificity and safety. Although most essential oils are exempt from registration, standardization and quality control are key issues for registration (Isman 2000; Koul et al. 2008). Moreover, EO chemical composition can negatively affect their application in operative conditions, since EO-based insecticides generally show high volatility and poor solubility in water (Moretti et al. 2002).

In this context, the development of a stable formulations containing EOs is a pivotal requisite for the application of these technique in field conditions. Nanoemulsions are defined as emulsions (i.e. mixtures of two or more liquids that are normally immiscible) in which the micelles of the dispersed phase show nanometric dimensions. In this study, we developed and characterized a nanoemulsion oil in water (i.e. the dispersed phase was EO and the dispersion medium was water) of sweet orange (SOR) [*Citrus sinensis* (L.)] essential oil. Thus, the aim of this study was to assess the insecticidal activity of SOR-EO against two stored product pests, *Tribolium confusum* du Val and *Cryptolestes ferrugineus* Stephens, testing its toxicity as cold aerosol (i.e. cold fumigation) against adult insects.

2. Materials and Methods

2.1. Insects

The confused flour beetle *T. confusum* and the rusty grain beetle *C. ferrugineus* were reared for several generations in the Stored Products Laboratory of the Department of Agriculture on wheat flour mixed with yeast (10:1, w: w). The rearing conditions were: 25 ± 1°C, 65±5% r.h., with a

photoperiod of 16h:8h (L: D). To obtain adults of the same age, about 100 unsexed adults were placed inside 5 l glass containers each provided with 500 g of non-infested rearing medium. After 2 days the specimens were removed and the newly emerged adults (2–8 days old) were used in the trials. Insects were collected from cultures using a 450-µm sieve (Technotest; Modena, Italy) and a mouth aspirator.

2.2. EO extraction

SOR-EO was extracted from the fruit peels, pesticide-free certified (Capua SRL, Campo Calabro Italy). The essential oil was extracted with the cold pressing technique (Citroflor, Condofuri Marina, Italy) (Lahlou 2004), from fruits cultivated in Calabria (Italy) and harvested from November to March following the harvest calendar for the species.

2.3. Nanoemulsion formulation and characterization

TWEEN 80 (Polyoxyethylene (20) sorbitan monooleate) was purchased from Sigma-Aldrich (Italy). The EO-NPs were prepared following Werdin-González et al. (2011), with some modifications. In brief, EO was mixed with Tween 80 and stirred for 30 min. Then, nanoemulsion was realized using the mechanism of spontaneous emulsification that is created between an organic phase and an aqueous phase when they are mixed. Double-distilled water was added to the homogeneous solution of citrus essential oil and a hydrophilic surfactant and then stirred for 60 min. The oil in water nanoemulsion was composed by 5% Tween 80®, 15% essential oil and 80% water. Lastly, to reduce micelles, the formulation was sonicated using an ultrasonic immersion homogenizer, stored at 25±0.5 °C in an airtight container and used for the bioassay within the following 48h. In order to measure the characteristics of the realized nanoemulsion, qualitative analyses such as particle size, polydispersion and emulsion stability were measuredusing the Zetasizer Nano (Malvern®) instrument. The dimensional and polydispersion analyses were carried out in cuvettes, model DTS0012 in polystyrene latex at 25°C. The nanoemulsion was diluted in double-distilled water in a ratio of 1/200 and 1 ml of the diluted solution was inserted into the cuvette. The measurement of each sample involved 3 replicas of 14 cycles. Three samples were tested as replicates. The stability analysis (potential ζ) was carried out by inserting 730 µl of the diluted solution in DTS1070 polystyrene latex cuvettes and tested at 25°C. The measurement of each sample involved 3 replicas of 14 cycles. Three samples were tested as replicates.

2.4. Cold aerosol trials

Toxicity trials were carried out in laboratory conditions at 25 ± 1°C, 65 ± 5% r.h. with a photoperiod of 16h:8h (L:D). Test specimens were placed inside a Perspex cage (25 x 25 x 25 cm), presenting a hole on one side (highness from the bottom 20 cm; diameter 14 mm) where was allocated an aerosol glass ampule. A known quantity of SOR-EO nanoemulsion (2 mL for *C. ferrugineus* and 4 mL for *T. confusum*) was put inside the aerosol ampule, which was connected to an air delivery system blowing purified air at 2 L min−1 constant flow. The air flow was turned off when the ampule was empty. Tested insects were maintained inside the cage for an exposure time of 24 h. After exposure time, specimens were removed from the cage and gently placed in a clean glass Petri dish, in which was added a plastic container containing 1g of wheat flour mixed with yeast (10:1, w: w). The dosages tested against *C. ferrugineus* were: 300, 150, 75, 37.5, 18.75 and 9.38 ppm of SOR-EO. For every dose 3 replicates (i.e. 15 insects each) were performed. The mortality was recorded after 24 from the beginning of the cold aerosol treatment. The dosages employed against *T. confusum* were: 600, 300, 150, 75 and 37.5 ppm of SOR-EO. For every dose 3 replicates (i.e. 10 insects each) were performed. For T. *confusum*, the mortality was counted after 24 48, 72, 96 and 120h.

To exclude the impact of surfactant on insect mortality, control trials were carried out using formulations of Tween in water at the same concentrations tested as EO-nanoemulsions. In addition, additional control trials using only distilled water were performed for both *T. confusum* and *C. ferrugineus*.

2.5. Data analysis

Statistics were carried out using SPSS® V. 20 (IBM). The efficacy of the tested formulations was corrected for control mortality using Abbott's formula (Abbott 1987). Probit analysis was performed in order to estimate the median lethal concentrations for both tested insect species (LD50 and LD99).

3. Results

The average size of the developed formulation belonged to the nanometer scale (average size ± standard error= 230.3 ± 14.65 nm) (Figure 1), with low polydispersity index (Pdi 0.274). The stability over time of the tested formulation was confirmed by the relatively high zeta potential value obtained (ζ= 26.93) (Figure 2).

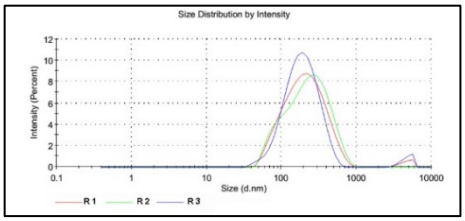

Fig. 1 Size and PdI values for the SOR-EO nanoemulsion

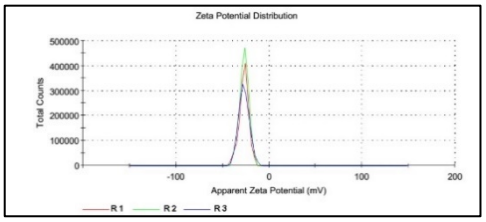

Fig. 2 ζ potential of the SOR-EO nanoemulsion

The efficacy of the tested formulation showed a dose-dependent response and the cumulated mortality of the exposed insects increased until 24h of exposure for *C. ferrugineus* (Figure 3) and until 120h for *T. confusum* (Figure 4). No mortality was recorded for control with distilled water, while little toxic activity was recorded for Tween solutions. From statistical analyses *T. confusum* adults proved less susceptible to the tested formulation than *C. ferrugineus* specimens. Lethal dose values for *C. ferrugineus* were LD50= 36.79 ppm and LD99= 209.7 ppm after 24h from the exposure. In contrast, the LD50 and LD99 values recorded for *T. confusum* at 24h from the exposure were 86.30 ppm and 631.14 ppm, respectively.

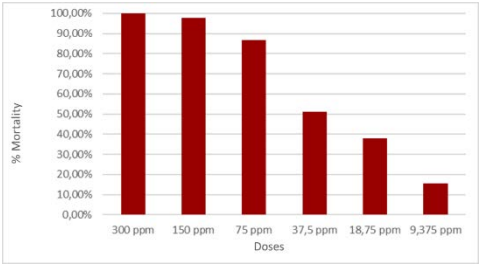

Fig. 3 Insecticidal activity of SOR-EO nan emulsion as cold aerosol against *C. ferrugineus* adults after 24h from the exposure.

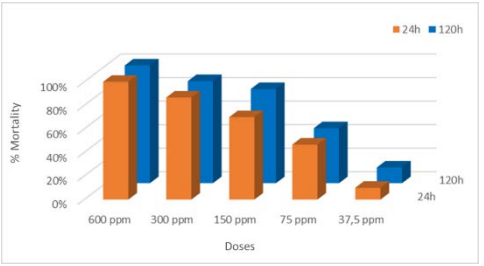

Fig. 4 Insecticidal activity of SOR-EO nanoemulsion as cold aerosol against *T. confusum* adults after 24h and 120h from the exposure.

4. Discussion

Several studies had demonstrated the fumigant activity of essential oils to several stored product pests (Polatoğlu and Karakoç 2016), highlighting higher susceptibility of adults than of pre-imaginal stages (Koul et al. 2008). Terpenes are key compounds for the bioactivity against insects, bacteria and fungi, acting as contact, fumigant and ingestion insecticides (Malacrinò et al. 2016; Prates et al. 1998). The formulation of EOs as nanoemulsions improves both the stability and effectiveness of botanical insecticides. Indeed, nano-formulations can solve problems related to EO volatility, poor water solubility, and the tendency to oxidize (Campolo et al. 2017; Werdin-González et al. 2014). Furthermore, these formulations are able to release the active compounds at the site of action

gradually (de Oliveira et al. 2014), and concurrently minimize the toxic effects on non-target organisms (Gogos et al. 2012).

Both the size and the polydispersion index obtained in our study are adequate for nanoemulsion and the zeta potential obtained can be considered an indicator of the extent of EO loading in the emulsion. Furthermore, our results highlighted the good insecticidal activity of the citrus peel essential oils against the stored product pests *T. confusum* and *C. ferrugineus*. In this study, we also tested a novel administration method of EOs, using cold fumigation as aerosol. Indeed, this promising technique may allow the development of efficient and effective control strategies based on the application of plant-derived compounds to protect stored products in the food industries. The results obtained in these trials, together with the availability of SOR-EO at reasonable cost, are promising for the potential development of new tools against stored product pests.

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Fogging loads of California fresh citrus for control of Asian citrus psyllid, *Diaphorina citri*

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Abstract

Contact insecticides are commonly applied as fogs to disinfest and disinfect spaces. Recently, these fogs have been adapted to treat commodity within the spaces, and much has been learned regarding the efficacy of this process. When considering fresh citrus in California, fogs are applied to control both insects and microbes. One insect pest, the Asian citrus psyllid (ACP), *Diaphorina citri*, is a quarantine pest in California and limiting its geographic distribution is a major goal of the California citrus industry. While a variety of phytosanitary measures can be used to control adult ACP once fruit is at a packing house, ultimately, a treatment must be developed to disinfest field-run fruit prior to its exiting the grove. High-pressure fogging with 1,100-L of an aqueous mixture containing 0.2% Evergreen® (6% pyrethrins & 60% piperonyl butoxide) and 0.5% (v/v) BreakThru® (polysiloxane surfactant) was explored in laboratory-, pilot-, and commercial-scale trials as an approach to disinfest a 48-bin trailer load of fresh citrus. Laboratory-scale studies were conducted to quantify, and subsequently model, insecticidal coverage as a function of temperature, surface area, droplet size, and fog volume. Results are discussed in the context of experimental variability across confirmatory trials and continued efforts to optimize the technical and economic feasibility of fogging as a postharvest control strategy.

Keywords: food security, food safety, pyrethrins, postharvest fogging

1. Introduction

Asian citrus psyllid (ACP), *Diaphorina citri*), which transmits citrus greening disease (Huanglongbing or HLB)-associated liberibacter ('*Candidatus* Liberibacter asiaticus; Las), has the potential to devistate the production of fresh citrus in California (Grafton-Cardwell et al. 2016). Moreover, the presence of ACP in the marketing channel can create a phytosanitary barrier for exports, which are key to the industries profitability. While ACP adults are removed from fresh citrus that has been subjected to cleaning and packing procedures standard to commercial production and distribution, State and Federal quaratines often restrict movement of fruit from ACP infested orchards to packhouses (CDFA, 2018).

Accordingly, a treatment must be developed to disinfest field-run fruit prior to its exiting the grove to control any incidental transportation of ACP and potential spreading of the insect and its associated disease. This work describes the development of a high-pressure fogging system using Evergreen® to control ACP in trailer loads of field-run fruit. The proposed treatment will reduce the number of psyllids in bulk citrus and reduce the insecticides applied to the grove, which will in turn improve worker safety, reduce environmental impacts, and improve IPM of ACP and other pests.

2. Materials and Methods

2.1. Chemicals

Evergreen® Pro 60-6 (McLaughlin Gormley King (MGK) Company, Minneapolis, MN), an aqueous mixture of 6% pyrethrins & 60% piperonyl butoxide, was sourced from Fruit Growers Supply (Exeter, CA) (EPA Reg. No. 1021-1770). BreakThru® S240, a polysiloxane surfactant (CA REGISTRATION #1051059-50001-AA), was sourced from Evonik Corporation (Hopewell, VA). Prior to dilution of the active and the surfactant, water was deionized using a Portable Deionizing System (ion exchange resin).

2.2. Insects, rearing, and infestation

The Asian citrus psyllid (ACP), *Diaphorina citri*, were reared on potted *Murraya koenigii* (L.) plants contained within ca. 0.5-m^3 rearing enclosures housed in an environmental room at the UC Riverside Insectary & Quarantine Facility set to 85 ± 2°C, 65% RH, and 16: 8 (L: D). Movement of the psyllids from the quarantine facility was permitted by CDFA (Permit # 3280)

To obtain an aliquot of adult ACP for efficacy studies, 10 specimens were consecutively aspirated into mesh cages using a customized arrangement of the aspirator and cage (Fig. 1). With respect to the commercial-scale trial conducted on 23 October 2017, two cage types were used. Cylindrical ~8-mL stainless-steel cages (30-mesh), as shown in Figure 1, were fabricated and following aspiration of the specimens, these cages were capped with a cork. Nylon mesh cages (3/4" diameter and 2.5" height) were fabricated by shrouding (Fig. 2A & 2B) – a wire cylinder (1/2" diameter and 2" height) with a square of nylon hardware cloth. After aspiration, the open end of the nylon cage was closed using a binder clip.

2.3. Commercial-scale fogging

At 07:00 PST on 23 October 2017 a 48-bin trailer load of field-run fresh navel oranges (ca. 56 to 88 size), sourced from Gless Ranch (Riverside, CA) arrived at Blue Banner Citrus in Riverside, California. Bins were off-loaded, numbered, and ACP specimens, which were caged ca. 0.5- to 1.5-h earlier, were buried throughout the bins at locations that were previously shown to have the relatively lowest piperonyl butoxide residues following a treatment (Fig. 3A). A total of 140 nylon cages were buried, 78 and 14 at low- and high-corner positions, respectively, and, 48 at the center of respective bins. To quantify residues throughout the load, and particularly those in proximity to nylon-caged specimens, nylon cages containing glass microfiber filter papers (1.6μm, 95 ± 1 mg, ~20 m^2/g, 4.7-cm diameter, 53 g/m^2 weight basis Whatman GF/A) were place next to nylon-caged specimens at each location. A stainless-steel cage containing ACP (used in previous experiments) was also placed at the center of respective bins. A total of 22 aliquots of ACP specimens and 12 nylon mesh cages as well as 10 "cage-less" 7-dram clear plastic aspirator vials, each of which contained a host leaf and a snap cap with an 8-mm diameter stainless-steel 100 wire mesh gas-portal, were buried throughout separate container of sourced fruit to serve as non-treated controls (Fig. 3B).

Within a de-greening room (V = 753.6 m^3; 21.3 l x 5.8 w x 6.1 h meters) at ca. 70°F, infested bins of fruit were re-oriented into the geometry of the truck load, two bins wide by two bins stacked, with a ca. 3-ft span between the 6th and 7th rows of bins (Fig. 4). The container of non-treated controls was transferred to an adjacent de-greening room, also set to ca. 70°F. The high-pressure spray system, designed and fabricated by Valley PackLine Solutions (Reedley, CA) was then situated around the load. Industrial Air Circulating Fans (34"- Fan Blade Dia, 17000 cfm Max Air Flow) were alternately arranged, directed laterally toward the center of each of the six 8-bin cubes comprising the load. An aqueous mixture (1,100-L) of ca. 0.1% Evergreen® (6% pyrethrins & 60% piperonyl butoxide) and 0.5% (v/v) BreakThru® (polysiloxane surfactant) was prepared in the reservoir of the spray system (note: Max label is 914mL per 290 gallons). The fans were turned on, and the aqueous solution was directed at 1000 psi to each of the six fans, outfitted respectively with a ¾"- steel manifold and a 45° fan nozzle that discharged into the airflow, ~ 6" below from the front of the fan. The de-greening room doors were shut, which marked the start of the treatment.

After the solution was delivered, the fans were turned off, 10 minutes elapsed to let the fog settle, and the de-greening room door was opened. The treated specimens were retrieved from the bins, along with the caged filter papers. Treated as well as non-treated control specimens were placed in bin- and location-specific Ziploc bags (separate from the caged filter papers that were organized similarly). Bagged-specimens were placed in a cooler and returned to the UC Riverside Insectary & Quarantine Facility for mortality evaluations (vide infra), which occurred at ca. 3-h following treatment.

2.4. Mortality evaluation

After returning the specimens, both treated and non-treated, to UC Riverside Insectary & Quarantine Facility the treatment, preparations were immediately made to evaluate mortality that resulted from the treatment. All specimens, less the non-treated controls specimens already in 7-dram snap cap vials, were transferred from nylon and stainless-steel cages into7-dram clear plastic "snap cap" cage modified with 8-mm diameter stainless-steel 100 wire mesh gas-portals on the cap (Fig. 5). A fresh lemon leaf was introduced into all plastic cages. Approximately 3 h following the treatment, all cages were visually inspected. Mortality was diagnosed by lack of motion and was calculated by subtracting the number of survivors from the number of treated specimens. Mortality of non-treated control specimens was treated numerically using Abbott's method (Abbott, 1925). Mortality, calculated as a percentage of the response per treatment, was expressed as a function of the number of specimens treated via probit analysis of Finney (1944 & 1977) at the 95% confidence level (CL), as further derived in Couey and Chew (1986) as well as Liquido and Griffin (2010).

3. Results

3.1. Commercial-scale fogging

The fogging process commenced at 10:30 AM and terminated at 12:10 PM. All but five non-treated control specimens survived, a single specimen did not survive in the plastic vial, while four did not survive in the nylon mesh. Only 2 specimens survived from 1,968 total treated, one specimen each from a stainless-steel and nylon mesh cage, both situated in the middle of the same bin on the top row, opposite the nearest fan. Using the statistical methods described above, the treatment resulted in 99.778% mortality (probit 7.85 at the 95% CL).

These results provide evidence to support the conclusion that adult ACP will be controlled in 48-bin trailer loads of fresh citrus subject to the high-pressure fogging treatment, at least when the volume of the load is ≥ 10% of the fogging enclosure. Although the 23 October 2017 commercial-scale trails were conducted in a de-greening room, analogues trials have been conducted on 48-bin trailer loads that were driven into a tent structure, as shown in Figure 6, methodology that is consistent with the need to disinfest field-run fruit prior to its exiting the grove.

Fig. 1 Method of collecting adult Asian citrus psyllid (ACP), *Diaphorina citri*, into 30-mesh stainless-steel cages using a standard mouth aspirator apparatus.

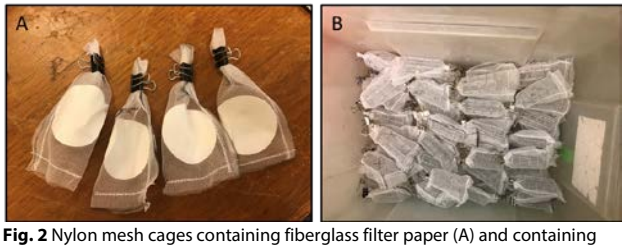

Fig. 2 Nylon mesh cages containing fiberglass filter paper (A) and containing ACP (B). Note that the open end of the nylon cage was closed using a binder clip.

Fig. 3 A bin filled with field run fruit showing placement of caged specimens in the middle position (A), a container filled with the same fruit that was used to analogously burry the non-treated, control ACP in the nylon mesh cages (B), the fruit were carefully positioned back atop the caged specimens (C), and the geometry of the trailer load was reconfigured within the degreening room (D).

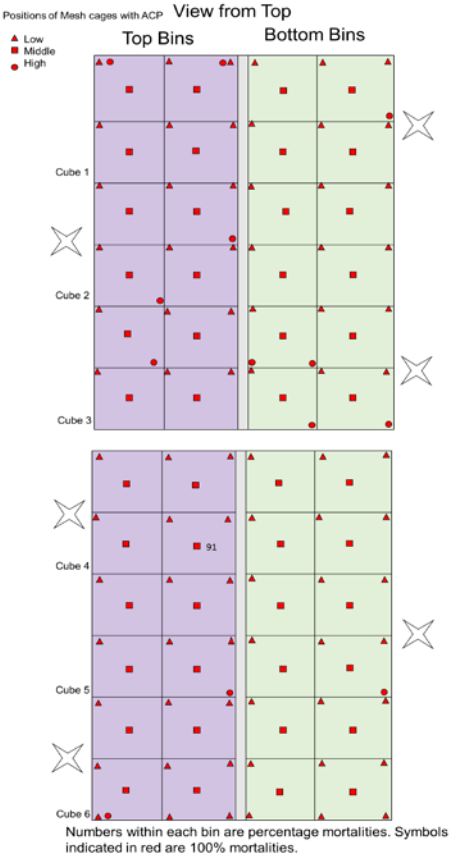

Fig. 4 Layout of 48-bin trailer loads of fresh citrus. Purple and green color indicate top- and bottom-stack positioning, respectively. Different shapes show location of cages within the bin (middle positon had both nylon and stainless-steel mesh cages). Note that the two survivors were found in the 8th row (top to bottom), one specimen each from a stainless-steel and nylon mesh cage, both situated in the middle of the same bin on the top row, opposite the nearest fan (denoted by stars).

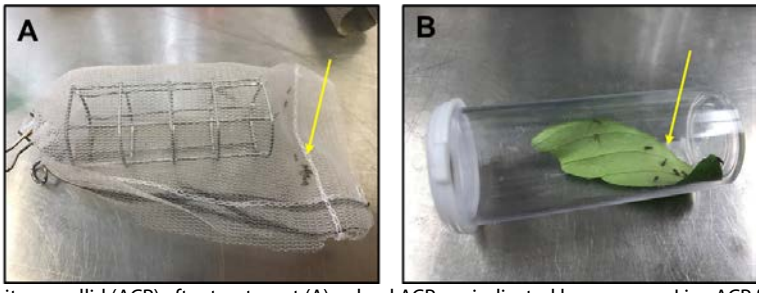

Fig. 5 Asian citrus psyllid (ACP) after treatment (A) – dead ACPs as indicated by an arrow. Live ACP feeding on lemon leaf in the non-treated controls caged in a plastic vial (B).

Fig. 6 The high-pressure fogging with an aqueous mixture containing 0.1% Evergreen® (6% pyrethrins & 60% piperonyl butoxide) and 0.5% (v/v) BreakThru® (polysiloxane surfactant) can be conducted on a 48-bin trailer load driven into a tent structure, methodology that is consistent with the need to disinfest field-run fruit prior to its exiting the grove.

4. Discussion

Future research effort will focus on minimizing the amount of fogging material required for efficacy, which will likely involve a transition to low-volume applicatiors, such as ultrasonic and thermal fogging technologies. Additionaly, treatment withina curtain-sided truck will be persued to further minimize the infrastructural requirement to tent the truck, while still remaining "mobile", within the grove. From a regulatory prespective, the proposed treatment represents a shift in paradign from the familiar pre- and post-harvest designations, and this divergence must be addressed at both the State and Federal levels. Considered a preharvest treatment, the application of Evergreen® is excempt from residue tolerance (USEPA and CA), has a 0-h postharvest interval, and a 12-h re-entry period for workers (CA). It is critical to note, however, workers will not be reuired to touch treated fruit after treatment, as it is already in field bins. While postharvest space foggings and direct applications to binned fruit on trucks are permitted postharvest treatments, with corresponding Personal Protective Equipment (PPE) and re-entry requirments, the proposed treatment occurs prior to washing and packing, which remove residues, contradicting the reationale for a postharvest tolerance. Regardless of the ultimate regulatory categorization, the proposed treatment could reduce the amount of insecticides applied to the grove, which will in turn improve worker safety, reduce environmental impacts, and improve IPM of ACP and other pests.

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Toxicity of fine powders, filter cake and Triplex against *Sitophilus zeamais* adults

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Abstract

Filter cake and Triplex are powdered by-products of aluminum sulfate and soap factories, respectively. There is limited data about the use of these powders as grain protectants. This study was aimed at determining contact toxicity of both powders against *Sitophilus zeamais*, a common pest of stored grains. Lethal concentration of both powders to *S. zeamais* was determined by exposing 10 adults for 12 h in 9 cm diameter concrete arenas inside Petri dishes dusted with filter cake (0 - 8 g/m^2) or Triplex (0 - 9 g/m^2). Lethal time was determined by exposing adults to 3 g/m^2 filter cake and 9 g/m^2 Triplex for 1 to 24 h. Each treatment was replicated 3 times. At the intended exposure time, adults were transferred to 150-ml round plastic containers with 30 g of wheat and held at 28 degree Celsius and 65% r.h. for 14 d to determine mortality. Adult progeny production was determined after 42 d. A 50% mortality of adults was obtained at 0.61 g/m^2 of filter cake and 1.61 g/m^2 of Triplex concentrations with a 12 h exposure. The corresponding effective concentrations for 50% reduction of progeny production were 0.18 g/m^2 of filter cake and 2.66 g/m^2 of Triplex. Lethal times for 50% mortality of adults after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex were 4.42 and 4.29 h, respectively. The corresponding effective times for 50% reduction of progeny production after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex were 1.74 and 2.34 h respectively. The overall result indicated that filter cake was highly toxic to *S. zeamais* than Triplex. Therefore, filter cake is a potential powder to be included in the integrated pest management practice in small holder farmers' storage structures after tested under real field conditions.

Keywords: Filter cake; Triplex; *Sitophilus zeamais*; Toxicity

1. Introduction

Grain losses due to insect pests in sub-Saharan Africa are very high, and the magnitudes of losses vary from country to country and from region to region (Abate et al., 2015). In countries like Ethiopia, about 80% of all grain produced is estimated to be stored at the farm or village level (Tadesse and Eticha, 2000). Grain storage losses in Ethiopia due to insect pests were estimated to be in the range of 10 to 21% (Abraham et al., 2008). A number of chemical insecticides used by Ethiopian small holder farmers to protect their grain in storage have been reported by several researchers (Abraham et al., 2008; Girma et al., 2008a,b; Hengsdijk and De Boer, 2017; Mengistie et al, 2017; Dessalegn et al., 2017). Recently, chemical pesticides, regardless of their inherent hazards, are used extensively in the fast changing agricultural sector of Ethiopia (Nigatu et al., 2016). However, Ethiopia is confronted with a number of problems related to unsafe handling of pesticide distribution and use, such as use of unsafe storage facilities, improper training on safe use of pesticides, and inadequate infrastructure to regulate safe use of pesticides (Mengistie et al., 2017). A survey done by Nigatu et al. (2016) on knowledge, attitude, and practices of farmers and farm workers in Ethiopia reported that 85% of farm workers (pesticide mixers/loaders, sprayers, and application supervisors) (n = 601) and 100% of female re-entry farm workers (harvesters, pesticide assessors, irrigation workers, irrigation supervisors, packing and sorting workers, transport/push car workers), (n = 275) did not receive pesticide-related training. In addition, 62% of farm workers did not shower after pesticide application, and none of the small-scale farm workers (n = 258) used personal protective equipment. A considerable increment in chemical pesticide usage intensity, illegitimate usage of DDT and Endosulfan on food crops, and direct import of pesticides without the formal Ethiopian registration process were also reported by Nigatu et al. (2016).

Therefore, there is a need to explore products that are safe and effective in controlling insects in smallholder farmers' traditional storages in Ethiopia. Two such products are filter cake and Triplex (Girma et al., 2008a,b; Tadesse and Subramanyam, 2018). Filter cake is a by-product of aluminum sulfate factory (Awash Melkassa Aluminium Sulphate & Sulfuric Acid Share Company, Melkassa Awash, Ethiopia (AMASSASC)). Triplex is a by-product of Mohammed International Development Research and Organization Companies (MIDROC) soap factory (Star Soap and Detergent Industries (SSDI Private Limited Company), Addis Ababa, Ethiopia). A study on elemental composition of both powders using energy-dispersive X-ray spectroscopy indicated that silicon and oxygen were dominant elements (Tadesse and Subramanyam, 2018). The same study showed 100% mortality when adults of *Sitophilus zeamais* Motschulsky were exposed to 7.5 g/m^2 of filter cake and 10 g/m^2 of both powders for 24 h on treated concrete arenas. Girma et al. (2008a) reported 92% mortality 3 d after *S. zeamais* were exposed to three genotypes of maize treated with 1, 2.5, and 5% (w/w) of filter cake. Similarly, a 0.25% (w/w) Triplex treated maize showed no significant mean percentage mortality of *S. zeamais* (93%) when compared with that of a synthetic insecticide, pirimiphos-methyl (100%), 7 months after treatment (Girma et al., 2008b). However, the toxicity of both products on multiple range of concentration and time was not known. Toxicity study is the investigation of either short or long-term toxic effects of a drug or chemical on animals (Saganuwan, 2017). Short-term toxic effect is determined using median lethal dose (LD_{50}), the dose required to kill half the members of a tested population after a specified test duration which was first introduced by Trevan in 1927 (Trevan, 1927) and revised many times (Saganuwan, 2017). It is frequently used as a general indicator of a substance's acute toxicity and lower LC_{50} is indicative of higher toxicity (Criswell and Campbell, 2013). Therefore, knowing the toxicity of filter cake and Triplex will help to use both products at the safer and effective level. This study was designed to determine the LC_{50}, lethal time (LT_{50}), effective concentration (EC_{50}) and effective time (ET_{50}) of filter cake and Triplex against *S. zeamais* economically, an important stored grain pest in Ethiopia (Abraham et al., 2008; Girma et al., 2008a,b; Tilahun and Hussen, 2014).

2. Materials and methods

2.1. Concrete-poured Petri dishes

Rockite®, a ready-to-mix concrete product (Hartline Products Co., Inc., Cleveland, Ohio, USA), was mixed with tap water in 2:1 ratio (grams to milliliter) to make a slurry. The slurry was poured into 9 cm diameter and 1.5 cm high plastic Petri dishes (Fisher Scientific, Denver, Colorado, USA). Slurry was poured to cover one half of the Petri dish's height. Slurry filled Petri dishes were allowed to dry on a laboratory bench for 24 h. Polytetrafluoroethylene (Insecta-a-Slip, Bio Quip Products, Inc., Rancho Dominguez, California, USA) was used to coat the inside walls of Petri dishes to prevent insects from crawling on sides of dishes.

2.2. Application of powders to concrete arenas and insect exposure

Brass frame, stainless steel wire cloth sieve with #80 mesh size (Seedburo Equipment Company, Des Plaines, Illinois, USA) were used to sift filter cake and Triplex powders. Concrete arenas of dishes were treated with the fines (particle size < 177 microns) at the following concentrations: 0 (untreated control), 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 g/m^2 of filter cake or 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 g/m^2 of Triplex. A laboratory strain of *S. zeamais* was reared at 28 °C and 65% r.h. on organic hard red winter wheat (Heartland Mills, Marienthal, Kansas, USA) of 12.5% moisture content (wet basis) at the department of Grain Science and Industry, Kansas State University. Adults of *S. zeamais* were separated from wheat using a 2.38 mm diameter round-holed aluminum sieve (Seedburo Equipment Company, Des Plaines, Illinois, USA). Ten unsexed newly emerged two weeks old adults were exposed to untreated concrete arenas (control) and arenas receiving each of the 9 concentrations of filter cake or Triplex for 12 and 24 h in an environmental chamber with 28 °C and 65% r.h. A separate set of experiment, 10 adults from the same culture were exposed to 3 g/m^2 of

filter cake or 9 g/m^2 of Triplex separately for 1,2,4,6, 8, 10,12, 14, 16, 18, 20, 22, 24 h to determine the lethal times of both powders. Each powder-concentration-time combination was replicated three times. After the intended exposure time period insects were transferred carefully with a camel's hair brush to 150 ml round plastic containers holding 30 g of cleaned, organic hard red winter wheat (Heartland Mills) of ~12.5% moisture content (wet basis). The plastic containers had perforated lids with wire-mesh screens to facilitate air diffusion. Containers were incubated on the same chamber at 28 °C and 65% r.h. After 14 d, wheat from each container was sifted using a 2.38 mm circular round-holed aluminum sieve to separate insects from wheat. Insects that did not respond when gently prodded by a camel's hair brush were considered dead. A third set of experiment with same concentrations and exposure times was set to determine adult progeny production 42 d, after exposure to filter cake or Triplex. Adult progeny produced was counted from each container and the 10 starting adults were subtracted.

2.5. Data analysis

The mean ± SE mortality of *S. zeamais* on untreated (control) (n = 3) arenas at all exposure times ranged from 0.0 to 0.33 ± 0.33 and 0.0 to 0.67 ± 0.33 for filter cake and Triplex, respectively. Therefore, mortality data of *S. zeamais* exposed to filter cake or Triplex were corrected for responses in the control treatment (Abbott, 1925). Probit analysis was used to generate probit regression estimates and lethal concentration (LC) or lethal time (LT) producing 50 and 99% mortality. Similarly, probit analysis was used to generate effective concentrations (EC) and effective times (ET) for the 50 and 99% reduction of progeny production. The LC_{50}, LT_{50}, EC_{50} and ET_{50} values of filter cake were compared to the corresponding values of Triplex using ratio tests (Robertson et al., 2007). All the data were analyzed using SAS software (SAS Institute, 2012). Differences between any two LC_{50} or LT_{50}, EC_{50} or ET_{50} values were considered to be significantly different ($P < 0.05$) if the 95% CL for the ratio did not include 1 (Robertson et al., 2007).

3. Results

3.1. Concentration and corrected mortality responses of S. zeamais

Mean ± SE percentage corrected mortality of *S. zeamais* adults exposed to 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 g/m^2 concentrations of filter cake at 12 h ranged from 41.58 ± 6.87 to 100 ± 0.00%. For 1, 2, 3, 4, 5, 6, 7, 8 and 9 g/m^2 concentrations of Triplex, the mortalities ranged from 20.96 ± 9.09 to 96.56 ± 3.44%. The corresponding mortalities of *S. zeamais* adults after exposure to 3 g/m^2 of filter cake or 9 g/m^2 of Triplex for 1,2,4,6, 8, 10,12, 14, 16, 18, 20, 22, 24 h ranged from 20.96 ± 6.87 to 100 ± 0.00% and 24.40 ± 3.44 to 96.56 ± 3.44%, respectively.

Table 1. Probit estimates and concentrations required for 50 and 99% mortality for *S. zeamais* of adults based on mortality assessment made 14 d after exposure to filter cake and Triplex for 12 h

Powder	*N*[a]	Mean ± SE		LC (95%CL) (g/m^2)		x^2(df)[b]
		Intercept	Slop	LC_{50}	LC_{99}	
Filter cake	180	0.63 ± 0.03	0.56 ± 0.07	0.61 (0.37 – 0.82)	7.54 (4.65 – 18.60)	11.01 (16)
Triplex	270	0.13 ± 0.05	0.84 ± 0.07	2.63 (2.12 – 3.11)	23.46 (15.88 – 44.05)	18.88 (25)

[a] N = total number of adults used to generate the probit regression estimates
[b] x^2- values for goodness-of-fit of model to data were not significant ($P > 0.05$)

Table 2. Probit estimates and times required for 50 and 99% mortality for *S. zeamais* of adults based on mortality assessment made 14 d after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex

Powder	*N*[a]	Mean ± SE		LC (95%CL) (g/m^2)		x^2(df)[b]
		Intercept	Slop	LC_{50}	LC_{99}	
Filter cake	240	-2.12 ± 0.32	2.72 ± 0.35	4.42 (3.47 – 5.26)	21.92 (16.74 – 33.56)	23.49 (22)
Triplex	390	-1.61 ± 0.24	2.00 ± 0.22	4.29 (3.14 – 5.34)	39.62 (29.87 – 60.30)	23.28 (37)

[a] *N* = total number of adults used to generate the probit regression estimates
[b] x^2- values for goodness-of-fit of model to data were not significant ($P > 0.05$)

The x^2 values for goodness-of-fit of the model to corrected mortality data were not significant ($P > 0.05$) (Table 1 and 2) indicating good fit of model to data. The lethal concentrations to kill 50% of *S. zeamais* adults exposed to filter cake and Triplex were 0.61 g/m^2 and 2.63 g/m^2, respectively. Triplex had significantly higher LC_{50} value than filter cake (ratio [95% CI] = 4.33 (2.85 – 6.57). The lethal times to kill 50% of adults exposed to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex were 4.42 and 4.29 h respectively. There was no significant differences between the LT_{50} value of Filter cake and Triplex (ratio [95% CI] = 0.97 (0.70 – 1.35).

Mean number of adult progeny produced ± SE by *S. zeamais* adults 42 days after exposure to 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 g/m^2 concentrations of filter cake at 12 h ranged from 0.00 ± 0.00 to 135.00 ± 27.78. The corresponding mean number of adult progenies produced ± SE were ranged from 1.00 ± 0.58 to 123.67 ± 34.76 for Triplex at 1, 2, 3, 4, 5, 6, 7, 8 and 9 g/m^2 concentrations. Similarly, Mean number of adult progeny produced ± SE by *S. zeamais* adults after exposure to 3 g/m^2 of filter cake or 9 g/m^2 of Triplex for 1,2,4,6, 8, 10,12, 14, 16, 18, 20, 22, 24 h ranged from 0.00 ± 0.00 to 111.33 ± 7.62 and 24.40 ± 3.44 to 96.56 ± 3.44%, respectively. The percentage reduction of adult progeny produced relative to the control treatment was used to fit the model.

Table 3. Probit estimates and concentrations required for 50 and 99% mortality for *S. zeamais* of adults based on mortality assessment made 7 d after exposure to filter cake and Triplex for 12 h

Powder	*N*[a]	Mean ± SE		LC (95%CL) (g/m^2)		x^2(df)
		Intercept	Slop	LC_{50}	LC_{99}	
Filter cake	180	1.41 ± 0.07	1.93 ± 0.28	0.18 (0.10 – 0.26)	3.00 (2.15 – 5.19)	6.31 (7)[b]
Triplex	270	-1.17 ± 0.28	2.76 ± 0.44	2.66 (1.98 – 3.30)	18.59 (11.91– 43.60)	470 (25)

[a] *N* = total number of adults used to generate the probit regression estimates
[b] x^2- values for goodness-of-fit of model to data were not significant ($P > 0.05$)

Table 4. Probit estimates and times required for 50 and 99% mortality for *S. zeamais* of adults based on mortality assessment made 7 d after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex

Powder	*N*[a]	Mean ± SE		LC (95%CL) (g/m^2)		x^2(df)[c]
		Intercept	Slop	LC_{50}	LC_{99}	
Filter cake	210	-0.82 ± 0.19	1.89 ± 0.25	1.74 (1.10 – 2.32)	17.49 (12.50 – 30.42)	132.51 (19)
Triplex	390	-1.08 ± 0.16	1.93 ± 0.16	2.34 (1.75 – 2.91)	22.31 (18.23 – 29.12)	200.52 (37)

[a] *N* = total number of adults used to generate the probit regression estimates
[c] x^2- values for goodness-of-fit of model to data were significant ($P < 0.05$)

The x^2 for goodness of fit of the model to percentage adult progeny reduction data collected 42 d after exposure to filter cake and Triplex were not significant (P > 0.05) (Table 3). The effective concentration to decrease 50% of adult progeny production for *S. zeamais* 42 d after exposure to filter cake and Triplex were 0.18 and 2.66 g/m^2, respectively. Triplex had significantly higher EC_{50} value than filter cake (ratio [95% CI] = 14.41 (8.83 – 23.51). The x^2 for goodness-of fit of the model to adult progeny reduction data collected 42 d after exposure to 3 g/m^2 of filter cake and 9 g/m^2 of Triplex were significant ($P < 0.05$) (Table 4), indicating poor fit of model to data. The corresponding effective times to reduce 50% of adult progeny production of *S. zeamais* after 42 d were 1.74 and 2.34 h for filter cake and Triplex, respectively. Triplex had significantly higher ET_{50} value than filter cake (ratio [95% CI] = 1.81 (1.12 – 2.92).

4. Discussion

Contact insecticides have been used to protect stored commodities from pests for a long period of time (Ebling, 1971; Headlee, 1924). Such products have large proportion of silica which has high capacity to absorb epicuticular lipid from insects' body (Ebling, 1971; Malia et al., 2016; Subramanyam and Roseli, 2000). Silicon and oxygen are the major components of filter cake and Triplex (Tadesse and Subramanyam, 2018) which makes them similar to other inert dusts.

Our data demonstrated contact toxicity of filter cake and Triplex through concentration and exposure time responses of *S. zeamais* adults. The mortality of *S. zeamais* increased with increasing concentration of filter cake and Triplex. Accumulation of powder over insect's body is directly proportional to concentration of the powder (Le Patourel et al., 1989), and insect behavior such as mobility. These factors cause adverse effects to exposed insects (Malia et al., 2016). A positive correlation between oil absorption and silicon dioxide concentration was reported by Filipović et al. (2010) after testing oil absorption capacity of silica powders prepared from sodium silicate solution with different concentration of silicon dioxide. This is supported in our study where adult mortality tended to increase with increasing concentration of filter cake and Triplex. The same phenomenon was reported by Tesfaye and Subramanyam (2018) after unsifted filter cake and Triplex were tested at 2.5, 3.75, 5, 7, 7.5 and 10 g/m^2 concentrations of filter cake or Triplex against *S. zeamais*.

The x^2 values for goodness-of fit of the model to our data to determine LT_{50} and ET_{50} was significant. These heterogeneous responses could be related to sex as unsexed adults were used in the experiment. Heterogeneous responses of insects were reported by several researchers when exposed to temperature (Mahroof et al., 2003), Spinosad and Chlorpyrifos-Methyl Plus Deltamethrin (Seghal et al., 2013), spinosad (Subramanyam et al., 2014) and chlorine-dioxide (Xinyi et al., 2017). The LC_{50}, EC_{50} and ET_{50} values of filter cake were three time less than that of Triplex. This was confirmed by the ratio test which showed significantly higher toxicity of filter cake to *s. zeamais* than Triplex. A 7.54 g/m^2 concentration of filter cake was effective to kill 99% of s. zeamais adults within 12 hours and 21.92 hours were required to get the same mortality when 3 g/m^2 concentration of filter cake was used. A similar concentration (7.5 g/m^2) of filter cake was reported by Tadesse and Subramanyam (2018) to kill 100% of *s. zeamais* adults exposed for 24 h. However, more than three times the concentration of filter cake was required to kill *s. zeamais* adults after exposure to Triplex for 12 h. This could be related to the carbon content (atomic percent = 39.43 ± 12.63) of filter cake in the form of calcium carbonate (Tadesse and Subramanyam, 2018). Calcium carbonate at 1 and 2% (w/w) applied to maize caused 70.2 and 84.2% mortality of *s. zeamais* adults, respectively, 15 d after treatment (Silva et al., 2004). Filter cake was also effective in suppression of progeny production with more than 3 times less concentration than Triplex.

In conclusion, 7.54 g/m^2 of filter cake was effective in killing adults of *s. zeamais*, an economically important species. It also completely suppressed adult progeny production, indicating adults were killed before they lay eggs into the kernel. Three-fold higher concentration was required to get similar result on Triplex treatments. This indicated that filter cake was highly efficacious to *s. zeamais* than Triplex. This work was done under laboratory conditions. Therefore, it needs to be confirmed under practical field conditions such as traditional storage structures of smallholder farmers.

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Efficacy of 10 dusts on life cycle of *Tribolium castaneum*

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Silica dusts have a long history of use in agriculture for insect control. But the product has several problems that limit its overall use and effectiveness. The present study is investigating a form of silica that has not been used in agriculture to date. The study has been comparing both hydrophilic and hydrophobic forms of the silica against traditional silica products such as Dryacide. The study

has focused on the key grain pest the red flour beetle (*Tribolium castaneum*) and explored the ability of the new form of silica to control the various beetle life stages. The results show that the efficacy of the new silica against larvae was nearly two-to threefold higher than that of adults. The results also show a clear difference in performance between hydrophilic and hydrophobic forms of the silica with hydrophobic forms outperforming the hydrophilic forms for the control of the red flour beetle.

Susceptibility of Stored Grain Insects to the Insect Growth Regulator Methoprene

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Abstract

The insect growth regulator (IGR) methoprene is labeled in the United States (US) for direct application to stored grain commodities, and as a residual surface treatment to empty grain bins and flooring surfaces inside indoor structures. Methoprene is also labeled in the US as an aerosol for use in indoor areas. One of the challenges in research with methoprene and stored product insects is through design of experiments that mimic how methoprene would be used in practical applications. Recent research with methoprene will be used to describe experimental designs to examine efficacy of methoprene when used as a grain protectant.

Keywords: stored products, IGRs, management, efficacy

1. Introduction

Insect pests can cause economic damage to stored grain commodities, especially in warm temperature areas and in the tropics. One of the components of integrated pest management (IPM) for stored grains is the use of protectant insecticides, which are applied as raw commodities are loaded into grain bins or elevator silos. Historically organophosphate insecticides were the primary protectants used by grain managers, but due to concerns with insecticide resistance, along with new regulations, some products in developed countries have been removed from the market (Daglish, 2008; Arthur, 2012). Today there is more emphasis today on using reduced-risk insecticides, including but not limited to pyrethrins, pyrethroids, insect growth regulators (IGRs) (Arthur, 2012), and biological insecticides such as spinosad (Hertlein et al, 2011, Nayak and Daglish, 2017).

Other components of IPM programs for stored grains include the use of aeration, which involves using low airflow rates to cool and modify a grain bin, thus limiting the growth of insect pests Arthur and Casada, 2016). It is not to be confused with grain drying, which utilizes much higher airflow rates to remove excess moisture from grains before they are stored (Navarro et al., 2012). Pre-binning cleaning and sanitation is also important, as the environment in and around grain storage sites can contain residual pockets of grain residues and spillages that will support insect pest development (see references in Arthur, 2018). Finally, the primary component for controlling stored grain insects is the fumigant phosphine. Concerns regarding the development of phosphine resistance (Lorini et al., 2007, Nayak et al., 2015, 2017, Afful et al., 2018) could lead to more extensive use of grain protectants. Thus, when designing experiments to evaluate efficacy of grain protectants, including reduced-risk adulticides and IGRs, there are multiple factors that must be considered

One IGR that is being used in the US as a grain protectant is methoprene, which is also labeled as a residual surface treatment and as an aerosol inside structures to control stored product insects. As an IGR, it does not kill adults, and it has limited efficacy against *Sitophilus species* (Lui et al, 2016). Methoprene can be used alone, or combined with other strategies, including the use of aeration (Arthur, 2016; Lui et al., 2016) and with the pyrethroid deltamethrin (Kavallieratos et al., 2015). Multiple factors should be considered when planning experiments utilizing methoprene as a grain protectant. This paper presents a review and discussion of some of those factors as they relate to

the use of methoprene alone and in combination with other strategies, and will not necessarily follow the standard format of a research paper, though data will be presented to illustrate specific concepts and ideas.

2. Three Simulated field trials

Here at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), we have grain bins of different storage capacities that can be used for studies. These bins are equipped with aeration systems that operate when temperatures fall below set thresholds, using a controller that operates as a thermostat when outside ambient temperatures fall below specified levels (Arthur and Casada, 2005, 2010, 2016). We have also conducted studies with university cooperators that also have small-scale storage bins equipped with similar aeration systems (Lui et al., 2016).

3. Field Trial One: Pitfalls with inconsistent natural populations

One of the problems with field trials involving methoprene is the difficulty assessing treatment effects by examining natural insect populations in treatment and control bins. The first example is a test in which wheat was treated with a target application rate of 2.5 ppm as it was loaded into each of two 110-metric ton (MT) capacity metal grain bins at the CGAHR, using a commercial application system. Two untreated bins served as controls. All bins had aeration systems that were set to operate at an approximate rate 0.206 m^3/min/MT when temperatures fell below 23.9, 15.6, or 7.2 °C (Arthur and Casada, 2005; 2010). After the bin was cooled to the desired temperature the aeration fans were turned off until the next cycle.

The treated and untreated wheat was loaded into the bins the first week of August. After the bins were filled, HOBO temperature cables (Onset Computers, Pocasset, MA, USA) were put into the bins at the North, South, and Center positions, at depths of 0.3, 0.9, and 1.8 m. In mid-August and at the end of the month, each bin was artificially infested with 500 adults each of *Rhyzopertha dominica* (F.), *Tribolium castaneum* (Herbst), and *Cryptolestes ferrigineus* (Stephens) to supplement natural populations. In late August insect pest populations in the bins were assessed by placing five plastic pitfall traps, at each cardinal position and in the center, left for one week, and then removed from the bin. Samples for bioassays, using only *R. dominica*, were also taken as well. Thereafter bins were sampled monthly during autumn except in December, and in January, February, March, and April of the following year (eight total). Data for live adult insects collected in pitfall traps were totaled for all five traps in the two untreated bins. This was also done for all traps in the treated bins as well. Data were compared by a t-test (Statistical Analysis System version 9.2, Cary, NC, USA). Temperatures in the bins were plotted using Sigma-Plot (Systat Software, Version 11, San Jose, CA, USA).

The only species collected in the pitfall traps were *C. ferrugineous*, *Ahasverus advena* (Waltl), *Typhea stercoria* (L.), and *T. castaneum* (Fig. 1). Insects were collected in early autumn, but populations in treated and untreated bins declined during the Winter and except for *C. ferrigineous* did not increase in the Spring. However, there were several sample points were populations were greater ($P < 0.05$) in untreated wheat versus the treated wheat. Temperatures for most of the storage period were below 15 °C, the lower developmental limit for most stored product beetles (Howe, 1965; Fields, 1991) (Fig 2.).

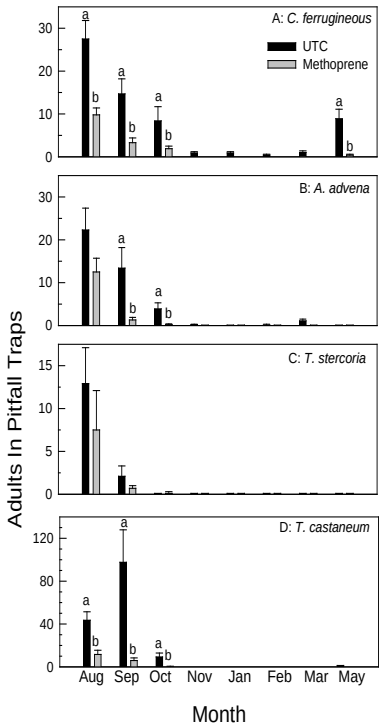

Fig. 1. Average number of adults of four insect species (A-D) collected from probe traps placed in bins with aeration alone (dark bar) versus bins with aeration + methoprene (grey barr), means denoted with different letters indicate significant differences ($P < 0.05$, Proc *t*-test, SAS). The sample in May was taken after aeration monitoring ceased.

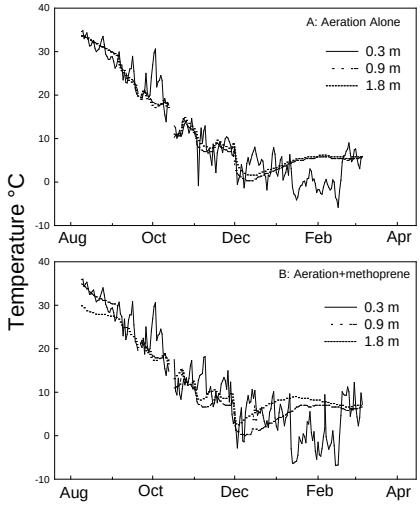

Fig. 2. Temperature at depths of 0.3, 0.9. and 1.8 m in wheat mass in 110 MT bins in wheat with aeration alone (A) and in wheat with aeration + 2.5 ppm methoprene (B).

The bioassay data gave a clearer picture of methoprene efficacy. These bioassays were conducted by placing 20 mixed-sex 1 to 2-week old adults on ca. 30 grams of wheat in a 37-ml capacity vial for two weeks, then removing the adults and holding the wheat in an incubator at about 27°C and 60% r.h. for about 6 weeks. At each sample point five 250-g samples were taken from each bin, and then subdivided into 30-gram lots for each of 5 vials. The remainder of the wheat was discarded. The wheat in the vials was warmed in the laboratory for several days before the parental adults were introduced. Thus, there were a total of 10 treated and 10 untreated samples. Average progeny production over all sample months was 99.9 ± 23.5, while progeny production in the methoprene bioassays averaged less than 0.1. This test indicated that strict reliance on natural populations of stored product insects may not be an appropriate method for assessing residual efficacy of grain protectants.

4. Field Trial Two: Supplemental introductions and sampling with traps and trier samples combined with bioassays

This test was conducted in sixteen 13.6 MT capacity metal bins, equipped with aeration systems, at the SPREC Research Center (Oklahoma State University, Stillwater, OK, USA). The test has been reported previously (Lui et al., 2016), so only the essential details will be summarized here. There were four treatments with four replications each: wheat with aeration alone at about 0.206 m^3/min/MT with no methoprene treatment, 1.0 ppm methoprene applied to the entire grain mass

with no aeration, aeration + methoprene applied to the entire grain mass, and aeration + 1.0 ppm methoprene applied only to the top 50-cm of the grain mass. For this test, grain temperatures were monitored and recorded hourly using an aeration control system from OPI Systems Inc. (Calgary, Alberta, CA). Fans were set to operate when outside air temperatures were 5°C lower than the grain temperature at 30 cm below the grain surface. This is different from the test above, where discreet aeration cycles were used.

Wheat was loaded into the bins in July. At 7, 14, 21, and 28 days after bins were filled, 100 mixed sex adults of each of the species described above were introduced into the bins. The bins were sampled at every two months after bins were filled, by placing one probe trap in each bin for seven days, then removing the trap and counting and separating adults by species. Grain samples of about 1 to 2 kg were also taken at the same time from the bins using a grain trier. Samples were also taken at 0.25, 4, and 10 months post-treatment for bioassays of *T. castaneum*, *R. dominica*, and *Plodia interpunctella* (Hübner). For the bioassays of beetles, 50 adults of each species were placed into 240-ml jars with about 100 g of wheat, then held in an incubator at 28°C and 60% r.h. *For P. interpunctella*, 20 eggs were placed on a mixture of ground and whole wheat (20 g each). The test ended after 10 months.

Total numbers of *T. castaneum*, *R. dominica*, and *C. ferrugineous* collected in the probe traps during the entire storage period are listed in Table 1 (complete data for bi-monthly bioassays given in Lui et al., 2016). Data were compared using Chi-Square analysis (SAS Institute). It is apparent that the most optimal treatment was the combination of the entire wheat mass treated with methoprene combined with aeration. Also, far more adults of the three species were collected in probe traps versus trier samples (Table 2). The probe traps provided a measure of relative abundance, and gave an indication of relative species susceptibility: *R. dominica* < *C. ferrugineous* < *T. castaneum*. However, it should be emphasized that the four separate introductions of the beetles helped to establish the populations, and strict reliance on natural populations may not have yielded successful results. Bioassay data for beetles (Table 3) and *P. interpunctella* (Table 4) also showed the residual efficacy of methoprene.

Table 1. Total numbers of three species collected from pitfall traps in wheat held under four treatment regimens from July to May in Stillwater, OK, USA (n=6). Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, $P< 0.05$)

Treatment	*T. castaneum*	*R. dominica*	*C. ferrugineous*
Aeration only	3138a	18ab	173a
Methoprene top+aeration	1868b	24a	89b
Methoprene total-no aeration	1143c	10b	80b
Methoprene total+aeration	564d	13ab	79b

Table 2. Total numbers of three species collected from trier samples in wheat held under four treatment regimens from July to May in Stillwater, OK, USA (n=6). Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, $P< 0.05$).

Treatment	*T. castaneum*	*R. dominica*	*C. ferrugineous*
Aeration only	36a	19a	96a
Methoprene top+aeration	9b	10ab	9b
Methoprene total-no aeration	4b	4b	2b
Methoprene total+aeration	4b	5b	4b

Table 3. Total numbers of progeny produced in bioassay samples from wheat held under four treatment regimens from July to May in Stillwater, OK, USA (n=6). Sum totals within columns followed by different lower-case letters are significantly different (Chi Square, $P< 0.05$).

Treatment	*T. castaneum*	*R. dominica*
Aeration only	659a	1694a
Methoprene top+aeration	0b	54b
Methoprene total-no aeration	0b	1c

Methoprene total+aeration	0b	1c

Table 4. Average percentage (mean ±SE) of adult *P. interpuctella* from eggs placed on wheat from four treatment regimens from wheat in Stillwater, OK, USA (n=3). Sum totals within columns followed by different lower-case letters are significantly different (*t*-test, $P< 0.05$).

Treatment	
Aeration only	98.7± 0.03a
Methoprene top+aeration	11.3± 5.48b
Methoprene total-no aeration	0
Methoprene total+aeration	0

5. Field Trial Three: Assessing residual efficacy assessed only with bioassays

Given the difficulty of determining methoprene efficacy based on actual infestation, another option for collecting data, and for determining species susceptibility, is to rely on bioassay data. This approach was utilized in Arthur (2016), a two-year study in which wheat, corn, rough rice, and brown rice was treated with 1.25 and 2.5 ppm methoprene, and stored for two years under natural conditions on the floor of a grain bin. The commodities were bioassayed every two months, using different insect species depending on the commodity. Data for rough rice and brown rice highlight the differences between grain commodities, in terms of assessing insecticidal efficacy. Data also show differences in susceptibility between two internal feeders, *R. dominica* and *Sitotroga cereaella* (Oliver). Adult females of both species lay eggs on the interior of the grain kernel, and the neonate larva bores inside the kernel, and completes development. Adult *R. dominica* chew their way out of the kernel, while adult *S. cereallella* push their way out through the feeding holes created by the larva. While *R. dominica* causes physical damage to the kernel, and *S. cerealella* does very little feeding, high populations of *S. cereallella* may still present a contamination issue.

The rough rice and brown rice was treated in August by treating four individual replicate lots of 11 kg with either 1.25 or 2.5 ppm methoprene, then storing the replicate lots in buckets held on the floor of an empty grain bin at the CGAHR. Every two months two aliquot samples of about 80 g each were taken from an individual replicate bucket, placed in a 120-ml capacity plastic vial and 10 parental adults of either *R. dominica* or *S. cerealla* was exposed on the aliquots from each treatment and on companion sets of untreated controls. The individual vials were held for 3 months at 27°C and 60% r.h. to determine progeny production.

There was very little development of *R. dominica* even on untreated rough rice, presumably because the husk offers protection from larval penetration. There was some development of *S. cerealla* on rough rice treated with the two methoprene rates, but far less compared to untreated controls. On brown rice, there was extensive progeny production of *R. dominica* in untreated controls, but none in treatments. As with rough rice, there was some progeny production in the treatments, but far less than the controls. The bioassay data were then used to compare percentage weight loss of samples, of *R. dominica* and *S. cerealella* feeding damage on brown rice. Progeny production was correlated with feeding damage for *R. dominica* but not for *S. cerealella*.

6. Conclusions

Data for these studies show there are multiple methods for assessing residual efficacy of methoprene, and other contact insecticides, when used as grain protectants. IPM in stored grains could be considered more as a multi-component management strategy, and grain protectants, especially reduced-risk products, could play a more important role in the future. However, when conducting experiments, many factors should be considered. In actual or simulated field tests, bioassays must be paired with grain sampling through probe traps and pheromone traps, as natural infestations may be inconsistent. Even seeding experimental bins with insects may not yield reliable results, as the introductions may not be successful, or the populations could decline and die out during winter months, especially in temperate climates. Bioassays offer the best option for evaluation of residual efficacy of methoprene. Relating progeny production to physical grain

damage, such as weight loss, frass production, and presence of insect-damaged kernels, should also be done as well. **8.**

7. Acknowledgements

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The US Department of Agriculture is an equal opportunity provider and employer**.**

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Comparative efficacy of spinetoram, chlorfenapyr, cypermethrin, beta-cyfluthrin against *Tribolium castaneum* (Herbst) and *Trogoderma granarium* (Everts)

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Post-harvesting losses is a critical component for filling the demands of ever increasing population. Because a large number of food security issues can be facing in the future. Current study was planned to probe the comparative insecticidal, growth inhibitory and feeding detterent activities of spinetoram, chlorfenapyr, cypermethrin and beta-cyfluthrin against *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst) under laboratory conditions. The insecticides were used at three different concentrations i.e., 5, 7 and 9ppm. Results revealed that maximum adult mean percent mortality of *T. castaneum* was recorded at highest concentration (9ppm) was 78.08% followed by 69.41% at 7ppm and 61.41% at 5ppm. In case of *T. granarium* at highest concentration (9ppm) the mortality was 72.58% followed by 64.08% at 7ppm and 55.33% at 5ppm. Results regarding growth inhibition showed that cypermethrin and chlorfenapyr gave highest values 28.77 and 23.78% for larval emergence inhibition. While beta-cyfluthrin gave lowest larval emergence inhibition against the *T. castaneum*, beta cyfluthrin gave 53.02% pupae inhibition. 50.26%, 48.66% and 46.48% pupae inhibition values were given by spinetoram, chlorfenapyr and cypermethrin, respectively. Adult emergence inhibition was highest 40.17% in case of cypermethrin followed by chlorfenapyr (30.60%). Similarly, the efficacy of all tested insecticides in term of feeding deterrence for both insects was cypermethrin > chlorfenapyr > spinetoram > beta-cyfluthrin.

Toxicity of four Cuban botanical derivatives against two stored-products coleopteran pests

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Abstract

Plants are a source of substances for protection of stored products. The Cuban flora has not yet been fully studied as a source of pesticides for postharvest protection, partly due to its great diversity. The toxicity of four Cuban plant derivatives against *Lasioderma serricorne* (F.) and *Sitophilus zeamais* Motschulsky was investigated. The anti-insect activity of the powders and the essential oil from plants belonging to Asteraceae, Fabaceae and Piperaceae was tested. Mortality and emergence of adult insects and the repellent effect of products were evaluated. Two products derived from *Piper aduncum* subsp. *ossanum*, caused high mortality (81,6 and 100%), reduced emergence (27,9 and 0,4%) and exhibited strong repellent activity on *L. serricorne*. Against *S. zeamais*, treatments with the highest mortality values were stems of *Lonchocarpus punctatus* (72,4%), seeds and stems of *Canavalia ensiformis* (64,9 and 69,9%), and leaves of *Tithonia diversifolia* (67,2%). The progeny production of *S. zeamais* was inhibited by powders of *L. punctatus* stems (31,8%), *C. ensiformis* seeds (40,5%), leaves (43,7%) and stems (30,6%), and *T. diversifolia* leaves (38,7%). The stems of *C. ensiformis*, leaves of *T. diversifolia* and *L. punctatus* had the highest repellent effect. These products have potential for small-scale treatments of grains for protection against both insects, and *P. aduncum* subsp. *ossanum*-based products to control *L. serricorne* infestation in tobacco. Identification of local candidates to develop effective and safe pesticides offers new alternatives to the Cuban agriculture in the control of storage pests.

Keywords: *Lasioderma serricorne*, *Sitophilus zeamais*, Fabaceae, Asteraceae, Piperaceae.

Introduction

Stored products of agricultural and animal origin are attacked by many species of insect pests causing quantitative and qualitative losses and insect contamination in food commodities is an important quality control problem of concern for food industries (Rajendran and Sriranjini, 2008). Storage insects cause significant losses for grain and legume producers, due to the reduction of the quantity and quality of food for domestic consumption and the value of the grain for sale in the

market (Jones et al., 2018). Concerns over the harmful effects of synthetic insecticides have stimulated interest in alternative pest management tactics including the use of botanical insecticides that provide novel modes of action against pests that have developed resistance against synthetic insecticides (Amoabeng et al., 2018).

Plants are a source of substances for protection of stored products (Ogendo et al., 2012). Plant parts and botanical derived products have gained importance because flora biodiversity has provided an excellent source of biologically active constituents or allelochemicals for use in traditional crop protection. In the tropics, plant biodiversity has provided an excellent source of allelochemicals for crop protection in traditional agriculture for centuries and crude local products were applied to crops and stored food grains to protect them from an array of pest species (Gahukar, 2014).

Cuba is considered as one of the most biodiverse countries in the world in terms of sheer numbers of species and has the richest plant biodiversity of all the islands in America, with an estimated 6,600 plant species of which 50% are endemic (González-Torres et al., 2013). The Cuban flora has not yet been fully studied as a source of pesticides for postharvest protection, partly due to its great diversity. To date, only a small fraction of the plant species has undergone systematic phytochemical or biochemical research, leaving valuable sources for commercial products undiscovered (Pino et al., 2013). The toxicity of four Cuban plant derivatives, *Tithonia diversifolia* (Hemsl.), *Lonchocarpus punctatus* Kunth, *Canavalia ensiformis* L. and *Piper aduncum* subsp. *ossanum* (C.DC.) Saralegui, against *Lasioderma serricorne* (F.) and *Sitophilus zeamais* Motschulsky was investigated.

Materials and Methods

Plant derived products (powders and essential oil)

Raw plant materials were collected from four botanical species belonging to Asteraceae, Fabaceae and Piperaceae in Mayabeque, Cuba (Table 1).

Tab. 1 Plant Material.

Plant Family	Plant species		Part of the plant
	Scientific name	Common name	
Asteraceae	*Tithonia diversifolia* (Hemsl.)	tree marigold, Mexican tournesol, Mexican sunflower, Japanese sunflower or Nitobe chrysanthemum	Leaf, stem, root
Fabaceae	*Lonchocarpus punctatus* Kunth	dotted lancepod	Leaf, stem, fruit
Fabaceae	*Canavalia ensiformis* L.	jack-bean	Leaf, stem, seed
Piperaceae	*Piper aduncum* subsp. *ossanum* (C.DC.) Saralegui	spiked pepper, canilla de muerto, guayuyo or platanillo de Cuba	Leaf

Asteraceae and Fabaceae plant materials were dried in an oven at 45 °C, then grounded into powder (< 1 mm) and sieved (250 µm). They were kept at room temperature in nylon bags and were identified according to the plant structures until use for bioassays. The essential oil of *P. aduncum* subsp. *ossanum* was extracted by hydrodistillation using a Clevenger type apparatus. After extraction, the essential oil was dried over anhydrous sodium sulphate and stored in a refrigerator at 4 °C. Two powder formulations, PAO-1 and PAO-2, were obtained from the fresh leaf powder and the essential oil of *P. aduncum* subsp. *ossanum*, respectively. The anti-insect effect of products from *P. aduncum* subsp. *ossanum* (PAO-1, PAO-2) and zeolite, as an inert material, was tested against *L. serricorne* on chickpea. The biological activity of the nine powders from the different parts of *L. punctatus*, *C. ensiformis* and *T. diversifolia* (Table 1) was evaluated against *S. zeamais* on maize. Mortality and emergence of adult insects and the repellent effect of products were evaluated.

Insects

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) was reared on maize (*Zea mays* cv. Manitou) in controlled conditions (25 ± 2 ºC, 70 ± 5 % of relative humidity and 12:12 light/dark photoperiod) in the Institute of Animal Science, Mayabeque, Cuba. *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) was maintained on chickpea (*Cicer arietinum* L.), at 25,7 ± 1,95°C and 76,4 ± 9,18% r.h. under a 16:8 light/dark photoperiod, at the Entomology Laboratory, Agricultural Pests Group, National Centre of Animal and Plant Health, Mayabeque, Cuba. Experiments were conducted using emerged adults (age < 10 days old) from laboratory colonies.

Effect on adult mortality and F1 adult *emergence*

The effect of the derived plant products on adult mortality and F1 adult emergence was evaluated by grain treatment test (Silva et al., 2003). The grains of chickpea or maize (100 g) were mixed thoroughly with the products (plant powder (1g), PAO-1 (1g), PAO-2 (2g) or zeolite (2g)) in 1L glass jars. Twenty couples of adult beetles (*L. serricorne* or *S. zeamais*) were introduced into each glass jars containing the different treatments. Untreated grains in glass jars, where twenty insects were also placed, acted as the controls.

Adult mortality was evaluated 15 days after jar infestation for both insects. They were considered dead when no movement was observed after carefully touching them with a dissection needle. The live and dead *L. serricorne* adults were also counted after 3, 6, 9, and 12 days.
The number of emerged F1 adults (F1 progeny) were counted 58 days and 55 days after infestation with *L. serricorne* and *S. zeamais,* respectively. The percentage of insect emergence was calculated..

Repellent effect of plant products to the two betle species

The effect of the tested materials applied to the grains on the behaviour of the insects was established by using an arena formed by five circular plastic boxes (8,5 cm of diameter, 1.5 cm of height). The central box was connected with the rest using plastic tubes disposed diagonally. The boxes with treated grains (the same concentration used in previos bioassay) and the control (untreated grain), were distributed in two symmetrically opposed boxes. In the central container, 50 adults of *L serricorne* or S. zeamais were released. After 24 hours, the number of insects was counted in each box and the index of repellence (IR) was calculated (Mazzonetto and Vendramim, 2003):
IR = 2G/(G + P)
Where,
G =percentage of insects in the treatment
P = percentage of insects in the control
The effect of the product was clasified: neutral if IR=1, attractive if IR>1 and repellent if IR<1.

Experimental design and statistical analysis

For both bioassays, all treatments were arranged in a completely randomized design in the laboratory and kept at the same conditions asthe colonies. Each treatment was replicated four (adult mortality and emergence bioassay) or three (repellency bioassay) times.

The mortality counts were corrected with Abbott's formula (Abbott, 1925). All data were subjected to analysis of variance (ANOVA) and the means of treatments were compared by Duncan's Multiple Range Test at p < 0,05. The Statistical Analysis System (SAS Version 9.2) was used.

Results

The two products derived from *P. aduncum* subsp. *ossanum* caused high mortality and reduced F1 emergence of *L. serricorne* (Table 2). The highest values of mortality and reduction of adult emergence were observed with PAO-2; although, in the variable mortality, no significant differences were found with the other treatments.

Tab. 2 Effect of products from *Piper aduncum* subsp. *ossanum* on mortality and F1 emergence of the *Lasioderma serricorne* adults.

Treatment	Mortality (%)*	No. of emerged insects (Mean ± EE)*	Emergence (%)
PAO-1	81,58[a]	87,25 ± 37,7[b]	27,88
PAO-2	100[a]	1,25 ± 0,75[a]	0,40
Zeolite	94,74[a]	50 ± 21,46[b]	15,97
Control		313 ± 25,89[c]	100

*15 days, four replicates of 20 insects in each replication, means in the same column with letters in common do not differ significantly (Duncan's Multiple Range Test, p > 0.05)

Contact toxicity assayed by coating chickpea grains showed that both products caused mortality of *L. serricorne* and it was time of exposure dependent (Figure 1). PAO-2 had the fastest action, causing the highest number of deaths (43.33%) after three days. Treatment with PAO-1 induced death to increase from the ninth day (46%). Mortality due to the zeolite increased from the sixth day, with values higher than those obtained with the other treatments after nine days.

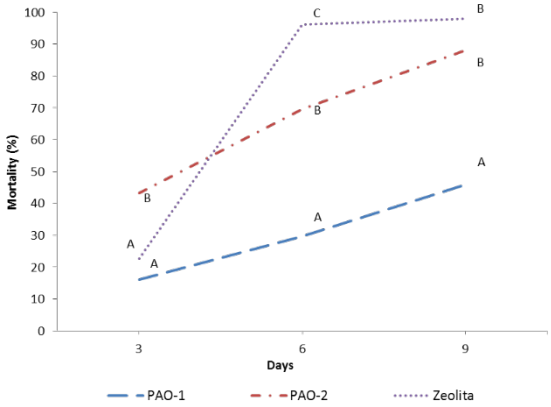

Fig. 1 Mortality of *Lasioderma serricorne* produced by products of *Piper aduncum* subsp. *ossanum* and zeolite after different periods of treatment.

All the products tested on *L. serricorne* showed a repellent effect, with IR values less than one (Table. 3). The best result was obtained applying PAO-1 with a value of 0.27.

Out of the nine treatments tested against *S. zeamais*, six surpassed 40% of mortality and five presented values below 50% of emergence from F1 in comparison to the control (Table 4).

Tab. 3 Effect of products from *Piper aduncum* subsp. *ossanum* on behavior of the *Lasioderma serricorne* adults.

Treatments	Index of repellence (IR)	Classification
PAO-1	0,27	Repellent
PAO-2	0,80	Repellent
Zeolite	0,68	Repellent

The treatments with the highest mortality values were stems of *Lonchocarpus punctatus*, seeds and stems of *Canavalia ensiformis*, and leaves of *Tithonia diversifolia*. Regarding the percent of F1 reduction, the best treatments were powders of: *L. punctatus* stems, *C. ensiformis* seeds, leaves and stems, and *T. diversifolia* leaves.

Tab. 4 Effect of powders from *Tithonia diversifolia, Lonchocarpus punctatus* and *Canavalia ensiformis* on mortality, F1 emergence and behavior of the *Sitophilus zeamais* adults.

Treatment	Mortality (%)*	Emergence (%)	Index of repellence (IR)**

T. diversifolia (leaf)	67,20[a]	38,7[fe]	0,47
T. diversifolia (stem)	45,87[b]	50,8[de]	0,72
T. diversifolia (root)	22,10[cd]	78,6[b]	1,12
L. punctatus (leaf)	25,16[cd]	60,12[cde]	0,48
L. punctatus (stem)	72,36[a]	31,79[f]	0,71
L. punctatus (fruit)	19,49[ed]	82,12[b]	0,82
C. ensiformis (leaf)	46,81[b]	43,75[fde]	0,84
C. ensiformis (stem)	69,93[a]	30,56[f]	0,67
C. ensiformis (seed)	64,93[a]	40,48[fde]	0,93

15 days, four replicates of 20 insects in each replication, means in the same column with letters incommon do not differ significantly (Duncan's Multiple Range Test, $p > 0.05$)
[**]Classification neutral if IR=1, attractive if IR>1 and repellent if IR<1

The index of repellence attained values below the unit for all of tested materials against *S. zeamais*, except for roots from *T. diversifolia* (Table 4). The leaves of *T. diversifolia* and *L. punctatus* and the stems of *C. ensiformis* had the highest repellent effect.

Discussion

Botanical derivatives, with biological activity against insects, include compounds with behavioral actions – those causing repellence, feeding deterrence or oviposition deterrence – and those with physiological actions – those causing acute toxicity, developmental disruption or growth inhibition. It is not uncommon for a plant secondary compound to have both behavioral and physiological effects in one insect species, or different effects in different species (Isman, 2014).

The products of *P. aduncum* subsp *ossanum* demonstrated the anti-insect properties of the plants of the Piperaceae family. According to Silva et al., (2003), 40% mortality must be achieved to consider a treatment promising. The activity of the *P. aduncum* essential oil was studied against *Tenebrio molitor* L and *Callosobruchus maculatus* (F.), it showed insecticidal activity against both insects (Fazolin et al., 2007, Pereira et al., 2008). The *P. aduncum* subsp. *ossanum* is an endemic subspecie, whose production of secondary metabolites is different from that of the plants of this species growing in other geographical regions, even in the same country (Pino et al., 2011). To the best of our knowledge, the toxicity of its products against *L. serricorne* has not been previously reported.

From a chemical point of view, the *P. aduncum* subsp. *ossanum* essential oil (active ingredient of PAO-2) is composed mainly of sesquiterpenes, monoterpenes and oxygenated compounds; the main components of this oil were camphene, camphor, piperitone and viridiflorol (Pino et al., 2011). These compounds and some essential oils containing them were toxic to stored-product insect pests; for instance, *Sitophilus granarius* L and *Rhyzopertha dominica* F. showed very high susceptibility to camphor (Pérez et al., 2010). This monoterpenoid may be one of the components responsible for the observed biological activity of PAO-2 on *L. serricorne*.

Against *S. zeamais*, treatments that caused at least 40% mortality of adults or at least 50% reduction of the F1 emergence in relation to the untreated control were considered promissory (Silva et al., 2003).

Our results confirmed the findings of other workers that reported the Asteraceae family as a promising source of metabolites, with insecticidal activity against *S. zeamais*. Grainge and Ahmed (1988) reported the contact toxicity of the compounds present in leaves of *T. diversifolia* against *S. zeamais*. Mortality by the leaves was higher than the rest of the structures (stems and roots). These results were in agreement with those obtained in other studies, which reported substances with insecticidal activity in leaves of this plant against *Dysdercus cingulatus* (Fab.), *Plutella xylostella* L., *Spodoptera exempta* (Walker) and *Tribolium castaneum* Herbst (Grainge and Ahmed 1988). The highest value of insect mortality, reached with the powders of the leaves, could be due to the higher concentration of α-amino groups, which was made evident by the more intense color observed for this plant structure sample in the phytochemical screening (González et al., 2010). Other groups of compounds responsible for this effect could be phenols, tannins and triterpenes (González et al.,

2010). The *T. diversifolia* appears among the plants from which crude preparations are recommended for insect control in different tropical regions (Isman, 2014).

No references of the effect of *L. punctatus* on *S. zeamais* were found according to the reviewed literature. The insecticide effect observed with the powders from the stems of *L. punctatus*, could be associated to the action of compounds or mixture of compounds belonging to the groups found in the phytochemical screening of this material, such as the α-amino groups, phenols, tannins, triterpenes/steroids, and alkaloids (González et al., 2011). The insecticidal effect of the raw meal of seeds of *C. ensiformis* against *Sitophilus oryzae* L. was reported by Zamora (2005), but no information was found regarding the effect of the powder of the seeds and the other studied parts of this plant against *S. zeamais*.

The Fabaceae family, represented in this research by *L. punctatus* and *C ensiformis,* comprises a wide group of species with insecticide activity. Among them, the most popular belong to the genera *Lonchocarpus*, *Derris* and *Tephrosia* (Gahukar, 2014; Isman, 2014). The substances from these genera act topically and by ingestion; besides, they are characterized by exerting their insecticide effect through the inhibition of the mitochondrial respiration (Grainge and Ahmed 1988; Koona et al., 2007). There are several species of this family with insecticidal action in which the presence of alkaloids and tannins is reported (Grainge and Ahmed 1988). According to Pérez and Iannacone (2006), the alkaloids is a group of very active compounds that block the transmission of the motor nerve, causing relaxation and flaccid paralysis of the skeletic muscle in the body of some insects, indicating that these compounds could be related to the insecticide action observed. In this family of plants, the non-protein amino acids are also reported (Udedibie, 2001) and they have a double function: defense and storage of nitrogen. Probably, the mortality produced by the seed powder of *C. ensiformis* was due to the presence of these compounds. The toxic effect is produced by their structural analogy with the essential amino acids, when they are misincorporated in the formation of enzymatic proteins or neurotransmitters (Ramos et al., 1998).

Besides, the acute insecticide effect on adults of *L. serricorne* and *S. zeamais*, it was necessary to assess the effect of the powders on the F1 emergence because of the high rate of their reproduction. The tested treatments that had the highest mortality in the adult insect of *L. serricorne* and *S. zeamais* also provoked the lowest emergence; the decrease of the adult emergence could be a method of effective control. The results obtained in the reduction of the emergence could be due to different causes; it could be related to an initial insecticidal effect and/or to deletorius physiological changes that affect reproduction or growth (Napoleão et al., 2013, Gahukar, 2014, Isman, 2014). The immediate death of the females and/or males may disrupt mating and thus the egg laying is reduced. A female may die before ovipositing the normal number of eggs, may survive treatment and become sterile or lay eggs that do not hatch. Other aspects that could affect reproduction are associated with the behavior. These active compounds could hamper the movement and encounter of the male with the female by occupying the space between the grains affecting the mating, or that the female by finding the grain covered with the powder does not receive the stimulus necessary for egg laying, a situation that could be caused by the allomones of the plant that are released to the environment where the grains are found (Shenk and Kogan 2003). Also, the death of the insects in the immature stages hampers the insect emergence. Other studies are required to determine the actual factors involved in the reduction of the F1 progeny by the evaluated products.

Additionally, all the botanical derivatives, tested on *L. serricorne* or *S. zeamais*, showed a repellent effect, except *T. diversifolia* roots. The results suggested that PAO-1 would have greater application in pest control for its effect on insect behavior to prevent grain infestation. The repellent effect could be determined by the presence of secondary metabolites, volatile substances that may be present in the plant parts studied (Regnault-Roger et al., 2004). When the insects detect these substances, they cause an effect on their behavior and evoke them to move away from product (Pérez et al., 2007). The attractive action of *T. diversifolia* root powder could be explained by the diversity of groups of secondary metabolites detected in the different tissues of this species under the conditions in which it was collected (González et al., 2010).

No available information was found about the repellent effect of the studied plants against *S. zeamais* or *L. serricorne*. But it is known that farmers sow *C. ensiformis* plants as intercrop and barrier to avoid the attack of pests (Arim et al., 2006; González et al., 2009). Also, *T. diversifolia* repellent action on the herbivorous *Atta* sp has been reported (Medina et al., 2009).

Further work is in progress to isolate and identify the insecticidal and repellent constituents of the plants studied. The sustainable use of these plants in the storage pest management is possible; they are cultivated or abundant wild species in Cuba. However, their practical application will require subsequent environmental and economic assessment. These products have potential for small-scale treatments of grains for protection against both insects. The *P. aduncum* subsp. *ossanum*-based products may also be used to control *L. serricorne* infestation in tobacco. The identification of local candidates to develop effective and safe pesticides offers new alternatives to the Cuban agriculture in the control of storage pests.

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Activity of two deltamethrin formulations on different surfaces against rice weevil, *Sitophilus oryzae* (L.)

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Abstract

Several methods are used to control stored product insects. The spraying of empty structures with insecticides, prior to the introduction of produce, is an important method for preventing development of insects. It is known that insecticide activity varies according to the various sprayed surfaces. In this study, the activity of deltamethrin was examined on concrete and plastic surfaces. Deltamethrin is a synthetic pyrethroid, active by contact against a variety of insects; applied in Israel in two formulations: KESHET 2.5% EC and BUNGY 1.5% SC (ADAMA Makhteshim Ltd.). Adults of the rice weevil, *Sitophilus oryzae* (L.), served as target insect in all experiments. The research was carried out in plastic Petri dishes and in Petri dishes with layer of concrete. Deltamethrin (KESHET 2.5% EC) was applied in water solution in doses of 0.02, 0.1, 0.5 g/m^2. Without concrete, complete mortality of *S. oryzae* was obtained at a concentration of 0.02 g/m^2, whereas in concrete plates, no mortality was found in all 3 concentrations. In contrast, deltamethrin (BUNGY 1.5% SC) in doses of 0.1 g/m^2, caused 100% mortality with and without concrete layer. The same results were found in the commercial warehouse. No difference in efficiency was found between the spraying methods: airbrush (Sparmax DH-125) or dripping by pipette. The results show that the efficacy of warehouse spraying by deltamethrin depends on its formulation.

Keywords: deltamethrin, stored product insects, treated surfaces, concrete, suspension, emulsion.

1. Introduction

The control of stored product insects, which cause serious damage to stored agricultural produce, is achieved by a combination of several methods. The use of protectants is an important and widespread method for preventing the development of stored product insects as an integral part of pest control practice. Protectants are applied by spraying incoming grains and empty warehouses, prior to produce introduction; in order to disinfest insect population on surface and cracks. Using residual materials can reduce and slow insects' infestation and reduce or eliminate the need for further control treatments. Surface treatments are applied as liquid contact insecticides on different types of surfaces (Arthur and Subramanyam, 2012). Floor and walls of grain warehouses are usually made of concrete. The surface of concrete is porous and alkaline, so when the insecticides are applied to concrete, hydrolysis and rapid degradation occur (Arthur, 1994; Jain and

Yadav, 1989). Hence, insecticides efficacy may depend on its formulation. Deltamethrin is synthetic pyrethroid, exceptionally potent against the whole spectrum of stored product pests (Snelson, 1987). Using deltamethrin can be effective as grain protectant or as surface treatment, singly or in combination with other insecticides, to control stored product insects. Contact toxicity of deltamethrin was found highly effective against *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), and *Rhyzopertha dominica* (Fab.) (Paudyal et al., 2016). Several studies which examined deltamethrin efficacy on various surfaces reported that wettable powder (WP) formulation was more effective than emulsifiable concentrate (EC) (Jain and Yadav, 1989). Deltamethrin is applied in Israel by two formulations: KESHET 2.5% EC and BUNGI 1.5% SC (ADAMA Makhteshim Ltd.). In this study, the activity of these two formulations was examined on concrete and smooth surfaces, under laboratory conditions and in commercial warehouses.

2. Materials and methods

Insects

Sitophilus oryzae (L.), *Rhyzopertha dominica* (Fab.) and *Tribolium castaneum* (Herbst) cultures have been reared under laboratory conditions for many years without any contact with insecticides. *S. oryzae* and *R. dominica* were reared on wheat grain, *T. castaneum* was reared on wheat flour. All these insects were maintained in 0.8L glass jars with paper covers and bred at 30±0.5^{0}C and 65±5% r.h. in dark.

Chemicals

The deltamethrin for the experiments was applied in two formulations: KESHET (2.5% EC, ADAMA Makhteshim Chemical Plants Ltd) and BUNGI (1.5% SC, ADAMA Makhteshim Chemical Plants Ltd).

Laboratory Experiment

In a laboratory experiment, deltamethrin formulations were examined in Petri dishes (diameter 9 cm). Plates without concrete (plastic) and plates with concrete were used. Individual concrete arenas in Petri dishes were made by mixing cement (42.5N II/A–LL) - sand - water (1:1:0.5). About 30 grams of the mixture was added in each Petri dish. Four days later, plates with concrete layers were ready to be used. The required concentrations of insecticides were obtained by dilution by tap water. About 0.62 ml of mixture (100 ml/m^2) was sprayed by airbrush (Sparmax DH-125), or dripping by pipette (approximately 40 drops per plate) to each Petri dish. Tap water was used as control.

For studying the effect of alkaline concrete, flush water from fresh concrete surface was used. 15 ml of tap water were added into each Petri dish with a concrete layer, and after one hour the water was removed from the dishes, with a pipette. About 10 ml of flush water was obtained from each Petri dish.

In all laboratory experiments, ten adults of *S. oryzae, R. dominica* or *T. castaneum* were exposed in Petri dishes with/without concrete layer, without food, in each probe. The plates were stored in the dark at 30±0.5^{0}C and 65±5% r.h. Insect mortality was recorded after 2 and 24 hours of exposure. Each treatment was replicated 3 times.

Warehouse experiments

For several years, local grains are stored in warehouses in the south of Israel. Before inserting the new wheat harvest, crop residue was removed from the warehouses and a mixture of ACTELLIC 50 EC, KESHET 2.5 EC and water was sprayed in the warehouses. The concentration of ACTELLIC 50 EC in the mixture was constant (1.0%), and the concentration of KESHET 2.5 EC increased from 1.0 to 3.0%. In a certain year, during commercial treatment, the warehouses were sprayed with only 4% KESHET 2.5 EC in water; working mixture consumption at 100 ml/m^2. A day after the spraying, the effectiveness of the treatment was tested. Adults *S. oryzae* (10 insects in each probe) were put on

various treated structures, and their movements were limited within lower part of Petri dishes which were attached to the surface with duct tape (14 probes). After 24 hours, the insects were transferred to Petri dishes which were put in incubator and their mortality was recorded the following day.

To test the efficiency of the spraying in storage facilities with two different deltamethrin formulations, 14×14cm squares (~0.02m^2) were marked on concrete walls and insecticides were sprayed by airbrush (Sparmax DH-125) on the marked squares. Ten adult *S. oryzae* were transferred onto the treated squares. The movement of insects was limited to a net that was attached with duct tape. After 24 hours, the insects were transferred from concrete walls to Petri dishes and their mortality was recorded. Each treatment was replicated 3 times.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to compare percent of insect mortality. The Tukey Multiple Range test was used to compare mean values. (JMP Pro 13.0.0., 2016).

3. Results

Exposing stored product insects to KESHET 2.5% EC in concentration 20 mg/m^2, caused after 24 hours of exposure the following results: 100% mortality of *S. oryzae* and *T. castaneum*, and 23.3% mortality of *R. dominica*; and in concentration 100 mg/m^2 - 100% mortality of *S. oryzae, R. dominica* and *T. castaneum* (Tab. 1).

Tab. 1 Contact effect of 2.5 EC KESHET on plastic surface against stored product insects.

Spray*	Dose of deltamethrin g/m^2	Mortality (%), after 24 hours exposure		
		S. oryzae	*T. castaneum*	*R. dominica*
0.8% KESHET 2.5EC	0.02	100 A	100 A	23.3±8.8 B
4.0% KESHET 2.5EC	0.1	100 A	100 A	100 A
20.0% KESHET 2.5EC	0.5	100 A	100 A	100 A
Control	0	0 C	0 C	3.3±3.3 C

* - Tap water was used for dilution of commercial concentrates of deltamethrin and as control. 0.62ml of mixture (100 ml/m^2) was transferred at dripping by pipette (approximately 40 drops per plate) to each plastic Petri dish.

** - Ten adult insects were introduced in each sample. Each treatment was triplicate. Values are the means ± SEM. Values represented with the same letter are not significantly different one from the other.

In the commercial treatment in the warehouse, after 24 hours exposure, mortality of *S. oryzae* in KESHET 2.5EC depended on the surface type and on the location of treatment in the warehouse. Mortality on galvanized steel in all tests was 100%; mortality on concrete was 20 -100%. Control mortality in the untreated warehouse was 0 - 20%.

In the laboratory test on concrete surface, KESHET 2.5% EC was not effective, even in deltamethrin rate of 500 mg (AI)/m^2. In contrast, BUNGI (1.5% SC, ADAMA Makhteshim Chemical Plants Ltd), in concentration of 100 mg (AI)/m^2 caused 100% mortality of *S. oryzae, R. dominica* and *T. castaneum*. No significant difference in efficiency were found between the spraying methods: airbrush (Sparmax DH-125) or dripping by pipette (approximately 40 drops per plate) (Tab. 2).

In the warehouse test, on concrete surface, KESHET 2.5% EC in concentration 100 mg (AI)/m^2 was not effective. However, BUNGI (1.5% SC, ADAMA Makhteshim Chemical Plants Ltd) in same concentration caused 100% mortality of *S. oryzae* (Tab. 3).

Tab. 2 The effect of two deltamethrin formulations on concrete against stored product insects.

Spray*	Mortality (%)** after 24 hours exposure		
	S. oryzae	*T. castaneum*	*R. dominica*

	Dose of deltamethrin g/m²	Airbrush	Pipette	Airbrush	Pipette	Airbrush	Pipette
4.0% KESHET 2.5EC	0.1	3.3±3.3 B	0 B	0 B	0 B	-	-
20.0% KESHET 2.5EC	0.5	0 B	16.7±16.7 B	0	3.3±3.3 B	-	-
6.7% BUNGI 1.5SC	0.1	100 A	100 A	100 A	100 A	100 A	90.0±5.8 A
33% BUNGI 1.5SC	0.5	100 A	100 A	100 A	100 A	100 A	93.3±3.3 A
Control	0	3.3±3.3 B	0 B	0 B	0 B	0 B	0 B

* -Tap water was used for dilution of commercial concentrates of deltamethrin and as control. 0.62ml of mixture (100 ml/m²) was transferred on each prepared Petri dishes with concrete by dripping by pipette (approximately 40 drops per plate) or by spraying with airbrush.

** - Ten adult insects were introduced in each sample. Each treatment was triplicate. Values are the means ± SEM. Values represented with the same letter are not significantly different one from the other.

Tab. 3 The effect of two deltamethrin formulations in dose 0.1 g/m² against *S. oryzae* on concrete wall in warehouse.

Spray*	Mortality (%)** 24 hours after exposure	
	2 hours of exposure	24 hours of exposure
4.0% KESHET 2.5EC	6.7±6.7 B	13.3±8.8 B
6.7% BUNGI 1.5SC	100 A	100 A
Control	6.7±6.7 B	11.2±0.7 B

* - Tap water was used for dilution of commercial concentrates of deltamethrin and as control. 2.0 ml of mixture (100 ml/m²) was sprayed by airbrush on concrete wall in surface area, at size of 14×14 cm. The temperature in warehouse was 5 – 15⁰C.

** - Ten adult insects were introduced in each sample. Each treatment was triplicate. Values are the means ± SEM. Values represented with the same letter are not significantly different one from the other.

For studying the effect of alkaline concrete, flush water from fresh concrete surface and KESHET 2.5% EC in low concentration 10 mg (AI)/m² were used. Influence of concrete alkaline on efficacy of 2.5EC KESHET was found.

Tab. 4 Influence of concrete alkaline on effectivity of 2.5EC KESHET against *S. oryzae*.

Spray*	Surface	Mortality (%) ** 24 hours after exposure 2 hours of exposure	24 hours of exposure
0.4% KESHET 2.5EC in tap water	**Plastic**	83±3.3 AB	96.7±3.3 A
0.4% KESHET 2.5EC in concrete water* **	**Plastic**	0 C	73±3.3 B
0.4% KESHET 2.5EC in tap water	**Concrete**	0 C	0 C

* - 0.62ml of mixture (100 ml/m²) was sprayed by airbrush to each Petri dishes with/without concrete; dose of deltamethrin 0.01 g/m²

** - Ten adult insects were introduced in each sample. Each treatment was triplicate. Values are the means ± SEM. Values represented with the same letter are not significantly different one from the other.
*** - pH concrete flush water 12.5.

4. Discussion

Deltamethrin is a broad-spectrum synthetic pyrethroid insecticide with contact activity that is widely used to control stored product insects. In this study, emulsifiable concentrate of deltamethrin (KESHET 2.5% EC, ADAMA Makhteshim Chemical Plants Ltd), which were applied on Petri dishes, in concentration 20 mg/m^2, was very effective .

According to Paudyal et al. (2016), who used technical grade deltamethrin in ethanol on glass Petri dishes, LD_{95} for *S. oryzae* was 123.6 mg/m^2 (70.9–288.3), for *R. dominica* it was 121.3 mg/m^2 (74.4–233.6) and 57.8 mg/m^2 (43.2–83.0) for *T. castaneum*. The differences in the efficacy of deltamethrin are due probably to the fact that in our study we used a commercial product, rather than a technical grade deltamethrin, and insect's strains variability.

In our warehouse experiment, the efficacy of commercial treatment by spray KESHET 2.5% EC in rate of 100 mg (AI)/m^2 on concrete, against *S. oryzae*, varied in different parts of the warehouse.

Suspension concentrate formulation of deltamethrin (Centynal™) and chlorpyrifos-methyl + deltamethrin (Storcide™ II) are used in USA (Sehgal and Subramanyam, 2014, Sehgal et al., 2014); mix pirimiphos-methyl (ACTELLIC 50 EC) + deltamethrin (KESHET 2.5 EC) - are used in Israel.

In our laboratory experiments on concrete, KESHET 2.5% EC was not effective. In contrast, the new commercial formulation of deltamethrin, BUNGI (1.5% SC, ADAMA Makhteshim Chemical Plants Ltd), in concentration of 100 mg (AI)/m^2 caused 100% mortality of *S. oryzae, R. dominica* and *T. castaneum*. Similar results were obtained when testing the two formulations of deltamethrin in a warehouse.

It is a known fact that pyrethroid efficacy may be dependent on formulation. Jain and Yadav (1989) reported differences in efficacy of an emulsifiable concentrate (EC) and wettable powder (WP) formulations. In the same study, adult insects exposed to deltamethrin were applied at 10 mg (AI)/m^2 on various surfaces including concrete. WP formulation was more effective than the EC formulation on concrete.

According to Arthur (1997), 0.05% deltamethrin dust has potential to knockdown or kill *T. castaneum* and *R. dominica* after 24 h exposure on concrete, and has potential as a residual treatment in food storage facilities. However, use of dust and WP is inconvenient in practice. Suspension combines effectiveness of dust/WP and all the performance benefits of emulsions. Suspension concentrates of deltamethrin: Centynal (Wellmark Internatonal, Schaumburg, IL, 37mg (AI)/ml)) and Suspend® SC 4.75% AI, were effective against stored product insects. Centynal at 20 mg (AI)/m^2 on concrete was effective against adults of *R. dominica*, *T. castaneum* and *Oryzaephilus surinamensis* (L.) causing 100% mortality of laboratory strains (Sehgal and Subramanyam, 2014); Suspend® SC 4.75% AI at 24 mg (AI)/m^2 on concrete caused 100% mortality of adults and larvae of laboratory strains of *Trogoderma granarium* (Everts) (Ghimire et al., 2017).

Factors such as surface activity, surface pH, porosity and transfer from carrier to insect may play a role in the different effectiveness of two deltamethrin preparations on concrete. It was observed, that there was an influence of concrete alkaline on efficacy of 2.5EC KESHET. However, the decline in efficiency of KESHET 2.5% EC on concrete cannot be explained by basic pH only. The results show that the efficacy of deltamethrin treatment depends on its formulation and on the sprayed surfaces.

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Evaluation of two new insecticide formulations based on inert dusts and botanicals against four stored-grain beetles

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Extended Abstract

Diatomaceous earth (DE) is toxic to insects because it absorbs their cuticular waxes causing insects to die from desiccation. DEs are obtained from geological deposits around the world, are skeletons of diatoms, and are mainly made up of SiO_2, with very low mammalian toxicity They can be applied with approximately the same technology as other powder insecticides (Korunic, 1998; Subramanyam and Roesli, 2000). Several DEs are effective at doses of 500 ppm or higher. However, these doses cause unwanted effects on grain quality and flowability and their application for direct mixing with grain has limited acceptance by the grain industry (Korunic et al., 1996; Subramanyam and Roesli, 2000). Therefore, it is essential to develop formulations that are effective at lower doses of DE. One of the solutions is to combine DE with other substances, primary with botanicals (Athanassiou and Korunic, 2007; Athanassiou et al., 2009). Our objective was to develop effective insecticides using as much as possible Generally Recognized as Safe (GRAS) compounds that would not significantly reduce grain flow or test weight.

We developed two new insecticide formulations that combine: diatomaceous earth (DE), silica gel, pyrethrin, piperonyl butoxide (PBO) and dill essential oil (F2Z) or with these same ingredients and disodium octaborate tetrahydrate (F3DOTZ). Silica gel (Sipernat® 50 S) is synthetic amorphous silicon dioxide and, has similar mode of action as DE. Amorphous silicon dioxide is (GRAS) and, is used as a food additive. Pyrethrin is one of the most common botanical insecticides. To prevent the recovery of insects after the treatment, the pyrethrin formulations contain a synergist, most frequently piperonyl butoxide (PBO) (Ware and Whitacre, 2004). Dill essential oil is extracted from the seeds or leaves/stems of the dill (*Anethum sowa* and *A. graveolens*). Dill oil is known as a natural synergist for pyrethrin (Liu et al., 2014). Disodium octaborate tetrahydrate (DOT) is a naturally occurring mineral salt commonly called borate or sodium borate. It is used to treat lumber and other wood products to control fungi, termites, and other wood infesting pests (Ware and Whitacre, 2004). DOT is effective against *Sitophilus oryzae* (L.) and does not reduce bulk density as much as DE reduces bulk density (Korunic et al., 2017).

Sitophilus oryzae L., *S. granarius* (*L.*) (Coleoptera: Curculionidae*),* and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and one external feeder*, Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae*)* were held on clean wheat (13.5% moisture) treated with different doses of the insecticides and held at 28 ± 1ºC and 60 ± 5 % RH for 3, 5 and 7 days, and then held for progeny emergence. In the series of three experiments both formulations were applied either as dusts or as wettable powders, F2Z at 150 ppm and F3DOTZ at 200 ppm.

After 3 days, with powders and wettable powder of F2Z and F3DOTZ formulations there was 100% mortality for *S. oryzae* and *R. dominica*. After 7 days, the mortality of *T. castaneum* was from 96 to

100%. All formulations completely reduced the progeny (100%). In another experiment, 75, 125, 175 and 225 ppm of F2Z and F3DOTZ formulations and 300, 400, 500 and 600 ppm of diatomaceous earth alone (Celatom MN 23) were tested. After 3 days, with 175 ppm of F2Z and F3DOTZ caused adult mortalities from 99 to 100%. The DE alone at 300 ppm after 3 days only caused 80% mortality of *S. oryzae*, 60% mortality of *R. dominica* and no mortality for *S. granarius* and *T. castaneum*, and the progeny of *S. granarius* was reduced by 77%, *S. oryzae* by 78%, *R. dominica* by 92% and *T. castaneum* by 100%.

The effective concentrations of 150 ppm of F2Z, 200 ppm of F3DOTZ and 300 ppm of DE reduced bulk densities by 4.9, 3.4 and 6.4 kg/hL, respectively.

These new formulations were effective at controlling insects better than DE alone, yet do not reduce the bulk density as much as DE alone. Further testing is required to determine if these formulations should be brought to market; duration of efficacy, cost of formulations, testing for their effect on non-target organisms, human safety and effect on end-use quality.

Keywords: diatomaceous earth, synergy, natural, mortality, bulk density

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Protecting Stored Maize Grain Against the *Sitophilus Zeamais* with Rice Husk Ash

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Abstract

Maize weevil (*Sitophilus zeamais*) is an important insect that affect the maize grain on the field and in storage. There are several ways of controlling this insect but the most commonly used is the use of chemicals. Although these chemicals are very effective, they are often expensive and not available to poor rural farmers resulting in high post-harvest losses of their harvested grains. In this study, the potential of using rice husk ash (RHA) as a protectant against maize weevil during storage was investigated. Cultured maize weevils were introduced into 400g of maize admixed with RHA at concentrations of 5g, 10g and 20g. A control set-up of both Actellic 50EC and no RHA was set-up to compare the effect of the ash treatments on weevil mortality, re-emergence and grain damage. The treatments were replicated and set-up in the lab at room temperature condition. Results showed that, 100% mortality was observed for the Actellic 50EC treatment 5days after application. However, there was no significant difference ($p>0.01$) after 60 days of storage between the 20g RHA application and the Actellic 50EC relative to weevil mortality, emergence and grain damage. With the 20g RHA admixture recording the highest mortality and suppression effect on adult weevil emergence as well as the lowest grain damage, the use of RHA can provide a significant economic advantage to farmers for storage of maize in tropical developing countries if reliable recommendations on application rate can be made for the protection of stored maize.

Keywords: Maize storage; maize weevil; rice husk ash; protectant, mortality

Introduction

Being an integral component of staples in Sub-Saharan Africa, maize is considered as an essential source of cash for farmers. It is well known to be one of the few crops that have profound effects on the livelihoods of people in Sub-Saharan Africa. There is no doubt that in Ghana, more than half of the area of land allocated for cereal production is being used for maize production (FAOSTAT, 2010). The adverse effect of climate change has led to the decrease in food output, of which maize constitute a significant part. This has caused a negative effect on the socio-economic lives of farmers. There is therefore, the need for innovative and well-adaptable methods for conserving the scarce food which is being produced by farmers from this part of the world.

In Ghana, maize like all other cereals, are stored in bags. In as much as initial pest infestation may occur in the field, the environmental conditions prevailing in traditional storage systems favour the survival and emergence of storage pests such as weevils. Indeed, weevils are often identified as one of the major problems causing loss of stored grain in Africa. An estimated percentage of 5 to 10 % maize is lost due to weevil attack (Booysen, 1983; Mashingaidze, 1994; Gadzirayi *et. al.,* 2006). This poses a risk to food security in Ghana and other developing countries in Sub-Saharan Africa.

The use of synthetic insecticides has proven to be effective as reported by Ogunleye and Adefemi (2007); Obeng-Ofori and Amiteye (2005) and Asawalam et al. (2007). In Ghana, two main chemicals: aluminium phosphide (phostixin) and actellic 50EC are used conventionally to treat/protect stored maize against the maize weevil. However, major drawbacks to the use of such insecticides such as the development of insect resistant strains and their toxic residues getting into food of animals and man are limiting their usage by farmers and other target customers. Again, the inimical macro-economy has resulted in high prices for these products and has therefore, sprouted the need for identifying other methods of preserving maize grains. Alternative methods such as the use of powdered plant parts and plant extracts have yielded positive results in comparison to the use of some synthetic insecticides (Cobbinah and Appiah-Kwarteng, 1989; Jembere *et al*., 1995; Lajide et al, 1998; Asawalam and Adesiyan, 2001; Asawalam *et al.,* 2007 and Udo, 2011). Aside these alternatives being eco-friendly, they are cheaper than chemical insecticides. Also, their availability for use is very easy since the materials used are readily available at farming areas.

The use of rice husk ash in storing maize for protection against storage pests is eminent in Ghana. However, information regarding its usage and effects on its protection of stored maize against *Sitophilus zeamais* attack is unknown. Therefore, there is the need for more study to be done in this direction. The present work therefore was designed to investigate the effectiveness of rice husk ash (RHA) in controlling *Sitophilus zeamais* in stored maize grain as compared to Actellic %0EC.

Materials and Methods

Culture of maize weevils

Species of *Sitophilus zeamais* was obtained from a sample of maize from the Ayigya market of Kumasi in Ashanti Region, Ghana. The insects were kept in Kilner jars at room temperature in the Entomology laboratory of the Agricultural faculty, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The weevils were cultured for a period of 12 weeks.

Preparation of ash

Rice husk was collected from a rice farm at Besease, a rice farming community in Ashanti Region, Ghana. The husk was burnt into ash using a gasifier. On cooling, the ash was pulverized and kept in air tight bags at room temperature.

Moisture content determination

The maize variety, *Obaatampa*, was obtained from the Ministry of Food and Agriculture, MOFA, Kumasi at a moisture content of 13%. This was confirmed using the oven method (ISO 6540-1980).

Samples and treatments

Visual inspection was made to remove infested maize from the sample in use. 400g of uninfested maize was weighed into 15 plastic containers. Different concentrations (1.25%, 2.5% and 5% wt/wt maize grains) of rice husk ash were introduced in nine of the containers containing 400g of the maize samples. Each treatment, T5, T10, T20, TCH and TC representing 1.25 % of RHA, 2.5 % of RHA, 5% of RHA, Chemical (Actellic 50 EC) application and Control (no chemical/ash applied) respectively, were replicated three times. 20 species of the cultured *Sitophilus zeamais* (10 males and 10 females) were introduced into each container using forceps.

Experimental setup

The complete randomized design as shown in Tab. 1, with five treatments and three replicates were used in setting up experiment.

Tab. 1 Experimental setup in laboratory

1 **T5**	2 **T10**	3 **TCH**	4 **T20**	5 **TC**
6 **T10**	7 **TCH**	8 **T20**	9 **TC**	10 **T5**
11 **T20**	12 **TC**	13 **T5**	14 **T10**	15 **TCH**

Mortality, progeny and damage assessment

Number of dead weevils in each container was recorded after the first five days of set-up and for every other week for two months. Mortality rate was then calculated using Equation 1 by Asawalam (2007).

$M_r = \frac{d_w}{d_w} \times 100$ ..... Equation (1)

Where

M_r-mortality rate

D_w–total number of weevils

D_w-number of dead weevil

Percentage damage made on maize by the weevils was calculated using Equation 2 as used by Asawalam (2007).

$\text{Damage } (\%) = \frac{number\ of\ damage\ grains}{total\ number\ of\ grains\ in\ containe} \times 100$ ..... Equation (2)

Weevil Perforation Index (WPI) is a damage assessment in which the number of perforations in treated grains is compared to that of the control. Equation 3 was used to determine WPI during the experiment.

$WPI = \frac{Nps}{Npc} \times 100$ ..... Equation (3)

Where

WPI- Weevil Perforation Index

Nps - Number of perforated seeds with treatment.

Npc - Number of perforated seeds in the control.

WPI > 50 = negative protectant of plant material tested (i.e. enhancement of infestation by the weevil)

WPI < 50 = positive protectant (i.e. prevention of infestation by the weevil)

Data analysis

Using an analysis of variance, the number of dead weevils was investigated to determine whether there were any significant differences in the numbers of live and dead weevils as well as the quality

of maize grain used. A significance level of 1% was used for all analysis. A one-way ANOVA was set up for the investigation.

Results

Mortality and suppression of adult emergence

The comparison of the S. zeamais mortality in the four treatments plus the control is shown in Tab. 2. A significant difference ($p<0.01$) was recorded by the treatments on the average number of dead weevils monitored every other week in the storage containers for a period of 2 months. Apart from the control, all treatment recorded more than 80 % mortality. TCH had great effect of 100 % on the mortality of S. zeamais. This was followed by T20, T10 and T5 of mortality rates 98 %, 95 % and 90.9 % respectively. TC witnessed an uncontrollable emergence of adult weevils with a mortality of 52.9 %. Comparing the results from TCH to T20 and T10 treatments, it was observed that the effect on population growth of the weevils can be likened to that of TCH since there was no significant difference ($p>0.01$) between the two rice husk ash treatments and the chemical treatment.

Tab. 2 Mortality of S. zeamais from maize with different treatments

Treatment	Mortality*
TC	52.9a
TCH	100bc
T5	90.9d
T10	95c
T20	98c
L.S.D.	8.09

* Results are means of four replicates of twenty insects each. Mean values with same variabls indicates no significant difference ($p > 0.01$)

Damaged grains

The number of damaged grains recorded after the two months of storage is shown in Fig. 1. 100 grains sample was taken from each of the 15 containers for this analysis. TC had the greatest damage of 13 damaged grains. This was followed by T5, T10 and T20 with 8, 6.3, 4.3 and 2 damaged grains respectively. This shows that the ash as compared to the chemical also had an adverse effect on the weevils making them uncomfortable, hence less damage on grain. Similar results were witnessed by Mazarin et. al. (2016); Otitodun et. al., (2017) and Goudoungou et. at., (2018).

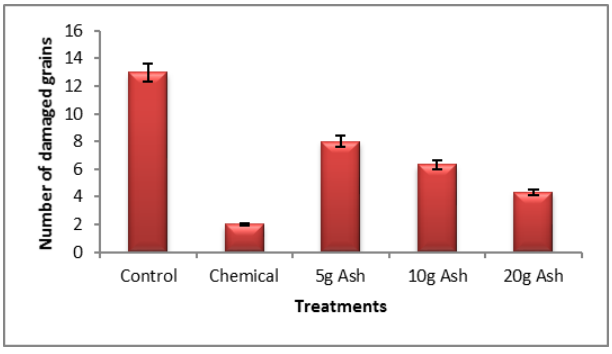

Fig. 1 Effect of different treatments on the number of damaged grains by *S. zeamais*

Weevil Perforation Index (WPI)

Weevil Perforation Index directly relates to weight loss and damage assessment. All treatments other than TC had WPI < 50 resulting in positive protectant of maize grains from *Sitophilus zeamais* attack. Hence, all tratments had the capabilities of preventing maize from the infestation by *S.*

zeamais. TCH had the least WPI of 15.38 followed by T20, T10 and T5 of 33.31, 40.69 and 58.54 WPI respectively. TC had WPI of 100 which means that it is ineffective or inefficient to store maize without any treatment (Asawalam et al., 2007).

Tab. 3 WPI of different treatments by S. zeamais

Treatment	WPI
TC	100
TCH	15.38
T5	48.54
T10	40.69
T20	33.31

Discussion

It is clear that the rice husk ash treatment used for the study gave positive results in terms of protecting maize grains from the attack of *S. zeamais*. Evidently, the rice husk ash treatments had an obvious effect on the population growth rate of the weevils, their re-emergence, WPI and the damage caused by *S. zeamais* to the maize grain. It was observed that, high application rate of 20g rice husk ash per 400g maize sample had a low mortality, low WPI and less damage to grains as it significantly suppressed the emergence of adult *S. zeamais* when compared to the control.

Results suggest that the weevils would prefer to avoid maize grains treated with the ash. The results showed that the ash had some degree of insecticidal activities. Insecticidal property of any plant material would depend on the active constituents of the plant material. Okonkwo and Okoye (1996); Ogban *et. al*. (2016) reported that *P. guineense* contains piperine and chavicine which are insecticidal. Idoko and Adesina (2012) also indicated that piperidine and alkaloids as the major active components in *P. guineense* seeds and suggested that *S. zeamais* development was adversely affected by grains treated with these powders than the control. The ability of these plant powders to cause mortality of *S. zeamais* adults on maize grains can be attributed to contact toxicity of the powders on the weevil. Though the lethal action of rice husk ash on maize weevils was not investigated, Otitodun et. al., (2017) reports that the main ingredient of rice husk ash which is silica (SiO_2) accounts for 87.1 % of the total content and has almost the same composition as diatomaceous earth which is effective in controlling pests of stored grain.

The rice husk ash is reported to contain large number of needle-like particles that are probably obtained from the setae covering the outer surface of the rice hull. These needle-like particles may have caused some skin irritations to the weevils which might cause death. It is believed that inert substances like the rice husk ash generally cause a loss in body moisture due to the presence of silica.

Conclusion

From this study, it can be concluded that rice husk ash has the potential in controlling the *S. Zeamais* in stored maize. It was observed that after the 120-125 days of storage, the incidence of weevils was lower in the containers with the higher concentration of ash (20g). This indicates that there is an increasing suppression on the population growth of the weevils with increasing concentration. This may be attributed to the toxic effect and unfavourable conditions created in the containers with the higher levels of rice husk ash. Hence, rice husk ash has the potential of controlling the damaging effects of *S. Zeamais* on maize grains in storage. Due to its eco-friendly and indigenous nature, rice husk ash can be adopted by small-scale farmers in rural communities in Ghana and some parts of Sub-Saharan Africa.

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Effectiveness of binary combinations of *Plectranthus glandulosus* leaf powder and *Hymenocardia acida* wood ash against *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Abstract

Combinations of botanicals could enhance biological activity against insects. This in turn, will reduce amount of botanical used in storage protection. In this issue, the bioassay was carried out on *Sitophilus zeamais* to assess the effectiveness of binary combinations of *Hymenocardia acida* wood ash and *Plectranthus glandulosus* leaf powder regarding adult toxicity, progeny inhibition, and reduction of damage and germination ability. *Plectranthus glandulosus* leaf powder, *H. acida* wood ash and their binary combinations significantly induced mortality of *S. zeamais* adult (P< 0.0001). The higher mortality rate was achieved by the highest content (40 g/kg) of *H. acida* wood ash (94.66%) and 25PG75HA (94.59%) within 14 days of exposure. The combinations of *P.*

glandulosus leaf powder with *H. acida* at different proportions produced different interactions. The combination made up by 75% of *P. glandulosus* leaf powder with 25% of *H. acida* wood ash produced synergistic effect whereas that made up by 50% of each of two powders had antagonistic effect in weevil mortality. The three combinations of *H. acida* and *P. glandulosus* significantly reduced the progeny production. In term of inhibition of F_1, the combination 25PG75HA revealed more effective than the two other. The grain damage and population increase were significantly reduced. In general, the non-infested maize grain had a good germination rate than the infested ones. The treatments did not have negative effect on seed germination. From These results, the two powders and their binary combinations could be used to reduce grain infestation by insect while taking in account the proportions of insecticidal powders implied in the combination.

Keywords: *Sitophilus zeamais*, *Hymenocardia acida*, *Plectranthus glandulosus*, wood ash, leaf powder, binary combinations

Introduction

Cereals constitute the group of most consumed grain in Sub-Saharan African especially in sahelian zones. In these zones, cereals are very interesting according to its conservation ability. Cereals are easily conserved compared to the others food products, and also less demanding in term of storage technology, which can be self-made. The conserved and protected seeds permit availability of grains throughout the year thereby contributing to food security. Maize remains the most cultivated and most consumed cereal in Africa. The production of maize is done in the short period of the year whereas its commercialisation and consumption establish along the year. This makes imperative the storage and the protection of this grain. The insufficiencies of different storage methods in developing countries have not stopped to cause grain losses and this in unacceptable proportions (Gwinner etal., 1996).

During storage, maize grain is highly devastated by several pests, especially insect pests that are at the origin of the majority of damage occurring in the stored food products. Temperature and high humidity of the tropical climate favour proliferation of insects and micro-organisms which, in order to survive; devour the food products causing enormous damage (Ngamo and Hance, 2007). Maize grain does not escape to insect attack during storage. Among the insect, maize grain pest, *Sitophilus zeamais* is the most detrimental. This pest causes quantitative and qualitative damage on stored maize. In this condition, the protection of this grain according to its multiple uses becomes a major necessity for food security. Damage caused by *S. zeamais* on maize could be reduced through chemical, biological, physical control and host plant resistance, which are important components of integrated pest management strategies. However, the use of synthetic residual chemicals dominates in Cameroon and other African countries. These chemicals, although effective, cause many environmental problems such as pollution, diseases and resistance in pests (Subramanyam and Hagstrum, 1995; Park etal., 2003). Furthermore, most farmers in Africa are resource-poor and have neither the means nor the skills to obtain and handle pesticides appropriately. Therefore, an environmentally safe and economically feasible pest control practice needs to be available.

Botanicals are products based on parts, powders, extracts or purified substances of plant origin. They are generally assumed to be more biodegradable, leading to less environmental problems. *Plecthrantus glandulosus* Hook leaf (Ngamo etal., 2007a; Nukenine etal., 2007) and wood ash (Ntonifor etal., 2001, Mulungu etal., 2010; Moyin-Jesu, 2010; Singh, 2011.) could stand out as good candidates for environmentally friendly control of storage beetle pests under Cameroonian conditions. *P. glandulosus* is an annual, glandular and strongly aromatic herb, used in folk medicine for the treatment of colds and sore throat in the Adamawa region of Cameroon (Ngassoum etal., 2001). The insecticidal properties of products from *P. glandulosus* have shown good insecticidal properties against stored maize grain pests (Nukenine etal., 2007; 2010; Ngamo etal., 2007b; Goudoum etal., 2010). Many authors have reported the effectiveness of wood ash as a grain protectant (Golob etal., 1982; Firdissa and Abraham, 1999; Akob and Ewete, 2007; Oguntade and Adekunle, 2010; Gemu etal., 2013). The insecticidal efficacy of *Hymenocardia acida* wood ash needs to be determined since it is one of the plants which the wood is most used as firewood and charcoal in traditional kitchens in the northern part of Cameroon. Combinations of wood ash with *P.*

glandulosus leaf powder could enhance biological activity against insects. This in turn, will reduce both the amount of botanical and wood ash used in storage protection. Data concerning the effectiveness of the binary combinations between *H. acida* wood ash and *P. glandulosus* leaf powder are not available, although farmers mix dusts like wood ash with plant materials in stocks. As the stored grain in traditional facilities is used as seeds, the determination of the influence of grain protectant on seed germination is imperative.

Therefore, the objective of this study was to assess the effectiveness of binary combinations between *P. glanduosus* leaf powder and *H. acida* wood ash regarding adult toxicity, progeny production, population growth, grain damage and germination.

Materials and MethodsSource of maize grains

The variety of maize used during all experimentation was Shaba, this provided by IRAD Wakwa in the Adamaoua region of Cameroon. Before experimentation, broken grains, the pieces of stone, sand and others foreign materials were removed from the stock. Then the maize was kept in the freezer at –20°C for 14 days to allow its disinfestations. After disinfestations and 14 days of acclimatisation, the maize was ready for use as substrate for insect rearing and bioassays.

Insect rearing

Adults of *S. zeamais* were obtained from a colony maintained in rearing since 2005 in the Applied Chemistry Laboratory of the University of Ngaoundere. The weevils were reared on disinfested maize in 900 ml glass jars and kept under fluctuating laboratory conditions of 23.08 ± 2.05° C and 74.67 ± 14.36%). The culture was maintained and used as source of *S. zeamais* for bioassays.

Plants

Stems and branches of *H. acida* were collected in Ngaoundéré, Adamaoua region of Cameroon (latitude 7°25' North and longitude 13°35' East, altitude of 1151 m above sea level). The identity of the plant was confirmed at the Cameroon National Herbarium, where voucher samples were deposited. *H. acida* is registered on number 50114/HNC. Woods were air-dried until moisture was completely lost and burnt separately in a traditional kitchen normally used in the region. The obtained ash was sieved and packaged in glass jars, labelled and kept in a freezer (at – 4°C) until subsequent use in the bioassays.

Leaves of *P. glandulosus* were collected in July 2012 in Ngaoundere located inVina Division, Adamawa region of Cameroon (latitude 7°25' North and longitude 13°35' East, altitude of 1151 m above sea level). The identity of the plant was confirmed at the Cameroon National Herbarium on number 7656/SRF. The leaves were dried at room temperature for seven days, and then crushed until the powder passed through a 0.20 mm sieve. Wood ash and leaf powder of the two plants were mixed in the following proportions to constitute the different binary combinations:

25 % *P. glandulosus* leaf powder and 75% *H. acida* wood ash: 25PG75HA;
50% *P. glandulosus* leaf powder and 50% *H. acida* wood ash: 50PG50HA;
75 % *P. glandulosus* leaf powder and 25 % *H. acida* wood ash: 75PG25H

Toxicity and F_1 progeny bioassays

The toxicity bioassay was carried out under ambient laboratoryconditions. Four concentrations for each combination were considered. The masses of 0.25; 0.5; 1 and 2 g of *P. glandulosus* leaf powder and *H. acida* wood ash and their binary combinations were separately added to 50 g of maize in glass jars to constitute, respectively the contents of 5; 10; 20 and 40 g/kg. Then, the insecticidal materials plus grain were thoroughly mixed by manual shaking. The controls consisted of substrate without insecticidal products. A set of 20 insects of mixed sexes and 7 to 14-days-old were added into the jars containing the treated or untreated grains. All treatments were replicated four times. Mortality was recorded 1, 3, 7 and 14 days post-infestation.

The co-toxicity coefficient per *P. glandulosus* leaf powder–*H. acida* wood ash mixture was calculated according toSun and Johnson (1960). After the 14 days mortality recordings, all insects and products were discarded. The grains were left inside the bottles and the counting of F_1 adults was carried out once a week for 5 weeks (Nukenine etal., 2007).

Damage and germination tests

Four rates of the binary combinations (5, 10, 20 and 40 g/kg) were mixed with 150 g of maize grain as described above. Fifty unidentified sex weevils (7-14 days old) were introduced into each jar. Each treatment had four replications. After three months, the live weevils and dead ones were counted. Damage assessment was performed by counting and weighingthe number of damaged and undamaged grain using the method of Adams and Schulten (Adams and Schulten, 1987).

Seed germination was tested using 30 randomly picked grains from non-perforated grains after separation of the perforated from the non-perforated in each jar. Also, the non infested treated seeds were used to assess the effect of binary mixtures on germination ability. The number of germinated seeds was recorded after 10 days (Rao etal., 2006).

Data analysis

Abbott's formula (Zar, 1999) was used to correct for control mortality before Analysis Of Variance (ANOVA) and probit analysis. Data on cumulative corrected mortality, reduction in F_1 progeny, damage, weight loss and germination percentage were arcsine-transformed [(square root(x/100)] and the number of F_1 progeny was log-transformed (x + 1). The transformed data were subjected to the ANOVA procedure using the Statistical Analysis System (SAS Institute, 2003; Finney, 1971). Tukey's test (P = 0.05) was applied for mean separation. Probit analysis (Finney, 1971; Abbott, 1925) was conducted to determine lethal dosages causing 50% (LC_{50}) and 95% (LC_{95}) mortality of *S. zeamais* at 1, 3, 7 and 14 days after treatment application. The probit analysis was also used to determine the effective content causing 50% (EC_{50}) reduction of F_1 progeny.

Results

Plectranthus glandulosus leaf powder, *H. acida* wood ash and their binary combinations significantly induced mortality of *S. zeamais* adult. This mortality increased with content and exposure time (Tab. 1). Low variation in term of efficacy was observed amongst the five tested powders. In general, this variation became highly significant (P< 0.0001) within 7 and 14 days. The higher mortality rate was achieved by the highest content (40 g/kg) of *H. acida* wood ash (94.66%) and 25PG75HA (94.59%) within 14 days of exposure. *P. glandulosus* leaf powder induced low mortality compared to the other products at all exposure periods. The mortality rate of 57.24% was recorded with *P. glandulosus* leaf powder at its highest content (40 g/kg) within 14 days of exposure. Low mortality rate was recorded at 5 g/kg for the different powders. However, this lowest content (5 g/kg) induced significant mortality with increasing of exposure time. In term of induced mortality, the different products can be ranked as follows: *H. acida* wood ash > 25PG75HA > 50PG50HA > 75PG52HA > *P. glandulosus* leaf powder.

The lethal content of different powders and their combinations reduced; when the exposure period increased (Tab.2). The combinations of *P. glandulosus* leaf powder with *H. acida* at different proportions produced different interactions. The combination made up by 75% of *P. glandulosus* leaf with 25% of *H. acida* wood ash produced synergistic effect whereas that made up by 50% of each of two powders had antagonistic effect.

Tab. 1 Cumulative mortality of *Sitophilus zeamais* adult induced by *Plectranthus glandulosus* leaf powder and *Hymenocardia acida* wood ash and their binary combinations (t = 22.76 ± 2.02° C; r.h. = 69.87 ± 9.93%)

Content (g/kg)	Products *P. glandulosus*	*H. acida*	25PG75HA	50PG50HA	75PG25HA	$F_{(4; 15)}$
1 day						
0	0.00 ± 0.0aA	0.00 ± 0.0cA	0.00 ± 0.00cA	0.00 ± 0.0cA	0.00 ± 0.0bA	-

5	3.82 ± 2.4aB	2.50 ± 1.4bcB	8.75 ± 3.1bcAB	11.25 ± 1.2bAB	21.25 ± 5.9abA	5.1*
10	6.25 ± 2.4aB	5.00 ± 8.7abB	22.50 ± 4.3abAB	20.00 ± 2.0bAB	28.75 ± 6.6aA	5.6*
20	7.50 ± 2.5aB	8.75 ± 1.2aB	25.00 ± 6.1abAB	20.00 ± 3.5bAB	36.25 ± 9.4aA	6.8*
40	16.25 ± 0.9aAB	12.50 ± 1.4aB	36.25 ± 5.5aAB	36.25 ± 5.5aAB	41.25 ± 8.5aA	5.0*
$F_{(4; 15)}$	2.48ns	19.66**	15.83***	40.80***	11.78**	
3 days						
0	0.00 ± 0.0cA	0.00 ± 0.0cA	0.00 ± 0.0cA	0.00 ± 0.0bA	0.00 ± 0.00bA	-
5	6.25 ± 3.1bcB	21.08 ± 3.7bAB	22.90 ± 7.5bAB	37.04 ± 4.4aA	25.06 ± 4.1aA	6.1*
10	11.38 ± 3.1abB	24.57 ± 2.0abAB	42.17 ± 7.2abA	40.92 ± 4.2aA	38.82 ± 8.0aA	6.2*
20	17.83 ± 5.0aB	33.66 ± 2.0aAB	44.42 ± 5.6abA	46.81 ± 7.3aA	40.07 ± 8.0aAB	3.9*
40	21.58 ± 3.9aB	37.41 ± 4.6aAB	57.57 ± 5.8aA	56.45 ± 6.2aA	52.83 ± 7.8aA	7.0*
$F_{(4; 15)}$	12.10***	66.26***	28.53***	40.77***	22.16***	
7 days						
0	0.00 ± 0.00cA	0.00 ± 0.00bA	0.00 ± 0.0bA	0.00 ± 0.00dA	0.00 ± 0.00bA	-
5	7.77 ± 2.6bB	63.55±12.8aA	36.55 ± 10.9aAB	43.71 ± 3.2cAB	36.92 ± 3.1aAB	5.9*
10	14.15 ± 3.2abB	72.69 ± 8.6aA	53.15 ± 9.6aA	58.92 ± 1.3bA	49.27 ± 5.4aA	10.6***
20	20.59 ± 3.7abB	77.96 ± 8.6aA	57.16 ± 9.1aA	69.72 ± 5.3aA	49.42 ± 7.0aA	11.6***
40	28.16 ± 4.7aC	87.11 ± 4.9aA	68.35 ± 4.4aAB	76.76 ± 1.0aA	56.14 ± 4.0aB	27.7***
$F_{(4; 15)}$	16.00***	18.27***	21.54***	202.13***	57.03***	
14 days						
0	0.00 ± 0.00dA	0.00 ± 0.00bA	0.00 ± 0.00cA	0.00 ± 0.00cA	0.00 ± 0.0cA	-
5	19.54 ± 2.6cB	69.08 ± 10.8aA	68.13 ± 10.3bA	63.16 ± 5.3bA	56.07 ± 3.9bA	7.9**
10	33.82 ± 3.5bB	82.60 ± 7.9aA	82.09 ± 3.6bA	80.92 ± 4.6aA	66.89 ± 5.8abA	15.3***
20	40.33 ± 4.1bC	90.64 ± 6.2aA	93.13 ± 1.41aA	82.17 ± 2.7aAB	72.44 ± 4.1abB	27.0***
40	57.24 ± 2.7aC	94.66 ± 3.7aA	94.59 ± 2.1aA	90.42 ± 1.3aAB	80.85 ± 1.4aA	47.3***
$F_{(4; 15)}$	106.53***	26.03***	65.27***	105.05***	122.33***	

Means ± S.E. followed by the same capital letter in a line and the same lowercase letter in a column do not differ significantly at P< 0.05 (Tukey's test), Each datum represents the mean of four replicates of 20 insects each.

ns: P > 0.05, *: P < 0.05, **: P < 0.001, ***: P < 0.0001.

The three combinations of *H. acida* and *P. glandulosus* significantly reduced the production of progeny compared to the control (Tab. 3). From the application of 5 g/kg (lowest content), the number of emerging adults was highly reduced. The highest inhibition of emerging insects was recorded at the highest content (40 g/kg) of the three combinations made up by *P. glandulosus* leaf powder and *H. acida* wood ash; 25PG75HA, 50PG50HA and 75PG25HA inhibited adult F_1 progeny production by 95.49, 83.39 and 80.92, respectively. Generally, the combination 25PG75HA was revealed more effective than the two other (50PG50HA and 75PG25HA). This combination recorded the lowest EC_{50} (2.55 g/kg) whereas the highest EC_{50} was achieved by 75PG25HA (10.69 g/kg).

Tab. 2 Lethal contents and co-toxicity coefficients of binary combinations (t = 22.76 ± 2.02° C; r.h. = 69.87 ± 9.93%)

Products	Slope	R^2	LC50(95% FL) (g/kg)	LC95(95% FL) (g/kg)	Co-toxicity coefficient (CTC)	Significance of CTC	χ2
3 days							
P. glandulosus	0.98 0±0.25	0.973	213.69 (87.60; 2902)β	—			1.50ns
H. acida	0.569±0.20	0.966	135.32 (50.48; 18401)β	—			0.292ns
75PG25HA	0.728 ± 0.19	0.923	34.119 (21.81; 99.05)	6180 (766.378; 4210605)β	547.111	synergistic	0.622ns
50PG50HA	0.525 ± 0.19	0.924	25.27 (14.68; 132.30)	—	21.414	antagonistic	0.349ns
25PG75HA	0.925 ± 0.19	0.904	24.774 (17.92; 42.31)	1485 (377.425; 38229)β	601.378	synergistic	1.342ns
7 days							
P. glandulosus	0.908 ± 0.23	0.999	166.041 (73.016; 1706)	—			0.121ns

H. acida	0.826 ± 0.21	0.988	1.946 (0.280; 3.910)	190.821 (73.336; 3007)β			0.323ns
75PG25HA	0.483 ± 0.19	0.848	18.166 (9.275; 69.952)	—	41.394	antagonistic	0.564ns
50PG50HA	0.946 ± 0.19	0.973	6.798 (3.711; 9.538)	372.549 (138.058; 3695)β	56.589	antagonistic	0.217ns
25PG75HA	0.851 ± 0.19	0.942	10.816 (6.810; 15.197)	928.122 (249.182; 26174)β	23.896	antagonistic	0.545ns
14 days							
P. glandulosus	1.088±0.19	0.976	28.645 (21.379; 46.464)	928.767 (306.688; 9366)β			0.826ns
H. acida	1.268±0.24	0.929	1.936 (0.618; 3.316)	38.341 (25.041; 91.006)			0.195ns
75PG25HA	0.769 ± 0.19	0.981	3.036 (0.605; 5.481)	416.348 (124.349; 15575)β	212.073	synergistic	0.085ns
50PG50HA	1.00 ± 0.22	0.861	1.967 (0.459; 3.634)	86.344 (44.754; 415.547)β	3.937	antagonistic	0.986ns
25PG75HA	1.381 ± 0.25	0.896	2.175 (0.837; 3.509)	33.773 (23.036; 70.093)	116.067	additive	0.583ns

ns: P > 0.05; *: P <0.05, β : the LC values were obtained by extrapolation, # : the Fudicial limit values for LC could not be computed due to very low variations in mortality among the different contents of insecticidal material.

All the treatments significantly reduced grain damage and population increase, compared to the control (Tab. 4). And, the reductions of grain damage and population growth were dose-dependent. The number of insects, grain damage and weight loss decreased when the concentration of powders increased. Concerning the different parameters, a difference was observed in term of effectiveness according to the combination. The number of insects was also considerably reduced. Even at the lowest content (5 g/kg), the three combinations revealed very effective; the grain treated with 25P75HA recorded 21.92 % damaged grain and 3.22 % weight loss whereas the non-treated grain recorded 49.61 % damaged grain and 12.81 % weight loss. At their highest content level (40 g/kg), the damage was almost completely suppressed. 25PG75HA revealed more effective compared to the other combinations.

The germination rate varied with treatment. In general, the non-infested maize grain had a good germination rate than the infested ones (Tab. 5). In non-infested grain, the germination percentage was almost the same; it varied according neither to the combination nor to the content. But, with maize grain infested by *S. zeamais*, the germination rate increased with ascending the dosage. The germination percentage of infested maize grain was highest at the highest content (40 g/kg). Without insect, the germination was significantly higher even without insecticidal powder (94.33 %) whereas with insect the germination rate was the lowest one (21.67 %).

Tab. 3 Progeny production of *Sitophilus zeamais* in maize treated with binary mixtures (t = 22.76 ± 2.02° C; r.h. = 69.87 ± 9.93%)

Content	25PG75HA	50PG50HA	75PG25HA	$F_{(2; 9)}$
	Mean number of F1 adult progeny			
0	42.50 ± 1.71aA	42.50 ± 1.17aA	42.20 ± 1.17aA	—
5	14.25 ± 0.85bB	23.00 ± 2.86bAB	28.50 ± 4.65abA	5.05*
10	9.25 ± 1.65bcB	15.75 ± 1.65bcAB	23.75 ± 3.79bA	7.97*
20	4.75 ± 1.44cdB	8.25 ± 0.63cdAB	14.50 ± 3.80bcA	4.34*
40	2.00 ± 0.82dB	7.00 ± 0.82dA	8.00 ± 1.08cA	12.40*
$F_{(4; 15)}$	145.94***	70.41***	16.23***	
	Inhibition of adult emergence relative to control (%)			
0	0.00 ± 0.00dA	0.00 ± 0.00dA	0.00 ± 0.00dA	—
5	66.51 ± 1.23cA	45.92 ± 6.42cAB	33.27 ± 10.56cB	5.47*

10	78.32 ± 3.87bA	62.88 ± 3.97bAB	44.53 ± 8.27bcB	8.66*
20	88.91 ± 3.47abA	80.42 ± 2.05aAB	66.60 ± 7.45aB	5.31*
40	95.49 ± 1.74aA	83.39 ± 2.32aB	80.92 ± 3.17aB	9.88*
$F_{(4; 15)}$	233.90***	86.76***	19.96***	
EC_{50} (95%FL) g/kg	2.55(1.29; 3.76)	5.57(3.54; 7.39)	10.69(6.74; 15.09)	

Means ± S.E. followed by the same capital letter in a line and the same lowercase letter in a column do not differ significantly at P< 0.05 (Tukey's test). *: P < 0.05, ***: P< 0.0001.

Tab. 4 Population increase of *Sitophilus zeamais* and grain damage recorded in stored maize treated with the binary combinations (t = 22.76 ± 2.02° C; r.h. = 69.87 ± 9.93%)

Content (g/kg)	**Products**					
	25PG75HA	**50PG50HA**	**75PG25HA**	**25PG75HA**	**50PG50HA**	**75PG25HA**
	Number of live insects			Number of dead insects		
0	240.00 ± 12.2a	240.00 ± 12.2a	240.00 ± 12.2a	50.50 ± 8.5a	50.50 ± 8.5a	50.50 ± 8.5a
5	54.75 ± 25.1b	118.50 ± 12.5b	170.00 ± 7.07	69.02 ± 7.5a	58.00 ± 8.9a	63.50 ± 8.4a
10	36.50 ± 4.6b	102.25 ± 2.2bc	128.25 ± 13.4b	69.00 ± 1.3a	62.02 ± 2.7a	64.75 ± 2.6a
20	38.75 ± 7.1b	89.25 ± 5.7bc	72.75 ± 2.5c	59.12 ± 1.4a	75.50 ± 1.5a	82.00 ± 12.7a
40	26.50 ± 7.27b	68.00 ± 10.0c	44.75 ± 11.6c	58.50 ± 0.9a	71.50 ± 2.5a	53.75 ± 5.5a
$F_{(4; 15)}$	45.08***	51.25***	58.05***	2.32ns	3.02ns	2.22ns
	Grain damage			Weight loss (%)		
0	49.61 ± 4.54a	49.61 ± 4.54a	49.61 ± 4.54a	12.81 ± 1.49a	12.81 ± 149a	12.81 ± 1.49a
5	21.92 ± 3.59b	28.02 ± 1.55b	36.81 ± 1.97b	3.22 ± 0.82b	5.07 ± 1.10b	5.87 ± 1.16b
10	18.20 ± 2.54b	25.13 ± 1.43b	30.41 ± 0.72b	3.02 ±1.36b	4.15 ± 0.91b	3.85 ± 1.14b
20	17.25 ± 2.60b	20.12 ± 3.01b	27.95 ± 0.68bc	2.94 ± 1.29b	3.92 ± 1.00b	3.43 ± 0.23b
40	13.56 ± 1.17b	17.20 ± 3.2b	17.60 ± 2.0c	1.54 ± 1.1b	3.09 ± 0.7b	2.79 ± 0.6b
$F_{(4; 15)}$	22.25***	18.21***	23.68***	13.83***	13.86***	15.93***

Means ± S.E. followed by the same lowercase letter in a column do not differ significantly at P< 0.05 (Tukey's test); ns: P > 0.05, *: P < 0.05, ***: P < 0.0001.

Tab. 5 Germination of stored grains treated with binary combinations of *Hymenocardia acida* wood ash with *Plectranthus glandulosus* leaf powder and infested and non-infested by *Sitophilus zeamais* in laboratory conditions (t = 22.76 ± 2.02° C; r.h. = 69.87 ± 9.93%)

Content (g/kg)	**Products** **25PG75HA** Non-infested	**50PG50HA**	**75PG25HA**	**25PG75HA** Infested	**50PG50HA**	**75PG25HA**
0	94.33 ± 1.3a	94.33 ± 1.3a	94.33 ± 1.3a	21.67 ± 2.1d	21.67 ± 2.1d	21.67 ± 2.1c
5	91.08 ± 0.2a	90.83 ± 1.5a	94.83 ± 1.9a	84.67 ± 0.8c	81.67 ± 3.1c	78.33 ± 0.9b
10	92.00 ± 0.8a	94.50 ± 1.8a	92.83 ± 0.8a	88.33 ± 1.7bc	84.17 ± 2.5bc	83.33 ± 1.3ab
20	92.17 ± 0.7a	94.17 ± 1.5a	93.42 ± 1.6a	92.33 ± 0.8ab	93.33 ± 1.3a	86.67 ± 2.4ab
40	91.17 ± 0.3a	92.08 ± 0.7a	92.20 ± 1.7a	97.50 ± 1.6a	95.00 ± 1.7a	90.00 ± 2.3a
$F_{(4; 15)}$	2.72ns	1.3ns	0.41ns	430.01***	180.43***	218.78***

Means ± S.E. followed by the same lowercase letter in a column do not differ significantly at P< 0.05 (Tukey's test); ns: P > 0.05; ***: P< 0.0001.

Discussion

The binary mixtures of *P. glandulosus* leaf powder and *H. acida* wood ash provoked significant mortality of *S. zeamais*. The combination of insecticidal materials has the advantages to increase efficacy by complementing the bio-efficacy of the individual products and simultaneously lowering their doses on the one hand, broadening the spectrum of activity and reducing the chance of resistance development, on the other hand (Das, 2014). However, with mixtures, negative effects

can also occur such as reduction of efficacy, phyto-toxicity and incompatibility problems between materials (Regupathy, 2004). The combinations of 75% of *P. glandulosus* leaf powder with 25% of *H. acida* on *S. zeamais* mortality produced synergistic effect, whereas combination made up by 50% of *P. glandulosus* leaf and 50% *H. acida* wood ash induced antagonistic effect within 14 days, it produced a significant synergism. In general, the mixtures composed by different insecticidal materials improved in efficacy. The additive effect was also observed, the effect of two materials is equal to the sum of each component given alone (1+3=4), which was observed in the present study by the combination 25PG75HA (25% *P. glandulosus* leaf powder and 75% *H. acida* wood ash) within 14 days of exposure.

The proportions of two products used in combinations can produce different performances according to the involved proportions. The combinations of 75PG25HA and 50PG50HA produced respectively synergistic and antagonistic effect. The same tendency concerning the variations in efficacy for different proportions of same products was observed by Ntonifor etal. (2010); these authors found that the combinations of *Syzygium aromaticum* (L.) (Myrtaceae) and *Cyperus aequelis* (Vahl) (Cyperaceae) at the proportions of 0:2, 0.5:1.5, 1:1, 2:0 (g:g) induced 36.3%, 93.8%, 98.8%, 100% mortality of *C. maculatus*, respectively, within 3 days of exposure.

The three binary combinations of *P. glandulosus* leaf powder and *H. acida* wood ash considerably inhibited the production of *S. zeamais* progeny. In addition to increasing mortality, the combinations of these products have an effect on *S. zeamais* development. The presence of *P. glandulosus* leaf powder in combinations may potentiate the effect of ash. There are physical and chemical action, which are the desiccation by ash and poisoning by the chemical compounds contained in *P. glandulosus* leaf. Mixtures can disturb or delay the development of larvae in adults. Karso and Al Mallah (2014) found that the mixture of soya oil and Acetamprid pesticide gave the highest average mortality of *Trogoderma granarium* larvae and which varied according to the proportions.

The combinations of insecticidal materials improve the protection of stored grain by reducing the qualitative and quantitative losses. The reduction of damage and the suppression of *S. zeamais* population growth were positively correlated. Combinations of *H. acida* wood ash with *P. glandulosus* leaf powder at different proportions considerably reduced damage, by lowering the number of perforated grains and weight loss, and at the same time by inhibiting the population increase. Hill (1990) reported that wood ash was useful as a physical barrier on the grain. However, it can also possess various chemical properties according to its botanical source. *P. glandulosus* leaf, thanks to its chemical compounds controlled the proliferation of insect, which explain the efficacy of combinations in short storage period. When the storage period increased, the efficacy decreased by loss of their volatile compounds which confer its toxicity against insects. Similar findings were reported by Mwangangi and Mutsiya (2013), who showed that the efficacy of *Ocimum basilicum* Linnaeus (Lamiaceae) powder deteriorated the fastest leading to 80, 77, 44, 20 and 15 % mortality over 0, 7, 14, 21 and 28 days of storage.

In many African countries, stored grains provide not only grains for food but also seeds for planting. The untreated maize in presence of insects recorded the week germination rate. In this case the seed loss their germination ability due to the high *S. zeamais* infestation that lays its eggs on grain. The larvae develop and feed inside the grain by consuming the germ of the seed, thus diminishing the viability of the seeds. Usha Rani and Devanand (2011) found that seed germination was significantly reduced when untreated maize seeds were exposed to *S. oryzae* and *T. castaneum*. Higher levels of the products improved their ability to protect grain, leading to a greater germination capacity. The different powders did not present any adverse effect on maize seed germination.

In the present study, no adverse effect was observed on germination ability. But, some findings reported the inhibiting effect of some plant extracts on seed germination (Chung and Miller, 1995; Bustos-Figueroa etal., 2009). The application of lower concentrations of *Murraya koenigii* Linnaeus (Rutaceae) and *Capsicum annuum* Linnaeus (Solanaceae) extracts caused a normal germination, but

the same plants at higher concentrations caused 30-35% inhibition of seed germination. Bustos-Figueroa etal. (2009) observed that the leaf powder of *Peumus boldus* used alone or mixed with lime did not affect the percentage of maize seed germination. This corroborates our findings about the germination rate recorded with the treatments. Higher germination rate recorded by the combinations 25% of *P. glandulosus* leaf powder with 75% of wood ash could be due to the higher content of ash in the combination. According to Philogène (1972), the ash does not affect germination but could enhance growth because of the cations that it contains. *H. acida* wood ash contains high quantity of Ca, K, P, Na, Fe, which are important for plant growth. Parimelazhagan and Francis (1999) found that leaf extracts of *Cerastium viscosum* Linnaeus (Caryophyllaceae) increased seed germination and improved seedling development of rice seeds. In general, grains in storage facilities lost their viability and germination chances as the post-harvest storage period increases (Hedimbi *et al.*, 2012). That could explain the loss of viability partly even when the seeds do not have damage. The combinations protected the maize grains against the destruction of their germination capacity by weevils and they did not influence negatively seed germination.

The binary combinations of *P. glandulosus* leaf powder and *H. acida* wood ash at different proportions effectively protect maize grain against infestation by *S. zeamais* in storage. The binary combinations permit to the maize grains to conserve their viability without affecting negatively germination rate. The beneficial effect of the combinations could be enhanced by using the appropriated proportions. Then, other proportions in combination of the two powders need to be tested in order to find out the most efficient combination. Further studies need to be carried out concerning mammalian toxicity that could be attributed to the use of these products in grain storage. Also, the investigations need to be undertaken in order to assess the effect of these powders on the organoleptic and technological properties of treated grains.

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Comparative Lethality of Rice Husk Ash and a Diatomaceous Eartht Adults of Four Storage Beetles

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Abstract

Lethality of rice husk ash (RHA) and a diatomaceous earth (SilicoSec) (DE) to adults of *Sitophilus zeamais*, *S. granarius*, *Lasioderma serricorne* and *Callosobruchus maculatus* was investigated under controlled conditions of 25 ± 2° C and 60 ± 3% relative humidity. Each product was tested at 0.05 g to 0.5 g/20 g of grain respectively in glass Petri dishes against 20 adults of each beetle. Adult mortality was observed up to 10 days post treatment. RHA/DE mixtures (1:1, 3:1 and 1:3 ratios) were also tested at 2% of grain weight. Additionally, RHA and DE were tested at low dosages (0.01 g to 0.04 g/20 g) against adults of *C. maculatus* alone. The DE generally produced significantly higher mortality of all the adult storage beetles and at earlier observation times, than RHA at the lower dosages (< 0.2 g). Adult mortality produced by RHA and DE in *S. zeamais* and *S. granarius* increased with increase in dosage from 0.05 g to 0.5 g. The RHA/DE mixtures generally produced similar mortality of all the adult storage beetles irrespective of post-treatment exposure time. The *S. zeamais* and *S. granarius* were generally more tolerant to the DE and RHA treatments than *L. serricorne* and *C. maculatus*. Percentage mortality of *C. maculatus* adults when DE was applied at low dosages (0.01 g to 0.04 g) was generally higher than RHA applied at similar dosages, up to 3 days-post treatment. All treatments produced 100% mortality of *C. maculatus* adults 4 days-post treatment. The data further confirm the efficacy of DE and RHA as insecticidal dusts at the dosage rate of 0.5 g or more per kg of grain.

Keywords: Rice Husk Ash; diatomaceous earth (Silico Sec); lethality; storage beetles

1. Introduction

In developing countries losses caused by insect pests may reach 6.5% or more of stored grain (Raju, 1984), making control imperative. Control of these insects by synthetic chemical insecticides is effective, but has several drawbacks such as increasing costs, inconsistent supplies and hazards to man and the environment (Ofuya, 2003). Inert dusts such as ash and diatomaceous earths may be suitable alternatives to contact insecticides from the point of view of resource poor farmers (Stathers et al., 2008). Rice husk ash appears to be especially effective in the control of stored products insect pests (Tee, 1981; Ofuya and Adler, 2014). Diatomaceous earth is an inert dust of almost pure amorphous silicon dioxide and made up of fossilized diatoms; and has been variously applied for the management of stored-product pests with good results (Shah and Khan, 2014; Perisic, 2018). The main ingredient of rice husk ash is silica (SiO_2), accounting for more than 90% of the total content, and therefore similar in composition as diatomaceous earth. However, direct comparison of any diatomaceous earth and rice husk ash in stored products protection against insect infestation has scarcely been reported. This paper reports the results of a study comparing the lethality of rice husk ash and a diatomaceous earth, Silico Sec to adults of four storage beetles namely *Sitophilus zeamais* Mots. *S. granarius* L., *Lasioderma serricorne* F. and *Callosobruchus maculatus* F.

2. Materials and Methods

The study was carried out at the Federal Research Centre for Cultivated Plants, Institute of Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany under controlled conditions of 25 ± 2° C and 60 ± 3% relative humidity.

Insects

The storage insects tested in the study are the cowpea seed beetle, *Callosobruchus maculatus* Fabricius, the maize weevil, *Sitophilus zeamais* Mots., the granary weevil, *S. granarius* L. and the cigarette beetle, *Lasioderma serricorne* Fabricius. Their cultures are maintained at Institute of

Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany using standard procedures (e.g. Tofel *et al*., 2015). The *C. maculatus* was tested using blackeye cowpea whilst *S. zeamais*, *S. granarius* and *L. serricorne* were tested on yellow maize.

Paddy Husk Ash (RHA)

Paddy husk was obtained from a processing mill in Emure in Ekiti State, Nigeria (7.4500° N, 5.4667° E) and rice variety was Igbemo local grown by communities around the metropolis. Paddy husk was first pulverized in an electric blender into coarse powder which was thereafter converted to ash material in electric oven at 550° C for three hours. The husk ash was pulverized in a laboratory mill into a fine powder with particle size of ≤ 150 µm using a British standard sieve (Ofuya and Dawodu, 2002). The ash powder (approximately 100 g) was then put in a plastic container with tight fitted lid.

Diatomaceous Earth (DE)

The diatomaceous earth (DE) used was SilicoSec, a natural silica powder obtained from processed fossilized diatoms. It is composed of 96% amorphous SiO_2 with particle size between 13 µm to 15 µm (Erb-Brinkmann, 2000).

Effect of high dosages of RHA and DE on mortality of adult beetles

Twenty unsexed adults of *C. maculatus* (< 2 days old), *L. serricorne* (< 1 week old), *S. zeamais* (< 2 weeks old) and *S. granarius* (< 2 weeks old) were separately dusted by shaking with either rice husk ash powder (RHA) or SilicoSec (DE) in clear glass Petri dishes (9.0 cm diameter) containing 20 g of cowpea seeds for *C. maculatus* and maize grain for the other beetle species. Each product was tested 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 g respectively. There was a control treatment with neither RHA nor DE. Adult mortality was observed daily for up to 10 days. The experiment was replicated three times.

Effect of combining RHA and DE on mortality of adult beetles

Twenty unsexed adults of *C maculatus* (< 2 days old), *L. serricorne* (< 1 week old), *S. zeamais* (< 2 weeks old) and *S. granarius* (< 2 weeks old) were separately dusted by shaking with mixtures of RHA and DE in three ratios (1:1, 3:1 and 1:3) in clear glass Petri dishes (9.0 cm diameter) containing 20 g of cowpea seeds for *C. maculatus* and maize grain for the other beetle species. Each mixture was tested at 0.4 g. There was a control treatment with no protectant. Adult mortality was observed daily for up to 10 days. Each treatment was replicated three times.

Effect of low dosages of RHA and DE on mortality of *C. maculatus* adults

Twenty unsexed adults of *C maculatus* (< 2 days old) was dusted by shaking with either rice husk ash powder (RHA) or SilicoSec (DE) in clear glass Petri dishes (9.0 cm diameter) containing 20 g of cowpea seeds. Each product was tested 0.01, 0.02, 0.03 and 0.04 g respectively. There was a control treatment with neither RHA nor DE. Adult mortality was observed daily for up to four days and each treatment was replicated three times

Data analysis

Data were analyzed using the SigmaStat® 3.5 software (Systat Software GmbH, Germany). Mortality data, where necessary, were corrected as recommended by Abbott (1925). Percentage data were arcsine transformed and subjected to one-way analysis of variance (ANOVA. Where the ANOVA indicated significant difference between treatments, least significant difference (LSD) method was used to separate the means at 5% level of probability.

3. Results

Three-days post treatment *S. zeamais* and *S. granarius* suffered higher mortality in DE treated seeds than in the RHA treated seeds with each dosage except with 0.5 g of ash that produced a higher kill

of *S. zeamais* than the DE counterpart (Table 1). Five-days post treatment mortality of *S. zeamais* was generally similar to RHA and DE treatments, but DE treatments produced significantly higher kill of *S. granarius* with the 0.05 g and 0.1 dosages in comparison with the RHA counterparts. The trend in adult mortality observed 7 days post treatment was similar to that recorded 5 days post treatment. Ten-days post treatment mortality of *S. zeamais* was generally similar with RHA and DE treatments, but DE treatments produced significantly higher kill of *S. granarius* with the 0.05 g, 0.1 and 0.2 dosages in comparison with the RHA counterparts. Both RHA and DE produced higher adult mortality in *S. zeamais* and *S. granarius* with increase in dosage from 0.05 g to 0.5 g.

For 1-day post treatment all the DE dosages (0.05 g to 0.5 g) produced 100% mortality in adults of *L. serricorne* and *C. maculatus* which was significantly higher than mortality produced by similar dosages of RHA except 0.4 g and 0.5 g.

for *L. serricorne* and 0.3 g, 0.4 g and 0.5 g for C. *maculatus* (Table 2). For 2-days post treatment the DE dosages also produced 100% mortality in adults of *L. serricorne* and *C. maculatus* which was significantly higher than mortality produced by similar dosages of RHA except 0.3 g, 0.4 g and 0.5 g for both beetle species. Similarly, for 3-days post treatment all the treatments produced 100% mortality in *L. serricorne* and *C. maculatus* except in the case of *C. maculatus* exposed to 0.05 g RHA where 81.7% mortality was recorded.

Mean % mortality of *S. zeamais* and *S. granarius* was not significantly different irrespective of the ratio of mixing RHA and DE (1:1, 3:1 or 1:3) for use against these insects, 3-days post treatment except with the 3:1 ratio against *S. granarius* where 16.7% mortality was recorded (Table 3). At 5-days post treatment the RHA/DE (3:1) produced significantly the highest of 76.7% of S. *granarius*. A similar trend was observed at 7 and 10-days post treatment.

The 1-day post treatment the RHA/DE mixtures produced similar mortality of *L. serricorne* (ranging from 23.3% to 30.0%) which was significantly lower than mortality produced by the same mixtures in *C. maculatus* (ranging from 60.0% to 75.0%) (Table 4). A similar trend was observed at 2 and 3-days post treatment. At 5-days post treatment, all the RHA/DE mixtures produced 100% mortality in both *L. serricorne* and *C. maculatus*. Mean % mortality of *C. maculatus* adults when DE was applied at low dosages (0.01 g to 0.04 g) was significantly higher than RHA applied at similar dosages during 1, 2 and 3-days post treatment except RHA applied at 0.04 g which produced 100% mortality 3-days post treatment as in DE treatments (Table 5). All the treatments produced 100% mortality of *C. maculatus* adults at 4-days post treatment. For the RHA treatments, mortality increased significantly with increase in dosage except on the 4th day post treatment.

Table 1. Mortality of adults of *S. zeamais* and *S. granarius* in RHA and DE applied at different dosages

Protectant/ Insect	Dosage (g/20 g of grain)	Mean % mortality in: 3 days	5 days	7 days	10 days
RHA/*S. zeamais*	0.05	13.3 ± 3.33ab	28.3 ± 10.93abc	41.7 ± 4.41ab	61.7 ± 7.27bc
	0.1	30.0 ± 2.89cd	46.6 ± 1.67cdef	65.0 ± 2.89cdefg	86.7 ± 1.67efg
	0.2	36.7 ± 1.67cd	65.0 ± 7.64fghi	66.7 ± 4.41defgh	96.7 ± 1.67g
	0.3	43.3 ± 8.33def	66.7 ± 10.14fghi	76.7 ± 10.14efghij	93.3 ± 4.41fg
	0.4	63.3 ± 1.67ghi	73.3 ± 1.67ghi	100.0 ± 0.00k	100.00 ± 0.00g
	0.5	76.7 ± 10.93ij	95.0 ± 5.00j	100.0 ± 0.00k	100.00 ± 0.00g
DE/*S. zeamais*	0.05	21.7 ± 1.67bc	30.0 ± 5.77bcd	51.7 ± 4.41bcd	70.0 ± 2.89cd
	0.1	35.0 ± 2.89cd	51.7 ± 3.33defg	70.0 ± 2.89defghi	95.0 ± 2.89fg
	0.2	41.7 ± 8.33def	63.3 ± 1.67fghi	76.7 ± 4.41efghij	96.7 ± 3.33g
	0.3	70.0 ± 2.89ghi	75.0 ± 2.89hij	86.7 ± 4.41ghijk	100.0 ± 0.00g
	0.4	76.7 ± 1.67ij	81.7 ± 3.33ij	100.0 ± 0.00k	100.00 ± 0.00g
	0.5	90.0 ± 2.89j	98.3 ± 1.67j	100.0 ± 0.00k	100.00 ± 0.00g
RHA/*S. granarius*	0.05	0.0 ± 0.00a	6.7 ± 1.67a	21.7 ± 6.01a	26.7 ± 3.33a
	0.1	10.0 ± 2.89ab	15.0 ± 2.89ab	43.3 ± 13.02abc	48.3 ± 10.14b
	0.2	21.7 ± 6.67b	40.0 ± 5.00cde	55.0 ± 2.89bcde	88.3 ± 3.33fg
	0.3	36.7 ± 8.82cd	50.0 ± 5.77cdefg	76.7 ± 6.01efghij	90.0 ± 2.89fg
	0.4	55.0 ± 2.89efg	71.7 ± 4.41ghi	88.3 ± 4.41hijk	100.00 ± 0.00g

	0.5	60.0 ± 2.89fgh	68.3 ± 1.67fghi	90.0 ± 2.89ijk	100.00 ± 0.00g
DE/*S. granarius*	0.05	31.7 ± 1.67cd	50.0 ± 2.89cdef	63.3 ± 4.41bcdef	73.3 ± 3.33cde
	0.1	56.7 ± 4.41efgh	68.3 ± 4.41fghi	76.7 ± 3.33efghij	81.7 ± 1.67def
	0.2	30.0 ± 2.89cd	56.7± 1.67efgh	78.3 ± 4.41fghijk	96.7 ± 1.67g
	0.3	56.7 ± 4.41efgh	68.3 ± 4.41fghi	86.7 ± 1.67ghijk	100.0 ± 0.00g
	0.4	71.7 ± 1.67hi	76.7 ± 3.33hij	91.7 ± 1.67ijk	100.00 ± 0.00g
	0.5	68.3 ± 7.27ghi	75.0 ± 5.00hij	93.3 ± 1.67jk	100.00 ± 0.00g
LSD 0.001		15.39	21.71	21.94	14.91

Along each column means bearing similar letters are not significantly different

4. Discussion

The results of this study showed that DE was generally more toxic to *S. zeamais* and *S. granarius* than RHA. DE produced 100% mortality in adults of these two beetles 10 days post-treatment at the dosage of 0.3 g or more per 20 g of grain whereas it required 0.4 g or more of RHA to achieve the same level of mortality. Similarly, the DE was generally more toxic to *L. serricorne* and *C. maculatus* than RHA. It was further observed that mortality of *C. maculatus* adults when DE was applied at low dosages (0.01 g to 0.04 g) was generally higher than RHA applied at similar dosages. Demissie et al. (2008) reported that diatomaceous earth was superior to wood ash in the control of *S. zeamais*. Our results may support the assertion by Shah and Khan (2014) that DE is probably one of the most efficacious natural dusts used as an insecticide. Sadeghi et al. (2012), however, did not record overwhelming superiority in lethality of Sayan®, a DE, to adults of six stored products insects including *S. zeamais*, *L. serricorne* and *C. maculatus* when compared with bran and sawdust. The DE used in this study is SilicoSec, composed of 96% amorphous SiO_2 with particle size between 13 µm to 15 µm (Erb-Brinkmann, 2000). Sayan® DE formulation contains 92% SiO_2 and an average particle size of 50 µm (Sadeghi et al., 2012). Differences in chemical and physical properties of insecticidal dusts can influence their efficacies (Dawodu and Ofuya, 2002; Olotuah et al., 2010; Shah and Khan, 2014).

Species variation in susceptibility to DE and RHA treatment was clearly observable in this study. Adults of *S. zeamais* and *S. granarius* were less susceptible to DE and RHA than those of *L. serricorne* and *C. maculatus*. For example, irrespective of dosage DE killed all introduced *L. serricorne* and *C. maculatus* adults within 1 day post treatment whereas a DE dosage of 0.4 g or more per 20 g of grain required 7 days to kill all introduced *S. granarius* and *S. zeamais* adults. Also, whilst 0.4 g dosage of RHA killed all introduced *L. serricorne* and *C. maculatus* adults within 2 days post treatment, the same dosage of RHA required 10 days to kill all introduced *S. granarius* and *S. zeamais* adults. Observations that stored products insects show a wide range of susceptibility to inert dusts have been reported by some other workers (Athanassiou et al., 2005; Sadeghi et al., 2012; Doumbia et al., 2014). Differences in susceptibility to inert dusts by insects could be due to size, quantitative or qualitative differences in cuticular lipids, differences in agility through grain, behavioural responses to the dusts or resistance to desiccation (Shah and Khan, 2014).

Ofuya and Adler (2015) observed that DE could be mixed with insecticidal plant powders without jeopardizing its lethality against four different adult storage beetles. Indeed mixing with DE was thought to have putatively increased the lethality of *Piper guineense* Schum & Thonn dry fruit and rice husk powders to the adult beetles. Ofuya et al. (2015) reached a similar conclusion. However, the results of this study indicate that there may be no advantage in mixing DE and RHA for stored products protection against insect infestation in terms of adult mortality. The DE and RHA may not have been physically homogeneous partly due to inherent differences in particle size. RHA has been reported to contain a large amount of needle-like particles presumably derived from setae covering the outer surface of the rice husk which may putatively trigger a physical reaction on the integument of insects that eventually results in their

death (Ofuya and Adler, 2014). It is hereby hypothesized that DE may have obliterated the activity of these needle-like particles in the RHA/DE mixtures, thus decreasing the ability to cause death of the insects.

Overall, data had been provided that further confirm the efficacy of DE and RHA as insecticidal dusts at the dosage rate of 0.5 g or more per kg of grain. The DE was observed to be generally more lethal to the beetles than RHA. *S. zeamais* and *S. granarius* were generally more tolerant to the DE and RHA treatments than *L. serricorne* and *C. maculatus*. For *C. maculatus* there is the possibility of achieving good control at lower dosage rate of DE and RHA of less than 0.5 g per kg of grain.

Table 2. Mortality of adults of *L. serricorne and C. maculatus* in RHA and DE applied at different dosages

Protectant/Insect	Dosage (g/20 g of grain	Mean % mortality (± SE) in: 1 day	2 days	3 days
RHA/*L. serricorne*	0.05	25.0 ± 2.89b	80.0 ± 2.89b	100.0 ± 0.00b
	0.1	31.7 ± 4.41b	83.3 ± 4.41b	100.0 ± 0.00b
	0.2	68.3 ± 4.41cd	93.3 ± 1.67c	100.0 ± 0.00b
	0.3	75.0 ± 2.89de	100.0 ± 0.00d	100.0 ± 0.00b
	0.4	93.3 ± 1.67fg	100.0 ± 0.00d	100.0 ± 0.00b
	0.5	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
DE/*L. seericorne*	0.05	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
	0.1	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
	0.2	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
	0.3	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
	0.4	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
	0.5	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
RHA/*C. maculatus*	0.05	10.0 ± 5.77a	55.0 ± 2.89a	81.7 ± 4.41a
	0.1	33.3 ± 8.82b	81.7 ± 1.67b	100.0 ± 0.00b
	0.2	33.3 ± 4.41b	90.0 ± 2.89c	100.0 ± 0.00b
	0.3	55.0 ± 5.77c	100.0 ± 0.00d	100.0 ± 0.00b
	0.4	83.3 ± 1.67ef	100.0 ± 0.00d	100.0 ± 0.00b
	0.5	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
DE/*C. maculatus*	0.05	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
	0.1	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
	0.2	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
	0.3	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
	0.4	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
	0.5	100.0 ± 0.00g	100.0 ± 0.00d	100.0 ± 0.00b
LSD 0.001		13.99	6.60	4.11

Along each column means bearing similar letters are not significantly different

Table 3. Mortality of adults of *S. zeamais* and *S. granarius* in RHA and DE in mixed formulations but applied in a single dosage of 2% of protected grain weight

Insect	Ratio (RHA:DE)	Mean % mortality (± SE) in: 3 days	5 days	7 days	10 days
S. zeamais					
	1:1	40.0 ± 7.64b	48.3 ± 10.14ab	63.3 ± 6.00a	76.7 ± 4.41a
	3:1	46.6 ± 4.41b	56.7 ± 3.33b	75.0 ± 2.89b	85.0 ± 2.89ab
	1:3	43.3 ± 8.82b	48.3 ± 8.33b	56.7 ± 4.41a	76.7 ± 4.41a
S. granarius					
	1:1	31.7 ± 7.27ab	50.0 ± 2.89ab	78.3 ± 1.67b	90.0 ± 2.89b
	3:1	16.7 ± 3.33a	38.3 ± 4.41a	68.3 ± 4.41ab	83.3 ± 3.33ab
	1:3	43.3 ± 3.33b	76.7 ± 3.33c	90.0 ± 2.89c	100.0 ± 0.00c
LSD 0.05		15.62	15.34	10.00	8.40

Along each column means bearing similar letters are not significantly different

Table 4. Mortality of adults of *L. serricorne* and *C. maculatus* in RHA and DE in mixed formulations but applied in a single dosage of 2% of protected grain weight

Insect	Ratio (RHA:DE)	Mean % mortality (± SE) in: 1 day	2 days	3 days	5 days

L. serricorne					
	1:1	26.7 ± 7.27a	50.0 ± 2.89ab	75.0 ± 2.89a	100.0 ± 0.00
	3:1	23.3 ± 6.00a	45.0 ± 5.00a	75.0 ± 2.89a	100.0 ± 0.00
	1:3	30.0 ± 5.77a	55.0 ± 2.89b	83.3 ± 4.41a	100.0 ± 0.00
C. maculatus					
	1:1	66.0 ± 3.33bc	90.0 ± 2.89c	100.0 ± 0.00b	100.0 ± 0.00
	3:1	60.0 ± 5.77b	85.0 ± 2.89c	100.0 ± 0.00b	100.0 ± 0.00
	1:3	75.0 ± 2.89c	93.3 ± 1.67c	100.0 ± 0.00b	100.0 ± 0.00
LSD 0.05		13.61	8.04	7.47	Ns

Along each column means bearing similar letters are not significantly different

Table 5. Mortality of adults of *C. maculatus* in RHA and DE applied at different low dosages

Protectant	**Dosage (g/20 g of grain**	**Mean % mortality (± SE) in: 1 day**	**2 days**	**3 days**	**4 days**
DE					
	0.01	86.7 ± 3.33e	96.7 ± 3.33e	100.0 ± 0.00c	100.0 ± 0.00
	0.02	100.0 ± 0.00f	100.0 ± 0.00e	100.0 ± 0.00c	100.0 ± 0.00
	0.03	100.0 ± 0.00f	100.0 ± 0.00e	100.0 ± 0.00c	100.0 ± 0.00
	0.04	100.0 ± 0.00f	100.0 ± 0.00e	100.0 ± 0.00c	100.0 ± 0.00
RHA					
	0.01	6.7 ± 1.67a	35.0 ± 2.89a	83.3 ± 4.41a	100.0 ± 0.00
	0.02	15.0 ± 2.89b	50.0 ± 2.89b	81.7 ± 4.41a	100.0 ± 0.00
	0.03	35.0 ± 2.89c	71.7 ± 1.67c	93.3 ± 1.67b	100.0 ± 0.00
	0.04	41.7 ± 3.33d	83.3 ± 4.41d	100.0 ± 0.00c	100.0 ± 0.00
LSD 0.05		5.64	6.17	5.64	Ns

Along each column means bearing similar letters are not significantly different

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Effects of different inert dusts on *Sitophilus oryzae* and *Plodia interpunctella* during contact exposure

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Abstract

The use of natural inert dusts against storage insect pests is increasing recently, as an alternative to conventional insecticides. Laboratory study was carried out to evaluate the contact effect of three inert dusts, diatomaceous earth (DE), kaoline (KA) and vermiculite (VE), at rates 5, 7.5, 10, 15 and 20 gm^{-2}, against adults of *Sitophilus oryzae* (L.) and larvae of *Plodia interpunctella* (Hubner). Insect mortality was evaluated 1, 2, 3 and 7 days after the exposure. Insect mortality varied depending on the species, concentrations and exposure periods. The DE and KA caused 86.7-98% mortality of *S. oryzae* after 2 days of exposure at the highest rates, while at 5 and 7.5 gm^{-2}, 100% mortality was achieved only after 7 days. The highest rates of inert dusts caused 42-50% (DE) and 60-75% (KA) mortality of *P. interpunctella* larvae only after 7days. The mortality of moths increased gradually with the concentration and 100% was achieved 3 days after the contact with DE and KA (10, 15 and 20 g m^{-2}). However, inert dusts induced faster pupation of *P. interpunctella*, while adult emergence was reduced and adults had smaller body-sizes, compared to control. The VE caused relatively low mortalities (7-11% of *S. oryzae* adults and 5-8% of *P. interpunctella* larvae) at all tested rates during the entire experiment. Our results have shown good insecticidal effect of DE and KA against *S. oryzae* and *P. interpunctella* at 10, 15 and 20 gm^{-2}. These products could therefore be used by small-scale farmers to protect stored grains against insect pest infestation.

Key words: Inert dusts, *Sitophilus oryzae, Plodia interpunctella*, contact exposure, diatomaceous eartth

Introduction

In recent years, the use of contact insecticides and fumigants for controlling storage pests is under increasing restriction due to the presence of residues in food and development of insect resistance (Collins, 2000; Kljajić and Perić, 2005). These shortcomings have stimulated the need for testing and evaluation of non-toxic methods that can replace conventional insecticides in stored grains (Arthur, 1996). Recently, physical control methods, like the use of inert dusts, have become prominent (Field and Korunić, 2002). These materials are classified into different groups depending on their composition and particle size and include materials such as diatomaceous earth, silicophosphate, rock phosphate, sand, kaolinite, clay etc. (Golob, 1997). There is a growing interest especially in desiccant or absorptive dusts, among which, diatomaceous earth is the most widely used in practice worldwide (Golob, 1997; Korunić, 1998a; Subramanyam and Roesli, 2000) and in commercial storages in the developed world. On the other hand, non-silica dusts and those composed of coarse grain silicates, such as kaoline and sand, have been used traditionally as grain protectants by small-

scale farmers in the developing world (Golob, 1997). Inert dusts, regardless of the group, can control a variety of common storage insect pests. Korunić (2013) reported that there are three areas of DE use: a) admixture with grain, b) as structural treatment on walls and floors and c) addition of DE to the surface of grain bulks. However, inert dusts, primarily DE, was found to reduce the grain bulk density, affects the flow characteristics of bulk grain (flowability), and also leaves visible dust residues (Subramanyam et al., 1998; Golob, 1997; Korunić et al., 1998b) and heath concerns (Korunić, 2016). Thus, DE is highly recommended for surface treatments and in general, it is thought that DE should be used primarily as a preventive measure for grain protection and not as a curative measure. In Australia, preparation based on DE (Dryacide) is frequently applied as a structural treatment in empty storages, and on grain bulk surfaces (Aleen, 2001). This work aimed to assess the contact efficacy of three different inert dusts (diatomaceous earth, kaolin and vermiculite) against *S. oryzae* adults and *Plodia interunctella* larvae, as additional preventive measure during storage.

Material and methods

Laboratory studies were conducted to evaluate the contact effect of three inert dusts: diatomaceous earth-DE (commercial preparation SilicoSec, produced by Biofa, uncalcinated diatomite), kaoline clay - KA and vermiculite dust - VE as contact insecticides against *Sitophilus oryzae* adults and 3rd instar larvae of *Plodia interunctella*. Inert dusts were applied on glass Petri dishes (surface area, 153.5 cm^2) at rates 5, 7.5, 10, 15 and 20 gm^{-2}. The dusts were dispersed over the glass surface and 20 (2-4-weeks old) adult weevils or 3th instar larvae of *P. interunctella* were put into dishes. Mortality was evaluated after 24, 48, 72 h and 7 days of exposure. Clean Petri dishes served as the control. The LC_{50} and LC_{90} were calculated using Probit analysis in SPSS 21.

Results

The effect of DE, KA and VE applied at 5, 7.5, 10, 15 and 20 gm^{-2} on *S. oryzae* adults are presented on Figs. 1-3. In all the inert dust treated jars, insect mortality increased with the increase in concentration and exposure period. After 24 h, DE caused significant mortality at rates 10 (68%), 15 (73.5%) and 20 gm^{-2} (98%), respectively. The mortality increased after 48h of exposure and ranged from 45% at the lowest rate (5 gm^{-2}), 67% at 7.5 gm^{-2} to 100% at the highest rates. After 72 h, 100% mortality was achieved in treatments with 10, 15 and 20 gm^{-2} of DE, while satisfactory mortality was also obtained in treatments with 5 and 7.5 gm^{-2} DE (68 and 93%, respectively). After 7 days of exposure mortality was 88-100%. The difference between mortalities depending on the type of inert dust and rates, within the same exposure period (24, 48, 72 h and 7 days) was statistically highly significant (F=57.66**; 102.12**, 93.02** and 145.29**, respectively, p<0.01). earth.

The KA caused significant mortality after 24h only at rates 10, 15 and 20 gm^{-2} (75, 98 and 100%, respectively). However, after 48 h of exposure, the increase in mortality was recorded at all rates, namely 53, 65, 89, 97 and 100%, respectively, while after 72 h it ranged from 97.8-100%. The total mortality (100%) was caused by KA after 7 days for all the rates applied. Like in the case of DE, the difference between mortalities caused by different inert dusts and rates, was statistically highly significant within the same exposure period i.e. after 24, 48, 72 h and 7 days (F=103.09**; 123.54**, 107.23** and 225.62**, respectively, p<0.01).

VE caused low mortality of *S. oryzae* adults irrespective of the exposure periods. After 7 days, mortality ranged from 10-17.5% depending on the rate of VE applied. The difference between mortalities was not significant at all exposure periods (F=7.11NS; 0.96NS, 0.74NS and 14.01NS, respectively, p>0.05).

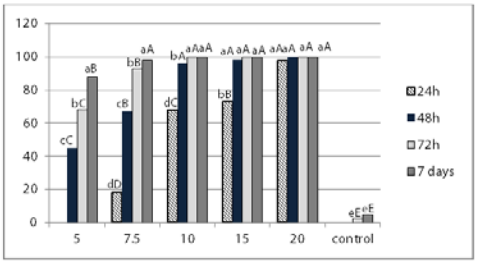


Fig. 1 The effect of DE on *S. oryzae* adults depending on the concentration and exposure period (small-case letters present differences within treatment between different days after exposure, upper-case letters present differences within day of exposure between different concentrations).

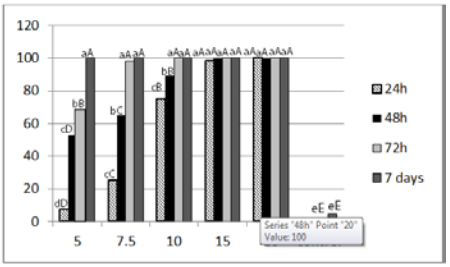


Fig. 2 The effect of KA on *S. oryzae* adults depending on the concentration and exposure period (small-case letters present differences within treatment between different days after exposure, upper-case letters present differences within day of exposure between different concentrations)

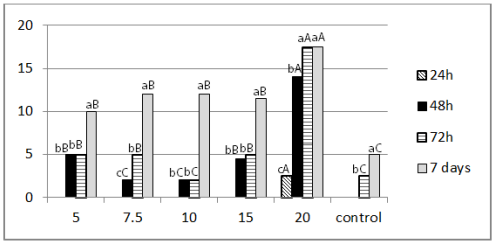
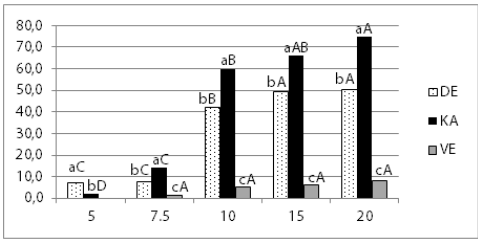


Fig. 3 The effect of VE on *S. oryzae* adults depending on the concentration and exposure period (small-case letters present differences within treatment between different days after exposure, upper-case letters present differences within day of exposure different concentrations)



Graph. 3. The effect of DE, KA and VE on *P. interpunctella* larvae depending on the concentration after 7 days (small-case letters present differences within treatment between different inert dusts, upper-case letters present differences within day of exposure between different concentrations).

According to Probit analysis (Tab. 1), the lowest LD_{50} and LD_{90} values were achieved by KA (6.63, 9.66, respectively) indicating at higher toxicity of kaoline to *O. oryzae* adults compared to DE (10.15, 16.52, respectively).

Table 1 Toxicity of tested inert dusts

Inert dust	LC_{50}	LC_{90}	Fiducial limits	Slope	Relative potency
DE	10.15	16.52	9.98-14.13	-9.83	65.32
KA	6.63	9,66	4.95-10.71	- 6.43	100.0
VE*	/	/	/	/	/

*Probyt analysis was not performed for VE due to the lack of relevant data;
Relative potency = (LC50 of the most toxic inert dust / LC50 of candidate inert dust) × 100

The effects of DE, KA and VE applied at 5, 7.5, 10, 15 and 20 gm^{-2} on *P. interpunctella* larvae are presented in Fig. 4. The highest rates of DE (10, 15 and 20 gm^{-2}) caused significant mortality of *P. interpunctella* larvae only after 7days, 42-50%, and the differences between the number of dead larvae was highly significant only after the last exposure period (F=9.12NS; 4.11NS; 0.79NS and 307.54**, respectively, p>0.05/p<0.01).

Discussion

According to El-Sayed, lower concentrations (0.1 and 0.2% w/w) of DE caused low mortality (16.7, 32.0%) of *S. oryzae* adults after 24 h of exposure while after 48 h the mortality increased (86.7 to 100%), regardless on the concentration. These results are in accordance with the results of this work. The high efficacy of DE (SilicoSec used in this work), was also proved by Korunić et al. (2011) reporting that this preparation was the most effective against *S. oryzae* of all other tested types of diatomaceous earth.

The results of this work are partially in agreement with those of Permual and Patourel (1990) who found that *S. oryzae* was relatively tolerant to activated kaolin (8% w/w) when exposed for 72 h on treated paddy. After 96 h mortality was 90%, and after 7 days the complete mortality was reported by mentioned authors, which was also proven in our work. Similar results were presented by Swamiappan et al. (1976) who report that kaoline clay activated by acid and heat treatments caused 100% mortality of several storage pests among which *S. oryzae* L. within 24 h, even at the minimal dose of 10 mg per Petri dish. Present findings are in agreement with observations of Verma et al. (1976). According to Jadhav (2006) kaoline was more effective than other tested materials (sand, sawdust, ash, Neem seed dust etc.), which was also proven in this work. The opposite results than those obtained in this work, were presented by El-Sayed et al. (2010) stating that, in general, DE was more effective than the kaolin against *S. oryzae*.

Results of Vukajlović et al. (2018) indicate that DE, originating from Serbia, exhibited low larvicidal efficacy against *P. interpunctella* 5th larval instar, (23.5-34.5%), while high insecticidal potential was expressed on 3rd and 4th instars larvae. Subramanyam *et al*. (1998) tested the efficacy of DE product Insecto against *P. interpunctella* 5th larval instar. In the same application rates as in our study authors reported that efficacy was significantly lower (10-70%) compared to the efficacy on 1st larval instar (99.5-100.0%), indicating at higher susceptibility of younger larvae to insecticidal effects of DE products than mature larvae. Susceptibility of insects to DEs can be attributed to their anatomy and physiology. Smaller insects are more susceptible because their surface area in relation to their body volume is larger than in bigger insects and therefore they lose great amounts of water from their body (Korunić, 1997). Results of many studies confirm that efficacy of inert dusts increases with the duration of exposure (Athanassiou et al., 2008; 2014; 2016; Kljajić *et al*., 2011; Andrić *et al*., 2012), which was also proven in this work.

The highest rates of KA (10, 15 and 20 gm^{-2}) caused significant mortality (60-75%) of *P. interpunctella* larvae only after 7 days of exposure ($F=115.07^{**}$, $p<0.01$). However, VE caused relatively low mortalities (5 to 8%) to *P. interpunctella* larvae at all tested rates during the entire experiment. The difference between mortalities in different exposure periods (24, 48, 72 h and 7 days) was not significant ($F=11.04^{NS}$; 4.50^{NS}, 0.79^{NS} and 7.11^{NS}, respectively, $p>0.05$).

The mortality of moths increased gradually with the increase of concentration and 100% was achieved 72 h after the contact with DE and KA at 10, 15 and 20 gm^{-2}. Also, a faster pupation was registered for larvae exposed to inert dusts, while adult emergence was reduced and adults had smaller body-sizes, compared to control. Shah and Khan (2014) also report that almost all tested larvae made the silken-web cocoon around their body and fastly entered the diapause or changed into the pupa stage after only two days after the exposure to maize kernels treated with 1.5 gkg^{-1} of DE. The physical properties of DE induced faster than usual pupation or diapause. The authors explain that in this way, larvae protect themselves from different negative effects of DE, such as abrasion of the cuticle, absorption of cuticular waxes from the epicuticle surface, damage to the digestive tract, blockage of the spiracles and tracheae, surface enlargement combined with dehydration and repellence caused by the physical presence of the dust.

As proven in this work, storage insects express different susceptibility to inert dusts due to morphological, physiological and ecological characteristics of each species. All insect characteristics which affect the efficacy of dusts are related to a mode by which insects sustain optimal content of water in the organism, because it has been confirmed that insects with thinner and gentler wax

layer are more susceptible to inert dusts, as insects whose body surface is smaller. Also, it was found that insects from different parts of the world show different susceptibility to dusts (Golob, 1997; Korunić, 1998a; Subramanyam and Roesli, 2000; Vayias *et al*., 2009). Among the representatives of Coleoptera order the most susceptible to diatomaceous earth are species from the genus Cryptolestes, somewhat less from Sitophilus and Oryzaephilus genus, while *R. dominica* and species from the genus Triboliumare the least susceptible (Korunić, 1997; Arthur, 2002; Athanassiou *et al*., 2007).

The results of this work indicate at good potential of DE and KA (10, 15 and 20 gm^{-2}) to be used as a surface treatment in grain stores for prevention of infestation by S. oryzae adults. However, since the contact effect on *P. interpunctella* larvae was not satisfactory within the first days of exposure, the prevention from this pest should not be relied only on the application of inert dusts as surface treatments, but other measures should be involved as well.

Acknowledgement

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Biopesticidal potential of green chemicals against *Callosobruchus analis* (f.) (Coleoptera: Bruchidae)

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Abstract

Pulses have 20-27% proteins which is 2- 3 times higher than traditional cereals. These constitute the main source of proteins for developing countries, like India where per capita consumption of the animal protein is low, thus they are rightly considered the **poor man's meat.** India is largest pulse consumer, importer and producer country of the world occupying an area of 228.47 lakh hectares with the production of 17380 million tones every year. With the United Nations declaration of 2016 as International Year of Pulses to replace the social evil of malnutrition by legume, the research pertaining to the biology and bio intensive management of bruchids pests has become increasingly important. Therefore, laboratory bioassay of essential oils which are regarded as "Green Chemicals" extracted from *Zanthoxylum armatum* DC., *Rabdosia rugosa* Wall. ex Benth, *Artemisia maritima* Linn. and *Colebrookea oppositifolia* Sm. by hydro distillation was carried out against *Callosobruchus analis* (F.) to evaluate biopesticidal potential in terms of oviposition and progeny deterrence and ovicidal activities. There was a significant difference in the number of eggs laid on treated and control sets and among the different treatments of essential oils. *Z. armatum* at 100 µl/ml allowed the bruchid to lay only 19.15±3.6 eggs as compared to 82.35±4.5 in control and proved to be most effective treatment with 76.74% oviposition deterrence. *R. rugosa* and *A. maritima* oil were found most effective in reducing the egg hatchability to 48.00±3.2 and 49.52±2.2% respectively at a lowest dose of 10 µl/ml. Egg hatching inhibition percentage increased with an increase in concentration of all the treatments. *R. rugosa* oil at 100 µl/ml proved to be most effective in reducing the adult emergence with 85.48% progeny deterrence followed by *A. maritima* showing 81.67% deterrence. All the tested essential oils revealed a wide range of bioactivities against the bruchid pest.

Keywords; Ovipositon deterrence, essential oils, bruchid pest, progeny emergence, ovicidal activity.

Introduction

Bruchids attack cereals and pulses both in fields and store and responsible for 10- 15% loss along with a germination inability varying from 50- 92% (Adugna 2006). The cow pea weevil *Callosobruchus analis* (F.) (Coleoptera: Bruchidae) a pest of economic importance for stored-leguminous grain worldwide (Southgate 1979, Rehaman 1989, Khandwe *et. al,* 1997 and Shafique and Ahamad, 2002). Due to the persistence usage of the synthetic insecticides, there is need to unveil the bio-pesticides and insecticides, which tend to be specific on the target species and biodegradable and less toxic to mammalian species. In the search for alternatives to conventional fumigants, essential oils, now designated as "green chemicals" extracted from aromatic plants have been widely investigated. Essential oils have the bioactive fraction of plant extracts (Shaaya *et al,.* 1991; 1997; Roger and Hamraoui 1997). They have potential as fumigants, ovicides, insect growth regulators and lethal against wide range of insect pests (Roger, 1997 and Shaaya *et al.,* 1997). The present study aimed to investigate the oviposition deterrence, ovicidal effects and progeny deterrence of four essential oils extracted from *Zanthoxylum armatum* DC., *Rabdosia rugosa* Wall. ex Benth, *Artemisia maritima* L. and *Colebrookea oppositifolia* Sm. against bruchid pest *Callosobruchus analis* (F.) a pest of stored legumes worldwide.

Material and methods

Leaves of *R. rugosa, C. oppositifolia, A. maritima* and *Z. armatum* were dried in shade and grounded followed by hydro-distilled in Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample in 1:10 plant material/water volume ratio for 4 hrs distillation. Extracted oil was stored in a refrigerator at 4^0C for further analysis. Cultures of *C. analis* were maintained in the laboratory on cowpeas, in glass containers with their open mouth covered with muslin cloth. Initially, forty pairs of 24 hours old adults were placed in a jar containing host seeds. Experiment was designed by following the method of Kumar *et al.* (2008). Fifty seeds of chickpea filled in glass conical flask were treated separately with different doses of the oils. After 24 hours, 6 males and 6 female bruchids were introduced in each Petridish separately. Mortality of insect was recorded and the number of eggs laid on treated and control seeds were enumerated after ten days of oviposition.

The % deterrency of oviposition was calculated according to the equation:

$$\text{Deterrency \%} = \frac{\text{NC - NT}}{\text{NC}} \times 100$$

Where NC is the number of eggs laid on control seeds, and NT is the number of eggs laid on treated seeds.

The number of eggs laid by gravid females were enumerated and exposed to different doses of essential oils. After an exposure period of 24 hours the eggs were observed for hatching after 8 to 10 days. Percentage egg hatching was calculated as:

$$\text{Egg hatching \%} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs}} \times 100$$

Numbers of unhatched eggs in each Petri dish were counted and the percent mortality of egg was calculated by Abbott's formula

Five gm of food media for each insect species was treated separately with different doses of oils. The seeds were then transferred into Petridishes and 6 pairs of *C. analis* (male and female of almost equal age) were introduced into them. The mortality of insects was observed and % progeny deterrence was calculated according to the equation:

$$\text{Deterrency \%} = \frac{\text{NC - NT}}{\text{NC}} \times 100$$

where NC is the number of adults emerged from control, NT is the number of adults emerged from treated food media.

Results

Different doses of essential oils reduced the fecundity of female *C. analis* as compared to control where maximum egg laying was recorded 82.35±4.5. At 100 µl/ml the essential oil of *Z. armatum* proved to be the most effective treatment with 76.74% oviposition deterrence. *R. rugosa* oil at a concentration of 50 µl/ml exhibited a high deterrent activity of 70.57% followed by *A. maritima* showing 67.15% deterrence. *C. oppositifolia* resulted in least oviposition deterrence. (Table 1).

Table 1. Oviposition deterrence of *C. analis* under different doses of four essential oils.

	Doses µl/ml	Oviposition deterrence (%)
Z. armatum	10	56.18(36.08 ±1.6)[a]
	30	65.81(28.15±2.2)[b]
	50	73.09(22.16±4.1)[c]
	100	76.74(19.15±3.6)[c]
R. rugosa	10	54.87(37.16±4.1)[a]
	30	59.65(33.22±2.5)[a]
	50	70.57(24.23±1.2)[c]
	100	75.68(20.02±2.5)[c]
A. maritima	10	53.75(38.08±1.9)[a]
	30	57.00(35.41±3.8)[a]
	50	67.15(27.05±2.4)[b]
	100	73.18(22.08±1.1)[c]
C.oppositifolia	10	51.11(40.26±4.1)[a]
	30	53.70(38.12±1.2[ja
	50	62.10(31.21±3.6)[b]
	100	68.16(26.22±2.1)[b]
Control		(82.35±4.5)[ab]

Values are mean ($n = 3$) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

The results revealed that *A. maritima* and *R. rugosa* oil were most potent in reducing the egg hatchability to 49.52±2.2 and 48.00±3.2 % respectively at a l dose of 10 µl/ml whereas, egg mortality was calcu 40.94±1.2 and 39.07±2.4% respectively. *Z. armatum* at a dose of 100 µl/ml obtained 55.70±4.8% egg mortality. *C. oppositifolia* was least toxic than any other resulted in a low mortality of 44.63±1.5% against eggs of *C. analis* even at a highest dose of 100 µl/ml. (Table 2).

Table 2. Ovicidal action of essential oils against *C. analis eggs*.

	Doses µl/ml	% Hatching	% Corrected mortality
R. rugosa	10	48.00±3.2[a]	40.94±1.2[b]
	30	40.16±1.2[c]	50.59±2.8[a]
	50	28.00±4.6[b]	65.55±1.4[c]
	100	20.20±1.2[d]	75.14±2.2[d]
A. maritima	10	49.52±2.2[a]	39.07±2.4[b]
	30	40.60±1.1[c]	50.04±1.9[a]
	50	40.00±2.8[c]	50.78±3.6[a]

	100	28.32±4.1[b]	65.15±1.2[c]
Z. armatum	10	57.68±3.2[bc]	29.03±4.4[ab]
	30	48.72±2.2[a]	40.05±2.2[bc]
	50	48.28±1.4[a]	40.60±1.2[bc]
	100	36.00±1.9[b]	55.70±4.8[a]
C. oppositifolia	10	70.08±1.1[de]	13.77±2.2[cd]
	30	65.28±2.3[de]	19.68±3.1[cd]
	50	60.40±2.8[cd]	25.68±4.8[ab]
	100	45.00±3.6[a]	44.63±1.5[bc]
Control		81.28[ab]	--

Values are mean (n = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

R. rugosa at 100 µl/ml proved to be most effective with 85.48% progeny deterrence followed by *A. maritima* oil resulted in 81.67% deterrence as compared to control (55.65±5.8). A dose of 50 µl/ml of *Z. armatum* oil resulted in 67.33% deterrent activity while *C. oppositifolia* was found to be least effective resulted in 63.89% deterrent activity even at a highest dose of 100 µl/ml (Table 3).

Table 3. F_1 progeny deterrence of *C. analis* under variable doses of essential oils.

Essential oils	Doses µl/ml	Progeny deterrence (%)
R. rugosa	10	63.48(20.32±2.2)[a]
	30	68.73(17.40±4.8)[a]
	50	81.76(10.15±1.1)[b]
	100	85.48(8.08±3.5)[b]
A. maritima	10	55.97(24.50±1.2)[a]
	30	63.46(20.33±3.4)[a]
	50	78.22(12.12±2.2)[b]
	100	81.67(10.20±1.9)[b]
Z. armatum	10	45.94(30.08±2.8)[c]
	30	54.30(25.43±3.6)[c]
	50	67.33(18.18±4.5)[b]
	100	72.47(15.32±2.8)[b]
C. oppositifolia	10	36.17(35.52±2.1)[d]
	30	45.39(30.39±1.8)[c]
	50	54.19(25.49±3.2)[c]
	100	63.89(20.09±2.8)[a]
Control		**(55.65±5.8)[ab]**

Values are mean (n = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

Discussion

There was a significant difference in the number of eggs laid on treated and control and among the different treatments of essential oils. The ability of essential oils and monoterpenoids to reduce fecundity in *Acanthosceildes obtectus* has been already reported (Roger and Hamraoui, 1995). At 100 µl/ml *Z. armatum* allowed the bruchid to lay only 19.15±3.6 eggs as compared to maximum egg laying of 82.35±4.5 in control and proved to be most effective treatment with 76.74% oviposition deterrency. *R. rugosa* and *A. maritima* oil also showed a remarkable acitivity significantly deterring the majority of females from egg laying on seeds than control. *Berberis lycium* in acetone the oviposition in *C. chinensis*was decreased from 38.40±0.81 in control to 11.06±0.65 in 20 per cent concentration and from 38.40±0.65 in methanol control to 9.46 in same concentration of methanol extract. The oviposition was reduced from 37.85±1.10 in control to 6.93±0.49 in 20 per cent roots extract of *B. lycium* in acetone and from 35.50±0.40 in methanol control to 8.60±0.41in same concentration in methanol (Thakur and Devi, 2016). In a similar study Shukla *et al.* (2011) recorded that 0.1µl/ml essential oil of *Callistemon lanceolatus* showed 96% deterrency followed by *Lippia alba*

oil (66.8%) and 1,8-cineole (65.8%). Similarly, *R. rugosa* oil at a concentration of 50 µl/ml exhibited a high deterrent activity of 70.57% followed by *A. maritima* showing 67.15% deterrence. *R. rugosa* and *A. maritima* oil were most potent in reducing the egg hatchability to 48.00±3.2 and 49.52±2.2% at a lowest dose of 10 µl/ml whereas 81.28% egg hatching was recorded in control. *C. oppositifolia* essential oil was least toxic than the others producing a low mortality of 44.63±1.5% against eggs of *C. analis* even at a highest dose of 100 µl/ml. The ionic surfactant at concentrations of 5 and 10 µL showed 68 to 88% mortality in *C. analis* and 63 to 76% in *Sitophilus oryzae* L. respectively after 24 hours of treatment (Brari and Thakur, 2016). The oil vapours diffused into eggs and affected the physiological and biochemical process associated with embryonic development. *R. rugosa* oil at 100 µl/ml resulted in 8.08±3.5 progeny production for *C. analis* while in controls the adult emergence was 55.65±5.8. *Z. armatum* also resulted in a significant progeny reduction even at a dose of 50 µl/ml with a progeny deterrence of 67.33% for *C. analis.* In related studies *Chenopodium* and *Clausena* oils checked more than 84% of adult emergence of both bruchids *C. analis* and *C. maculatus* at different doses (Pandey *et al.,* 2011).

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Effectiveness of Essential Oils from Ngaoundere, against Post-Harvest Insect and Fungal Pests of Maize

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Abstract

Successful storage of harvest is a matter of utmost importance in the Sudano-Guinean agro-ecological zone where intense cultivation takes place only once a year. Poor and rudimentary drying/storage methods, high relative humidity as well as inaccessibility to the chemical pesticides leave stored maize at the mercy of insect and fungal attack. Insect attack favours secondary attack by fungi; both leading to a fall in the nutritional, sanitary and organoleptic qualities of the stored maize. Thus, poor peasant farmers are left with the choice of locally available botanicals as alternatives to chemical pesticides. It is against this backdrop that this study seeks to determine the insecticidal efficacy of essential oils from the leaves of *Chenopodium ambrosioides* and *Cupressus sempervirens* together with their 50/50 binary combination against the maize weevil, *Sitophilus zeamais,* and the fungi: *Rhizopus stolonifer* and *Aspergillus flavus* on stored maize. Insect mortality and progeny inhibition and the inhibition of fungal invasion were evaluated. Pesticidal activities of both essential oils increased with ascending dose of application. 200 µL/kg of the binary combination caused 100% mortality within 14 days and it completely inhibited progeny production in the weevil. The mixture of the two oils showed additive effects against the weevils and fungi. The two essential oils in isolation significantly inhibited fungal spore invasion in 21 days of storage although *A. flavus* was less susceptible than *R. stolonifer*. Therefore both plants could provide active botanical pesticides against *S. zeamais* and fungal pests in stored maize.

Key words: botanical, essential oil, fungal spore, stored maize pests, food security

1. Introduction

Sub-Saharan Africa is the most vulnerable region in the world with the average amount of food available per person per day being 1,300 calories compared to the world wide average of 2,700 calories (FAO, 2013). In 2012, maize had a yield of 70,076,591 tons in Africa (FAOSTAT, 2015). It is grown in diverse agro-ecological zones and farming systems, and consumed by people with varying food preferences and socio-economic background (Langsi *et al.,* 2017a). Cameroon with agriculture as its backbone has about 70% of its active population involved in agriculture, which contributes to about 25% of the GDP (FAO, 2008). Stored maize, especially in regions of high humidity is highly prone to attack by insect and fungal pests. High humidity and water content favour fungal growth (Pitt and Hocking, 2009). The most prolific insect is *Sitophilus zeamais* which bores holes and creates hotspots suitable for fungal growth. The fungi now produce mycotoxins thereby lowering the quality and also rendering it hazardous for consumption (Rashad *et al.*, 2013).

Plants which make excellent leads for new pesticide development (Napoleao *et al.,* 2013) could be used. Essential oils from plants generally contain chemicals which have both curative and protective potentials on stored products (Hamdani *et al.,* 2015). *Chenopodium ambrosioides* L. (Amaranthaceae) and *Cupressus sempervirens* L. (Cupressaceae) locally used as botanicals were chosen for this work. *Ch. ambrosioides* L. (Amaranthaceae) is a plant whose powders have been studied against *Sitophilus*

zeamais Motschulsky for Toxicity, oviposition suppression, ovicidal and larvicidal effects (Abiodoun *et al.,* 2010; Ntonifor *et al.,* 2011; Tapondjou *et al.,* 2002). Tapondjou *et al.* (2002) did *in-vitro* toxicity and progeny control effects using the essential oils while with *Cu. sempervirens* L. (Cupressaceae), mortality, progeny and Repellency effects have also been studied (Achiri *et al.,* 2015, Tapondjou *et al.,* 2005) on *S. zeamais* Motschulsky. Essential oils have also been proven to have antimicrobial and positive food technological potentials such as: *in-vivo* effectiveness and anti-oxidant properties of *Ocimum grattisimum*, *Lippia rugosa* and *Xylopia aethiopica* on *Aspergillus flavus* on maize fungi (Tatsadjieu *et al.,* 2010), antibacterial property of methanol, ethanol and hexane extracts (Sati *et al.,* 2015), significant antifungal activity of *Cu. Sempervirens* (Amri *et al.,* 2013).

The main objective of this work was the use of essential oils of *Ch. ambrosioides* L. and *Cu. sempervirens* L. available locally to control maize weevils (*S. zeamais* Motschulsky) and fungi (*Rhizopus stolonifer* and *Aspergillus flavus).* And specifically to evaluate their effectiveness on insect: Mortality, progeny production and fungal invasion *in-vivo* on treated maize grains.

2. Materials and methods

Insects and maize substrate

2.1. Test maize

The Acid Tolerant Population (ATP) variety of maize was collected from farmers in Big Babanki (North West Region, Cameroon) and identified in the cereals unit of the Institute of Agricultural Research for Development, IRAD, Bambui. The moisture content of maize used in bioassays was determined to be 12.669% (AFNOR, 1982). Weevils were obtained from laboratory stock cultures from the Crop Protection Laboratory of the Institute of Agricultural research for Development, IRAD, Bambui.

2.2. Collection of plant material and extraction of essential oils

Fresh green leaves of *Ch. ambrosioides* and *Cu. sempervirens* were collected from the University of Ngaoundere between December 2015 and February 2016, shade dried on laboratory benches (17.3–28.8°C), and hand crushed to get powder. The unseived powder was then packaged in black polythene bags and used for essential oil extraction.The essential oils were extracted by hydrodistillation with the help of a Clevenger apparatus and dried over anhydrous Sodium Sulphate. It yielded 0.812% (wt.wt) for *Ch. Ambrosioides* and 0.697% (wt/wt) for *Cu. Sempervirens*. Essential oils were analysed for component identification using an Agilent Technologies 6850 gas chromatograph coupled with a mass detector 5973 and a 7683B Series Injector autosampler. The essential oil was diluted with hexane which was injected in splitless mode at 200 °C. Components were separated in the oven following a temperature gradient starting from 50 °C and kept for 7 min; then raised to 300 °C (10 °C/min) and kept at this temperature for 4 min. Helium was used as carrier gas and retention indices were calculated according to Kovats, for alkanes C9-C24 compared with those reported by Adams (2007).

2.3. Bioassays

All bioassays were carried at a temperature of: 17.3–28.8°C and relative humidity: 56.3–97.8% from May to September 2016. Twenty five g of maize grains were placed in 500 mL glass jars. Aliquots of both essential oils and their 50/50 binary combination were applied to the maize grains at the following dosages 0 µL (control), 25 µL/kg, 50 µL/kg; 100 µL/kg, 200 µL/kg (diluted in 1mL acetone). All treatments were replicated 4 times. The maize-essential oil-acetone mixture was then hand shaken to permit complete coating of the maize by the essential oils and later left open for about 45 minutes on laboratory shelves to permit complete evaporation of the solvent. Afterwards, 20 adult (less than 7 days old) *S. zeamais* of mixed sex were separately added into each jar and kept on laboratory shelves. Insect mortality was recorded 1, 3, 7 and 14 days post treatment and percentage

insect mortality was corrected using the Abbott (1925) formula. All tests were carried at temperature: 17.3–28.8°C and relative humidity: 56.3–97.8%

On the 14th day post-infestation, the remaining live insects were removed and the different jars containing grains were kept under the same experimental conditions. The recording of F1 progeny was done once a week for 5 weeks commencing 6weeks post-infestation (Nukenine *et al.,* 2007). Percentage reduction in adult emergence or inhibition rate (% IR) was calculated as:

$$\%\boldsymbol{IR} = \frac{\mathbf{(Cn - Tn)x100}}{\mathbf{Cn}}$$

Where C_n is the number of newly emerged insects in the untreated (Control) jar and T_n is the number of insects in the treated jar.

2.4. Fungal invasion tests

Visibly contaminated stored maize samples surface sterilized using 2% $NaOCl_{(aq)}$ (Hocking *et al.,* 2006). Whole grains were placed directly on solidified PDA (potatoes dextrose agar: 200 g potato infusion, 20 g glucose, 15 g agar, pH of 5.6 with HCl) supplemented with Chloramphenicol (60 µg/mL) medium. The fungi were identified with the help of Domsch *et al.* (1980) manual.This involved treatment of self-contaminated maize grains with essential oils at varying concentrations and stored for a given duration. The method by Aoudou *et al*., (2012) was used. In order to homogenise the water content, the maize grains were soaked in sterile distilled water for 24 hours then dried in an oven at 45°C for 24 hours. Twenty five grams of maize grains were measured and put in 300mL glass jars on whose lids had been attached with the use of plaiting thread, 12 cm^2 Whatman no. 1 filter papers. The sealed containers containing the grains were sterilised by autoclaving at 1 atmosphere, 121°C for 15 minutes. Isolates of *Rhizopus stolonifer* and *Aspergillus flavus* were cultured on a PDA/chloramphenicol medium until the development of mature spores. With the aid of a haemocytometer, a 10^5 spores/mL suspension was prepared. In aseptic conditions, the sterile grains were inoculated with 2 mL of spore suspension (10^5spores/mL) of each fungus species. The containers were sealed and allowed at ambient conditions for three days to allow spore invasion. The filter papers were then soaked with different concentrations of essential oil (0, 20, 40, 60 and 80 µL/Kg) and sealed. Storage was maintained at ambient conditions for 30 days while growth inhibition was accessed every 7 days beginning from day 1 (Chatterjee, 1990). The percentage of contaminated grains was calculated using the formula:

$$\text{Percentage of contaminated grains } (\%\text{CG}) = \frac{\text{NCG}}{\text{TNG}} \times 100$$

Where: NCG = number of contaminated grains; TNG = total number of grains.

2.5. Statistical Analysis

Adult mortality was corrected relative to natural mortality in the controls using Abbott (1925) formula. Data on mortality and progeny production was transformed by using √(x + 0.5), then later ANOVA was done using statistical package for social sciences (SPSS) software. Tukey test (HSD) was used for mean separation for both weevil and fungal data. The dose–mortality response was analyzed by probit analysis (Finney 1971) using the maximum likelihood estimation. The co-toxicity coefficient per *Ch. ambrosioides* and *Cu. sempervirens* mixture was determined using the formula by Sun & Johnson (1960).

3. Results and Discussion

In both oils, monoterpenes hydrocarbons were predominant (Table 1). Monoterpene hydrocarbons constituted 69.2% in *Cu. sempervirens* and 79.87% in *Ch. ambrosioides*. The greatest in *Ch. ambrosioides* being 4-carene (46.32%). Only monoterpenes were found. However, in *Cu. sempervirens* monoterpene hydrocarbons predominated followed by the hydrogenated monoterpenes, the oxygenated sisqueterpenes and last by the oxygenated sisqueterpenes. Of all

the compounds identified α -pinene was the most concentrated (20.10 %) followed by β -phellandrene (6.94%). All sisqueterpenes were below 1% in concentration.

Tapondjou *et al.* (2002) instead found more elevated proportions of Cymol (50%) and terpinene (37.6%) in *Ch. ambrosioides* harvested from Mbouda in the West Region, Cameroon. We also noted the presence of lower proportions of ascaridole (0.86%) same as Rafaela *et al.* (2014) found in Brazil (0.87%) but lower than that found in samples by Ali *et al*. (2016) (Yemen) and Tapondjou *et al.* (2002).

With *Cu. Sempervirens,* Tapondjou *et al.* (2005) instead noticed the absence of cymol, the *Cupressus* essential oil also showed an elevated presence of hydrogenated monoterpenes. A similar chemical composition rich in α-pinene (27.5 to 35.8%), α-cedrol (7.7 to 19.3%), δ-3-carene (5.8 to 13.2%) was found by Amri *et al.* (2013) in Tunisia. While Mazari *et al.* (2010) in Algeria also found that the majority of the compounds found are hudrocarbon monoterpenes. Tapondjou *et al.* (2005) also found α –pinene to be the most abundant component.

The essential oils and their binary combination caused significant the mortality of adult *S. zeamais* relative to the control (Table 2). Mortality rates were generally higher for *Ch. ambrosioides* than for *Cu. Sempervirens* and the binary combination at the dosages of 50 and 100 uL/kg, irrespective of the exposure period. However, at the highest dosage level of 200 uL/kg, *Ch. ambrosioides* essential oil and the binary combination caused greater mortalities than *Cu. Sempervirens* within the first 24 hours of exposure. Only the binary combination achieved 100% mortality with 14 days of exposure at the 200 ul/kg dosage level although this was similar to the 95.83% mortality caused by *Ch. ambrosioides* essential oil. Langsi *et al.* (2017b) found 100% mortality with *Ch. Ambrosioides* and the 50:50 binary combination of *Chenopodium ambrosioides* and *Cupressus sempervirens* within 72hours of exposure.

Table 1: Chemical compositions of *Cupressus sempervirens* and *Chenopodium ambrosioides* essential oils

N°	KI	Name	Percentage (%)	
			Cu. sempervirens	*Ch. ambrosioides*
Monoterpene hydrocarbons			69.2	79.87
	935	α –Pinene	17 .59	/
	1008	3-carene	25 .91	/
	1012	4-carene	/	46.32
	1017	p-cymene	/	32 .62
Oxygenated Monoterpenes			16.95	1.55
	1260	Ascaridole	/	0.86
Sisqueterpene hydrocarbons			4.21	0.00
Oxygenated Siqueterpenes			0.7	0.00
Total			91 .34	81.42

Table 2: Corrected cumulative mortality (Mean ± S.E) of *Sitophilus zeamais* due to treatment of maize grains with essential oils of *Chenopodium ambrosioides, Cupressus sempervirens* from Ngaoundere and their 50:50 binary combination

Exposure Period (days)	Content (µL/kg)	Mortality *Ch. ambrosioides*	*Cu. sempervirens*	50 :50 Combination	$F_{(2, 9)}$
	00	0.00 ± 0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
	25	0.00±0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
1	50	5.00 ±0.00Aa	7.50±1.44ABb	2.50± 1.44Aa	54.000***
	100	61.25 ±3.15Bb	13.75 ±2.39Ba	20.00 ±2.04Ba	456.000***
	200	73.75±4.73Cb	33.75±2.39Ca	72.50 ±4.79Cb	634.776***

$F_{(4, 15)}$		204.992***	64.403***	165.321***	
	00	0.00 ± 0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
	25	0.00±0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
3	50	15.00 ±2.04Bb	10.63 ±0.47Ba	7.70 1.49Aa	140.522***
	100	72.50±4.79Cc	17.75 ±1.32Ba	33.36 ±3.41Bb	348.703***
	200	80.00±3.54Cb	69.61 ±3.56Ca	82.17±7.71Cb	455.209***
$F_{(4, 15)}$		198.000***	286.881***	83.283 ***	
	00	0.00 ± 0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
	25	0.00±0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
7	50	17.14 ±2.89Bc	20.75 ±1.95Bb	17.83±4.22Ba	123.536***
	100	82.65±2.55Cc	21.10 ±3.88Ba	43.62 ±1.62Cb	900.446***
	200	92.02±1.50Db	74.61±6.25Ca	98.75 ±1.25Db	1640.870***
$F_{(4, 15)}$		602.138***	80.529***	388.999***	
	00	0.00 ± 0.00A	0.00 ± 0.00A	0.00 ± 0.00A	/
	25	0.00 ± 0.00Aa	1.32 ±1.32Aab	2.63 ±1.52Ab	3.857*
14	50	19.08±3.31Ba	24.02 ±1.82Ba	40.20±3.65Bb	197.281***
	100	93.20±2.59Cc	25.88 ±4.30Ba	48.03 ±2.25Bb	923.394***
	200	95.83±1.39Cb	80.70±5.40Ca	100.00±0.00Cb	2646.476***
$F_{(4, 15)}$		611.088***	101.609***	401.344***	

P = 0.05 (Chi-square test). (P<0.01); ***: very highly significant (P<0.001).

The co-toxicity indices indicated that the combination of the two essential oils resulted in additive effect vis-a-vis *S. zeamais* mortality on the treated maize grains for all time-points (Table 3). With additive effects, it has been proven that combinations of insecticidal materials have the advantages to increase the efficacy by complementing the bio-efficacy of the individual products and simultaneously lowering their use on the one hand and broadening the spectrum of activity and overcoming pest resistance to individual pesticide on the other hand (Das, 2014).

Table 3: Lethal contents and co-toxicity coefficients of binary combinations on the mortality of *Sitophilus zeamais* due to treatment of maize grains with essential oils of *Chenopodium ambrosioides, Cupressus sempervirens* from Ngaoundere

Period of exposure	Product	LC_{50} (µL/kg)	Co-toxicity index	Interpretation
	Ch. ambrosioides	114.48		
1 Day	*Ch. sempervirens*	244.58	103.19	Additive
	50:50 combination	151.14		
	Ch. ambrosioides	91.15		
3 Days	*Ch. sempervirens*	167.15	97.09	Additive
	50:50 combination	121.51		
	Ch. ambrosioides	76.31		
7 Days	*Ch. sempervirens*	138.18	106.47	Additive
	50:50 combination	92.35		
	Ch. ambrosioides	67.60		
14 Days	*Ch. sempervirens*	121.05	116.60	Additive
	50:50 combination	74.40		

All the essential oils and their binary combination significantly inhibited progeny production. These inhibitions all increased with dose of administration (Table 4). Apart from *Cu. sempervirens* with 43%, even the lowest concentrations of all the oils inhibited progeny production by more than 50% relative to the control. The 50/50 binary combination at 200 µL/kg, completely supressed progeny production. This was followed by *Ch. ambrosioides* with 99% while *Cupressus* had 95% inhibition to progeny production.

Table 4: F_1 progeny (Mean ± S.E) of *Sitophilus zeamais* due to treatment of maize seeds with essential oils of *Chenopodium ambrosioides, Cupressus sempervirens* from Ngaoundere and their binary combination

Product Content (µL/kg)	*Ch. ambrosioides* F_1	% reduction of F_1	*Cu. sempervirens* F_1	% reduction of F_1	50/50 binary combination F_1	% reduction of F_1

00	27 ± 1.29d	0.00 ± 0.00a	27±1.29d	0.00 ± 0.00a	27±1.29d	0.00 ± 0.00a
25	13±0.41c	51.31±3.82b	17±1.29c	36.04±7.53b	8.75±0.48c	67.46±1.92b
50	11±0.41b	58.90±3.02b	11±0.96b	56.61±5.71c	4.25±0.48b	84.10±2.08c
100	7±0.41b	74.01±0.52c	9.50±0.65b	64.48±3.48c	2.75±0.48ab	89.78±1.82c
200	0.5 ± 0.29a	98.00±1.16d	1.75±0.48a	93.23±2.16d	0.00 ±0.00a	100±0.00d
$F_{(4, 15)}$	213.222***	259.538***	89.987***	56.791***	247.261***	704.133***

Means ± S.E. in the same column for the same category of insecticide, followed by the same letter do not differ significantly at $P = 0.05$ (Tukey test). Each datum represents the mean of four replicates of 20 insects each. ***: very highly significant ($P<0.001$).

Percentage inhibition generally increased with dose administered. They are shown in Figures 1 (a, b and c). The 80 µL/L dose of *Ch. ambrosioides* gave 88% inhibition of *Rhizopus* growth in the first week. In all the doses administered, inhibition decreased from a maximum in 7 days to less than 10% in 21 days (Figure 1a). From Figure 1.b, it is noticed that *Cu. sempervirens* at 80 µL/L gave 55% inhibition of *Rhizopus'* spore germination and all the different concentrations dropped to zero within 21 days. The 50:50 binary combination however was more efficient than both plants used separately. Its 80 and 60 µL/L doses gave respectively 96 and 90% inhibition to *R. stolonifer* spore germination. Essential oils are very well known for their bactericidal, bacteriastatic, virucidal, fungicidal activity due to their medicinal properties against the wide range of pathogenic microorganisms (Akthar *et al.* 2014, Ambindei *et al.,* 2017). However, the spectrum of antimicrobial activity is dependent on the tested pathogens, measurement conditions and the source of the antimicrobial compounds (Turgis *et al.,* 2009).

Figures 2 (a, b and c) show the efficiency of essential oils from Ngaoundere on *Aspergillus flavus.* From the results, *Aspergillus* was more sensitive to the essential oils of both plants and their binary combination than *Rhizopus.* In figure 2.a, the 80 µL/L content gave 100% inhibition in seven days of exposure while the lower doses all gave inhibitions more than 60%. With *Cu. Sempervirens* percentage inhibition did not go beyond 65% for all the doses administered on *Aspergillus*. And all the percentage inhibitions dropped to zero within 21 days of exposure (Figure 2.b) all these results on *Aspergillus* are in agreement with those of Mahmood *et al.* (2013). With the 50:50 binary combination, percentage inhibition of 100 was gotten after 7 days with the 80 and 60 µL/L essential oil concentrations on *Aspergillus*. The percentage inhibitions dropped to zero within 21 days of exposure. This was followed by the 40 and 20 µL/L with respectively 90% and 80%. However, all inhibitions fell to zero after 21 days of exposure (Figure 2.c). The presence of phenolic compounds in the different essential oils renders them good antifungal agents. Thymol, linalool, carvacrol and eugenol are indications of an outstanding antifungal potential (Hyldgaard et al., 2012, Ambindei *et al.,* 2016, 2017)

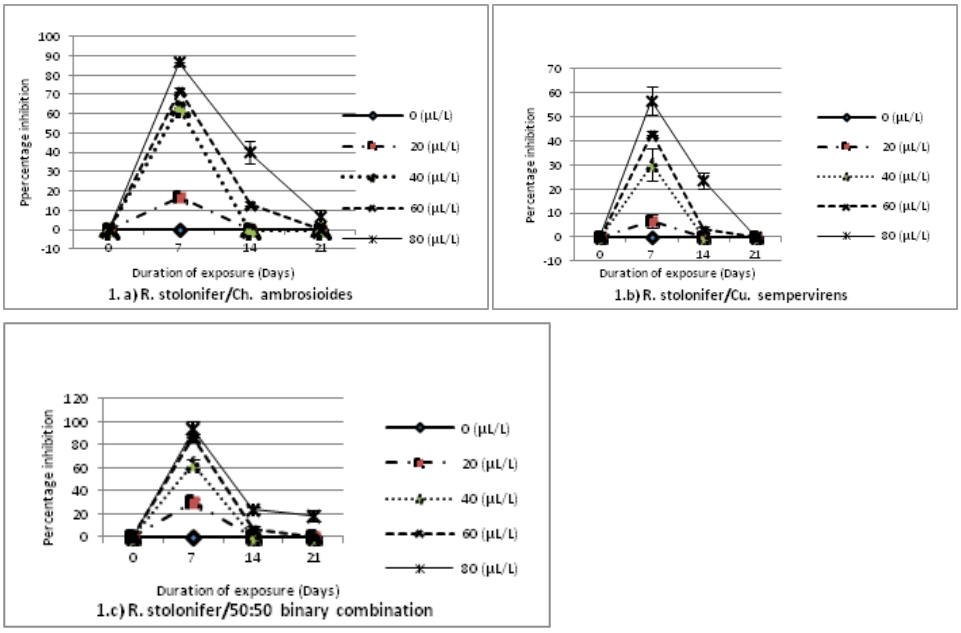

Figure 1: *In vivo* percentage inhibition of *Rhizopus stolonifer* growth on contaminated maize as a result of treatment with essential oils of *Chenopodium ambrosioides, Cupressus sempervirens* and their 50/50 binary combination from Ngaoundere.

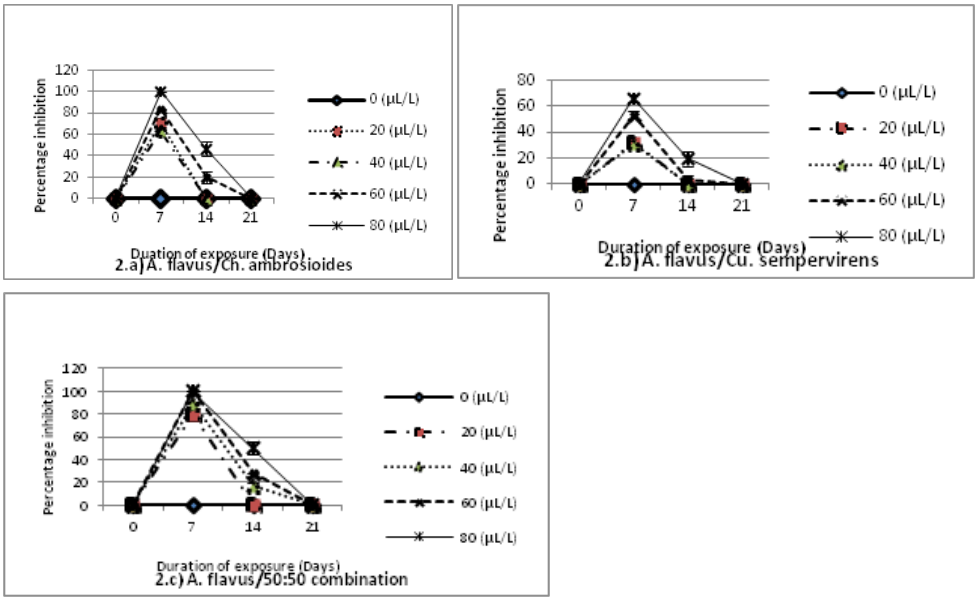

Figure 2: *In vivo* percentage inhibition of *Aspergillus flavus* growth on contaminated maize as a result of treatment with essential oils of *Chenopodium ambrosioides, Cupressus sempervirens* from Ngaoundere and their 50/50 binary combination

Both essential oils were found to be rich in α –pinene and cymol, while *Chenopodium* contains ascaridole; which are all reference fungicides and insecticides (Mazari *et al.,* 2010; Yang *et al,* 2007; Demirci *et al.,* 2007). P-cymene and p-cymeene found in both oils, on their own, are not an excellent

antifungal agent (Bagamboula *et al.,* 2004), but will boost the activities of components with functional side groups (Rattanachaikunsopon and Phumkhachorn, 2010).

Essential oils of *Ch. ambrosioides* and *Cu. sempervirens* available locally presented additive insecticidal and fungicidal efficacy against both weevils and fungi. The ability to control the proliferation of *S. zeamais, A. flavus* and *R. stolonifer* in stored maize by these essential oils is dose dependent and increases with period of exposure to the pesticide. Therefore both pesticides stand highly recommended due to their insecticidal and progeny control effects as well as their ability to inhibit fungal spore germination on stored maize.

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Insecticidal contact toxicity of several essential oils against stored product pests

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Abstract

Results of laboratory bioassays in Petri dishes on evaluation of contact toxicity of *Illicium verum*, *Artemisia absinthium* and *Abies sibirica* essential oils (EOs) against larvae of khapra beetle, *Trogoderma granarium* Ev., adults of grain weevil, *Sitophilus granarius* L., and rice weevil, *Sitophilus oryzae* L., and confused flour beetle, *Tribolium confusum* Duv., and larvae and adults of the lesser mealworm, *Tenebrio molitor* L., are presented. EOs commercial samples from retail pharmacy were tested at doses 0.01, 0.25, 0.50, 0.75 and 1.00 µl/cm^2. A treated Petri dish surface treted with acetone was used as a control. The experiment was carried out in triplicate. Mortality of insects was assessed after 1, 3, 6 and 24 hours post exposure. After exposure insects were placed into untreated Petri dishes for 3 days. The main components of the *A. absinthium* EOs are thujil alcohol (19.65%), phellandrene (16.71%), borneol (12.1%) and thujone (11.55%) was found. The major component of *I. verum* EOs was anethole (98.64%). Isobornyl acetate (57.25%), α-pinene (13.55%) and limonene (10.62%) were found as the main components of *A. sibirica* EOs. *S. oryzae* and *S. granarius* were most sensitive to each EO. *I. verum* EOs was the most effective and caused 100% mortality of each insect at the dose 0.25 µl/cm^2.

Keywords: essential oils, stored product pests, contact toxicity, *Illicium verum*, *Artemisia absinthium*, *Abies sibirica*

Introduction

Aluminium and magnesium phosphides formulations for phosphine fumigation and chemical protectants based on deltamethrin and pirimiphos-methyl for spraying are allowed for grain disinfection in Russian Federation. Regular and non-alternative application these formulations could cause development of resistance in major stored product pests (Nakakita & Winks, 1981; Mordkovich, 2003, Holloway et al., 2016) and also accumulation of pesticide residues in food commodity. Therefore, it is necessary to apply the same effective but more environmental friendly not chemical synthetic formulations for stored product protection. Essential oils (EOs) could be used as alternative formulation for management of stored product pests.

Materials and Methods

Test Insects

The insects used in bioassays were adults of *S. granaries*, *S. oryzae*, *T. confusum*, larvae and adults of *T. molitor* and larvae of *T. granarium*. *S. granaries* and *S. oryzae* were reared on *Triticum aestivum* whole grain in 25±1°C and 70±5% relative humidity (r.h.). *T. confusum* and *T. granarium* were reared on *T. aestivum* crushed grain in 27±1°C and 70±5% r.h. *T. molitor* was reared on *T. aestivum* crushed grain with *Zea mays* whole corn grain in 25±1°C and 70±5% r.h. The rearing conditions were darkness.

Essential oils and GC/MS spectrometry analysis

Commercial samples of *I. verum*, *A. absinthium* and *A. sibirica* EOs from retail pharmacy were tested. The obtained oils were subjected to GC/MS analysis using an Agilent 7890A gas chromatograph (Agilent Technologies, USA) equipped with a Agilent 5975C mass spectrometer, fitted with a DB17MS capillary column (30 m × 0.25 mm; 0.25 µm film thickness). Temperature was kept at 50 °C for 2 min and programmed to reach 200 °C (a rate of 10 °C/min), then to reach 260 °C (a rate of 20 °C/min) and held at this temperature for 4 min. Helium was used as the carrier gas at the rate of 1.18 mL/min. The samples were injected at the injector temperature of 240 °C.

Experimental procedure

A direct contact application assay (Qi and Burkholder, 1981; Broussalis et al., 1999) was used to evaluate the insecticidal activity of the tested essential oils. Five concentrations (640, $16{\cdot}10^3$, $32{\cdot}10^3$, $48{\cdot}10^3$ and $64{\cdot}10^3$ ppm) of each EO were prepared using acetone as a solvent. Aliquots of 1 ml of each concentration correspond to the respective 0.01, 0.25, 0.50, 0.75 and 1.00 µl/cm^2 were placed on the surface of Petri dish (9 cm diameter, ~ 64 cm^2). After evaporation of the solvent, 20 adults of *S. granaries*, *S. oryzae*, *T. confusum* and 20 larvae of *T. granarium* and 10 adults and larvae of *T. molitor* were separately introduced on the treated Petri dish's surface. The experiment was carried out in 25±1°C and 70±5% r.h. and a photoperiod of 10/14 L/D during the entire period. Control sets were made, where the same number of insects were placed in Petri dishes with surface treated with acetone only. The experiment was carried out in triplicate. The mortality was assessed after 1, 3, 6, and 24 hours (h) of exposure under a Zeiss stereomicroscope (Stemi-2000, Carl Zeiss Microscopy GmbH, Germany). Then, after exposure, insects were moved to untreated Petri dishes and were hold there during 3 days. The viability of insects was estimated every day during this period. The data were subject to a two-way ANOVA. A 5% probability level was used for individual pairwise comparisons by the made Tukey-Kramer's HSD test.

Results

Chemical composition of the test oils

Data of the chemical composition analysis (Table 1) revealed that the *A. absinthium* EO contains, mainly thujil alcohol (19.65%), phellandrene (16.71%), borneol (12.1%) and thujone (11.55%).

Isobornyl acetate (57.25%), α-pinene (13.55%) and limonene (10.62%) were found as the main components of *A. sibirica* EO, while anethole (98.64%) was the main compound in the essential oil of *I. verum*.

Tab. 1 Chemical composition of the tested essential oils

Compound	RT	Concentration (%)		
		A. absinthium	***I. verum***	***A. sibirica***
α-Pinene	4.091	4.29	-	13.55
Camphene	4.558	1.76	-	2.22
Phellandrene	5.117	16.71	-	0.80
Carene	5.634	7.01	-	8.91
d-Limonene	6.033	3.98	-	10.62
p-Cymene	6.480	7.80	-	0.91
1,8-Cineole	6.733	1.74	-	0.41
Terpinolene	7.250	-	-	3.68
Linalool	7.577	1.75	-	-
Thujil alcohol	8.111	19.65	-	-
β-Thujone	8.256	11.55	-	-
Isoborneol	8.950	1.11	-	-
Borneol	9.172	12.10	-	-
Camphor	9.285	3.58	-	-
Estragole	10.146	-	1.36	-
Isobornyl acetate	10.826	5.14	-	57.52
Anethole	11.780	-	98.64	-
Total, %	-	98,17	100	98,62

Contact Activity

Results show (Tables 2-4) that the test essential oils showed variant degrees of toxicity, where the *I. verum* EOs was the most toxic, followed by *A. absinthium* and *A. sibirica*. Essential oil of *I. verum* at the dose 0.25 µl/cm^2 caused 100% mortality of *S. oryzae* and *T. confusum* and also significant mortality (96,7%) of *S. granaries* after 24 h exposure. In addition, the total mortality of adults and larvae of *T. molitor* were found on 1 and 3 days during post-exposure period. *Trogoderma granarium* larvae were also sensitive to *I. verum* at the dose 0.25 µl/cm^2, where percentage of killed insects 98% was recorded on third day during post-exposure period. Essential oil of *A. absinthium* was the less toxic, where 100% mortality of tested pests were found after application at the dose 0.5 µl/cm^2. *A. sibirica* displayed the least contact activity. In this case, total mortality of insects was found to the dose 0,75 µl/cm^2. *S. oryzae* and *S. granarius* were the most sensitive to each tested essential oil. Larvae of *T. molitor* were the most resistance to *A. absinthium* and *A. sibirica* EOs. Larvae of *T. granarium* were the most resistance to *I.verum* EO.

Tab. 2 Mean ±SE mortality (%) of insects exposed to *A. absinthium* EO at different doses after 24 h exposure. For all Tables, means within columns followed by different lower-case letters represent significant differences between doses, means followed by capital letters represent significant differences between species ($P < 0.05$).

Dose (µl/cm^2)	Stored product pest					
	S. granarius	***S. oryzae***	***T. confusum***	***T. molitor* (larvae)**	***T. molitor* (adults)**	***T. granarium***
0.01	0.0 ± 0.0bA	0.0 ± 0.0bA	3.3 ± 5.8cA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0cA
0.25	95.0 ± 8.7aA	96.7 ± 5.8aA	40.0 ± 20.0bC	13.3 ±11.5bD	66.7 ± 23.1bB	40.0 ± 10.0bC
0.50	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	86.7± 11.5aA	100.0 ± 0.0aA	83.3 ± 11.6aA
0.75	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	73.3± 30.5aB	100.0 ± 0.0aA	100.0 ± 0.0aA
1.00	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	93.3± 11.5aA	100.0 ± 0.0aA	100.0 ± 0.0aA
Control	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0cA

Tab. 3 Mean ±SE mortality (%) of insects exposed to *I. verum* EO at different doses after 24 h exposure

Dose (µl/cm²)	Stored product pest *S. granarius*	*S. oryzae*	*T. confusum*	*T. molitor* (larvae)	*T. molitor* (adults)	*T. granarium*
0.01	0.0 ± 0.0bA	0.0 ± 0.0bA	6.7 ± 11.6bA	0.0 ± 0.0cA	0.0 ± 0.0cA	0.0 ± 0.0cA
0.25	96.7 ± 5.8aA	100.0± 0.0aA	100.0± 0.0aA	46.7 ± 25.2bC	73.3 ± 5.8bB	46.7 ± 20.2bC
0.50	100.0± 0.0aA	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	90.0 ± 0.0abA	86.7 ± 10.4aA
0.75	100.0± 0.0aA	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	93.3 ± 5.8aA	100.0 ± 0.0aA
1.00	100.0± 0.0aA	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA	100.0 ± 0.0aA
Control	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0cA

Tab. 4 Mean ±SE mortality (%) of insects exposed to *A. sibirica* EO at different doses after 24 h exposure

Dose (µl/cm²)	Stored product pest *S. granarius*	*S. oryzae*	*T. confusum*	*T. molitor* (larvae)	*T. molitor* (adults)	*T. granarium*
0.01	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0cA
0.25	100.0± 0.0aA	100.0± 0.0aA	31.7 ± 17.6bB	0.0 ± 0.0bC	26.7± 30.6cBC	43.3 ± 28.9bB
0.50	100.0± 0.0aA	100.0± 0.0aA	91.7 ± 7.6aA	93.3± 11.6aA	60.0 ± 20.0bB	100.0 ± 0.0aA
0.75	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	93.3± 11.6aA	73.3 ± 23.1abA	100.0 ± 0.0aA
1.00	100.0± 0.0aA	100.0± 0.0aA	100.0 ± 0.0aA	100.0± 0.0aA	93.3 ± 11.6aA	100.0 ± 0.0aA
Control	0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0bA	0.0 ± 0.0cA	0.0 ± 0.0cA

Discussion

The composition of tested *I. verum* and *A. sibirica* essential oils in the present study showed a similarity to that reported in the literature (Tuan and Ilangantileke, 1997; Singh et al., 2006; Matsubara et al., 2011). For example, according to Singh et al. (2006), major component of *I. verum* EOs was trans-anethole (85-90%). Some differences were observed in the composition and abundant compounds of *A. absinthium* EO (Lawrence, 2006; Gandomi Nasrabadi et al., 2012). For example, myrcene (21.5 %), thujyl alcohol (18.9 %), sabinene (17.3 %), α-thujone (7.4 %) and camphor (5.5 %) were found to be the main components in *A. absinthium* essential oil growing in Russia (Lawrence, 2006). These differences in essential oil compositions might arise from several environmental (climatical, seasonal, geographical) or genetic differences and different chemotypes and the nutritional status and the extracted part of the plant.

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Toxicity of extracts derived from different parts of cassava plant, *Manihot esculenta* Crantz to four major coleopteran pests of stored-products

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Abstract

Fumigant toxicity of insecticidal principles extracted from tuber rind, fresh leaf, fresh leaf with petiole, and dried leaf of cassava (var. M4) was studied against four major stored-product insect pests *viz. Sitophilus oryzae (*L.*)*, *Rhyzopertha dominica* (F.*)*, *Tribolium castaneum* (Herbst) and *Callosobruchus chinensis* (L.) under laboratory conditions (28±2ºC, Rh. 75±5%). Mortality of the test insects varied with respect to extracts collected from different parts of the plant, and time of exposure. Extract collected from cassava rind recorded the highest toxicity. *Callosobruchus chinensis* was highly susceptible and showed immediate knockdown effect to the active principles extracted from tuber rind, fresh leaf, fresh leaf with petiole, twig and semi-dried leaf. The extract collected from various parts of plant caused 100% mortality of *R. dominica* at 1 hour after treatment (HAT), but the same collected from tuber and dried leaves did not show any toxic effect. Mortality of *S. oryzae* was 100% at 1 HAT with tuber rind extract, but no response was observed from the extract collected from semi-dried leaf, twig, and leaf with petiole. No fumigant action was observed in all the four coleopteran pests exposed to the extract collected from dried leaves. The study revealed that fresh leaf and tuber rind are good sources for the extraction of biofumigant against major coleopteran pests, however dried leaves are unfit for same purpose.

Key words: Cassava, extracts, Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum, Callosobruchus chinensis.

Introduction

Insect pest infestation is a major problem in the storage of cereals and pulses. Realising the fact that indiscriminate application of synthetic pesticides has created major challenges to man and ecosystem, there is a global concern to contain pests using non-chemical methods, particularly to lessen the pesticide residues in food (Flinn and Hagstrum, 2001). Fumigation is an effective method to protect stored-products from insect infestation. The commonly used fumigant like aluminium phosphide, ethylene dibromide and methyl bromide are associated with health and environmental pollution, and also many of the stored- product pests have developed resistance against synthetic fumigants (Zettler, 1982). Phillips et al. (2001) opined that exposure to low or sublethal doses pose an increased risk in phosphine resistance. Opit et al. (2012) reported high levels of resistance in several strains of major storage pests to phosphine in the USA.

The use of natural compounds in place of synthetic insecticides is an alternative strategy to reduce environmental pollution, and to preserve non-target organisms. Phytochemicals have been suggested as the alternatives to synthetic insecticides as they are the storehouse of a wide range of bioactive chemicals (Wink, 1993). Plant products are inexpensive products for the management of stored-grain pests (Mishra et al., 2012b), and are potentially suitable as vital components in integrated pest management strategies (Saxena, 1989; Schmutterer, 1992). The insecticidal activity of many plant derivatives against several stored-product pests has been demonstrated (Malik and Mujtabe Naqvi, 1984; Singh, S. 2017).

Cassava (*Manihot esculenta* Crantz), originally from Amazonia, is a woody shrub extensively cultivated as an annual crop in tropical and subtropical regions that provides the staple food of an estimated 800 million people worldwide. Although the leaves of cassava are rich in proteins, minerals, and vitamins, the presence of antinutrients and cyanogenic glucosides are the major drawbacks to human consumption. However, cyanogen can play a pivotal role in pest management.

Desmarchelier and Ren (1996) has patented cyanogen as a fumigant to replace methyl bromide in a variety of applications. Being a good source of cyanogen, cassava was used for the isolation of insecticidal molecules for the development of a fumigant against the stored-product pests.

Materials and Methods

Insects

Colonies of four beetle species were reared under laboratory conditions (Temp. 28±2ºC; Light and Dark photo period 12:12). *Sitophilus oryzae*, *Tribolium castaneum* and *Rhyzopertha dominica* were reared on whole grain wheat, and *Callosobruchus chinensis* was reared on cowpea seeds in plastic containers (100 g capacity).

Sample preparation

Twigs, leaves, leaves with petiole, semi-dried and dried leaves, tuber flesh and tuber rinds of cassava variety, M4, were collected from the experimental field of ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, India for the isolation of insecticidal molecules. Each sample (200 g) was macerated and distilled in 500 ml of distilled water. The distillates were collected 25 ml each, and thus four fractions were collected for bioassay on the selected four weevil species.

Bioassays

The bioassay revealed that first fraction of the extract was highly toxic to the four species of weevils exposed; hence this fraction was used for the subsequent toxicity study. Plastic tubes of 3 ml capacity were loosely impregnated with cotton and each tube was separately loaded with 1 ml of the extract. The tubes were perforated around with a needle to ensure the dissipation of biofumigant. The tubes were introduced separately into plastic containers of 250 ml capacity wherein the test insects were released. Cotton impregnated with distilled water was run as control. To retain the biofumigant within the experimental containers, these were closed airtight. All the treatments were replicated three times. Mortality of *S. oryzae*, *R. dominica* and *T. castaneum* was recorded at 1, 2 and 12 hours after treatment (HAT), whereas *C. chinensis* was observed at 2, 3 and 5 minutes after treatment as these are highly susceptible to the fumigant.

Statistical analysis

The percentage of mortality was determined and transformed to arcsine values for analysis of variance (ANOVA) using SAS (version 9.3). Treatment means were compared using least significance difference (LSD) at $p < 0.05$ level of significance. .

Results

The extracts collected from cassava tuber rind, fresh leaf, fresh leaf with petiole, twig and semi-dried leaf were highly toxic to *R. dominica* (Table 1). Mortality of *R. dominica* was 100% at 1 HAT in all the treatments, except with dried leaf. The extract collected from cassava tuber flesh was significantly less toxic to *R. dominica* than all other treatments. No mortality was observed in the control and treatment with dried leaf extract.

Mortality of *S. oryzae* at 1 HAT was 100% due to the treatment of extract collected from tuber rind, but there was no difference in the rate of mortality between the extracts of fresh leaf and semi-dried leaf. In all the treatments, except tuber flesh and dried leaf extracts, over 70% mortality of *S. oryzae* was noticed, however no mortality was observed with the treatments of extracts collected from tuber flesh and dried leaf.

Table 1. Mortality of *Rhyzopertha dominica* and *Sitophilus oryzae* due to the fumigant action of cassava extract

Treatment	*Rhyzopertha dominica* (Hours after treatment)	*Sitophilus oryzae* (Hours after treatment)

	1	2	12	1	2	12
Tuber rind	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)
Fresh leaf	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	86.7 (68.9)	86.7 (68.9)	100.0 (89.6)
Fresh leaf with petiole	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	73.3 (59.2)	78.3 (62.5)	88.3 (70.1)
Twig	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	73.3 (68.9)	86.7 (68.7)	91.7 (76.1)
Semi-dried leaf	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	86.7 (68.9)	90.0 (74.9)	96.7 (83.6)
Tuber flesh	26.7 (30.8)	26.7 (30.8)	26.7 (30.8)	0 (0.4)	0 (0.4)	0 (0.4)
Dried leaf	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)
Control	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)
(0.05)			3.3			5.3

Values in parenthesis are arc sine transformed values

The extracts collected from tuber rind and from semi-dried leaf were shown significantly higher toxicity to *T. castaneum* than the other treatments (Table 2). Mortality of *T. castaneum* was 100% at 1 HAT due to the treatment with tuber rind extract. The extract of semi-dried leaf recorded higher toxicity than fresh leaf with petiole, and twig extracts. In all the treatments, except the extracts of tuber flesh and dried leaf, 100% mortality was observed at 12 HAT

Preliminary study revealed that *C. chinensis* was highly susceptible to the bioactive components extracted from cassava, hence the mortality was observed at 2, 3 and 5 minutes after treatment. Immediate knockdown effect of the test insect was noticed due to the treatment with the extracts of tuber rind, and semi-dried leaf. Except the extract from tuber flesh and dried leaf, higher mortality was also observed in all other treatments.

Table 2. Mortality of *Tribolium castaneum* and *Callosobruchus chinensis* due to the fumigant action of cassava extract

Treatment	*Tribolium castaneum* (Hours after treatment)			*Callosobruchus chinensis* (Minutes after treatment)		
	1	2	12	2	3	5
Tuber rind	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)
Fresh leaf	50.0 (45.0)	56.7 (48.8)	100.0 (89.6)	93.3 (77.6)	100.0 (89.6)	100.0 (89.6)
Fresh leaf with petiole	78.3 (62.9)	86.7 (72.7)	100.0 (89.6)	90.0 (74.9)	93.3 (77.6)	100.0 (89.6)
Twig	61.7 (52.1)	70.0 (65.7)	100.0 (89.6)	93.3 (77.6)	96.7 (83.6)	100.0 (89.6)
Semi-dried leaf	88.3 (77.7)	93.3 (80.9)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100(89.6)
Tuber flesh	0 (0.4)	0 (0.3)	0 (0.3)	0 (0.4)	0 (0.4)	0 (0.4)
Dried leaf	0 (0.4)	0 (0.3)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)
Control	0 (0.4)	0 (0.3)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)
CD (0.05)		8.3			5.5	

Values in parenthesis are arc sine transformed

Discussion

The use of chemical fumigants for the management of stored-product pests is highly discouraged due to multiple reasons. Aulicky and Stejskal (2018) reported that although fumigation with phosphine proves satisfactory control of major stored-product pests, its effect sharply declines with the increasing distance from the fumigated spot. In the present investigation, the extracts collected from different parts of cassava was found toxic to *R. dominica*, *S. oryzae*, *T. castaneum* and the toxicity was extremely high as in the case of *C. chinensis.* Cassava tuber rind extract recorded the highest toxicity among the extracts from other parts of the plant. Burns et al. (2012) observed that concentration of cyanide in the tuber rind was much higher than that of the tuber parenchyma and leaves. Aggarwal et al. (2001) reported the fumigant action of essential oil from *Artemisia annwa* against *C. maculatus*, *R. dominica* and *S. oryzae*. Fumigant action of extracts collected from semi-dried leaf was significantly higher than that of fresh leaf; this may be due to the increase in concentration of active principles in leaf due to the loss of water during slow drying. The extract collected from fully dried leaves did not show any toxicity to all the test insects, which is a clear indication that the active principles are fumigant in nature. Plant extracts and essential oils are

known to possess insecticidal, ovicidal and repellent activities against various stored-product insects (Desmarchelier, 1994: Shaaya et al., 1997). El- Nahal et al., (1989) reported that adults of *C. chinensis*, *S. granarius*, *S. oryzae* were susceptible to the essential oil from *Acorus calamus,* but *T. castaneum* and *R. dominica* were tolerant to all doses and exposure time. They also reported that the exposure period is the most important factor affecting the efficiency of the vapours than dosage. Leaf, bark and seed extracts of *Aphanamixis polystachya* have been reported to exert repellent and insecticidal effects against *C. chinensis* (Talukder and Howse, 1994). Several natural products including principles from many spices, herbs and medicinal plants are known to have a range of useful biological properties against insects (Tripathi et al., 1999a, b). Insecticidal activity of *Cinnamomum* cassia bark, *Illicium verum* fruit and *Foeniculum vulgare* fruit was reported by Kim et al., (2003).

Although phytochemicals are reported to have very good insecticidal action against stored-product pests, poor extractability and cumbersome techniques in the isolation of active principles are the bottlenecks in its commercial availability in the markets. In the present study, cassava bio-wastes, which are either underutilized or thrown as waste, are promising source for the extraction of biofumigant.

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Entomocidal, repellent, antifeedent and growth nhibition efects of different plant extracts against *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera)

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ABSTRACT

In present investigation, toxic, repellent, antifeedent and growth inhibition effects of five different plant extracts: *Melia azedarach, Pegnum hermala, Salsola baryosma, Azadirachta indica* and *Zingiber officinale*, were evaluated on different life stages of *T. castaneum*. The highest mortality (10.14%) was observed with *A. indica* and the minimum mortality (0.67%) was invoked by *Z. officinale* treatment. Similarly, *A. indica* showed highest repellent effect compared to rest of the plants. Feeding deterrence was highest (90.15%) with *S. baryosma* treatment, followed by *P. hermala* (84.85%), *M. azedarach* (80.19%), *A. indica* (73.48%) and *Z. officinale* (57.58%). The extracts inhibited the growth of *T. castaneum*. Inthe case of *A. indica,* the lowest numbers of larvae (32.67), pupae (16.33) and adults (11.33) emerged at 15% concentration, while the highest emergence of larvae (80.33), pupae (75.00) and adult (71.00) were observed for *Z. officinale*. The other three plant extracts had moderate regrowth inhibition on the beetle. Overall, *A. indica* extract was found to be the most effective while, *Z. officinale* extract was least effective against the beetle. This study can be very helpful in future when the use of plant extracts become common and available to the farmers as an alternative to synthetic pesticides.

Key words: Mortality, repellency, antifeedant effect, growth inhibition, plant extracts, *T. castaneum*

Introduction

Food security is an emerging threat to world's expanding population. Stored grain insect pests are severely damaging our valuable products like wheat. These stored grain pests are responsible for about 10% loss of cereals all over the world (Danahaye *et al*., 2007). According to Matthews, (1993) 10-40% losses per annum have been estimated globally due to the infestation of these insects. These losses can reach 9% in developed and up to 20% in the developing countries throughout the world (Phillips and Throne, 2010).

Among the stored product insect pests, *T. castaneum* is the pest of economic importance throughout the world which cause serious damage to stored products (Arbogast, 1991). It is one of the major pests of wheat flour (Howe, 1965). Both larvae and pupae are responsible for the losses. In case of severe destruction, flour may be converted into greyish and moldy produce with pungent off-smell. Thus, the commodity becomes unfit for human use (Atwal and Dhaliwal, 2002). It can cause economic loss of 40% of wheat flour (Ajayi and Rahman, 2006).

Widespread and indiscriminate use of pesticides is posing a serious threat to the environment and human health (Subramanyam and Hagstrum, 1996). Consequently, the use of biopesticide for the control of stored commodity pests has received considerable attention throughout the world (Faraz

et al., 2015). Bio pesticides have potential to replace the use of conventional insecticides (Regnault-Roger *et al*., 2002). Plant extracts contain several compounds which are responsible for the control of insect pests of stored grain (Rahman and Schmidt, 1999). Several plant derivatives have been investigated to have mortality effect on stored product pests (Mukherjee and Joseph, 2000) and a number of plants of Pakistan have been known for their repellency to stored grain pests (Verma *et al.,* 2000). Some of the plants show repellency and growth inhibition effects (Kanvil *et al*., 2006).The five plants which were tested in this research are abundantly available and possess numerous medicinal properties (Iqbal *et al*., 2010). *Azadirachta indica* and *M. azedarach* are two closely related species of Meliaceae and are known in India and Pakistan for more than 2000 years. It is best known to have various medicinal properties including toxic and repellent effect. *Melia azedarach* (dhrek) has been found to have insecticidal, antifeedant, growth regulatory activities (Nakatani *et al*., 2004; Al-Rubae, 2009). They contain azadirachtin, meliacarpinin, bakayanin and triterpenoids (Vishnukanta, 2008). The pharmacological effects of *Pegnum hermala* are found to be due to the presence of alkaloids which include: β-carboline, harmine, harmaline, harmalol, harman, peganine, dipeganine, isopeganine, deoxypeganine and quinazol (Fathizad *et al*., 2007; Asgarpanah and Ramezanloo, 2012). *Zingiber officinale* (ginger) has beautiful fragrance and bitter taste (Jean, 1995). The compounds which are responsible for bitter taste are gingerol, zingiberene, zingerone and shogaol. Kaempferol and quercetin are the flavonoids that are present in the various parts of *Salsola baryosma*(khar booti) (Kaur and Bains, 2012).

The current study was conducted to assess the toxicity repellency, the antifeedent behavior and the growth inhibitory action of five different plant extracts namely *Azadirachta indica, Melia azedarach, Pegnum hermala, Salsola baryosma* and *Zingiber officinale*) against *T. castaneum* using acetone as solvent.

Materials and Methods

Collection and Rearing of Insects

The heterogeneous population (larvae and adults) of *Tribolium castaneum* was collected from grain market, poultry litter and stores of Rehman Farms and J. K. Agricultural Farms located at Faisalabad District, Punjab, Pakistan. The insects were reared in plastic jars (1.5 kg capacity) containing wheat flour which had been sterilized at 65^{0}C for 30 minutes before putting into rearing jars. In the rearing jars, 50 adults of *T. castaneum* were released on wheat flour. After 5 days the parent adults were removed from the rearing jars and culture medium containing eggs of the beetles were kept in incubator (Sanyo M.I.R 254) at optimum conditions (30±2°C and 65±5% r.h.) for getting homogenous population.

Collection and Preparation of Plant Materials

Fresh leaves of Dhrek, *Melia azedarach,* and Neem, *Azadirachta indica, s*eeds of Hermal, *Pegnum hermala,* stem of Khar booti, *Salsola barysoma* and rhizome of Ginger, *Zingiber officinale* were collected from different farms and fields located at District Faisalabad. The different plant parts were washed and after drying in shade for 5 days the leaves, seeds, stems and rhizomes were ground using electric grinder (Pascall Mortar grinder, Machine no. 20069) into fine powder. The powder was used to prepare extract using acetone as a solvent. In 250 ml flask, 50 gram of plant powder was soaked in 100 ml of acetone. The flasks were placed on rotary shaker at 220rpm for 24hr and then filtered. The filtrate thus obtained was used to make different concentrations for the various experiments. Three concentrations (5, 10 and 15%) of plant extracts were prepared in acetone (solvent). To make 5% solution, 5 ml of stock solution was added to 95 ml acetone and for 10% solution 10ml stock solution was added to 90 ml acetone and for 15% solution 15 ml stock solution was added t0 85 ml acetone.

Toxicity and Progeny Emergence of Plant Extracts

The diet incorporation method was used to estimate the efficacy of the different plant extracts in terms of beetle mortality and growth inhibition. The three concentrations of plant extracts were applied to 100 g of wheat flour along with an untreated check. About 30 adults of *T. castaneum* were put into the vials and the treatments were replicated three times using Completely Randomized Design. The mortality of the beetles was recorded after 24, 48 and 72 hrs. The survivors of *T. castaneum* from the above experiment were released on fresh wheat grains to determine progeny emergence. The number of beetles that emerged was recorded after 30 and 60 days. Corrected mortality was calculated using Abbot's formula given below:

$$\text{Corrected Mortality (\%)} = (Mo-Mc)/(100-Mc) \times 100$$

Where, Mo = observed mortality

Mc = control mortality

Percent inhibition rate of progeny was calculated using following formula:

$$\text{Percent Inhibition Rate (\%)} = (Cn-Tn)/Cn \times 100$$

Where, Cn = no. of progeny in control jars

Tn = no. of progeny in treated jars

Repellent Effect of Plant Extracts

The repellent effect was determined using area preference method. After cutting the filter papers into two equal halves, one half of each paper was treated with plant extracts. After evaporation of excess solvent, the two halves were joined together and placed in petri dishes. 50 adults of *T. castaneum* were released in the center of treated filter papers. Repellency was recorded after 12, 24 and 48 hrs.

Percent repellency was calculated following the formula used by Guruprasad and Pasha (2014).

$$\text{Percent Repellency (\%)} = (Nc-Nt)/(Nc+Nt) \times 100$$

Where, Nc = No. of insects in control half

Nt = No. of insects in treated half

Antifeedant Effect of Plant Extract

The jars containing 20 g of treated grains were kept in open environment for 24 hrs in order to allow the excess acetone to evaporate. After 24 hrs, thirty insects were released in each jar. The jars were weighed to take the initial reading. After 7, 14 and 21 days' interval the weight loss was recorded to check the antifeedant effect of the extracts.

$$\text{Feeding Deterrence Index (\%)} = ((C-T))/((C+T)) \times 100$$

Where, C = food consumed in control jars

T = food consumed in treated jars

Growth Inhibitory effect of Plant Extracts

Thirty adults of mixed sexes of *T. castaneum* were released into the jars containing wheat flour treated with the different concentrations of the five plant extracts. The number of larvae emergence, pupae transformation and adult emergence was recorded after 15 days for larvae, 25 days for pupae and 40 days for adult.

Statistical Analysis

Data were subjected to Analysis of Variance using STATISTICA 6.0 as one factor complete randomized design. Means were compared using Tukey's HSD test at 0.05 probability level, to check the significant difference among treatments.

Results

Toxicity of Plant Extracts against *Tribolium castaneum*

In general, all the extracts showed low toxicity to *T. castaneum* as the highest percent mortality of 10.14% was recorded in the diet treated with extract of *A. indica* (Table 1). In all the diets treated with each of the five plant extracts, percent mortality was time and dose dependent as it increased with the increase in concentration and exposure time (Table 1). In diets treated with the extracts of *A. indica, M. azedarach*, *S. baryosma*, *P. hermala* and *Z. officinale,* percent mortality ranged from 1.33-10.14%, 1.33-9.46%, 0.00-8.79%, 0.00-6.76% and 0-6.08%, respectively (Table 1). For example, in diets treated with the extract of *A. indica*, the highest mortality 10.14% was recorded at maximum concentration of 15% after 72hrs while the lowest mortality of 1.33% was recorded at 5% after 24 hrs of exposure time.

Tab. 1 Comparison of mean mortality of *Tribolium castaneum* treated with different plant extracts

Time	Conc.	Mortality (%) ± S.E.				
(hrs)	(%)	***A. indica***	***M. azadirach***	***S. baryosma***	***P. hermala***	***Z. officinale***
	5	1.33±0.66e	1.33±0.66e	0.00±0.66e	0.00±0.66d	0.00±0.66c
24	10	2.67±0.67de	2.00±0.65de	1.33±0.66de	0.67±0.66d	0.00±0.67c
	15	3.33±0.67cde	3.33±0.66cde	2.00±0.67cde	1.33±0.65d	0.67±0.66c
	5	3.35±0.65cde	2.68±0.67de	2.01±0.67cde	1.34±0.67d	1.34±0.67bc
48	10	5.37±0.65bc	4.69±0.67bcd	3.35±0.67bcd	3.35±0.67c	2.01±0.67bc
	15	7.38±0.66b	6.04±0.66bc	4.02±0.66bc	5.37±0.66ab	3.35±0.66b
	5	4.73±0.68cd	3.38±0.68cde	3.38±0.68bcd	3.38±0.68c	2.03±0.67bc
72	10	7.44±0.68b	6.76±0.68ab	5.41±0.68b	4.73±0.68bc	3.38±0.68b
	15	10.14±0.67a	9.46±0.65a	8.79±0.67a	6.76±0.67a	6.08±0.68a

Repellency of Plant Extracts against *Tribolium castaneum*

All the plant extracts were highly repellent to *T. castaneum* and repellency was dose and time dependent as it increased with the increase in concentration and exposure time. (Table 1). Percent repellency ranging from 50.67-89.33%, 56.00-85.33%, 58.67-89.67%, 60.00-89.33% and 56.00-82.67% were recorded in diets treated with the extracts of *A. indica. M. azedarach*, *P. hermala. S. baryosma* and *Z. officinale*, respectively. (Table 2). The highest percent repellency of 89.67% was invoked by the extract of *P. hermala* and the lowest by *M. azedarach* at the highest concentration of 15% and 48 hours exposure time (Table 2).

Tab. 2 Comparison of (%) mean repellent effect of different plant extracts *against Tribolium castaneum*

Time	Conc.	Repellency (%) ± SE				
(hrs)	(%)	***A. indica***	***M. azadirach***	***S. baryosma***	***P. hermala***	***Z. officinale***
	5	50.67±1.33g	56.00±1.31f	60.00±1.33f	58.67±1.33f	56.00±1.30f
12	10	58.67±1.30f	60.00±1.30f	70.67±1.30e	68.00±1.33e	64.00±1.28e
	15	66.67±1.37e	73.33±1.28cd	81.33±1.27bcd	74.00±1.31cd	72.00±2.67cd
	5	60.00±1.35f	60.00±1.33f	72.00±1.27e	60.67±1.35f	68.00±1.33de
24	10	72.00±1.35d	68.00±1.33e	78.67±2.67cd	76.67±1.37bc	72.00±1.33cd
	15	80.00±1.33bc	76.00±1.31bc	85.33±1.33ab	80.33±1.40b	77.33±1.35ab
	5	76.00±1.33cd	70.67±1.31de	76.00±1.33de	70.00±1.41de	74.67±1.33c
48	10	81.33±1.31b	78.67±1.48b	82.67±2.67bc	78.00±2.67bc	77.33±1.33ab
	15	89.33±1.30a	85.33±1.41a	89.33±2.67a	89.67±2.67a	82.67±2.67a

Progeny Inhibition and Feeding Deterrence Effect of Plant Extracts against *Tribolium castaneum*

Both percent inhibition and feeding deterrence were time and dose dependent as they increased with the increase in concentration and exposure time (Tables 3 & 4). Percent inhibition ranging from 59.08-89.70%, 52.30-81.62%, 48.36-79.10%, 3.76-75.15% and 49.80-80.06% were recorded in diets treated with the extracts of *A. indica. M. azedarach*, *S. baryosma, P. hermala.*and *Z. officinale*, respectively. (Table 3). The highest percent inhibition of 89.70% was recorded at the highest *A. indica* extract concentration of 15% after 60days and the lowest percent inhibition of 75.15% was invoked by the extract of *P. hermala.*

Similarly, percent feeding deterrence effect ranged from 51.45-73.48%, 53.31-85.61%, 66.39%-90.15%, 43.98-84.85% and 25.30-57.58% in diets treated with extracts of *A. indica. M. azedarach*, *S. baryosma, P. hermala.*and *Z. officinale*, respectively. (Table 4). The highest percent feeding deterrence of 90.15%) was recorded at the highest concentration of 15% of *S. baryosma* after maximum exposure period of 21days and the lowest percent deterrence of 57.58% was recorded in diets treated with the same concentration and exposure period (Table 4).

The results depict that the highest inhibition of larvae, pupae and adults was observed at the highest concentration of 15% (Table 5). The highest number of larvae (111.00) emerged in control treatment. Lower numbers of larvae (32.67, 38.00, 52.67, 57.33 and 71.67) emerged in diets treated with *A. indica,M. azedarach, S. baryosma, P. hermala and Z. officinale,* respectively (Table 5). Similarly, lower numbers of pupae (16.33, 14.67, 32.67, 39.00 and 57.33) emerged in diets treated with *A. indica M. azedarach, S. baryosma, P. hermala* and *Z. officinale* respectively compared with control in which 110 pupae emerged (Table 5). In the case of adults the highest number (110 adults) emerged in the control treatment compared with lower number of adults (11.33, 10.67, 26.00, 32.00 and 47.33) that emerged in diets treated with extracts of *A. indica, M. azedarach, S. baryosma, P. hermala* and *Z. officinale* respectively (Table 5).

Tab. 3 Comparison of (%) mean population inhibition of *Tribolium castaneum* treated with different plant extracts

Time	Conc.	Population Inhibition (%) ± SE				
(days)	(%)	***A. indica***	***M. azadirach***	***S. baryosma***	***P. hermala***	***Z. officinale***
	5	59.08±0.21f	52.30±0.21e	48.36±0.22f	43.76±0.21f	49.89±0.21f
30	10	62.36±0.22e	55.58±0.22d	51.42±0.22e	45.29±0.21e	51.42±0.22e
	15	65.43±0.22d	59.30±0.21c	54.27±0.21d	47.92±0.22d	59.74±0.21d
	5	86.83±0.05c	79.52±0.10b	75.75±0.05c	72.04±0.06c	76.35±0.05c
60	10	88.08±0.06b	80.06±0.10b	77.96±0.06b	74.13±0.06b	78.32±0.05b
	15	89.70±0.05a	81.62±0.06a	79.10±0.06a	75.15±0.05a	80.06±0.06a

Tab. 4 Comparison of (%) mean feeding deterrence effect of different plant extracts against *Tribolium castaneum*

Time	Conc.	Deterrence Effect (%) ± SE				
(days)	(%)	***A. indica***	***M. azadirach***	***S. baryosma***	***P. hermala***	***Z. officinale***
	5	51.45±0.93f	53.31±0.99f	66.39±0.93d	43.98±1.86g	25.30±0.93f
7	10	58.92±0.94de	60.78±0.94e	68.25±0.90d	52.38±1.86f	33.71±0.94e
	15	63.59±0.93cd	64.52±0.93de	73.86±0.95c	62.65±0.93d	42.11±0.94d
	5	57.80±0.86e	65.55±0.86d	74.16±0.86c	57.80±0.93e	37.12±0.86e
14	10	64.69±2.27c	72.27±0.68c	77.61±0.83bc	65.55±0.86d	43.15±0.87d
	15	70.71±1.86ab	80.19±0.86b	79.33±0.83b	72.44±0.86c	49.18±0.83bc
	5	67.42±0.76bc	78.03±0.75b	80.30±0.77b	70.45±0.75c	46.97±0.75c
21	10	70.45±0.75ab	80.30±0.77b	87.12±0.73a	77.27±0.77b	52.27±0.77b
	15	73.48±0.76a	85.61±0.77a	90.15±0.71a	84.85±0.74a	57.58±0.77a

Growth Inhibitory Effect of Different Plant Extracts against *Tribolium castaneum*

Tab. 5 Comparison of mean growth inhibitory activities of different plant extracts against *Tribolium castaneum*

Plants	Conc. (%)	Larvae No.	Pupae No.	Adults No.
	5	53.00±0.57h	32.67±0.88h	26.67±1.00h
	10	45.67±0.57i	25.67±0.88i	20.00±0.66i
	15	32.67±0.33k	16.33±0.33k	11.33±0.67j
A. indica	0	111.00±0.33a	110.00±0.33a	110.00±0.33a
	5	54.00±0.57h	38.67±0.57g	34.33±1.00g
	10	46.33±0.33i	23.67±0.33j	23.67±0.66h
	15	38.00±0.33j	14.67±0.33l	10.67±0.33j
M. azadirach	0	111.00±0.57a	110.00±0.88a	110.00±0.33a
	5	62.33±0.57f	47.33±0.88f	42.67±1.00f
	10	57.33±0.33g	40.67±0.33g	35.33±0.33g
	15	52.67±0.33h	32.67±0.33h	26.00±0.33h
S. baryosma	0	111.00±0.31a	110.00±0.33a	110.00±0.57a
	5	73.00±0.51d	59.33±0.88d	55.00±1.00d
	10	66.33±0.57e	50.67±0.33e	43.67±0.57f
	15	57.33±0.33g	39.00±0.33g	32.00±0.33g
P. hermala	0	111.00±0.33a	110.00±0.57a	110.00±0.57a
	5	80.33±0.57b	75.00±0.88b	71.00±1.00b
	10	77.00±0.33c	69.33±0.58c	61.33±0.57c
	15	71.67±0.57d	57.33±0.33d	47.33±0.33e
Z. officinale	0	111.00±0.33a	110.00±0.33a	110.00±0.33a

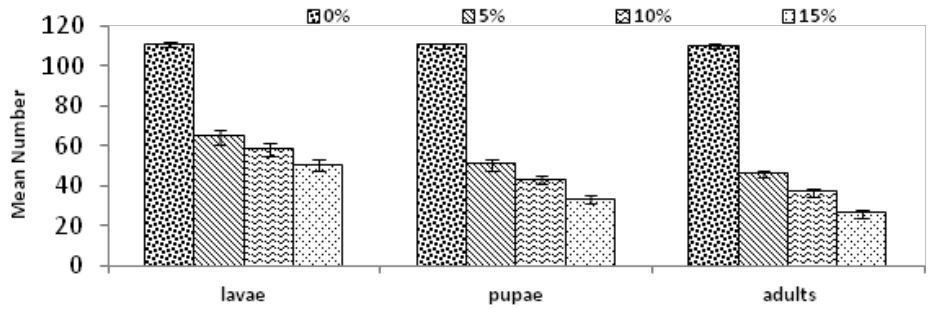

Fig. 1 Effect of different concentrations of plant extracts on the growth parameters of *T. castaneum*

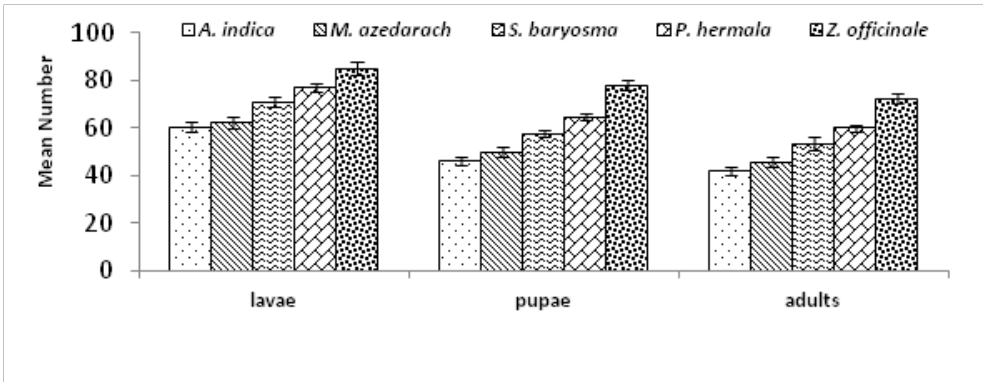

Fig. 2 Effect of different plant extracts on the growth parameters of *T. castaneum*

Discussion

The plant products have growth regulatory effects on different pests particularly red flour beetle, *T. castaneum* (Joseph *et al.,* 1994; Haque *et al.,* 2000).The most effective plant was *A. indica* in our study. It is known that *A. indica* contains bioactive compounds like azedarachtin. This compound affects the growth behavior and reproduction of stored grain insect pests (Pascual *et al*., 1990; Mordue (Luntz) and Blackwell, 1993; Singh, 1993). *A. indica* interrupts the normal development of larvae and adults as well as metamorphosis of insects (Koul *et al*., 1987). *A. indica* also significantly reduces the emergence of adults of *T. castaneum* and weight of the adults (Anonymous, 1992). The emergence of larvae, pupae and adults is affected by the change in dose rate of the plant extracts (Sagheer *et al*., 2011). Reduced growth of the *T. castaneum* was observed with the use of different plants including *P. heramala* (Jbilou and Sayah, 2006). *Melia azedarach* has been found to have insecticidal, antifeedant, growth regulatory activities (Nakatani *et al*., 2004; Al-Rubae, 2009)

In our study, all the plant extracts showed very low toxicity against *T. castaneum*. According to Fang *et al*. (2002) *Tribolium* sp. are the most tolerant among all stored grain insect pests so they are more difficult to control. Plant extracts contain several compounds which are responsible for the control of insects (Rahman and Schmidt, 1999). Several plant derivatives have been investigated to have mortality effect on stored product pests (Su, 1990; Desmarchelier, 1994; Schmutterer, 1995; Mukherjee and Joseph, 2000). Plant extracts reduce the release of phosphate ions for the production of energy during transphosphorylation reactions (Senthil-Nathan *et al*., 2004; Senthil-Nathan *et al.,* 2006). Anita *et al*. (2012) reported the dose-dependent insecticidal activity of *Eucalyptus globulus* against *T. castaneum*.

In the present study, neem extract was proved most effective in term of mortality. Neem possesses toxic effect against *T. castaneum* (Ahmed *et al.,* 2000). Many compounds in neem have been reported for their toxic effects the most potent is azadirachtin (Mordue and Blackwell, 1993; Lin-er *et al*., 1995). As a polyphagous pest, *T. castaneum* has been controlled successfully by the use of different insecticides (Okonkwo and Okoye, 1996; Islam and Talukdar, 2005). However, this pest have developed resistance against these pesticides (Guedes *et al*., 1997). Some plants have toxic effect against *T. castaneum* (Tripathi *et al*., 2000). The efficacy of neem against insect pests varies with the change in the storage atmospheres and storage period. Reduced activity of neem as a bio pesticide has been reported when it was stored in sun. Hence, the bioactivity of neem was higher when it was stored for two weeks than when it was stored for 4-6 weeks (El Shafie and Almahy, 2012). The activity of the neem increased 10 times at low temperature (Kabaru and Mwangi, 2000). To use the plant extracts as bio pesticides, farmers should be well aware of its production as well as application (Hellpap and Dreyer, 1995).

A number of plants of Pakistan have been known for their repellency against grain pests (Jilani *et al*., 1991, 1993; 2000; Verma *et al.,* 2000). Few plants show repellency and growth inhibition effects (Kanvil *et al*., 2006). Neem possesses repellent, antifeedant, oviposition inhibition and deterrent effects (Lale and Abdulrahman, 1999; Weathersbee and Tang, 2002; Liang *et al*., 2003; Hou *et al*., 2004). Jilani *et al*., (1984) reported the repellent effects of 30 plant extracts including *P. harmala* against *T. castaneum*. Diwivedi and Kumari (2000) reported the repellent action of different plant extracts prepared in acetone and petroleum solvents against *C. chinensis*.

Trematerra and Sciarretta (2002) reported that the mortality of the insects by plant extracts is due to its repellent and feeding deterrent effects. They also reported that the alkaloids present in the plants affect the chemoreceptors of insects thus inhibiting food consumption. Azadirachtin excites the deterrent cells of chemoreceptors and inhibits the sugar receptors thereby preventing the insects from feeding (Mordue-Luntz *et al*., 1995).

Neem also significantly reduce the emergence of adults of *T. castaneum* and weight of the adults (Anonymous, 1992). The emergence of larvae, pupae and adults is affected by the change in dose rate of the plant extracts (Sagheer *et al*., 2011). Reduced growth of the *T. castaneum* was observed with the use of different plants including *P. heramala* (Jbilou and Sayah, 2006).

The results of the study has shown that plant extracts can be used as a component of an IPM strategy for the control of insects both in the crops growing in the field and stored commodities. The use of plants as an insect control strategy can be a better substitute to synthetic insecticides.

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Toxicity and repellence of *Citrus jambhiri* Lush rind essential oil against maize weevil (*Sitophilus zeamais* Motschulsky 1855) (Coleoptera: Curculionidae)

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Abstract

Rind of matured fruits of *Citrus jambhiri* Lush was hydro-distilled to obtain essential oil (EO) which was subjected to Gas chromatography-Mass spectrometry (GC-MS) analysis. The EO was evaluated for fumigant toxicity (at 27-107µL/L air) and repellence against maize weevil (*Sitophilus zeamais* Motschulsky). Area preference methodology was used to evaluate the repellence of the EO at 0.15-0.9 µL/cm^2, while isopropanol served as control for both bioassays. The experiments were set up in a completely randomized design and data were subjected to analysis of variance and probit analysis. Fifty-two compounds were identified in the EO with the

predominant compounds being α-terpineol (8.03%), citral (7.00%), 4-terpineol (6.52%), caryophellene (4.58%), cis-geraniol (4.44%), citronellal (4.38%), β-bisabolene (4.01%) and n-hexadecanoic acid (4.70%). Others were α-bergamotene (3.74%), lemonol (3.23%), precocene I (3.33%) and β-copaene (3.09%). Toxicity progressed with EO dose and exposure period and application of EO at 80 and 107µL/L air caused significantly higher mortality (33.75-100.00%) than isopropanol (0.00-22.50%). Lethal time for 50% assayed weevil (LT_{50}) for the EO application at 107 µL/L air {7.51 (6.95-8.13) h} was significantly lower than the values obtained for 27 and 53 µL/L air {44.78 (27.49-312.61) and 21.87 (11.91-45.96) h, respectively}. EO caused significantly higher repellence (75.00-90.00%) than control (15.00%) at 24 hours after treatment. The results indicate that *C. jambhiri* rind EO has prospects as effective biorational formulation for control of maize weevil.

Keywords: *Citrus jambhiri,* Essential oil, Fumigant toxicity, GC-MS, Lethal time, Maize weevil

1. Introduction

Maize weevil (*Sitophilus zeamais* Motschulsky 1855) (Coleoptera: Curculionidae) is a cosmopolitan tropical postharvest pest attacking cereals, processed tubers and their products (Haines, 1990; Babarinde et al., 2008, 2013). Its infestation can cause quantitative loss like weight reduction, or qualitative losses like reduction oF aesthetic/market value, nutritional values and loss of seed germination ability, especially when weevils feed on the seed's embryo. Its control with synthetic chemicals, although very effective, can cause several economic, ecological and health risks (Arthur, 1996); hence the need for bio-rational control options for the pest. Several formulations of botanicals had been studied (Dales, 1986), but essentials oils (EOs) are receiving renewed attention due to the fact that they are effective at low concentrations, even without direct contact with the target organisms (Babarinde *et al*., 2015). Due to the multiple bioactive compounds present in EOs and their multiple sites and modes of action, the tendency for a pest to develop resistance against EOs is comparatively low (Venkitanarayanan *et al.,* 2013)

Several authors have worked on different *Citrus* species as protectants of stored produce against postharvest pests (Don-Pedro, 1996; Dutra *et al*., 2016; Fouad and Camara, 2017; Lu, 2017; Oboh *et al*., 2017). The need to convert agricultural waste product into useful by-products is a major concern in many developing countries. This is primarily because of poor waste management practices peculiar to the region. *C. jambhiri* rind usually constitute a a nuisance compared to other agricultural wastes that can be converted to positive use like animal feed, for instance. Therefore, any positive use of the waste product will be embraced by rural dwellers to whom its handling is an ecological or economic burden. To a very great extent, evaluation of the insecticidal properties of *C. jambhiri* seems to be scarce in the literatures. Different parts of *C. jambhirir* have differs uses. In India, its ground root is orally administered to control vomiting (Tiwari *et al.,* 2017) In Nigeria, it is formulated into concoction for medicinal purposes like tooth whitening and human weight loss. The fruit is also used for making local juice. Despite its several uses, the rind id usually thrown away after peeling without any productive utilization. In some rural areas of south western Nigeria, *Citrus* species rinds are often dried and put on indoor charcoal fire to produce smoke which acts either as toxicant or repellent to mosquitoes This ethno botanical practice was one of the major thrusts for this research. The study was therefore designed with the following objectives (a) To evaluate the fumigant toxicity and repellence of *Citrus jambhiri* rind EO against *Sitophilus zeamais,* and (b) To identify the chemical components of the EO using Gas chromatography-Mass spectrometry.

2. Materials and methods

2.1 Insect culture

Sitophilus zeamais was reared on Tsolo variety maize under laboratory conditions of 28±2°C temperature and 70±3% relative humidity as described by Babarinde *et al.* (2008).

2.2 Essential oil Extraction and Chromatographic analysis

Rinds of freshly harvested *C. jambhiri* fruits were manually removed and pounded in a mortal with the aid of a pestle. EO was extracted from the rind using hydro distillation method (British

Pharmacopoeia, 1988; Babarinde *et al*., 2017b). The EO was subjected to Gas chromatography-Mass spectrometry (GC-MS) using the following procedures. EO (1.0 µL) was injected into a GC-MS machine (GCMS-QP2010SE®, a product of Shimadzu, Kyoto, Japan), equipped with an AOC-20i auto sampler and a split injector (split ratio 1:50). The description and conditions of the GC-MS machine are as follow. Column used: Optima® 5MS (a product of Macherey – Nagel, USA) (30 m × 0.25 mm internal diameter × 0.25 µm film thickness) coated with 95% dimethylpolysiloxane 5% diphenyl packing materials; helium was the carrier gas at 56.2 kPa inlet pressure and 36.2 cm/s linear velocity, 3 and 0.99 ml/min purge flow rate, respectively. Oven temperature began at 60 °C and ramp of 10 °C/min up to 180 °C held for 2 min, and subsequent increase to 280 °C with a 15 °C/min heating ramp at 280 °C for 4 min. Injection temperature was 250 °C. The MS operating conditions were as follows: ionization with an ion trap detector in full scan mode under electron impact ionization (EI) at 70 eV, ion source temperature 200 °C; interface temperature 250 °C, scan range, 40–700 m/z. The identification of the components was done as earlier described (Adams, 2001; Joulain and Koenig, 1998: Babarinde *et al*., 2017b).

2.3 Entomological bioassays

2.3.1 Fumigant toxicity bioassay

Varying doses of *C. jambhiri* EO (27, 53, 80 and 107µL/L air) were separately dissolved in 0.2 mL isopropanol and applied to 8 cm^2 Whatman filter paper attached to the inner surface of the cork of 750 mL capacity fumigation chamber. Isopropanol (0.2 mL) served as control. Twenty 1-3-day old mixed sex *S. zeamais* adults were introduced into the fumigation chamber and covered. Mortality data were collected at 1, 3, 6, 12, 24 and 48 h after treatment (HAT). The weevils were adjudged to be dead when they were unable to move their body parts after a gentle shaking of the fumigation chamber.

2.3.2 Repellence bioassay

Area preference methodology was adopted for the repellence bioassay. Whatman filter paper (9 cm diameter) was used following the method of McDonald *et al*. (1970), which was modified by Zhang *et al*. (2015) and Babarinde *et al*. (2017b) using the following doses: 0.15. 0.30. 0.45 and 0.90 µL/cm^2. Each dose was separately dissolved in 0.2 mL isopropanol, while isopropanol served as control. Twenty insects similar in all respects with those used for fumigant toxicity bioassay were introduced into the repellence chamber. Numbers of insects on the treated (Nt) and untreated (Nc) discs of the filter paper were counted at 24 hours after treatment and percentage repellence (PR) was calculated using the formula:

$$PR= \{(Nc-Nt)/(Nc+Nt)\} X 100$$

2.4 Experimental design statistical analyses

The experiments were set up in completely randomized design and data were subjected to analysis of variance (ANOVA), while significant means were separated using Studentized Neuman Keuls (SNK) post-hoc test at 5% probability level. Lethal times (LT_{50} and LT_{90}) for each of the EO doses were determined using probit analysis. All statistical analyses were done with the aid of SPSS software package version 16 (SPSS, 2006).

3. Results

3.1 Chromatographic analysis

A total of 52 chemical compounds were identified in *C. jambhiri* rind EO. Among the identified chemical groups were monoterpenoid and sesquiterpenoid compounds (hydrocarbon and oxygenated or alcohols), fatty acids and aldehydes. Major compounds were α-terpineol (8.03%), citral (7.00%), 4-terpineol (6.52%), citronellal (4.38%), β-bisabolene (4.01%) and n-hexadecanoic acid

(4.70%). Others were caryophellene (4.58%), lemonol (3.23%), precocene I (3.33%) and β-copaene (3.09%) (Table 1).

3.2 Entomological bioassays

At 1 Hour after treatment (HAT), there was no mortality due to the applied treatments. Observed mortality in all EO doses (5.00-100.00%) was significantly higher than mortality observed in isopropanol (0.00-22.50%). At 3 HAT, treatment had no significant (df=4,19; F=3.29; p=0.051) effect on weevil's mortality; thereafter, toxicity increased with increase in EO doses. At 6 HAT, mortality observed when 80 µL/L air (33.75%) and 107 µL/L air (40.00%) was significantly (df=4,19; F=10.317; p<0.0001) higher than the observed mortality when lower doses of EO and isopropanol were applied (0.00.16.25%). At 12-48 HAT, all EO doses caused significantly (p<0.0001) higher mortality (28.75-100.00%) than 5.00-22.5% mortality observed in isopropanol (Table 2).

The results of probit analysis followed similar pattern as ANOVA results. LT_{50} for EO applied at 107 µL/L air {7.51(6.95-8.13) h} was significantly lower than the values for 53 µL/L air {21.87(11.91-45.96) h} and 27 µL/L air {44.78(27.49-312.62) h}. LT_{90} values followed the same pattern; application of EO at 107 µL/L air had significantly lower value {11.7(10.82-12.85) h} than 18.33(13.02-42.72) - 87.53(53.25-804.05) h observed in other EO doses (Table 3). All EO doses caused significantly (df=4, 19; F=5.173; p=0.008) higher percentage repellence (75.00-90.00%) than isopropanol (15.00%) (Fig. 1).

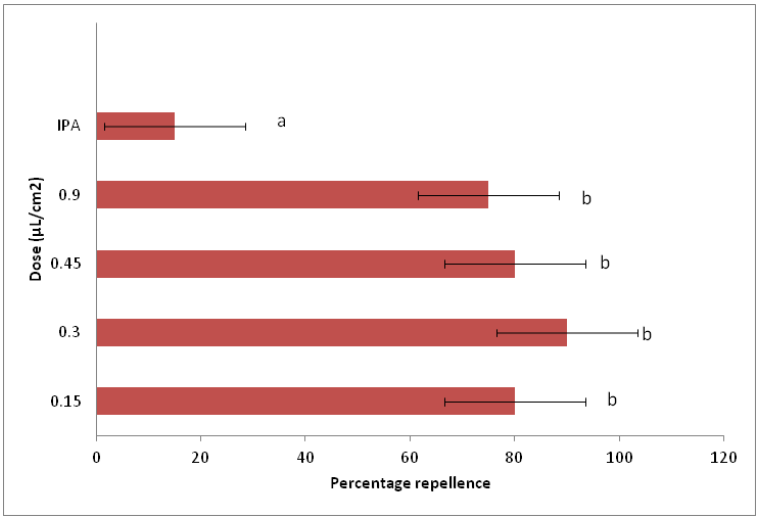

Fig. 1: Percentage repellence of *Citrus jambhiri* rind essential oil against *Sitophilus zeamais*

Means with the same letters of alphabet are not significantly different using SNK at 5% probability level.
IPA: Isopropanol (used as spreading agent for the essential oil and control).
ANOVA Results: df=4, 19; F=5.173; p=0.008

Tab. 1: Chemical composition of *Citrus jambhiri* rind essential oil

S/N	Retention Time	Name	% Composition
1.	4.215	1-nonanol	0.89
2.	4.303	4- pentadecyne, 15 chloro	0.8
3.	4.431	4- terpineol	6.52
4.	4.684	α- terpineol	8.03
5.	5.002	Citronellal	4.38
6.	5.137	Cis- geraniol	4.44

7.	5.349	Decanol	1.97
8.	5.441	Lemonol	3.23
9.	5.574	Alfol 10	1.73
10.	5.636	Cyclohexene, 2-ethenyl-1,3,3-trimethyl	3.8
11.	5.774	Allyl trisulphide	0.31
12.	5.863	Mentha-1,8-dien-7-yl acetate	0.65
13.	6.047	P-menth-3-ene, 2-isopropenyl-1-vinyl-,(15,2R)-(-)	1.72
14.	6.283	(-) Carvone	2.11
15.	6.440	Citral	7.0
16.	6.726	Perillaaldehyde	1.30
17.	6.978	Cyclohexene, 1-ethenyl-1-menthyl-2,4 bis(1-methyle nemy)-(15-1-apha)	1.8
18.	7.182	Neryl acetate	3.19
19.	7.366	α-bergamotene	3.74
20.	7.472	Genanyl acetate	2.44
21.	7.578	Caryophyllene	4.58
22.	7.975	Acetic acid, chloro- decyl ester	0.64
23.	8.062	Humulene	1.07
24.	8.298	β-bisalolene	4.01
25.	8.434	β-copaene	3.09
26.	8.629	Precocene 1	3.33
27.	8.727	β—sesquiphollendene	0.22
28.	9.371	Squalene	0.57
29.	9.649	Nerolidyl acetate	2.27
30.	10.019	Dodecanoic acid	0.51
31.	10.985	Spathulenol	0.45
32.	11.241	Viridiflorol	0.42
33.	11.372	α- bisabolol	0.65
34.	11.856	Farnesol	0.15
35.	12.036	(E)- stilebene	0.35
36.	12.256	3-methyldiadamenthane	0.21
37.	12.491	2,6,10 Trimetnyl-2,6,9,11-dodecatetraenal	0.44
38.	13.077	Isocarveol	0.22
39.	13.382	Eicosqunoic acid	0.14
40.	13.648	Spiro (andnot-5-ene-17	0.08
41.	13.851	Methyl 16-hydroxy-hexadecanoate	0.06
42.	14.249	n-hexadecanoic acid	4.7
43.	14.424	(6E)-nerodidol	0.64
44.	14.932	Dimethoxybicyclo(3.3.1) nonane-2-4-dione	0.51
45.	15.052	2-4-diflorobenzene,1-benzylosy	0.29
46.	15.234	Phytol	0.23
47.	15.483	Oleic acid	3.37
48.	15.615	Octadecanoic acid	3.34
49.	16.699	Dipalmitin	0.69
50.	18.000	Palmitin,2-mono	2.15
51.	19.208	Oleic acid chloride	1.21
52.	19.367	α-monostearin	1.0

The components are listed in ascending order of retention time

Percentage composition is the percentage peak area relative to total peak area obtained from total ion chromatogram peak report

Tab. 2: Percentage mortality of *Sitophilus zeamais exposed to Citrus jambhiri* rind essential oil in fumigant bioassay.

	Duration after treatment (h)					
Treatment (µL/L air)	**1**	**3**	**6**	**12**	**24**	**48**
Isopropanol	0.00±0.0	0.00±0.0	0.00±0.0a	5.00±3.5a	10.00±5.8a	22.5±4.3a
27	0.00±0.0	5.00±3.5	15.00±3.5a	28.75±3.1b	35.00±2.9b	46.25±3.8b
53	0.00±0.0	0.00±0.0	16.25±3.8a	45.00±7.4c	63.75±10.3c	90.00±4.6c
80	0.00±0.0	3.75±1.25	33.75±8.9b	72.50±2.5d	93.75±6.3d	100.00±0.0c
107	0.00±0.0	7.50±1.4	40.00±4.1b	88.75±2.4e	100.00±0.0d	100.00±0.0c
ANOVA	-	df=4,19	df=4,19	df=4,19	df=4,19	df=4,19
Result		F=3.29	F=10.317	F =63.379	F=39.398	F=116.621
		p=0.051	p<0.0001	p<0.0001	p<0.0001	p<0.0001

Values are means of four replicates ± S.E. Means followed by the same letters of alphabet are not significantly different using SNK at 5% probability level.

Tab. 3: Lethal time (h) of *Citrus jambhiri* rind essential oil against *Sitophilus zeamais.*

Dose (µL/L air)	LT_{50}(FL)	LT_{90}(FL)	X^2	P	DF	Slope
27	44.78(27.49-312.62)	87.53(53.25-804.05)	31.617	0.001	4	-14.483
53	21.87(11.91-45.96)	40.93(27.75-109.19)	53.135	<0.0001	4	-15.116
80	10.51(6.18-19.23)	18.33(13.02-42.72)	42.93	<0.0001	4	-13.633
107	7.51(6.95-8.13)	11.7(10.82-12.85)	6.26	0.041	4	-13.084

FL: Fiducial limits

4. Discussion

The result of the GC-MS analysis of C. jambhiri rind oil shows that the oil was predominated by terpenoid compounds. Apart from the terpenes, other chemical groups identified were aldehydes, alcohols and fatty acids. Monoterpenes and sesquiterpenes have been associated with the bioactivity of EO against many invertebrate pests (Obeng-Ofori and Reichmuth, 1999; Yildrim *et al.,* 2013; Saad *et al.*, 2018). Previous studies (Usman *et al.*, 2016; Fouad and Camara, 2017) on chemical components of different parts of *Citrus* species show the dominance of terpenoid compounds. The variations in the constituents of EOs are attributable to the difference in the activity of the synthases that mediate the formation of the compounds from their respective precursors (Degenhardt, 2009). Basically, however, the disparity in the chemical composition of any plant could be the differences in the species studied and the environmental factors involved in its cultivation (Jemaa *et al.,* 2012; Fouad and Camar, 2017).

The toxicity of *C. jambhiri* EO against *S. zeamais* conforms to recent studies (Campolo *et al.*, 2014; Dutra et al., 2016; Heidari *et al*., 2017; Oboh *et al*., 2017) on the bioactivity of EO obtained from *Citrus* species against stored product pests. According to Don-Pedro (1996), toxicity of *Citrus limon* against *Callosobruchus maculatus, S. zeamais* and *Dermestes maculatus* depended on strong fumigation action. Kumar and Tiwari (2016) reported the fumigant toxicity of *Citrus reticulata* against *Sitophilus oryzae. Apart from Citrus* species*,* other botanical EOs which have been reported to be toxic or repellent against *Sitophilus species* include Hoslundia opposita *(Babarinde* et al., 2017a), *Lippia javanica (Kamanula et al.,* 2017), *Teucrium capitatum* and *Salvia pomifera* subsp. c*alycina (Koutsaviti* et al., 2018). Although the mechanism of toxicity of the EO against *S. zeamais* was not covered in the scope of this study, fumigant toxicity of another EO (*X. parviflora)* against *C. maculatus* was suggested to be due to the inhalation of the EO which led to neurotoxicity and eventual mortality (Babarinde *et al*., 2015). The fumigant toxicity of *C. jambhiri* implies that the EO can be used to protect infested maize against the damage of *S. zeamais*.

Whereas Dutra *et al.* (2016) classified the repellence of the EO from four *Citrus* species against *C. maculatus* as neutral; our study shows *C. jambhiri* to be repellent against *S. zeamais.* The disparity in the result of Dutra *et al.* (2016) and the present study was due to the differences in the studied Coleoptera species and their physiological responses to the exposures to the chemical compounds in the different EOs. Repellence of *Citrus reticulata* EO *against Cryptolestes ferrugineus has been reported by* Lu (2017). The repellence of the *C. jambhiri* rind EO against *S. zeamais* implies that the EO

is effective to prevent re-infestation after an initial effective control of *S. zeamais* in stored maize. It also indicates the ability of the EO to prevent the infestation of non-resident pest population (Lale and Alaga, 2001; Babarinde *et al*, 2014).

5. Conclusion

C. jambhiri EO was effective as a toxicant and repellent against *S. zeamais*. With the appreciable number (52) of identified chemical compounds in the EO, the tendency of the development of resistance against the EO by maize weevil is low. Therefore, the EO can be incorporated into Integrated Weevil Management scheme. Since citrus rind is often thrown away after peeling, the results of this research have established a potential of the waste product of *C. jambhiri* in the pest control segment. The scope of the study did not extend to the evaluation of the bioactivity of the prominent chemical constituents, we therefore suggest that the observed bioactivity of the EO could be due to the combined effects of the chemical constituents identified in *C, jambhiri.*

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Binary mixture efficacy of NeemAzal and *Plectranthus glandulosus* leaf powder against cowpea and maize weevils

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Abstract

The aim of this study was to determine the insecticidal efficacy of mixture of NeemAzal a commercial neem product and *Plectranthus glandulosus* leaf powder against *Callosubruchus maculatus* and *Sitophilus zeamais*. Mixed at various proportions (100 + 0, 75 + 25, 50 + 50, 25 + 75 and 0 +100%, these powders were tested on adult mortality, inhibition of offspring production and their persistence on *C. maculatus* and *S. zeamais*. All the mixed NeemAzal and *P. glandulosus* caused significant mortality to adult *C. maculatus* and *S. zeamais*. No

significant difference was observed among the mixed powders that were subjected to the three mixture proportions regarding the mortality they caused to the weevils. The mixed 75% NeemAzal + 25% *P .glandulosus* of powder led to a higher mortality (100%) of both insect species, three (5 g/kg) days post exposure. The three days LC_{50} values decreased with ascending proportion of NeemAzal in the mixture from 3.21 g/kg (25% NeemAzal + 75% *P. glandulosus*) to 0.24 g/kg (75% NeemAzal + 25% *P. glandulosus*) in *S. zeamais*. In *C. maculatus,* the opposing effect was observed. The number of F_1 progeny produced reduced significantly ($P \leq 0.01$) in both insect species with the mixture proportion of botanicals. The mixtures reduced better the adult progeny production than the botanicals applied alone. The 75% *P. glandulosus* + 25% NeemAzal persisted well on grains up to 180 days for all dose levels. Powder from NeemAzal and *P. glandulosus* leaves stand as good candidates to protect maize and cowpea against the infestation of *S. zeamais* and *C. maculatus* respectively during storage. Mixing these products could not be advantageous since the binary mixture gave similar result as when they were applied alone.

Key words: Callosobruchus maculatus, Sitophilus zeamais, mixture, Plectranthus glandulosus, NeemAzal, efficacy

Introduction

Crop production plays major importance in the livelihood of people all over the world and particularly in developing countries where it is considered as the principal activity on which most economies depend on (Gustavsson et al., 2011. Paradoxically many farmers loss heavy quantity of cultivated plants because of the attack by insect pests. *Sitophilus zeamais* and *Callosobruchus maculatus* are the most important pests stored maize and cowpea respectively with great losses mostly in tropical countries(Guèye et al., 2011). To reduce post-harvest losses, different methods of grain protection are used by small holder farmers as well as at the industrial level (Isman, 2006). However, over the past decades, synthetic chemical insecticides have played a significant role in modern agricultural pest management (Guo *et al.,* 2014). Their repeated use over the years has led to the evolving of resistance in pest populations and fostered environmental and human health concerns (Ofuya, 2003). These problems have highlighted the need for the development of new types of selective insect-control alternatives (Lee et al., 2001), which combine broad spectrum action against stored product insect pests with low toxicity to non-targeted organisms, but at the same time also readily available and affordable to the small-scale grower (Nukenine et al*.,* 2007). Nowadays, products from plant origin are recommended as alternative to the hazardous synthetic chemicals. *Plectranthus glandulosus* leaf powder and essential showed greater insecticidal efficacy against adult insects of stored cereals (Nukenine et al*.,* 2011).The commercial NeemAzal powder which is constituted of the mixture of diatomaceous earth with azadiracthin was effective against the maize weevil (Nukenine et al., 2011). To promote the use of safer NeemAzal or *P. glandulosus* leaf powder combined with good efficacy in stored product protection, the mixture of these powders to reduce the quantity applied need to be reconsidered. The aim of the present work is to determine the insecticidal efficacy of mixture of NeemAzal a commercial neem product and *P. glandulosus* leaf powder against *C. maculatus* and *S. zeamais***.**

Material and Methods

The leaves of *P. glandulosus* were collected around Ngaoundere (Quartier Champ de prière) (latitude 7°22′ North and longitude 13°34′ East, altitude of 1,100 m.a.s.l.), located in the Adamawa region of Cameroon. The plants were less than one-year old and only the green leaves were harvested from plants which were yet to attain the flowering stage. The collected leaves were sun-dried (29 ± 4°C). Dried leaves were hand crushed. The crushed leaves were ground into powder using a Bosch Universal grinder (model MUM 6012, Remscheid, Germany) until the particles passed through 0.1 mm mesh sieve. NeemAzal powder, was provided by Trifolio-M GmbH, Lahnau, Germany.

The parent adults of *S. zeamais* and *C. maculatus* were obtained from colonies maintained at JKI, Institute for Ecological Chemistry, Plant Analysis and Stored Products Protection, Berlin since 1968 and 2011, respectively. *S. zeamais* were reared on maize (Ricardino variety) while *C. maculatus* were reared on cowpea (Black eyes Perou).

NeemAzal powder were mixed with *P. glandulosus* leaf powder in the proportions of 100/0, 75/25, 50/50, 25/75 and 0/100% in glass jars. Each glass jar was shaken with a bidimensional mixer (Gerhardt, Dreieich, Germany) for 5 hours to ensure uniform mixture of the powders. The masses 0.125, 0.25, 0.5, 0.75 and 1 g were separately introduced to 50 g of maize or cowpea in 250 ml glass jars to give the doses of 2.5, 5, 10, 15 and 20 g/kg of maize or cowpea. Controls consisted of grains devoid of the plant powders. Each binary mixture was tested on *S. zeamais* and *C. maculatus* for adult toxicity, progeny production. The persistence test was performed with 75% *P. glandulosus* + 25% NeemAzal.

The co-toxicity coefficient of powder mixture was used to determine their responses: A co-toxicity coefficient of less than 80 is considered as antagonistic, between 80 and 120 as additive, and higher than 120 as synergistic (Sun and Johnson 1960; Islam *et al.,* 2010). If a mixture (M) compounds of two parts (A and B), and both components have LC_{50}, then the following formulas are used (A serving as standard):

Toxicity index (TI) of A = 100

$$\text{Toxicity index (TI) of B} = \frac{\text{LC50 of A}}{\text{LC50 of B}} \times 100$$

$$\text{Actual TI of Mixture} = \frac{\text{LC50 of A}}{\text{LC50 of M}} \times 100$$

Theoretical TI of M = TI of A × percentage of A in M + TI of B × percentage of B in M

$$\text{Co-toxicity coefficient} = \frac{\text{Actual TI of M}}{\text{Theoretical TI of M}} \times 100$$

If one component of the mixture alone (for example B) causes low mortality at all doses (< 20%), then the co-toxicity coefficient of the mixture was calculated by the formula: Co-toxicity coefficient = LC_{50} of A alone/LC_{50} of A in the mixture × 100.

Data on % cumulative corrected mortality and % reduction in F_1 progeny were arcsine [(square root(x/100)] transformed and the number of F_1 progeny produced were log (x + 1) transformed to homogenise the variance. The transformed data were subjected to the ANOVA procedure using the Statistical Analysis System (SAS Institute, 2008). Tukey (HSD) test (P = 0.05) was applied for mean separation**.**

Results

All the different combinations of NeemAzal and *P. glandulosus* generally caused significant mortality to adult *C. maculatus* and *S. zeamais* compared to the control (Tables 1and 2). The increase in mortality with ascending dose levels and time exposure was much more pronounced within three days post exposure than thereafter, irrespective of mixture proportions and insect species. Overall, mixture proportions generally had no effect on the mortality of the two insect species caused by the mixed *P. glandulosus* leaf powder and NeemAzal. However, the combination 25% NeemAzal + 75% *P .glandulosus* powder tended to be less potent to both insect species, since the lowest tested powder dose of 2.5 g/kg caused lower than 100% mortality to *C. maculatus* (6-d) and *S. zeamais* (7-d) for this combination while the other two combination proportions caused complete mortality . The highest tested dose (20 ml/kg) achieved complete mortality of both weevils three days post exposure for all mixtures, except the 25% NeemAzal + 75% *P. glandulosus* leaf powder which caused a maximum mortality of 87.50% in *S. zeamais*.

Table 1: Corrected cumulative mortality (mean ± SE) of *Callosobruchus maculatus* exposed to binary combinations of NeemAzal and *Plectranthus glandulous* leaf powder

Doses (g/kg)	Proportion of powders in mixture/Mortality (mean ± SE)[†]					
	100% *P. gland*	75% *P. gland* + 25% NeemAzal	50% *P. gland* + 50% NeemAzal	25% *P. gland* + 75% NeemAzal	100% NeemAzal	$F_{(4, 15)}$ [‡]
			3-d			
0	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
2.5	0.00 ± 0.00^{bC}	77.50 ± 2.23^{bB}	86.25 ± 2.39^{bAB}	93.75 ± 1.25^{bA}	87.50 ± 4.33^{bAB}	208.65^{***}
5	0.00 ± 0.00^{bC}	85.00 ± 2.89^{bcB}	97.50 ± 2.50^{aA}	100 ± 0.00^{aA}	96.25 ± 2.39^{abA}	143.47^{***}
10	0.00 ± 0.00^{bC}	96.25 ± 2.39^{abAB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	91.25 ± 2.39^{abB}	264.67^{***}
15	3.75 ± 1.25^{abB}	95.00 ± 3.54^{abA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	95.00 ± 2.04^{abA}	102.92^{***}
20	5.00 ± 2.04^{aB}	98.75 ± 1.25^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	98.75 ± 1.25^{aA}	165.62^{***}
$F_{(5, 18)}$ [‡]	5.51^{**}	222.49^{***}	795.68^{***}	6265.00^{***}	247.17^{***}	
			6-d			
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
2.5	0.00 ± 0.00^{eC}	92.50 ± 3.23^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	314.11^{***}
5	13.75 ± 1.25^{dB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	3941^{***}
10	21.25 ± 1.25^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	5310.1^{***}
15	31.25 ± 2.39^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	825^{***}
20	42.50 ± 3.23^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	690.67^{***}
$F_{(5, 18)}$ [‡]	230.45^{***}	936.60^{***}	$-^{***}$	$-^{***}$	$-^{***}$	

[†] Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).
[‡] ** $P < 0.01$; *** $P < 0.001$. *P. gland = Plectranthus glandulosus.*

The toxicity parameters of the binary mixture of *P. glandulosus* and NeemAzal powders to *C. maculatus* and *S. zeamais* are given in (Table 3). The 3-d LC_{50} values decreased with ascending proportion of NeemAzal in the mixture from 3.21 g/kg (25% NeemAzal + 75% *P. glandulosus*) to 0.24 g/kg (75% NeemAzal + 25% *P. glandulosus*) for *S. zeamais*. With *C. maculatus,* the opposite effect was observed, the LC_{50} values increased as the quantity of NeemAzal in the mixture increased. When the proportion of NeemAzal was ≥ 50% the LC_{50} and LC_{95} LC were not estimated due to complete adult mortality. The slopes seemed similar (1.24 – 1.51) for all the combinations of the powders in *S. zeamais* while they decreased (18.82 – 1.45) with increase in the quantity of *P. glandulosus* in the mixture. All the estimated co-toxicity coefficients were less than 80.

The number of F_1 progeny and the percentage inhibition of the progeny of *C. maculatus* and *S. zeamais* that emerging from grains treated with different combinations of NeemAzal and *P. glandulosus* at different doses are shown in Table 4. The number of emerging F_1 progeny reduced significantly ($P \leq 0.01$) in both insect species with ascending of the botanicals. The binary combinations of the powders reduced progeny emergency more than each botanical applied alone, with NeemAzal being more potent than *P. glandulosus*. All the binary combinations of the powders completely suppressed progeny production in *S. zeamais*. On cowpea 97.77%, 97.15% and 66.78% inhibition of *C. maculatus* emergence were recorded respectively with the combinations 25% *P. glandulosus* + 75% NeemAzal, 50% *P. glandulosus* + 50% NeemAzal and 75% *P. glandulosus* + 25% NeemAzal at the highest tested dose of 20 g/kg.

Table 2: Corrected cumulative mortality (mean ± SE) of *Sitophilus zeamais* exposed to binary combinations of NeemAzal and *Plectranthus glandulous* leaf powder

Doses (g/kg)	Proportion of powders in mixture/Mortality (mean ± SE)†					
	100% *P. gland*	75% *P. gland* + 25% NeemAzal	50% *P. gland* + 50% NeemAzal	25% *P. gland* + 75% NeemAzal	100% NeemAzal	$F_{(4, 15)}$ ‡
			3-d			
0	0.00 ± 0.00^{c}	0.00 ±0.00^{d}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ± 0.00^{d}	
2.5	0.00 ± 0.00^{cC}	45.00 ± 9.35^{cB}	80.00 ± 10.80^{bA}	88.75 ± 3.15^{bA}	63.75 ± 2.39^{cB}	28.23***
5	1.25 ± 1.25^{cD}	60.00 ± 7.36^{bcC}	91.25 ± 4.27^{abAB}	97.50 ± 1.44^{aA}	80.00 ± 2.04^{bB}	65.36***
10	5.00 ± 0.91^{bcD}	73.75 ± 5.15^{abC}	100 ± 0.00^{aA}	95.00 ± 2.04^{abAB}	87.50 ± 5.20^{abBC}	56.93***
15	13.75 ± 2.39^{abC}	88.75 ± 1.25^{aB}	95.00 ± 0.00^{abAB}	98.75 ± 1.25^{aA}	93.75 ± 1.25^{aAB}	190.33***
20	21.25 ± 3.75^{aC}	87.50 ± 3.23^{aB}	97.50 ± 1.44^{abAB}	100 ± 0.00^{aA}	95.00 ± 2.04^{aAB}	96.72***
$F_{(5,18)}$ ‡	18.04***	37.24***	64.95***	525.81***	183.60***	
			7-d			
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	
2.5	30.00 ± 3.54^{cC}	92.50 ± 4.33^{aAB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	82.50 ± 2.23^{cb}	51.35***
5	76.25 ± 3.75^{bB}	98.75 ± 1.2^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	95.00 ± 2.04^{bA}	25.93***
10	80.00 ± 5.40^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	98.75± 1.25^{abA}	20.70***
15	97.50 ± 2.50^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	1^{ns}
20	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	–
$F_{(5,18)}$ ‡	156.37***	477.74***	–***	–***	578.26***	
			14-d			
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
2.5	51.25 ± 13.90^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	12.30***
5	85.00 ± 5.40^{bAB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	7.71**
10	93.75 ± 3.75^{ab}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	2.93ns
15	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	–
20	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	–
$F_{(5,18)}$ ‡	39.71***	–***	–***	–***	–***	

† Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ** $P < 0.01$; *** $P < 0.001$.

P. gland = *Plectranthus glandulosus.*

Table 3: Toxicity of binary combinations of NeemAzal and *Plectranthus glandulosus* leaf powder at different proportions to adult *Callosobruchus maculatus*

Insects/ product proportion	Slope ± S.E	R^2	LC_{50} (95% FL)[a]	LC_{95} (95% FL)[a]	Co-toxicity coefficient
C. maculatus					
			3 days		
100% *P.gland*. + 0% NeemAzal	3.49 ± 1.57	0.69	55.80 (31.40 - 4.43E^{5})	11.00 (8.22 -17.78)	
75% *P.gland*. + 25% NeemAzal	1.45 ± 0.25	0.94	0.81 (0.27 - 1.39)	11.00 (8.22 -17.78)	0.20
50% *P.gland*. + 50% NeemAzal	3.20 ± 0.84	0.73	1.15 (0.39 -1.66)	3.76 (3.14 - 5.29)	0.07
25% *P.gland*. + 75% NeemAzal	18.82 ± 1.62	0.58	2.07	2.53	0.17
0% *P.gland*. + 100% NeemAzal	0.70 ± 0.40	0.52	0.04	10.34	
			6 days		
100% *P.gland*. + 0% NeemAzal	1.97 ± 0.26	0.97	25.01 (20.11 – 35.01)	173.84 (96.60 -471)	
75% *P.gland*. + 25% NeemAzal	19.13 ± 1.09	0.58	0.10	2.56	0
50% *P.gland*. + 50% NeemAzal[£]	-	-	-	-	-
25% *P.gland*. + 75% NeemAzal[£]	-	-	-	-	-
0% *P.gland*. + 100% NeemAzal[£]	-	-	-	-	-
S. zeamais					
			3 days		
100% *P.gland*. + 0% NeemAzal	2.84 ± 0.52	0.84	40.23 (29.25 – 80.77)	171.52 (84.11 -872)	
75% *P.gland*. + 25% NeemAzal	1.51 ± 0.19	0.97	3.21 (2.30 - 4.05)	39.22 (26.90 - 71.3)	1.57
50% *P.gland*. + 50% NeemAzal	1.33 ± 0.43	0.72	0.51	8.90	5.17
25% *P.gland*. + 75% NeemAzal	1.24 ± 0.34	0.72	0.24 (0.01 - 0.74)	5.13 (3.20 - 8.13)	7.63
0% *P.gland*. + 100% NeemAzal	1.42 ± 0.31	0.96	1.39 (0.69 – 2.07)	19.68 (14.14 -34.30)	
			7 days		
100% *P.gland*. + 0% NeemAzal	1.45 ± 0.25	0.94	3.56 (1.04 – 5.66)	13.02 (8.05 -107.92)	
75% *P.gland*. + 25% NeemAzal	2.95 ± 1.08	0.72	0.81 (0.03 - 1.45)	2.96 (2.05 - 4.20)	2.75
50% *P.gland*. + 50% NeemAzal[£]	-	-	-	-	-
25% *P.gland*. + 75% NeemAzal[£]	-	-	-	-	-
0% *P.gland*. + 100% NeemAzal[£]	2.45± 0.49	0.82	1.05 (0.45 – 1.56)	4.92 (3.97- 6.75)	-

[a] FL = Fiducial limits; [£] Toxicity parameters were not determinate due to 100% mortality

Table 4: Progeny production of *Callosobruchus maculatus* and *Sitophilus zeamais* in grains treated with binary combinations of *Plectranthus glandulosus* leaf powder and NeemAzal

Insects/ doses (g/kg)	Proportion of powders in mixture					
	100% *P. glandulosus*	75% *P. gland* + 25% NeemAzal	50% *P. gland* + 50% NeemAzal	25% *P. gland* + 75% NeemAzal	100% NeemAzal	$F_{(5, 19)}$ ‡
Number (mean ± SE) of F_1 adult progeny †						
C. maculatus						
0	443.50 ± 15.61^{a}	439.50 ± 13.36^{a}	439.50 ± 13.36^{a}	439.50 ± 13.36^{a}	439.50 ± 13.36^{a}	0.02^{ns}
2.5	409.50 ± 3.01^{aA}	299.75 ± 36.67^{abB}	35.25 ± 1.65^{bD}	30.75 ± 3.88^{bD}	97.00 ± 8.60^{bC}	146.42***
5	355.75 ± 13.77^{abA}	250.50 ± 43.84^{bcA}	27.50 ± 1.71^{bcB}	20.50 ± 3.77^{bcB}	58.00 ± 12.46^{bcB}	68.11***
10	283.75 ± 31.76^{abA}	201.50 ± 23.06^{bcA}	18.00 ± 4.04^{cdB}	18.00 ± 2.08^{bcB}	35.50 ± 8.53^{cdB}	69.85***
15	260.25 ± 50.34^{abA}	144.50 ± 13.32^{cB}	12.75 ± 1.55^{dC}	11.00 ± 2.27^{cC}	17.25 ± 4.17^{dC}	44.10***
20	206.50 ± 56.16^{bA}	146.00 ± 5.48^{cA}	12.50 ± 1.55^{dB}	9.75 ± 2.17^{cB}	11.75 ± 1.49^{dB}	21.26***
$F_{(5,18)}$ ‡	4.43**	15.84***	647.93***	407.35***	143.42***	
S. zeamais						
0	51.25 ± 0.95^{aB}	69.25 ± 2.10^{aA}	62.75 ± 1.65^{aA}	63.75 ± 2.95^{aA}	65.25 ± 2.53^{aA}	9.79**
2.5	36.75 ± 2.25$^{a\,A}$	0.75 ± 0.75^{bC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	6.00 ± 0.71^{bB}	194.11***
5	22.00 ± 4.71^{bA}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	4.50 ± 1.32^{bcB}	40.93***
10	6.00 ± 0.91^{cA}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	1.25 ± 0.48^{bcB}	41.44***
15	3.00 ± 0.71^{cA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{cB}	28.37***
20	1.75 ± 1.03^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	2.92^{ns}
$F_{(5,18)}$ ‡	63.83***	570.87***	1442.77***	465.57***	277.34***	
Percentage (mean ± SE) reduction in adult emergence relative to control †						
C. maculatus						
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	
2.5	7.88 ± 3.21^{cdC}	31.84 ± 8.08^{cB}	91.97 ± 0.35^{cA}	92.99± 0.93^{bA}	77.84 ± 2.26^{cA}	73.68***
5	19.35 ± 4.99^{bcC}	3.00 ± 9.96^{bcB}	93.71± 0.51^{bcA}	95.34 ± 0.85^{abA}	86.76 ± 2.96^{bA}	47.23***
10	36.24 ± 6.45^{abC}	56.16 ± 5.17^{abA}	95.83 ± 1.04^{abB}	95.93 ± 0.37^{abB}	91.75 ± 2.17^{abB}	60.26***
15	41.93 ± 10.52^{abC}	67.28 ± 2.01^{aB}	97.11 ± 0.24^{aA}	97.46 ± 2.27^{abA}	96.00 ± 1.07^{aA}	38.86***
20	54.18 ± 12.18^{aB}	66.78 ± 0.74^{aB}	97.15 ± 0.35^{aA}	97.77 ± 0.58^{abA}	97.31 ± 0.37^{aA}	20.07***
$F_{(5,18)}$ ‡	13.11***	35.05***	1947.02***	1245.03***	338.90***	
S. zeamais						
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00d^{d}	
2.5	28.14 ± 4.90^{cC}	98.96 ± 1.04^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	90.68 ± 1.43^{cB}	148.34***
5	56.71 ± 9.53^{bC}	100 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{a}	93.21 ± 1.89^{cB}	41.78***
10	88.30 ± 1.78^{aC}	100 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	98.05 ± 0.78^{bB}	44.25***
15	94.08 ± 1.43^{aB}	100 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00± 0.00^{aA}	45.92***
20	96.65 ± 1.96^{a}	100 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	2.98^{ns}
$F_{(5,18)}$ ‡	86.50***	922.23***	∞***	∞ ***	579.71***	

† Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; P < 0.05).
‡ ns P > 0.05; *** P < 0.001.
P. gland = *Plectranthus glandulosus.*

Figure 1 shows the results of the persistence of the mixture of 75% *P. glandulosus* + 25% NeemAzal on *C. maculatus* and *S. zeamais*. The efficacy of the mixture varied significantly (P < 0.001) with the ascending dose but not with the storage interval as the efficiency persisted up to the 180-d storage interval. For this binary combination, the mortality caused to *S. zeamais* and *C. maculatus* at the 180-d storage interval did not differ from the observed mortality at the 0-d storage interval (P > 0.05) for all the dose levels.

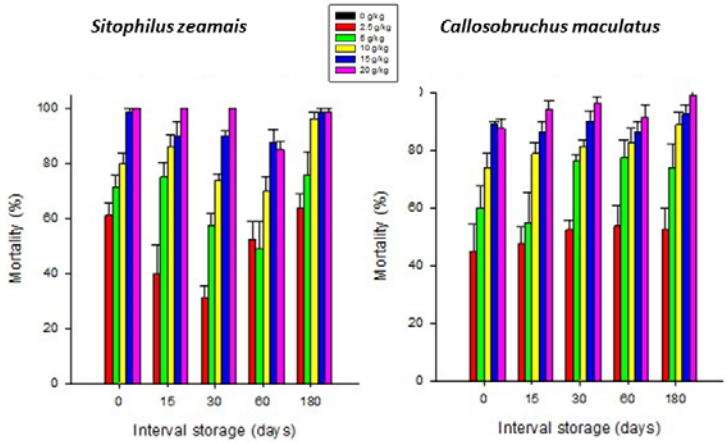

Figure 1: Corrected cumulative mortality of *Callosobruchus maculatus* and *Sitophilus zeamais* exposed in grains treated with the combinations of *Plectranthus glandulosus* with NeemAzal powders at different storage intervals

Discussion

The mixture of NeemAzal and *P. glandulosus* leaf powder was also antagonistic regarding the mortality they caused to *C. maculatus* and *S. zeamais*. In isolation, NeemAzal caused greater mortality to both insects than *P. glandulosus*. The mixture of *Vernonia amygdalina* and neem powder was antagonistic with respect to insecticidal efficacy (Akunne et al., 2013). The NeemAzal used in the present study was produced by incorporating azadirachtin into silica gel. The mortality observed with NeemAzal could largely be due to the presence of silica gel compared to that of azadirachtin (Ogemah, 2003). Silica gel acts by desiccation, as the insects move through grains, they pick up the powder on their cuticle which leads to the absorption of the cuticular waxes from the epicuticule surface of the insect, thus enhancing the rate of desiccation (Prasantha, 2003). Ulrich and Mewis (2000) showed that combinations of diatomaceous earth (Fossil shield (1 gm/kg) and a commercial neem product NeemAzal (1 gm/kg) resulted in higher mortality of the weevils. Since NeemAzal contains silica gel, the mixture of this powder with Fossil shield implies the doubling of the concentration of diatomaceous earths, which resulted in higher mortality in the study of Ulrich and Mewis (2000).

It could also be concluded that the binary mixtures at different proportion levels of the powders from NeemAzal and *P. glandulosus* has various effects on adult emergence. the mixture of NeemAzal and *P. glandulosus* reduced almost completely the emergence of adult insects when the rate of NeemAzal ≥ 25%. Nukenine et al., (2011) reported that under fluctuating conditions, NeemAzal powder registered similar results on *S. zeamais.* It seems that the silica gel absorbed the water contained in grains which affected the development of the weevils. Before treatment the moisture content of the grains was above 12% and after F_1 progeny evaluations, this value decreased to less than 10%. When the moisture content of the grains is less than 10%, the development of immature stages of both insect species is hindered.

NeemAzal contains silica gel and for this reason, the activity of its mixture with *P. glandulosus* was more or less constant up to 180 d compared to the 70% reduction in the efficacy of *P. glandulosus* alone. Silica gel is an inert durst and does not contain volatiles like *P. glandulosus,* which loses its active ingrediants with time. The activity of *Ocimum basilicum,* an aromatic plant of the Lamiaceae family like *P. glandulosus on S. zeamais* mortality declined most 0 (80% mortality) and 28 d (15%

moratlity) (Mwangangi and Mutisya, 2013), which is in conformity with the results of the present work.

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Effects of chlorpyrifos-methyl and pirimiphos-methyl applied with 5°C temperature on *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in wheat grain

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Abstract

The effects of the insecticides chlorpyrifos-methyl and pirimiphos-methyl in combination with low temperature treatment at 5°C were tested in the laboratory to improve the existing pest management programs for *S. oryzae* control. Adults were released into wheat grain pretreated with three insecticide doses: 0.08, 0.12 and 0.16 mg/kg of chlorpyrifos-methyl, and 0.125, 0.19 and 0.25 mg/kg of pirimiphos-methyl, and exposed to 5°C temperature over intervals of 5, 6, 7 and 8 days. Mortality after low temperature only, insecticides only, and their

combinations, was assessed after 1, 2, 7 and 14 days of recovery at 25±1°C and 60±5% r.h., as well as their impact on F1 progeny production/reduction (PR) after 8 weeks. The combined application of 0.16 mg/kg chlorpyrifos-methyl and 5°C temperature (5-7 days of exposure) caused a significantly higher mortality of *S. oryzae* after 1, 2, 7 and 14 days of recovery than the activity of low temperature alone, as well as the combined application of 0.12 mg/kg chlorpyrifos-methyl and 5°C after 7 and 14 days of recovery. Adults mortality and progeny reduction of *S. oryzae* was ≥92% after 14 days of recovery from an interaction of 0.16 mg/kg dose of chlorpyrifos-methyl and exposure for 6 days to 5°C, as well as all doses in combination with 7 days exposure to 5°C. Combined application of 0.25 mg/kg pirimiphos-methyl and 5, 6 and 7 days of exposure to 5°C caused a significantly higher mortality of *S. oryzae* after 7, 2 and 1 day of recovery, respectively, compared to temperature-only exposure. High mortality (91-95%) and progeny reduction >92% were caused by the same pirimiphos-methyl rate in combination with 6 and 7 days of exposure to 5°C after 7 days of recovery as the combination of 5, 6 and 7 days of exposure to 5°C after 14 days of recovery.

Keywords: *S. oryzae*, chlorpyrifos-methyl, pirimiphos-methyl, 5°C temperature, combined effects.

1. Introduction

Since the 1960s, residual grain protectants, chiefly organophosphorus and pyretroid insecticides, have been used in management programs for insect pest control in stored raw agricultural commodities. Protectants are usually applied when commodities are loaded into storage, and residues from this single application are expected to protect grain throughout the storage period (Arthur, 1996). Malathion, first registered in the United States in 1958, received extensive use as a storage spray and grain protectant (Arthur and Subramanyam, 2012). The use of malathion decreased significantly after control failures in stored grain (because the extensive use of this compound has resulted in a worldwide resistance of several species). Thus, malathion has been replaced by other organophosphorous insecticides, such as chlorpyrifos-methyl and pirimiphos-methyl (Boyer et al., 2012). Chlorpyrifos-methyl and pirimiphos-methyl are the most common organophosphate insecticides, and both are used as chemical protectants of stored grain throughout the world, either alone or in combination with some pyirethroid compound, e.g. chlorpyrifos-methyl plus deltamethrin in the United States (White and Leesch, 1996; Arthur and Subramanyam, 2012). This class of insecticides is favored for use in stored grains because of relatively low mammalian toxicity and suitable degradation rates that are directly influenced by temparature and product moisture content (White and Leesch, 1996). Compared to contact insecticides, treatments with extreme temperatures as a physical method of controlling stored-product insects have a number of advantages: they leave no residues on products, they are effective against populations resistant to contact insecticides, and the risk for operators is minimal (Fields, 1992, 2001; Burks et al., 2000; Beckett and Morton, 2003; Hagstrum and Subramanyam, 2006; Beckett et al., 2007; Fields et al., 2012). Over the past three decades, low temperatures have been largely used to disinfest either commodities or storage facilities from stored product pests, or for quarantine purposes (Fields, 1992, 2001; Donahaye et al., 1995; Burks et al., 2000; Loganathan et al., 2011; Fields et al., 2012).

Another area of potential benefits from low temperature treatments is their combination with some other pest control methods, such as chemical protectants as part of an integrated approach to manage stored products pests and their resistance to insecticides (Kljajic et al., 2014). Cold pre-treatment at -5°C of adults of granary weevil, *Sitophilus granarius* (L.), has consistently increased the insecticide efficacy of deltamethrin, compared to an unexposed weevil population. Deltamethrin toxicity to field and selected populations after 24 h recovery, following exposure to -5 °C, was 12.1 and 11.0 times higher, respectively, while it was 6.9 and 36.6 times higher, respectively, after 72 h of recovery (Kljajic et al., 2014).

However, insufficient information is available about the influence of the lethal supra-optimal extreme temperature of 5°C on insecticide effectiveness. Research of the interactions between insecticides and temperature in recent years have been mostly based on testing temperatures that ranged from 10 to 30 °C. Organophosphate compounds were found to be more toxic at higher temperatures (≥ 30 °C), in contrast to pyrethroids, which were more toxic at lower temperatures (≤

20 °C) (Tyler and Binns, 1982; Watters et al., 1983; Thaung and Collins, 1986; Subramanyam and Cutkomp, 1987; Longstaff, 1988; Subramanyam and Hagstrum, 1996; Fleurat-Lessard et al., 1998). Wilkin et al. (1999) exposed *Sitophilus oryzae* (L.) adults to treated wheat for five days and reported pirimiphos-methyl (4.0 mg/kg) effectiveness of 64 % at 5 °C, and 100% efficacy at 10° C, revealing the insecticide's higher effectiveness at higher temperature. However, almost none of these studies examined how the combination of treatments affected the survival of insects at low temperatures. However, the effects of acclimation on their survival at low temperatures are well documented (Andreadis and Athanassiou, 2017).

Working on potential improvements of current pest management programmes for *S. oryzae* control, we focused our present laboratory study on examining the effects of a combination of 5° C temperature with the contact insecticides/grain protectants chlorpyrifos-methyl and pirimiphos-methyl on a lab population of rice weevil, *Sitophilus oryzae* (L.) in wheat grain. The effects on its progeny production/ reduction in F1 generation were also examined, and all data compared with the effects of extreme temperature and independent insecticide treatments.

2. Materials and methods

Test insects and insecticides applied

Adults of a laboratory population of *S. oryzae*, reared at the institute were used as test insects in experiments as described by Harein and Soderstrom (1966) and Davis and Bry (1985). The weevils were reared on whole soft wheat grain of 12% moisture, in 2.5 L glass jars at 25 ± 1°C temperature and 60 ± 5% relative humidity. Unsexed 2-5 week old adults were used in all trial variants. The following insecticide products were used in tests: chlorpyrifos-methyl (Reldan EC 40 with 400 g/L a.i., Dow AgroSciences, Austria) and pirimiphos-methyl (Actellic EC 50 with 500 g/L a.i., Galenika-Fitofarmacija, Serbia).

Bioassays

Moisture content in the soft wheat grain variety NS 40 S was 11.0 ± 0.5% and was measured by a DickeyeJohn Mini GAC (DickeyeJohn Co., USA) device before the experiment. Based on a preliminary experiment, three application rates were determined for each insecticide, i.e. chlorpyrifos-methyl (0.08, 0.12 and 0.16 mg/kg) and pirimiphos-methyl (0.125, 0.19 and 0.25 mg/kg), as well as four intervals of exposure to 5° C temperature: 5, 6, 7 and 8 days, in order to test the combined activity of the insecticides and low temperature. Four standard solutions were prepared for each insecticide and diluted into dose series, so that each insecticide dose was used for four treatments (replications) of 500 g lots (one treatment included a single replication of each interval of grain exposure to 5° C temperature: 0, 5, 6, 7 and 8 days. Each 1000 mL glass jar was filled with 500 g of wheat grain and treated with 5 mL of water solution of one of the insecticides, or 5 mL of water for control grain. After hand shaking the treated wheat grain for 30 s, it was mixed on a rotary shaker for 10 min. Plastic 200 mL bottles were then filled with 50 g of treated wheat grain and untreated control grain, and 25 *S. oryzae* adults, previously acclimed in a refrigirator at 15 °C over 24 h, were added into each bottle and the bottle was topped with cotton cloth and fixed with a rubber band. The lots representing each time interval were then independently placed in an incubator (LE-519, MRC, Israel) set to 5 ± 0.5 °C temperature. Temperature in all trial variants was recorded by a data logger (Kestrel 4000, USA). The bottles had a completely randomized arrangement in the incubator. They were transferred after each interval to laboratory conditions (25 ± 1° C and 60 ± 5% r. h), and lethal effects were determined after 1, 2, 7 and 14 days. The same procedure was used to determine the independent effects of both insecticides. After the last assessment, insects were seaved out (seive density 2.0 mm) to enable F1 progeny counts eight weeks after the adults made contact with treated wheat grain and the bottles were kept in the laboratory at 25 ± 1° C and 60 ± 5% r. h.

Data analysis

Mortality data were analyzed using one-way ANOVA. Means were separated by Fisher's LSD test at P=0.05 (Sokal & Rohlf, 1995). *S. oryzae* progeny production/reduction in wheat grain was determined using the formula PR (%) = (K-T) 100/K (Tapondjou et al., 2002), where K - number of progeny in the untreated control, and T - number of progeny in treatments.

3. Results

In the variant without exposure to 5°C, none of the adults in grain treated with all rates of chlorpyrifos-methyl and pirimiphos-methyl (Tab. 1) died after 1 day of contact, while 1% and 5% mortality, respectively, occurred only under the highest doses of chlorpyrifos-methyl and pirimiphos-methyl (0.16 and 0.25 mg/kg, respectively) after 2 days of exposure. Adult mortality increased significantly after 7 and 14 days of contact with both insecticides, the highest being after 14 days of contact with 0.16 mg/kg chlorpyrifos-methyl (87%), and 0.25 mg/kg of pirimiphos-methyl (91%). Average counts of *S. oryzae* progeny in wheat decreased nominally with increasing rates of the tested insecticides. The highest number of progeny (around 530 and 400) and lowest progeny reduction (10.5 and 32.1%) were detected after treatment with the lowest rates of chlorpyrifos-methyl and pirimiphos-methyl (0.08 and 0.125 mg/kg, respectively), while the highest rates of chlorpyrifos-methyl and pirimiphos-methyl (0.16 and 0.25 mg/kg) allowed the lowest average number of progeny (207 and 232, respectively), i.e. the greatest progeny reduction (65.0 and 60.8%, respectively).

Tab. 1. Effects of chlorpyrifos-methyl and pirimiphos-methyl applied alone on *S. oryzae* in wheat grain.

Insecticide	Dose (mg/kg)	Mortality (%±SE) after exposure (days)				Mean No. of progeny (±SE)	PR[b] (%)
		1	2	7	14		
CPM	0.08	0.0±0.0a[a]	0.0±0.0a	0.0±0.0a	2.0±1.0a	530.0±123.6d	10.5
	0.12	0.0±0.0a	0.0±0.0a	43.0±2.1b	46.0±1.9b	227.5±159.5c	61.6
	0.16	0.0±0.0a	1.0±0.5b	78.0±3.0c	87.0±2.6c	207.5±44.2b	65.0
PM	0.125	0.0±0.0a	0.0±0.0a	4.0±1.4a	9.0±2.6a	402.5±213.3c	32.1
	0.19	0.0±0.0a	0.0±0.0a	40.0±0.8b	61.0±2.4b	375.0±118.5c	36.7
	0.25	0.0±0.0a	5.0±1.3b	87.0±1.7c	91.0±3.3c	232.5±61.8b	60.8
Control	0.00	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	592.5±149.1d	-

[a] For each insecticide separately, means within columns followed by the same letter are not significantly different, Fisher's
LSD test at P > 0.05; b Progeny reduction; CPM- chlorpyrifos-methyl; PM- pirimiphos-methyl

The mortality of S. oryzae adults after 1 and 2 days of recovery from exposure to 5° C temperature in grain untreated with insecticides (Tab. 2) ranged from 15- 83% and 26-86%, respectively, depending on exposure duration, and increased after 7 and 14 days of recovery to 41-96%. Regardless of the period of recovery, a significant increase in adult mortality occurred after 7 and 8 days of exposure to 5° C (43-96%), compared to the shortest exposure periods of 5 and 6 days (15-49%), and 7 and 8 days of exposure (43-74% and 83-96%), while no statistical difference was detected between the shortest exposure periods of 5 and 6 days (15-42% and 20-49%).

In all investigated combinations of insecticide treatment with 5° C temperature (Tab. 2), adult mortality significantly increased with the duration of exposure to extreme temperature, with increasing insecticide doses, and duration of insect exposure to treated wheat grain. Excepting the 8 day exposure, which caused high adult mortality (83-100%) and progeny reduction (92-100%) regardless of recovery period and insecticide, both independently and in all combinations, high adult mortality (>89%) and high progeny reduction (>92%) was also revealed for the combination of 0.16 mg/kg chlorpyrifos-methyl and 6 days of exposure to 5° C temperature, after 14 days of recovery, and for all combinations of chlorpyrifos-methyl treatment and 6 and 7 days of exposure to 5° C after 7 and 14 days of recovery. Adult mortality (>91%) and progeny reduction (>92%) were also high in the combination of the highest dose of pirimiphos-methyl (0.25 mg/kg) with 6 and 7

days of exposure to 5° C after 7 days of recovery, and with 5, 6 and 7 days of exposure to 5° C after 14 days of recovery.

Tab.2. Combined effects of chlorpyrifos-methyl and pirimiphos-methyl, and 5°C temperature on *S. oryzae* in wheat grain after 1, 2, 7 and 14 days of recovery/exposure.

Exposure (days)/ Dose (mg/kg)	Mortality (% ± SE)				Mean progeny production (±SE)	PR[b] (%)	PR[c] (%)
	1	2	7	14			
Chlorpyrifos-methyl							
5 d							
0.00	15.0±2.6ab[a]	26.0±2.4bc	41.0±3.4bc	42.0±4.0bc	226.0±27.2c	-	61.9
0.08	22.0±1.3cd	27.0±1.3bc	35.0±1.0ab	38.0±1.3ab	285.7±36.7d	-26.4	51.8
0.12	14.0±1.0ab	20.0±1.4ab	25.0±1.7a	31.0±1.9a	313.5±75.3d	-38.7	47.1
0.16	26.0±2.6cd	41.0±2.5d	76.0±1.8e	83.0±1.5ef	128.7±31.3b	43.0	78.3
6 d							
0.00	20.0±0.0ab	29.0±1.5bc	46.0±3.3c	49.0±2.6c	219.2±11.1c	-	63.0
0.08	18.0±2.6ab	15.0±1.3a	40.0±0.8abc	46.0±2.1bc	213.7±50.2c	2.5	63.9
0.12	9.0±2.6a	24.0±1.4bcd	64.0±1.6d	65.0±1.7d	190.0±29.4c	13.3	67.9
0.16	21.0±1.3c	31.0±1.0c	84.0±0.8ef	92.0±0.8fg	45.5±16.7a	79.2	92.3
7 d							
0.00	43.0±2.6d	56.0±1.8e	74.0±1.0de	74.0±1.0de	119.5±52.0b	-	79.8
0.08	55.0±1.3e	61.0±1.3ef	89.0±2.7fg	92.0±1.8fg	30.0±24.6a	74.9	95.0
0.12	79.0±1.3e	79.0±1.0g	97.0±1.0gh	100g	20.0±37.4a	83.3	96.6
0.16	64.0±1.6e	66.0±2.1f	89.0±2.2fg	93.0±1.3g	38.0±39.5a	67.6	93.5
8 d							
0.00	83.0±2.5f	86.0±2.4gh	96.0±0.8gh	96.0±0.8g	47.5±33.6a	-	92.0
0.08	94.0±0.6f	95.0±0.5h	99.0±0.5gh	100g	8.0±16.0a	83.2	98.6
0.12	83.0±2.7f	83.0±2.7g	97.0±1.0gh	100g	27.0±31.3a	43.2	95.4
0.16	88.0±0.8f	92.0±0.8h	100h	100g	0.0±0.0a	100	100
Pirimiphos-methyl							
5 d							
0.00	15.0±2.6a	26.0±2.4b	41.0±3.6bc	42.0±4.0bc	226.0±27.2de	-	61.9
0.125	12.0±0.8a	12.0±0.0a	26.0±3.1a	29.0±2.6a	293.7±138.0e	-30.0	50.4
0.19	12.0±0.8a	16.0±0.8a	33.0±3.2ab	35.0±2.9ab	220.2±111.4de	2.5	62.8
0.25	16.0±1.1a	26.0±1.7b	87.0±2.5fg	95.0±1.0gh	90.7±45.1bc	59.8	84.7
6 d							
0.00	20.0±0.0a	29.0±1.5bc	46.0±3.3c	49.0±2.6c	219.2±11.1de	-	63.0
0.125	16.0±2.2a	20.0±0.8ab	45.0±1.3c	48.0±1.8c	152.2±30.1cd	30.6	74.3
0.19	21.0±2.4a	37.0±1.7cd	62.0±2.4d	66.0±1.7d	163.7±56.2cd	25.3	72.4
0.25	19.0±1.0a	41.0±1.9d	91.0±1.7gh	93.0±1.3fgh	43.0±54.9ab	80.4	92.7
7 d							
0.00	43.0±2.6b	56.0±1.8ef	74.0±1.0e	74.0±1.0de	119.5±52.0bc	-	79.8
0.125	47.0±1.0b	52.0±0.8e	78.0±2.1ef	83.0±3.5ef	152.2±46.3cd	-27.4	74.3
0.19	60.0±1.6c	63.0±2.1f	85.0±1.5efg	86.0±1.7fg	102.0±55.4bc	14.6	82.8
0.25	63.0±1.7c	73.0±2.2g	92.0±0.8gh	92.0±0.8fgh	43.0±7.5ab	64.0	92.7
8 d							
0.00	83.0±2.5d	86.0±2.4h	96.0±0.8gh	96.0±0.8gh	47.5±33.6ab	-	92.0
0.125	93.0±1.3d	91.0±1.3h	99.0±0.5h	100h	0.0±0.0a	100	100
0.19	89.0±2.1d	91.0±2.2h	100h	100h	0.0±0.0a	100	100
0.25	87.0±1.0d	88.0±0.8h	100h	100h	0.0±0.0a	100	100

[a] For each insecticide separately, means within columns followed by the same letter are not significantly different, Fisher's LSD test at P > 0.05.

[b] Progeny reduction compared to control 1 (weevil progeny in untreated wheat grain exposed to 5°C).

[c] Progeny reduction compared to control 2 (weevil progeny in untreated wheat grain not exposed to 5°C).

The lowest doses of both insecticides (0.08 and 0.12 mg/kg of chlorpyrifos-methyl and 0.125 mg/kg of pirimiphos-methyl) stimulated progeny production of treated parents by 26-38.7%, compared untreated wheat exposed to 5° C temperature over 5 days, and 5 and 7 days, respectively.

4. Discussion

Our results showed that the mortality of *S. oryzae* lab adults exposed to 5 °C temperature in untreated cold grain increased with exposure duration (5-8 days) and recovery duration (1-14 days), while weevil mortality after 7 and 8 days of exposure and 7 days of recovery were roughly the same as they were after 14 days of recovery (74% and 96%, respectively). Experimental data have shown variable effects of low temperatures on this species. A survey by Fields (1992) of the then available

data on lethal effects of low temperatures on *S. oryzae*, cited a 50% survival of adults exposed for 10-16 days to 4.5 °C temperature, while other data showed no (0%) surviving adults after exposure to the same temperature, or survival of 4-6% adults after 14 days of exposure to 4.4 °C, the data depending on conditions in each particular experiment and on acclimation. On the other hand, when Pražić Golić et al. (2013) tested lethal effects of 5° C temperature in wheat grain on *Sitophilus* species, they found that *S. oryzae* adults mortality after 1-7 days of recovery from exposure to 5 °C for 5, 6, 7 and 8 days was 27-58, 33-61, 58-90 and 64-93%, which is similar to the present research data.

Our data on *S. oryzae* mortality caused by the insecticides chlorpyrifos-methyl and pirimiphos-methyl without cold treatment at 5°C showed that mortality increased with insecticide concentration and exposure interval, which generally agrees with many other studies on *S. oryzae* and some other stored-product insect pests (Wilkin et al., 1999; Arthur et al., 2004; Kavallieratos et al., 2009; Kljajić and Perić, 2009; Andrić et al., 2011; Athanassiou et al., 2008). In our present study, the highest mortality of *S. oryzae* adults of 87 and 91% was detected after 14 days of exposure to wheat treated with 0.16 mg/kg chlorpyrifos-methyl and 0.25 mg/kg pirimiphos-methyl, respectively. Some earlier studies had shown high efficacies (100%) of chlorpyrifos-methyl at 1.0 mg/kg and 5.0 mg/kg rate against *S. oryzae* after 7 and 14 days of exposure, respectively (Fleurat-Lessard et al., 1998; Daglish, 2008) and high efficacies (100%) of pirimiphos-methyl at 1.0 mg/kg and 4.0 mg/kg rates against *S. oryzae* after 7 days exposure (Huang and Subramanyam, 2005; Rumbos et al., 2013). In our research, 0.25 mg/kg pirimiphos-methyl caused 0.0, 5.0, 87.0 and 91.0% adult mortality after 1, 2, 7 and 14 days, respectively. In a study with the same exposure intervals conducted by Rumbos et al. (2013), a double dose of pirimiphos-methyl (0.5 mg/kg) caused a significantly lower mortality, 0.4, 1.2, 66.6 and 69.2%, respectively. The inconsistent data may be attributed to a higher susceptibility to insecticides of our lab population.

Generally, very little is known about the effects of insecticide treatments of storage insects in combination with extreme low temperatures in wheat grain. The results in our present study on wheat grain showed that the exposure of *S. oryzae* adults to combinations of insecticide treatment and 5° C cold treatment over a period of 7 days caused, in almost all cases, a mortality that significantly exceeded the independent activities of either component, i.e. insecticide or low temperature. For example, regardless of the recovery period (1-14 days), Weevil mortality was 55-92%, 79-100% and 64-93% in grain treated with 0.08, 0.12 and 0.16 mg/kg chlorpyrifos-methyl, respectively, while adult mortality in wheat treated only with the insecticides was 0-2%, 0-46% and 0-87%, respectively, and 43-74% in untreated wheat after exposure at 5°C. Similarly, each individual component (insecticide and temperature) an interaction with 0.125, 0.19 and 0.25 mg/kg of pirimiphos-methyl and 7 days exposure to 5° C caused a statistically significant difference in adult mortality, i.g. mortality in wheat treated with pirimiphos-methyl was regardless of the recovery period 47-83%, 60-86% and 63-92%, respectively, while mortality in wheat treated with the insecticides only was 0-9%, 0-61% and 0-91%, respectively. After exposing *S. oryzae* (L.) adults to treated wheat for five days, Wilkin et al. (1999) found that pirimiphos-methyl applied at 4.0 mg/kg rate and 5 °C temperature achieved 64 % efficacy, while our 16 times lower dose (0.25 mg/kg) caused 4 times lower mortality (16 %), which demonstrates how the insecticide application rate greatly determines the effects of combined application of insecticide and low temperature.

As data on the effectiveness of insecticides as grain protectants vary, Subramanyam and Roesli (2000) insisted on the importance of checking their effects on progeny production of various storage insects. High progeny reduction of *S. oryzae*, >92%, was reported after the application of 0.16 mg/kg chlorpyrifos-methyl in combination with 6 days of exposure to 5° C, and all application rates of that insecticide (0.08, 0.12 and 0.16 mg/kg) in combination with 7 days of low temperature exposure. The same rate of progeny reduction was detected for the combination of the highest dose of chlorpyrifos-methyl (0.25 mg/kg) and 6 and 7 days of exposure to 5° C. Individual exposure of *S. oryzae* adults to 5° C over 8 days caused 92% progeny reduction. However, the highest doses of both chlorpyrifos-methyl and pirimiphos-methyl (0.16 and 0.25 mg/kg) caused a significantly lower

progeny reduction in the laboratory, 65.0 % and 60.8 %, respectively. Kljajić and Perić (2010) found that contact of laboratory *S . granarius* weevils with sublethal doses of pirimiphos-methyl caused increases in progeny counts of 40%, while contact of weevils selected with a sublethal dose of chlorpyrifos-methyl caused 127% increase. In our present experiment, the lowest doses of chlorpyrifos-methyl, 0.08 and 0.12 mg/kg, combined with exposure to 5°C over 5 days, compared to the control not treated with insecticides but exposed to 5°C, increased the number of progeny 26.4 and 38.7%, respectively, while the lowest dose of pirimiphos-methyl (0.125 mg/kg) applied with 5°C temperature over 5 and 7 days, resulted in 30% and 27.4% increase, respectively. The data in our own and some earlier studies show evidently that there is a risk of lower or recommended insecticide doses, applied either alone or in combination with other control methods, becoming sublethal to stored product insects in certain situations, which may then further reflect on their survival and progeny production.

In conclusion, our findings show that the existing pest management programmes for *S. oryzae* control in wheat grain can be significantly improved through highly effective combinations of lower doses of the insecticides chlorpyrifos-methyl (0.08-0.16 mg/kg) and pirimiphos-methyl (0.25 mg/kg) with cold treatment at 5°C for 7 days.

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Residual efficacy of deltamethrin applied on porous and non-porous surfaces against *Sitophilus granarius* (L.), *Plodia interpunctella* (Hübner) and *Blattella germanica* (L.)

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Abstract

Residual efficacy of the insecticide deltamethrin, EC formulation with 25 g/L AI + 225 g/L PBO (synergist piperonyl butoxide), against lab populations of *S. granarius* and *P. interpunctella* by applying product water solutions (12.5 mg AI/m^2) to porous surface, and against *B. germanica* by applying them to non-porous surface,

was investigated in laboratory (at 25±1°C and 55-60% r.h.). The mortality of cockroach adults on deposits aged 0, 14, 30 and 45 days was estimated after 30 minutes of their contact with the treated surfaces, and additional 24 h and 48 h of recovery, while the mortality of stored-product insects (adults or larvae) on 0, 7, 14 and 30 days old deposits was estimated after 2, 7 and 14 days of exposure to treated surfaces and additional 7 days of recovery. Mortality of cockroaches in all variants was 100%, except on 45 days old deposit and after 24 h of recovery, when it was 97%. Deltamethrin caused 0% weevil mortality after 2 days of exposure to deposits of all ages (0-30 days), while *P. interpunctella* larval mortality was 87-93%. However, mortality was 100% after 7 and 14 days of weevil/moth exposure in all variants of deposit ages and/or additional 7 days of recovery. The results show that deltamethrin applied to porous and non-porous surfaces is a highly effective insecticide for weevil/moth and cockroach control, and it showed a good residual activity for up to 30 and 45 days, respectively.

Keywords: *S. granarius*; *P. interpunctella*; *B. germanica*; Deltamethrin; Residual efficacy.

1. Introduction

The granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) and Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Phycitidae) are very important insect pests of stored plant products, which are able to cause major losses unless controlled. Also, the German cockroach *Blattella germanica* (L.) (Dictyoptera: Blattellidae) is a widespread urban pest which is frequently present in storages, stored-products and food processing facilities (Hill, 1990; Ebeling, 1991; Rees, 2004; Almaši, 2008; Stejskal et al., 2015).

The use of contact (residual) insecticides, besides sanitation of storage ambient and surfaces, is the most important step in prevention and control of pests (Kljajić, 2008; Arthur, 2012; Jankov et al., 2013; Rumbos et al., 2014). Several products, mostly in the class of organophosphate insecticides and synthetic pyrethroids, have been registered worldwide (Arthur and Subramanyam, 2012; MacBean, 2012) and in Serbia (Team of editors, 2016) as plant protection products (PPPs) and/or biocide products (BP) (WHO, 1999 and 2006).

The residual efficacy of contact insecticides on treated surfaces is known to depend on the type of insecticide, its formulation, type of surface (e.g. metal, wood or concrete), species of stored product insect or duration of exposure (Arthur, 1996; Athanassiou et al., 2013; Jankov et al., 2013; Rumbos et al., 2014). The present study therefore focused on examining the residual efficacy of deltamethrin (with piperonyl butoxide synergist), EC formulations, applied at the recommended rate against stored-product insect pests, represented by *S. granarius* and *P. interpunctella* on porous surface, and an urban pest, *B. germanica* on non-porous surface after different periods of contact with treated surface.

2. Materials and Methods

Test insects and insecticides

Laboratory populations of test insects were reared in an insectary at 25±1°C temperature and 60±5% relative humidity (r.h.). Adults of the *S. granarius* were reared in 2.5L glass jars containing whole grain soft wheat of 12% moisture content as described by Harein and Soderstrom (1966) and Davis and Bry (1985). Indian meal moths *P. interpunctella* were reared on a diet containing corn meal (flour), ground wheat flour, honey and glycerol as described by Boles and Marzke (1966). German cockroaches *B. germanica* were reared in 5.0 L glass jars with cardboards inside, mainly containing coarse wheat meal and/or pelleted food, as described by Morgan (1985).

Two-to-four weeks old *S. granarius* unsexed adults, third instars (L_3 stage) of *P. interpunctella* larvae and one week old *B. germanica* adults, were used in the bioassays.

An insecticide (EC formulation) based on deltamethrin 25 g/L AI, with the synergist piperonyl butoxide 225 g/L, (Kontakt, Galenika-Fitofarmacija a.d., Serbia) was tested.

Bioassay

For stored-product insects, the residual efficacy tests were performed using a methodology described in PP 1/202 (1) and PP 1/204 (1) (OEPP/EPPO, 2004). Porous surface, made by plates of burned bricks (33 x 33 cm) of around 1 m^2, was cleaned, washed and dried before treatment. Water solutions of the insecticide deltamethrin were made immediately before treatment (49.5 mL water and 0.5 mL product). After stirring, the 1 m^2 plate surfaces were treated with 50 mL of water solutions of deltamethrin (12.5 mg AI/m^2) using a low-pressure sprayer. The procedure was repeated (two treatments) with fresh water solutions of the same insecticide. Untreated control surface was sprayed with water only (50 mL/m^2) following the same procedure. Temperature in the facility was 25±1°C and 60±5% r.h. throghout the experiment.

After deposits were dried (0 days deposit age), three glass rings (h=25 mm, R=55 mm) were placed to represent each treatment, each species and each duration of exposure. About 0.25 g of coarse wheat meal was placed into each ring and spread over ring area before 20 adults of *S. granarius* and 10 larvae of *P. interpunctella* were inserted (3 x 2 repetitions per each test species and each exposure). Ring edges were coated with paraffin after insect insertion and the rings lidded with plastic sieves to prevent insects escape. Insect mortality on deposits of different age (0, 7, 14 and 30 days) was estimated after 2, 7 and 14 days of insect exposure to treated porous surface and 7 days of recovery on untreated coarse wheat meal in the laboratory.

For cockroaches, according to principles described by Busvine (1971) and WHO (1999),

tests were performed on non-porous surface made of tile plates (33 x 33 cm) of around 1 m^2, previously cleaned, washed and dried. The plates were treated with 50 mL of water solutions of deltamethrin (12.5 mg AI/m^2) using a low-pressure sprayer. The procedure was repeated (two treatments) with fresh water solutions of the same insecticide. Untreated control surface was sprayed with water only (50 mL/m^2). After deposits were dried (0 days deposit age), 10 adults of *B. germanica* were put on treated surface and covered with three glass Petri dishes (h=2 cm, R=15 cm) (3 x 2 repetitions). Insect mortality on deposits of different age (0, 14, 30 and 45 days) was estimated after 30 minutes (non-choice exposure), and 24 and 48 hours of lab recovery with addition of coarse wheat meal.

Data analysis

Mortality data were initially corrected as suggested by Abbott (1925) and then analyzed using one-way ANOVA. Means were separated by Fisher's LSD test at P=0.05 (Sokal & Rohlf, 1995). Before analysis, mortality percentages were transformed using *arcsine*. However, untransformed means and standard deviations are shown in tables.

3. Results

Sitophilus granarius

The residual efficacy of deltamethrin after 2 days of adult exposure to porous surface was 0%, on deposits of all ages (0-30 days) (Table 1). After 7 and 14 days of exposure, deltamethrin efficacy was 100%, also on deposits of all ages (0-30 days). After seven days of recovery on untreated wheat in the laboratory, the mortality of adults in all treatment variants was 100%, including the variant of 2 days of exposure.

Plodia interpunctella

After 2 days of larval exposure to treated porous surface, deltamethrin achieved the highest efficacy (100%) on the 0-day deposit, while on 30 days old deposit it was 90%, with statistically significant differences (Table 2). After 7 and 14 days of larval exposure to deltamethrin deposits, residual efficacy were 100% in all treatament variants.

Tab. 1 Residual efficacy of deltamethrin (12.5 mg AI/m²) against *S. granarius* after 2, 7 and 14 days of adult exposure to treated porous surface. For each exposure period separately, means within columns followed by the same letter are not significantly different, Fisher's LSD test at *P*>0.05. Where no letters exist, no significant differences were recorded.

Deposit age	**Residual efficacy (% ± SE)**	
(days)	**Before recovery**	**After 7 days of recovery**
After 2 days of exposure		
0	0.0 ± 0.0	100.0 ± 0.0
7	0.0 ± 0.0	100.0 ± 0.0
14	0.0 ± 0.0	100.0 ± 0.0
30	0.0 ± 0.0	100.0 ± 0.0
After 7 days of exposure		
0	100.0 ± 0.0	100.0 ± 0.0
7	100.0 ± 0.0	100.0 ± 0.0
14	100.0 ± 0.0	100.0 ± 0.0
30	100.0 ± 0.0	100.0 ± 0.0
After 14 days of exposure		
0	100.0 ± 0.0	100.0 ± 0.0
7	100.0 ± 0.0	100.0 ± 0.0
14	100.0 ± 0.0	100.0 ± 0.0
30	100.0 ± 0.0	100.0 ± 0.0

Tab. 2 Residual efficacy of deltamethrin (12.5 mg AI/m²) against *P. interpunctella* after 2, 7 and 14 days of larval exposure to treated porous surface. For each exposure period separately, means within columns followed by the same letter are not significantly different, Fisher's LSD test at *P*>0.05. Where no letters exist, no significant differences were recorded.

Deposit age	**Residual efficacy (% ± SE)**	
(days)	**Before recovery**	**After 7 days of recovery**
After 2 days of exposure		
0	100 ± 0.0 a	100.0 ± 0.0
7	96.7 ± 0.3 ab	100.0 ± 0.0
14	98.3 ± 0.3 a	100.0 ± 0.0
30	90.0 ± 0.3 b	100.0 ± 0.0
After 7 days of exposure		
0	100.0 ± 0.0	100.0 ± 0.0
7	100.0 ± 0.0	100.0 ± 0.0
14	100.0 ± 0.0	100.0 ± 0.0
30	100.0 ± 0.0	100.0 ± 0.0
After 14 days of exposure		
0	100.0 ± 0.0	100.0 ± 0.0
7	100.0 ± 0.0	100.0 ± 0.0
14	100.0 ± 0.0	100.0 ± 0.0
30	100.0 ± 0.0	100.0 ± 0.0

Blattella germanica

After 30 minutes of cockroach exposure to treated non-porous surfaces, deltamethrin achieved the highest paralysis (100%) on 0-45 days old deposits (Table 3). After 24 and 48 h of cockroaches recovery, deltamethhrin caused 100% adult mortality in all treatament variants, except in the variant of 24 h of recovery from exposure to 45 days old deposit when it was significantly lower, 97%.

Tab. 3 Paralysis/mortality (%) of *B. germanica* after 30 minutes of adult exposure to non-porous surface treated with deltamethrin (12.5 mg AI/m^2) and 24 h and 48 h recovery. For each exposure period separately, means within columns followed by the same letter are not significantly different, Fisher's LSD test at $P>0.05$. Where no letters exist, no significant differences were recorded.

Deposit age	Paralysis/mortality (% ± SE) after		
(days)	30 min exposure	24 h recovery	48 h recovery
0	100.0 ± 0.0	100.0 ± 0.0 a	100.0 ± 0.0
14	100.0 ± 0.0	100.0 ± 0.0 a	100.0 ± 0.0
30	100.0 ± 0.0	100.0 ± 0.0 a	100.0 ± 0.0
45	100.0 ± 0.0	97.5 ± 0.5 b	100.0 ± 0.0

4. Discussion

In reviewing analysis by Arthur (2012) we concluded that aerosols and contact insecticides are becoming good alternatives to the fumigant methyl bromide in flour mills, food production facilities and food warehouses. A number of later studies showed good potential of various contact insecticides after application on different surfaces in control of stored-product insect pests: e.g. malathion, pirimiphos-methyl and lambda-cyhalothrin against rice wevil *Sitophilus oryzae* (L.) (Jankov et al., 2013), spinetoram against stored-product beetle species (Vassilakos et al., 2014), cypermethrin and pirimiphos-methyl against *S. granarius* and *P. interpunctella* (Andrić et al., 2014) and two formulations of pirimiphos-methyl (EC and CS) against *S. granarius,* lesser grain borer *Rhyzopertha dominica* (F.) and confused flour beetle *Tribolium confusum* (Jacquelin duVal) (Rumbos et al., 2014).

The pyrethroid deltamethrin has been classified as a highly applicable insecticide, and is used for control of many arthropods, including cockroaches that are important in public health (Baur, 1991; WHO, 1984 i 2006; Mac Bean, 2012; Team of editors, 2016). Also, international research (Arthur 1997a,b; Kavallieratos et al., 2016) and studies in Serbia (Kljajić and Perić, 2006; Andrić et al., 2010; Pražić Golić et al., 2015) on the effects of different formulations of deltamethrin have shown that it is a highly effective insecticide in control of different stored-products species and cockroaches having notable residual efficacy (Arthur, 1997a,b and 2018; Kljajić and Perić 2009; Sims et al., 2010; Paudyal et al., 2016).

In our tests with laboratory populations of the stored-product insect pests *S. granarius* and *P. interpunctella*, deltamethrin applied to porous surface at a rate of 12.5 mg AI/m^2 reached its highest efficacy (100%) on up to 30 days old deposits after 7 and 14 days of adult and larval exposure before and after 7 days of recovery, including the 2 days exposure, only after 7 days of recovery. Also, in tests with *B. germanica*, in all treatament variants after 30 minutes of adults exposure to treated non-porous surface, deltamethrin achieved the highest paralysis of 100% on up to 45 days old deposits, and 100% adult mortality after 24 h and 48 h of recovery, except in the variant of 24 h of recovery after exposure to 45 days old deposit (97%). The results of our study showed that the pyrethroid deltamethrin with synergist piperonyl butoxide has high residual efficacy against the stored-product insects *S. granarius* and *P. interpunctella* after application on porous surface, and against the urban pest *B. germanica* on non-porous surface. These findings contribute significantly to the existing IPM programmes, showing that deltamethrin can be used simultaneously as a PPP and BP insecticide.

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Insecticidal efficacy of abamectin against red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae): influence of dose, exposure interval, relative humidity and temperature

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In this communication, the insecticidal efficacy of Abamectin against *Tribolium castaneum* adults were evaluated in two sets of bioassays: In the 1st series of experiment, the effect of temperature was assessed on wheat treated at 0.01, 0.10, 0.25, 0.50, 0.75, 1.00 and 1.50 mg kg-1 at 20°C and 30°C, and 65%rh. The moisture content of the wheat ranged between 11.8 and 12.1%. Spraying was performed using a Badger 100 artists' airbrush (Franklin Park, IL, USA) on a stainless-steel tray in which 1 kg of wheat containing 5% (weight:weight) broken kernel was treated with 5 mL of an aqueous solution containing the appropriate volume of the EC formulation corresponding to each dose. Treated and untreated grains were kept into incubators at 30°C for 6 h to remove the excess moisture. After that, the grains were kept at 20 and 30°C and 65% rh in temperature-controlled incubators (Binder Model: KB 720) for 24 h for acclimatization to experimental conditions. For the experiment, eight samples of 60 g were obtained from each treated or untreated lots for each temperature and put into a cylindrical plastic vial (7 cm long × 5 cm diameter). Sixty (1-2 wk old) adults were placed in each vial containing treated wheat. There were 8 replicates for each exposure period and control. Adult mortality were recorded on 7th, 14th, and 21st d after treatment by reintroducing alive adults in the same vial while discarding the dead ones. At the end of the 21th d of exposure, all dead and alive adults were discarded, and the vials containing wheat only was returned to corresponding temperature-control.

The effectiveness of Spinetoram against red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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The data were analyzed, after arcsine transformation, with GLM-repeated measures (ANOVA) at a significance level of P less than 0.05 using Statistica version 7, and the means were compared with Tukey's HSD test. Arcsine transformed means were back transformed for presentation. Mortality was proportional to dose rate and exposure period. At 1 ppm dose rate, mortality at the end of 7th day was recorded as 34,44% and increased to 52.59% after 15 day of exposure. Mortality response was more pronounced at/above 1 ppm. Thus at 15th day of exposure, mortality rates were calculated as

3.33%, 52.59%, and 99,26 for 0,01, 1, and 10 ppm dose rate, respectively. F1 development was also proportional to dose rate and the population growth was suppressed by 99% and 100% at 5, and 10 ppm, respectively. Results show that the spinetoram can be effectively used for the control of stored grain insects.

The effectiveness of Spinetoram against maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae): influence of dose, exposure interval, and temperature

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In this research, the effectiveness of Spinetoram was investigated against *Sitophilus zeamais* at three temperatures of 20, 25, and 30°C and 65%RH. Radiant 120 SC was selected to test the efficacy of Spinetoram. The formulation was applied to maize at the rates of 0.00, 0.01, 0.10, 0.25, 0.50, 0.75, 1.00, 2.00, 5.00, and 10.00 mg/kg using 9 replicates each. 50 g samples of treated maize were separately put into small PVC vials along with 30 adults. Mortality of insects were observed at 1st, 2nd, 3rd, 7th, 14th, 21st and 28th days after setting up the experiment. At the end of final count at 28th day, all individuals were removed and the test vials containing maize only were additionally kept for 50 days to determine the F1 development. Mortality rates increased along with temperature and exposure time. At the dose of 1 mg/kg, 66.68% and 97,08% adult mortality were obtained at 20 and 30°C, respectively. Similarly, at 25°C at the dose of 5 mg/kg, adult mortality were 71,89%, 98,89%, and 100% for 7, 14, and 21 days of exposure, respectively.

Session 8

Postharvest Pest Management and Extension in Developing Countries

Postharvest knowledge, perceptions and practices of African small-scale maize and sorghum farmers

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Abstract

Due to a single annual food production season in southern Africa, small-scale maize and sorghum farmers store grain until the next harvest. The farmers' postharvest knowledge, perceptions and practices (KPP) is important in reducing postharvest losses (PHLs); a key component of household food and nutritional security. Using random sampling, 310 farmers from two districts of Zimbabwe with contrasting agroecologies and agricultural systems (maize and sorghum) were interviewed to assess their KPP on post-production aspects. Maize and sorghum grain were stored in new and recycled polypropylene bags (93.5% and 42.6%) placed in ordinary rooms (44.5% and 27.1%), brick store houses (28.4% and 54.2%) and traditional huts (23.2% and 16.1), respectively. Farmers recognised field infestation as important source of insect infestation in sorghum (60%) but not in maize (21.3%). Synthetic commercial grain protectants were used more on maize (90.2%) than on sorghum grain (63.2%). Majority of farmers (> 75%) perceived these insecticides as both effective and safe to use. Farmers' household reserved grain ran out before the next harvest and was supplemented through buying grain or mealie-meal with cash, or exchanging grain with labour or livestock. Postharvest information and training were scarce in both systems. The study provides important information to extensionists, policy makers, development agents and researchers for reviewing and benchmarking extension services and farmer training requirements to effectively accelerate progress towards PHL reduction and contribute to household and national food and nutritional security.

Keywords: stored maize and sorghum grain; post-production practices, knowledge and perceptions; small-scale farmers; household survey.

Introduction

Maize is the staple crop in Southern Africa with dietary, economic, social and political importance (Mvumi et al., 1995; Tefera, 2012). Similarly, sorghum is one of second most important cereal staples in semi-arid areas of the region (Taylor, 2003). Stored product protection of these staple cereals is a key component of food and nutritional security largely missing in Africa (World Bank, 2011; Tefera, 2012). This is especially critical to more than 70% of southern African population that depend on unimodal rainy season for rain-fed crop production (Abbass et al., 2014). This means that the major cereal staple grain is harvested only once per year and farmers have to rely on their storage techniques and knowledge to preserve reserves during the long off-season lean-supply-high-demand period before the next harvest. Small-scale farmers are custodians of their household food harvesting, processing, storage and budgeting throughout this non-productive season.

Research has shown that grain is most vulnerable to rodent, moulding and insect pest attack during the storage period. In Southern Africa alone, independent reports from APHLIS and Worldbank show postharvest losses between 10 – 20% (World Bank, 2011; APHLIS, 2014) which is worth about

US$4 billion dollars (World Bank, 2011). After maturity, maize or sorghum grain undergoes various processing steps along the harvesting and postharvest chain of activities. Therefore, farmers' knowledge, perceptions and practices along this post-production chain are strong determinants of the level of losses incurred on-farm (Abbass et al., 2014). Since losses occur at various stages of the post-production chain (Tefera, 2012; APHLIS, 2014), it is imperative to study farmers' practices throughout the various post-production stages to identify major loss points that need redressing.

It has been more than two decades since the last published study that focussed on small-scale farmers' postharvest practices in Zimbabwe which revealed a lot of knowledge gaps (Mvumi et al., 1995). The objective of the current study was not only to compare and contrast the postharvest knowledge, perceptions and practices of maize and sorghum-based farming systems, but also to establish knowledge gaps considering the advances made in developing postharvest technologies in the last two decades. This is important to establish whether the status quo research and extension systems are effective in disseminating postharvest information for future curriculum and policy adjustments.

Materials and Methods

Study sites

The study was conducted in two districts in the semi-arid Insiza District (S20º.54'14.00"; E29º. 27'89.00") Matebeleland South province and the semi-humid Murehwa district (S17°. 64'99.97"; E31°.78'33.30") in Zimbabwe from March to June 2013. Insiza district is a sorghum-producing area, which typically receives an average rainfall of 450-650 mm annual rainfall while Murehwa district is a maize-producing area with a comparably higher average annual rainfall of 650-1,000 mm. Murehwa and Insiza districts lie in Zimbabwe agro-ecological zones II and V, described as intensive and extensive farming regions respectively due to huge differences in rainfall patterns and aridity (Mugandani et al., 2012; Muhoyi et al., 2014). This separates them into maize and sorghum farming areas respectively.

The study approach and data collection

A standard structured coded questionnaire was used for data collection using face to face interviews during the harvesting season (April-May 2014). In each district, four wards (Wards 1, 2, 17 & 19 for Insiza district and 8, 13, 16 and 28 for Murehwa district) were selected using a purposive sampling technique in consultation with agricultural extension staff. Prior to this study (2012 and 2013 storage seasons), under the same grant (UZ-RUFORUM postharvest project grant) farmers from wards 17 & 19 (Insiza District) and 13 & 28 (Murehwa District) were trained on good postharvest practices including pesticide use practices, proper mixing of grain and grain protectants, proper grain storage and insect pest identification and basic ecology. One hundred and fifty-five households were interviewed across all four wards (75 from trained wards and 75 from untrained wards and 3 reserve households for discard questionnaires) in each district, giving a total of 310 respondent households for the study. For the trained wards, households were purposely sampled following the list of participants (attendance lists), while a random sampling (every 5th household) was used for the untrained wards. Both researchers and trained extension staff conducted the interviews in local languages; Shona and Ndebele for Murehwa and Insiza districts respectively. In addition, field observations were used to collect anecdotal evidence where possible; farmers were asked to show researchers grain protectant containers, traditional harvesting equipment and/or techniques. Data were entered in CSPro 6.1 software for Windows 7 and IBM SPSS Statistics 23 was used for statistical analysis. Cross tabulation was used to determine associations between categorical factors and variables of interest through Wald's χ^2 tests at 95% Confidence Interval (CI).

Results

Demographics

More females (62.6%) were observed participating in maize grain postharvest than males (37.4%) whereas more males (68.4%) participated in sorghum postharvest activities (Tab. 1).

Tab. 1. Socio-economic characteristics of farmers from Murehwa and Insiza districts

Characteristic	District	
	Murehwa (maize)	*Insiza (sorghum)*
Sex (%)		
Male	37.4	68.4
Female	62.6	31.6
Age (years)		
≤ 40	13.2	10.0
40 – 60	48.7	55.5
> 60	38.2	35.5
Education (%)		
No school	3.3	8.4
Primary	62.5	55.5
***ZJC**	7.2	6.5
Ordinary level	27.0	4.5
Tertiary	0	3.9
Land sizes (acres)		
≤ 2	7.7	34.8
2.1 – 5	72.3	25.8
> 5	38.2	39.3
Farming experience (years)		
≤ 10	18.7	7.8
10 - 15	19.4	58.7
> 15	60.0	35.5
Cannot remember	1.9	-

*Zimbabwe Junior Certificate of Education (equivalent to Form 2 or two years of secondary education).

There were more middle aged farmers in Insiza compared to Murehwa. Trends in education were generally similar except that more farmers attained the generally recognised Ordinary level in Murehwa than in Insiza district. Land sizes are much bigger in Insiza (Tab. 1), but Murehwa (60.0%) farmers have more years (> 15 year) of farming experience compared to the Insiza farmers (35.5%).

Knowledge

In both districts, postharvest information was scarce and for the remote sorghum district, the government extension services were the major source of information (94.4%) compared to maize (68.7%) which supplemented by NGO trainings (22.7%) as well as private companies and research institutions (5.3%). Nevertheless, postharvest information was generally not recognised and not perceived as important by farmers from both systems. All farmers knew about grain varietal susceptibility to insect pest attack (85.5%) and (60.0%) for maize and sorghum respectively. However, farmers reported continuously using the more susceptible varieties for reasons of high yields and seed availability. More maize farmers (50.3%) knew about the newly arrived pest, the larger grain borer *Prostephanus truncatus* (Horn) compared to sorghum farmers (29.7%). However, the awareness was spatial depending on the wards that were trained ($\chi^2_{(8.1)} = 42.12$, $p < 0.001$) (maize) and ($\chi^2_{(8.1)} = 97.89$, $p < 0.001$) (sorghum) and gender in maize ($\chi^2_{(2.1)} = 13.69$, $p = 0.001$) but not sorghum) ($\chi^2_{(2.1)} = 1.73$, $p = 0.422$). This awareness was however, independent ($P > 0.001$) of education level, age and time of farming experience in both systems. Most farmers just knew the name 'LGB' but could not physically identify the pest. Almost all maize farmers believed that LGB was absent from their stores (78.1%) compared to about half (51.6%) in sorghum (the rest did not

answer the question or were not sure) although anecdotal evidence showed that some of the maize farmers had the LGB in their stored grain but unaware of it.

We assessed if farmers in trained wards had changed some of their traditional 'methods' as a result of the training. Tab. 2 shows the farmers' responses from both maize and sorghum

Tab. 2. Postharvest aspects changed by farmers after in the UZ-RUFORUM trained wards compared to farmers from non-trained wards

Did you improve the following	**Maize**			**Sorghum**		
aspects after training?	**Yes*	*No*	*ǂNo response*	**Yes*	*No*	*ǂNo response*
Cutting time	59.3	3.5	37.2	67.7	0.6	31.6
Drying method	58.4	1.8	39.8	60.0	0	40.0
Drying time	72.6	2.7	24.8	59.4	0	40.6
Dehusking time	56.6	2.7	40.7	N/A	N/A	N/A
Threshing/Shelling method	54.0	6.2	39.8	52.9	0	47.1
Threshing/Shelling time	54.9	3.5	41.6	67.7	0	21.8
Types of pesticides	61.9	2.7	35.9	74.2	0	25.8
Moisture testing	60.2	2.7	37.2	63.2	0.6	36.1
Grain treatment time	67.3	2.7	30.1	67.7	0	32.3
Grain treatment method	60.2	2.7	37.2	78.1	0	21.8

*Trained wards, 13 & 28 (maize); 17 & 19 (sorghum)
ǂNon-trained wards, 8 & 16 (maize); 1 & 2 (sorghum)

In both systems, most farmers reported having changed their timing and methods of some postharvest practices (see Tab. 2). Most of these farmers were from the wards previously trained in on good postharvest practices as confirmed by the positive significant association between the trained wards and changing a postharvest practice both in maize ($\chi^2_{(8.1)} = 47.89$, $p < 0.001$) and in sorghum ($\chi^2_{(8.1)} = 44.12$, $p < 0.001$) farming systems. Considerable proportion of 'no responses' to this question came from the –non-trained wards, since they mostly were not aware of any training.

Practices

Farmers harvested both maize (97.1% and sorghum (94.2%) between March and May every year. Due to relatively high rainfall, 83.6% of maize farmers harvested ≥ 0.5 tonnes, compared to 62.5% of farmers harvesting the same quantity of sorghum. From the field, maize was mainly transported using scotch carts (40.6%), wheelbarrows (31.3%), head-carrying (9.4%) or hired trucks (7.8%) and transport was reported as one of the major challenges. However, most sorghum farmers (78.7%) did not respond to how they carried their produce. Corresponding to their yields, more maize farmers (38.4%) retained more grain (300 – 1000kg) after harvest compared to sorghum respondents (29.7%) who retained the same amount of grain (Fig. 1). However, more sorghum farmers preferred to store more than a tonne of grain (Fig. 1) compared to maize farmers.

Although farmers in both districts did not generally sell their retained grain, the family reserved grain ran out more for sorghum (56.8%) than maize (37.4%) although sorghum farmers stored more. Reserved maize grain started running out starting in November (19.4%), peaking up in December (32.3%) and subsiding gradually in March, whereas for sorghum, grain started running out in March (19.7%) and peaking up in April (27.4%) and subsiding in May (9.4%) every season. Almost equal proportion of farmers supplemented their grain mainly by buying grain with cash from other farmers (35.5% and 37.1%) or buying mealie-meal (27.4% and 31.9% sorghum) for maize and sorghum respectively (Fig. 2). In the maize based system, exchanging grain with labour was more prominent (21.0%) whereas Non-Governmental Organisation (NGO) rations were perculiar in sorghum (6.0%) systems. Exchanging grain or mealie-meal for livestock was less common in both districts (see Fig. 2).

Before threshing and storage, sorghum farmers tested grain for storable moisture content mainly through biting (56.8%), crushing between fingers (29.0%) and by easiness of threshing (8.4%).

Although maize farmers also largely depended on biting (41.3%), a significant proportion also used experienced eyes (24.5%), the kernel sound method (14.8%), crushing (9.7%) and the salt method (6.5%). Maize grain was mostly stored in polypropylene bags (94.8%) which were new (23.2%), recycled (16.1%) or a mixture of both (54.2%). Most of these bags (78.7%) were placed on stones/bricks/timber dunnage in ordinary rooms (44.5%), brick and motor store houses (28.5%) or in traditional huts (23.0%) also used as cooking houses or 'kitchens'. However, only 45.8% of sorghum grain was stored in polypropylene bags in ordinary rooms, most of the farmers (54.2%) stored bulky grain in brick and motor storehouses (54.2%). Therefore, most farmers still largely stored their maize grain in in polypropylene bags (94.8%) in in ordinary rooms as previously reported by Nyagwaya et al. (2010). Unlike in maize, most sorghum farmers (54.2%) preferred storing their grain in bulk inside raised brick and motor storehouse compartments (54.2%) referred to as 'granaries'. Fewer farmers (45.8%) used polypropylene bags for grain storage.

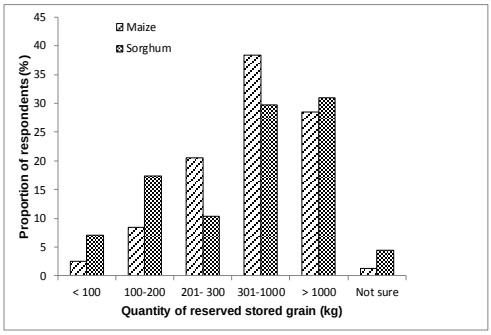

Fig. 1. The quantities of household consumption grain reserves stored by farmers in Murehwa (maize) and Insiza (sorghum) districts after a normal harvesting season.

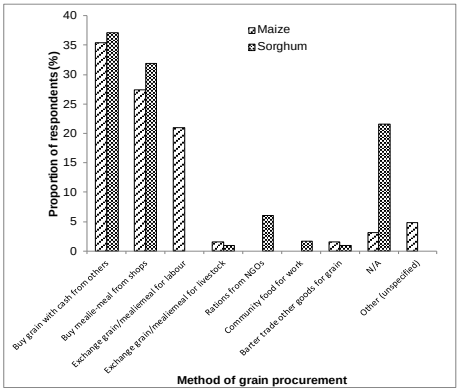

Fig. 2. Farmers methods of household grain procurement in Murehwa (maize) and Insiza (sorghum) districts.

The storage facility was significantly influenced by location (wards) ($\chi^2_{(12, 1)} = 65.146$, $p < 0.001$) and sex ($\chi^2_{(4, 1)} = 23.103$, $p < 0.001$). Commercial chemical grain protectants were mostly used (90.2%) to protect maize than sorghum (63.2%) grain (Fig. 3).

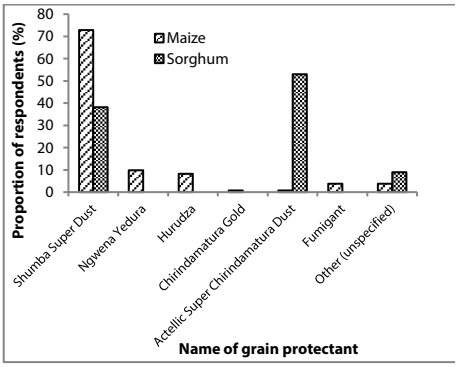

Fig. 3. Types of insecticides used by farmers within the previous five years in Murehwa (maize) and Insiza (sorghum) districts.

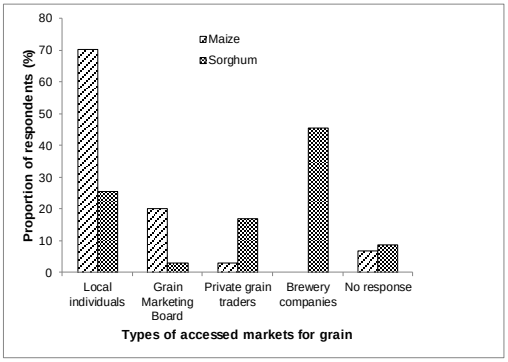

Fig. 4. The types of markets that farmers accessed by the farmers for selling their sorghum and maize grain in Insiza and Murehwa districts respectively

Farmers purchased commercial grain protectants from the local agro-dealer shops (78.0% and 67.9%) respectively for the maize and the sorghum farming systems. Only two insecticides were dominant in the sorghum area; Shumba Super Dust® (fenitrothion 1% + deltamethrin 0.13%) and

Actellic Super Chirindamatura Dust® (pirimiphos-methyl 1.6% w/w + permetrin 0.3% w/w. On the contrary, six registered grain protectants were recorded in the maize farming district, including Hurudza Grain Dust® (fenitrothion 1.7% w/w + deltamethrin 0.05% w/w) and Ngwena Yedura® (pirimiphos-methyl 2.5% w/w + deltamethrin 0.2% w/w) including the two already recorded for sorghum (Fig. 3). More maize farmers knew and had access to more pesticides than sorghum farmers because of their proximity to major cities. Farmers from both farming systems perceived chemicals as safe (94.7% and 76.9%) and effective (77.2% and 75.4%) for maize and sorghum respectively. In the contrary, a significant number of maize farmers (40.6%) reported that insect pests remained a problem post-chemical grain treatment compared to sorghum (23.9%), citing chemical ineffectiveness (15.5%) and improper insecticide use, which was more pronounced in maize (53.4%) than in sorghum (11.6%). Traditional grain protection methods were very minimal, 3.8% and 4.5% in both maize and sorghum respectively. The major postharvest pests for sorghum were quelea birds, *Quelea quelea* L. (81.9%), *Sitophilus species* (90.7%) and rodents (3.2%); while in maize, the major insect pests were *Sitophilus species* (91.0%). The larger grain borer was mentioned as a major pest by only 1.5% farmers in maize while *Tribolium casteneum* (1.3%) was mentioned by few farmers in sorghum.

Perceptions

Farmers perceived insect infestation as originating from the field (81.3%) from March and April when maize is between the hard dough stage and physiological maturity (63.2%). On the contrary, although sorghum farmers equally acknowledged field infestation initiating from the field (95.3%), they believed that infestation started much earlier (milky dough stage) (79.4%) from February to March than observed in maize. Correspondingly in storage, insect pests were first seen earlier (August) (8.4%) on sorghum grain and later (September) (10.3%) on maize. Likewise, peak insect pest populations were reported to occur earlier (September – December) (73.9%) for sorghum and slightly later (October- December) (63.3%) for maize. Farmers recognised and perceived pre-harvest field infestation as an important source of insect inoculum in stored sorghum (60%) but not in maize (21.3%). Apart from insect pests (91.0% and 67.1% for maize and sorghum; respectively), farmers from sorghum farming systems perceived labour shortage during harvesting time (56.8%), domestic and wild animals (11.0%) and poor prices (7.7%) as major postharvest challenges. In maize, most farmers perceived labour shortages at shelling (23.9%) and transport challenges from the field (12.9%) as major challenges while the bulk of farmers (45.8%) perceived having no notable challenges during the harvesting process. Types of markets accessed by the farmers differed with the type of grain that they produced (Fig. 4).

Most maize sold their maize grain to local individuals (70.3%) within their communities compared to sorghum (25.7%), whereas most sorghum was sold to the brewery companies (45.7%). Few farmers, 20.3 (maize) and 2.9 (sorghum) sold their grain to the government parastatal Grain Marketing Board (GMB) (see Fig. 4).

4. Discussion

More females participated in maize grain postharvest than males whereas more males participated in sorghum postharvest activities than females. This is attributed to ethnical differences in gender roles between the two tribes where grain processing is more of a female role (Mvumi et al., 1995; Manda and Mvumi, 2010) in some areas (tribes) than others in Zimbabwe. In addition, the maize district, Murehwa, is nearer to urban areas (Harare and Marondera) where males migrate to seek formal employment in the cities, leaving females in charge of farming activities whilst Insiza district is far from such urban areas. On the other hand, there were more middle aged farmers in Insiza compared to Murehwa, this may be explained by more early adolescent cross border migration to South Africa in Insiza district which borders Southern part of Zimbabwe with South Africa. Trends in education were generally similar except that more farmers attained the generally recognised Ordinary level in Murehwa than in Insiza district; again this is attributable to early adulthood

migration to South Africa before completing school dominant in Insiza District. Land sizes were much bigger in Insiza (Tab. 1) because farmers were resettled in this area by the Government of Zimbabwe whereas Murehwa was a traditional tribal trust land (generational landholdings) where land has been inherited from forefathers and partitioned continuously to current generations over the years, thus gradually dwindling in percapita landholdings as populations increased. This also explains why farmers have more years of farming experience in Murehwa than in Insiza, because they have traditionally been on the same land.

Although almost equal number of farmers from both maize and sorghum systems preferred to store 300-1000kg of grain as reserves for family consumption, more sorghum farmers preferred to store more than a tonne of grain compared to maize farmers. This is because the sorghum producing area is generally arid with low and unpredictable rainfall patterns and farmers store more grain to cushion against frequent droughts, whereas, in Murehwa district, farmers are cautious not to store more than a tonne to avoid storage losses due to insect pests, rodents and thieves. This compels farmers to sell part of their grain early, mainly to local individuals in exchange for cash, livestock or labour. The parastatal responsible for buying the grain, normally offers too low prices to attract both maize and sorghum farmers. Most sorghum farmers rely on the brewery companies (especially Ingwebu Breweries®) for market, which in some cases provides them with inputs under contract farming. Although most farmers from both farming systems stored considerable amount of grain, most farmers ran out of grain before the next harvest without extra income sources for cash requirements to buy grain. New postharvest technologies that could increase the stored grain per household have been introduced in some parts of Zimbabwe (Chigoverah and Mvumi, 2016; Mlambo et al, 2017), most farmers have limited access to this information including new storage technologies and the high cost associated with new technologies (Mvumi, 1997; Nukenine, 2010).

The significant positive correlation between trained wards and changes in postharvest practices and knowledge about the P. truncates suggests that our farmer trainings in the previous 2 seasons were to a larger extent, effective. Majority of farmers from untrained wards in both districts were not aware of any postharvest training. This showed that there is need for huge investment in farmer training to cover as many farmers in as many areas as possible since farmers do not seem to freely share postharvest information even when trained (Mvumi, 1997). The scarcity of postharvest information also shows that dynamics and modern changes in postharvest activities, grain protectants, innovations, stored product insect ecology and research have not been closely followed up by the relevant postharvest technical back-stopping support to improve and update farmers and extension staff knowledge and skills on stored product pest control (Larsen and LilleØr, 2014, Chigoverah and Mvumi, 2016). In addition, postproduction activities have comparably received little institutional and extension efforts (Mvumi, 1997; Abass et al., 2014), limited research investment and limited publication of research results, especially in Southern Africa (Mvumi, 1997; Larsen and LilleØr, 2014; Mvumi and Stathers, 2015; Affognon et al., 2015).

In the maize-based farming system, we recommend the introduction of chemical-free hermetic storage systems such as hermetic bags, cocoons and metal silos to address storage pests and pesticide mis/overuse challenges. Hand-operated shellers and cheap produce transporting systems are also necessary to reduce labour bottlenecks during shelling and transportation for both systems. In the sorghum farming system, we recommend early harvesting to avoid extensive bird damage (Mutisya et al., 2016). There is also need to explore the possible use of new bird-scaring techniques such as the Unmanned Aerial Vehicles (UAV) popularly known as 'drones' to reduce losses by quelea birds. However, benefit-cost analysis will be essential to determine viability of such investments and the potential for wide-spread use of the technology under small scale conditions.

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Evaluation of five storage technologies to preserve quality composition of maize in Nigerian markets

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Abstract

Maize needs to be stored using good and safe postharvest management measures that will maintain the quality as at harvest. Insects and moisture must be controlled in storage to ensure quality and methods to achieve this, such as the use of reduced-risk measures were evaluated in this study, conducted February–December 2016. The efficacy of Bularafa diatomaceous earth (DE), *Piper guineense* (Botanical), PICS bags, ZeroFly® bags and permethrin (Rambo™) in preserving maize quality in Nigerian markets was assessed. A sixth treatment comprised maize in untreated polypropylene bags. Study locations were in four markets in Ibadan, Ilorin and Oyo towns. Each market had a storehouse, which contained experimental 100-kg bags. In each storehouse, each technology

had six bags, which were all sampled monthly except in PICS treatment where six bags were destructively sampled every four months. Data taken in February) and December) showed that quality of maize in PICS bags was best having the lowest percentage of insect damaged kernels, numerical based (%IDKNB), — 0.01 ± 0.01and 0.02 ± 0.01; %IDKWB— weight based were 0.00 ± 0.00 and 0.00 ± 0.00); % weight loss (0.01 ± 0.01; 0.01 ± 0.01), % number of discolored maize (0.02 ± 0.01; 0.01 ± 0.01) and % seed germination (96.77 ± 0.53; 98.37 ± 0.35) respectively. Treated and untreated maize had mean aflatoxin levels below limit of detection of 5 ppb in February and December (0.47 and 1.66), respectively and their proximate composition were within ranges reported in literature. By December, untreated maize had the highest %IDKNB (1.42 ± 0.22), %IDKWB (1.07 ± 0.18), % weight loss (0.36 ± 0.07) and lowest % seed germination (88.09 ± 0.98) when compared to the evaluated storage technologies Therefore, these five technologies can be incorporated in integrated management of storage insect pests in storehouses.

Keywords*:* Insect damage, Weight loss, Aflatoxin, Seed germination, Proximate composition.

Introduction

Maize (*Zea mays* L.) is an important cereal which ranks third in global production/consumption after wheat and rice (Asghar et al., 2010). It is a major cereal for livestock feed and human nutrition. It is also an important raw material for several agro-based industries (Akande and Lamidi, 2006). In addition, in a market that is not controlled, the value of any surplus maize in good condition tends to rise during the off-season period (Nagaraj et al., 2016); meaning maize can be an important cash crop. Maize has the disadvantage of being harvested in the wet season; hence, prone to damage by microorganisms in addition to the problems of insect infestation.

Sitophilus zeamais is a serious cosmopolitan field-to-store pest of maize in tropical and subtropical regions (Hagstrum et al., 2012). Damage caused by the insect becomes noticeable after the adult insect makes holes and deposits its eggs within the hole. Developmental stages of the insect take place within the grain after which the adult weevil bores its way out, leaving a characteristic emergence hole on the grain (Rees, 2004). In developing countries, maize production and consumption often falls below demand as a result of post-harvest losses due to storage pests and other agents (Aulakh et al., 2013). Post-harvest losses due to *S. zeamais* have been reported as an important constraint to grain storage in Africa (Edelduok et al., 2015), these losses threaten household food security and reduced market returns making stakeholders seek any type of option for protecting their grain during storage (Stathers et al., 2008). Despite success in controlling insect pests using synthetic insecticides, their persistence in the environment, the toxic residues they leave in food and development of resistance by insect pests require that more reduced-risk alternatives be sought (Ileke and Oni, 2011).

During storage, grain quality can remain at the initial level or decline to a level that may make it unacceptable for both food and planting purposes. This decline is due to many determinants: adverse environmental conditions during seed production and storage, pests (insects, rodents and micro-organisms), high moisture content, mechanical damage during threshing, long duration of storage, bad packaging, pesticides and biochemical injury of grain tissue (Jyoti and Malik, 2013).

Moreover, the change in color of seed grains, protein and carbohydrate degradation, and the production of mycotoxin reduce the quality of stored grains, and endanger human health (Pimentel et al., 2011). The preservation of quality and nutritional value of grain during the period of storage depends not only on the conditions of production and harvesting, but also on the maintenance of appropriate storage conditions of the grain.

Presently in Nigeria, control of insect pests in stored food products, maize inclusive, is by the use of synthetic insecticides which have some hazards such as pollution of the environment, toxic residues in stored grains, development of resistance in target species, pest resurgence, lethal effects on non-target organisms, direct toxicity to users and other health hazards in addition to the high cost of the insecticide cum inadequate skills in application, (Adedire et al., 2011). However, there are reduced-risk technologies such as botanicals (e.g. *Piper guineense* (Schum & Thonn), diatomaceous earths (DE), Purdue Improved Crop Storage (PICS) bags and ZeroFly® Storage Bags (hereafter referred to as ZeroFly bag) that are available in sub-Saharan Africa and can be used for proper preservation of

grains without the negative effects associated with pesticides. Botanical such as *P. guineense,* which are inexpensive, relatively safe, and poses little or no hazard to human health and the environment have been used for postharvest insect pest control in grains (Asawalam et al., 2007; Otitodun et al, 2015).

Despite the fact that there are reduced-risk technologies available for grain preservation in sub-Saharan Africa, there is little information available on how effective these technologies are for preservation of grains such as maize in storage. This means it is important to evaluate the effectiveness of these reduced-risk technologies in the field. Therefore, the objective of this study was to evaluate five storage technologies — a botanical (*P. guineense*), Bularafa DE, PICS, ZeroFly® bag and permethrin (Rambo™) — to preserve the quality of maize in Nigerian markets.

Materials and Methods

Study Sites

The experiment was conducted during the period February–December 2016 in storehouses located in three grain markets — Eleekara market in Oyo and Arisekola market in Ibadan, Nigeria and Old Ago market in Ilorin, North Central Nigeria.

Maize Samples

Maize used for the experiment was obtained from Ijaye Farm Settlement in Akinyele Local Government Area, Ibadan Oyo State. The variety known as SWAN 2 is widely grown in Southwest Nigeria; Farmers in the settlement had applied aflasafe™ in fields used to produce maize for this study. Aflasafe contains a mixture of four atoxigenic strains of *Aspergillus flavus* of Nigeria origin (Agresults Online). Initial maize moisture content was determined by the ASABE. Maize was fumigated before use to ensure that there was no field to store transfer of insect pests. Fumigation was conducted at the Nigerian Stored Products Research Institute (NSPRI), Ilorin, Nigeria.

Storage Technologies (treatments)

Maize was stored for 11 months using five storage technologies — ZeroFly bags, PICS bags, diatomaceous earth (Bularafa DE), a botanical (*P. guineense*) and permethrin (Rambo™) — to assess their effectiveness in controlling stored product insect pests and were compared with untreated control.

Methodology

In each storehouse six 100-kg maize-filled bags were assigned to ZeroFly, DE, Botanical, Rambo and untreated control; each stack of six bags was on a separate pallet. Sixteen bags were assigned to PICS arranged on four pallets. Bags on pallets were arranged in such a way that they formed two layers. The pallets for each treatment were placed 1 m apart from each other. There were forty-six 100-kg bags per storehouse

The experimental design used was randomized complete block design (RCBD) with four replications and six sub-replications. Each market represented a replication.

Samples of maize were obtained using a 1.2-m open-ended Trier (grain probe) (Seedburo Equipment, Chicago, IL) with six openings, total of 700 g was taken from each bag during each sampling event.

In the PICS treatment, six bags were destructively sampled every 4 months — 1 bag with a sensor and 5 bags without sensors. The six bags to be sampled were randomly selected at the beginning of the study using randomization software

Seed germination, insect damaged kernels, weight loss and maize discoloration were determined monthly, Nutritional composition of maize (Proximate analysis) was determined at 4 months' intervals while aflatoxin levels were determined at the begining and end of the study.

Statistical Analyses

Statistical analyses were performed with SAS Version 9.3 (SAS Institute, Cary, NC). Treatment effects were assessed using analysis of variance methods (PROC MIXED). A repeated measures model in a randomized complete block design was utilized, with market as the blocking factor and month as the repeated factor. An autoregressive covariance structure was used to model the correlations within treatment and across months. An analysis of the aflatoxin level was conducted with the use of a square root transformation. A square root transformation was used to correct for heterogeneous variances and the lack of normality of the count response variable. The simple effects of treatment given month were assessed with protected planned contrasts (SLICE option in an LSMEANS statement). In the case of percent insect damaged kernel, weight loss, seed germination, discoloured grain and proximate composition, data analyses were conducted with the use of an arcsine transformation to stabilize variances but untransformed percentages are reported.

Results

Results presented on the study are in two categories; study involving four treatments (Botanical, DE, Rambo and ZeroFly) and study involving five treatments (Botanical, DE, PICS, Rambo and ZeroFly).

Percent number of insect damaged kernels (%IDKNB), weight of insect damaged kernels (%IDKWB), weight loss (%WL), seed germination (%SG), discolored grains (%DG), aflatoxin levels (AF) and proximate composition of maize preserved in Nigeria market with four treatments

In the study involving 4 treatments, mean values for %IDKNB, %IDKWB and %WL was significantly low in Rambo treatment by December, followed by DE, ZeroFly and Botanical compared to untreated control with the highest mean values (1.42 ± 0.22; 1.07 ± 0.18 and 0.36 ± 0.07), respectively (Fig 1A–C). Furthermore, in December, mean %SG was significantly high in all treatments (>96%) compared to untreated control with significantly low value — 88.09% (Fig. 2). With respect to %DG from March to December, ANOVA result shows no significant interaction between month and treatment and treatment alone (P>0.05) while significant difference occured in %DG within the months (Tab. 1).

Percent number of insect damaged kernels (%IDKNB), weight of insect damaged kernels (%IDKWB), weight loss (%WL, seed germination (%SG), discolored grains (%DG), aflatoxin levels (AF) and proximate composition of maize preserved in Nigeria market with five treatments

In the study involving 5 treatments, mean values of %IDKNB, %IDKWB and %WL were significantly low by December in PICS treatment, followed by Rambo, DE, ZeroFly and Botanical compared to untreated control with the highest mean values (1.42 ± 0.22; 1.07 ± 0.18 and 0.36 ± 0.07), respectively (Fig 3A–C). Also in December, mean %SG was significantly high in all treatments (>96%) compared to untreated control with significantly low value — 88.09% (Fig. 4A); significantly low mean values of %DC was recorded in PICS (Fig. 4B); aflatoxin estimates in maize samples from all treatments and untreated control was below 2ppb (Fig. 4C): Proximate composition values was within recommended range for maize (Tab. 2; Fig 5A–C).

Tab. 1. ANOVA for main effects treatment and month, and interaction (*) for percent discolored grains (% DG).

Variable	Source	Four treatments			Five treatments		
		df	***F***	***P***	***df***	***F***	***P***
% DG	Treatment	4, 12	0.38	0.819	5, 18	3.06	0.036
	Month	7, 309	0.91	0.013	3, 198	93.98	<0.001
	*	28, 309	0.91	0.599	15, 198	2.21	0.007

Tab. 2. ANOVA for main effects treatment and month, and interaction (*) for maize proximate composition.

Variable	Source	Proximate Composition		
		df	*F*	*P*
Moisture	Treatment	5, 15	5.82	0.004
	Month	3, 174	222.84	<0.001
	*	15, 176	3.62	<0.001
Energy	Treatment	5, 15	5.50	0.005
	Month	3, 180	7.57	<0.001
	*	15, 181	1.71	0.052
Crude Fibre	Treatment	5, 15	0.23	0.943
	Month	3, 176	10.02	<0.001
	*	15, 176	1.94	0.023
Fat	Treatment	5, 18	2.42	0.076
	Month	3, 171	5.98	0.001
	*	15, 172	3.10	0.000
Ash	Treatment	5, 118	3.92	0.003
	Month	3, 205	7.83	<0.001
	*	15, 206	2.19	0.008
Protein	Treatment	5, 15.2	0.75	0.598
	Month	3, 254	26.86	<0.001
	*	15, 270	1.08	0.374
Carbohydrate	Treatment	5, 64.8	1.59	0.174
	Month	3, 260	28.40	<0.001
	*	15, 270	1.15	0.312

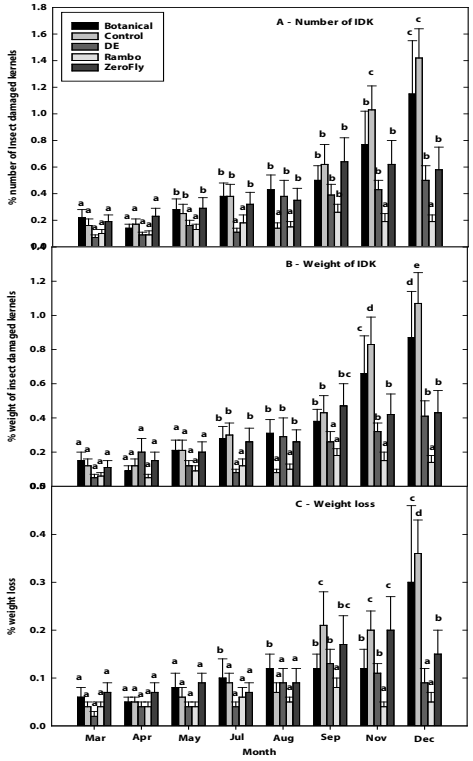

Fig. 1. Percent number of insect damaged kernels (A), percent weight of insect damaged kernels (B) and percent weight loss (C) of maize preserved with four treatments in Nigerian markets.

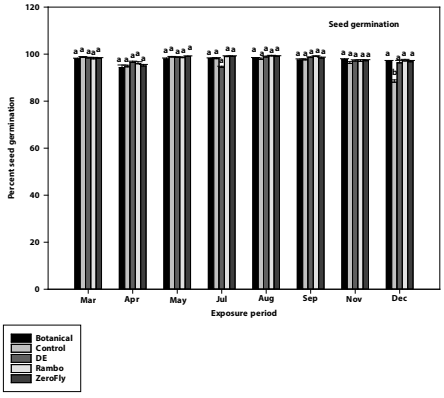

Fig. 2. Percent seed germination of maize preserved with four treatments in Nigerian markets.

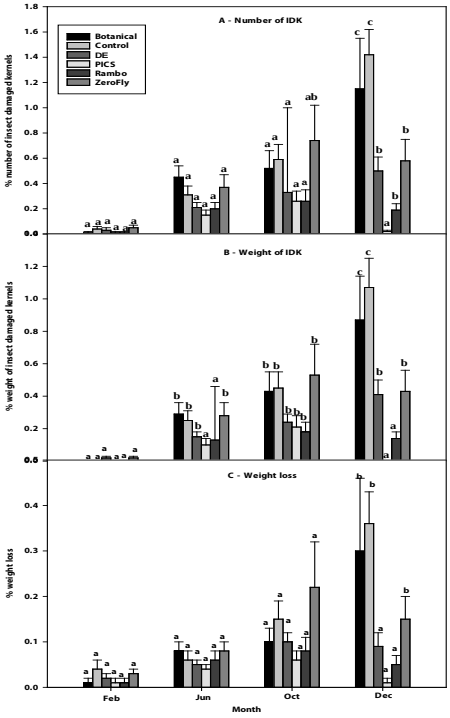

Fig. 3. Percent number of insect damaged kernels (A), percent weight of insect damaged kernels (B) and weight loss (C) of maize preserved with five treatments in Nigerian markets.

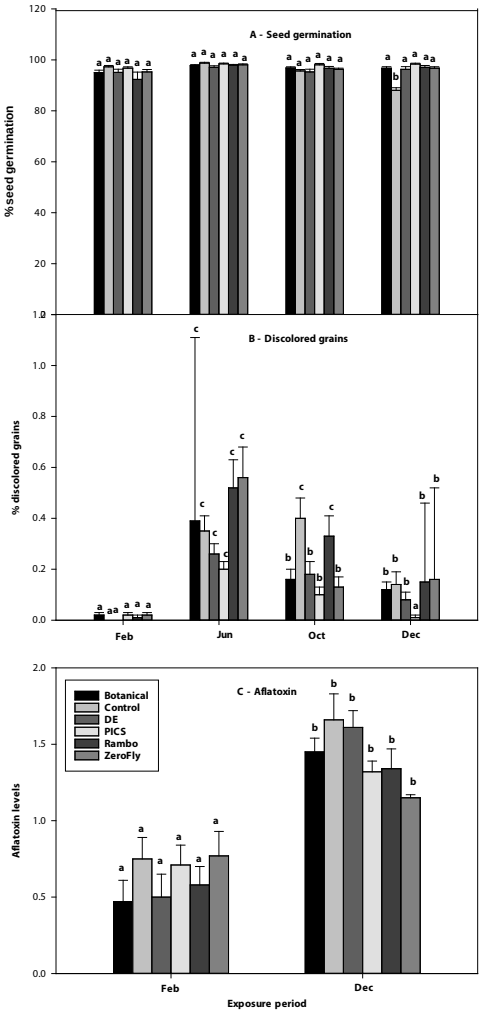

Fig. 4. Percent seed germination (A), percent discoloured grains (B) and mean aflatoxin (C) of maize preserved with five treatments in Nigerian markets.

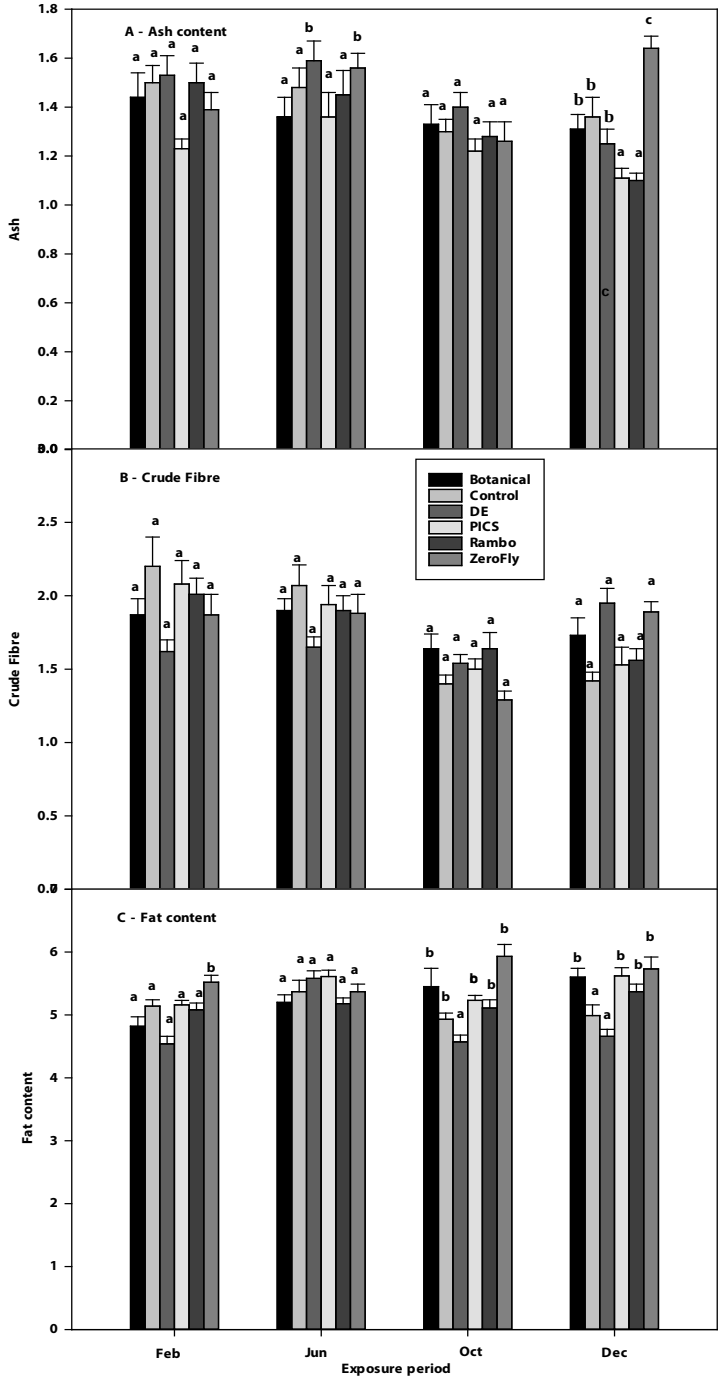

Fig. 5. Ash content (A), Crude fibre (B) and Fat content (C) of maize preserved with five treatments.

Discussion

Quality of stored maize is dependent on activities of associated insect pests. It is their feeding activity that brings about losses such as insect damaged kernels, loss in grain weight, reduced seed viability and mold growth amongst others. Grading is therefore based on these physical parameters (World Bank and FAO, 2011).

Our investigation showed that insect damaged kernels and weight loss tend to increase as month of storage increases. This could be attributed to increase in insect activities with length of storage time. This observation corroborates the report by Reed et al., (2007) that insect infestation can result, not only in grain damage as understood by shorter storage times, but can also affect the actual weight of the grain, leading to lower market prices and reduction in nutritional value of the grain.

In the five treatment study, PICS had the lowest % IDKNB, % IDKWB, weight loss and moisture content by December, after 11 months of storage (0.02, 0.00, 0.01 and 8.6%). This result confirmed earlier observations reported by Baoua et al., (2014) and Mutungi et al., (2014) that grain damage due to insect and grain weight loss is relatively lower in PICS compared to other tested grain bags because oxygen level has been depleted. High seed germination was also maintained.

In the four treatment study, Rambo treatment had the lowest % IDKNB, % IDKWB, weight loss and moisture content by December, after 11 months of storage (0.19, 0.14 and 0.05% respectively) compared with botanical, DE and ZeroFly. This could be attributed to the active ingredient Permethrin, which has been reported to affect insect nervous system by creating multiple potentials across the membrane and disrupt signal transmission in the insect (Bonny et al., 2014).

In both studies, ZeroFly bags had significantly lower values for percent IDK and weight loss with increasing storage period compared with untreated control. For example, by December in the study involving four treatments, IDKNB, IDKWB and weight loss for ZeroFly was 0.58, 0.43 and 0.15% compared to untreated control with 1.42, 1.07 and 0.36% respectively. This could be attributed to reduction in insect activities as reported by Paudyal et al., (2017) that ZeroFly bag can cause direct effects on knockdown and mortality and sub lethal effects such as reduced progeny production.

Other treatments like DE and botanical also affected insect activities in one way or the other, which invariably led to reduction in IDK, weight loss and maintained seed germination compared to untreated control. Rajapakse, 2006 reported that major action of plant powder against adult insect is through either fumigation or direct contact. Although storage of botanical treated maize in polythene lined bags could possibly improve its efficacy. Diatomaceous earth on the other hand, works by physical abrasion and adsorption of the epicuticular wax of insects by silica (Athanassiou and Steenberg, 2007; Otitodun et al., 2015).

Aflatoxin is one of the most common and important mycotoxins found in maize (Suleiman et al., 2013). Despite the fact that a significant increase was observed in aflatoxin levels from February through December, the values were all below detection limit of 5 ppb for maize meant for further processing (USDA-GIPSA, 2015).

The proximate composition results are in agreement with reports by some scientists. For example, in our study, the moisture content was observed to increase with month of storage; this consequently led to increase in both percent number and weight of IDK in control treatment. This observation corroborated the report by Child, (2007) that when a combination of favorable factors leads to increased insect development there is a co-related increase in the damage to materials through eating, despoiling, burrowing and other activities. Additionally, high moisture content leads to storage problems, respiration and reduction in germination (Suma et al., 2013). The percentage ash content observed in our study ranged between 1.10–1.59%. This is in agreement with the range of 0.70–2.50% reported from different maize hybrids in Nigeria (Keshun, 2009). The percentage protein recorded was found closely related to those reported on different maize varieties in Nigeria. In 2005, Ijabadeniyi and Adebolu reported protein content of three maize varieties grown in Nigeria within the range of 7.71–14.60%. Although some values were found a little higher and can be attributed to environmental factors. Yadav and Yadav, (2002) from their

investigation reported fat content from stored maize to range between 3.98–5.45%. This confirmed the fat content range of 4.54–5.73% recorded from all the treatments and control in our study to be adequate; samples from ZeroFly bags had the highest fat content (5.73%) by December. The crude fibre value of 1.29–2.20% obtained from all samples is in agreement with the report on stored maize by Aminogo and Oguntunde (2000) where they observed crude fibre to be in the range of 0.8–2.35%. Maize is known and reported to be high in carbohydrate and as such, it is a good source of calories (Nuss and Tanumihardjo, 2011). Carbohydrate content of the maize studied was found to be within the range of 72–73% and corroborate report on stored maize varieties by Mlay et al., (2005) and 69.67–74.55% as reported by Ullah et al., (2010).

In developing countries, especially in Nigeria, where maize production is mostly by low-resource and unskilled farmers, affordable and easy to use storage measures needs to be advocated to reduce postharvest losses. Based on results from our study, using better agricultural practices and adequate storage technologies can significantly reduce quality losses attributed to insect activities and help strengthen food security, poverty alleviation and increase returns to small holder farmers and aggregators. Therefore, use of easy to apply and reduced-risk storage technologies such as botanicals (*P. guineense*), diatomaceous earths (Bularafa DE), PICS bag, and ZeroFly bag are recommended to small and medium holder farmers and grain aggregators for storage of maize in Nigeria. However, further work would be required to access the duration of insecticidal action of botanical treated maize stored in polythene lined polypropylene bags.

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Evaluation of the suitability and optimal use of postharvest storage bag technologies and a combination thereof for maize storage in Nigeria.

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Abstract

The severity of postharvest losses varies considerably depending on storage method and prevalence of storage insect pests known to bore into storage bags. Polypropylene (PP) bags used by smallholder farmers in Nigeria do not provide effective protection for stored produce due to insect boring activities. Deltamethrin incorporated polypropylene, ZeroFly® (ZF) and ZeroFly® Hermetic Storage Bags are technologies with potential to improve protection of stored food commodities against insect attack. Therefore, a 12-month study was conducted in Ibadan, Nigeria to determine the suitability and potential of combined postharvest bag technologies involving ZeroFly® (hermetic and non-hermetic) bags for smallholder farmers when exposed to *Sitophilus zeamais* and *Prostephanus truncatus* infestation pressure. Cleaned but un-fumigated 50-kg lots of maize were used to fill bags in each of the following 8 treatments — PP and ZF bags alone, diatomaceous earth-treated maize in PP and ZF bags, single and double hermetic liners in ZF bags, single hermetic liner in PP bags and lastly PICS bags. Results obtained over a 12-month period showed infestation by *S. zeamais*, *Tribolium castaneum*, *Cryptolestes ferrugineus* and *Liposcelis* spp and abundance of insect increased with storage period in PP and ZF bags without liner. The percentages of insect damaged kernels by number (IDK) were higher in PP and ZF bags without liner and were 5.4 and 16.9%, respectively; in ZeroFly bags with hermetic liners, these values were ~ 0.5%. The PP and ZF bags without liner also had higher weight loss values of 1.4 and 6.7%, respectively compared with ZeroFly bags with hermetic liners and PICS bags which had a relatively lower weight loss of ≤0.2%. These results indicate that the ZeroFly Hermetic bag mitigates insect infestations, thereby offering a suitable alternative towards achieving significant reduction in postharvest losses during storage.

Keywords: maize storage, ZeroFly® Hermetic bag, smallholder farmer, postharvest loss, integrated pest management

1. Introduction

Maize (*Zea mays* L.) is the most widely cultivated staple food that plays a key role in the food security and economic well-being of sub-Saharan Africa (SSA) population (Abate *et al.,* 2017). With more than 5 million ha of land planted with maize annually, Nigeria is the second largest producer after South Africa, in the continent of Africa and produces nearly 8 million tons annually (Abate *et al.,* 2015). The bulk of this production in Nigeria is by smallholder farmers who face numerous challenges after their grains are harvested from the field (Abdoulaye *et al.,* 2016). Postharvest storage has been indicated as a major constraint in the maize sector in West Africa (Baoua *et al.,* 2014) and every year across sub-Saharan Africa, unacceptable levels of food loss continue to occur (Costa, 2014). These losses are dependent not only on the management practices of the farmers but also on environmental conditions and prevalence of post-harvest insect pests. The larger grain borer (LGB) (*Prostephanus truncatus* (Horn); Coleoptera: Bostrichidae) along with maize weevil (*Sitophilus zeamais* Motschulsky; Coleoptera: Curculionidae) are the major insect threats to stored maize in Africa (Holst, 2000). Losses due to post-harvest pests of maize are estimated to average between 20 and 30% after 3 months of storage (Boxall, 2002). For *S. zeamais* and *P. truncatus* infestations, losses

of 21.5% have been estimated after 6.5 months of storage in woven polypropylene bag (Baoua *et al.,* 2014). Currently, the control of stored grain insect pests is predominantly by the use of chemical insecticides including fumigants (Ceruti *et al.,* 2008); this is the case in Nigeria and worldwide. Storage pests are fast developing resistance to phosphine (Lee *et al.,* 2001). Alternative control methods to reduce insecticide persistence or toxic residues in food and pest resistance are being sought (Ileke and Oni, 2011) and have encouraged the return and development of inert dust formulations (Korunic, 1998). Treatment with diatomaceous earth (DE) dust is an efficient insect control technique in integrated pest management programs of stored grain (Ceruti *et al.,* 2008). As DEs are inert, they offer long term effectiveness, are safe for consumption, and do not adversely affect grain quality (Korunic *et al*., 1996). Polypropylene woven storage bags remain the conventional method of maize storage for smallholder farmers in Nigeria, which shows that bag technologies are culturally acceptable (Abdoulaye *et al.,* 2016). Due to the boring activities of insects into materials such as plastic films, adults of *P. truncatus* (Hodges, 1986; Ramirez Martinez and Silver, 1983) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) (Riudavets *et al.,* 2007) may find it easy to penetrate untreated bags used to protect stored grains and cause damaging infestations. Consequently, the use of insecticide-treated bags has been recognized as a possible alternative to direct treatment of grains (Kavallieratos *et al.,* 2017). The deltamethrin incorporated polypropylene (PP) bag, ZeroFly® Storage Bag, is a new technology used to reduce postharvest losses caused by stored-product insect pests. Additionally, the ZeroFly® Hermetic Storage Bag is a novel technology that protects grains and seeds stored in it against both external and internal insect attacks. The ZeroFly® Hermetic Storage Bag is made of an outer woven deltamethrin incorporated polypropylene bag and an 80-µm thick multilayered recyclable mixed polymer hermetic inner-liner with a gas barrier. The use of hermetic grain bags to preserve durable commodities such as grains is practiced in several countries (De Bruin *et al.,* 2012). Hermetic storage bags such as the Purdue Improved Crop Storage (PICS) and SuperGrain bags have been evaluated in previous studies and found to be effective technologies for protection of stored grains (Baoua *et al.,* 2013; William *et al.,* 2017).

Based on the information provided above, a 12-month study was conducted to investigate the suitability and optimal use of combined postharvest bag storage technologies (hermetic and non-hermetic) in the presence of external *S. zeamais* and *P. truncatus* infestation pressure. This study was conducted in a storehouse, located within Arisekola-Bodija market (7°25′59 N, 3°54′43 Ë) Ibadan, Oyo State, Nigeria during the period February 2017 to January 2018.

2. Materials and Methods

2.1. Maize

A yellow variety maize called 'Swan 2' sourced from a single local farm at the Ijaye Farm Settlement (07°42′13 N, 3°44′10 E) Ibadan, Oyo State was used in order to ensure uniformity of maize used for the study. The moisture content of the maize ranged between 11–13% based on measurements by the John Deere moisture meter (SW08120, Illinois, US).

2.2. Treatments

The maize used was cleaned and un-fumigated. Batches of 50 kg of maize were placed individually in bags according to the various treatments as follows: i) three polypropylene (PP) bags filled with untreated maize; ii) three PP bags filled with Insecto® (Insecto Natural Products, Costa Mesa, California, USA) diatomaceous earth–treated maize (hereafter this treatment is referred to as PPDE bags). The diatomaceous earth (DE) was admixed at a rate of 50 g per 50–kg bag of maize (i.e. at a rate of 0.1% w/w or 1,000 ppm) (Nwaubani *et al*., 2014); iii) nine triple layer PICS bags filled with untreated maize; iv) nine PP bags, each with a single hermetic liner (80 µm thick) filled with untreated maize (hereafter referred to as PP + 1 liner bag); v) three deltamethrin incorporated PP bags (ZeroFly® Storage bags) filled with untreated maize, hereafter referred to as ZF bags; vi) three

ZeroFly storage bags filled with Insecto® diatomaceous earth-treated maize filled (hereafter this treatment is referred to as ZFDE bags); vii) nine ZeroFly storage bags, with each having two PICS bag hermetic inner liners, filled with untreated maize (hereafter referred to as ZF + 2 liners bags) and viii) nine ZeroFly storage bags, with each having a single hermetic liner, filled with untreated maize (hereafter referred to as ZF + 1 liner bag).

2.3. Set up of treatments

For the different treatments there were three sub-replicates for each sampling event. Each stack of three or nine bags was placed on a wooden pallet (1.5 m x 1.5 m); pallets for the different treatments were a minimum distance of 2 meters apart from each another. Treatments involving ZeroFly storage bags were arranged on one side of the storehouse whereas PP bags of maize were set up on the opposite side to prevent ZF bags from influencing conditions immediately around PP bags. Altogether, there were forty-eight 50–kg bags of maize placed in the storehouse. Treatments in this study were not replicated but there are plans to conduct another two replicates in short order.

2.4. Storehouse infestation with Prostephanus truncatus and Sitophilus zeamais

Eight vials containing 100 g of maize each were infested with 20 unsexed, newly emerged adults of *P. truncatus* and *S. zeamais* separately in the laboratory and cultured for 1.5 months. The vials containing the adults and presumably the immature stages were placed in the storehouse, near pallets containing the eight treatments to create the required pest pressure on the storage bags. This procedure was repeated every 4 months.

2.5. Sampling and data collection

Bags of maize were sampled at the beginning of the study in February 2017. Monthly sampling was thereafter conducted on all 3 bags of each non-hermetic treatment (PP, PP + DE, ZF, and ZF + DE) from March 2017 to January 2018. For the hermetic treatments, three bags from each of the nine bags were randomly selected and destructively sampled after 4, 8 and 12 months — these treatments were PICS, PP + 1 liner, ZF + 1 liner, and ZF + 2 liners.

2.5.1. Moisture content

Moisture content in each bag sampled was estimated using four moisture measurement methods namely; i) a low cost moisture meter by the USDA-ARS Center for Grain and Animal Health Research, Manhattan KS, referred to in this study as the PHL meter, ii) a GIPSA-approved method (GAC 2100 Agri, DICKEY-john Corp., Auburn, IL.), iii) a commercial meter, the John Deere Moisture Chek PLUS, model SW08120 (AgraTronix Streestboro, Ohio), and iv) a standard oven-dry test method (ASABE standards). The average of three different readings with each meter, from each bag was calculated.

2.5.2. Grain sampling

A brass grain probe (1.2-m open-ended trier) (Seedburo Equipment, Chicago, IL) was used to sample maize and a composite sample of ~ 350 g was taken from each bag and placed in a labeled Ziploc plastic bag for analyses. For each of the samples collected, data on number of insects of each species, percentage number and weight of insect damaged kernels (IDK), weight loss, seed germinability were taken. All maize samples collected were analyzed at Entomology Research Laboratory, Department of Zoology, University of Ibadan, Nigeria. The samples were sifted using a U.S. Standard #10 sieve (2 mm openings) (Seedburo Equipment, Chicago IL) on to a tray to recover both live and or dead insects. The species of insects were identified using a tripod magnifying lens and their numbers were recorded. A 250–g sub-sample was poured on a tray and kernels were examined visually. Kernels with insect exit holes or perforations were separated from undamaged kernels and the numbers in each category were recorded. Percentage of insect damaged kernels

(IDK) by numerical basis (IDKnb) and by weight basis (IDKwb) were calculated per 250-g sample (FAO): the method of Quitco and Quindoza (1986) was used:

Percentage IDK (nb) = $\frac{Nd}{Total\ grain\ count} \times 100$; Percentage IDK (wb) = $\frac{Wd}{250} \times 100$

Where, Nd = Number damaged grain, Wd = Weight of damaged grain

2.5.3. Weight loss (%)

Weight loss due to insect damage was determined using the "count and weigh" method (Gwimmer *et al.,* 1996) and calculated as: % Weight loss = $\frac{[(Wu \times Nd) - (Wd \times Nu)]}{Wu \times (Nd + Nu)} \times 100$

Where, Wu is the weight of undamaged kernels, Nu is the number of undamaged kernels, Wd is the weight of damaged kernel and Nd is the number damaged.

2.5.4**.** Seed germinability (%)

Germination tests were conducted using the method of Baoua *et al.* (2014) with little modification — from each 250–g maize sub-sample previously referred to above, one hundred seeds were picked and 25 seeds were then placed in each of four petri-dishes that had moistened cotton sheets at their bases. The seeds were re-wetted and percent germination was determined after 5–7 days based on the number of seeds that sprouted.

Data were summarized using the SPSS version 20 software to evaluate means of the various response variables.

3. Results

The lack of replication in this study means analysis of variance (ANOVA) could not be conducted. Therefore, information presented in the results section describes only numerical differences between means based on three sub-replicates of each treatment, for each sampling event and not statistical differences. Information in this section also highlights notable patterns in the various response variables that were investigated.

3.1. Moisture content

There was a ≤4% offset in the MC measure by the PHL, JD and GAC2100 meter compared to the oven-dry reference test. MC measurement for the PHL meter ranged from 11–15%; JD meter ranged from 12.5–15.3%; Oven-dry test ranged from 10.6–13% and GAC 2100 meter ranged from 11–14.4% based on data collected during the 12 months of the study, in all the treatments.

3.2. Insect infestation level

3.2.1. Sitophilus zeamais

The pattern for numbers of *S. zeamais* found in the different treatments during the study are shown in Figs. 1 and 2. Live weevil population grew progressively in the PP bags but grew more in the ZF bags after 5 months of storage (during and after June). The number of live *S. zeamais* was higher in ZF bags where 124 insects were found in October but this number decreased to 52 in January (Fig. 1A). There were no live adult *S. zeamais* in the PPDE and ZFDE samples during the first eight months of the study. In the ZFDE treatment, numbers of live *S. zeamais* did not increase substantially during the entire storage period. In the case of PPDE treatment, numbers of live weevil in bags did not exceed 2 during the period February to October, but the number increased numerically to 31 at the end of the experiment in January. On the other hand, the numbers of dead *S. zeamais* were numerically higher in the ZF bags than in the other three treatments except in October where more dead insects were found in PP bags (Fig. 1B). An average of 33 dead weevils per sample were found in ZF bags at the end of 12 months of storage, in January 2018 (Fig. 1B).

For the hermetic treatments, very low numbers of *S. zeamais* were found and did not exceed a mean of 4 in any of the four treatments (Fig. 2A). In the PICS bags, 3.7 live *S. zeamais* per sample were found after 12 months of storage (Fig. 2A). The mean number of dead *S. zeamais* in all the hermetic treatments during the 12 months of storage was ≤1 (Fig. 2B).

3.2.2. Tribolium castaneum

The pattern for numbers of *T. castaneum* found are shown in Figs. 3 and 4. In the PP treatment, the numbers of live *T. castaneum* were much higher during the period October to January (Fig. 3A). The numbers of live *T. castaneum* were lower in the PPDE, ZF and ZFDE bags in all the storage periods and did not exceed the highest number found of 4.3 insects per sample. The mean number of live insects per sample throughout the storage period was 4 in PPDE treatment whereas live insect was below 1 in ZF and ZFDE treatments, respectively (Fig. 3A). Expectedly, higher numbers of dead insects were found in PP bags during all the storage periods (Fig. 3B).

For the hermetic treatments, very low numbers of *T. castaneum* were found and did not exceed a mean of 6 in any of the four treatments (Fig. 4A). In the PICS bags, the number of live *T. castaneum* increased from 3.7 in September to 6 per sample in January (Fig. 4A). The mean number of dead *T. castaneum* in all the hermetic treatments during the 12 months of storage was 4 (Fig. 4B).

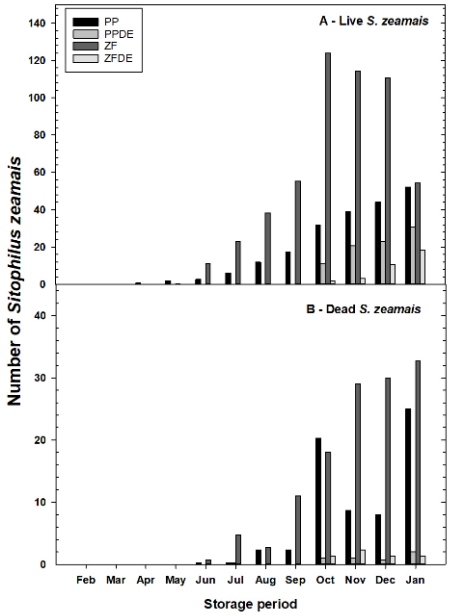

Fig. 1: Number (mean) of live (A) and dead (B) *Sitophilus zeamais* per 250 g of maize kernels in the non-hermetic treatments (PP, PPDE, ZF and ZFDE) sampled at monthly intervals over 12 months.

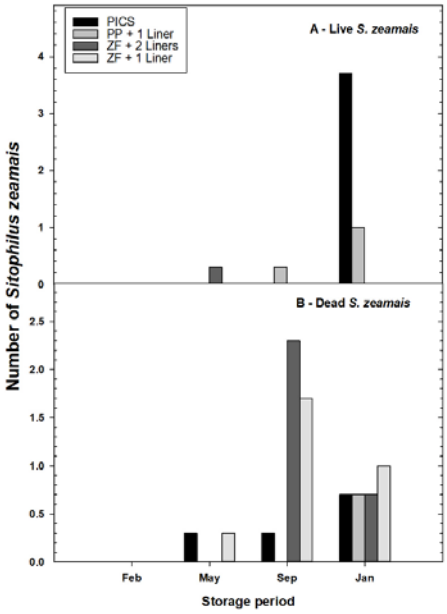

Fig. 2: Number (mean) of live (A) and dead (B) *Sitophilus zeamais* per 250 g of maize kernels in the hermetic treatments (PICS, PP + 1 Liner, ZF + 2 Liners and ZF + 1 Liner) sampled every four months.

3.2.3. Cryptolestes ferrugineus

The pattern for number of *C. ferrugineus* in non-hermetic treatments is shown in Fig. 5. There were no live *C. ferrugineus* from samples in all the four treatments during the first 5 months of storage in May. During the period August to January, more insects were found in the ZF treatment than in the other three treatments, and the number increased markedly from 47 in November to 69 in January (Fig. 5A). In contrast, there was no live or dead *C. ferrugineus* in all the different hermetic treatments during the entire storage period.

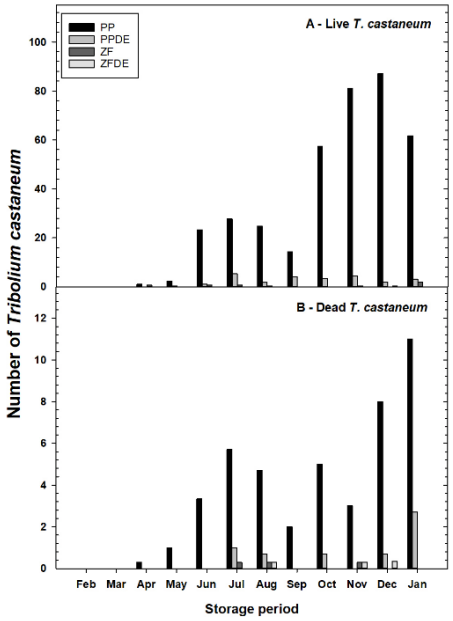

Fig. 3: Number (mean) of live (A) and dead (B) *Tribolium castaneum* per 250 g of maize kernels in the non-hermetic treatments (PP, PPDE, ZF and ZFDE) sampled at monthly intervals over 12 months.

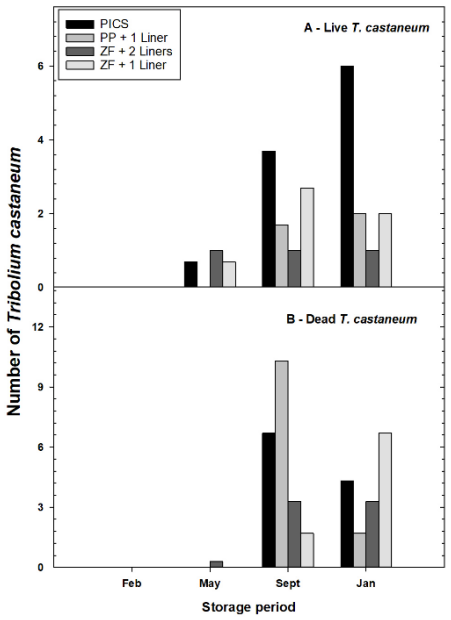

Fig. 4: Number (mean) of live (A) and dead (B) *Tribolium castaneum* per 250 g of maize kernels in the hermetic treatments (PICS, PP + 1 Liner, ZF + 2 Liners and ZF + 1 Liner) sampled every four months.

3.2.4. *Liposcelis* spp.

The pattern for numbers of *Liposcelis* spp. found in the different non-hermetic treatments is shown in Fig. 6. Lower numbers of live *Liposcelis* spp. were found in the PP treatment during the entire storage periods. Among the other three treatments, the ZFDE treatment had the highest number of live insects in July (29) but this decreased to 8 in December (Fig. 6A). *Liposcelis* spp. were not found in all the hermetic treatments.

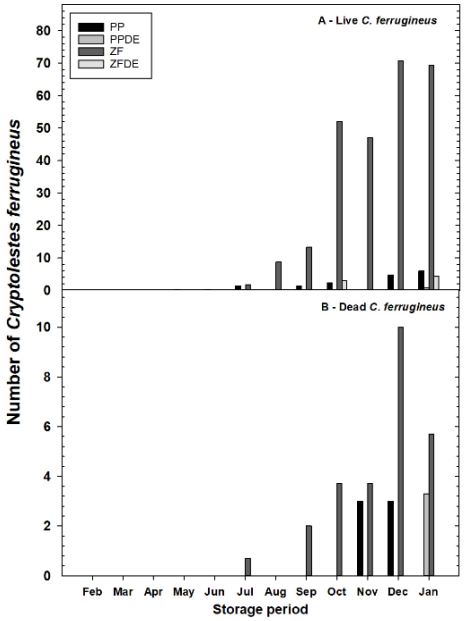

Fig. 5: Number (mean) of live (A) and dead (B) *Cryptolestes ferrugineus* per 250 g of maize kernels in the non-hermetic treatments (PP, PPDE, ZF and ZFDE) sampled at monthly intervals over 12 months.

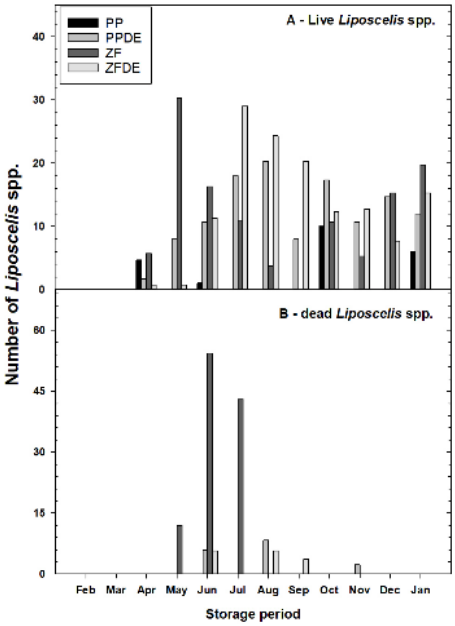

Fig. 6: Number (mean) of live (A) and dead (B) *Liposcelis* spp. per 250 g of maize kernels in the non-hermetic treatments (PP, PPDE, ZF and ZFDE) sampled at monthly intervals over 12 months.

3.3. Percent Insect Damaged Kernels (% IDK)

The patterns for % IDK, by both numerical basis (IDKnb) and weight basis (IDKwb), are shown in Figs. 7 and 8. In the ZF treatment, IDKnb markedly increased from 0.2% in March to 16.9% in January (Fig. 7A). In the case of PP treatment, there was consistent increase in IDKnb from 0.4% per 250 g maize sample in May to 5.4% in January. In the PPDE and ZFDE treatments, IDKnb did not exceed 1.9 and 0.8%, respectively, over 12 months of storage (Fig. 7A). The IDKwb values were highest in the ZF treatment followed by PP treatment, and were 10.9% and 5.3%, respectively, at the end of storage in January 2018 (Fig. 7B).

For the hermetic treatments, IDKnb and IDKwb values were below 1% in all the four treatments (Fig. 8). With the exception of May, generally, ZF + 2 liners and ZF + 1 liner treatments had the lowest IDKnb and IDKwb values (Fig. 8A).

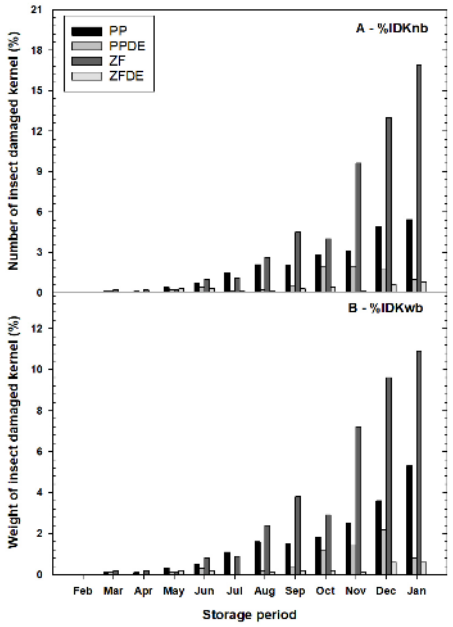

Fig. 7: Percentage number (A) and weight of insect damaged kernels (IDK) (B) (mean) per 250 g of maize kernels in the non-hermetic treatments (PP, PPDE, ZF and ZFDE) sampled at monthly intervals over 12 months.

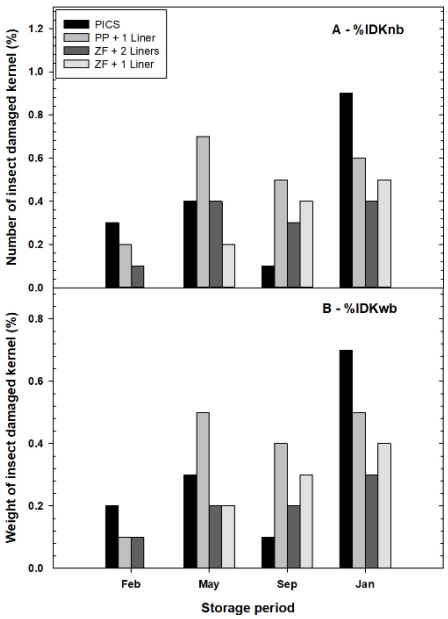

Fig. 8: Percentage number (A) and weight of insect damaged kernels (IDK) (B) (mean) per 250 g of maize kernels in the hermetic treatments (PICS, PP + 1 Liner, ZF + 2 Liners and ZF + 1 Liner) sampled every four month

3.4 Grain weight loss

The general trend from July 2017 to January 2018 was for percentage weight loss to be highest in the ZF treatment followed by PP treatment (Fig. 9). In the ZF treatment, percentage weight loss increased substantially from 0.2% in August to 6.7% in January (Fig. 9). In the PP treatment, weight loss did not exceed 0.5% after 8 months of storage in September but increased to 1.4% at the end of the study in January (Fig. 9). Low weight loss values were found in the hermetic treatments with PP + 1 liner, ZF + 2 liners and ZF + 1 liner bags mostly having a low value of 0.1% and loss did not exceed 0.2% on average in the PICS treatment (Fig. 10).

3.5 Seed germination (%)

The initial mean germination rate for all the treatments (hermetic and non-hermetic) was 97.5% (Figs. 11and 12). At the end of storage period, germination rates in ZF (87%) and PP (91.3%) were relatively the lowest compared to other treatments (Fig. 11). After 12 months, mean germination rates of samples collected from the hermetic treatments was similar to that obtained at the start of experiment (97%) (Fig. 12).

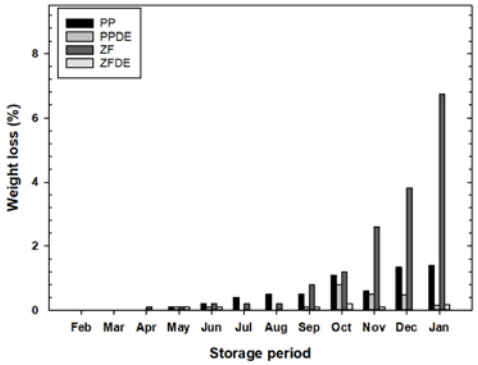

Fig. 9: Percentage weight loss (mean ± SE) per 250 g of maize kernels in the non-hermetic treatments (PP, PPDE, ZF and ZFDE) sampled at monthly intervals over 12 months.

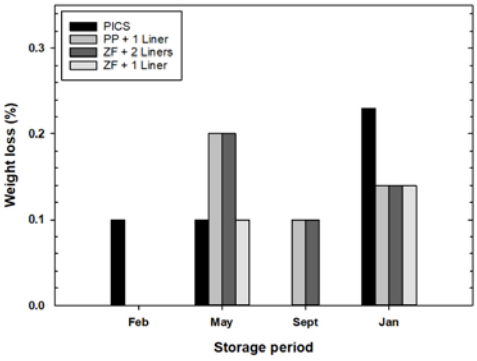

Fig. 10: Percentage weight loss (mean ± SE) per 250 g of maize kernels in the hermetic treatments (PICS, PP + 1 Liner, ZF + 2 Liners and ZF + 1 Liner) sampled every four month

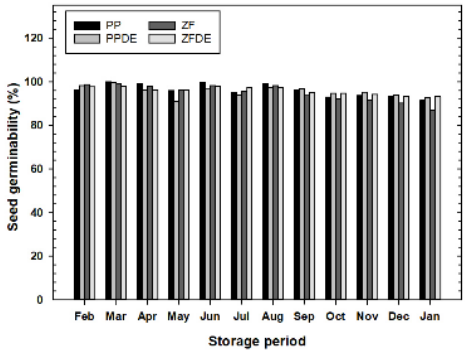

Fig. 11: Percentage seed germinability (mean) per 250 g of maize kernels in the non-hermetic treatments (PP, PPDE, ZF and ZFDE) sampled at monthly intervals over 12 months.

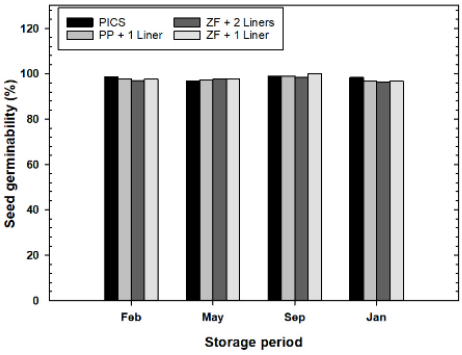

Fig. 12: Percentage seed germinability (mean) per 250 g of maize kernels in the hermetic treatments (PICS, PP + 1 Liner, ZF + 2 Liners and ZF + 1 Liner) sampled every four months

4. Discussion

Among the non-hermetic treatments, our data revealed that PPDE and ZFDE treatments were effective in reducing insect infestation and damage levels compared to the control treatment (untreated maize in PP bags). Maize treated with Insecto DE provided better protection for stored maize from *S. zeamais* infestation for up to 8 months during storage. In the PPDE and ZFDE treatments, mean weight losses of ≤1% and ≤0.2% respectively were observed after the 12-month storage period. Other studies have shown that stored grain insect pests can be controlled by commercially available DE formulations (Ashraf *et al.,* 2016). In the non-hermetic and no DE treatment, *S. zeamais* was the major damaging pest resulting in high IDK levels of 5.4% and16.9% after 12 months of storage in PP and ZF bags, respectively. The ZF treatment (untreated maize in ZeroFly bags) was effective for up to 4 months of storage (June), but thereafter, levels of insects such as *S. zeamais* and *C. ferrugineus* increased markedly. Data obtained from this study correspond to those of (Paudyal *et al*., 2017) who found high insect infestation levels in ZeroFly bags after 4 months of storage. The high level of infestation in ZeroFly bags after 4 months of storage may be due to pre-infestation of maize during bagging by insects at the egg or larval stage — maize used in this study was un-fumigated. Given that the newly-harvested grains can be infested after storage (Vela Coiffier *et al.,* 1997; Hagstrum, 2001), it is recommended by the manufacturer of ZeroFly storage bag that

the grains be pre-fumigated because the bags are designed to give protection to commodities by preventing the entry of insect pests, thereby facilitating preservation of cereal grains and grain legumes. Additionally, as a result of the *S. zeamais* infestation pressure in the storehouse, it is possible that the repeated sealing and unsealing of the ZeroFly bags during grain sampling might have compromised the deltamethrin barrier and consequently allowed easy access of insects into the bags (Paudyal *et al*., 2017). In this study, *S. zeamais* was the major damaging pest eventually causing weight loss of 1.4% and 6.7% after 12 months of storage in PP and ZF bags, respectively (January 2018 weight losses). Comparatively, in all the hermetic treatments, insect infestation was effectively suppressed resulting in fewer insect damaged kernels and lower weight losses due to the low oxygen environment created by the hermetic technologies. Also few live insects were found in maize treated with DE dust throughout the storage period, this may be a result of the killing action or repellency of the diatomaceous earth.

One of the main purposes of storing grain is to ensure seed availability and viability, both of which are important to farmers. In all the eight treatments, germination rates of maize stored in all the hermetic treatments for 12 months were similar to that observed at the beginning of the study. This is consistent with the data obtained by Yakubu (2012) who stored maize hermetically for a period of one year and concluded that the hermetic conditions contribute to preservation of seed quality. The lowered oxygen concentration within the bags thus does not appear to affect the viability of maize seed (Baoua *et al.,* 2014). On the other hand, germination rates of seeds in the PP and ZF bags were greatly reduced at the end of the 12-month storage period. This is most likely a result of higher insect infestation.

Conclusion

The suitability and performance of hermetic storage bag technologies used in this study in mitigating insect infestation and preserving maize was much better than the non-hermetic methods over the 12-month storage period. Based on data from this study, hermetic storage technologies are effective and need to be more widely adopted for improved food quality and security.

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Insecticide treated packaging for the control of stored product insects

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Abstract

Improper or poor post-harvest handling and storage of stored grains contributes significantly to product loss, and bagged stored grain presents an option for safe storage and handling. Bagged grain is intended to maintain quality and safety, while protecting it from infestations. The objective of this research was to determine the effect of deltamethrin-treated packaging material on adults and larvae of common stored product pests. Adults or larvae of several species of stored product insects were exposed to deltamethrin-treated packaging for time intervals ranging from 1 h to 4 weeks. The percentage of affected*Prostephanus truncatus, Callosobruchus maculatus* and *Rhyzopertha dominica* adults was < 98% after 60 minutes of exposure to treated packaging. Mortality of adult *Trogoderma granarium* was about 33% after 1 day of exposure, and increased to 93% after 7 day of exposure. Direct mortality of *T. granarium* larvae exposed to the deltamethrin-treated packaging for 8 h was about 15%, but increased to 50% when larvae were exposed for 72 h.*Tribolium castaneum, Oryzaephilus surinamensis,* and *Trogoderma inclusum* larvae continually exposed to the deltamethrin-treated packaging resulted in > 96% larval death within 1-2 weeks.The major primary stored product insects were highly susceptible to the deltamethrin-treated storage bags, but there was variation in susceptibility between species

and life stages tested. The deltamethrin-treated storage bags can offer protection of bagged grains and be used as a preventative measure to reduce infestations during storage.

Keywords: treated packaging, deltamethrin, stored product insects, affected adults

1. Introduction

Stored product insects are a common and persistent problem in grain storage, milling, and, warehouse facilities. *Rhyzopertha dominica* F., lesser grain borer, *Callosobruchus maculatus* F., cowpea weevil, *Tribolium castaneum* (Herbst), red flour beetle, *Prostephanus truncatus* (Horn), larger grain borer, and *Trogoderma spp.* are major stored product species found or associated with stored grains (Rees, 2004; Hagstrum et al., 2012). The improper handling and storage of grains impacts the quantitative and qualitative attributes of stored grain. Traditional integrated pest management (IPM) techniques depend on a series of management evaluations, decisions, and controls in order to provide the best control method (US-EPA, 2017). For IPM of food materials, prevention is one of the most important IPM strategies.

In recent years, use of insecticide-treated packaging has gained interest and proven effective against stored product insects. Use of insecticide-treated packaging can be easily incorporated into existing IPM programs and functions as aprevention techniquefor finished product. Previous research in the area of insecticide treated packaging has focused on the insect growth regulator methoprene and the contact insecticide deltamethrin. Both insecticide treated packaging materials are highly effective against a variety of stored product insects such as *T. castaneum, P. truncatus, Sitophilus* spp., and *R. dominica*. (Kavallieratos et al., 2017; Paudyal et al., 2017a, 2017b; Scheff et al., 2016, 2017). However, there is still a need for research examining the effects of treated packaging materials on more pest species, including evaluations against different life stages, and more information on the impact of exposure duration to better understand the effectiveness of this pest management tool. The objective of this research was to determine the effect of deltamethrin-treated packaging material on adultsand larvae of common stored product pests.

2. Materials and Methods

Experiments with larvae and adult*Trogoderma granarium*Everts, Khapra beetle, were conducted at the USDA-Animal and Plant Health Inspection Service (APHIS)-Center for Plant Health Science and Technology (CPHST), Otis Laboratory, in Buzzards Bay, MA, USA, in the insect quarantine facility. All other insect species were evaluated in experiments conducted at the USDA-Agricultural Research Service (ARS)-Center for Grain and Animal Health Research in Manhattan, Kansas, USA.

Deltamethrin-treated woven polypropylene storage bag material (ZeroFly® Storage bags) was used in the experiments (Vetergaard Frandsen, Lausanne, Switzerland). The deltamethrin concentration of the treated storage bags was 3g/kg or 3000 ppm (Vestergaard, 2015). Untreated control material consisted of a laminated woven packaging material containing no insecticide, as described by Scheff and Arthur (2017). Bioassay arenas used in all experiments were created as described by Kavallieratos et al. (2017) and Scheff and Arthur (2017). In brief, packaging material was cut into ~9 cm circles and affixed to thebottom of 100 x 20 mm plastic Petri dishesusing adhesive caulking. The interior sides of the Petri dishes were coated with Fluon® (polytetrafluoroethylene, Sigma-Aldrich Co., St. Louis, MO, USA) to confine insects to the bottom of the dish.

2.1 Adults

Cohorts of 10 adults of *P. truncatus, C. maculatus*, and *R. dominica* were exposed to treated packaging and monitored every 15 minutes for up to 4 h, and the time until 100% of adults were observed as being affected was determined. Affected insects were those where the adults could not remain upright and exhibited uncoordinated movement for five or more seconds, were turned on their backs and displayed loss of appendage control and tremors, or only exhibited movement in legs, antennae or mouthparts when probed by a fine-hair brush. Additional testing on mortality of adults exposed to deltamethrin-treated material is currently being conducted. Adults of *T.*

granarium were continually exposed to treated or untreated packaging materialas described for *P. truncatus, C. maculatus,* and *R. dominica*, and observed after 1, 2, 5 and 7 daysof exposure on the packaging material to determine the percentage of affected and dead adults at each time. The time to 100% of adults that were affected in 15 minute increments is currently being conducted.

2.2. Larvae

The effects of deltamethrin-treated packaging material on larvae was conducted as either continually exposing larvae on treated or untreated surface (continual exposure assay) or a short term exposure period of ≤4 days on treated or untreated material (short-term assay).

The continual exposure assays were conducted for *T. granarium, Trogoderma inclusum* LeConte, larger cabinet beetle, *T. castaneum* and *Oryzaephilus surinamensis* (L.), sawtoothed grain beetle. Ten individual larvae were added to deltamethrin-treated or untreated arenas along with ~1 g of diet and continually held on each area and monitored for adult emergence, up to 8 weeks. Six replicates were used. Short-term exposures were conducted for *T. castaneum, T. granarium,* and*T. inclusum* as described by Scheff et al. (2017). Briefly, 50 larvae of each species, six replicates, were exposed to deltamethrin-treated or untreated packaging material, and ten larvae were removed from the arenas after 0.3, 1, 2, 3, and 4 days and placed onto an untreated plastic Petri dish along with ~1 g of diet and held for adult emergence for 8 weeks. Two larval sizes for *T. granarium* and *T. inclusum* were used. Small larvae were <3 mm and largelarvae were >4 mm (Anthanassiou et al., 2015).

3. Results

3.1 Adults

The time requiredfor 100% adults tobe affected varied among species tested. The order of susceptibility was *P. truncatus* >*R. dominica* >*C. maculatus.* All adult*P. truncatus* were affected after 30 m of exposure to the deltamethrin-treated packaging material, while only 83% of*R. dominica* and 30 % of *C. maculatus* were affected.*R. dominica* needed 45 minutes of exposure for 100% of adults to be affected and 75 minutes of exposure was needed for*C. maculatus.*
There was 100% affected or dead adult *T. granarium* after 1 day of exposure to deltamethrin-treated packaging and the percentage of mortality increased with longer exposure periods as the affected adult *T. granarium* succumbed to the effects of the deltamethrin. After 7 dayd of continual exposure, 93% of adults were dead.

3.2 Larvae

Continual exposure of *T. granarium* larvae to treated packaging material resulted in approximately95 and 97% mortality for small (<3 mm) and large (> 4mm) larvae, respectively, while on untreated material mortality was <4% for both larvae sizes. Likewise, *T. castaneum* had <7% adult emergence and *O. surinamensis*had 0% adult emergence when continually exposed at two different temperatures, 27 or 32°C, and all the larvae died. Continual exposure of *T. inclusum* larvae to treated packaging material resulted in 10% adult emergence for large larvae held at 32°C, and <3% for small larvae at 27 or 32°C and large larvae at 27°C. Larval death ranged from 65-100% among small and large larvae held at 27 or 32°C. However, roughly 25% of large *T. inclusum*larvae held at 32°C were alive after 8 weeks of continual exposure and 22% of small larvae at 27°C were still alive.
Short-term exposure of large*T. granarium* larvae, > 4mm, resulted in adult emergence ranging from 61-34% among the exposure periods. Larval death ranged from 15-50% depending on exposure time. As exposure time to deltamethrin-treated packaging material increased, the percentage of emerged adults decreased and the most effective exposure period was 3 days. We observed a significant reduction in adult emergence for *T. castaneum* larvae exposed to the deltamethrin-treated material for >3 days at 32°C. Adult emergence was 62% after 3 days of exposure and 50% after 4 days of exposure to treated material. Similar to *T. granarium,* the precentage of small *T. inclusum* larvae, < 3 mm, exposed to deltamethin-treated material at 32°C that emerged as adults

was reduced as exposure time increased. After 0.33 days of exposure, 72% of larvae emerged as adults but only 42% of larvae emerged as adults after 4 days of expsoure to treated material.

4. Discussion

Results of this study show there is variationin the susceptibility among life stages andstored product insectspecies, and a strong effect of exposure duration on susceptibility and lethality. We first observed that exposure to the deltamethrin-treated packaging material yielded affected adult stored product insects within 60 minutes. The exposure time required to yield affected adults reported here was similar to previous research by Kavallieratos et al. (2017), in which the order of susceptibility based on knockdown and mortality during 5 dayexposures to deltamethrin-treated material was*T. variabile* >*P. truncatus* > *R. dominica* >*T. castaneum*. Kavallieratos et al. (2017) found out that all *P. truncatus* and *R. dominica*were knocked down after 60 minutes of exposure. In our experiements, we were able to deterime time to knockdown or affected, was less than 60 minutes for both species.

Larvae of several different stored product insects showed susceptibility to treated packaging. Among all species tested, adult emergence was <10% and larval death was 100% for *O. surinamensis* and large *T. inclusum* larvae at 32°C. Scheff et al. (2016, 2017) observed differences in susceptibility to methoprene-treated packagaing between *T. castaneum* and *T. variabile* aftercontinual exposure for egg-to-adult development. The differences observed in the previous studies and the current study could be due to physiological differences between species and pubescenceof larvae. *T. inclusum* and *T. granarium* larvae are covered in fine hairs, which could reduce the precentage of body surface exposed to the treated packaging. Scheff et al. (2017) also observed longer exposure periods to methoprene-treated packaging material, decreased the percentage of normal adult emergence from exposed larvae of*T. castaneum* and *T. variabile.* We observed similar results for *T. granarium, T. castaneum,* and *T. inclusum* when exposed to deltamethrin.

Our study demonstrated that some major primary stored product insects were highly susceptible to the deltamethrin-treated storage bags among various life stages. The use of these deltamethrin-treated storage bags can offer protection of bagged grains and be used as a tool in the integrated pest management approach to stored grains.

Acknowledgement

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The US Department of Agriculture is an equal opportunity provider and employer.

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Field studies with insecticide treated packaging for the control of stored product insects

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Abstract

Food Security is an issue that will impact everyone by 2050 it is projected there will be a global crisis unless action is taken. The ZeroFly® Storage Bag is a new combination of key technologies developed to reduce post-harvest losses. It contains an insecticide, Deltamethrin that is incorporated within the polypropylene yarns woven into a storage bag. The level of insecticide residue found on grains stored for up to two years in ZeroFly® Storage Bag are below CODEX & EPA maximum residue levels. This technology can be combined with natural rodent repellent compounds and the multilayer hermetic liners, meaning these bags can adhere to and improve on currently accepted practices and requires limited behavior change for the user. Studies show that the ZeroFly® Storage Bag can effectively control key stored product insects. The presentation will explore the current scale-up efforts and strategies of distribution planned throughout Africa and Asia, this would also include an assessment of the broader impact of ensuring the most appropriate combinations of technologies reach the most vulnerable groups.

On-Farm Comparison of Different Postharvest Storage Technologies for effectiveness in pest management in a Maize Farming System of Tanzania Central Corridor

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Abstract

Seven methods for storing maize were compared with traditional practice of storing maize in polypropylene bags. Twenty farmers managed the experiment under their prevailing conditions for 30 weeks. Stored grain was assessed for damage every six weeks. The dominant storage insect pests identified were the Maize weevil (*Sitophilus zeamais*) and the Red flour beetle (*Tribolium castaneum*). There was no significant difference ($F = 87.09$; $P < 0.0001$) in insect control and grain damage between hermetic storage and fumigation with insecticides. However, the insecticide treated polypropylene yarn (ZeroFly®) did not control insect infestation of grain for the experimental period under farmers' management. Grain damage was significantly lower in hermetic storage and fumigated grain than ZeroFly® and polypropylene bags without fumigation. No significant difference in grain damage was found between airtight treatment alone and when combined with the use of

insecticides. During storage, *S. zeamais* was predominant and could be of more economic importance than *T. castaneum* as far as maize damage is concerned. Even though ZeroFly®, and polypropylene bags without grain treatment did not control storage pests, farmers still prefered this cheap technology. Hermetic storage techniques can be recommended to farmers without the use of insecticides provided they are inexpensive, and the proper application of technologies is ensured.

Key words: Maize Farmers; Hermetic storage; Grain damage; Food loss; Insect damage

1. Introduction

Maize is one of the crops most severely affected by Post harvest Losses (FAO, 1998; Abass et al., 2014). Major losses of stored maize are caused by insect pests especially the larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), the Red flour beetle (*Tribolium castaneum*), and the Maize weevil (*Sitophilus zeamais* Motschulsky) (Coleoptera: Curculionidae) (Golob and Hanks, 1990). If the grain is dried to an appropriate moisture level of 12–13% storage insects can be controlled effectively with fumigants such as Phostoxin (Hodges, 1986). In Tanzania, farmers are allowed to use Phostoxin if supervised by authorized extension agents, but the effectiveness of such arrangements at the community level is yet to be ascertained. Farmers widely use a mixture of Pirimiphos-methyl (Actellic) and Permethrin, commercially sold as Actellic Super (local name: *Shumba*) but farmers are often unable to verify the genuineness of some local brands.

More recently hermetically sealed containers are being promoted in Africa to control storage insect pests, based on the oxygen depletion mechanism that rapidly occurs in the containers, causing an increase in CO_2 concentration and death of the pests (Yakubu et al., 2011; Murdock et al., 2012; Baoua et al., 2013; de Groote et al., 2013; Moussa et al., 2014, Chigoverah and Mvumi, 2016; Likhayo et al., 2016; Midega et al., 2016). Metal silos, plastic barrels and flexible hermetic storage systems, such as Purdue Improved Crop Storage (PICS) bags, super grain bags (SGB), Zerofly bags, cocoons, and others, are being tested to control storage insect pests in different African countries (Quezada et al., 2006; Phiri and Otieno, 2008; Baoua et al., 2013, 2014; Jones et al., 2014). However, the potential adaptability of the technologies and their acceptance by farmers as alternatives to the use of insecticides is required. This study was conducted in Tanzania to determine the relative effectiveness of different hermetic storage materials under actual on-farm conditions and farmers' management practices and elucidate sociocultural evidence on their acceptability.

2. Materials and Methods

2.1 Description of the experimental sites

Twenty farmers in four villages located in three agro-ecologies (Southern Guinea Savannah, Northern Guinea Savannah, and Semi-arid Sudan Savannah) within two regions of Tanzania (Dodoma and Manyara) were involved in the experiment. The relative humidity (H_{in}), and temperature (T_{in}) inside the storage facilities were monitored using electronic data loggers *(Dickson TK550 model)*.

2.2 Experimental set up

Shelled maize with natural insect infestation was stored in eight different storage treatments as follows.

Metal silo hermetic: Hermetic storage of untreated maize using a metal silo filled to 90% of the 500 kg capacity.

Metal silo phostoxin: Hermetic storage using a metal silo filled to 90% of the 500 kg capacity with a Phostoxin-treated grain (active ingredient is aluminum phosphide, 57% w/w).

Plastic barrel hermetic: Hermetic storage of untreated grain using a plastic barrel (a flat-topped 50-liter high-density polyethylene container) filled to 90% of its capacity.

Plastic barrel Photoxin: Hermetic storage of Phostoxin-treated grain using a plastic barrel filled to 90% of its capacity.

PICS: Hermetic storage of 100 kg of untreated grain using two 100-kg Purdue Improved Crop Storage (PICS bags, described by de Groote et al., 2013) purchased from Pee-Pee Tanzania Ltd, Tanga, Tanzania.

ZeroFly®: Storage of 50 kg of untreated grain using a ZeroFly® storage bag (non-hermetic; polypropylene bag with deltamethrin insecticide incorporated at the rate of 3 g/kg ± 25%) purchased from Vestergaard, Lagos, Nigeria, and shipped by airfreight to Tanzania. Four 50-kg bags were used.

PP Shumba: Storage of 100 kg of grain treated with Actellic Super® (Pirimiphos-methyl 16 g/kg plus Permethrin 3 g/kg) in polypropylene (PP) bags (non-hermetic). This is the common farmers' practice known as *Shumba* in Tanzania. Two 100-kg bags were used.

PP without treatment: Storage of untreated grain in polypropylene (PP) bags (non-hermetic) commonly used to transport and store grain. Two 100-kg bags were used (control).

2.3 Grain sampling and field assessments

Sampling: A representative sample (1 kg) from each treatment was collected at 6-week interval, transferred into a labeled paper bag, sealed, and then transported to the laboratory for further analysis. All samples were stored at ambient conditions until processed.

Grain moisture (GM) and Bulk Density (BD): Samples were tested for percentage grain moisture (GM), and bulk density (BD; g/cm^3) using a hand-held grain moisture tester (Dickey-John GAC ® Plus, Illinois, USA).

2.5 Laboratory assessment

Insect counts: The type and population of insects were visually evaluated in the laboratory following the method described by Ng'ang'a et al. (2016).

Grain assessment: In the laboratory, samples were visually examined for broken and damaged grain (DG) using the 1000 grains count. The percentage DG was calculated following the formula described by Boxall (1986). Weight loss (WL) was calculated as shown by Njoroge et al. (2014).

2.6 Farmers' perceptions of the storage technologies

At the end of the experiment, the participating farmers (20 respondents: 6 female, 14 male; 70% aged between 40 and 60 years) were asked to rate the storage technologies according to their perceptions about effectiveness to prevent grain loss and how the farmers liked the storage technologies.

2.7 Data analysis

Data were entered into an Excel spreadsheet and analyzed using SAS® version 9.4 (SAS Institute, Cary, NC). To determine means and frequencies to explain the data pattern. A stepwise multiple comparisons GLM procedure was used to determine the pattern of differences in the samples. Significant differences in storage parameters were concluded when the coefficient of the interaction term was significant at $P < 0.05$, $P < 0.01$, or $P < 0.001$ as the statistical significance levels. Additionally, standard errors were calculated and used as means separation tests.

3. Results and Discussion

3.1 Relative humidity and temperature conditions during the experiment

Average relative humidity (H_{in}) inside four selected treatments representing insecticide treated and untreated maize inside all polypropylene bags (including ZeroFly), PICS bags, all metal silos, and all

plastic barrels during the entire period of storage was 60.35±0.97, 66.24±1.14, 68.50±0.27, and 66.7±1.24 respectively. Similarly, average temperature (T_{in}) condition inside the containers was 29.65±0.51, 25.02±0.23, 25.57±0.03, and 25.07±0.20 for all PP bags without treatment (including ZeroFly), PICS, all metal silos, and all plastic barrels, respectively.

Maize stored in hermetic storage containers had a higher GM content than in non-hermetic bags (ZeroFly®, PP *Shumba,* and PP bags without treatment; Fig. 1).

The moisture content of grain stored in non-hermetic conditions (ZeroFly®, PP bags) reduced until week 18 of storage and increased slightly afterward. The moisture content of grain in hermetic conditions increased slightly during storage. These values were significantly higher than the moisture content of the maize stored in the non-hermetic facilities (especially the PP bags).

3.3. Bulk Density (BD) of stored grain

The BD of stored grain decreased from the start of storage until storage Week 6 in all the storage conditions (Fig. 2). The BD of the grain in ZeroFly® and PP bags decreased during storage.

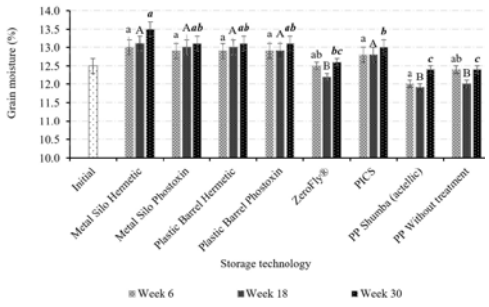

Fig 1: Percent (Mean±SE) grain moisture in the storage technologies over 30 weeks of storage. Foot note: Significant difference between means at Week 6 denoted by different lower-case letters (F=5.04, P<0.0001), significant difference at Week 18 denoted by different upper case letters (F=11.46, P<0.0001), significant difference at Week 30 denoted by different bold lower case letters in ***italics*** (F=7.69, P<0.0001).

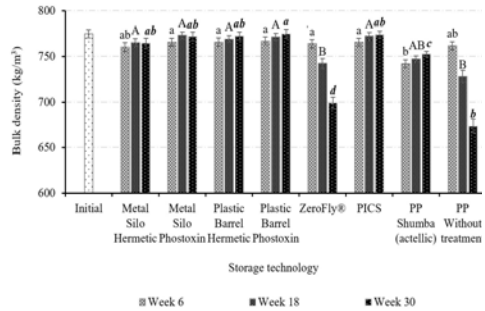

Fig. 2: Grain bulk density ((mean ± SE) in the storage technologies over 30 weeks.
Significant difference at Week 6 denoted by different lower case letters (F=3.16, P=0.0038), significant difference at Week 18 denoted by different upper case letters (F=11.23, P<0.0001), significant difference at Week 30 denoted by different bold lower case letters in ***italics*** (F=49.85, P<0.0001).

3.4 Insect population

Two major maize spoilage insects were identified: *S. zeamais* and *T. castaneum*. We did not find *P. truncatus* throughout the storage period. The population of live adult *S. zeamais* in the grain increased rapidly in ZeroFly® and PP bags (Fig. 3) but reduced in all the airtight containers. The insect was completely absent in PP *Shumba* (Actellic).

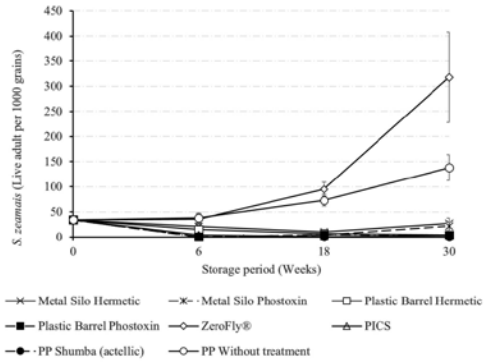

Fig 3: Number (mean ± SE) of live *S. zeamais* adult polupation in the storage technologies over 30 weeks.

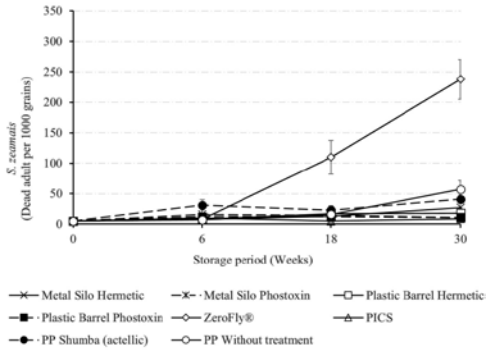

Fig. 4: Number (mean ± SE) of dead *S. zeamais* adult population in the storage technologies over 30 weeks.

At the end of storage the number of dead *S. zeamais* adults was highest in ZeroFly®, and in PP bags (Fig. 4). PICS had the lowest number of dead *S. zeamais* adults, significantly fewer than in other treatments (F = 28.01; $P < 0.0001$). Adult *T. castaneum* was not detected at the time of storage but later detected during storage (Fig. 5&6). At week 30 of storage, the population of live *T. castaneum* adults was low in all airtight containers and insecticide-treated grain while it was significantly higher in ZeroFly® and PP bags(F = 33.98; $P < 0.0001$). Dead adult of *T. castaneum* was found in ZeroFly® and PP bags, maximum of one was found in hermetic storage containers and also in grain treated with insecticides.

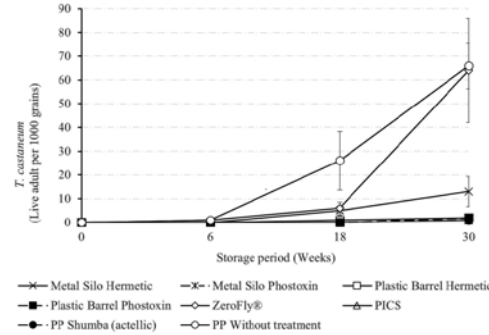

Fig. 5: Number (mean ± SE) of live *Tribolium Castaneum* adult population in the storage technologies over 30 weeks.

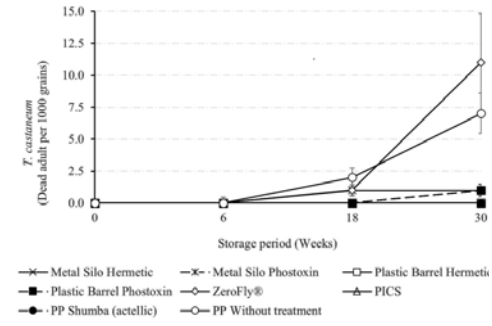

Fig. 6: Number (mean ± SE) of dead *Tribolium Castaneum* adult population in the storage technologies over 30 weeks.

3.5 Grain damage (DG)

Fig. 7 reveals very crucial patterns of insect behavior concerning the destruction of grain and the consequent food loss during storage if farmers would adopt the various storage technologies tested. The results reveal the implication of poor shelling methods that break the grain before storage and how this could accentuate insect damage. Significant difference at Week 6 denoted by different lower case letters (F=3.17, P=0.0037), significant difference at Week 18 denoted by different upper case letters (F=25.06, P<0.0001), significant difference at Week 30 denoted by different bold lower case letters in ***italics*** (F=89.09, P<0.0001).

During storage, there was an initial decrease in DG at week 18 after which DG values increased.

The increases were highest in PP bags (PP without treatment) and in ZeroFly® bags at week 30 of storage, and were significantly higher (F = 87.09; $P < 0.0001$) than the DG percentage in all the other

storage treatments. No significant differences were observed among the remaining treatments irrespective of the use of insecticide whether combined with hermetic storage or not.

3.6 Weight Loss (WL)

The consequence of the increase in DG and other factors was that average WL in PP bags (PP without treatment) and ZeroFly® bags continuously increased but did not change significantly in any of the remaining storage treatments (Fig. 8). At week 30 of storage, WL was significantly higher ($F = 10.31$; $P<0.0001$) in untreated grain stored in ZeroFly® (8.1 ± 0.6%) and PP bags (11.6 ± 1.7%) than in PP bags with insecticide treatment (PP *Shumba*; 4.4± 0.2%). There was hardly any WL in PP *Shumba* and the hermetic treatments.

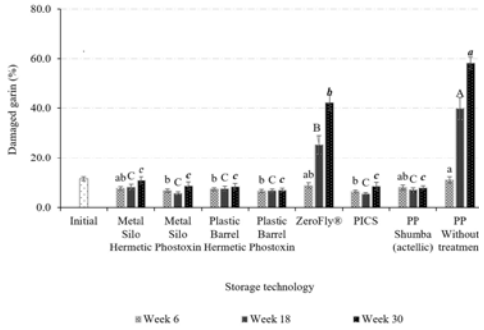

Fig. 7: Percent (±SE) of damaged grains in the storage technologies over 30 weeks

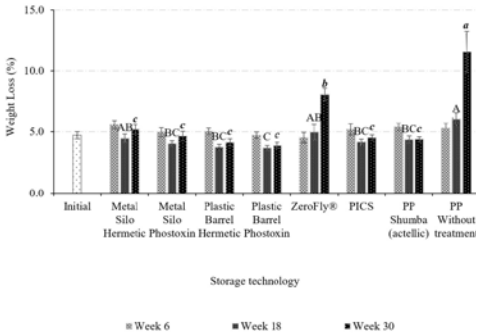

Fig. 8: Percent (±SE) grain weight loss during 30 weeks of storage No significant difference between means of weight loss (%) at Week 6 ($F=0.99$, $P=0.4379$), significant difference at Week 18 denoted by different upper case letters ($F=3.74$, $P<0.0005$), significant difference at Week 30 denoted by different bold lower case letters in ***italics*** ($F=10.31$, $P<0.0001$).

3.7 Agents of grain loss

Considering a batch that was damaged at base condition (before storage), the most critical causes were calculated to be grain breakages (32.2%), fungi infection (31.8%), and damage by insects (24.1%). After storage, the most economically important damage agents were insects: 25.4% for plastic barrel Phostoxin, 90.8% for ZeroFly®, and 91.4% for PP bags without treatment. Fungal coloration appears to constitute an important agent of grain defects in the hermetic containers. The increase in moisture in hermetic storage could promote fungi growth. Since a large insect population would cause more damage, therefore, preventing an increase in insect population is a critical factor for reducing DG and WL. In addition, *S. zeamais* seems to be more economically important in the Central Corridor of Tanzania than *T. castaneum* concerning damage to stored maize grain and food losses.

3.8 Farmers' perception

Farmers rated the hermetic storage technologies without insecticide application (metal silo hermetic, plastic barrel hermetic and PICS) as the most effective ways to control storage pests. However, contrary to trial results, PP *Shumba* was not rated as effective. Farmers also liked the same hermetic technologies best. Metal silos were preferred to plastic barrels.

Even though PP bags without treatment did not control storage pests, farmers still liked them as this was a cheap technology. PP *Shumba*, and above all ZeroFly® bags were liked the least. Farmers indicated that the PP *Shumba* treatment was not liked because it altered the taste of the grain. Field

observations revealed that farmers who store their maize with insecticide avoid using such grain as much as possible for household consumption but prefer to sell it.

4.0 Conclusion

This study showed that hermetic storage techniques could be used to store grain for 30 weeks without a significant effect on the quality and germination of the grain. Storage of maize treated with Actellic Super in PP bags, a traditional practice in Tanzania, was effective in controlling insect damage. However, for public health reasons, the application of insecticides to staple food should be avoided especially in locations where trained personnel to supervise the use of insecticides are absent. Hence hermetic storage without the application of insecticides is preferred, but the storage materials need to be made affordable to the smallholders. Sound handling and management of the technologies by farmers must also be ensured, i.e., proper placement and hermetic sealing of lids should be ascertained; insect infestation from the field should be as low as possible; grain must be properly dried before storage, and re-infestation during the intermittent opening of airtight containers should be prevented as much as possible.

Acknowledgement

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Quality and mycotoxin contamination of maize stored in air-tight containers in rural farm stores: data from two semi-arid zones in Kenya and Tanzania

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Abstract

Hermetic containers have been promoted in recent years for chemical-free grain storage among smallholder farmers. In the context of grain quality, the influence of maize storage and pre-storage practices (harvesting time, dehusking, drying, and shelling method) on performance of air-tight bags was investigated in the semi-arid regions of south eastern Kenya and northern Tanzania. Completely randomised trials were conducted in farmer-own stores; shelled maize was filled in air-tight bags or woven polypropylene (PP) bags and stored for 30-35 weeks. Insect damage, physical grain quality, mould infection were evaluated at 6-7 weeks intervals, and mycotoxin contamination was examined at onset, mid, and end of storage. Maize stored in hermetic bags was generally free from insect infestation, while PP bags permitted profuse build-up of insect populations causing grain damage of up to 82%. Total aflatoxin contamination of maize stored at moisture content below 14% increased significantly in the PP bags (5 - 8 folds) but not in the air-tight ones. Harvesting, drying and shelling practices significantly influenced the quality of maize stored in hermetic bags, resulting in sorting losses of 6-23 kg/100 kg after 6-8 months of storage. Since sorting is an important operation for improvement of food value and market quality, such losses would significantly lower the benefits of air-tight storage. Pre-storage practices of sorting, cleaning and moisture verification by farmers have impact on overall performance of air-tight storage.

On-Farm Maize Insect Pest and Mycotoxin Levels in Ghana

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Abstract

Maize post-harvest losses are perennial in Ghana but reliable comparative information on on-farm losses of maize produced in the Middle and Northern Belts of Ghana is lacking. Two studies were conducted from September 2015 to February 2016 to identify factors contributing to on-farm losses of maize in these two Belts. In the Northern Belt, the study was conducted in six communities including Adubiyili, Diari, Pong-Tamale, Savelugu, Toroyili and Zamnayili; and in the Middle Belt, in Ejura, Sekyedumase and Amantin communities. Moisture content, percent weight loss, percent insect damaged kernels (IDK) on numerical basis (%IDK_{nb}) and percent IDK by weight basis (%IDK_{wb}), insect pest abundance, and mycotoxin levels were estimated. Moisture content values of maize at pre-harvest and heaping stages in all nine communities were below 15%. *Sitophilus zeamais*, *Sitotroga cerealella*, *Cathartus quadricollis*, and *Carpophilus dimidiatus* were found to attack maize on-farm in communities in the Middle Belt, but no adult insect pests were collected on pre-harvested maize in the Northern Belt. The %IDK_{nb} values on-farm in all nine communities were < 2% per 250 g. Mean aflatoxin levels below 15 ppb were obtained from pre-harvested maize in both regions but levels above 15 ppb were obtained from heaped maize on-farm. Fumonisin levels of maize were below 4 ppm on pre-harvested and in heaped maize in both regions. Results show that heaping maize on-farm increases aflatoxin levels beyond the acceptable threshold level and should not be practiced.

Keywords: insect infestation, aflatoxin, fumonisin, maize post-harvest loss, food security

1. Introduction

In Ghana, maize accounts for 50–60% of cereal production, with major producing areas located within the middle to southern parts of the country (CSIR-SARI/AGRA, 2014). Maize post-harvest losses in Ghana are estimated to be 35% (Edusah, 2007) which is a threat to food security. The major causes of maize post-harvest losses are grain moisture content, environmental conditions and biological agents, mainly insect pests and mold. Insect pests cause both qualitative and quantitative losses to maize and can facilitate dissemination of fungal spores (Hell et al., 2010). Mold and fungal infections can lead to mycotoxin contamination and this may occur throughout the maize value chain (Enyiukwu et al., 2014). From 2015 to 2016, separate surveys were conducted in the Middle and Northern Belts of Ghana to assess post-harvest losses of maize on-farm and at post-drying stages in order to identify mitigating strategies. This paper sought to provide comparative information on the factors contributing to post-harvest losses of maize on-farm in the two Belts (Middle and Northern Belts).

2. Materials and Methods

In the Middle Belt of Ghana, the study was conducted in three principal maize growing districts of Ejura, Sekyedumase and Amantin. The study covered the periods September–mid-October 2015 and late-December 2015–mid-February 2016. These periods corresponded with harvest time for the major and minor cropping seasons, respectively. A three-factor factorial-RCBD design of the factors cropping season, district and sampling stage with 2x3x3 levels (two cropping seasons: major and minor; three districts: Ejura, Sekyedumase and Amantin; and three sampling stages: field, ground-pile and post-drying) was used. Farmers within sampling location represented replications for the district in a cropping season. White maize varieties (Obatanpa,aborohomaand OBT) the most widely cultivated varieties in the Middle Belt of Ghana were sampled from selected farmers. Samples were taken from 51 maize farms. The three sampling stages were assigned to each randomly selected maize farm for a maize cropping season. In the Northern Belt, the study was conducted in farms in two districts of Central Gonja and Savelugu-Nanton in the Tamale area of Northern Region, Ghana. The locations selected were Adubiyili, Toroyili and Zamnayili in Central Gonja district; Diari, Pong-Tamale and Savelugu in Savelugu-Nanton district. Five farmers were chosen from each location. This study spanned the period October to December 2015 in the Northern Region, and this period corresponded with harvest time for the single maize season in the Northern Belt. In both belts, field sampling was performed by sampling mature cobs either before or during harvesting. From each farm 30 cobs were randomly collected from three parts of the field. Cobs from each part of the farm were de-husked and kept in three sealed and labelled plastic bags (39 cm x 25 cm) with each bag containing 10 de-husked cobs. De-husking was carefully done to avoid escape of insects on cobs. Cobs in each bag were hand shelled into a basin, mixed thoroughly and 500 g weighed out for moisture content, insect and aflatoxin and fumonisin data. Maize samples for mycotoxin test were kept in a portable cooler to reduce further growth and development of fungi. For ground-pile/heaping stage sampling, similar protocols as the field sampling stage were used for sampling and data collection, however, samples were taken from heaps of cobs piled on the ground in the farm fields after harvest. With respect to post-drying sampling stage, maize was sampled after cobs had been mechanically shelled and kernels sun-dried for storage or market. Dried maize grains either on tarpaulin or in store were divided into three different sections. A 2-kg sample was taken from each division by sub-sampling ten 200 g of maize from a sampling section. Three 500 g sub-samples were taken from each of the three 2 kg samples, kept in sealed and labelled plastic bags for data collection. Mycotoxin (aflatoxin and fumonisin) analyses were performed using AgraStrip® WATEX (aflatoxin) and AgraStrip® Total Quantitative Fumonisin (COKAS3000A) test kits provided by Romer Labs®, Inc. Union, MO, USA. In both tests, sample grinding, extraction, solute preparation and test procedures were done in accordance with the manufacturer's instructions (Romer Labs Methods, romerlabs.com). Complete details of these surveys can be found in Danso et al. (2017).

Statistical analyses were performed with SAS Version 9.4 (SAS Institute, Cary, NC). Data were analyzed by location (district) because interest was primarily understanding the effects of stage and cropping season on response variables whose data were collected.

3. Results

In both Belts, moisture content of maize at all three sampling stages was < 15%. In the Middle Belt, *Sitophilus zeamais* (Motschulsky), *Sitotroga cerealella* (Olivier), *Cathartus quadricollis* (Guerin-Meneville) and *Carpophilus dimidiatus* (Fabricius) were the dominant insect species that were recovered from maize on-farm but in the Northern Belt, there were no insect pests on maize at the field stage and only a few larvae were found in heaped maize. In both Belts, mean percentage insect damaged kernels (%IDK_{nb}) recorded across locations were below the 5% threshold set by Ghana Standard Authority (GSA) for commerce. The %IDK_{nb} values on-farm in all nine communities were < 2% per 250 g and mean maize weight losses of < 1% were recorded in all the communities. In the Middle Belt, mean %IDK_{nb} recorded across the locations in both cropping seasons ranged between 0.35–0.82% while 0.35–1.82% were recorded in the Northern Belt. However, in both Belts, %IDK_{nb} was significantly lower at field stage than at both the heaped and post-drying stages. Mean aflatoxin levels below 15 ppb were recorded from pre-harvested maize in both Belts but levels above 15 ppb were recorded from heaped maize on-farm. Fumonisin (ppm) levels of maize were below the recommended threshold of 4 ppm on pre-harvested and in heaped maize in both Belts.

4. Conclusion

The data presented in this comparative study show that more insect pests attack pre-harvested maize in the Middle Belt compared to the Northern Belt of Ghana. Interestingly, comparable insect damage and weight loss of maize occur in both Belts. Data also show that heaping maize on-farm increases aflatoxin levels beyond the acceptable threshold level and should not be practiced.

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Insect pests of post-harvest storage in promising crop sectors in Burkina Faso: current concerns and prospects for solutions

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Abstract

Effective post-harvest management of crops could significantly contribute to food security by improving the availability and quality of food. In Burkina Faso, new concerns have emerged as a result of the growing importance accorded to sesame (Sesamum indica), roselle (Hibiscus sabdariffa), "zamnè" (Acacia macrostachya), and sorghum (Sorghum bicolor). The insects identified as storage pests on these crops belong mainly to the order Coleoptera and families of Chrysomelidae, Curculionidae and Bostrichidae are the most representative. The studies carried out allowed a better knowledge of the pests as well as their economic importance. Losses due to insects, estimated up to 100% depending on the crop and protection methods used, are frequently observed after a few months of grain storage. Several alternatives to the use of chemicals including biological control, biopesticides and hermetic storage are being promoted. The triple bagging technology is one of the promising alternatives that can adapt to the post-harvest storage of a wide range of crops. Despite its proven effectiveness for several commodities, there is need to verify its efficiency against a diversity of insect pests with differing behaviour and evolution. The importance of the challenges is such that the strategies to be implemented must be conceived in a comprehensive, integrated approach, even at the regional scale.

Keywords: Post-harvest concerns, Promising crop sectors, Grain beetles, Storage alternative methods, capacity building, Burkina Faso, regional integrated strategies.

Introduction

Persistent food insecurity in sub-Saharan Africa is a complex and multifactorial phenomenon (FAO, 2017). While it is true that food insecurity is related to the inadequacy of agricultural production in general, it is also important to recognize the role of inappropriate post-harvest management practices. In 2011, post-harvest losses were estimated by FAO and the World Bank in sub-Saharan Africa at around $ 4 billion a year for an estimated total annual production of $ 27 billion (World Bank, NRI and FAO, 2011). In addition, it is estimated that lost food could meet the minimum annual needs of at least 48 million people.

In Burkina Faso, agriculture is booming with growing interest in some crop sectors. To overcome food and nutritional insufficiency and reduce poverty, it is important to support the efforts made by farmers and government policies to increase crop productivity through appropriate post-harvest management strategies. The promising sectors mainly include food crops, legumes and/or oil crops, which are increasingly considered as cash crops. For the most part, these crops are important for food security of the majority of the population in the country and are an essential element of the livelihoods of smallholders.

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important cereals in the semi-arid tropics providing a major source of dietary energy and protein for nearly a billion people living in semi-arid areas (Belton and Taylor, 2004; Awika and Rooney, 2004). In Burkina Faso, sorghum serves as the major staple food crop with a total annual production of 1,435,640 tons in 2015-2016 (INSD, 2016). Burkina Faso is the third largest African producer of sorghum after Nigeria and Sudan (FAOSTAT, 2016). Cowpea (*Vigna unguiculata* L. Walp.) is the most widely grown legume in Burkina Faso. All the parts of the plant are useful but the seeds, by their richness in proteins (23-30%), play an exceptional role as food. Burkina Faso is the third largest African producer with a production of 571,304 tons in 2015-2016 (FAOSTAT, 2016). "Zamnè" (*Acacia macrostachya*) is an example of a non-timber forest product of food interest in Burkina Faso. The nutritional value of seeds makes them the foods of

choice to produce nutritional improvement formulations (Hama-Ba et al., 2017). They contain nutrients that are essential for human health (Sawadogo et al., 2011). The increasing interest in this wild legume is such that «zamnè» is used in the preparation of prestigious dishes served in receptions of religious and customary ceremonies (Ganaba, 1997) where they are consumed in the form of ragout and dishes associated with cereals. (Hama-Ba et al., 2017). In economic terms, seed marketing generates substantial income for the rural population (Hagberg et al., 1996). Most of the production comes from harvests in the wild, but the importance of the plant has allowed the development of a research program to facilitate its wider domestication. Sesame (*Sesamum indicum* L) is an oilseed crop grown in several countries around the world for its seeds that are traded internationally (Amoukou et al., 2013). In Burkina Faso, sesame production has increased significantly in recent years, from 25,060 tons in 2005-2006 to more than 321,837 tons in 2014-2015 (FAOSTAT, 2016). This places Burkina Faso among the top three sesame producing countries in Africa after Sudan and Ethiopia and is the third largest agricultural export product after cotton and peanut (INSD, 2016). Unfortunately, since that date, the sector has been experiencing difficulties which are dropping production, which in 2016 was only 235,079 tons (INSD, 2016). In Burkina Faso, peanut, (*Arachis hypogeae*) is both an important food crop and a cash crop. Peanut production is becoming increasingly important with an average production increase of 9.7% compared to the five-year average, which makes a total production of 365,887 tons of unshelled peanut in 2016 (FAOSTAT, 2016). In West Africa and particularly in Burkina Faso, Roselle (*Hibiscus sabdariffa L*. Malvaceae) is receiving increasing attention as a crop with potential for making great socio-economic impacts. The Sabdariffa variety, the most important one, is grown for the production of calices, which are used in the preparation of Bissap, a high nutritional value and commercial drink (Egharevba and Law-Ogbomo, 2007; Lepengue et al., 2007; Sanou et al., 2004; Babajide, 2004).

Despite their importance, the seeds of all the above mentioned plants face post-harvest storage and management constraints, leading to variable post-harvest losses negatively impacting their availability, accessibility, and income that could result from their commercialization. In this paper, we focus on the problems, their causes, their impact and we will end up giving an overview of the solutions that are currently available or expected.

Major storage insect pests and damage caused

Post-harvest losses are correlated with economic losses since grain quality is an important determinant of market prices (Langyintuo et al., 2003). The above described crops are generally producing grains that have in common to be attacked by several species of insect pests (Tab. 1). We will describe for each of them the insects as well as the damages associated with the level of losses when possible.

Sorghum

An inventory of insects associated with sorghum storage in Burkina Faso revealed that fourteen species divided into eight families of Coleoptera and two families of Lepidoptera were recorded on the sorghum stores (Waongo et al., 2015). The most representative families included Bostrichidae, Silvanidae, Tenebrionidae, Cucujidae and Curculionidae. The Family Gelechiidae dominated the Order Lepidoptera (Waongo et al., 2015). Rhyzopertha dominica (Bostrichidae), representing 39.53% of the total number of individuals collected was the more important species responsible for the grain damage. Insects of the R. dominica species develop and feed on sorghum grains. This biological activity results mainly in a loss in weight and a production of flour from perforated and crushed grains (Waongo, 2016). After 6 months of storage in polypropylene bags, the perforated grains are estimated to 11.73% for a total grain loss of 2.87% (Lankoandé, 2017).

Cowpea

Several species of the subfamily Bruchinae (Chrysomelidae), and specifically Callosobruchus maculatus Fab., have been identified as insect pests of stored cowpeas in Burkina Faso (Ouédraogo

et al., 1996, Sanon et al., 2005). The overlapping generations of flightless form C. maculatus during cowpea post-harvest storage in West Africa are responsible of grain damage (Ouédraogo et al., 1996). Infested seeds become increasingly hollow resulting in weight loss and perforation, adult insect emergence holes at the end of larval growth. Previous studies have shown that farm storage for six months was accompanied by 70% seed infestation and about 30% weight loss and virtually unfit for consumption (Singh and Jackai, 1985).

Tab. 1. Summary information on the major insect pests and their damage in stored grains for crops under consideration, Burkina Faso.

Crops	Major insect pests	Damage caused	References
Sorghum (Sorghum bicolor L. Moench)	Rhyzopertha dominica Fabricius Oryzaephilus mercator Fauvel Cryptolestes ferrugineus Stephens Sitophilus zeamais Motschulsky Sitotroga cerealella Olivier Tribolium spp	Weight loss, crushed grain, flour	Waongo (2016) Waongo et al. (2015)
Cowpea (Vigna unguiculata L. Walp)	Callosobruchus maculatus Fab. Bruchidius atrolineatus Pic.	Weight loss, seed perforation	Sanon et al. (2005) Ouédraogo et al. (1996)
Peanut (Arahis hypogeae L.)	Caryedon serratus Olivier Tribolium castaneum Herbst Oryzaephilus surinamensis Linnaeus Plodia interpunctella Hubner	Peanut pod perforation, Aflatoxin	Ouédraogo et al. (2017)
"Zamnè" (Acacia macrostachya Reichenb. ex.De.)	Bruchidius silaceus Fahraeus Caryedon furcatus Anton & Delobel Bruchidius spp	Weight loss and seed perforation	Unpublished data
Sesame (Sesamum indicum L.)	Ephestia cautella Walker Tribolium castaneum Herbst Tribolium confusum Duval	Rancidity of seeds, mycotoxins	Sanou et al. (2011)
Roselle (Hibiscus sabdariffa L.)	Spermophagus niger Motschulsky	Seed perforation and weight loss	Koussoube et al. (2016) Sanon et al. (2017)

Peanut

Sampling of peanut stores in the farm environment identified four families of insects (Ouédraogo et al., 2017) including three families of Coleoptera, Tenebrionidae, Chrysomelidae, Silvanidae and a family of Lepidoptera (Pyralidae). Among these insects, Caryedon serratus (Chrysomelidae, Bruchinae) seems to be the predominant and the most damaging species to stored peanuts (Ouédraogo et al., 2017). Monitoring peanut stores in several localities in western Burkina Faso showed a variation in the level of infestation according to localities and a rapid increase during storage. For example, in the locality of Toussiana, the rate of perforation of the peanut pods was almost zero at the beginning of storage but this increased significantly during storage to 53.7, 81.8, 89.8 and 100% after 2, 3, 4 and 6 months of storage, respectively. Another consequence of peanut

infestation by insects is the association of insect attacks with the production of aflatoxin, which causes severe food poisoning.

The "zamnè"

Recent studies of Acacia macrostachya grain storage have identified six insect pest species belonging to three families, Chrysomelidae (Bruchinae), Tenebrionidae and Silvanidae. The last two families contain mostly secondary pests. The subfamily Bruchinae, with 3 species, represents about 98% of insects collected in stores. These are Bruchidius silaceus, Bruchidius spp. and Caryedon furcatus and responsible for the damage (Sanon, Personal observation). The main damage caused by these pests to A. macrostachya includes weight loss and seed perforation.

Sesame

From the samples of sesame seeds collected from storage, two species of Coleoptera/Tenebrionidae (*Tribolium castaneum* and *T. confusum*) and one species of Lepidoptera/Pyralidae, *Ephestia cautella* were identified as storage pests (Sanou et al., 2011). The infestation of sesame during storage by these insects causes a rancidity of the seeds rendering them unfit for human consumption. Insects also cause the loss of germinability and promote the establishment and development of mycotoxins (Sanou et al., 2011) whose consequences remain to be clarified.

Roselle

Spermophagus niger (Coleoptera: Chrysomelidae: Bruchinae) is the main pest of roselle seeds stored in Burkina Faso (Koussoubé et al., 2016, Sanon et al., 2017a). Roselle infestations by S. niger occur in the field at calyx maturity with an estimated infestation rate of 67% of the samples examined. Like other Bruchinae, development takes place in the seeds and successively passes through an egg stage, four larval stages and a pupal stage (Sanon et al., 2017a). At the beginning of grain storage, infested seeds generally had only one insect emergence hole, with seed perforation rates ranging from 1.8% to 4 % depending on their origin (Sanon et al., 2017a).

Insect control strategies during post-harvest storage

The need to protect grain during post-harvest storage is imperative to ensure the availability and quality of food commodities that are limited resources. Increasingly, crops grown for export must also meet post-harvest quality standards to compete in the international market. Although synthetic insecticides have played a key role in post-harvest grain storage, their use by farmers is nowadays criticized worldwide (Williamson et al., 2008). Furthermore, a survey of cowpea traders in Burkina Faso found that 77% of the insecticides used were neither registered nor intended for food preservation (Zongo et al., 2015). The consequences of inappropriate usages of chemicals include health and environmental risks (Idrissi et al., 2010; Zongo et al., 2015), and sometimes induce resistance in storage insect pests populations (Leontieva et al., 2006; Oyedemi et al., 2006; Opit et al., 2012). To minimize the negative effects of chemicals, many studies have investigated some components of integrated pest management with a view to developing alternatives to systematic chemical control. In Burkina Faso, studies carried out on post-harvest storage of the crop sectors considered in this paper can be grouped into three categories: biological control, botanicals, and hermetic storage.

Biological control has often been considered at the experimental level without a method of producing biological control agents and large-scale application being developed. Studies on the biological control potential of the main cowpea pest, C. maculatus, have identified the parasitoid Hymenoptera Dinarmus basalis Rond. (Pteromalidae) and Uscana lariophaga Stephan (Trichogrammatidae) as the best biocontrol agents (Sanon et al., 1998; Amevoin et al., 2007) without cost-effective methods of mass production of these biocontrol agents being available. The recent discovery of four (4) families of Parasitoids, Eulophidae, Pteromalidae, Eupelmidae and Eurytomidae associated with Bruchinae pests of A. macrostachya (Personal communication) opens interesting

perspectives of research on the possibilities of biological control of these insects. Although an important component of integrated pest management, success stories of biological control under stored food conditions are rare.

The use of botanicals is by far the most explored field of research in Burkina Faso. The data collected mainly concern cowpeas and peanuts. In Burkina Faso, since the early 2000s, several scientific investigations have focused on the control of C. maculatus using botanicals considered as promising and safe alternatives to chemicals (Sanon et al., 2017b). Extensive studies were carried out on six (6) plant species from three families including Capparaceae, Lamiaceae and Verbenanceae, through bioassays on C maculatus and cowpea storage trials. The set of data analyzed shows that several plants materials, including powders, crushed plants and essential oils (EO) were active against eggs, larvae and adults of C. maculatus, through dose-dependent mortality responses. However, EO extracted from native aromatic plants have yielded the most promising results, specifically EO from Ocimum canum (Lamiaceae) appeared as the best candidate control agent (Sanon et al., 2017). With regard to peanuts, Ouédraogo et al (2016) also tested several essential oils on eggs and adults of C. serratus. The results also showed a variable dose dependent biological activity depending on the origin of the essential oil. The current stage of research on essential oils is the conduct of toxicity tests towards humans and animals and the optimization of their use in storage conditions. For example, it is envisaged to have the essential oils carried by supports which would facilitate the treatment and improve the remanence (Ilboudo et al., 2015).

Hermetic storage is an old technique but more recently revalorized through triple bagging technology, known as PICS bags (www.ag.purdue.edu/ipia/pics). Since previous research of Sanon et al. (2011) who validated the effectiveness of a triple bag prototype in post-harvest storage of cowpeas in Burkina Faso, the effectiveness of triple bagging has been demonstrated for many commodities in Africa and around the world. Grains that are effectively preserved with triple bagging include other legumes such as bambara groundnut, peanut or pigeon pea (Baoua et al., 2014a; Sudini et al., 2015; Vales et al., 2014) cereals such as maize, rice, sorghum, wheat (Njoroge et al., 2014; Williams et al., 2017; Martin et al., 2015; Baoua et al., 2014b, 2016; Lankoandé, 2017); roselle seeds (Amadou et al., 2016). Promising crop sectors such as sesame and "«zamnè»" have been the subject of very few studies regarding post-harvest management strategies. Moreover, recently, the use of insecticides on sesame above the maximum residue limits has resulted in seizures and destruction of export grains from Burkina Faso (Personal Communication), anything that calls for greater vigilance in the production and post-harvest management of this important cash crop. In the meantime, advice is provided to producers who also have the opportunity to use or experiment with airtight containers such as PICS bags.

Prospects of future research

Post-harvest issues are a global concern. Limiting post-harvest losses throughout the food chain must be an important part of food strategies to achieve food and nutrition security. In addition, effective post-harvest management, limiting losses and improving the quality of food, can help reduce poverty through increased incomes. The diversity and the multitude of the species of insect pests of grains in the described crop sectors highlights the predominant role of this taxonomic group in the occurrence of losses in post-harvest. That is why it is crucial to find the most appropriate solutions while minimizing the use of chemicals. Concerns about crop sectors such as sesame, roselle and «zamnè» are relatively new and deserve rapid action to address them. The components of IPM already tested on other models could also be extended to these emerging sectors. In view of the proven effectiveness of triple bagging for the preservation of several grain commodities, this technology should be quickly tested and validated for the above mentioned sectors. However, the different insects identified have different ecological and biological requirements, which necessarily involve a permanent evaluation of the technology vis-à-vis the targets for efficient and sustainable post-harvest management. Any control strategy, to be effective, should be part of a comprehensive

integrated pest management approach based on the natural potential offered at national and regional levels.

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Abundance and diversity of arthropod pests infesting stored maize in smallholder farmers and traders systems highlight critical points for pest management in Uganda

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Abstract

Knowledge of the diversity of arthropod pests infesting stored maize value chain in Uganda is very scanty to guide the development and implementation of management strategies. From a cross-sectional study conducted in north western, eastern and central regions of Uganda during 2017/2018, the diversity and economic importance of storage arthropod pests of maize in farmer storage, trader/retailer stores in villages and townships, and in milling and processing facilities is presented. A total of 11 insect pests were recorded feeding internally and externally on stored maize. Rhyzopertha dominica, Sitophilus zeamais and Sitophilus oryzae were the primary insect pests followed by Tribolium spp., Cryptolestes spp., Sitotroga cerealella, and Oryzaephilus mercator. The highest insect diversity and damage was recorded when maize was stored with husked cobs in farmers' houses, a practice farmers use to store seed for next planting. Meanwhile the distribution pattern of the pests in trader/retailer stores in villages and townships, and in milling facilities indicate waves of insect infestation occurring with stocks of grain being brought in storage. The maize grain at the peak of harvesting was in excellent quality but later stocks brought in several months after harvest were infested with diverse insects. Re-drying at farm level and use of chemical dusts at trader/retailer stores in villages and townships were the most common pest management practice. However, the lack of a differentiated market, whereby better quality would fetch premium price, discourages investment to reduce postharvest losses. Subsequently, most farmers sold their grains immediately after harvest and most traders sold their stocks as soon as there was the next bulk buyer. The critical point for pest management is at farm level where pest diversity and damage is greatest, and at the village/ township stores where the grain may be held in anticipation of improvement in price.

Introduction

Maize is the important cereal crop grown in most parts of the Uganda for food, feed and income, (Asea. et al, 2014). The maize sub-sector in Uganda is estimated to provide a livelihood for about 3 million farm households, close to 1,000 traders and over 20 exporters (UBOS, 2017; UBOS and MAAIF. 2011). As elsewhere in eastern and southern Africa, maize is increasingly the staple food of choice in many parts of the country, providing over 40% of the calories consumed in both rural and urban areas (Mason and Smale, 2013; Byerlee et al., 1994). Nutritionally, maize whole grain of 100g contain 10g of protein (poor in trytophan and rich in leucine) and 4gm fat, and provitles 360 calories; the germ (12% by weight of the whole grain)) contains 22% of the total protein and 80% of the oil (Nuss and Tanumihardjo, 2010; Shah et al., 2016).

Smallholder farmers dominate maize production in Uganda, generally characterized by small farm acreage (0.5- 5 ha) (MAAIF, 2013), low yields (1.0 -1.8 MT/ha) and high production costs and consequently low returns. Unfortunately, poor post harvest handling and storage practices have led to low grain quality reducing potential income for smallholder farmers by ~$300/ha per annum and limiting market volume potential. The inefficient traditional drying methods, high moisture levels and lack secure storage have led to low quality grain susceptible to fungal diseases and insect pests. It is estimated that 5-15 % of the maize produced each year is lost over a period of 3-6 months during storage as a result of storage pest damage (Affognon et al., 2015). Elsewhere, comprehensive lists of insect fauna infesting maize are available, for example Ethiopia (Tadese 1996; Walker and Boxall 1974.), Malawi (Schulten, 1974), Saudi Arabia (Rostom, 1993). However, very little work has been conducted in Uganda to understand the population structure of insect fauna infesting stored maize along its value chain in order to guide the development of management strategies.

Materials and Methods

A survey was conducted between December 2017 and March 2018 when there was grain in storage and when infestation and grain damage levels were most likely to be serious after maize grain spending at least 5 months in storage since harvest. A total of 150 farmers, 30 trader/retailer stores in villages and townships, and 15 maize milling facilities were randomly selected from north western, eastern and central regions of Uganda, and sampled for presence of storage pests. From a maize storage facility at each sampling point, grain was collected from the top, on the sides, in the centre, and at the bottom, combined and a subsample of 100g of grain taken. The subsamples were each put in a sealed plastic-bottles punctured with pinholes and labelled with sample number. At each sampling point, specific information such name of farmer/ trader/retailer/mill, date of sampling, time of grain spend in storage, treatments to control pest infestations, and which pests among those sighted or otherwise the respondent perceived as most important, were recorded.

In the laboratory, each grain sample was sieved over a 2 mm mesh sieve and all fractions were examined. Insects were removed, counted and grouped according to order or genus and were preserved dry as pinned collections or in 75% alcohol. The different fractions of grain samples were reconstituted, re-bagged, and were held at room temperature to determine any internal infestation or parasitism. After about one month, these samples were re-examined and any emerged insects were recorded as previously. Based on their relative abundance in grain samples, the status of insects of each species was noted as being: very common when they were numerous and recorded from all samples; common when they were few but recorded from all samples; uncommon when they were few in number and recorded from some of the samples only; uncertain when it was not possible to handle for counting (for example mites); and unusual when the species is not known as a pest of stored products and was recorded from one or two samples only. The samples of insects and other arthropods obtained during both inspection periods are kept at the School of Agricultural Sciences Makerere University for further confirmation of identification.

Results and Discussion

The species of arthropods recorded on stored maize are listed in Tab. 1. Five species of Coleoptera and three species of Lepidoptera were preliminarily identified at the School of Agricultural Sciences Makerere University. Among Coleoptera, *Sitophilus* spp., *Tribolium* spp., *Rhizopertha dominica* and *Cryptolestes* spp. were the most common and widespread pests. One species of Coleoptera was vaguely identified as *Prostephanus truncatus* (large grain borer) but only one insect in one sample was recovered. The species of Lepidoptera that were recovered from the maize samples included *Sitotroga cerealella*, *Ephestia cautella* and *Plodia interpunctella*. These were dominant but only widespread in maize grain samples collected from village trader/retailer/milling premises. There were many other coleopteran pests, possibly mites, thysanura and diptera that were recovered in store refuse within the farmer stores and village trader/retailer/milling premises that were considered mostly secondary pests, and were not identified.

Sitophilus spp. was the most common and perhaps the most destructive of all the insect pests recorded (Tab. 2). Most of the pest species were recovered from grains that have been broken or damaged by *Sitophilus* spp., *S. cerealella* and *R. dominica* that are the primary pests, and thus considered secondary pests. These species that were recovered are cosmopolitan pests in stored products and not unique to Uganda. However, nationwide surveys may be necessary to determine the diversity of species of pests associated with stored maize and other stored produce in Uganda. This study did not attempt to estimate the exact losses caused by the major pest species in maize. It is important to determine the losses associated within the different types of stored produce so as to guide development of integrated pest management system.

Tab. 1: List of arthropod pests and their status in maize stored at on-farm and in village trader/retailer/milling premises in north western, eastern and central regions of Uganda between December 2017 and March 2018

Insect species*	Incidence and Spread		
	Central	North western	Eastern
Coleoptera			
Cyptolestes spp	+	++	+++
Oryzaephilus spp	+	+	+
Rhizopertha dominica	+	+++	+++
Sitophilus spp	+++	+++	+++
Tribolium spp	++		+++
Prostephanus truncatus		?	?
Lepidoptera			
Ephestia cautella	+	+++	++
Plodia interpunctella	+	++	+++
Sitotroga cerealella	+	+++	+++
Others		++	+++

Re-drying at farm level is the common practice for managing pests, as most farmers reported that it reduces insect populations, with many of the winged insects flying away and the coleopteran insects crawling away from the grain under the hot sun. However, this practice exposes the grain to re-infestation, and increased damage in continued storage. The lack of a differentiated market, whereby better quality would fetch premium price, discouraged farmers to invest in postharvest loss reducing technologies. Depending on the quantities produced and the desperate need of income by the farmer, most farmers to sell their grains immediately after harvest whereby the drying process may also be hurried through. Subsequently poor quality produce enters the trading component of the value chain and thus the commodity fetching very low prices. At trader/retailer stores in villages and townships, grain was not held at all early in the season due to demand, and most traders sold their stocks as soon as there was the next bulk buyer. This was also a way to build capital to buy more grain later in the season. Where grain was held for some time in anticipation of improvement in price, there was no partitioning of the maize grain brought into storage the peak of harvesting that was in most cases in excellent quality from the later stocks brought in several months after harvest that were infested with diverse insects. At trader/retailer stores in villages and townships, chemical dusts either spread on the bags or admixed with grain before bagging was the most common pest management practice. No milling premise was observed to hold stocks of maize grain. The pests recovered within these sampling points were in grain dust on the floors, walls and roofs of the premises.

Tab. 2: Mean number (per 100g) of insect pests recorded from stored maize samples collected at on-farm and in village trader/retailer/milling premises in north western, eastern and central regions of Uganda between December 2017 and March 2018

	Central	North western	Eastern
Coleoptera			
Sitophilus spp	85.3 ± 7.6	63.1 ± 6.8	51.4 ± 6.8
Rhizopertha dominica	33.1 ± 7.2	37.0 ± 3.2	82.4 ± 6.8
Tribolium spp	10.8 ± 2.6	50.0 ± 1.7	19.5 ± 3.7
Cyptolestes spp	7.4 ± 2.1	12.1 ± 1.6	23.4 ± 2.0
Lepidoptera			
Sitotroga cerealella	2.5 ± 1.1	1.1 ± 0.3	6.2 ± 0.9
Plodia interpunctella	3.8 ± 1.5	5.9 ± 0.6	0.7 ± 0.2
Ephestia cautella	5.6 ± 1.6	3.4 ± 1.2	2.7 ± 1.6

± SE

Based on the insect species diversity, dominancy, spread, and greatest potential for damage, the critical point of pest management is at the farm level. Postharvest control of insect pests should be an integral part of maintaining safe, high quality, abundant produce domestically and for trading in the country. For example, the necessity to variously demonstrate appropriate postharvest technologies such as mechanical crop shelling, solar dryers, and improved storage (e.g. hermitic bags and insecticide incorporated polypropylene bags) to farmers and traders. In order to promote adoption of these technologies, there is need for behaviour change communication campaign, and to conduct short-term trainings to create awareness and build a cadre of local postharvest specialists among extension workers as part of long-term capacity building in on-farm grain handling and storage; stored grain management; and application of basic food safety principles. There is also need to improve the operation environment, for example, strengthening farmer associations/community-based organizations to increase access to postharvest tools, equipment, packages, supplies; and facilitate development of well-structured commodity value chains and postharvest management and handling operations in the country. These associations/community-based organizations would establish postharvest service centres and Agribusinesses which offer a combination or all of the following services: harvesting, de-husking, drying, shelling, cleaning, storage, provision of agricultural inputs, and credit facilities. These postharvest handling services could make it possible to produce large quantities of consistently high quality commodities which would then facilitate value addition and bigger profit margins for the smallholder farmers. At trader/retailer stores in villages and townships, there is need for improved sanitation in the stores, and ability to rotate the produce in store according to the "first in - first out" principle in order to prevent over-storage.

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Potential of Essential Oils from four Cameroonian Aromatic plants used in Integrated Protection of Stored Products programs

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Abstract

The efficacy of essential oils extracted from fruits of *Piper capense* and *Xylopia parviflora*, and roots of *Echinops giganteus* and *Mondia whitei* were evaluated against *Acanthoscelides obtectus* and fungi isolated from bean seeds in laboratory conditions in Cameroon. The essential oils were extracted by water-distillation and their chemical composition identified by Gas Chromatography-Flame Ionization Detection (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS). Toxicity assays of essential oils against *A. obtectus* were carried out by fumigation in which insect pests were exposed fumes of the essential oils, and mortality recorded after 6, 12, and 24 hours. Additionally, the toxicity by contact of the essential oils was evaluated through coating grains with essential oils or impregnating the essential oils onto the filter paper, allowing the insects to physically get in contact with the essential oil, and assessing weevil mortality recorded after 1, 2, 3, and 4 days. The essential oils from *P. capense* and *X. parviflora* consisted mainly of hydrocarbon monoterpenes (56.5% and 50.0% respectively), whereas the essential oils from *E. giganteus* was mostly constituted of sesquiterpenes (94.3%) in which the tricyclic compounds are more abundant. A major compound identified in the essential oil from *M. whitei* was 2-hydroxy-4-methoxy-benzaldehyde (81%). The essential oil from *X. parviflora* was the most effective as contact and fumigant against *A. obtectus,* causing 100% mortality within 1 day at low lethal concentrations. On the other hand, the essential oil from *M. whitei* exhibited the best anti-fungal activity. These essential oils could play an important role in pest protection of stored beans and reduce the risks associated with use of synthetic insecticides especially in low income small holder farming systems.

Keywords : Essential oil, Insecticide, Fungicide, biopesticides, Integrated Pest Management

Background

Bean crops (Phaseolus vulgaris L.) occupy a prominent place in medium and large farming units in Cameroon (Pessoa et al., 2016). Most of the time, the availability of this crop depends on, among other factors, strict quality control, timely harvesting, and appropriate storage. During storage, bean seeds may be destroyed by insects mainly Acanthoscelides obtectus SAY which consume substantial quantities of the beans, and their respiration increases temperature and intergranular humidity which in turn facilitates fungal growth (Rupolho et al., 2006) and production of mycotoxins. Stored grain pest infestation is controlled by various methods amongst which the application of chemical pesticides remains the most effective. However, because of the negative side effects of most synthetic insecticides on environment and human health, alternative control methods are gaining importance. Over the last decade, essential oils from plant origin and other botanicals (plant powders, plant extracts and non-volatile oils) have been developed as potential alternatives for pest control. They are often of low mammalian toxicity, readily biodegradable and pose low danger to the environment if used in small amounts (Regnault-Roger et al., 2002). This research evaluated the insecticidal and fungicidal activities of essential oils from fruits of Piper capense and Xylopia parviflora, and roots of Echinops giganteus and Mondia whitei against Acanthoscelides obtectus and fungi isolated from bean seeds. These plants were selected among others because they are locally available and used as spices in some camerounian traditional foods.

Materials and methods

Toxicity assays of essential oils extracted from Piper capense, Xylopia parviflora, Echinops giganteus *and* Mondia whitei against *the bean weevil (*Acanthoscelides obtectus*)*

Adult insects were obtained from stock cultures maintained in the laboratory and reared on common bean grains. They were kept in a controlled chamber under a 10-h light, 14-h dark photoperiod at 26.08 ± 0.2 °C and 70.8 ± 0.4% relative humidity (RH). After two weeks of oviposition, parent insects were removed by sieving and the glass bottles containing bean were held under the same conditions until the emergence of F1 progeny. For activity testing, one or two days old adult of unsexed insects were used. The different plant materials were collected from Dschang market where they are sold as spices. Essential oils of *Piper capense* and *Xylopia parviflora* fruits, of *Echinops giganteus* and *Mondia whitei* roots were extracted by hydro-distillation and analyzed by Gas Chromatography-Flame Ionization Detection (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS). Toxicity assay of essential oils against *A. obtectus* was carried out by fumigation in which insect pests were exposed fumes of the essential oils, and mortality recorded after 6, 12, and 24 hours. Additionally, the toxicity by contact of the essential oils was evaluated through coating grains with essential oils or impregnating the essential oils onto the filter paper, allowing the insects to physically get in contact with the essential oil, and assessing weevil mortality recorded after 1, 2, 3, and 4 days. Percent mortality was calculated using the Abbott correction formula for natural mortality in controls (Abbott, 1925). The Bliss (1938) method based on the regression of the probits of mortalities (Finney, 1971) was used to determine the 50% lethal concentration (LC50) based on the decimal logarithms of the oil concentrations. After the above contact toxicity tests, the remaining living adults were removed, discarded and the glass jars containing beans were kept under the same experimental conditions until the emergence of F1 progeny. The emerged F1 insect's was counted to evaluate the effect of essential oils on the progeny production of *A. obtectus* adults. Percentage of reduction in progeny production was calculated and the damaged seeds were weighed to assess weight loss.

Evaluating antifungal activity of essential oils extracted from Piper capense, Xylopia parviflora, Echinops giganteus and Mondia whitei against different fungi isolated from market bean seeds

Thirty-six samples of bean were collected from three markets in Dschang town, at the rate of 12 from each market. To isolate post-harvest pathogens, 10 seeds of each sample were placed in Petri dishes on potatoes dextrose agar (PDA) for 7 days at 21±2 °C. The pure isolated fungi were morphologically identified according to the documented keys in fungal identification (Champion, 1997; Mathur and Kongsdal, 2003) and were subcultured and stored in a fridge (4 °C) until needed. Among these, 8 filamentous fungi (*Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Fusarium moniliforme, Fusarium nivale, Fusarium solani, Fusarium crookwellense,* and *Penicillium* sp) were used to evaluate the antifungal activity of essential oils. The antifungal activity of the essential oils was performed following the procedure described (Ngono et al., 2000). The minimal inhibitory concentration (MIC) which inhibits the visible growth of fungi was recorded. For confirmation of the fungistatic or fungicidal activity, all wells showing no visible growth after 7 days were subcultured onto potatoes dextrose agar medium and incubated at 25°C for 10 days and the minimal fungicidal concentration (MFC) was recorded as the lowest concentration where no fungal growth was observed.

Results

Chemical composition of the essential oils

The essential oils of *P. capense* and *X. parviflora* consisted mainly of hydrocarbon monoterpenes (56.5% and 50.0% respectively), followed by hydrocarbon sesquiterpenes (17.8%) for *P. capense* and oxygenated monoterpenes (20.7%) for *X. parviflora*, whereas *E. giganteus* was mostly constituted of sesquiterpenes (94.3%) in which the tricyclic compounds are more abundant. A major compound, 2-hydroxy-4-methoxy-benzaldehyde (81%) was identified in the essential oil of *M. whitei* (Tab. 1).

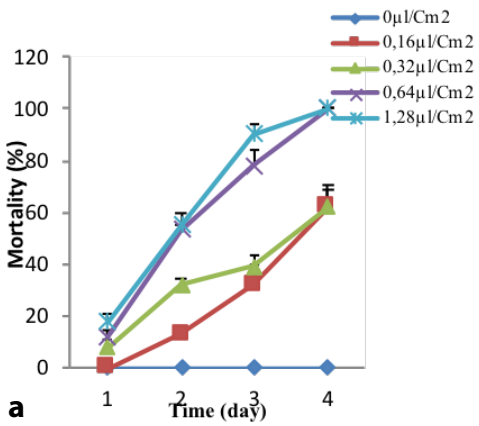
Tab. 1. Chemical composition of the essential oil from *Echinops giganteus, Piper capense* and *X. parviflora*.

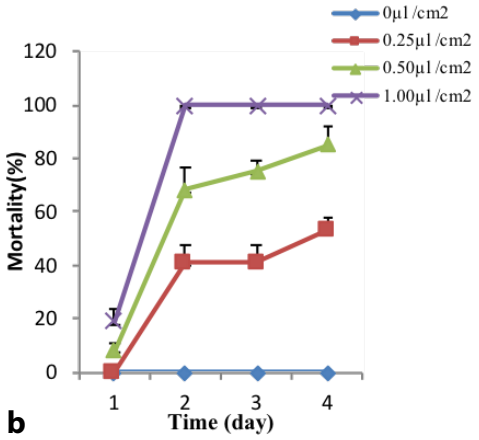
	Essential oils (%)		
Compound name	Echinops giganteus	P. capense	Xylopia parviflora
4-hydroxy-4-methyl-2-pentanone	/	/	0.3
α-thujene	/	0.6	0.1
α-pinene	/	8.9	10.3
camphene	/	0.6	3.3
sabinene	/	10	/
β-pinene	/	33.2	34
myrcene	/	0.9	0.1
δ-3-carene	/	0.5	/
p-cymene	/	0.4	1.3
limonene	/	1.8	0.6
1,8-cineole	/	0.4	1.7
Cis-sabinene hydrate	/	0.3	/
linalool	/	1.3	0.9
α-campholenal	/	/	0.2
trans-pinocarveol	/	0.6	5.0
camphor	/	0.2	0.4
pinocarvone	/	0.1	0.4
borneol	/	0.6	0.5
terpinen-4-ol	/	1.2	0.8
p-cymen-8-ol	/	0.5	0.1
α-terpineol	/	0.6	1.0
myrtenal	/	0.3	2.5
myrtenol	/	0.4	4.6
verbenone	/	/	0.5
isobornyl acetate	/	2.0	1.0
7-epi-silphiperfol-5-ene	3,5	/	/
α-cubebene	/	0.4	0.3
cyclosativene	/	0.2	0.5
modheph-2-ene	3,0	/	/
α-copaene	/	0.6	0.5
silphiperfol-6-ene	23,0	/	/
α-isocomene	2,4	/	/
β-cubebene	/	1.2	0.3
β-elemene	/	1.0	0.5
β-isocomene	2,1	/	/
(E)-caryophyllene	6,3	6.3	0.2
β-copaene	/	0.1	1.1
Gamma-elemene	/	0.3	/
6,9-guaiadiene	/	0,2	/
α-humulene	2,0	1.1	0.1
(E)-β-farnesene	/	0.1	/
germacrene D	0,3	3.8	/
β-selinene	/	0.3	0.1
trans-muurola-4(14), 5-diène	/	0.1	0.1
α-muurolene	/	0.6	0.6
silphiperfolan-6-α-ol	1,0	/	/
cameroonan-7-α-ol	7,1	/	/
silphiperfolan-7-β-ol	2,5	/	/
trans-calamenene	/	/	1.4
δ-cadinene	0,3	0.8	/
hedycaryol	/	/	1.2
germacrene B	/	0.6	/
silphiperfolan-6-β-ol	1,7	/	/
(E)-nerolidol	/	1.5	/
prenopsan-8-ol	3,2	/	/
caryophyllene oxide	/	2.8	2.1
presilphiperfolan-8-ol	22,7	/	/
salvial-4(14)-en-1-one	/	0.2	0.3
humulene epoxide II	/	0.3	0.8
1,10-di-epi-cubenol	0,1	0.4	2.2
caryophhyla-4(12),8(13)-dien-5-ol	/	0.1	0.7
epi-α-muurolol	0,4		
cubenol	/	0.3	0.8
α-muurolol	0,1	0.4	2.1
α-cadinol	0,4	/	/
cis-calamenen-10-ol	/	/	0.3
trans-calamenen-10-ol	/	/	0.2
eudesma-4(15),7-dien-1-β-ol	/	0.3	1.0
curcuphenol	0,4	/	/
manoyl oxide	/	/	1.0
phyllocladene	/	/	0.2

Toxicity of extracted essential oils to Acanthoscelides obtectus

The toxicity of essential oils applied on filter paper was dose-dependent because weevil mortality increased with concentrations of the oils, except for *M. whitei* which did not show any activity against *A. obtectus* (Fig.1). Essential oil extracted from *X. parviflora* fruits was the most effective at all concentrations evaluated. At the highest concentrations (0.31 and 0.47µl/cm2), it caused 100% mortality within the first day, whereas the essential oils from *E. giganteus* and *P. capense* caused weevil mortality after 2 and 4 days exposure, respectively. The LC50 of essential oils extracted from *E. giganteus, P. capense* and *X. parviflora* when used as physical contact poisons against *A. obtectus* were 0.35 µl/cm2, 0.31µl/cm2 and 0.17µl/cm2, respectively.

After 24h, the lowest concentration of all fumigated essential oils had strong toxic action (between 98 and 100% mortality) against *A. obtectus* (Fig. 2). Between 6h and 12h, the mortality induced by all the concentrations was significantly different ($P<0.05$). The LC50 values after 12h were 0.15 µl/cm3, 0.08 µl/cm3 and 0.05µl/cm3 for *M. whitei, P. capense* and *X. parvflora*, respectively. The essential oil from *E. giganteus* was not effective against *A. obtectus* at all the tested concentrations when used as a fumigant. The mortality of *A. obtectus* due to fumigation with essential oils also increased with the dosage of oil and the exposure time. Essential oil *of X. parviflora* was the most effective against A. *obtectus* on beans. Indeed, its highest doses (0.04 and 0.06µl/g) caused 100% mortality on the first day. The LD50 of the essential oils calculated after 2 days of exposure when used as fumigants against *A. obtectus* adults were 0.80µl/g, 0.60µl/g and 0.19µl/g for *M. whitei, P. capense* and *X. parvflora*, respectively.





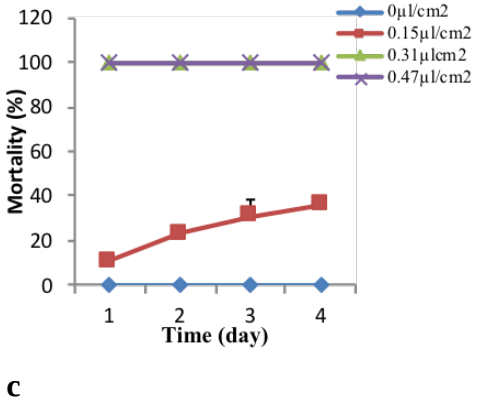
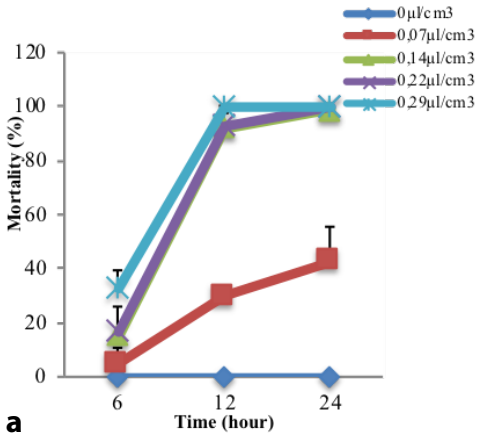
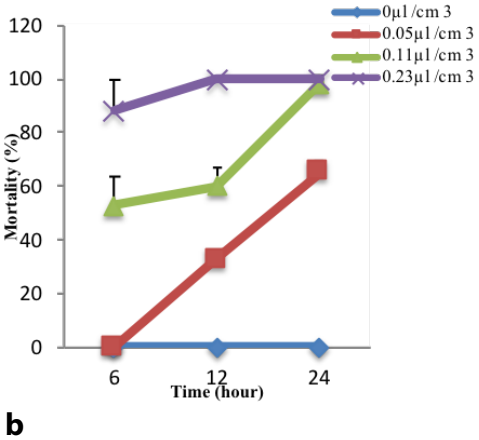


Fig 1: Mortality percentage (CM±SD) of *Acanthoscelides obtectus* as a function of time and concentration of essential oils of *Echinops giganteus* (a), *Piper capense* (b) and *Xylopia parviflora* (c) on filter paper. CM: corrected mortality; SD: standard deviation





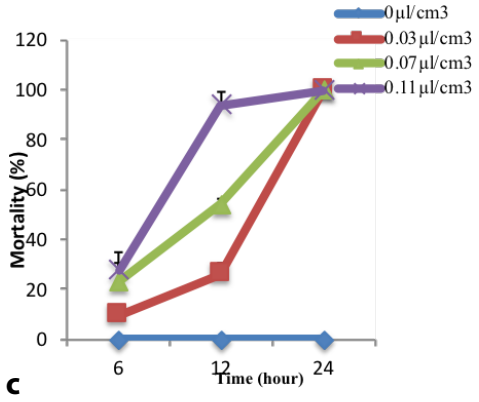


Fig 2: Mortality percentage (CM±SD) of *Acanthoscelides obtectus* as a function of time and concentration of essential oils of *Mondia whitei* (a), *Piper capense* (b) and *Xylopia parviflora* (c) fumigated. CM: corrected mortality; SD: standard deviation.

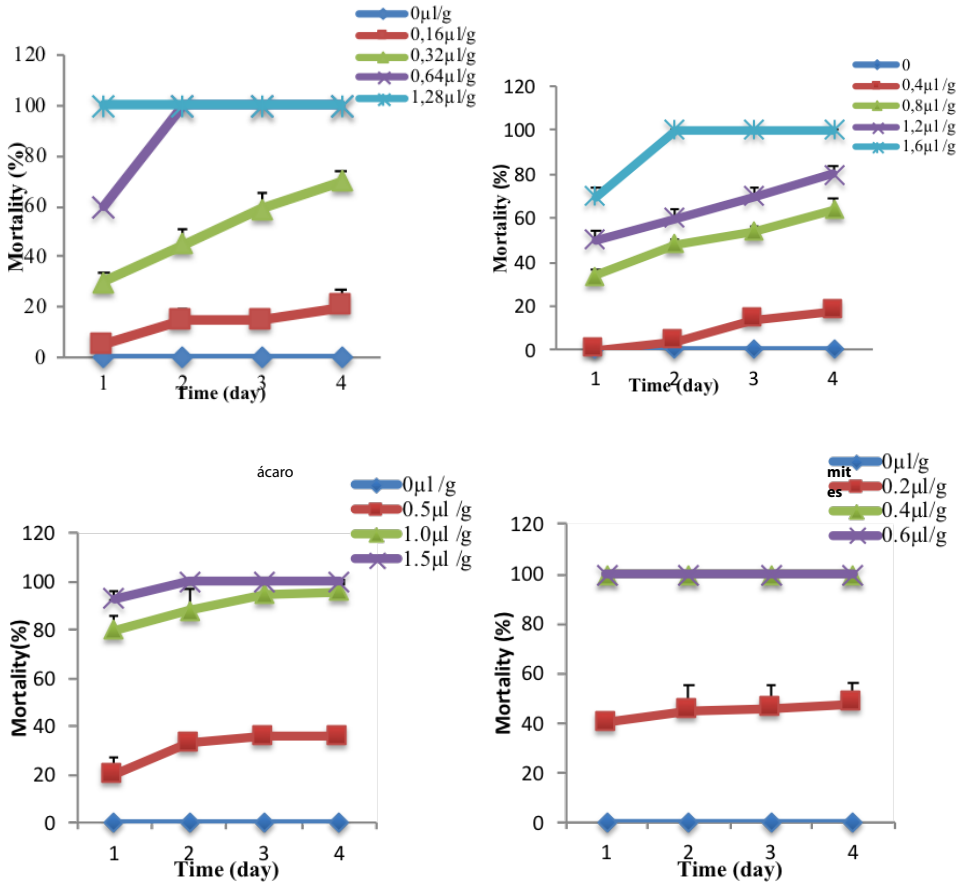


Fig 3: Mortality (CM±SD) of *Acanthoscelides obtectus* as a function of time and concentration of essential oils of *Echinops giganteus* (a), *Mondia whitei* (b), *Piper capense* (c) and *Xylopia parviflora* (d) on bean grains. CM: corrected mortality; SD: standard deviation

Effect of essential oils on F1 progeny production of Acanthoscelides obtectus infesting stored beans

All doses of essential oils caused significant reduction in F1 progeny of *A. obtectus* (Tab. 1). No progeny emerged in grains treated with the highest dosages of the four essential oils. Subsequently, all the doses of the oils reduced the grain weight loss, since it is proportional to the number of insects that emerge.

Tab. 2: Effect of dosage of essential oils of *Echinops giganteus*, *Piper capense, Mondia whitei* and *Xylopia parviflora* on F1 progeny production in stored beans and weight of beans after emergence.

Plant	Dose (µl/g of grains)	Number of emerged insects (F1)	Percentage of inhibition of adults at F1	Weight of bean after emergence
Echinops giganteus	0.00	273.00±88.29a	00.00	46.30±0.03a
	0.16	65.00±6.82b	76.19	48.12±0.02b
	0.32	10.00±4.92b	96.32	49.98±0.01c
	0.64	0.00 ± 0.00c	100.00	50.00 ± 0.00c
	1.28	0.00 ± 0.00c	100.00	50.00 ± 0.00c
Mondia whitei	0.00	220.00±9.50a	00.00	46.22± 0.22a
	0.4	178.00± 4.65b	19.09	47.12± 0.06b
	0.8	112.00 ± 1.50c	49.09	48.60 ± 0.12c
	1.2	24.00± 3.36d	89.09	49.86 ± 0.04d
	1.6	0.00 ± 0.00e	100.00	50.00 ± 0.00e
Piper capense	0.00	73.00±43.85a	0.00	48.33 ± 0.97a
	0.5	43.00 ± 34.68a	41.09	49.08± 0.84a
	1.0	0.00 ± 0.00b	100.00	50.00 ± 0.00b
	1.5	0.00 ± 0.00b	100.00	50.00 ± 0.00b
Xylopia parviflora	0.0	331.00 ± 45.23a	0.00	46.84 ± 0.36a
	0.2	299.00± 84.62a	10.21	46.94 ± 1.23a
	0.4	00.00 ± 0.00b	100.00	50.00 ± 0.00b
	0.6	00.00 ± 0.00b	100.00	50.00 ± 0.00b

Anti-fungal activity of extracted essential oils

Several fungal species were isolated and identified on bean seeds collected in Dschang town; the main species were *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, penicillium sp., Rhizopus stolonifer, Rhizoctonia solani* and *Mucor* sp. The genus *Aspergillus* is most prevalent (58%) followed by the genera *Fusarium* (16%), *Penicillium* (12%), while the genera *Mucor* (6%), *Rhizopus* (3%), *Rhizoctonia* (2%) and others (3%) were the least prevalent.

Mondia whitei essential oil showed the best antifungal activities (MIC = 0.06 – 1.02 mg/ml). Among the eight fungal species tested, *A. niger* was the most sensitive to the treatment, while *Penicillium* sp., *F. oxysporum* and *F. moniliforme* were the most resistant. *Piper capense*, *D. glomerata* and *X. parviflora* showed low antifungal activity on all fungal isolates tested with MIC values between 4.1 and 16.32 mg/ml while *E. giganteus* did not show any antifungal activity.

Tab. 3: Minimal Inhibitory Concentrations (MIC) and Minimal Fungicidal Concentrations (MFC) results for antifungal activity of essential oil from *Mondia whitei.*

	Mondia whitei		**Mancozeb**	
Fungi species	**CMI(mg/ml)**	**CMF (mg/ml)**	**CMI(mg/ml)**	**CMF(mg/ml)**
A. flavus	0.51	0.51	0.51	1
A. niger	0.06	0.51	0.51	1
F. solani	0.25	0.51	0.25	1
F. nivale	0.51	1.02	0.25	1
Penicillium sp.	1.02	2.04	0.25	1
F. oxysporum	1.02	2.04	0.12	1
F. crookwellense	0.51	2.04	0.12	1
F. moniliforme	1.02	1.02	0.51	1

Conclusion

A good level of control of *A. obtectus* was achieved with essential oils of *E. giganteus*, *M. whitei*, *P. capense* and *X. parviflora*, which successfully reduced *A. obtectus* progeny production and bean loss. At the same time, the antifungal activity of these essential oils gives new opportunity for the control

of bean pathogens. Overall, essential oils extracted from these spices could play an important role in stored bean protection and reduce the risk associated with the use of synthetic insecticides.

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Sustained effective use of phosphine in stored product protection in India: Role of UPL Limited

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Phosphine has a predominant role in stored products protection in India since more than 4 decades. Its use has gained further prominence ever since methyl bromide has been withdrawn (except QPS applications) on environmental concerns. Accordingly, the use of phosphine is being expanded to QPS treatment of certain commodities. Phosphine has several merits and as a stored product fumigant. However, there is a concern about occasional failure to achieve desired 100% mortality of insect pests during phosphine treatment in the country. Hence the factors contributing for control failures have been identified. Also there are reports about need to improve existing fumigation practices and to create awareness about the required parameters to ensure successful treatments. In this context UPL Limited, a leading manufacturer of metal phosphide formulations in the world took important steps: A. To create awareness about proper sealing of fumigation enclosures, phosphine dosage, exposure period and target terminal concentration parameters B. To impart practical demonstrations to the stakeholders in across the country details of phosphine fumigation workshops, demonstrations and industry & end user & farmers interactions conceptualized, funded and executed by UPL Limited in coordination with other lead agencies, will be discussed. Furthermore, focus on the use of on-site phosphine generators which has the advantage of rapid generation and even distribution of the gas facilitating successful treatments by way of demonstration to different end users has also been presented.

Recent Developments in the Global Application of ECO2FUME® and VAPORPH3OS® Phosphine Fumigants

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Abstract

ECO2FUME® (2% phosphine, 98% CO2 by weight) and VAPORPH3OS® (99.3% phosphine average by weight) are cylinderized gas formulations of phosphine that have achieved significant growth in commercial applications for the disinfestation of food and non-food commodities in the last two decades. The expansion in the global application of these two cylinderized phosphine fumigants is driven by increasing concern for safety, efficacy, unreacted powdered residue and disposal associated with aluminium phosphide tablets, which are promoted as alternatives to methyl bromide, and the concern of insect resistance to phosphine in both developed and developing countries. This paper describes recent developments in the global application of ECO2FUME® and VAPORPH3OS® in terms of commercial in-transit fumigation of grains and logs in ships, fumigation of export distiller's dried grain with solubles (DDGS) in containers and shiphold, best practices in the management of phosphine resistance of insects in grains, and establishment and application of quarantine and preshipment (QPS) phosphine fumigation protocols for selected fresh fruits, vegetables, dried fruits and cut flowers as an alternative to methyl bromide. The growing issue of powdered residue from unspent aluminium phosphine tablets and the use of cylinderized phosphine as an effective solution are discussed.

Keywords: ECO2FUME®, VAPORPH3OS®, cylinderized phosphine, Horn Diluphos System, fumigation applications, aluminium phosphide tablets issue, alternative to methyl bromide, phosphine resistance management

1. Introduction

Among the existing fumigants commercially available in the market, phosphine is considered the most cost-effective and widely used fumigant for protection against stored product pests worldwide. Although using phosphine in a solid metal phosphide formulation has drawbacks --it is slow acting, self-ignites when exposed to air, and requires deactivation and disposal of unspent residue--these disadvantages have been overcome with the introduction of ECO2FUME® and VAPORPH3OS® cylinderized phosphine fumigants.

ECO2FUME® is a cylinderized formulation of a nonflammable, ready-to-use liquefied gas mixture of 2% phosphine and 98% carbon dioxide (CO2) by weight. It is packaged in high-pressure aluminum or steel cylinders with a net fumigant weight of 31 kg and 620 grams of phosphine. Though the phosphine/CO2 mixture is liquefied inside the cylinder, it is converted immediately to gaseous form upon release into the atmosphere. The formulation requires simple dispensing equipment made of either a stainless steel double-braided hose or a high-pressure hydraulic hose designed to deliver the fumigant as quickly or slowly as required by individual applications. VAPORPH3OS® is 99.3% average phosphine purity by weight and is designed for use with approved blending equipment for safe on-site dilution with CO2 or air in nonflammable proportions. It comes in steel cylinders with a net fumigant weight of 22 kg. Due to the larger amount of phosphine contained in a cylinder, VAPORPH3OS® is most suitable for larger volume applications for which it is not practical to store, handle or transport large numbers of cylinders; for price-sensitive applications such as grains; and for locations that conduct frequent fumigations (Tumambing et al., 2012).

ECO2FUME® and VAPORPH3OS® offer numerous benefits. The conduct of fumigation can be completed safely because it is applied externally to the fumigation structure, thereby removing confined space entry, reducing worker exposure and eliminating retrieval of 3 – 5% unspent powdered residue associated with metal phosphides (van Graver 2001). Due to its gaseous form, there is no residue that requires waste deactivation or disposal. When phosphine gas is vented into the atmosphere during aeration, it will react with oxygen in the air and, in the presence of sunlight, will readily convert to phosphoric acid. It is environmentally friendly as it is non-ozone depleting and does not contribute significantly as a greenhouse gas. It has a non-phytotoxic property and, therefore, does not damage sensitive commodities such as cut flowers, fruits and vegetables during fumigation. Required fumigation time is considerably faster using a phosphine gas than a metal phosphide formulation because the gas mixture can be easily applied to quickly distribute and uniformly achieve the target concentration. Compared to methyl bromide, the dose level for phosphine gas is at least 20 times less in terms of g/m3 application rate for phosphine

environmental and safety benefit. Additionally, phosphine gas is more penetrating and has extremely active molecules, which will enable the gas to distribute quickly and uniformly inside the fumigation structure without the help of a blower. Better gas distribution results in more effective control of target insects. Target concentration can be maintained quickly and safely anytime during the fumigation by top-up, which also decreases the amount of phosphine that needs to be applied.

Through the years and over nearly two decades, ECO2FUME® and VAPORPH3OS® have achieved significant growth in commercial application for the disinfestation of food and non-food commodities. The expansion in the global application of these two cylinderized phosphine fumigants has been driven by increasing concern for safety, efficacy, unreacted powdered residue and disposal associated with aluminium phosphide tablets; alternatives to methyl bromide; and the spread of insect resistance to phosphine in both developed and developing countries.

Application for In-Transit Fumigation of Grains and Logs in Ships

In-transit fumigation of grains and logs in ships has been practiced for several years around the world using aluminum phosphide tablets or blankets. However, the use aluminum phosphide in ships has been associated with risk of ignition, fire and explosion, particularly during aeration or gas venting due to ingress of high-humidity air and exposure of still-active fumigant inside the hold, which leads to quicker generation of phosphine gas, exceeding the low flammability limit. Additionally, the use of aluminum phosphide tablets leaves unspent powder residue that contaminates the fumigated commodity in the ship.

Due to safety issues and residue contamination of fumigated commodities in ships, in recent years ECO2FUME® and VAPORPH3OS® have been utilized in commercial fumigation of grains and logs in ships during in-transit journeys at sea. In Turkey, ECO2FUME® is being used for in-transit fumigation of exported corn and soy beans in the Black Sea region. A typical cargo ship with approximately 45,000 m3 volume capacity containing about 30,000 tons of corn and soy bean can be fumigated while in transit for 14 days using a phosphine dose of 400 ppm. As shown in Fig. 1, the complete setup for dispensing ECO2FUME® involved the following (Goztas et al., 2015):

1. A perforated drainage pipe was placed at the bottom of the ship hold.
2. The drainage pipe and a recirculation pipe were connected via a “T” connection.
3. A blower was attached to the recirculation pipe to ensure movement of the gas.
4. After loading of the commodity, the recirculation pipe was placed on top of the commodity.
5. ECO2FUME® cylinders were secured and connected to the system via a standard manifold (Fig. 2).
6. ECO2FUME® was then dispensed into the hold.

In Australia, VAPORPH3OS® in combination with the Horn Diluphos System (HDS) has been used for in-transit fumigation of export grains destined to the Middle East as a mitigation measure. The option of using VAPORPH3OS® instead of aluminium phosphide tablets was prompted when the importing country rejected fumigated grains contaminated with powdered residue. The fumigation protocol and overall setup of gas dispensing and gas distribution is similar to ECO2FUME® except for using VAPORPH3OS® and an HDS fumigation machine. Fig. 3 and 4 show a setup using VAPORPH3OS® and HDS 200 inside a trailer and dispensing the gas through a flexible distribution hose into a hatch in the shiphold (courtesy of Ball, 2016).

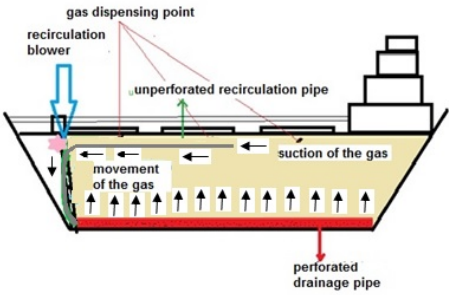

Fig. 1 Schematic of setup of ECO2FUME® during gas dispensing and distribution inside the shiphold.

Fig. 2 Eight ECO2FUME® cylinders secured and connected in manifold during gas dispensing through the hatch.

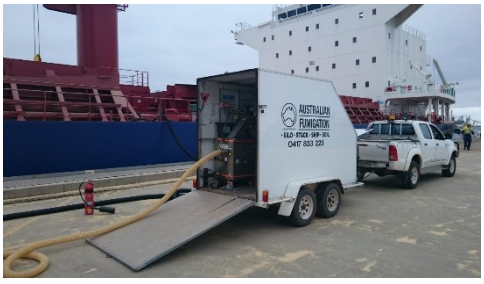

Fig. 3 VAPORPH3OS® and HDS 200 inside a trailer ready for dispensing gas into a shiphold.

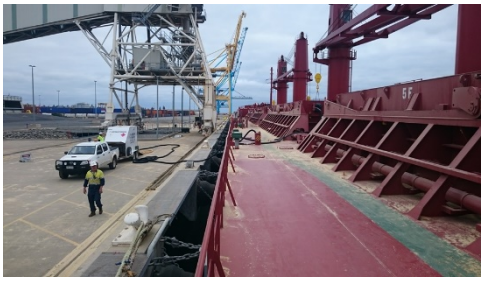

Fig. 4 Setup of flexible gas distribution hose between the trailer and hatch in a shiphold.

For additional safety when using ECO2FUME® and VAPORPH3OS®, guidelines on the use of pesticides applicable to fumigating cargo in ships set by the International Maritime Organization (IMO 2010) are being followed. IMO guidelines have specific instructions for pre-inspection of shipholds for the presence of cracks, and gas monitoring for the presence of gas leaks outside the shiphold and inside the cabin of the crew.

Fumigation of logs with VAPORPH3OS® and HDS destined to China has been practiced in Uruguay in the past two years. Previously, China has accepted the use of phosphine from aluminium phosphide tablets and blankets for in-transit fumigation of logs using a fumigation protocol of 2 g/m3 of phosphine as an initial dose at the wharf and a second dose of 1.5 g/m3 of phosphine as a top-up 5 days later while on the ship to maintain a phosphine concentration of 200 ppm for 10 days (Zhang and van Epenhuijsen, 2005). Chinese authorities have accepted the use of VAPORPH3OS® for in-transit fumigation of logs from Uruguay. In the case of VAPORPH3OS® the fumigation time is cut in half to 5 days using a one-off dose of 3.5 g/m3 of phosphine conducted while the ship is at wharf. With the use of VAPORPH3OS® and HDS, fumigation of logs is accomplished safer and quicker, eliminating the risk of having a fumigator on board to open the hatch and do complete top-up 5 days into the sea journey. The added benefit of using VAPORPH3OS® for in-transit fumigation of logs is the elimination of unspent powdered residue from aluminium phosphide tablets, which is currently not accepted by Chinese authorities.

Fumigation of Export Distillers Dried Grain with Solubles

Distillers dried grain with solubles (DDGS) as a by-product of ethanol production from corn is produced in large quantities in the US and became popular as a feed ingredient for export. However, in the past, as the Vietnamese quarantine authority banned the export of DDGS from the US due to

repeated insect infestation when the grain reached the destination port in Vietnam. The insect infestation issue was resolved with the establishment of fumigation protocols for phosphine gas for complete treatment against insect pests. With new protocols in place, the Vietnam Plant Protection Department lifted the ban on imported DDGS from the US effective September 2017 (Trung, 2017). The new protocols combine phosphine concentration, exposure time and temperature, as indicated below:

- 750 ppm for 3 days at >20°C
- 750 ppm for 4 days at 15 - 20°C
- 750 ppm for 5 days at 10 - 15°C

These protocols are applicable to both DDGS in shipping containers and larger bulk quantities in a shiphold with a gas recirculation system.

Best Practices in the Management of Insect Resistance to Phosphine in Grains

Insect resistance to phosphine has become a major concern in many countries where grains are harvested and stored for a period of 6 months or longer under high temperature and relative humidity favorable for insect growth and reproduction. The phosphine resistance issue emerged due to the use of leaky structures and poor fumigation practices associated with aluminium phosphide tablets. Aside from the common strong resistant strains of the lesser grain borer (Rhyzopertha dominica), rice/corn weevil (Sitophilus sp.), red flour beetle (Tribolium castaneum) and saw toothed grain beetle (Oryzaephilus surinamensis) another strongly resistant rusty grain beetle (Cyptolestes ferrugineus) is added to the list. This particular insect pest is a concern in Australia, China, India, Thailand, Vietnam, the Philippines and Brazil, among other countries. A series of efficacy studies and field validation trials conducted by the Postharvest Grain Protection Unit of the Queensland Department of Primary Industries have identified the strong resistant rusty grain beetle and established effective fumigation protocols for complete treatment of this insect pest (Kaur and Nayak, 2014; Nayak and Kaur, 2016). The fumigation protocols for the strong resistant rusty grain beetle are shown in Tab. 1 and are now part of the ECO2FUME® and VAPORPH3OS® label in Australia.

Tab. 1 Fumigation protocols for the strong resistant rusty grain beetle as part of the ECO2FUME® and VAPORPH3OS® Australian label

Commodity	Minimum Application Rate (g/m3) & Minimum Phosphine Concentration (ppm)		
Temperature	0.5 g/m3 (360 ppm)	1.0 g/m3 (700 ppm)	1.4 g/m3 (1000 ppm)
20 – 24°C	30 days	23 days	na
25 – 29 °C	27 days	18 days	12 days
30 - 34°C	na	na	10 days
35°C or higher	na	15 days	6 days

In the US, the USDA Agricultural Research Station conducted phosphine resistance management studies entitled "Technical Framework for Using Cylinderized Phosphine for Managing Phosphine Resistance" and presented the following findings (Walse et al., 2017).

Resistant pests can be effectively controlled with the right dose of phosphine.

- More is not always better - there is a "Sweet Spot" Phosphine concentration, such as 500 – 1000 ppm for lesser grain borer and red flour beetle.
- Narcosis threshold for eggs of major grain insects treated with phosphine is generally ≥ 1000 and ≤ 2000 ppm depending on the insect species.
- Phosphine concentration higher than the threshold concentration will result in narcosis, and much longer time is required to kill the egg stage.
- As shown in Fig. 5, the sweet spot phosphine concentration for the egg stage of susceptible and resistant strains of red flour beetle (RGB) is similar; however, eggs of resistant strains take

relatively longer time to kill compared to eggs of susceptible strains at a given phosphine concentration.

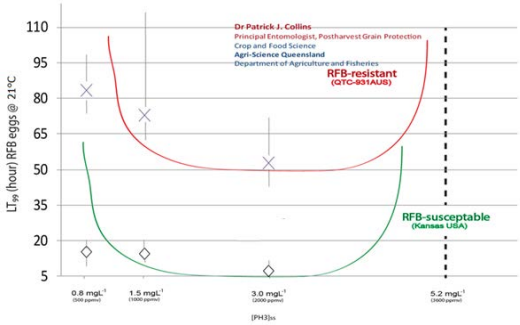

Fig. 5 Time in hours to reach LT99 of susceptible and resistant strains of red flour beetle (RGB) at different phosphine concentrations and 21°C temperature.

Phosphine fumigation protocols for quarantine and pre-shipment

Over the years, phosphine fumigation protocols have been developed in different countries as alternatives to methyl bromide for quarantine and pre-shipment application for selected fruits and vegetables, cut flowers, dried fruits, pine timber and associated nematodes, coffee and cocoa beans. These protocols were established under lab and field scale efficacy studies as conducted by accredited research institutions in different countries. Tab. 2 shows the different phosphine gas fumigation protocols for QPS treatment of selected commodities and corresponding research institutions.

Tab. 2 Phosphine gas fumigation protocols for QPS application of selected exported and imported commodities in different countries.

Commodity	Plant Pest Type	Phosphine Conc. (Minimum)	Exposure Time	Temperature	Reference
Pineapple	Purple scale, Citrus mealy bug	1400 ppm	24 hours	5 oC or higher	NPQS Korea 2015
Citrus	Queensland fruit fly (Bactrocera tyroni)	1400 ppm	48 hours	23 – 25°C	Williams 2000
Citrus	Citrus red scale	1500 ppm	48 hours	5°C	USDA ARS 2014
Mango	Fruit fly	1400 ppm	24 hours	26 - 33°C	NPQS Sri Lanka 2017
Bitter Gourd	Melon fly	1400 ppm	24 hours	26 - 33°C	NPQS Sri Lanka 2017
Cut Flowers (chrysanthemum, rose, lily)	Western flower thrips, two spotted spider mites, cotton aphids	1400 ppm	24 hours	8oC or higher	NPQS Korea 2015
Dracaena house plants	Purple scale, aphids, white fly, scales	1400 ppm	24 hours	15 oC or higher	NPQS Korea 2015
Mushrooms	Lycoriella mali	1400 ppm	24 hours	5 oC or higher	NPQS Korea 2015
Timber pine Pine Nut pine	Pine weevil, white ant, Bursaphelenchus xylophilus, Monochamus alternatus, Monochamus saltuarius	2800 ppm	5 days	5 oC or higher	NPQS Korea 2015
Pineapple	Planococcus minor	200 ppm	7 hours	26 – 30 oC	BIOTROP 2012
Mangosteen	Planococcus minor	200 ppm	7 hours	26 – 30 oC	BIOTROP 2012
Orchids	Planococcus minor	200 ppm	7 hours	26 – 30 oC	BIOTROP 2012
Dried Fruits	Ephestia CautellaPlodia Interpunctella	1000 ppm	24 hours	20 - 27°C	Ankara Univ. 2013
Dates	Ephestia Cautella Red flour beetle Saw toothed grain beetle	700 ppm 1000 ppm 1500 ppm	72 hours 48 hours 24 hours	30°C or higher	ARC Egypt 2013

Conclusions

1. ECO2FUME® and VAPORPH3OS® phosphine fumigants offer advantages over methyl bromide in terms of lower dose for phosphine environmental and safety benefits, greater gas penetration and quicker gas distribution into bulk commodities without the need for recirculation blower and without the need for a heater/vaporizer to dispense the fumigant in form.
2. ECO2FUME® and VAPORPH3OS® phosphine fumigants offer greater benefits over metal phosphide than metal phosphide formulation in terms of reduced worker exposure, no need of deactivation and disposal in unreacted residue, non-phytotoxic to sensitive commodities and quicker fumigation time.
3. The application of these two cylinderized phosphine fumigants has expanded to commercial fumigation of grains and logs in ships during in-transit journeys at sea and fumigation of export distiller's dried grain with solubles (DDGS) in containers and shipholds.
4. The emergence of phosphine resistance insects particularly in grains and oilseeds can be better addressed with the use of cylinderized phosphine fumigants due to its ability to be controlled and maintained at higher dose and longer exposure time.
5. There is sweet spot phosphine concentration that should be used for effective control of resistant insects. Above the sweet spot phosphine concentration narcosis effect could set in resulting in much longer time to kill the egg stage.
6. Phosphine gas fumigation protocols have been developed as alternative to methyl bromide for quarantine and pre-ship treatment of selected fruits and vegetables, cut flowers and nursery trees, dried fruits and pine timber and associated nematodes.

Disclaimer

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Effects of Myristica fragrans and Alpinia conchigera oils against *Callosobruchus maculatus*

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Efficacy of *Myristica fragrans* and *Alpinia conchigera* oils were evaluated against *Callosobruchus maculatus* at Post-harvest Technology on Field Crops Research and Development Group, Post-harvest and Processing Research and Development Office during October 2014 to September 2015. Seed of *M. fragrans* and rhizomes of *A. conchigera* were extracted the essential oils. It was identified the chemical composition by GC-MS which 10 and 12 constitutes were found on *M. fragrans* and *A. conchigera* oils. The major component of *M. fragrans* and *A. conchigera* oils were sabinene and 1,8-cineole, respectively. Contact toxicity assay on filter paper of both essential oils, the LC50 value of *C. maculatus* adults when treated with *M. fragrans* oil at 72 h were 4.6 µL/cm2 while 1.7 µL/cm2 for *A. conchigera* oil. Furthermore, the number of laid egg and adult progeny production of *C. maculatus* were inhibited by treated with *M. fragrans* and *A. conchigera* oils at 8 and 10% under laboratory condition. In additions, the efficacies of both essential oils were conducted for 6 months at warehouse of Lopburi Agricultural Research and Development Center. The results showed that insect pests and natural enemies were more found in the mung bean treated with *M. fragrans* oil than *A. conchigera* oil and *C. maculatus* was the major pest. Furthermore, *C. maculatus* was found on mung bean that coating with *M. fragrans* oil than *A. conchigera* oil. Both essential oils were control insect pests for 1 month.

Insecticidal and larvicidal activities of Cinamic acid esters isolated from Ocimum gratissimum L. and Vitallaria paradoxa leaves against Tribolium castaneum Hebst (Coleoptera:Tenebrionidae)

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Insect pest of stored products is one major threat to food safety globally. Various techniques are being employed to address these pest problems. Pest management using botanicals have been widely practiced in recent times. The natural compounds present in these botanicals have been known to be responsible for the protection they offer against insect pests. Some of these compounds may act as single compounds to produce an effect or may be synergistically effective. In the present study using a bioassay guided approach, two cinnamic acid derivatives; Methyl cinnamate and Sitosterol cinnamate were isolated from the leaves of O. gratissimum and V. paradoxa respectively. Adults and a week old larvae of T. castaneum were dipped in the samples and transferred into clean petri dishes containing wheat flour and observed for mortality or larval growth activity. These compounds show high levels of insecticidal, larvicidal and larval growth inhibition activities against T. castaneum. The LC50 of methyl cinnamate was determined to be 26.92 mg/mL against the adult, 8.31mg/mL against the larvae whiles the LC50 of sitosterol cinnamate was determined to be 6.92 mg/mL against the adult and 3.91 mg/mL against the larvae. Generally, the susceptibility of adult T. castaneum to these cinnamic acid esters can be directly associated with the concentration as well as time of exposure to the compounds. Several studies have confirmed the safety of cinnamic acid esters by evaluating acute toxicity, skin irritation and genotoxicity and therefore can be used safely for stored product protection.

Assai (Euterpe oleracea Mart.) fruit: Green method development by Andiroba oil (*Carapa guianencis* L.) for Hemiptera control

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The assai (*Euterpe oleracea* Mart.), in Portuguese açaí, is a Brazilian fruit grown mainly in the Amazon forest (Northern region) and Cerrado (Northeastern region) which has a quite high staple & economic importance to the country. It is part of the region's culture and the fruit is consumed in salty dishes (mixed with cassava or tapioca flour and also with fried fish) by the natives. Although the main consumption is in those regions, its frozen pulp is the best-known worldwide available product (exported to the United States & European Union, mainly) and has increased in recent years. Apart from Amazon forest natives high lipid and protein food source, assai fruit is rich in antioxidants (anthocyanins & flavonoids), with high levels of vitamin C and fibers - that makes it highly consumed by the youngsters and sports people (as diet supplement - frozen pulp / ice-cream). Despite that, insects (Hemiptera *Triatoma brasiliensis*) infestation with subsequent disease development caused by *Trypanosoma cruzi* (parasite present in the mosquitoes faeces) may take place at the assai fruit stage. Considering the lack of information on assai fruit contamination and mosquito (parasite vector) control, the current study aimed to develop a green method through Andiroba oil (*Carapa*

guianencis L.) to control/repel that Hemiptera from the fruits (thus replacing chemical insecticides exposure). Andiroba oil and assai fruits utilized were from Belem city, Para state, Northern region of Brazil. They were divided into 2 main groups: treated (Treated Group - TG) and not treated (Control - C). The TG was sub-divided into TGI, TGII, TGIII, TGIV and TGV for the application of oil at different concentrations (10, 25, 30, 50 e 100%, respectively) and time of exposure (n=3). After oil treatment under controlled environment, the assai fruits insects were left standing 24 h, with their behavior variation observed (each 2 h) and the most effective concentration registered by decreasing order of efficacy was selected. As expected, on the assai surfaces`, the insect movements (distance) and speed reduced with the percentage of dead ones reaching to 100% as the oil concentration raised. The Andiroba oil green method could be a safe treatment to be utilized for assai insect infestation (instead of chemical insecticides) as the whole fruit is utilized n the de-pulping process.

Colour changes in pulses fumigated with different metal phosphide formulations

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Abstract

Many phosphine-emitting products are used globally to control insect pests in dried vegetables, grains and pulses. However, variation in phosphide formulations is associated with colour change in many pulses. This study evaluated the effect of fumigation using Mg_3P_2 containing ammonium carbamate; AlP containing no ammonium carbamate and pure ammonium carbamate on colour of diferrent pulses. Different pulses showed different reactions towards fumigation with phosphine. A distinctly darker discolouration was observed in broad beans and lentils when fumigated with ammonium carbamate containing Mg_3P_2 and pure ammonium carbamate, whereas there were no apparent colour changes in white kidney beans, soybeans and green peas. The use of ammonium carbamate-free AlP resulted in no changes in any of the pulses. Therefore, formulation type of the phosphine product plays a major role in the visible colour change of the pulses.

Keywords: phosphine, ammonium carbamate, pulse varieties, colour change

Introduction

In many fields of stored product protection one of the most important substances in use worldwide to control stored product pests is the fumigant phosphine. The most established method of distribution is the use of solid-based aluminium phosphide and magnesium phosphide products in the form of tablets, pellets, bags or plates. After distribution, existing stored product moisture or ambient moisture contribute to the release of the actual active substance itself: phosphine gas. The advantages of this gas are its excellent penetrative ability and its extraordinarily high rate of efficiency against all stock-damaging insects. In this respect, all developmental stages of storage insect pests can be easily controlled; these properties even deal reliably with those developmental stages living hidden within the stored products.

Phosphine also has favourable properties regarding innocuity and the formation of no residues in treated food and animal feed products. The gas has no negative impact on the treated products and volatilizes quickly after use. Therefore, no residues are to be expected in fumigated goods. Besides, quality parameters such as germability or taste are not negatively influenced by the gas. However, according to oral reports, colour changes have arisen in different types of pulses after fumigation with metal phosphide products. This present study emonstrates the extent phosphine is may be responsible or the circumstances under cwhich certain changes in product colour may be triggered by substances emitted from the product formulation enhancers.

Materials and Methods

Five types of pulses were selected for the studies: green peas (*Pisum sativum*), broad beans (*Vicia faba*), lentils (*Lens culinaris*), white kidney beans (*Phaseolus vulgaris*) and soybeans (*Glycine max*)

while the fumigants used were: MAGTOXIN Pellets (active substance Mg_3P_2, contains ammonium carbamate) at a dosage of 3.3 g PH_3 / m^3, DETIA-GAS-EX B (active substance AlP, contains no ammonium carbamate) at a dosage of 3.3 g PH_3/m^3 and pure ammonium carbamate.

The bioassays involving ammonium carbamate were carried out in a glass dessicator (diameter 320 x height 235 mm) with a perforated plate. A dish filled with ammonium carbamate was placed at the bottom of the dessicator below the perforated plate, and petri dishes containing the pulses were placed on the perforated plate. This arrangement allowed ammonia vapour escaping from the ammonium carbamate to circulate around the perforated plate unhindered. The dessicator was kept closed for 8 days. In parallel, fumigation assays using metal phosphide products were carried out in two fumigation chambers each with a volume of 0.5 m^3. The relevant amount of fumigant was introduced together with the dishes filled with the pulses. The designated period of exposure time was 8 days. The test conditions were 20°C and 65% relative humidity.

Results

After treating the various pulse seeds with ammonium carbamate (and the ammonia released from it), distinct colour changes were observed in some of the pulse varieties. Whilst white kidney beans, soybeans and green peas didn't show any apparent changes, a distinctly darker discolouration was observed in broad beans and lentils in comparison with the untreated pulses.

In the fumigation trials both products used achieved the expected maximum phosphine concentration of between 2,000 – 2,500 ppm after 48-60 hours. After the exposure time of eight days, the ammonium carbamate containing product MAGTOXIN Pellets resulted in the same colour changes which were observed when using pure ammonium carbamate. Again, broad beans and lentils were distinctly dark discoloured after the treatment whereas no changes were evident in the white kidney beans, soybeans and green peas. In contrast, the use of ammonium carbamate-free DETIA-GAS-EX B resulted in no changes in any of the pulses.

Discussion

The most important active ingredient worldwide used for controlling stored product pests is phosphine gas. Through its positive properties with regard to eco-toxicity as well as its good penetration properties and the associated excellent effectiveness against stored product pests, phosphine has become indispensable for successful stored product protection over decades. The fumigant is also known as a substance, which has no serious effects on the treated goods with regard to residues and quality.

The present trials therefore raised the question as to whether phosphine actually is responsible for apparent colour changes in pulses following their fumigation with metal phosphide products; or whether other substances in the composition of metal phosphide formulations might be held responsible for such changes.

Consideration should be given in particular to the ammonium carbamate often deployed in compressed formulations used to improve compressability and to regulate the outgassing behavior. This ammonium carbamate leads to the release of ammonia during the degassing process.

The results of comparable trials using pure ammonium carbamate as well as metal phosphide formulations with and without ammonium carbamate clearly show that to a large degree of certainty it may be assumed that the colour changes are caused by ammonia and not by phosphine.

Whereas no changes occured in the pulses following fumigation using the ammonium carbamate-free metal phosphide product 'DETIA-GAS-EX B', dark discolouration in various pulses was observed following treatment with the product containing ammonium carbamate "MAGTOXIN Pellets" as well as following treatment with pure ammonium carbamate.

RYMAN (2017) reported similar results after having treated various pulses with metal phosphides which contained or did not contain ammonium carbamate. After treatment with formulations

containing ammonium carbamate on a few pulses, he likewise reported dark changes in colour whereas none of the varieties displayed changes after having been treated with the ammonium carbamate-free product DEGESCH PLATE.

It is known that especially tannic woods display discolouration in the presence of ammonia and on contact with the substance darken in colour (SELL AND KÜHNE, 1967). This circumstance is consciously utilized, for example when "smoking" oakwood, in order to modify the colour of the wood (MARQUARDT, 2005).

MARQUARD (1998) describes that pulses might also contain tannic acid and tannins. The amount can vary from species to species and from type to type and ranges from more or less no tannic acid up to 4.5% in the dry weight of the pulses.

In this respect, it can therefore be assumed that the colour changes in individual pulses are caused by the reaction of the ammonia from metal phosphide formulations containing ammonium carbamate with the tannin contained in the various pulses.

In order to avoid visual changes in the form of dark discolouration in pulses after treatment with metal phosphide formulations the use of formulations which do not contain any ammonium carbamate is recommended for such treatment.

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The Postharvest Education Foundation's Role in Reducing Postharvest Losses

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Abstract

The Postharvest Education Foundation (PEF) was founded to address postharvest losses through education and training. Postharvest expertisewas identified as a key weakness in many developing countries. The PEF provides innovative programs that motivate and empower people to reduce food losses and waste. At the heart of the PEF is a structured e-learning program that provides a practical curriculum to address the causes of postharvest losses, as well as methods to minimize these losses for horticultural crops and staple foods. E-learning is an efficient and cost-effective way to reach interested parties globally, and keeping costs low enables PEF to train and mentor a large number of candidates in developing countries. The curriculum entails several assignments and participants can conduct these assignments on a crop of their choice, making the training relevant to their situations. Most of the 154 people who have completed the program have in turn trained hundreds of farmers, traders and marketers in their own regions inhandling fresh produce, crop storage, and food processing, thereby delivering maximum impact with minimum input. In addition to its e-learning program, the PEF provides education on improved technical practices along the postharvest chain and on extension education. This training includes a wide range of topics from measuring postharvestlosses to designing demonstrations on storage, pest management, packaging and temperature management, from building and using low cost cold storage systems to calculating return on investment of changes in handling practices. The PEF also provides advice on designing postharvest training and service centers. This information is available on the organization's website. In addition, mentoring is provided through social media sites, continuing with the philosophy of providing distance education and training.

Keywords: E-learning, fresh produce handling, storage

Introduction

The Postharvest Education Foundation (PEF,www.postharvest.org) was founded as a non-profit organization in 2011 by Dr. Lisa Kitinoja with the assistance of a small group of like-minded colleagues. The aim of the PEF is toprovide innovative programs that motivate and empower people to reduce food losses and waste through education and training. At the heart of the PEF is a structured e-learning program to train participants in how to teach farmers, traders, processors and marketers on handling produce and crops after harvest to maintain quality and reduce postharvest losses for horticultural crops and staple foods. In addition the PEF provides postharvest tool kits, mentoring, access to information through its website, and practical education via hands-on workshops and conferences.

E-learning program

E-learning is an efficient and cost-effective way to train and mentor a large number of candidates in improved postharvest technologies, extension skills and outreach practices. Participants in the e-learning program include trainers in non-governmental organizations, governmental employees, horticulture companies, extension workers, research scientists, postharvest professionals and graduate students, and are predominantly located in developing countries (Tab. 1). Most of the 154 graduates of the PEF postharvest e-learningprogram have in turn trained farmers, traders and marketers in their region, extending the impact of the program.

From 2011 to 2016 the 'Global Postharvest E-learning Program' was offered as a mentor-guided learning program. Participants enrolled in January and worked through the assignments at their own pace using a crop of their choice. This allowed those working or studying fulltime to participate and complete the program by the end of December. As of November 2016, the PEF Postharvest E-learning Manual was posted online so individuals or groups can participate at no cost, and on their own schedules (Kitinoja, 2016).

The course consists of 12 chapters and up to 12 assignments (some are optional) as summarized here:

1. Introduction to the PEF training of postharvest trainers e-learning program and the manual
2. Assessing the learning needs, skills and experience of the postharvest trainer/extension worker (Assignment 1)
3. Investigating available resources in the field of postharvest technology (Assignment 2)
4. Performing a commodity systems assessment (CSAM) and identifying the causes and sources of postharvest losses and quality problems for any crop of interest (Assignment 3) (Fig. 1) (La Gra et al., 2016)
5. Identifying and prioritizing research, extension and advocacy needs for the crop based on the CSAM report (Assignment 4)
6. Assessing the suitability of 'best postharvest practices' and appropriate technologies for their respective communities (Assignment 5)
7. Determining the costs and benefits of using improved practices and technologies (Assignment 6) (Tab. 2 and 3)
8. Designing postharvest demonstrations for local farmers, traders, processors and marketers (Assignment 7)
9. Setting measurable goals and objectives for a postharvest training program (Assignment 8)
10. Using postharvest extension methods, simple postharvest tools and basic equipment for quality assessment and as training aids (Assignment 9)
11. Designing local postharvest training and extension programs for various audiences (Assignment 10)
12. Evaluating the effectiveness of postharvest training programs (Assignment 11).

There is an optional assignment (12) of designing a Postharvest Training and Services Center (PTSC) (Kitinoja & Barrett, 2015).

Conducting a CSAM (Fig. 1) helps to highlight crop-specific needs for training, research and advocacy. For example a CSAM conducted on French beans grown in Rwanda by Kangondo (2017) reached the following recommendations for growers, the government and other stakeholders on training, research and advocacy needs.

Training needs:

- Best agricultural practices to increase the quality and quantity of French bean production.
- Postharvest handling, especially after harvesting and during transportation.
- Evaluating affordable storage and transportation solutions for this perishable crop.
- Food safety.

Research needs:

- Evaluating new varieties, in particular, varieties suited to the Rwandan climate and those resistant to pests and diseases,
- Processing methods to prevent waste if the beans are not consumed fresh.

Advocacy needs:

- The government should assist, or facilitate private investors to assist, the industry in developing processing facilities for adding value and minimizing waste.
- Improved infrastructure including roads and constructing collection centers near production areas.

Assignment 6, where costs and benefits of adopting a new practice are evaluated, is a valuable exercise. Tab. 2, prepared by an e-learner, demonstrates the cost of constructing a Zero Energy Cool Chamber (ZECC) for storing passion fruit (Nantambi, 2016). The result was much lower water loss resulting in greater salable weight, and better quality fruit. In addition, the use of the ZECC strengthened the bargaining power of the small farmer and extended their revenue period providing them with a market advantage. The construction of a ZECC takes a few hours of time and labor (neither of which were included in Tab. 2) but the relative profit of this new practice compared to the current practice is clear (Tab. 3).

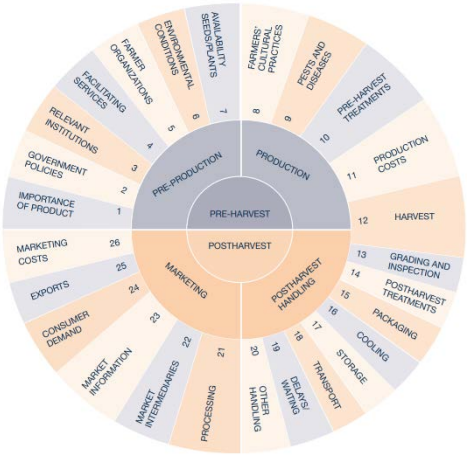

Fig. 1 The 26 principal components of the Commodity Systems Assessment Methodology (CSAM) presented in a figure (LaGra et al., 2016).

Tab. 1 Global impact of the Postharvest Education Foundation e-learning program as indicated by number of graduates per country as of end of 2017 and those currently enrolled.

Country	Graduates	Currently enrolled
Bangladesh	3	
Benin	2	
Bhutan	7	
Botswana	1	
Burkina Faso		30
Cambodia	4	
Cameroon	3	
Chile	1	
Egypt	1	
Ethiopia	15	5
Germany	1	
Ghana	12	2
India	8	
Indonesia	1	
Iran	1	
Kenya	13	1
Lebanon	1	
Malawi	1	
Malta	1	
Namibia	1	
Nepal	2	
Nigeria	5	12
Pakistan	5	1
Peru	1	
South Africa	1	1
Rwanda	14	10
Sri Lanka	1	
Suriname		1
Tanzania	25	
Togo	2	
Uganda	7	1
USA	12	1
Zambia	2	
Total	**154**	**65**

Tab. 2 Costs (in Ugandan shillings USh) of building a Zero Energy Cool Chamber (ZECC) in Uganda measured by an e-learner (adapted from Nantambi, 2016).

ZEEC requirements	Quantity	Unit price	Total (USh)	Equivalent in US$
Clean sand (kg)	700	87	60900	16.92
Burnt bricks	800	200	160000	44.44
Plastic crates	6	27000	162000	45.00
Thatch (bundles)	10	20000	200000	55.56
Spades	2	15000	30000	8.33
Bush knives	2	10000	20000	5.56
Small buckets	4	8500	34000	9.44
Poles	6	9500	57000	15.83
Hessian cloth (m)	2.5	20000	50000	13.89
Basins (medium)	2	5000	10000	2.78
Nails and binding wire			60000	16.67
Total			843900	234.42

Tab. 3 Postharvest cost/benefit analysis (in Ugandan shillings USh) of using a Zero Energy Cool Chamber (ZECC) when storing on passion fruit in Uganda as performed by an e-learner (adapted from Nantambi, 2016).

Factors	Current Practice	Equivalent in US$	New Practice	Equivalent in US$
Situation	Stored open rooms at ambient temperatures		Stored in a ZECC at 95% RH and 13-15C	
Costs				
Fruit amount	10 sacks @ 5000/sack		10 sacks @ 5000/sac	
Fruit weight (kg)	1000 kg		1000 kg	
Fruit cost	USh 50000	$13.89	USh 50000	$13.89
Cost of practice			USh 843900	$234.42
Total costs			USh 893900	$248.31
Loss	40%		3%	
Fruit available for sale	600 kg		970 kg	
Value/kg	USh 3000	$0.83	USh 5000	$1.39
Market value	USh 1800000	$500.00	USh 4850000	$1,347.22
Profit on first load	USh 1750000	$486.11	USh 3956100	$1,098.92
Profit on subsequent loads	USh 1750000	$486.11	USh 4800000	$1,333.33

Other roles of the PEF

In addition to the e-learning program the PEF provides access to a wide range of postharvest information. Training materials are available on the website and are used by those involved in extension work and training of farmers, produce handlers and small-scale food processors. The resources site of the webpage provides white papers on relevant topics such as the use of returnable plastic crates (Kitinoja, 2013), and measuring postharvest losses of fruits and vegetables (Kitinoja and Kader, 2015).

A postharvest toolkit is available at a discounted price and includes a pulp temperature thermometer, produce caliper and gauges, pH and chlorine test strips, color charts for produce maturity and quality assessment, produce knife, refractometer and digital scale. A smaller version of the Postharvest toolkit is given to those that complete the e-learning program. The PEF website provides links to videos on using these postharvest tools in the white paper on 'Creating fruit and vegetable postharvest videos' (Barrett, 2014).

In addition, the website provides instructional videos and designs for building solar dryers, evaporatively cooled storage structures, cold rooms, hermetic storage of grains, transportationoptions and reduced energy use, to name a few. PEF e-learning graduates also provide links to their journal articles, research publications and extension materials e.g. 'Mycotoxins contamination in maize alarms food safety in sub-SaharaAfrica' (James &Zikankuba, 2018), 'Commodity systems assessment methodology of postharvest losses in vegetable Amaranths: The case of Tamale, Ghana'(Osei-Kwartenget al., 2017), Vegetable handling, distribution, and wholesale profitability in "Abinchi" night market, Kumasi-Ghana (Zu et al.,2014) and 'Zero Energy Cooling Technology for Storage of Cavendish Banana Fruits' (Abdul-Rahaman et al., 2015).

The PEF organizes postharvest workshops for e-learners who successfully complete their online programs, offers long-term mentoring for participants in e-learning programs via social networking websites, conducts short courses, study tours, and workshops and provides advice and guidance for establishing local postharvest training and services centers. As a consequence, postharvest training and services centers have been established in several African countries including Arusha, Tanzania (Kitinoja & Barrett, 2015) as well as 16 additional sites in Tanzania as a component of the Market Infrastructure, Value Addition and Rural Finance support project (World Bank), and in Guinea, BurkinaFasoand Rwanda as part of Feed the Future projects managed by the Horticulture Innovation Lab.

Conclusions

The impact of the PEF in training is not limited to the e-learning graduates but has benefited those trained by graduates, plus the many users of the PEF website. This impact was achieved with a small budget by passionate andcommitted professionals. Although the focus of The Postharvest Education Foundation is on fruits and vegetables, similar principles can be applied to improved

handling, drying, packing, pest protection, storage and processing of grains and cereals.

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Evaluation of Plastic and Steel Bins for Protection of Stored Maize against Insect Infestation in Ghana

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Abstract

Maize is a staple food in Ghana where there is ever increasing demand for its use to also support poultry and livestock production. However, post-harvest loss of maize is high in Ghana. This study evaluated the effectiveness of plastic and steel bins as bulk storage structures to reduce maize post-harvest loss in Ejura, Ghana during the period from February 2016 to January 2017. Maize pre-disinfested with a solar biomass hybrid dryer was stored in the following treatments: i. a white 7-ton plastic bin filled with untreated maize, ii. agreen 7-ton plastic bin filled with untreated maize, iii.a 6-ton Kikapu steel bin filled with untreated maize, iv. six 50-kg polypropylene (PP) bags filled with maize treated with Betallic Super (80 g pirimiphos-methyl and 15 g permethrin per liter as an emulsifiable concentrate (EC)), and v. six 50-kg PP bags filled with untreated maize ascontrol. Moisture content, insect pests, insect damaged kernels (IDK), grain weight loss, aflatoxin and fumonisin levels data were collected monthly. *Sitophilus zeamais*, *Triboliumcastaneum, Cathartusquadricollis*, and *Cryptolestesferrugineus* were the dominant insect species collected from maize samples. At the end of 12 months of storage, % IDK in the control was >17% while IDK values in the other treatments were <3%. Mean grain weight losses of <1% were recorded in the bin treatments. Mycotoxin levels in the control were above the allowable threshold of 15 ppb. Our data suggest that use of plastic and steel bins has potential to reduce post-harvest loss of maize during storage.

Key words: Storage bin**,** post-harvest loss, aflatoxin, fumonisin, grain storage.

1. Introduction

Maize is a staple food for about 1.2 billion people in sub-Saharan Africa (SSA) (IITA, 2009). In Ghana, there is ever increasing demand for its use to support poultry and livestock production. However, measurable quantitative, qualitative, and economic losses of maize grain occur along the post-harvest system (Tefera, 2012). And, the Food and Agriculture Organization of the United Nations and World Bank data estimated that post-harvest loss (PHL) of cereals in SSA ranged between 5–40%, and worth approximately $4 billion (Zorya et al., 2011).

Insect pest infestation constitutes the major threat; these infestations can cause losses of approximately 20–50% of stored maize in most African countries (CABI, 2012). Although synthetic insecticides can be effectively used to manage stored-product insect pests, the majority of resource-poor farmers in developing countries do not use these chemicals because of inability to afford them, along with the associated environmental and health concerns among others. Therefore, farmers resort to the use of traditional storage techniques including bag storage, and warehouses (FAO, 1994; Adejumo and Raji, 2007). However, bag storage is the most preferred storage technique in developing countries (Koona et al., 2007; De Groote et al., 2013) even though post-harvest losses in bagged commodities are high.

Therefore, there is a need to explore the use of alternative technologies for the protection of grains. In Ghana, little research has been undertaken in bulk storage of maize in plastic and steel hence this study which evaluated the effectiveness of two 7-metric ton plastic storage bins (white and green color) and a 6-metric ton steel bin to protect maize against infestation by stored grain insect pests as compared to farmers' current practice of using PP bagswith or without pesticide.

2. Materials and Methods

This study was conducted in Ejura, located in the Middle Belt of Ghana. The site selected for the study was near a maize market where insect pressure was expected to be high. The study spanned the period from February, 2016 to January, 2017. Maize pre-disinfested with a solar biomass hybrid dryer was stored in the following storage types as experimental treatments: i. a 7-ton white plastic bin filled with untreated maize, ii. a 7-ton green plastic bin filled with untreated maize, iii.a 6-ton Kikapu steel bin filled with untreated maize, iv. six 50-kg polypropylene (PP) bags filled with maize treated with Betallic Super (80 g pirimiphos-methyl, and 15 g permethrin per liter as an emulsifiable concentrate (EC), and v. six 50-kg PP bags filled with untreated maize (control).

No insect pest-control measures were conducted during the 12-month storage period of the study. The experimental design used was completely randomized design (CRD). However, the bin treatments could not be replicated because of budgetary constraints arising from the cost of plastic and steel storage bins and quantities of maize used. However, there were six replicates for the bag treatments. White maize variety "Obaatanpa" sourced from a single farmer was dried to a moisture contentof 12.5% using a solar biomass hybrid dryer. Initial and monthly maize samples were collected from storage bins and bags. Three out of six bags from the control and Betallic-treated maize were randomly selected and 250-g sample taken from each bag during each sampling month. For the storage bins, 250 g samples were taken from three different sections along a vertical profile (top, middle and bottom sections). In each section of the profile, grains were collected from six random positions. Moisture content (MC) and temperature of maize in each selected bag and each section of the storage bins were determined using a John Deere moisture meter and PHL moisture meter developed by the USDA-ARS. Maize samples were transported to the insect laboratory of the Department of Crop and Soil Sciences of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana in 17-liter Koolatron® 12-V Compact Portable Electric Cooler for mycotoxin analyses. Insect species and numbers and percentage insect damaged kernels (%IDK) were also determined. Mycotoxin (aflatoxin and fumonisin) analyses were performed using a standard Romer Labs test kit (romerlabs.com). Weight loss due to insect damage was assessed using the count and weigh method of Harris and Lindbald (1978) and Boxall (1986) as:

$$\text{Weight loss (\%)} = \frac{[(WuxNd)-(WdxNu)]}{Wux\,(Nd+Nu)} x\ 100$$

Where, W_u= Weight of undamaged grain, N_u= Number of undamaged grain, W_d= Weight of damaged grain, and N_d= Number of damaged grain.

Statistical analysis was not performed on the data because the storage bins were not replicated. Only means and standard error were calculated using SAS version 9.4 (SAS Institute, Cary, NC).

3. Results

The results showed that initial mean moisture content (MC) of maize in bins was 15.9–18.0% compared to the bags (10.1–10.5%); however, MC in the bins declined to 12.6–12.9% in March. Grain MC subsequently fluctuated in all the treatments reaching 14.0–14.3% and 13.8% in the bins and bags, respectively at the end of the experiment. No live insect species was observed during the first 6 months of storageprobably because pre-storage disinfestation was effective against the adults and re-infestation was virtually nil? Temperaturesrecorded were between 26.6 and 39.7 °C whiles relative humidity ranged 22.9-73.1%. The dominant insect species found after the sixth-month period were *Sitophilus zeamais*Motschulsky, *Triboliumcastaneum*Herbst, *Cathartusquadricollis*Guerin-Meneville, and *Cryptolestesferrugineus*Stephens. *Sitophilus zeamais* was the most dominant insect species with a mean total 34.3 ± 6.7 per 250 g in the control; other treatments had < 4 per 250 g) (Table 1). The highest total number of *C. quadricollis* (10.83 ± 3.55 per 250 g) was recorded in the Betallic treatment while the bin treatments had <1.00 per 250 g. With the exception of the white plastic bin which had 1.17 ± 0.41 live *Cryptolestesferrugineus*per 250 g, all other treatments had < 1.00. Percentage insect damage kernels (% IDK) was highest in the control (17.9 ± 5.2) than the other treatments (<3%) (Fig. 1), which is below the 5% threshold set by Ghana Standard Authority (GSA) for commerce (Reference). Mean grain weight losses recorded in the bin treatments were < 0.5% throughout the storage period. However, ~1.6% mean grain weight loss was recorded in the control (Fig. 1). Mean aflatoxin levels of maize in the storage bins were below 15 ppb which is the safe threshold set by GSA (year?). However, in the control, mean aflatoxin level was over 47 ppb at the end of the study (Fig. 2). Similarly, mean levels of fumonisin in the control was 5.3 ppm which is above the safe threshold of 4 ppm; mean levels of fumonisin in the bins and the betallic treatment was < 2 ppm.

Tab. 1. Mean total number (±SE) of *Sitophilus zeamais*(SZ), *Triboliumcastaneum* (TC), and *Cathartusquadricollis* (CQ) per 250 g of maize sampled from five storage types in Ejura, Ghana from September to January.

Storage type	SZ	TC	CQ
Control	34.28 ± 6.75	2.28 ± 0.68	1.28 ± 0.45
Betallic	0.28 ± 0.14	0.11 ± 0.08	10.83 ± 3.55
White plastic bin	0.94 ± 0.26	0 ± 0.0	0.78 ± 0.47
Green plastic bin	1.72 ± 0.38	1.06 ± 0.32	0.22 ± 0.13
Steel bin	1.11 ± 0.34	2.83 ± 0.69	0.06± 0.06

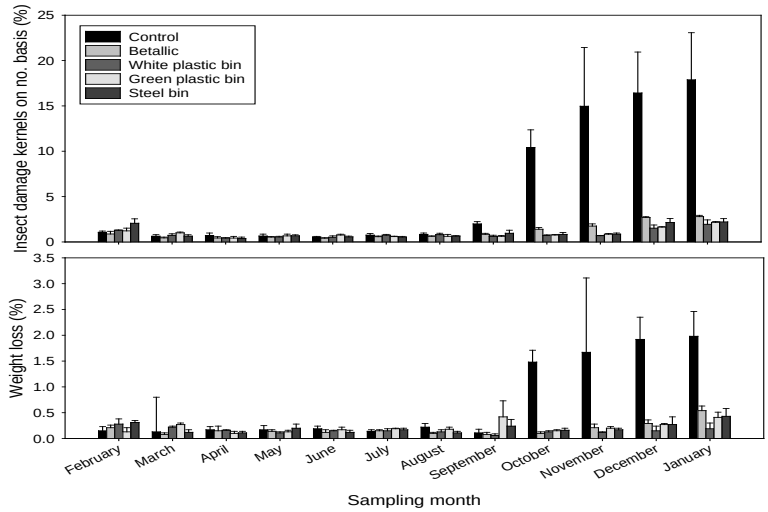

Fig. 1.Percentage insect damage kernels on number basis and percentage weight loss (Mean ± SE) per 250 g of maize obtained from five storage types in Ejura, Ghana.

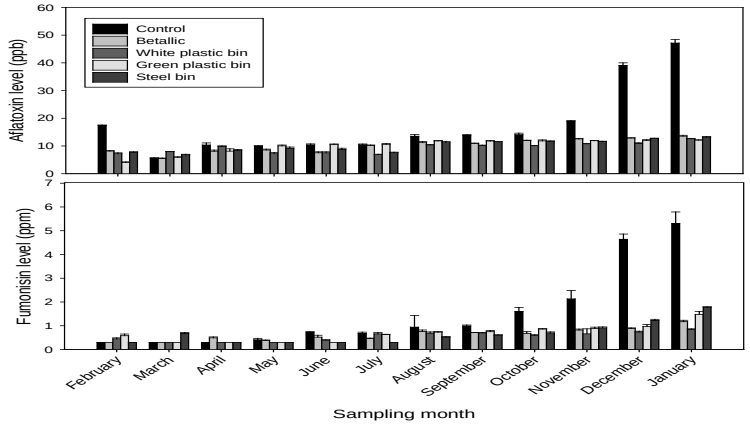

Fig. 2. Aflatoxin (ppb) and fumonisin (ppm) levels (Mean ±SE) in maize sampled from five storage types in Ejura, Ghana.

4. Discussion

The results of this study demonstrate that storage bins can keep the quality of maize for a reasonably longer period of time than the PP bags if they are properly designed and managed. It must be noted that during sampling a lot of insects which otherwise might have entered to infest maize inside the bins were found dead on the lid on the plastic storage bins**.**

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Insect infestation and quality loss of major stored products in Ghana

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Post-harvest losses are economically significant in Ghana for a broad range of commodities, resulting in a substantial negative impact on food security and livelihoods. Maize grains are the main food crops that provide staple diet for the majority of the population. A nation-wide survey was conducted in the three different geographical zones of Ghana (Northern savannah, the semi-deciduous middle belt and the coastal zones) to determine insects infesting major staples and evaluate the damage and losses caused. At each sampling, 1 kilogram of grain was sieved. Insects, frass and grains were collected separately. A random sample of 100 grains was taken from each sample for the determination of moisture content, percentage damage, weight loss and the number of insects per kilogram. The Thousand grain mass method was used to determine dry-weight loss. The levels of grain damaged were significantly different among the samples. Maize from markets in the Central region recorded the highest mean damages (14% and 17%) while the least (0%) was from Tinga in the Northern region. *Sitophilus zeamais* was the predominant insect in all maize stores and farms across the country. Its damage was lower than that caused by *Prostephanus truncatus*. Several parasitoid Hymenoptera, and an anthocorid predator were also collected in this survey. The parasitoids will be identified to species level to help us understand their biology and consequently develop rearing models for mass release to curb the injuriousness caused.

Session 9
Integrated Pest and Resistance Management

Star Wars in food stores – automated detection, determination and laser elimination of insect pests

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In a project supported by funds of the German government (PT BLE), we test a mobile camera system, scanning surfaces in storage warehouses or food processing industry. If insects are detected they are compared with morphological data in store to decide if the detected individual is a target pest. In case a target pest is determined with high probability, a laser beam is directed to the target to eliminate the insect by heat. The concept is to develop a system that is able to learn and identify more and more different species over time. First aims of the project are to improve reliability of species detection and identification in contrast to the grain with different light spectra and camera parameters. Reaction tests under different light conditions of the two exemplary insects grain weevil *Sitophilus granarius* (Col., Curculionidae) and Indianmeal moth *Plodia interpunctella* (Lepid., Pyralidae) will be carried out. Further the project will investigate laser beam wavelengths and intensities not damaging surfaces and items beneath or next to targets. The system could be utilized to support IPM in well-sealed structures for storage or processing of food and feed stuffs.

Keywords: camera insect surveillance, light, laser control, Sitophilus, Plodia.

1. Introduction

Stored product insects may be found in a given premise for three different reasons:

1. The insects came with infested raw materials, palettes or machinery.
2. The insects have been present for some time and developed in residual products accumulated in unattended areas of the building or within the machinery.
3. The insects found a way to enter from outside, attracted by light, temperature, moisture or volatiles.

In any case it is worth-while to determine the cause why insects can be found inside a storage or food processing facility in order to improve the situation and to reduce the frequency of insects as potential contaminants of food. If insects invaded a premise in infested raw materials, packaging materials such as card-board boxes, tarpolin, palettes, or in infested machinery an improved inspection of all goods prior to bringing them in would be advisable (Adler 2015).

A residual infestation in the building can be detected by regular inspection or by heat-treatments when insects start leaving their favorite hiding spaces. To prevent the immigration of insects from outside one needs to check the seal of windows and doors, the quality of gaskets between frames and movable parts. The air movement could be important, too, as around openings to the outside a reduced pressure in the building could prevent the development of a gradient of attractive odours and thus the orientation of stored product pests (Adler 2016). However, no preventive measure except low temperatures can keep premises permanently insect-free. If insects entered in spite of preventive strategies, they should be detected and controlled as early as possible. One study

developed a laser system called "photonic fence" to identify, track, and shoot down small flying insects in the wild (Keller 2016).

Our project outlined here aims to test an automatized camera surveillance of surfaces, combined with a determination of insect genera and a method of pest control using laser beams as a physical control device. Such an automated surveillance could be used in the reception of raw products and storages of finished products, in areas without laborers or at hours when there is no production. Ideally, an insect could be detected and controlled prior to oviposition or other damage.

Questions to be answered are:

1. At which wavelength and light intensity stored product insects are not disturbed in their normal activity but can easily be detected by camera surveillance?
2. Can the utilized optical system detect and determine stored product insect genera with sufficient accuracy?
3. Is the coordination between optic detection and laser control sufficiently fast and accurate for pest-control?
4. Can laser beams be used for insect pest control without damaging goods or surfaces next to the target?

2. Materials and Methods

In a first study the following species resembling crawling beetles and flying moth are tested:

Granary weevil *Sitophilus granarius* (L.) (Col., Curculionidae)

Indianmeal moth *Plodia interpunctella* (HÜBNER) (Lep., Pyralidae).

We test at which wavelengths and light intensities insect movement is not influenced by a sudden flash from a certain angle, critical wavelengths and light intensities will be determined. Specimens for testing come from our own laboratory culture at defined conditions and on defined substrates. All experiments will be carried out in a stationary testing environment. Later on, a mobile camera detection unit will be used.

The principle of the recognition software is based on pattern analysis (Deep learning) and is illustrated in figure 1. The motion patterns of insects on grain surface, under the different light wavelengths and intensities are recorded in high resolution pictures and videos. The comparison of the pictures results in a probability value for a certain pest insect. The image acquisition of the grain surface and pattern matching are running permanently, creating an automated surveillance system. If a pest insect is determined with a minimal probability of 80 %, laser control is initiated and the process is also transmitted via a data interference to a central pest monitoring center. Is the pest insect on the image not in the database, a new species connected with image and video data can be entered and saved as specimen for the new species. Hence, the pest insect database can be extended.

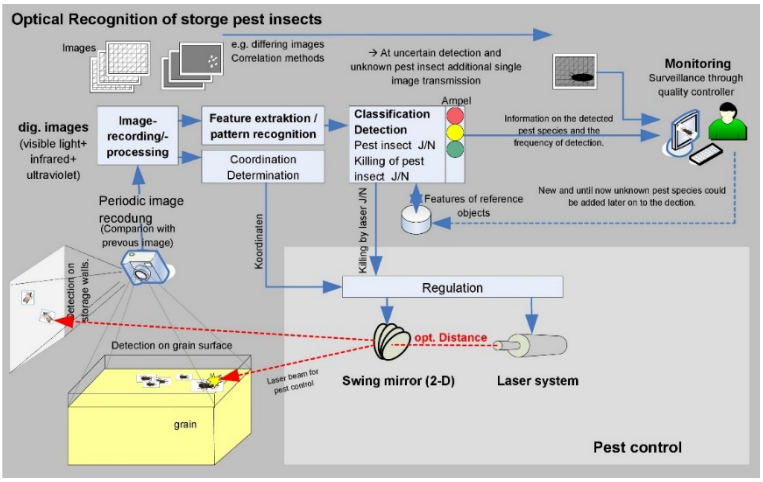

Fig. 9 Scheme of the complete system with detection and pest control

3. Outlook

This study will evaluate the probability of the early detection and pest control and will be a foundation to develop a prototype system. The detection of pest insects facilitates the installation of a model database under diverse environmental conditions and camera angles. The database will be improved if the information of different capture systems (storages) with diverse conditions and will be saved here altogether. With this detection unit a centralized monitoring can be established to lower the personnel costs for inspections and to facilitate a prompt action. Industry partners are integrated early on, starting in the development stage, to guarantee the incorporation of commercial demands. With the results of this study, an industry partners can develop a suitable scanner (light source & camera) in the evaluated spectral region. Preprocessing of video data could be implemented in the camera hardware, hence the transmission of big data amounts (video data) could be minimized drastically.

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Web-Based Phosphine Fumigation Monitoring with Active Sensor Validation Confirms Lethality in Stored Grains

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Abstract

The predominant measurement technologies for fumigation gases over the past 60 years include colorimetric tubes, photoionization detectors, and electrochemical sensors. Their limitations and inaccuracies are well documented. Spectros Instruments has shown non-dispersive infrared monitoring (NDIR) to be a superior analytical tool for the practical measurement of fumigation gases as shown in Table 1. Any compliant fumigation

monitor must be accurate, reliable and affordable. Stored Product Protection has additional requirements in remote regions such as Central China and Western Australia. In these cases, the value of real time access via the internet to fumigation data collected with NDIR Technology from a remote location adds heretofore unknown benefits. Allocation of manpower and materials resources are optimized by access to information about fumigant gas levels in grain storages via the internet. Data is automatically transferred to a central database that can be accessed in real-time from any location with internet access. Intelligent monitors with built-in diagnostics tracking barometric pressure, temperature, sample flows and detector voltages are described. This data collection, data warehousing and reporting platform maintains measurement traceability to certified compliance with secure, encrypted electronic notebook format. Knowing REAL phosphine concentrations allows informed decisions to be made to achieve required CxT and avoid situations leading to target pest phosphine resistance.

Key words: NDIR, phosphine resistance, fumigation monitors, phosphine, internet, remote sensing

Introduction

Stored Product Protection requires a compliant fumigant to be applied as a gas and achieve penetration within the grain mass. Control of insect populations necessitates precise phosphine fumigation control and accurate gas concentration measurements. Phosphine has achieved premier status as the fumigant most used worldwide. It is inexpensive, offers good results when used correctly and leaves no residues but also has unique requirements for accurate measurement.

Currently, stored grain is heavily reliant on phosphine to eradicate infestations. The warmer climates have increased likelihood of more widespread insect occurrence in stored grains. Countries such as Australia have used phosphine since the 1950s. As the need for low chemical residues on grains was mandated on international markets through the 1980s; phosphine became the viable solution and its use increased significantly through the 1990s. World-wide, some estimate that phosphine is used over 80% of the time in grain storage/pest control applications.

Phosphine Resistance

Along with the increased use of phosphine there has been a well-documented increase in the frequency of global resistances of major target pests. This resistance to phosphine is a major challenge to the worldwide grain market. Insect resistance to phosphine occurs because of improper application of the product usually applied as aluminum phosphide tablets under various trade names. In grain storages these react with moisture in the air to release phosphine gas. The gas moves around by diffusion and in air currents inside the silo. Phosphine leaks in non-gas tight silos are quite common.

The widespread use of phosphine gas fumigation in unsealed silos in farm, merchant, and bulk handling facilities has significantly contributed to insect resistance to phosphine fumigations. Frequent exposure of insect populations to sub-lethal dosages allows some insects with a new resistance gene to survive treatment and continue breeding, passing on their resistance. Repeat fumigations favour the insects that carry the resistance gene by allowing them survival, but killing normal, susceptible insects.

When strongly resistant insects are present, phosphine fumigation in an unsealed silo will have virtually no effect on the insects. One key to success is the ability of a silo to pass a pressure test. Compliant Silos that can be sealed well enough will hold the required concentration of phosphine for long enough to kill all stages of the insects, including resistant insects.

Monitoring Phosphine Fumigation Gas Concentrations

Accurate measurements of phosphine gas concentrations will increase the likelihood of successful fumigations. A precise, measured concentration level is desired. Dosage IS NOT concentration! Monitoring done correctly helps avoid situations where either too little or too much gas is used. Real-time web-based diagnostics of measured physical parameters confirms proper monitor

performance and in turn defends traceability to compliance in matters of concentration documentation. (Figure 1.)

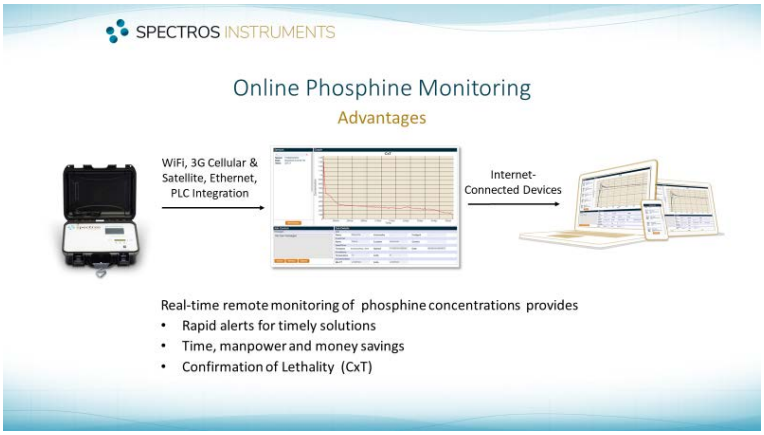

Figure 1: Spectros Instruments Phosphine Monitors Onboard Active, Real-Time Diagnostics assures correct monitor performance and validates phosphine fumigation data

Infrared Spectroscopy measures absolute physical constants and compensates for changes in temperature; barometric pressure; relative humidity as well as other interfering gases. The Spectros Instruments Phosphine Web Based Monitors provide communication protocols (3G cellular, WiFi, ethernet, Modbus RTU) for remote collection, organization, and reporting of fumigation data that the phosphine monitor collects as well as any alerts generated. Goals of increased efficiency, secured electronic records, compliance proof (traceability) and financial savings have been realized. Confidence in target CxT is achieved. (Figure 2.)

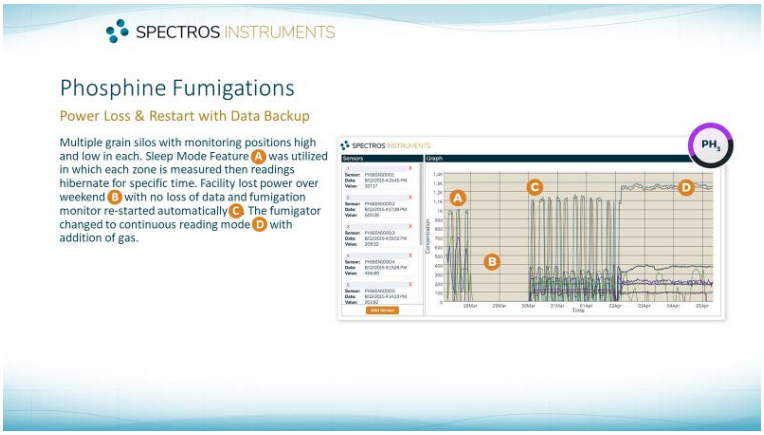

Figure 2: Spectros Instruments Architecture for secure web portal communication, warehousing & analytics of phosphine fumigation data

Table 1 History of Fumigation Gases Monitored by Spectros Instruments

Year	Fumigation Gas	Development Partners
1996	Ethylene Oxide	Johnson & Johnson
1998	Phosphine	Lorillard; RJR
2004	Sulfuryl Fluoride	Dow Chemical
2005	Methyl Bromide	USDA APPROVED
2009	Ethanedinitrile	Linde

Conclusions

Accurate, traceable to compliance and accessible phosphine concentration web monitoring provides immediate actionable data (**Figure 3)** to deliver safeguards that address potential insect resistance. If implemented, these demonstrable advantages allow an expanding global market to reasonably rely on a higher quality, uninterrupted supply chain for stored grain stuffs. Data accuracy, warehousing and easy access of data is key for informed decisions.

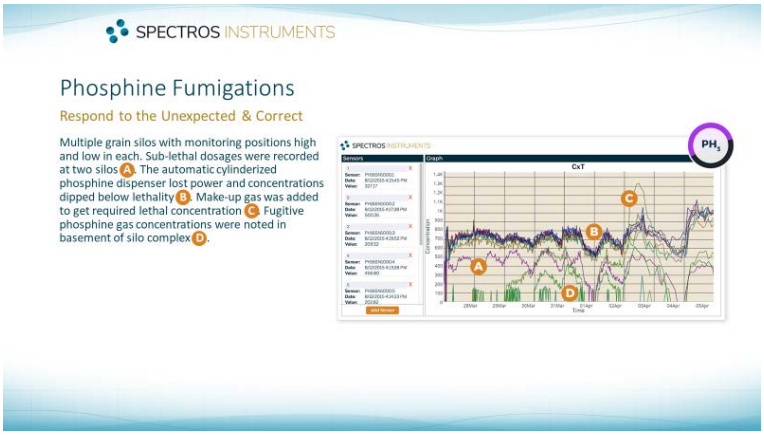

Figure 3: Sixteen-position web-based phosphine fumigation at a grain processing facility. Each line (trace) represents one sampling point of gas concentration vs. time and details proactive corrections avoiding a fumigation failure.

Qualitative Discussion about Reducing Grain Postharvest loss with Mobile storage in Ghana, West Africa

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Abstract

Farming sustainably and protecting gross harvest production correctly provides growers with "health care, school fees and peace-of-mind" (net benefits). Reducing Postharvest and input loss sustains the components of agriculture's triple-bottom-line which are "accessible nutrition, reduced green-house emissions, and foreign exchange reserves". Lacking storage that stops grain PHL, agriculture suffers critical problems like the Aspergillus fungi that leaves grain contaminated with invisible aflatoxin that growers cannot consume or market. The objective of the Ghana pilot study was to understand why new ideas/findings like, applying biologicals to the soil before harvest, gross production inputs, virtual markets and especially the spread of stationary grain warehouses have failed to improve the net benefits of farming or agricultures' triple-bottom-line in sub-Saharan Africa. Qualitative comparison methods were used to identify roadblocks to improvement as scientific monitoring and storage eliminate grain Postharvest loss on the drylands in many parts of the world. Observations suggest net benefits are being ignored as reviews and assessments of primitive or council storage exchange scientific rigor for Stationary Warehouse Prejudice. Scientific rigor illuminates how the qualitative cost of aflatoxin, and quantitative expense of pests, recycling plastic, and empty stationary warehouses impact end-user-cost per unit stored per month. We conclude that Postharvest loss is expensive, and that relatively inexpensive mobile metal storage assets would improve net benefits and the triple-bottom-line.

Key words: grain, aflatoxin, storage, postharvest loss, triple-bottom-line.

1. Introduction

Staple, pulse, and legume (grain) farming means harvesting sustainably as much as possible from

production inputs, arable land (ecosystem services), and protecting what is harvested correctly so surplus provides for "health care, school fees and peace-of-mind" (net benefits). Reducing "Postharvest loss sustains the important components of agriculture's triple-bottom-line which are "accessible nutrition, reduced green-house emissions, and foreign exchange reserves".

Of course, not all sub-Saharan Africa (SSA) farm production is the same, so it is impossible to lump all Postharvest loss together. Some Postharvest loss is of "fruits, vegetables, and meat" (dense nutrition) and some is dry, high calorie and protein grain. However, grain provides most of the calories that power animal and human hard labor to "plant, grow, harvest, thresh, clean, dry, aggregate, store, monitor and process" grain and densely nutritious food. At the farm level, especially in the field, many biotic pests like fungi, insects, rats, birds, or abiotic groundwater, flooding, wildfire, and theft are difficult or impossible to control without protective storage. Historically Postharvest loss means SSA grains are contaminated by rats, insects, and fungi that cause Postharvest loss like aflatoxin. Aflatoxin "increases morbidity and mortality" (IARC, 2016) and small-scale grain growers' cannot safely consume or market grain.

Development often confronts Postharvest loss with production packages that temporarily increase gross grain production. For example, guaranteeing a price 10% above market premium for all compliant product. When there is typically a great deal of Postharvest loss, first season sourcing from local growers' results in 70% out of tolerance product, mostly from aflatoxin. When a lab test determines contamination, grains are simply turned back, and growers must fend for themselves. Some contaminated product goes to animal and fish feed formulators, which take half of the rejected product. Same growers then sell another quarter to mill operators, who do not test or care about quality. The remainder is consumed by growers (Lamb, 2017).

Objectives

Our objectives were to strengthen knowledge about why the spread of inputs and stationary warehouse storage for surplus grain have failed to reduce Postharvest loss. For example, knowing why International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, 2017) says application of biologicals like harmless strains of Aspergillus fungi to the soil have had very limited success reducing SSA Postharvest loss in storage, is critical (Kumar, 2017). Exposing this aspect of Postharvest loss would help development experts guide research and outreach by HarvestPlus, International Fertilizer Development Corporation (IFDC), International Institute for Tropical Agriculture (IITA), Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), Ghana's Social Enterprise Development Foundation (SEND-Ghana), Adventist Development and Relief Agency (ADRA), Mennonite Economic Development Associates (MEDA) and Center for Agricultural Rural Development (CARD) for example.

2. Materials and Methods

The three qualitative comparison methods were:

- evaluate the scientific rigor used to assess storage by organizing, reviewing, and comparing research
- field test mobile storage (Fig. 1) by observing adaptive learning at four of many locations
- identify any potential roadblocks for growers' rights to reduce Postharvest loss with mobile storage.

Figure 1. Mobile metal storage has utility for storing many crops, monitoring and primary proccesing surplus grain. Image: Unknown and modified by author.

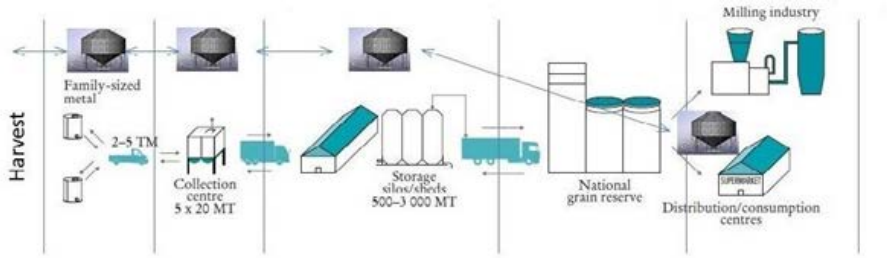

Figure 2. Mobility lets growers optimize storage at many locations. Image: FAO 2015 D. Mejla and modified by author.

3. Results

Qualitative comparison is relevant as scientific improvements (Butler, 1907), research (Proctor, 1999), storage testing (Opit, 2016), and grain moisture content measurement (Armstrong, 2016) etc., permit judicious adjustments to the timing, choice, and intensity of control actions, timely chemical pest control measures, in grain storage. Adjustments that are expedited using "Integrated Pest Management" (IPM) are often not only the cheapest but also the most reliably efficacious of the possible options and reduce Postharvest loss to insignificant levels on the drylands of the world that provide SSA with a staggering 83% of the food it consumes, though SSA holds nearly 50% of the land available worldwide" (Juma, 2016).

The key results suggest that solutions to the aflatoxin challenge that plagues SSA farmers, other agri-entrepreneurs, and governments are ignored. Kaminski, (2014) describes how reviews and life-cycle assessments of African storage, exchange scientific rigor for "Stationary Warehouse Prejudice" (Adjei, 2017). While Kumar (2017), and Ampuko (2018) mention aflatoxin, they ignore innovation storage solutions that their "Archer Daniel Midland Institute for the Prevention of Postharvest Loss" at University of Illinois and "1st All Africa Postharvest Congress and Exhibition", at University of Nairobi, respective research organizations exposed for peer review in conference proceedings. These and other reviews also ignore how sealed storage requires additional stationary infrastructure (Fig. 1) to mitigate condensation, handling to monitor for grain damaging insects that bore into plastic bags, and that rodenticides are not chemical free and soft plastic needs to be recycled. Examples of life-cycle assessments are the World Food Program (WFP) Global Postharvest Knowledge Center (Rierson, 2017) which ignores that that growers and refugees are often tenure-insecure. Michigan Institute of Technology (MIT) Comprehensive Initiative on Technology (2016)

merely evaluates the end-user-cost per unit stored annually, assuming all storage is full for the same number of months, instead of a per unit per number of months perspective that illuminates the cost-effectiveness of storage that is full for longer periods.

During pilot study field tests, we observed Ghana's agricultural business environment and witnessed adaptive learning that suggested mobile storage addresses the needs of tenure-insecure growers. While field testing at four locations we observed that rights to storage shifted benefits to growers and away from patriarchs wielding land-tenure, opportunistic traders setting prices or councils that are unresponsive (Easterly, 2015).

Roadblocks that the pilot study identified were Stationary Warehouse Prejudice, Development Packages, and Purchase Price of mobile storage.

Stationary Warehouse Prejudice Roadblock

University of Ghana's Egyir (2017) questions the competence of most of the current stationary warehouse management to source working capital, or network to accomplish IPM. Lack of management means Postharvest loss increases with storage period and capacity. However, empty, or full, with or without market access, prejudice for stationary warehouses provide protocol fees or services that facilitate cooperation by grain councils in East Africa or Ghana. This kind of cooperation makes implementation of production package blueprints easy for any "non-Governmental Organization" (NGO), even though NGO cultural advisors know that Postharvest loss will continue to impact advisor extended families in rural communities.

Agribusiness lobbies globally to set the agricultural research and education agenda to facilitate profits through the sale of inputs like mechanization, seeds, fertilizers, and pesticides (gross production technologies). Input agribusiness sees little profit in the preservation of food once it is produced. In fact, SSA agribusiness profits from Postharvest loss in warehouses as this loss reduces the food supply thus creating the perception that inputs to plow new land (extensification) and/or for irrigation (intensification) are needed to produce more food (Wilson, 2016). SSA agribusiness ignores that when Postharvest loss drives intensification or extensification, ecosystems are degraded and soon limit net benefits and the triple-bottom-line.

SSA research and outreach assess grower-controlled storage with wheels, as disruptive and suggest already proven off-the-shelf "Mobile storage needs basic research and testing if we are to share/promote [for growers to evaluate] it widely" (Essegby, 2017). Agribusiness and councils lobby so research and outreach delay Postharvest loss solutions and so annual grain summits and USAIDs' ADVANCE Preharvest events or forums exclude innovation from local agendas. Excluding innovation from local agendas discourages grower evaluation of inputs and protocol.

Development Package Roadblock

Development often confronts PHL with gross production packages and support councils to blueprint stationary warehouses for average local production. As averages are rare, council managed warehouses are either almost empty or overflowing and often far away from Postharvest loss control locations. On the other hand, the dynamic nature of farming and chronic Postharvest loss it is risky for tenure-insecure growers to build and maintain warehouses at optimal locations.

Many development packages attempt to move growers up by implementing warehouse receipt systems (WRS). However, if the receipt system warehouse is "too far away or does not scale to production for cost-effective IPM" (unresponsive), WRS are soon "rusting" monuments to Postharvest loss (Armah, 2006). Kula (2017) suggests development experts should learn the lesson that WRS based on stationary warehouses do not even out supply or help growers (World Bank, 2013).

"Tackling [stationary] WRS Challenges" by Mugano (2017) explains precisely, the familiar lack of suitable infrastructure or requisite skills, legal and regulatory issues, missing or weak complementary market institutions, disabling elements in the policy environment that discourage key stakeholders especially bankers from financing agriculture in SSA.

An example of a Development package is Financing Ghanaian Agriculture Project (FinGAP) Incentive grants. FinGAP assists "Financial Organizations" (FO) to focus on areas that will never support commercialized farming where the "most vulnerable growers are" (World Vision). One of these FOs is the Center for Agricultural and Rural Development (CARD). Incentive grants allow CARD to provide credit-in-kind for inputs to approximately 10,000 vulnerable growers in exchange for bags of "maize, rice and soy" (produce). The credit-in-kind is more likely put to good use by growers for approved production practices than cash which could be diverted to unapproved uses. Middlemen from target districts assist CARD activities by delivering approximately 500 MT of loan repayment produce which is then aggregated, stacked on pallets and covered by tarps anticipating price appreciation. FinGAP reports that supporting FOs activities leads to increased gross production and 100% loan repayment.

Although easy to move pallets and tarpaulins are at first attractive option to stationary warehouses, they do not stop Postharvest loss from flourishing throughout the stack during the 6-8 months the repayment produce anticipates price appreciation. Ground water and termites weaken pallets and allow sacks to contact fungi in the soil. Manually removing/replacing the tarpaulins daily is needed to prevent condensation that allows fungi, insects, rats, and birds to feast. CARD's capacity to move up above grants and sustain the triple-bottom-line is limited by Postharvest loss (Shukla, 2017).

The surplus grain that remains with CARD growers at the farm level or council district warehouses will likely be rewetted (Trenk, 1970) and allow Postharvest loss like aflatoxin to impact the most vulnerable children (Cardwell, 2014). Postharvest loss is not approved production practice as the net benefits of credit-in-kind inputs are diverted to pests, middlemen and councils.

Development packages that use gross production to ignore the impact of Postharvest loss, miss an opportunity to approve storage practices so that net benefits drive the triple-bottom-line without further use of land, water, and other agricultural inputs (APHLIS, 2015).

Purchase Price Roadblock

Even though the cost of any metal grain storage decreases with increases in capacity and the number of months that capacity is full, the up-front purchase price of metal storage is a roadblock for grower storage rights that reduce Postharvest loss.

4. Discussion

Rights that secure access to land or tenure, reduce the risk to resources invested to build and maintain stationary storage like warehouses. However, SSA growers are often tenure-insecure. Lacking storage that meets their needs, growers are forced to sell quality surplus early or suffer significant Postharvest loss (Lipinsky, 2013).

North Carolina State University (NCSU, 2018) focus group discussion suggests crops are not stored in the field for fear of theft. As a result, farmers only harvest volumes that they can carry in any one day. Considering that the main means of transportation was by head, the amounts that can be transported within a given period is limited. As a result, the produce may be exposed to rewetting in case of rains. These findings seem to imply that a transport intervention that parks cost-effectively to store aggregated quality while heads, wagons or trailers haul heavy loads may go a long way in reducing the losses that occur at harvest before or as crops leave the fields.

Opportunistic traders or middlemen know growers lack storage and set low prices. Low prices reduce the net benefits of inputs like hard labor, ecosystem services, and especially gross production inputs like "FarmerLine (sms information), Tuluu (virtual market), AgriCorp (education), Oikocredit (micro-finance), Area Yield Index Crop (insurance), Hello Tractors and Solar powered irrigation (mechanization), HarvestPlus Biofortification (improved crop varieties), IFDC (fertilizers), IITA's AflaSafe fungi (biologicals)" and other process improvements like commodity marketing. The result is the tenure-insecure grower may experience "market failure" (Jones, 2011) after investing inputs, selling grain low, and then buying similar grain back at a higher price. Or, if the grower attempts to

gain the advantage by controlling assets that store grain in bulk, sack, or airtight metal can, hard plastic drum or soft plastic bag, they may "challenge the tradition" of patriarchs (Bott, 2005). In SSA the grower invests the important inputs and gets just enough to survive but not enough to move up, as "Postharvest and input loss" (PHL), middlemen and councils divert significant net benefits.

Simply, harvesting grain without storage means PHL is chronic and invisible aflatoxin stops growers from setting, or modifying, their own goals, so two farms with identical climates and soils may be managed with different aims to achieve the diversity needed to sustain the triple-bottom-line (FAO, 2015).

If SSA development experts realized that grain PHL is an integral part of the SSA agricultural system (Boa, 2016), innovative grain storage would initiate the 'golden age' of SSA agriculture (Pearce, 2016). The Great Grain Bin Adventure (Butler, 1907) is an example which justifies many calls for proposals that specify food chain policy innovations, as there are few positive outcomes if aflatoxin means small-scale grain growers cannot safely consume or market grain (Mendoza, 2016).

Solutions to the Roadblocks and PHL

When scientific rigor quantifies the role PHL played during "decades of grain net yield increases in other parts of the world, to keep SSA grain agriculture less mechanized, low-yielding, and insecure" (Juma, 2016), accountable development packages will finance agendas that are responsive to grower net benefit and improve SSAs' triple-bottom-line.

On the drylands of SSA, output agribusiness like Cimbria and African Grain Care etc., have built, validated, tested, sold and maintained 1000s of stationary metal vented, raised sloping floor silos for utility storage. If the storage was mobile the same storage could be relocated at any PHL control point and provide the practical utility needed to support IPM practices. So, should be easy for research and outreach to understand how storage with wheels, just like just like agricultural wagons and trailers will likely improve tenure-insecure growers' net benefits. Storage with wheels can be leased. Leased and/or purchased mobile storage can be parked cost-effectively at dynamic PHL control points so utility like vents will cost effectively mitigate condensation; wide-opening roofs reduce the labor needed to aggregate quality and monitor insects while also stopping rewetting; sloping floors reduce cleaning requirements and rise above groundwater and rats secure net benefits. Incentives for FOs to address the purchase price of an approved practice like storing and marketing safe surplus will move growers up to economies of scale and attract the working capital of local banks that will sustain agricultures' triple-bottom-line (Mugano, 2017).

To help focus the qualitative discussion we wanted to assess options like the airtight metal can (Fig. 3) versus mobile utility using the Granary Selector 'app' developed by the Natural Resources Institute at University of Greenwich under a contract with the Swiss Development Agency (Tran, 2016). However, the app does not allow users to organize, review and compare storage factors like lease or mobile types. We addressed this roadblock to financing by organizing a comparison based on practical field handling (Text Box 1.), storage (Text Box 2.), and marketing (Text Box 3.).

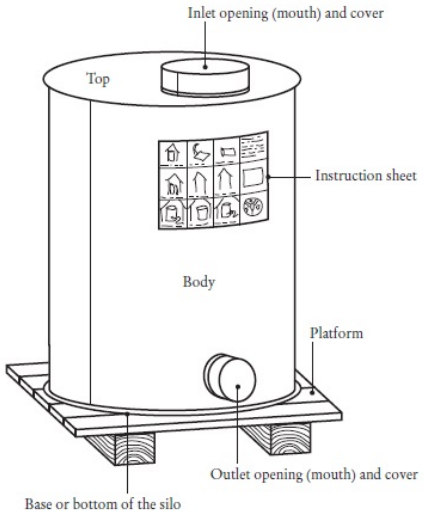

Figure 3. Airtight metal can capacities larger than 1.8 MT "become hard to operate" (George, 2011). Image: FAO 2015.

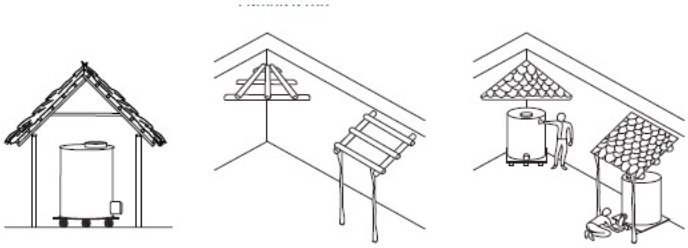

Figure 4. Airtight metal cans require additional infrastructure like floors, stationary platforms and roofs. Image: FAO 2015.

Text Box 1. Summary practical comparison of field handling to storage environments when significant PHL occurs (Lipinski, 2013). See Appendices A for detail.

Can (artisan, airtight, not for paddy rice)	Bin (mobile utility)
Field handling with cans prevents rodents, birds, insects, rain, and theft without walls. Cans do require platforms to exclude ground water and a roof to mitigate condensation caused by temperature fluctuations (day vs night). Cans do not allow air exchange and so condensation caused by temperature fluctuations can encourage fungi and insects and in turn lead to major losses in grain quality and volume. A hermetic or airtight seal is used to prevent fungi and insects. At first the cost of airtight insect and fungi control is low. However, the longer the grain is stored in airtight cans, the longer it will take for any metabolism to reduce the atmosphere. If the can is not filled, then the excess atmosphere may	Field handling mobile bins excludes rodents, groundwater, birds, rain, wild fire, theft and vents allow air exchange to mitigate condensation caused by temperature fluctuations (day vs night). Handling or "process solutions" (Rockefeller, 2015) have high utility when they mitigate fungi and insects by moving IPM into cropping systems for excellent value loss prevention/month/unit stored.

encourage any metabolism. Without the low oxygen atmosphere environment cans are less effective at suppressing insects and fungi.	

Text Box 2. Summary of storage where significant PHL impacts (Lipinski, 2013) the value of stored gross yield. A market-oriented growers' net benefits are a function of price seasonality, value loss prevention, and their opportunity cost of capital invested (Jones, 2011). See Appendices B for detailed practical comparison.

Can (in warehouse to stop condensation)	Bin (self-contained)
Storage will capture seasonality as IPM for value loss prevention/month/unit stored is good. However, artisan constructed cans with capacities larger than 1.8 MT "become hard to operate so this is the largest practical size" (George, 2011) and limits scaling for growers' cooperative storage.	Bins capture seasonality as leased capacity is a business expense and reduce the need for capital, transport, and tenure. Mobile storage is a value adding process solution, since utility with wide-opening-roofs mitigates abiotic and biotic problems with excellent IPM/month/unit stored.

Text Box 3. Summary estimation of the marketing incentives for optimal production as can or mobile bin utility counter act the "yield gap that may exist as the high costs of inputs or the low returns from intensification and/or extensification make it economically suboptimal to raise production to the maximum technically attainable" (Godfray, 2010). See Appendices C for detail.

Cans (in warehouses for primary processing)	Bin (primary processing)
Primary processing out to bowls, sacks, back to bulk and cleaning is assisted by gravity if the loaded cans are set up on platforms. Building and maintaining strategic roofs and platforms is capital that must be risked in anticipation of price appreciation and to ease primary processing. Maintenance of redundant roofs and platforms close to dynamic aggregation and marketing locations may limit surplus production.	Primary processing out by gravity matches demand for bowl, sack, bulk anywhere roads go. Leasing process solutions keeps maintenance costs per unit stored per month low, and reduce the scale needed to be economical. This primary processing utility is economical as units move when empty, and park cost effectively where storage is needed.

Conclusion and recommendations

PHL limits the net benefits that storage should provide grain growers and SSA agriculture is therefore insecure and production sub-optimal.

SSA research or outreach should conclude that PHL is expensive and recommend that relatively inexpensive storage assets should meet growers' needs, as well as democratize food supply decisions.

The pilot study recommends mobile utility be reviewed objectively and compared with roadblocks so

- growers have many IPM alternatives
- abiotic and biotic PHL becomes insignificant
- agricultures' triple-bottom-line benefits growers in an inclusive manner.

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Appendices

Appendices A.

Detail practical comparison during field handling to storage environments when significant PHL occurs (Lipinski, 2013) detail.

Can (artisan, airtight, not for paddy rice)	Bin (mobile utility)
Field handling with cans prevents rodents, birds, insects, rain, and theft without walls. Cans do require platforms to exclude ground water and a roof to mitigate condensation caused by temperature fluctuations (day vs night). Cans do not allow air exchange and so condensation caused by temperature fluctuations can encourage fungi and insects and in turn lead to major losses in grain quality and volume. A hermetic or airtight seal is used to prevent fungi and insects. At first the cost of airtight insect and fungi control is low. However, the longer the grain is stored in airtight cans, the longer it will take for any metabolism to reduce the atmosphere. If the can is not filled, then the excess atmosphere may encourage any metabolism. Without the low oxygen atmosphere environment cans are less effective at suppressing insects and fungi. If necessary, grain in the cans can be fumigated, with a caution that fumigation should never be done in cans that are located inside of living spaces. Flexible capacity for field handling to storage is poor because cans do not store cobs, groundnuts in the shell or sacks. There are mechanical options to the manual labor typically used to load, but flat-bottomed cans require manual cleaning. Cans are suited to smallholder field handling to storage because scaling to harvest is only limited by the roofs and raised platform on floors growers are willing to invest. However, relative to the surplus storage at dynamic PHL control locations needed to impact foreign exchange, cans will soon limit growers' benefits.	Field handling mobile bins excludes rodents, groundwater, birds, rain, wild fire, theft and vents allow air exchange to mitigate condensation caused by temperature fluctuations (day vs night). Handling or "process solutions" (Rockefeller, 2015) have high utility when they mitigate fungi and insects by moving IPM into cropping systems for excellent value loss prevention/month/unit stored. Leases effectively scale without warehouses, so "Growers whose scale of operation is too small to be able to produce SAFE FOOD" (Cardwell, 2015) can move up by participating in cooperative storage. Field handling to storage is excellent because bins with utility also store cobs, groundnuts in the shell or sacks and combinations of sacks and bags by multiple growers. Loading utility can be either manual or mechanical and sloping floors reduce manual cleaning. Mobile utility bins are very well suited to field handling to storage because leases effectively scale (location and capacity) to prevent PHL and secure harvest regardless of transport or land rights. If the bins are purchased, they become "mobile assets" (Growing Africa, 2013). 15+ year life cycle assessments must consider assets with the utility to store inputs (seed and fertilizer) at planting, optimal aggregation locations, proximity for monitoring, primary processing, and self-cleaning features.

Appendices B.

Detailed comparison of storage where significant PHL impacts (Lipinski, 2013) the value of stored gross yield. A market-oriented growers' net benefits are a function of price seasonality, value loss prevention, and their opportunity cost of capital invested (Jones, 2011).

Can (in warehouse to stop condensation)

Storage will capture seasonality if local water tank artisans build cans so capital requirements are medium. However, if airtight cans are opened for monitoring or to add or remove portions, the hermetic atmosphere that prevents fungi and insects, must be restored by metabolism. The longer the grain is stored in hermetic cans, the longer it will take for metabolism to restore and maintain the hermetic atmosphere. If a can is not filled, then the excess atmosphere may prevent the creation of hermetic environment. Without the hermetic environment cans are less effective at suppressing for example, residual fungi. If necessary, grain in the cans can be fumigated, with a caution. Due to the limits of artisan construction, fumigation should never be done in cans that are located inside of living spaces. IPM for value loss prevention/month/unit stored is good. However, artisan constructed cans with capacities larger than 1.8 MT "become hard to operate so this is the largest practical size" (George, 2011) and limits scaling for growers' cooperative storage. Investment is required to maintain and monitor a low atmosphere environment, roofs and the raised platform needed for gravity assisted processing and cleaning. Infrastructure for can storage is fixed relative to where large and small harvests or floods may occur, and tenure-insecure growers are less likely to invest if they consider surplus storage too risky.

Bin (self-contained)

Bins capture seasonality as leases are a business expense and reduce the need for capital, transport, and tenure. Mobile storage is a value adding process solution, since utility with wide-opening-roofs mitigates abiotic and biotic problems with excellent IPM/month/unit stored as, if necessary utility can be easily fumigated. Purchasing storage, a "process solutions" like mobile utility "are innovative ways of providing collateral" (Growing Africa, 2013) because asset with mobile utility make sense for on-site storage, security and proximity that replaces PHL with marketing for growers' net benefits. 15+ year life cycle assessments must consider the protocol fees and services for storage rights that impacts foreign exchange.

Appendices C.

Detailed estimation of the marketing incentives for optimal production as can or mobile bin utility counter act the "yield gap that may exist as the high costs of inputs or the low returns from intensification and/or extensification make it economically suboptimal to raise production to the maximum technically attainable" (Godfray, 2010).

Cans (warehouses for primary processing)

Secure warehouses at markets offer good return even though cans are fragile and difficult to transport and require building and maintaining redundant stationary roofs and platforms. Roofs must allow access by ladder to the lid for aggregating in. Processing out to bowls, sacks, back to bulk and cleaning is assisted by gravity if the loaded cans are set up on platforms. However, if opened for monitoring, growers must restore the hermetic atmosphere and

Bin (primary processing)

Mobile bin marketing offers optimal returns. When mobile utility secures the hard labor required to aggregate harvest quality and control abiotic and biotic problems, moisture testing and using the Sun to cook insect pests (solarization) prior to storing become relevant. On the drylands at aggregation, humidity is low enough for applications of Diatomaceous Earth. After grain is stored, utility means aeration to condition, and wide-opening-roof features that ease monitoring and secure collateral. Utility nearby means aggregation, monitoring fumigation or marketing decisions become judicious and will reduce PHL, especially insects. SSA temperatures are consistent, and so endemic parasitic wasps are likely effective in a vented bin to control moths and beetles (biocontrol). Since the vented storage is located nearby, the labor required to monitor biocontrol is reduced.

Primary processing out by gravity matches demand for

maintain platforms and roofs against termites and rotting. Building and maintaining strategic roofs and platforms is capital that must be risked in anticipation of price appreciation and to ease primary processing. Maintenance of redundant roofs and platforms close to dynamic aggregation and marketing locations may limit surplus production.

bowl, sack, bulk. In other words, mobile utility means capacity for growers' and their cooperatives can be adding value adding anywhere roads go. Leasing process solutions keeps maintenance costs per unit stored per month low, reduce the scale needed to be economical and enable access at any temporal or spatial link in the value chain. Self-contained primary processing utility is economical as units that move when empty, will park cost effectively where storage is needed. Mixing leases and purchases scale capacity to growers' requirements at harvest, aggregation, storing and processing at markets. 15+ year life-cycle assessments should consider how bins move to where storage is optimal, so capital investment provides the triple-bottom-line.

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Utility of biotechnology based decision making tools in postharvest grain pest management: An Australian case study

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Abstract

A major concern for the Australian grain industry in recent years is the constant threat of resistance to the key disinfestant phosphine in a range of stored grain pests. The need to maintain the usefulness of phosphine and to contain the development of resistance are critical to international market access for Australian grain. Strong levels of resistance have already been established in major pests including the lesser grain borer, *Rhyzopertha dominica* (F.), the red flour beetle, *Tribolium castaneum* (Herbst), and most recently in the rusty grain beetle *Cryptolestes ferrugineus* (Stephens). As a proactive integrated resistance management strategy, new fumigation protocols are being developed in the laboratory and verified in large-scale field trials in collaboration with industry partners. To aid this development, we have deployed advanced molecular diagnostic tools to accurately determine the strength and frequency of key phosphine resistant insect pests and their movement within a typical Australian grain value chain. For example, two major bulk storage facilities based at Brookstead and Millmerran in southeast Queensland, Australia, were selected as main nodes and several farms and feed mills located in and around these two sites at a scale of 25 to 100 km radius were selected and surveyed. We determined the type, pattern, frequency as well as the distribution of resistance alleles accurately for two major pests, *R. dominica* and *T. castaneum*. Overall, this information along with the phenotypic data, provide a basis for designing key intervention strategies in managing resistance problems in the study area.

Keywords: phosphine**,** molecular platform, grain value chain, resistance management

1. Introduction

Protecting harvested grain from insect infestations is essential for facilitating domestic and international trade. In Australia, for example, the industry strictly adheres to a 'nil tolerance' principle for live insects to gain competitive advantage in international trade. Over the last decade, there has been significant progress in pest and resistance management in Australia in response to the development of high level of resistance to phosphine in key pest species, the primary fumigant used to disinfest stored grain (Nayak et al., 2013; Kaur and Nayak, 2015). While the alternative fumigants sulfuryl fluoride is being evaluated as a 'resistance breaker' to alleviate phosphine resistance problems (Nayak et al., 2016), efforts are ongoing to extend the usefulness of phosphine through development of higher application rates to control strongly resistant populations (Nayak et al., 2013; Kaur and Nayak, 2015).

In any resistance management program, key components include proper determination of strength of resistance and its distribution along the value chain, and appropriate and timely control of resistant populations. Researchers in Australia and India are collaboratively engaged in the

deployment of advanced molecular diagnostic tools to accurately quantify resistance to phosphine, assess risks along the grain value chain and to implement appropriate intervention strategies to manage them. It is important to note that Australia and India share similar sub-tropical and tropical climates conducive to insect infestations and both countries have a long history of use of phosphine to disinfest stored grain. Over the last decade, both countries have faced a constant threat to stored grain through widespread development of resistance to phosphine in key pest species, leading to risk to food security and market access. While losses to stored food grain due to insect problems are conservatively estimated around US$364 million (Boxall, 2001) in India, Australian losses are negligible. However, a 'nil tolerance' to live insects applies to all export and domestic grain trade, therefore, poor implementation of pest management practices can jeopardise the country's trade in grains worth AU$9 billion annually (https://www.graintrade.org.au).

Here we present a brief account of progress made in gathering critical resistance data in Australia using advanced molecular diagnostics. We used a molecular screening assay on pest populations collected along a pre-determined grain value chain that has two major bulk storage sites and numerous farms in Southeast Queensland. Our overall aim is to utilise the molecular resistance detection method as a decision-making tool for accurate determination of problematic sites within each node of the grain value chain and to facilitate timely implementation of resistance management tactics. The current study focuses on two major grain insect pests, the lesser grain borer, *Rhyzopertha dominica* (F.), and the red flour beetle, *Tribolium castaneum* (Herbst). Resistance data generated through both phenotypic and molecular methods are presented and discussed in the context of managing these two species.

2. Materials and Methods

2.1 Study sites and sample collection

Our area of focus was on a typical grain supply chain containing several grain handling nodes in the township of Millmerran, located in south-east Queensland, Australia. The supply chain contains a cluster of several on-farm grain storage silos, feed and stored product processing mills in and around two bulk grain depots, Millmerran, and Brookstead, each with the storage capacity of 30,000 tonnes (Figure 1). These depots are located 25 km apart and the distance between the farms and feed mills are approximately within 100 km. Grain samples were collected in a consistent pattern across all the selected nodes, representing the entire grain supply chain during 2017-18. For example, 3-5 sites within each node were selected, depending upon the storage size and the structure of the site. Within each site, 5-10 grain samples were collected, each weighing approximately 2 kg. The grain samples were screened in the laboratory for live adults and progeny (in the form of eggs and other immature life stages generated from the collected parent populations). Although several pest species were collected through this study, here we present data only on *R. dominica* and *T. castaneum*.

2.2 Phenotypic testing

The collected live adults were subjected to a phosphine discriminatory dose of 0.25 mg L^{-1} over 48 h for *R. dominica* and 20 h for *T. castaneum* to diagnose strongly resistant populations in each species as described previously for the resistance testing bioassays (Collins et al 2002 and Jagadeesan et al 2012). A cohort of approximately 150 insects were used for each site within each node for the resistance testing bioassays. In the case of eggs, all the eggs emerged as adults (progeny) after 6-8 weeks of incubation were fumigated. Both live and dead insects from the bioassays were subsequently preserved in 70% ethanol at -20˚C before DNA extraction and molecular resistance screening.

2.3 High throughput molecular screening of resistance alleles

2.3. 1 Genomic DNA extraction

Genomic DNA was extracted from individual insects using a modified Hotshot DNA extraction method described by Montero-Pau et al. (2008). Test insects in a 96 well PCR plate were lysed individually with 75 µL Alkaline lysis buffer (25 mM NaOH and 0.2 mM EDTA) (pH = 12) at 95 °C for 30 min, cooled down at 4 °C for 10 min and then neutralized by addition of 75 µL of 40 mM Tris–HCl (pH = 5). Samples were centrifuged and the supernatant containing *g*DNA from individual insects were stored at -20 °C for high-throughput sequencing. The susceptible and resistant reference strains in both *R. dominica* and *T. castaneum* were also included in each 96 well PCR plate in gDNA extraction for valid interpretation and used as positive controls.

2.3.2 Molecular resistance screening assay

The molecular assay is a genotyping-by-sequencing method comprising multiplex amplification and sequencing of the exons of the dihydrolipoamide dehydrogenase (DLD) gene of either *R. dominica* or *T. castaneum* (Schlipalius et al., 2012) using the Illumina Miseq™ next-generation sequencing platform. The assay encompasses nearly the entire protein coding sequence of the gene by employing multiple primer pairs to amplify the gene in segments that are subsequently sequenced together. The forward and reverse primers for each exon region were tagged with individual 10-mer index sequences during synthesis to facilitate bioinformatic sorting of the sequences to the individual from which they had been amplified.

Each forward primer was tagged to be specific to a 96-well plate that was assayed 96 tagged reverse primers were specific to individual wells of a 96 well plate. As a result, each DNA amplification product could be traced back to the plate and well in which it had been amplified.

2.3.3 PCR conditions to amplify multiple alleles

The PCR reactions utilised Terra™ PCR mix (Clontech), which amplifies directly from tissue and samples with high protein content. Each sample reaction contained: 3 µL template DNA (~5-10ng), 1X PCR Buffer, 10 µM of each primer (5 forward, 5 reverse), 0.6U Taq polymerase and water to a final volume of 22 µL. The PCR cycling conditions were: 98˚C for 2 min; 4 cycles of 98˚C for 15 s, 65˚C for 30 s, 68 ˚C for 60 s; with a final 36 cycles of 98˚C for 15 s, 55˚C for 30 s and 68˚C for 60 s.

Resulting amplicons were pooled and sent to the Australian Genomic Research Facility (AGRF) for sequencing on the Illumina MiSeq™ sequencing platform with the 250 bp paired-end read protocol.

2.3.4 Data processing and interpretation

Paired-end Miseq data were demultiplexed using CLC Genome Workbench V9.5.4 (CLCBIO) using the forward and reverse tags. The data for each sample was then aligned against a genomic reference sequence (JX434608 or KF032715) using the parameters: mismatch cost=2, insertion cost =2, deletion cost=3, length fraction=0.5. Variants were then called using the CLC Genome Workbench basic variant detection algorithm (minimum coverage=10, min frequency 25%, minimum variant count=2).

3. Results and Discussion

Our results on insect sampling clearly indicated the existence of both target pests, *R. dominica* and *T. castaneum* within the selected grain value chain at different density levels. For example, the average number of *R. dominica* per sample (1430.5) was higher than that of *T. castaneum* (728.5) (Tables 1 and 2). Comparison of number of insects identified in each grain handling node across the grain value chain confirmed that infestation *of R. dominica* was prevalent across the entire grain value chain, except for Depot 1 and the processing feed mill, whereas infestation of *T. castaneum* was prevalent only in farm storages (Tables 1 and 2). The average number of *T. castaneum* recorded

in the only mill sampled was double of that of *R. dominica* and far much higher than the *T. castaneum* collected on both depots (Tables 1 and 2).

Our phenotypic resistance screening in collected insect populations across the grain value chain identified three strongly phosphine resistant populations of *R. dominica* (one each from Depot 1, Depot 2 and Farm 1) and a single resistant population of *T. castaneum* (from Farm 1). The molecular screening for single nucleotide variants (SNV) (that confers strong resistance to phosphine), in both *R. dominica* and *T. castaneum* supported the results of phenotypic screening. In addition, the assay has identified two other strongly resistant *R. dominica* populations from Farm 2 and Farm 3, and one strongly resistant *T. castaneum* population from Farm 3, which were initially scored as 'not strongly resistant' in the discriminating phenotypic testing (Table 1). In total, molecular screening has identified three different SNVs, P>49>S, G>135>S and K>142>E in *R. dominica,* and a single SNV in *T. castaneum*, P>45>S (a homologue of P>49>S in *R. dominica*). These resistant alleles were also previously detected in farms and bulk storages in south east Queensland (Schlipalius et al., 2012, Kaur et al 2013), and bulk grain storages in India (Kaur et al 2015), and USA (Chen et al., 2015). Unlike our current high-throughput methods, however, the earlier studies relied on low-throughput DNA marker assays, targeting a specific resistance allele.

The accurate discrimination of multiple genotypes (*rr, rs and ss*) in each SNV (resistance allele) in selected populations of *R. dominica* and *T. castaneum*, identified allele frequency (R %) as well as percentage of actual carriers of resistance (R%) in each node (i.e. the proportion of individuals having at least one copy of resistant allele in the population). Comparison of resistance allele frequency between the grain handling nodes, indicated that frequency was higher in *R. dominca* in bulk storage depots (10.6-11.3%) compared to farms (2.5-3.95%) (Table 1). In an earlier study Kaur et al. (2013) estimated frequency of one specific variant, K>142>E in populations of *R. dominica* from farms in southern Queensland in 2011 using traditional CAPS (Cleaved Amplified Polymorphic Sequence) marker analysis, which showed a much higher range of allele frequency (3-26%) compared to the range established in our current study. Daglish et al (*in press,* in this Proceedings), using the same method estimated resistance allele frequency in *R. dominica* between (6.0-13.4%), that were trapped at the bulk storage depot sites of the same study site as ours, which supports the findings of the current study. In the case of *T. castaneum,* resistance was confined to farms, and frequency of resistance alleles remained relatively low (1.27-6.25%) (Table 2).

The observed variation in resistance allele frequency among the *R. dominica* and *T. castaneum* populations within the study area indicates that insect populations at each grain handling node have been exposed to differential selection pressure to phosphine. For example, the higher resistance observed in *R. dominica* at depots suggests that populations at this node might have had undergone stronger phosphine selective pressure than populations that were collected from farms and the feed mill. However, this trend was not observed with *T. castaneum* and, in fact, resistance in this species appeared to be prevalent only on farms. This difference, perhaps related to their inherent strength of expressing the resistance phenotype (Jagadeesan and Nayak, 2017) or probably related to species biology and habitat (Daglish et al., 2017). For example, Jagadeesan and Nayak (2017) showed that adults and eggs of strongly resistant *R. dominica* populations exhibit nearly 2-3 fold higher resistance level than that of *T. castaneum*. Thus, the fumigation strategies (concentration x exposure period) that are currently adopted at bulk storage depots to control resistant *R. dominica* could have been extremely high for *T. castaneum*, leaving no survivors after the phosphine treatment.

The grain value chain studied here is ideal in a sense that there has been a high degree of grain movement over the years between farms and bulk storage depots in the region. Our results led us to conclude that there is a high degree of possibility that resistance alleles, can migrate from farms to bulk storages and get exposed to higher selective pressures to phosphine. There is also the possibility of two way insect movement within the study area, which may aggravate the resistance problems in this region. Australian studies have demonstrated that *R. dominica* and *T. castaneum* flight occurs across the broader farming landscape (Daglish et al., 2017), and there is also the

potential for human-aided movement of insects along the supply chain (Hernandez Nopsa et al. 2015).

In conclusion, the current study established base-line information on pest populations and the type and frequency of phosphine resistance alleles for two key grain insect species in a typical grain supply chain in Australia using both phenotypic and molecular resistance tools. Currently, we are in consultation with industry collaborators for implementation of suitable intervention strategies for each grain handling node in a systemic pattern across our study area. We strongly believe that such approach will facilitate achievement of a sustainable pest and resistance management program for stored grains in Australia.

Table 1 Frequency of phosphine resistance in *R. dominica* within a selected grain value chain.

Sites	Insects collected	Phenotype scoring*	DNA analysed	Resistant alleles			Genotypes			R (%)	Carriers of R (%)
				P49S	*G135S*	*K142E*	*rr*	*rs*	*ss*		
Depot 1	52	SR	52	-	-	9	2	7	43	10.6	17.3
Depot 2	5234	SR	637	-	1	96	47	50	540	11.3	15.2
Farm 1	220	SR	40	1	-	1	0	2	38	2.5	5.0
Farm 2	3000	Not SR	152	1	-	8	3	6	141	3.95	5.92
Farm 3	1100	Not SR	156	1	2	6	3	6	147	3.85	5.77
Farm 4	376	Not SR	80	-	-	-	-	-	80	0	0
Feed mill	32	Not SR	32	-	-	-	-	-	32	0	0

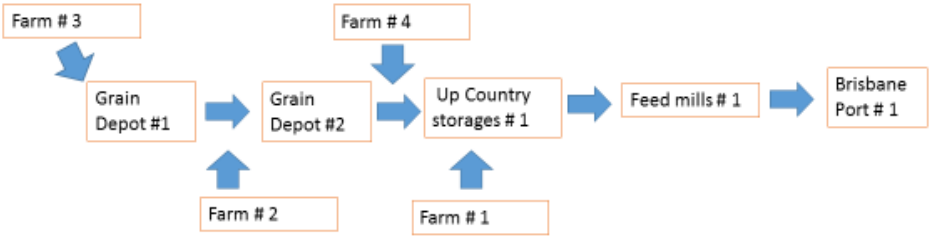
*SR – strongly resistant.

Table 2 Frequency of phosphine resistance in *T. castaneum* within a selected grain value chain

Sites	Insects collected	Phenotype scoring*	DNA analysed	Resistant alleles		Genotypes			R (%)	Carriers of R (%)
				P45S	*G131S*	*rr*	*rs*	*ss*		
Depot 1	25	Not SR	23	-	-	-	-	*23*	0	0
Depot 2	16	Not SR	15	-	-	-	-	15	0	0
Farm 1	199	SR	48	3	-	2	1	45	5.21	6.25
Farm 2	69	Not SR	24	-	-				0	0
Farm 3	4000	Not SR	315	4	-	0	4	311	0.63	1.27
Feed mill	62	Not SR	62	-	-	-	-	62	0	0

*SR – strongly resistant.

Figure 1. The model of bulk grain supply chain selected for this study.



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Australia's On-Farm Grain Storage Extension Project – a national initiative improving stored grain pest management and maintaining phosphine fumigation efficacy on-farm for the Australian grains industry.

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Abstract

Phosphine's continued use in Australia to control grain insect pests in on-farm and central storage systems is threatened through increased resistance in both frequency and strength in target insect pests. Effective fumigation combined with best practice integrated pest management is essential to the sustainability of grain biosecurity, food safety, quality assurance and market access for Australian post-harvest grain systems.

The National Stored Grain Extension Program (NSGEP) is an industry funded initiative developed to facilitate best practice in grain storage management within Australia's grains industry. The NGSEP uses a multi approach engagement strategy and a variety of adult learning principles and training techniques aimed at increasing awareness and knowledge to build capacity and support to enable farmers and industry to manage their grain storage systems and meet best practice and market requirements. These include: training workshops, field days, practical demonstrations, industry forums, multi-media and website development and building networks with grower groups, government agencies and agribusiness.

Various evaluation methods have shown that awareness and adoption of best practice in on-farm grain storage management has increased. Key outcomes include increased knowledge in insect identification and skills development and practice change in the management of grain hygiene, aeration, phosphine application, silo testing and planning of storage systems.

The NSGEP contributes to the positive on-going changes observed in Australia's on-farm grain storage systems, primarily through the specialized extension network of information, support and training provided that is highly regarded and in demand. It plays an instrumental role in building capacity and maintaining phosphine fumigation efficacy in the Australian grains industry.

Introduction

This paper reports on a Grains Research and Development Corporation (GRDC) sponsored project in extension for on-farm grain storage. The project was a national extension project, this paper focuses on the south eastern grain growing region of Australia, where the author is based and on-farm grain storage has increased significantly as a consequence of the deregulation of the domestic and export markets.

Phosphine fumigation is widely used to kill insect infestations in on-farm and central receival storages. When applied in unsealed storages poor results are typically attained which can lead to rejection at receival sites due to poor insect control and or detection of phosphine in the delivered grain. Use of phosphine in unsealed storages is a risk to the occupational health and safety of the user and increases resistance to phosphine. Safe phosphine application is essential to maintain the continued use of phosphine.

The project involved a multi approach to improve on-farm storage practises and the efficacy and safe application of phosphine for fumigation. The project consulted and worked with key industry stakeholders including state based departments of agriculture, research institutions, regulators, bulk handling authorities, silo and machinery manufacturers, training bodies, private agronomists and consultants, peak farmer representative bodies and farmer growing system groups.

Key elements of the project were delivery of best practise grain storage management and phosphine fumigation workshops and field days aligned to adult learning principles, information packages, media releases and communication, phosphine label changes and the introduction of an Australian Standard for gas-tight sealable silos.

Materials and Methods

The project involved using a multi approach to improve on farm grain storage management and phosphine fumigation practises including;

Workshops and field days were conducted for farmers, agribusiness and advisers on the principles of best management grain storage and fumigation practises. Grower, industry, silo and agricultural machinery networks were used to set up events in local areas. Events were advertised through these networks and local media and local grain storage issues were assessed to ensure the events were relevant to the immediate needs of participants as well as delivering the key messages around best practise fumigation and grain storage management.

Workshops and field days were developed and conducted using adult learning principles to create a positive learning environment for participants, build capacity in the industry to further support farmers and enable farmers and industry to implement ongoing changes in their grain storage systems. Typically the event would be for 3 to 4 hours depending on discussion and either started or finished with a meal and socialising to foster further discussion and knowledge transfer.

The events used a variety of delivery and training techniques, including auditory, sensory, visual and practical learning examples to cater for the different learning styles of the participants. Learning materials were supplied during the event and contact and further information details were given for participants to follow up on any questions.

Sessions would include presentations of best management practises combined with demonstrations and practical examples either at an on-farm grain storage or local grain storage site.

Group discussion and knowledge sharing was an integral component of each session, fostering learning and skills development for the participants.

A certified phosphine training course, "Responsible, Safe and Effective Use of Phosphine Generating Formulations on Farms" was developed with a chemical training provider (AusChem Training Inc) for on farm users. This course is available for training on-farm users of phosphine throughout Australia.

Best practise fumigation and grain storage management presentations were delivered at a variety of industry forums including GRDC Research and Grower updates, agribusiness and industry field days and seminars.

In consultation with industry a Strategy to manage resistance to phosphine was written to encompass the variety of grain storage systems in the Australian grain value chain. The strategy was written for use by small through to large central receival bulk handling authorities, grain growers and end users. This strategy has been adopted by the industry as a blueprint to manage phosphine resistance.

To support the training activities a variety of media articles were written and disseminated throughout local newspaper, journal and newsletter outlets. Articles were written in response to arising issues and as general information for growers and to promote best practise phosphine fumigation and management. A DVD was recorded on gas-tight sealed silos and phosphine use and distributed via the GRDC to all registered grain growers in Australia and uploaded onto YouTube and GRDC television. Radio interviews and discussions were also used to disseminate information and advice.

As an integral part of the nationwide project information packages and materials were developed and distributed through a variety of industry channels and were available at workshops and field days. Two written specifically for fumigant use were a GRDC factsheet called "Pressure testing sealable silos" and a booklet called "Fumigating with phosphine, other fumigants and controlled atmospheres. Do it right – Do it once".

Label changes to phosphine are currently being written to improve overall management of the product, safe use and to improve resistance management. Label changes have also been made to enable regulators to enforce breaches of the label, particularly in regards to what constitutes a suitable sealed structure.

In conjunction with SAI Global (formerly Standards Australia) the author convened a committee to write an Australian Standard for sealed silos. Prior to the Australian Standard (AS 2628-2010), there was no industry benchmark for sealed silos which growers could use to determine whether a silo they were purchasing was actually sealed and gas-tight when purchased. The committee was made up of representatives from State Department of Agriculture research scientists, the CSIRO, State and national farmer peak industry bodies and silo manufacturers. The committee consulted widely with industry, chemical registrants, the AVPMA and grower bodies and representatives.

All of these approaches were used to deliver a whole of industry extension program promoting best practise on-farm grain storage management and phosphine fumigation.

Results and Conclusions

Personal communication and anecdotal evidence in communication with the grains industry and value chain has demonstrated that on-farm grain storage management and the awareness and implementation of best practise phosphine fumigation has increased. Feedback from silo manufacturers has shown that growers are actively asking for Australian Standard compliant sealed silos when comparing and purchasing sealed silos. The Australian standard enables growers to purchase a sealed silo which meets a standard gas-tight pressure test, enabling them to have the correct system to fumigate. Prior to this standard being enacted growers found it difficult to benchmark silos in the marketplace, where a number of silo manufacturers claimed their silos were sealed but did not meet the standard pressure test.

The Australian standard alone does not ensure that efficacious phosphine fumigations can be administered; however it is the first step in ensuring a grower has the correct system in which to undertake an efficacious and safe fumigation.

Stakeholders across the grains value chain are asking for and disseminating best practise information through their networks and the many grower workshops and field days conducted through the national extension project and industry forums. Development of the various information packages covering best management practises for on-farm storage and fumigation provides a mechanism for growers and industry to support the training and knowledge development they have undertaken. The phosphine booklet "Fumigating with phosphine, other fumigants and controlled atmospheres" was a comprehensive and farmer friendly publication covering sealed storage management, silo testing and best practise fumigation. The Australian standard provided growers with the tools to select sealed storage, the extension program and information packages built on and supported best practise fumigation and grain storage.

At workshops and field days growers are taught the theory behind a successful fumigation and with practical demonstrations shown the features of a gas-tight sealed silo, how to maintain and replace seals and how to perform a standard pressure test to test whether they are sealed. A website www.storedgrain.com was developed to further provide a source for growers and industry to look for and download information.

Using mediums such as websites and the media allowed specific and timely information to be brought to the attention of growers and industry and to promote key messages when necessary. An example of this was a major media campaign using rural media and industry networks promoting and discussing the Australian standard for sealed silos, which was a great success and very quickly converted to growers actively asking whether silos being considered and or purchased met the standard.

Newspaper and newsletter articles and radio interviews are regularly released to promote best practise fumigation and grain storage practises when the information is timely and can be used to assist farmers in their storage management.

The accredited phosphine training module has had a minor uptake to date, largely due to growers still not being required to undertake training specific for the use of phosphine in all states except New South Wales. Currently New South Wales farmers are required to undertake phosphine training as a Work Cover (Occupational Health and Safety regulator) requirement. State regulators of chemical use are currently considering mandatory training for phosphine use, particularly in Victoria. The introduction of training is being considered as part of a response by regulators to the potential label changes for phosphine being proposed to the APVMA (Australian pesticides and veterinary medicines authority).

Overall the extension project has had a positive impact on improving the efficacy of phosphine fumigation in on farm fumigation. Growers are actively asking for Australian Standard Compliant sealed silos. Growers, industry and agribusiness are asking for and disseminating best practise information through their networks, and there has been a continuing demand for workshops and field days and addresses at industry forums.

Temporal and Spatial Patterns in Aerosol Insecticide Droplet Distribution: Modifying Application Strategies to Improve Coverage and Efficacy

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Abstract

With the phase-out of methyl bromide, treatment of food facilities with aerosol insecticides as part of management programs has increased. The physical layout of the structure, the distribution of equipment and other items within the space, and the application method and location may all cause spatial variation in how the insecticide is deposited, which can result in areas with insufficient or excessive amounts of insecticide applied. The impact of aerosol insecticide application position and dispersal method/formulation on the distribution of droplets was evaluated using a series of applications within the same flour mill room. The spatial pattern of droplet distribution and the effect of treatment on bioassay insects (*Tribolium confusum* Jacquelin DuVal) was evaluated. There was variation in aerosol concentration and droplet size distributions within room and application position had an impact on the spatial pattern of aerosol droplets. The further away and more obstructed by structural features a location was the lower the aerosol concentration, but concentration was also lower to the side and behind the release point. Evaluation of the temporal pattern in droplet deposition shows that most larger droplets settle out of the air relatively quickly, supporting that idea that shorter shutdown times are be possible. Efficacy was correlated with droplet concentration. The overall conclusion is that there can be considerable variation in distribution of aerosol insecticides and as a result considerable potential for improvement in the effectiveness of these applications.

Keywords: insecticide, aerosol, flour mill, *Tribolium confusum*, efficacy, spatial distribution.

1. Introduction

Some insecticides can be applied as aerosol treatments which involves atomizing the liquid insecticide and carrier and dispensing as small particles ranging in size size from 5 -50 µm. Use of aerosol applications using reduced risk insecticides such as pyrethinrs, pyrethroids, and insect growth regulators has increased with decreased use of structural fumigations in food facilities. Application of insecticides as aerosols offers the advantage over other spray methods in that more complete coverage of surfaces within a food facility can be obtained. However, information on the coverage that is actually achieved and the impact of variation in aerosol deposition on efficacy remains limited. Aerosol droplets have limited ability to penetrate into machinery or commodiites so they don't function like a fumigant and they also have limited ability to disperse under obstructions. This can generate areas with inadequate coverage with insecticide and can result in reduced efficacy (Campbell et al. 2014; Kharel et al. 2014; Arthur et al. 2018). Evaluations of spatial patterns in aerosol distribution can be done using bioassay insects (e.g., Campbell et al. 2014), but using particle size measuring equipment can improve our understanding of what is happening during aerosol treatments (e.g., Arthur et al., 2018). Understanding the spatial pattern in both aerosol droplet size and concentration is important given that droplet size impacts both dispersal but also efficacy, given that smaller droplet sizes tend not to be efficacious against insects (Arthur et al. 2014).

Previous research has shown that there is spatial variation in efficacy against stored-product insects within a facility, presumably due to uneven aerosol dispersal and deposition patterns (Arthur and Campbell 2008, Campbell et al. 2014, Arthur et al. 2018). When aerosol treatment with a combination of pyrethrin and insect growth regulator was applied from one location within a flour mill there were areas with high efficacy, typically in open areas in the center of the room, and areas with low efficacy, typically in corners, behind application point, obstructed areas, and locations farthest from application point and which had the most physical obstructions between point of release and where measurements were taken (Arthur and Campbell 2008; Campbell et al. 2014). As a result, a critical question is how can coverage of these treatments be improved so that get more even coverage and efficacy within a space. The aerosol formulation and delivery method are likely to be important variables, since velocity at release and droplet size distribution produced will impact the distribution and deposition of aerosols. Also, where the aerosol is applied within a space is also likely to impact coverage, given that barriers and distances that need to be traveled by droplets will vary with release point. In these tests, we evaluated the impact of application point and formulation type on coverage of aerosol treatments. Specifically, we used a combination of droplet size and concentration measurement and bioassay insects to evaluated spatial pattern in aerosol

applications when aerosols were applied from one of three locations or if aerosol application was split among all three locations.

2. Materials and Methods

Aerosol applications were conducted at the pilot scale flour mill at Kansas State University, on the third floor which is roughly L shaped, 13.5 x 21.0 m in main area and 7.5 x 6.5 m in the smaller offshoot of the main area (volume of approximately 1,504 m^3). Aerosol applications were applied by a commercial applicator using the label rates of (1) cylinderized formulation of a combination of pyrethrin and the IGR pyriproxyfen (TurboCide Py-75 with IGR, Chem-Tech Ltd., Des Moines, IA, USA) and (2) combination of pyrethrin (BP-100, BASF Corp., Research Triangle Park, NC) and the IGR methoprene (Diacon® IGR, Central Life Sciences, Schaumburg, IL, USA) applied using a portable handheld mechanical fogger hand applicator.

Each aerosol was applied from one of three locations within the mill, or the application was split equally among the three locations (Fig. 1). Treatments were replicated three times. Aerosol distribution was measured using bioassay dishes containing confused flour beetles, *Tribolium confusum*, and Aerodynamic Particle Sizer (APS) spectometer 3321 units (TSI Inc., Shoreview, MN, USA) placed at different locations within the mill (Fig. 1). After one hour of treatment, the room was vented and the bioassay insects collected and evaluated on whether they showed signs of being effected by the insecticide and then held for 14 days and assessed again and number alive, dead, or knocked down was recorded.

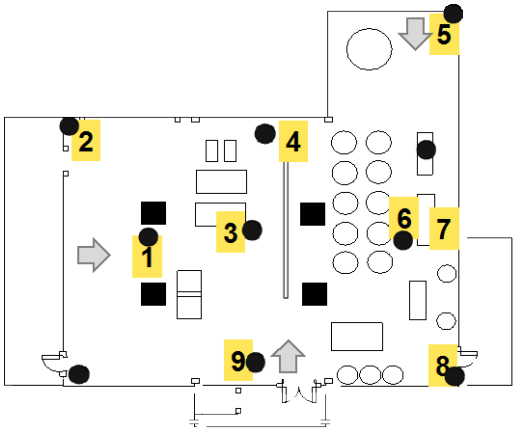

Fig. 1 Floor plan of the flour mill where the aerosol tests were conducted, with aerosol application release points and directions indicated with gray arrows, positions of the aerosol particle size analyzers indicated by numbers in yellow boxes, and positions of the bioassay dishes indicated by the black circles.

3. Results

Number of aerosol droplets, droplet size distribution, total concentration in air (mg/m^3), and estimated deposition on surfaces (ug/m^2) were calculated for each location/treatment combination. Example of the temporal and spatial pattern in total concentration and mean particle size is shown in Fig. 2. Total droplet concentration decreased with distance from application point and in more obstructed locations, and at all locations within the mill the total concentration had dropped to low levels after less than 20 min and remained unchanged until the end of the treatment. There was considerable variation in the estimated deposition on surfaces among locations, with greatest estimates near point of application and dropping as move further away or if more obstructed. Aerosol application location did impact which locations had higher concentrations of aerosol, but all application locations and formulations resulted in patterns of high and low deposition. Splitting

the application among three locations increased the number of locations with higher deposition but in all treatments had locations with low deposition.

There were significant differences in bioassay insect knockdown immediately after treatment and mortality after 14 days among application location/treatment combinations. Application location did result in differences in the pattern of efficacy but regardless of whether released from one of the three locations, or split among three locations, there continued to be zones where beetles survived treatments (Fig. 3).

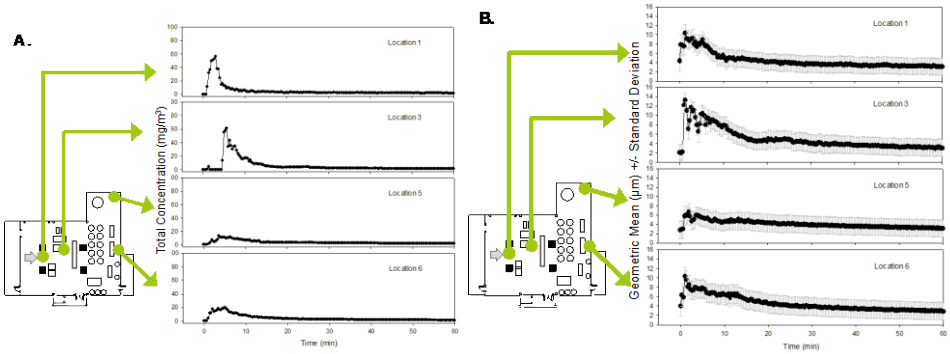

Fig. 2 Example of how total concentration (A.) and geometric mean droplet size (B.) varied among locations and changed over time after start of treatment, using results of one trial using the cylinderized formulation of pyrethrin and pyriproxyfen released at one location (only four the APS units shown for clarity).

4. Discussion

Results of this study show that there is spatial variation in the distribution of droplets that is impacted by release location and the insecticide formulation/application method. There was also a correlation between droplet deposition and efficacy using bioassay insects, suggesting that it is the droplet characteristics that are causing variation in efficacy. As expected, distance and physical barriers contributed to reduced droplet concentrations and droplet sizes, and were associated with lower efficacy. An exception to this pattern is that locations behind where the applicator stood often had reduced aerosol deposition and bioassay efficacy, suggesting that the release velocity of droplets resulted in limited drift of droplets back into the area of release. Unfortunately, none of the different application locations evaluated, including releasing aerosol split among all three locations, resulted in all locations having high efficacy. Further evaluation of other patterns of aerosol release and use of fans to facilitate movement of droplets is needed.

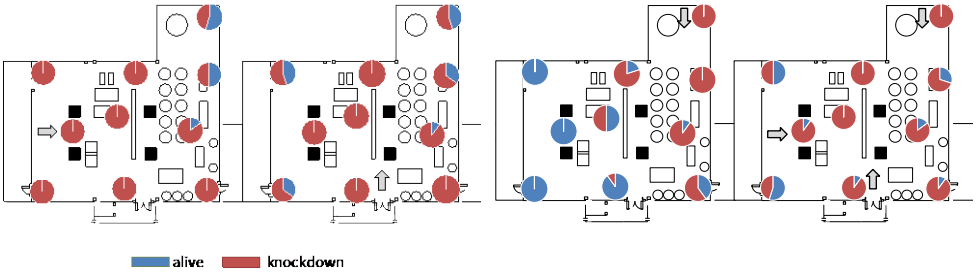

Fig. 3 Example of effect of application location on bioassay insects (*T. confusum*) immediately after exposure to aerosol at different locations, using results of one trial with cylinderized formulation of pyrethrin and pyriproxyfen. Each floor plan has an arrow to indicate the aerosol application location and direction and the pie charts represent the percentage of beetles alive or knocked down immediately after the treatment.

Our results bioassay results are meant to be used as indicators of aerosol concentration, not necessarily as an indicator of overall effectiveness of a treatment against a resident pest population. First, we did not include the impact of the insect growth regulator in the aerosol formulation. Initial evaluations indicate that because much smaller amounts are needed for efficacy that more consistent high efficacy is found using larvae exposed to surfaces at different spatial locations. Second, the spatial pattern of insects in the facility and how much of the population is hidden in areas aerosol cannot reach is not known. In most situations we would predict that large portions of the population will not be directly exposed to the droplets during an application. Contact with treated surfaces and materials after the aerosol application is likely to more important in terms of the overall impact of a treatment on the pest population.

Aerosol insecticide applications have tended to be a black box and little information was available on the impact of the treatments. Research presented here is part of a broader research effort to understand these treatments better, to make them more effective, and to be better able to predict the best strategies for using reduced risk aerosol insecticides.

Acknowledgement

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Technical improvement of the Detia Degesch Phosphine Tolerance Test Kit

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Abstract

Phosphine is the most important commonly used fumigant for the control of stored product insects in warehouses and processing facilities globally. However, the improper and extensive use has led to reduced susceptibility to phosphine for several insect species and strains in many parts of the world. To evaluate and quantify this phenomenon, Detia Degesch developed the Detia Degesch Phosphine Tolerance Test Kit (DDPTTK) more than 10 years ago. The use of DDPTTK is based on the exposure of the insects on a high concentration of phosphine (e.g. 3000 ppm) for short exposure periods (e.g. 8-15 min). This kit can be used on site by the fumigation and food industry, and can provide immediate results on the tolerance status of the insect strains that are to be treated. So far, the instructions of DDPTTK refer only to a six insect species. In this work, data for

the expansion of knowledge about other species is provided, in order to broaden the spectrum of cases where the kit can be used. Moreover, certain improvements for the use of the kit are introduced, i.e. practical recommendations on the procedure and safety instructions.

Keywords: stored product insects, laboratory species, tolerance to phosphine, fumigation.

1. Introduction

The determination of insects´ sensitivity status towards treatments with phosphine has been a widely discussed matter all over the scientific world. As there have been many approaches to determine tolerance or even resistence as part of monitoring programs or other projects, a great discussion about validity, comparability and as a result, tendency of the development of resistence has been unleashed.

But as a scientific discussion is ongoing on a very different level as the actual fumigation work, a gap has developed between the results of various testing approaches and storage protection itself.

Due to this reason, Detia Degesch has developed a simple and easy-to-use testing kit, which can be utilized on-site and by basically anyone. In this way, the fumigator can have a fast and uncomplicated answer to his question: is there anything suspicious about the pests in my commodity?

The scientific basis for determining susceptibility in stored product pest insects has been described by REICHMUTH (1997), who discovered a relation between activity in a phosphine containing atmosphere with 3,000 ppm and narcosis with the narcotical effect showing direct proportionality to mortality.

The endpoint to be evaluated by the user is quite simple: Do the insects still walk? Have they become inactive or uncoordinated? How many of my 20 insects overgo their indicated time-to-immobility?

As the kit was released for the first time in 2007, time has come to relaunch an updated version, as most of the data was outdated. The basis of the sensitivity determination has been originally derived from laboratory reared insects, without prior contact to phosphine. Thus, the endpoint to be monitored has shifted for some species. The aim was to use actual monitoring data from the project "Tolerance/resistence of stored product insect pests to phosphine monitoring in Europe", which is the first project of its kind in Europe (SAKKA et al. 2017, AGRAFIOTI et al. 2017).

While the first kit included monitoring advice for six species, the new version contains information about 13 different species (to be presented during the conference).

2. Materials and Methods

The kit includes the following components:

100 mL syringe
2 canula, 1 with a rubber hose
5 L flexible plastic canister
Lid including a septum
5 x 2 test kit pellets
Instructions for use, containing determination of dilution

Additionally and not included in the kit, measuring equipment to determine phosphine concentration is required. It is advisable to use a pump and measuring tubes with a measuring range up to 10,000 ppm. To determine the time, a stopwatch or any clock should be at hand. The procedure of testing is as follows:

- Unfold the plastic canister
- Add 50 mL of water
- Add two test kit pellets and close with the lid, shake carefully (waiting for pellets to be completely dissolved)
- Connect the measuring device with the canister by using the canula and the ruber hose to determine phosphine concentration

- Use the diagram to determine the diltution for a target concentration of 3,000 ppm in the syringe
- Remove syringe piston, add 20 adult insects into the syringe and put back the piston without damaging the insects
- Adjust air volume in the syringe first (see figure 1 and table 1)
- Connect syringe with the canister and fill the syringe up to 100 mL with phosphine
- Start the clock
- Oberserve the behaviour/activity of the insects

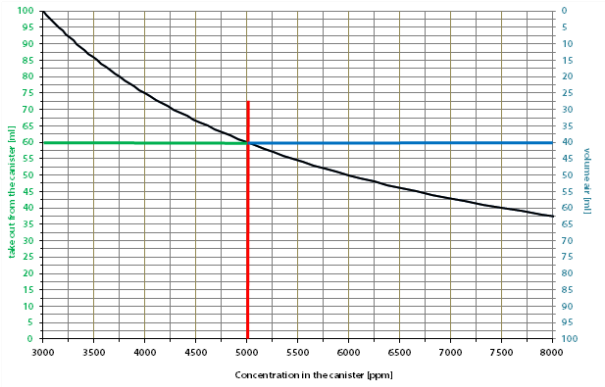

Fig. 1 Dilution determination scheme. To achieve 3000 ppm in the syringe, the concentration inside the canister has to be determined first. On the basis of this result, the volumes to be taken by the 100 mL syringe of normal air (first step) and phosphine from the canister (second) can be determined as follows: The red line symbolizes the measured concentration in the container. The point crossing the black line can than be used to draw lines in horizontal direction. Where the light blue line crosses the secondary x-axis, the required volume of air can be read off, while the green line crossing primary x-axis ascertains the volume of phosphine to be taken from the canister.

The endpoint to be determined shall be “walking” or not walking”. After the species specific time, the test can be terminated. To record the testing, the kit includes pre-printed forms, which are a useful overview, whether or not suspicious insects occur and to follow up on consequent fumigations (see table 2).

Tab. 1 Dilution scheme for desired syringe concentration of 3,000 ppm (testing concentration)

concentration in the canister (ppm)	take out from canister (mL)	volume air (mL)
3,000	100.0	0
3,250	92.3	7.7
3,500	85.7	14.3
3,750	80.0	20.0
4,000	75.0	25.0
4,250	70.6	29.4
4,500	66.7	33.3
4,750	63.2	36.8
5,000	60.0	40.0
5,250	57.1	42.9
5,500	54.5	45.5
5,750	52.2	47.8
6,000	50.0	50.0
6,250	48.0	52.0
6,500	46.2	53.8
6,750	44.4	55.6
7,000	42.9	57.1
7,250	41.4	58.6

7,500	40.0	60.0
7,750	38.7	61.3
8,000	37.5	62.5

Tab. 2 Example of pre-printed form for documentation of test results and fumigation details

Test Report Tolerance Kit		
1. General information		
Name of user:		
Country/region:		
Date:		
2. Tolerance test		
Pest:		
Phosphine concentration in container (ppm):		
Volume air (mL):		
Temperature during test:		
3. Active beetles after:		
5 min	20 min	45 min
10 min	25 min	60 min
15 min	30 min	90 min
4. Fumigation conditions		
Dosage for fumigation:		
Exposure time:		
Structure to be fumigated:		
Further comments:		

3. Results

Study data from a monitoring project to be published shows that immobilization of 100 % of all species is not a feasible endpoint from the biological point of view. Therefore, the immobilization of 19 out of 20 individuals during the species specific exposure time is enough to proof normal susceptibility.

After finishing the observation time (max. 90 min, or after the species specific determination time), the outcome has to be evaluated in a very simple way. If the test indicates a strongly tolerant strain, the key parameters for the scheduled fumigation need to be reconsidered and adjusted to the circumstances.

4. Discussion and Outlook

The Detia Degesch Tolerance Test Kit has been proven to be useful in various occasions as a small and simple tool to evaluate insects´ susceptibility status by any user. It can be seen as the basis for a proper and situation-based fumigation of the infested commodity or storage system.

To extend the possibilities, the tool will include a scientific protocol to be used by institutions in laboratories as well. Here, it has become more and more important to evaluate a factor scientifically known as delayed mortality. This has been in discussion to give a more detailed picture about phosphine induced mortality and will be part of a new research project. Furthermore, the simplicity of the kit is very handy for laboratories and institutions with high security status, as the small container enables a safe and clean use of the gas without demanding cylinder stored gas or others.

Acknowledgement

Detia Freyberg GmbH would like to thank the University of Thessaly for their engagement in the projects with and behind this testing method.

We also highly appreciate the cooperation with Control Union and AgroSpeCom for supporting the sampling of insects all over Europe.

Likewise, thank you to all participating pest protection companies, laboratories and end users for supplying the project with insect samples.

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From narcosis to recovery: development of a rapid diagnostic test for phosphine resistance

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Abstract

Hydrogen phosphide (PH_3) is the most commonly used gas for insect control in durable stored products. One of the quick diagnostic tests that are currently in use is the Detia Degesch Phosphine Tolerance Test Kit (DDPTTK), which has been developed by Detia Degesch GmbH (Laudenbach, Germany). DDPTTK provides a rapid evaluation tool for phosphine resistance, where insects are exposed in syringes that contain a high concentration of gas (e.g. 3000 ppm), while this gas is produced on site by adding tablets into a canister. We used DDPTTK to evaluate resistance of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) to phosphine. For this purpose, we followed a specific succession of observations on the exposed adults of this species, in an effort to set the scene for designing a rapid diagnostic tool for phosphine resistance, based upon quick bioassays. Two *T. castaneum* strains were used, one susceptible and one resistant to phosphine. Twenty adults of each of the populations (separate sets of adults each time) were placed in syringe of 100 ml under 1000 or 3000 ppm of phosphine. The insects inside the syringe were monitored at 15-min intervals, for a total period of 90 min, and classified as active, under narcosis and immobilized. After this period, all insects were removed from the syringe and placed in plastic petri dishes with a small quantity of wheat flour. The insects were classified again at the three categories above, after 2 h, 1 d, 2 d, 3 d and 7 d. Regarding the exposure period, at 1000 ppm, all adults of the susceptible strain were immobilized after 60 min of exposure, and remained at this condition until the end of the observation period. At the same concentration, the majority of adults of the resistant strain remained active until the end of the observation period. At 3000 ppm, for the susceptible strain, all adults became immobilized after 90 min observation. For the same concentration, the percentage of the adults of the resistant strain that were active was notably reduced in comparison with 1000 ppm. For the post-exposure period, at 1000 or 3000 ppm, for the susceptible strain, the number of adults that were immobilized reached 95 % after 7 d. At the same phosphine concentration, almost all of the adults of the resistant strain were active even at the 2 h post-exposure period, and practically remain at this condition until the end of the observation period. Our findings indicate that time-to-narcosis / immobilization is inversely proportional to time-to-recovery of the same individuals, and this characteristic can be also considered as an indicator for resistance.

Keywords: phosphine, narcosis, mortality, *Tribolium castaneum*, resistance, diagnostic tool

Diagnosis and indicators

Evaluation of resistance to phosphine and its diagnosis has been performed in many places of the globe, using different diagnostic tools. The most common diagnostic method is the Food and Agriculture Organization (FAO) protocol, which is based on the exposure of the insects for 20 h at concentrations that are generally fixed as "discrimination concentrations" per target species and usually fall within the range of 30 to 50 ppm (FAO 1975). Other tools include bioassays that last for longer, e.g. 3 d, and are carried out at higher concentrations, in order to quantify resistance, in contrast with the FAO method that is used to indicate the presence or absence of resistance (Nayak and Collins 2008, Kaur and Nayak 2014, Holloway et al. 2016, Collins et al. 2017, Koneman et al. 2017). In these bioassays, apart from the initial mortality, which is the mortality that is recorded after the exposure, delayed mortality is also estimated, given that surviving insects are likely to be affected at a later post-exposure stage, which usually is determined at 7 or 14 d after the exposure. This last indicator is generally considered more reliable than the initial mortality.

There is also one more indicator that is taken into account as a means of quantifying resistance: insect immobilization. Theoretically, insects that are susceptible to phosphine are immobilized faster than those that are resistant. This is a generally accepted rule, despite the fact that there are different theories suggesting that immobilization is not concentration-depended and that quick immobilization is not always reliable as an indicator of resistance. In this regard, additional experimental work is needed to underline the potentials of using immobilization as an indicator towards this direction.

Recovery after exposure

While there are many papers that are focused on the evaluation of immobilization or mortality after exposure to phosphine, there are disproportionally few works that examine recovery as an indicator of resistance. Quick recovery may suggest that the insects are resistant to phosphine; nevertheless, the "speed of recovery" here is crucial, and cannot be estimated in one observation interval alone. Recovery may be expressed more vigorously in the case of quick diagnostic tools, such as the Detia Degesch Phosphine Tolerance Test Kit (DDPTTK) (Steuerwald et al. 2006, Aulicky et al. 2015). In DDPTTK, the insects are exposed at high concentrations (usually 3000 ppm) for some minutes that are usually less than 15 and immobilization is used to indicate if the exposed insects are susceptible to phosphine. This method is very easy in its use, and practically this is the only method so far that can be used by fumigators, flour millers etc. on site, without the need to go to a specialized laboratory. In the current work, we used different populations of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), to illustrate immobilization and recovery patterns after short exposures to DDPTTK (data have been submitted for publication to a scientific journal and briefly described in the abstract above). In this effort, there is indeed a big difference regarding the "speed to immobilization" and the "speed to recovery" between strains that have different levels of susceptibility to phosphine. Hence, based on the current data, the theory "fast immobilization=slow recovery" may be true for the tested strains, and can be used further to understand resistance to phosphine and its diagnosis.

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Evaluation of tolerance/resistance to phosphine of stored product beetle populations from Europe, by using different diagnostic methods

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Abstract

We evaluated the susceptibility to phosphine in different populations originated from 14 European countries, by following different diagnostic protocols. In total, more than 200 populations were screened during these tests, classified to 9 beetle species: *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Lasioderma serricorne* (F.) (Coleoprtera: Anobiidae). The different bioassay-related diagnostic protocols that were followed were based on different exposure intervals and phosphine concentrations, ranging between 90 min and 4 d, and between 30 and 3000 ppm, respectively. Our results indicated that one of the populations that had been sampled from Europe was strongly resistant to phosphine. Moreover, the different protocols provide comparable results, which means that a standardized diagnostic can be further designed and adopted. Moreover, molecular assays indicated that the mutations P49S in *R. dominica* and P45S in *T. castaneum* are common among different populations, regardless of the degree of resistance to phosphine. Our results suggest that there are reliable quick tools for the evaluation of resistance to phosphine and that insect sampling in target areas should be conducted on a regular basis.

Keywords: Phosphine resistance, tolerance, fumigation, stored product beetles, protocols

1. Introduction

Phosphine fumigation is the primary fumigation tool to control stored product insects. Nevertheless, although phosphine has been proved effective against most major stored product insect and mite pests, its extensive use meets with several drawbacks (Benhalima et al., 2014). The main disadvantage on the use of phosphine is the development of tolerance/resistance by several stored product insect species. Actually, many species, such as the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) (Opit et al.; 2012), the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) (Konemann et al., 2017), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Ridley et al., 2012), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Daglish et al., 2014) and the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) (Saglam et al., 2015) have developed a

considerable level of resistance, and there are specific strains of these species that can survive concentrations that are considerably higher than the recommended application rates. Currently, resistance to phosphine is found in several parts of the world, such as Brazil (Lorini et al., 2007), USA (Gautam et al., 2016), Australia (Nayak et al., 2017), India (Kaur et al., 2015) while, for the majority of the species tested, eggs and pupae are considered the most tolerant life stages (Price and Mills, 1987; Rajendran et al., 2001; Ridley et al., 2012). However, there is still inadequate information on the evaluation of the level of tolerance to phosphine in Europe, despite the fact that phosphine is widely used in Europe.

There are different diagnostic tests have been widely used for the evaluation of phosphine resistance. The most widely accepted protocol for the evaluation of phosphine resistance is the Food and Agriculture Organization (FAO) method number 16 (Food and Agriculture Organization, 1975). This method uses a discriminating dose with a concentration based on the $LD_{99.9}$ for different stored product insects. In this concentration, the insects are exposed for 20h and after this interval, the exposed individuals are removed and mortality is recorded usually after a 14-d post-exposure period. Hence, survival after this interval is an indication of resistance in this test. A modification of FAO protocol is the Dose Response Protocol, known also as Dose Bioassay. This protocol is based on a different exposure period (3 days) in a range of concentrations. Another protocol is the one that has been developed by the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) for tobacco pests, were the insects are exposed to 200-700 ppm for 4-10 days (CORESTA, 2013). Moreover, a quick test has been developed by Detia Degesch (Steuerwald et al., 2006) which is based on the evaluation of the mobility of the insects after short exposures (usually 15 min or less) to high concentrations (e.g. 3000 ppm).

Apart from the "classic" protocols for the evaluation of phosphine resistance, molecular/genetic methods have been designed, with the use of PCR and molecular markers (Chen et al., 2015; Nguyen et al., 2016). According to literature, there are two major loci, *rph1* and *rph2*, that are responsible for strong resistance. When *rph1* and *rph2* loci are individually homozygous they confer weak resistance, but when they are both homozygous they confer strong resistance (Schlipalius et al., 2002; Jagadeesan et al., 2012; Nyugen et al., 2015; Nguyen et al., 2016). The stored product insects that have been genetically characterized with this method are *R. dominica* (Schlipalius et al., 2008), *T. castaneum* (Chen et al., 2015), *S. oryzae* (Nguyen et al., 2016) and *C. ferrugineus* (Tang et al., 2017).

The present study aims in investigating the tolerance/resistance of different populations of stored product insects in Europe with different evaluation methods. Preliminary investigations were also carried out to detect the genes that are responsible for phosphine resistance in some of these populations. Knowledge of phosphine resistance in different countries in Europe will provide the inferences necessary for improving fumigations and stored product protection measures in general.

2. Materials and Methods

2.1 Populations tested

A total of 500 samples of different commodities (e.g. rice, wheat, barley, chocolate) were collected during 2016-2018 from storage and processing facilities from 14 different European countries. The insects of each sample were identified isolated and transferred to 1L glass jars with commodity to initiate rearing. All rearings have been carried out at the Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly at 25° C, 65 % r.h., and continuous darkness. In this study, we present the results of 18 different populations of stored product insects, corresponding to nine different species: the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *S. oryzae*, *S. granarius*, *L. serricorne*, *T. castaneum* , *T. confusum*, *R. dominica* and *C. ferrugineus*. Only adults were used in the tests.

2.2 Methods for the evaluation of phosphine resistance

Resistance was tested using four different protocols. a) the FAO protocol, based on screening by exposure of the tested insects to 30 ppm for 20 hours, b) the Dose Response protocol, based on the exposure of the tested insects to phosphine to 50, 100, 200, 500, 700 and 1000 ppm for 3 days, c) the CORESTA protocol, based on the exposure of the tested insects to phosphine to 200ppm for 4 days, and d) the Detia Degesch Phosphine Tolerance Test Kit (DDPTTK), based on the exposure of insects at 3000 ppm for 90 minutes. For all protocols, after the termination of the exposure interval, the insects were classified as active, under narcosis or immobilized. Then, the exposed insects were transferred to a clear petri dish for additional 7 days, and then classification was made again. The whole procedure was repeated 9 times (three replicates of three sub-replicates).

2.2.1 FAO Protocol

Twenty (20) adults of the test species were placed in a 1.5 lt glass jar and exposed to phosphine concentrations of 30 ppm for 20 hours. After the termination of the exposure interval, active, under narcosis and immobilized insects were recorded and were transferred to a clean petri dish with food for 7 days. Then, delayed mortality or recovery were recorded.

2.2.2 Dose Response Protocol

The procedure was similar to that for the FAO protocol (including delayed mortality), while the tested adults were exposed for 3 d at 50, 100, 200, 500, 700 and 1000 ppm.

2.2.3 CORESTA

This protocol was based on the CORESTA guidelines, where 20 insects of the test species/population were placed in a 1.5 lt glass jar and exposed to 200 ppm concentration of phosphine for 4 days. After the exposure, active, immobilized and under narcosis insects were recorded, while if there were active insects, then the protocol was repeated with exposure of insects at 700 ppm for 10 days with new individuals (Guide 2 CORESTA).

2.2.4 Detia Degesch Phosphine Tolerance Test Kit (DDPTTK)

Twenty insects were placed in a syringe of 100ml and exposed to a concentration of 3000 ppm of phosphine for 5, 10, 15, 20, 25, 30, 45, 60 and 90 minutes. For strains that active insects were recorded until 90 minutes, the exposure time was extended to 270 minutes (recorded every 30 min). After each exposure interval, active, under narcosis and immobilized insects were recorded, and after the last exposure intervals, insects were transferred to clean petri dishes with food for seven days, to record the delayed mortality or recovery, as above.

2.3 Determination of the mutations that are related to phosphine resistance in *rph2* locus

For the molecular study, two populations of *T. castaneum* and three populations of *R. dominica* were used, based on earlier indications for their susceptibility to phosphine. Specific primers were designed for *T. castaneum* and *R. dominica*. A single band at ~1500-1600 bp was obtained for all the three insect species. PCR products were purified and sent for sequencing in Macrogen sequencing facility (Amsterdam, The Netherlands).

3. Results

Some levels of reduced susceptibility to phosphine, as compared with the laboratory populations, were recorded for many of the populations tested (Table 1). One hundred percent of active individuals were recorded in the population of *T. castaneum* and *C. ferrugineus*. After the exposure of 50ppm for 3 days, for one population of *C. ferrugineus* all (100 %) individuals were recorded as active. Moreover, some populations of *T. castaneum* and *R. dominica* had resistant individuals that could survive at 500 and 200ppm, respectively. Nevertheless, at the highest concentrations (700 -

1000 ppm) there was no survival for any of the populations tested. Moreover, active individuals were recorded after exposure to CORESTA protocol for only one population of *L. serricorne*. Finally, there were certain populations of different species (*S. oryzae* 3Tusc, *S. zeamais* Mach, *L. serricorne* E1, *T. castaneum* BTS and 3SP.18.1, *T. confusum* D1 and *C. ferrugineus* B1) that showed considerable percentages of active individuals after the exposure to DDPTTK.

The P45S allele of *rph2*, which is responsible for strong resistance, was detected from *T. castaneum* populations tested while P49S was detected in the case of R. *dominica*. All populations of *T. castaneum* and *R. dominica* tested are homozygous for the mutant allele, including the lab/susceptible ones, which the exception of *R. dominica* Inj which was found to be heterozygous.

Table 1: Percentage (% ± SE) of active adults of laboratory and field populations of different beetle species after the termination of a 7-d post-exposure period, following the exposure to phosphine under different protocols.

	Sample Code	Concentration of phosphine (ppm)/ exposure time (hours or days or minutes)								
		30ppm 20h	50ppm 3 days	100ppm 3 days	200 ppm 3 days	500ppm 3 days	700ppm 3 days	1000ppm 3 days	200ppm 4 days	3000ppm 90-270min
ae	3T	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	3Tusc	82.2 ± 6.8	18.5 ± 5.1	1.7 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 1.7
	lab	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
arius	3W	2.2 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ais	Mach*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.8 ± 2.2
corne	E1*	11.7 ± 5.2	2.5 ± 1.4	5.0 ± 0.0	1.25 ± 1.25	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 1.7	32.2 ± 9.6
	lab	1.1 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
neum	BTS	29.4 ± 12.4	29.4 ± 12.4	2.8 ± 2.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	90.6 ± 2.6
	3SP.18.1	100.0 ± 0.0	43.3 ± 8.4	6.7 ± 4.3	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	75.0 ± 2.9
	molab	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
usum	BPM	1.7 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	D1	0.6 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 0.8
	Lab	1.1 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
namensi	1W	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
inica	Inj	63.9 ± 9.5	25.7 ± 4.7	10.0 ± 2.9	1.7 ± 1.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	lab	16.1 ± 8.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
rugineus	B1*	100.0 ± 0.0	100.0 ± 0.0	89.4 ± 2.6	50.0 ± 0.0	15.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	83.3 ± 3.3

* these populations had been sampled from counties outside of Europe

4. Discussion

The results presented here indicate that there are some certain survival patterns in some of the populations tested after exposure to phosphine, but very few indications of possible strong resistance (i.e. populations that had survived after 3 d at 500 ppm). In a similar screening from Morocco, Benhalima et al. (2004) noted that all samples tested were phosphine resistant according to the FAO protocol. In the current work, there were some field populations that were susceptible to phosphine, e.g. *O. surinamensis* 1W and *S. oryzae* 3T. Bell et al. (1977) underlined that FAO protocol is a successful method of identifying resistant strains, while at higher doses of phosphine for 20h, the results from that study showed also resistance to some populations. In general, the FAO protocol could be used with success as a quick diagnostic tool to indicate possible resistance, but Dose Response at higher exposures can be performed to cross-check and quantify resistance. By using a similar approach, Konemman et al. (2017) reported that phosphine resistance in *C. ferrugineus* is common in Oklahoma. Specifically, at the discriminating dose of 56.2 ppm all field populations were resistant to phosphine with frequency that ranged between 6 and 100%. Nayak et al. (2013) also reported extremely high levels of resistance for populations of *C. ferrugineus* from Australia. In our study, one *C. ferrugineus* population was able to survive at 500ppm at the Dose Response test. The

current data set indicate that some of the populations that had been sampled from non-European areas, were much less susceptible to phosphine than the ones that had been collected from Europe.

Our study initially identified the presence of the P45S and P49S mutations that are related with phosphine resistance to *T. castaneum* and *R. dominica*, respectively. Genetic studies of phosphine resistance are focused especially to four major species: *T. castaneum*, *R. dominica, S. oryzae and C. ferrugineus*, and are based on the presence of two loci, *rph1* and *rph2* that are responsible for weak and strong resistance. Most studies were focus on *rph2* locus (Schlipalius et al., 2008; Kaur et al. 2013; Chen et al. 2015). More recently, Schlipalius et al. (2018) identified *rph1* locus for *R. dominica*, *S. oryzae*, *C. ferrugineus* and *T. castaneum*. They found one orthologous gene, a cytochrome b5 fatty acid desaturase (Cyt-b5-r), to be associated with the *rph1* locus in all four species. A more thorough research on these indicators will reveal the genetic basis for the resistance of different populations, in terms of frequency patterns in Europe and elsewhere.

In this work we performed a surveillance on the presence of resistance in populations that had been sampled from Europe and some comparable populations sampled from other areas. Our results showed no evidence of strong resistance in the European populations tested, whereas the common mutations that are related for phosphine resistance were identified for *T. castaneum* and *R. dominica*. Finally, we found that different protocols for the evaluation of resistance to phosphine, although they often provide dissimilar results, are comparable and could be revisited on the basis of designing a novel standardized protocol, which can be adopted further in laboratory trials and "real world" applications.

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Potential for using pheromone trapping and molecular screening in phosphine resistance research

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Abstract

Phosphine resistance monitoring typically involves bioassays of beetles from population samples collected from grain storage facilities. Insects are classified into susceptible or resistant phenotypes based on mortality or survival at one or more discriminating doses. Although valuable, phenotype testing has several drawbacks. First, phenotype testing needs live insects, and considerable effort is required to collect and maintain them before testing. Second, population samples may contain multiple genotypes expressing different levels of resistance that may not be distinguishable using discriminating dose bioassays. Third, collections are likely to be focussed around grain storages to maximise sampling success. Recent research shows that several key pests are actively dispersing through flight. The availability of commercial pheromone lures and recent advances in molecular screening provide an opportunity to provide information on resistance gene frequencies more broadly across the landscape. This approach is proving to be a valuable adjunct to traditional resistance testing in Australia.

Keywords: pheromones, DNA markers, traps**,** phosphine resistance, allele frequency

1. Introduction

The development of resistance in stored grain beetles threatens the efficacy of phosphine fumigation (e.g. Daglish et al., 2002; Lorini et al., 2007; Kaur and Nayak, 2015). Generally, monitoring for resistance involves bioassays of beetles from population samples collected from grain storage facilities, and insects are classified into different susceptible or resistant phenotypes based on mortality or survival at one or more discriminating doses (e.g. Benhalima et al., 2004; Daglish et al., 2014; Cato et al., 2017; Konemann et al., 2017). This approach, also known as phenotype testing, is valuable but has several drawbacks. The first drawback is that live insects are needed for testing, and considerable effort is required to collect and maintain them before testing. Another drawback is that population samples may contain multiple genotypes (including heterozygotes), expressing different levels of resistance, that may not be distinguishable using discriminating dose bioassays. Finally, collections are likely to be focussed around grain storages to maximise sampling success. Recent research shows that several key pests are actively dispersing through flight. The availability of commercial pheromone lures and recent advances in molecular screening provide an opportunity to provide information on resistance gene frequencies more broadly across the landscape. We demonstrate this using results from eastern Australia on the lesser grain borer, *Rhyzopertha dominica* (F.).

Phosphine resistance in *R. dominica* is conferred by two major genes (*rph1* and *rph2*) and there could be up to nine genotypes to be present in sampled populations (Schlipalius et al., 2002). Beetles that are homozygous for resistance at *rph1* alone are widespread in eastern Australia and exhibit weak resistance. Beetles that are homozygous for both *rph1* and *rph2* are much less common and exhibit strong resistance. While the FAO discriminating dose is useful for discriminating between susceptible and resistant beetles, using a discriminating dose to distinguish between weak (*rph1*) and strong resistance (*rph1* + *rph2*) is more difficult because of the overlap of the dose-response curves of beetles with weak resistance and those with strong resistance (Lorini et al., 2007). The dose-response curves of many of the other seven genotypes is not known, and further prediction of their responses is complicated by the semi-recessive nature of phosphine resistance.

The development of the capacity to screen with a molecular resistance marker for *rph2* means that individual beetles (including heterozygotes) can be tested for the presence of absence of *rph2* alleles (Kaur et al., 2013). Because resistance at *rph1* is already widespread, the presence of the resistance marker at *rph2* provides a strong indication that strong resistance is already posing a pest control problem or that it will soon emerge in insects at the site where it was found. An advantage of molecular testing over phenotype testing is that it does not need live insects, provided the DNA of the dead insects has been preserved. Traps baited with commercial aggregation pheromone lures for *R. dominica* have been used in Australian and North America to show that flying adults can be caught not only near grain storages but also many kilometres away (Edde et al, 2006; Mahroof et al., 2010; Ridley et al., 2016; Daglish et al., 2017). Also, such trapping has provided *R. dominica* specimens with intact DNA suitable for gene flow analysis (Ridley et al., 2016). A trapping program using aggregation pheromone lures is under way in southern Queensland, Australia, providing information on infestation pressure from flying *R. dominica*, and specimens for molecular resistance screening. This approach is proving to be a valuable adjunct to traditional resistance testing.

2. Materials and Methods

Trapping

A trapping program is under way around two large storage depots in southeast Queensland, Australia, hereafter referred to as Depot 1 and Depot 2, respectively. The two depots are about 25 km apart. Ten Lindgren four-funnel traps (Contech Inc, Delta, BC, Canada) were set up at each site, 50-100 m from the silos and sheds. Each trap is baited with an aggregation pheromone lure for *R. dominica* (Trécé Inc, Adair, OK, USA) and lures for two other species not covered here. A small

amount of propylene glycol in the trap collection containers serves as a preservative. A sub-set of trapped *R. dominica* is used for molecular screening (see below).

Genomic DNA extraction

Genomic DNA (gDNA) was extracted from individual insects using a modified Hotshot DNA extraction method described by Montero-Pau et al. (2008). Individual insects were placed in 96 well PCR plates and single insect was lysed in 75 µL Alkaline lysis buffer (25 mM NaOH and 0.2 mM EDTA) (pH = 12) at 95°C for 30 min, cooled down at 4°C for 10 min; then solution was neutralized by 75 µL of 40 mM Tris–HCl (pH = 5). Samples were centrifuged and the supernatant gDNA from individual insects were stored at -20°C. Susceptible (QRD14) and resistant (QRD569) laboratory strains were also included in each 96 well PCR plate in gDNA extraction for resistance marker visualisation and valid interpretation.

Resistance marker visualisation

The *rph2* marker used a previously designed QRD569_*rph2* PCR marker, which visualises a K142E substitution in dihydrolipoamide dehydrogenase (DLD) first observed in the strongly resistant laboratory strain QRD569, originally collected from Millmerran, Queensland (Schlipalius et al., 2002; Kaur et al., 2013). The PCR amplification was performed using a Terra™ PCR polymerase kit (Clontech Laboratories, Inc.) in a reaction volume of 50 µL containing 25 µL of 2×Terra™ PCR buffer, 1.5 µL of 10 µM forward (5_-CGTGACTTCCGATCCAGT-3_) and reverse (5_-ACACAGTGGTGAATTAGCGG-3_) primers, 1.0 µL of Terra™ PCR polymerase and 4.0 µL of gDNA stock. PCR conditions were: denaturation for 2min at 98°C, followed by 35 cycles of 98°C for 10 s, 60°C for 15 s and 68°C for 1 min and a final extension at 68°C for 1 min. Amplified 327 bp product was digested with 1 U of *Hpy*188III at 37°C for 2 h in a reaction volume of 10 µL containing 5 µL of PCR product, 1.5 µL of 10× buffer and 0.15 µL of 100× BSA. Digested product was visualised in 2% agarose gel electrophoresis. The susceptible allele in the PCR product showed two fragments, 135 bp and 192 bp fragments after digestion with *Hpy*188III, while the resistant allele showed no cleavage.

Total number of susceptible and resistant alleles in *R. dominica* populations from Depots 1 and 2 were scored and allelic frequency was calculated.

3. Results and Discussion

As an example, the trapping and screening results for *R. dominica* trapped between January and February 2018 are shown in Tab. 1. Pheromone trapping is proving to be an effective means of obtaining *R. dominica* adults for molecular screening in southern Queensland. This is expected based on similar trapping studies in Queensland using the same types of trap and pheromone lure (Ridley et al., 2016; Daglish et al., 2017). The number of beetles caught varied greatly between traps (8-34 at Depot 1 and 2-241 at Depot 2), showing that multiple traps should be deployed at sites to ensure sufficient beetles are available for screening and analysis. The trapping reported here occurred during summer so it is possible that trapping during colder months would yield fewer beetles (Ridley et al., 2016; Daglish et al., 2017). Lindgren funnel traps baited with aggregation pheromone lures have also been effective at trapping *R. dominica* in the USA (Edde et al, 2006; Mahroof et al., 2010) showing the potential for using trapping in other countries to obtain beetles for molecular screening.

Screening of *R. dominica* for the QRD569_*rph2* marker confirmed the presence of this resistance allele in beetles caught between January and February 2018 (Tab. 1). Despite being only 25 k apart there were large differences between the two depots. Allele frequency at Depots 1 and 2 were 6.0 and 13.4%, respectively. In comparison, Kaur et al. (2013) reported QRD569_*rph2* allele frequencies of 3-26% in populations of *R. dominica* collected from grain on farms in southern Queensland in

2011. Most of the beetles that were carrying the QRD569_*rph2* allele were heterozygotes, and it is unlikely that these would have been detected in phenotype tests because of the semi-recessive nature of phosphine resistance. Our screening results provide no information on resistance *per se*, but they do provide information on the frequency of one of the two alleles required for expression of strong resistance to phosphine. Changes in the frequency of resistance alleles shown through regular or periodic trapping and screening are likely to be correlated with changes in phenotypic resistance frequencies.

Beetles from a reference susceptible and strongly phosphine resistant strains were screened for the QRD569_*rph2* allele as well. DNA was extracted from 12 susceptible beetles and 12 strongly resistant beetles. PCR amplification was successful in all reference beetles. All susceptible beetles were homozygous susceptible for this allele while all resistant beetles were homozygous resistant for this allele, providing confidence in the screening. Although PCR amplification was successful in the reference beetles, this was not the case with many of the trapped beetles (Tab. 1). This shows the need to optimise methods to ensure that every trapped beetle can be genotyped.

Our results show that trapping with aggregation pheromone lures, followed by molecular screening for resistance alleles is providing information on resistance gene frequencies, and is proving to be a valuable adjunct to traditional resistance testing in Australia. The beetles caught in the traps could have been beetles flying away from the depots, beetles flying towards the depots, or a combination of both types of beetles. Thus, trapped beetles cannot be directly attributed to a storage site, unlike beetles collected by sampling directly from infested grain. Nevertheless, screening of trapped beetles can provide valuable information on background frequencies of resistance alleles. There are multiple resistance allele variants (Schlipalius et al., 2012), and the current results are for only one of these. The capacity to screen beetles for more of these variants would increase the information value of this approach.

Tab. 1 Screening for *rph2* phosphine resistance allele in *Rhyzopertha dominica* caught in southeast Queensland using traps baited with aggregation pheromone lures.

Depot	Trapped	DNA extracted	PCR amplified	Genotypes			*r* (%)	Carriers of *r* (%)
				rr	*rs*	*ss*		
1	154	96	50	0	6	44	6.0	12.0
2	706	264	71	2	15	54	13.4	23.9

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Screening of Phosphine Resistance in *Sitophilus oryzae* (L.) (Rice Weevil) Populations in Turkey

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In this study, the status and prevalence of phosphine resistance in *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) populations collected from Mersin and Konya Province in Turkey were investigated by conducting the discrimination concentration tests and the concentration–mortality bioassays. Discriminating concentration tests showed that 89.9 and 83.3 % populations of tested total *S. oryzae* populations collected from Mersin and Konya province respectively were resistance to phosphine, which reveals high prevalence of phosphine resistance in the insect sampling locations of both provinces. Moreover, discrimination low concentration (0.04 mg/l) tests indicated that 62.5 and 33.3% of total *S. oryzae* populations collected from Mersin and Konya province respectively had 90% or above survival rate, which showed that the frequency of high phosphine resistance in *S. oryzae* populations collected from Mersin province was higher than that in *S. oryzae* populations collected from Konya province. The concentration–mortality bioassays indicated that there were significant differences in resistance levels of *S. oryzae* populations collected from different provinces. Based on the resistance factors (RF) calculated by LC_{50} values *S. oryzae* populations from Mersin and Konya province were 102- to 104-fold and 38- and 81-fold resistance to phosphine compared with susceptible *S. oryzae* population, respectively. The highest level of phosphine resistance was determined in *S. oryzae* populations from Mersin province, followed by those from Konya provinces, respectively. These results indicated that *S. oryzae* populations from Mersin province had higher phosphine resistance than those from Konya Province. In conclusion, this study showed that high levels of phosphine resistance in *S. oryzae* populations collected from different grain storages in Mersin and Konya province of Turkey were prevalent.

Key Words: Phosphine resistance, *Sitophilus oryzae*, populations, discrimination concentration, Turkey

Introduction

Total global grain production is around 2.569 billion tons in 2015-2016 (FAO 2016). Turkey's total grain production is nearly 33.2 million tons in 2015-2016 (FAO 2016), presenting 1.29% of total global grain production. During storage, grains are attacked by a numerous pests, particularly insect species, which cause very serious quantitative losses and qualitative degradations. Pest control in any storage system depends on fumigation with either methyl bromide or phosphine. Phosphine is the primary fumigant used to protect the majority of grain and a variety of other stored commodities from insect pests (Chaudhry 2000; Wang et al. 2006). Attributes that contribute to wide- spread use of phosphine are that it is relatively inexpensive, easy to apply, leaves minimal residues and can be used in a wide range of storage types and commodities (Nayak and Collins 2008).

The lack of ideal airtight conditions for fumigation in leaky structures increases the frequency of control failures and thus increases the frequency of phosphine fumigation (Pacheco et al. 1990; Chaudhry 2000; Benhalima et al. 2004; Lorini et al. 2007). Phosphine fumigation is a long established effective method to control stored product insects, but its continuous and discriminate use has resulted in the evolution of resistant populations (Chaudhry 2000; Collins et al. 2005; Lorini et al. 2007; Pimentel et al. 2007). Repeated application of phosphine in poorly sealed warehouse resulting in under closing have been cited as the cause of the development of strong resistance (Friendship et al. 1986; Zeng 1999). Strong phosphine resistance in all key species of stored grains has been reported in a number of countries (Lorini et al. 2007; Pimentel et al. 2010; Opit et al. 2012; Daglish et al. 2014). The resistance of stored grain insect pests to phosphine was reported following a worldwide survey carried out by the Food and Agriculture Organization (FAO) of the United Nations in 1972-73 (Champ and Dyte, 1976) which detected resistance in 33 out of the 82 countries they surveyed involving 82 of the 849 population tested.

Strong resistance to phosphine was first recorded in *S. oryzae* in China in a 1995–1997 survey (Zeng 1999). In this survey, a resistance level 337 times that of a fully susceptible strain was observed. The resistance level of this species in India was reported in 1998 to have increased to 425 times that of a susceptible reference strain (Rajendran 1999). Weakly resistant *S. oryzae* is found at a high frequency in most regions of Australia, with strong resistance occurring sporadically in field collected strains (Emery et al. 2003). To date, there is only one study on the status of phosphine resistance in stored grain insect pests in Turkey, published by Koçak et al. (2015). In this study, four Turkish population of *T. castaneum* were tested through bioassays for determining phosphine resistance phenotypes and all population exhibited high level of phosphine resistance. There is very limited information on the status of PH_3 resistance in stored grain insect pests in Turkey. The objective of present study to determine resistance frequencies and the levels of phosphine resistance in *S. oryzae* adults collected from grain storage facilities in South and South-eastern region of Turkey.

Material and Methods

Test insects and insect collection

Sitophilus oryzae adults collected from two provinces of Turkey were used in the bioassays and were cultured in the laboratory on whole wheat at 26 ± 1ºC and 65 ± 5 % relative humidity (RH). Insect populations were taken in Mersin and Konya provinces of Turkey, which are located in Central Anatolian and South-eastern regions. For Mersin and Konya province, 18 and 6 *S. oryzae* populations were collected respectively. Wheat grains were taken in bulk with a spiral grain probe from ten different wheat storages per each province. Cylindrical plastic probe traps (STORGARD WB Probe II trap, TREECE, USA) were also used to detect adult stage of *S. oryzae* in bulk stored-grain. From June, 2016 up to November 2016, the probe traps were checked for adult beetles every month.

Phosphine fumigation procedures

One hundred fifty ml fumigation glass vials with the insects and 40 gr wheat, which are closed with aluminum cap with septa by using the crimper. Phosphine gas cylindrical tube (volume; %1 PH_3 + % 99 N_2 and latex rubber ball for gas sampling. Collecting phosphine gas from gas sampling ball by the syringe. Injection of phosphine gas into the fumigation glass vials for dosing. A GC with FPD detector for measuring phosphine concentration. Taking phosphine gas concentration in each vial and injection to GC sampling port. Fumigation jars held for 20 hours at 25 ± 1°C. Insects held for 14 d at 25 ± 1°C and 65 ± 5% RH

Phosphine resistance tests

Discrimination concentration tests

Discrimination concentration tests was used to determine whether the samples had detectable resistance and the frequency of resistance (presence of resistance) by FAO Protocol #16 FAO Protocol: *S. oryzae* adults exposed to low discrimination dose (0.04 mg/l-29 pm) phosphine for 20 h, at 25°C and 65% RH. Mortality determined after 2 wk. In order to classification resistance population weak or strong, high discrimination dose (0.20 mg/l-1436 ppm) for 20 h by method of modification of Daglish and Collins (1998) (Table 1). Mortality determined after 2 weeks. FAO Protocol recommends 3 replications for each treatment and each replicate 40 mixed sex adults.

Table 1. Interpretation of discrimination concentration test results

Low dose 0.04 mg/l*	High dose 0.20 mg/l**	Resistance classification
No survivors	No survivors	Susceptible
Survivors	No survivors	Weak resistance
Survivors	Survivors	Strong resistance

*FAO (1975) **Modification of Daglish and Collins(1998)

Concentration-mortality tests

In order to determine the levels of phosphine resistance in *S. oryzae* adults collected from grain storage facilities in Mersin and Konya provinces of Turkey, two populations for each province, which had the highest resistance frequency and one of phosphine-susceptible population were used for concentration-mortality tests. For concentration-mortality tests, each selected population was exposed to at least 6 to 8 different concentrations of phosphine for 20 h at 25°C and 65% RH. Mortality assessments were conducted at 14 day after phosphine fumigation. Bioassay procedures were similar to discrimination concentration tests.

Data processing and analysis

In all biological tests, numbers of dead and live insect obtained on the 14th day after the fumigations were counted and the survival rates were calculated. Analysis of variance (ANOVA) was used to analyze percentage survival data after arcsine transformation to normalize the data. Percentage survival was also adjusted for natural survival in controls using Abbott formula before analysis and was then analyzed using two-way analysis of variance (factors; application concentration and insect population). Differences between the means were determined using the Duncan's Multiple Range Test at the 5% significance level. In order to calculate the lethal concentration values (LC_{50} and LC_{99}) of the phosphine-resistant *S. oryzae* populations, the concentration-mortality data obtained were subjected to probit analysis using the POLO-PC (LeOra Software, 1994) program. The resistance factor (RF_{50}) for each phosphine-resistant population was obtained by dividing the LC_{50} value estimated for each insect population by the LC_{50} value of the phosphine susceptible population.

Results and Discussion

Discriminating concentration tests showed that 89.9 and 83.3 % populations of tested total *S. oryzae* populations collected from Mersin and Konya province respectively were resistance to phosphine, which reveals high prevalence of phosphine resistance in the insect sampling locations of both provinces. Moreover, discrimination low concentration (0.04 mg/l) tests indicated that 62.5 and 33.3% of total *S. oryzae* populations collected from Mersin and Konya province respectively had 90% or above survival rate, which showed that the frequency of high phosphine resistance in *S. oryzae* populations collected from Mersin province was higher than that in *S. oryzae* populations collected from Konya province.

The concentration–mortality bioassays indicated that there were significant differences in resistance levels of *S. oryzae* populations collected from different provinces. Based on the resistance factors (RF) calculated by LC_{50} values *S. oryzae* populations from Mersin and Konya province were 102- to 104-fold and 38- and 81-fold resistance to phosphine compared with susceptible *S. oryzae* population, respectively. The highest level of phosphine resistance was determined in *S. oryzae* populations from Mersin province, followed by those from Konya provinces, respectively. These results indicated that *S. oryzae* populations from Mersin province had higher phosphine resistance than those from Konya Province.

Conclusion

This study showed that high levels of phosphine resistance in *S. oryzae* populations collected from different grain storages in Mersin and Konya province of Turkey were prevalent.

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Co-fumigation with phosphine and sulfuryl fluoride: Potential for managing strongly phosphine-resistant rusty grain beetle, *Cryptolestes ferrugineus* (Stephens)

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Abstract

Populations of rusty grain beetle, *Cryptolestes ferrugineus*, have developed a very high level of resistance (1300×) to the fumigant phosphine (PH_3) in Australia. Resistant insects triggered control failures, threatening the country's annual grain market worth AU$8 billion. Although PH_3 protocols were amended to manage this new resistance, fumigation requires lengthy exposure periods which has practical difficulties. While there is no suitable replacement for PH_3, the current study explores potential approaches to enhance the efficacy of this fumigant. One possibility is co-fumigation of PH_3 with another complementary fumigant, sulfuryl fluoride (SO_2F_2 or SF), with the dual goals: enhanced efficacy and minimise use of both fumigants. A cohort of mixed age eggs and adults of PH_3-resistant *C. ferrugineus* was fumigated with PH_3 and SF individually, as well as in combination inside desiccators at 25°C and 60%RH for 168 h. Two doses below the maximal registered rates for SF (8.9 mg L^{-1}, equivalent to 1500 g hm^{-3}) and PH_3 (1.0 mg L^{-1}) were tested. Co-fumigation was performed simultaneously for 168 h. Our results revealed that, the mixture of 1.1 mg L^{-1} or 2.2 mg L^{-1} of SF and 0.5 mg L^{-1} of PH_3 over 168 h achieved complete control against resistant *C. ferrugineus* eggs and adults, whereas each of the tested doses failed individually. Our study confirms that SF and PH_3 enhance the efficacy of each other when used in combination, which holds great potential for managing resistant *C. ferrugineus*.

Key words: stored grain, phosphine resistance, sulfuryl fluoride, co-fumigants, resistance management

1. Introduction

Phosphine (PH_3), an effective fumigant is commonly used to disinfest stored grains and processed products from insect pests. However genetic resistance to this fumigant in insect pests is widespread and increasing (Schlipalius et al., 2012). For example, in Australia, populations of rusty grain beetles, *Cryptolestes ferrugineus* (Stephens), have developed a high level of resistance (1300×) to PH_3 and resistant insects require high concentrations (1 mg L^{-1}) and long exposure periods up to 14 days (Nayak et al., 2013). Thus, resistant insects of this species are a threat to grain industry as live insects of this species can jeopardy the country's access to international grain export markets worth of AU$ 8 billion annually. Although, new PH_3 protocols were developed (Kaur and Nayak, 2015) with higher PH_3 rates, there is an urgent need to find alternative pest control strategies that can enhance the efficacy of PH_3, specifically to shorten the fumigation period. One of such approaches is co-

fumigating PH_3 with another fumigant. Sulfuryl fluoride (SO_2F_2 or SF) is an ideal choice for co-fumigation as it exhibits complementary properties to PH_3.

Like PH_3, SF is a broad spectrum fumigant, that is currently being used as an alternative to PH_3, specifically to eliminate PH_3-resistant insects (Nayak et al., 2016). However, SF is a greenhouse gas (Tsai, 2010) and leaves fluoride residues on the treated materials (Sriranjini and Rajendran, 2008). It is also relatively expensive compared to PH_3. Therefore industry is receptive to strategies to minimise use of this fumigant on commodities. In this context, co-fumigation of PH_3 with SF would be of considerable interest for the grain industry as this approach aims to use low dose rates of both the fumigants over relatively short exposure periods. Such an approach may help industry not only to overcome PH_3-resistant insects but also minimise the usage of SF on treated commodities. Additional benefits from this approach may include, shorter fumigation periods, less treatment cost, and reduced selection pressure in insects to both fumigants.

Preliminary research on the efficacy of the PH_3 + SF mixture have indicated that both the fumigants at reasonably low concentrations, have enhanced the efficacy of each other (Misumi et al., 2010; Naito et al., 2006) against grain pests, including PH_3-resistant phenotypes (Jagadeesan et al., 2016b). However, these studies were conducted over short exposure periods (16-48 h) aiming to reveal the type of toxicity relationship between PH_3 and SF in the mixture and so no prior information is available in relation to developing co-fumigation protocols. Thus the present study was conducted to assess the efficacy of co-fumigation of PH_3 with SF against eggs and adults of PH_3-resistant *C. ferrugineus*. We have evaluated concentrations similar to field application rates in both the fumigants, over an exposure period of 168 h (7 days), towards developing a joint fumigation protocol, as a part of integrated pest and resistance management strategy.

2. Materials and Methods

2.1 Insect strain and life stages

A PH_3-resistant strain, QCF122 collected from Edgeroi, south east Queensland, was used in this study (Nayak et al., 2013). A cohort of 100 adult beetles of mixed age and sex, were released into 100 ml glass jar containing 50g of recommended dietary media (barley flour + 5% yeast) (Jagadeesan et al., 2016a) and allowed to lay eggs in the media for 3 days. Thereafter, the experimental jars containing parental adults and 0-3 day old eggs along with the dietary media were fumigated with selected PH_3, SF and the PH_3 + SF concentrations (Tab 1).

2.2 Fumigation bioassay

For both SF and PH_3, the derivation of the source gas, initial concentration measurement using gas chromatograph, and estimating the required volume of gas for achieving desired concentrations within the air-tight desiccators for bioassays were explained in detail previously (Jagadeesan and Nayak, 2017). The experimental jars containing eggs and adults were placed inside the desiccators and fumigated using gas-tight syringes. Two concentrations for each fumigant were selected based on their field application rates. This includes, 0.5 and 1.0 mg L^{-1} for PH_3, and 1.1 mg L^{-1} (187.5 g hm^{-3}) and 2.2 mg L^{-1} (375 g hm^{-3}) for SF. These concentrations were tested individually and in combinations as per the treatment structure explained in Table 1. The fumigation for individual treatments (PH_3 alone or SF alone), was performed independently over 168 h at 25°C and 60% RH, whereas co-fumigation by injecting required volume of PH_3 and SF into the air-tight desiccators simultaneously (at the same time) and the fumigation continued for 168 h. After the fumigations, the treated jars were aerated and shifted to controlled environment room for recovery at 25°C and 60% RH. The entire experiment was replicated twice and each treatment contained two technical replicates. The mortality of adults was recorded 48 h after the fumigation bioassay, whereas for eggs, mortality was recorded after 6 weeks by estimating per cent reduction in the emergence of F_1 adults in treated jars in comparison to the control.

3. Results and Discussion

As anticipated both of the tested concentrations of PH_3 failed individually to achieve complete control against eggs and adults of PH_3-resistant *C. ferrugineus* over 168 h at 25°C. A significant proportion of eggs (57 and 84.6%) and adults (2 and 47.6%) survived at 0.5 and 1 mg L^{-1} PH_3, respectively (Table. 1). In the case SF, although complete mortality in adults was achieved at both the selected doses (1.1 and 2.2 mg L^{-1}) individually, substantial proportion of eggs survived at these concentrations. For example, the egg mortalities were 83.6 and 98.8%, for 1.1 mg L^{-1} and 2.2 mg L^{-1}, respectively, confirming that these concentrations of SF failed to achieve complete control, individually (Table 1). Comparison of our results across SF alone and PH_3 alone treatments, clearly indicates that SF is effective against PH_3-resistant insect pests irrespective of the insect life stages and re-affirm our recent conclusion that PH_3 resistance does not confer cross resistance to SF in PH_3-resistant grain insect pests, including *C. ferrugineus* (Jagadeesan and Nayak, 2017).

Table. 1 Efficacy of co-fumigation of phosphine (PH_3) with sulfuryl fluoride (SF) against eggs and adults of rusty grain beetle, *Cryptolestes ferrugineus* at 25°C and 60% RH over 168 h (7 days)

Individual treatments (168 h)			**Mortality (mean ± SD) (%)**	
PH_3 alone (mg L^{-1})			Adults	Eggs
0.5			2.0 ± 1.3	57.0 ± 9.3
1.0			47.6 ± 13.3	84.6 ± 15.7
Control			0.0 ± 0.0	0.0 ± 0.0
SF alone (mg L^{-1})			Adults	Eggs
1.1			100 ± 0.0	83.6 ± 5.5
2.2			100 ± 0.0	98.8 ± 0.07
Control			0.0 ± 0.0	0.0 ± 0.0
Simultaneous co-fumigation (168 h)			**Mortality (mean ± SD) (%)**	
PH_3 (mg L^{-1})	+	SF (mg L^{-1})	Adults	Eggs
0.5	+	1.1	100 ± 0.0	98.9 ± 0.15
0.5	+	2.2	100 ± 0.0	100 ± 0.0
1.0	+	1.1	100 ± 0.0	100 ± 0.0
1.0	+	2.2	100 ± 0.0	100 ± 0.0
Control	+	Control	0.0 ± 0.0	0.0 ± 0.0

Examination of combination treatments, clearly showed that co-fumigation of PH_3 at 0.5 mg L^{-1} along with 2.2 mg L^{-1} of SF over 168 h was sufficient to achieve complete control against eggs and adults of strongly PH_3-resistant *C. ferrugineus* (Table 1). This is an important finding indicating that PH_3-resistant insects can effectively be managed by adopting a combination regime containing half of the maximal registered rate of phosphine with one fourth of maximal registered rate of SF over a standard exposure period of 7 days at 25°C. Similar enhancement in toxicity of the PH_3 + SF mixture was also observed against different life stages of maize weevil *Sitophilus zeamais* (Motschulsky) (Misumi et al., 2010; Naito et al., 2006) and granary weevil, *S. granarius* (L.) (Naito et al., 2006) over 48 hr at 15˚C, supporting the results of the present study. Currently, we are testing series of PH_3 and SF co-fumigation regimes, including the effective regime identified in this study on sequential pattern. In this, co-fumigation was achieved in two separate fumigations with SF first for 78 h followed by PH_3 for 78 h with a break period of 12 h for aeration. Preliminary results of this experiment suggest that both simultaneous and sequential co-fumigations are equally effective in enhancing the efficacy of PH_3 and SF. Overall, our study has confirmed that co-fumigation of PH_3 with SF, either simultaneously or sequentially enhances the efficacy of each other, and holds great potentials for managing PH_3-resistant grain insect pests.

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Response of *Callosobruchus chinensis* L. to plant extracts and to the parasitoid *Anisopteromalus calandrae*

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Abstract

Present investigation was carried out to elucidate the extracts of botanicals i.e., *Cichorium intybus, Glycyrrhiza glabra, Trachyspermum ammi* and *Terminalia chebula*, for their possible toxic effect against *C. chinensis* population. The results revealed that mortality was highest (94.649%) in case of *T. ammi* treatment, followed by *T. chebula* with mortality value 56.929%. Mortality was 52.363% where application of *C. intybus* was carried out. Minimum mortality (34.500%) was observed in *G. glabra* treated grains. A natural ecto-parasitoid, *Anisopteromalus calandrae* was used to manage *C. chinensis* population. *A. calandrae* male and female adults (5, 10 and 15 pairs) were released to analyze the parasitism efficiency. *A. calandrae* was reared in the laboratory on *C. chinensis* larvae. Honey was offered as a suitable food to parasitoid. The parasitism data was recorded after the adult emergence of brunchid beetles. The experiment conducted under Completely Randomized Design and results statistically evaluated using statistical software at 5% level of significance. *A. calandrae* parasitized both larval and pupal stages of *C. chinensis* and preferred 4th instar larvae of *C. chinensis*. Large amount of *A. calandrae* may efficiently control the *C. chinensis* population. As compared to control (1558.7 host adult), the minimum host emergence (699.00 host adult) was observed with high population density of *A. calandrae*. It was also

obvious from the results, that mortality was increased with the increase in concentration so, a direct dose-mortality response was observed.

Key words: Callosobruchus chinensis, Plant Extracts, Anisopteromalus calandrae, Mortality,

1. Introduction

Mungbean is highly infested by particularly three species included *Callososbruchus chinensis* (L.), *C. maculatus* (F.) and *C. analis* (F.); caused significant losses during storage (Angus, 2010). *C. chinensis* is a well known insect pest of stored mung bean, chickpea and other pulses. Use of synthetic pesticides is the main method to control the insect pest due to their high cost, environmental pollution and development of resistance in insects, alternative approaches have been developed to manage insect pest problems. To control the insects in this sense, essential oils are the best alternative (Perez *et al*., 2010).

Trachyspermum ammi is traditionally widespread used medicinal plant to treat various illnesses. The essential oil of this plant has antimicrobial activity (Kaur and Arora, 2009). Due to the insecticidal activities of *T. ammi*, its essential oil has been used against *C. chinensis* (Chaubey, 2011). *Glycyrrhiza glabra* has antifungal and antimicrobial efficiency. It has small cellular toxicity, anti-tumor and anti-virus (Wang *et al*., 2003). *Terminalia chebula* has antibacterial and anti-pathogenic potential (Malekzadeh *et al.,* 2001). The roots of *Saussurea lappa* have distinct antimicrobial and anti-inflammatory potential and used as a traditional drug for the treatment of several ailments (Pandey *et al*., 2006). *Cichorium intybus* is a popular folk medicinal plant used in curing the urinary tract inflammation, gallstones and liver disorders. It helps in maintaining healthy gastrointestinal tract and metabolism (Roberfroid and Slavin, 2000).

Anisopteromalus calandrae has a wide host range including *Sitophilus granarius* (Ghani, and Sweetman, 1955)*, Sitophilus oryzae* (Lucas and Riudavets, 2002), *Lasioderma serricorne* (Ahmed and Khatun, 1988) and *Rhyzopertha dominica* (Menon *et al.,* 2002). *Anisopteromalus calandrae* gave effective control for *C. maculatus* in Cameroon. It was used as an adult parasitoid and gave efficient results (Ngamo *et al.,* 2007). *Anisopteromalus calandre* ecological and biological investigation were made under laboratory condition and showed that it preferred 4th larval instars over pupa and then 2nd instars for parasitism (Kazemi *et al*., 2004).

In the light of above discussion the present study was carried out with the objective to develop environmentally friendly IPM, to check the biological activity of some plants including *Trachyspermum ammi, Glycyrrbiza glabra, Terminalia chebula* and *Cichorium intybus* and to evaluate the efficiency of an ectoparasitoid *A. calandre* to manage *C. chinensis* populations.

2. Materials and Methods

Collection and Rearing of Insects

Callosbruchus chinensis population was collected from grain market in Faisalabad. Insect population was reared on mung bean in sterilized jars which kept in the incubator at temperature 30±2ºC, 70±5% relative humidity and 12:12 L:D photoperiod to get the homogeneous population. Thirty insects were released in each jar which contains 500 g of mung bean. The jars were covered with muslin cloth so that to avoid insects escape. After five days adults were separated from the mung bean and the grains containing eggs were kept again in the incubator to get another generation. The grains containing adults were also kept in the jar to get homogenous population.

Preparation of Plant Extracts

Plant materials including *Trachyspermum ammi* (Ajowin), *Terminalia chebula* (Hararr), *Glycyrrhiza glabra* (Mulathi) and *Cichorium intybus* (Kasni) were purchased from a medicinal plant shop, Faisalabad. The material was cleaned to avoid contamination. The materials were grinded to get powder. The extraction of plant extracts was accomplished using rotary shaker by dipping 50 grams

of powder in 250 ml acetone. The extracts which were obtained were placed in clean bottles and stored in refrigerator.

Mortality Bioassay

The experiment was carried out in 60 small jars. Different concentrations of plant extracts were applied on the inner side of jar and allowed to get dry. Twenty adults of test insects were released in each jar and then the jars were covered with muslin cloth. Mortality of the adults was recorded three times after equal intervals of 24 hours.

Parasitism Bioassay

Anisopteromalus calandrae was reared on the adults and pupae of *C. chinensis*. The trial contained 36 jars with 20 g of mungbean grain. Thirty adult females of *C. chinensis* were released in each jar for egg laying. After one week the adults were removed and the eggs were placed in jars with grains till emergence. With the start of emergence, the parasitoids were introduced on cowpeas infested by *C. chinensis*. This allowed the synchronisation of the life cycles of the parasitoid and its host. The jars were placed in an incubator and the adult emergence of *C. chinensis* was checked after 27-42 days to record the parasitism data.

Statistical analysis

After the completion of the experiment the recorded data was analyzed using statistical software and the corrected mortality was measured using Abbott's formula. The data was analyzed using Completely Randomized Design and suitable statistic software.

3. Results

Effect of plant extracts against adult mortality of *Callosobruchus chinensis*

Results showed that impact of plants and duration of insects to plant extracts has a highly significant effect on mortality of *C. chinensis*. Interaction of plants and time and interaction of plants and concentrations also have significant impact on mortality. But concentrations, interaction of time and concentrations and interaction of plants, time and concentrations have no significant impact on mortality of test insect. Figure 1 shows the mean comparison of percent mortality of *C. chinensis* of various plant extracts. The results showed that maximum mortality (93.65%) was recorded of *T. ammi* extract and it was statistically different to *C. intybus, T. chebula* and *G. glabra* with percent mortality of 64.67, 63.67 and 44.42% was observed respectively. The results regarding mean comparison of percent mortality of *C. chinensis* at various time exposures revealed that maximum mortality (80.20 %) was recorded after 72 hours and it was statistically different to 48 and 24 hours with percent mortality of 69. 63 and 49.98% was observed respectively (Figure 2). Mean comparison of percent mortality of *C. chinensis* at various time exposures of plant extracts is given in Figure 3. The results showed that maximum mortality (69.33%) was observed at 5% concentration. It was statistically similar to 15 and 10% concentrations, where mortality was 65.46 and 65.01%, respectively.

The results regarding mean comparison of percent mortality of adults of *C. chinensis* of plant extracts and various time periods showed that the effect of interactions of plant extracts and various time exposures was significant (Figure 4). Similarly, the results in Figure 5 show that the effect of interactions of plant extracts and various concentrations was significant.

Maximum mortality (97.62%) was observed with *T. ammi* after 72 hours while minimum mortality (26.16%) was observed with *G. glabra* after 24 hours of exposure. In the interactions of plant extracts and concentrations, maximum mortality (98.21%) was observed with *T. ammi* at higher (15%) concentrations and minimum mortality (39.85%) was observed with *G. glabra* at 10% concentrations. With the interaction of exposure time and concentrations the maximum mortality

(83.92%) was recorded after 72 hours at 5% concentration. In plant, exposure time and concentrations interaction the maximum mortality was observed with *T. ammi* after 72 hours at 15% concentrations and minimum mortality (23.19%) with *G. glabra* after 24 hours at 15% concentration.

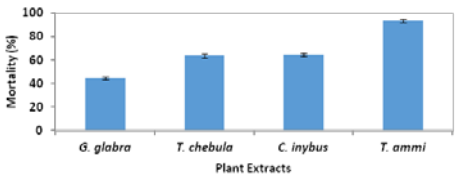

Fig. 1 Comparative effect of four plant extracts against mortality of adults of *C. chinensis*

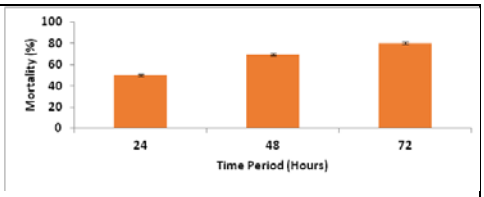

Fig. 2 Comparative effect of plant extracts on the mortality of *C. chinensis* at different time exposure

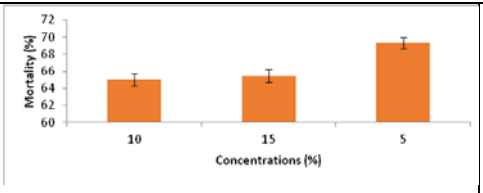

Fig. 3 Comparative effect of plant extracts on the mortality of *C. chinensis* at different concentrations

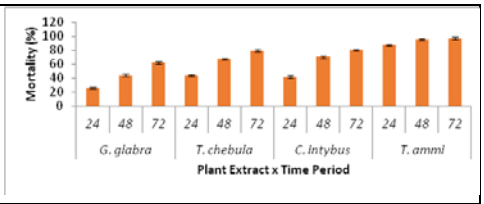

Fig. 4 Impact of interaction of plant extracts and time period on adult mortality of *C. chinensis*

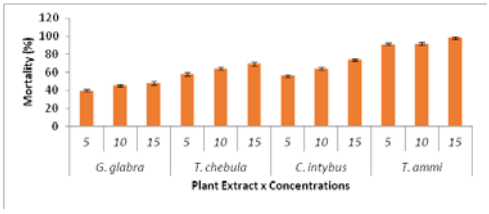

Fig. 5 Impact of interaction of plant extracts and contraptions on adult mortality of *C. chinensis*

Effect of Anisopteromalus calandrae on parasitism (%) of Callosobruchus chinensis

With respect to biological control, the response of *A. calandrae* was also observed. *A. calandrae* was released in three different treatments with 5, 10 and 15 pairs of the parasitoids on *C. chinensis*, and observations were made after 15 and 45 days.

After 15 days of Host Emergence

Results showed that impact of treatments of host insects to parasitoid has a significant effect on adult emergence of *C. chinensis*. Results in Table 1 show the mean comparison of adult emergence of *C. chinensis* in the presence of parasitoid. The finding revealed that the effect of parasitoid on adult emergence differed significantly. Minimum percent adult emergence (699.00) was observed with the release of fifteen pairs of parasitoid and it was statistically different from the other treatments. Maximum of percent adult emergence (1558.7) was observed in control. The trend of adult emergence in respect of parasitoid was mentioned in order to Fifteen pairs < Ten pairs < five pairs < Control.

After 45 days of Host Emergence

Results regarding after 45 days of host emergence revealed that the effect of parasitoid on adult emergence differed significantly among treatments (Table 1). Minimum percent adult emergence (17536.00) was observed with the release of fifteen pairs of parasitoid and it was statistically different from others. Maximum of adult emergence (36754) was observed in control. The trend of adult emergence in respect of parasitoid was mentioned in order to Fifteen pairs < Ten pairs < five pairs < Control.

Tab. 1 Comparative effect of different treatments on parasitism (%) of adults of *C. chinensis* after 15 and 45 days of host emergence

Treatment	Parasitism (%) After 15 days	Parasitism (%) After 45 days
Control	1558.7 a	36754 a
5 pairs	1132.0 b	31893 b
10pairs	961.7 c	24165 c
15 pairs	699.0 d	17536 d

Discussion

Overall results revealed that maximum percent mortality 93.65% of adults of *C. chinensis* was recorded with *T. ammi* and minimum 44.42% was observed with *G. glabra.* These results are in line with the findings of Pereira *et al.* (2008) who reported that the oils of *Piper aduncum*, *Lippia grcillis* and *Cymbopogon martinii* gave 100% mortality against *Callosobruchus maculatus*. Moreover, similar results of several plant extracts have been observed by Shimizu and Hori (2009) against *Callosobruchus maculatus*, while other studies show good efficacy of certain plant extracts for the control of *Callosobruchus* spp. (Roberfroid and Slavin, 2000; Wang *et al.*, 2003; Pandey *et al*., 2007).

Results regarding parasitism effect showed that after 15 days of host emergence, minimum adult emergence (699.00) was observed with fifteen pairs of parasitoid and maximum adult emergence (1558.7) was observed in control. At the same time, after 45 days of host emergence, minimum adult emergence (17536) was observed with fifteen pairs of parasitoid and maximum adult emergence (36754) was observed in control. However, from these results it was concluded that maximum parasitism was achieved at highest number of pairs of *A. calandrae* and after highest time interval (45 days) while at lower number of pairs and time interval test insect percent mortality was not sufficient. Our findings are also related to Utida (1943) who conducted as series of experiments with the same host-parasitoid complex, and found that the species can coexist for 50 generations. He described that *A. calandrae* showed functional response of type III. Our results are also in accordance with the findings of Ngamo *et al.* (2007) who reported a significant reduction in progeny emergence of *C. chinensis* due to the presence of *A. calandrae.* Previous studies have also provided similar findings of the effect of *A. calandrae* against *Rhyzopertha dominica*, *Sitophilus oryzae*, *Lasioderma serricorne* and *Tribolium confusum* (Mahal *et al.*, 2005, Ghrimire and Phillips, 2007, Belda and Riudavets, 2010).

From these results it is concluded that the use of plant extracts and bio-control agents could be a better alternative to our conventional synthetic insecticides and could be an integral part of stored grain IPM programs.

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Detection of hidden insect *Sitophilus oryzae* in wheat by low-field nuclear magnetic resonance

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Abstract

Insects, either adults or larvae, living inside grains are difficlut to detect but can cause enormous loss of grain. Therefore, we explored the use of low-field nuclear magnetic resonance (LF-NMR) techniques to detect *Sitophilus oryzae* hidden inside wheat. Significant difference in transverse relaxation times (T_2/ms) and the T_2 components proportion (P_2/%) was observed between wheat and *S. oryzae* at its four different growth stages (small larvae, large larve stage, pupal stage and adult stage). The transverse relaxation signals on the infested

wheat kernels varied with *S. oryzae* developmental stages. LF-NMR image of uninfested wheat were very different than infested wheat with the hidden insects at its four growth stages. Therefore, LF-NMR, as a novel non-destructive method, could be used to detect insects hidden in grains to take necessary management against pest damage to grains during storage.

Keywords Rice weevil (*Sitophilus oryzae*); Grain storage; Transverse relaxation signal; Infested wheat; Low field magnetic resonance imaging

1. Introduction

Insect infestation in stored grain is a worldwide problem. Insect pest infestations in grain are responsible for qualitative and quantitative losses of 5 - 10% grain losses in developed countries and 30 - 40% in some developing countries(Brader et al., 2002; Kumar and Kalita, 2017) ENREF_1. Insect detection is a prerequisite of Integrated Pest Management, a process often used to solve pest problems while minimizing risks to people and the environment. Many methods have been developed for the detection and monitoring insect infestations, including visual inspection, sampling & sieving, floating & cracking (Brader et al., 2002), trapping (Hagstrum et al., 1998), bioacoustic methods (Mankin et al., 2011), computer vision (Ridgway et al., 2002), near-infrared hyperspectral imaging (Kaliramesh et al., 2013; Singh et al., 2009), and soft X-rays (Karunakaran et al., 2003). Recently, some new techniques have emerged, such as microwave heating (Jian et al., 2015), zymography (Piasecka-Kwiatkowska et al., 2014), solid phase micro-extraction (Laopongsit et al., 2014), and biophotonic methods(Shi et al., 2016). However, most of these approaches cannot be commercialized due to various issue including throughput limits, unreliability, labour costs, time consuming and safety concerns. The most common used technique are sieving samples or the probe-and-trap methods. It is important to note that exceptions to the probe-and-trap include the United States, where visual images are used in combination with probes, and Canada, where the Berlese funnel method is mandated (Sabu et al., 2011). These time-consuming techniques have low accuracy, which detect adult insects and ignore larvae growing inside the kernels. Primary pests, such as the granary weevil, the rice weevil, the maize weevil, the lesser grain borer, and the Angoumois grain moth, cause most of the damage to grains in storage and transportation. The larvae of the primary insect pests live entirely inside the kernel and are hard to detect. Therefore, a rapid, simple, and accurate method for detecting internally feeding life-stages of insects in grains is highly desired by grain industry and inspection and quarantine branch.

Low-field nuclear magnetic resonance (LF-NMR) is a sensitive and non-destructive technique, and widely used to detect water characteristics in numerous systems (Greiff et al., 2014; Hills, 2006) ENREF_15. Typically it detects ingredients with high numbers of hydrogen protons after the food is processed or stored such as starch (Ritota et al., 2008), salted fish (Carneiro et al., 2016), blanching sweet corn (Shao and Li, 2012, 2013), ripening bananas (Raffo et al., 2005), and drying wheat (Ghosh et al., 2006). The difference in moisture is directly proportional to signal produced, which allows or the NMR to identify regions of higher moisture. Grain is typically stored at 12%-18% moisture, whereas the moisture content of insects about 65% (Shrestha and Baik, 2013), suggesting that NMR technology could be a promising approach to detect insects. The difference in moisture between insects and wheat kernels has been used to detect insects in the grain by electrical conductivity (Pearson and Brabec, 2007; Pearson et al., 2003) ENREF_23 ENREF_23. However, the sample must be milled before determination. It is a destructive method, whereas NMR require no sample destruction. Previously, only two studies using NMR were done on detecting insect infestations in grain (Chambers et al., 1984; Street, 1971). In those experiments, grain weevils (*Sitophilus granarius*) were detected at different stages of development inside of grain kernels by measuring chemical shifts using high-field NMR. Even though the technique showed promising results, research in using NMR for insect detection ceased, due to a lack of scientific expertise and high equipment costs. With the development of economical low-field NMR instrumentation, the use of low-field NMR for quality monitoring has recently become feasible in agri-food sciences. Therefore, we explore here the possibility with low-field proton NMR relaxation signal measurement

and magnetic resonance imaging (MRI) to detect hidden insect at different growth stages within wheat.

2. Material and methods

2.1. Insect sample preparation

Sitophilus oryzae was reared on whole wheat kernels in the National Engineering Laboratory of Grain Storage and Transportation, Nanjing, China. Approximately 500 adult *S. oryzae* were mixed with 1 kg of wheat (about 13.5%, w/w) in a wide-mouth glass bottle covered with a cotton cloth, and were maintained in a growth chamber at 29 ± 1°C and 65 ± 2% relative humidity. After 48 hours, all of the adults were removed from the bottle. *S. oryzae* at 4 different stages (small larvae, large larvae, pupae and adult as shown in Figure 1) were separated and collected from the infested wheat by a knife after 15, 20, 25 and 40 d, respectively. The samples were tested after collecting.

2.2. Infested wheat preparation

Approximately 400 adult *S. oryzae* were mixed with 20 g whole wheat for 48 h for infested wheat kernels. High population density of pests increases the probability of having infested kernels. Wheat kernels with single pest egg were collected under a microscope (Chongqing Guangdian Instrument Corporation Ltd., Chongqing, China). The infested kernels were divided into 2 g per jar, and cultured in a chamber at 29 ± 1°C and 65 ± 2% relative humidity. The samples were tested at 5, 10, 15, 20, 25 and 30 d. Some adults of *S. oryzae*, which emerged from the wheat, were removed before 30 d test.

2.3. Moisture content measurement

The moisture content of wheat kernels was determined using the whole-grain oven drying at 130 °C for 19 h (ASABE, 2009). About 1 g of *S. oryzae* at each growth stage was dried at 70 °C for 48 h to a constant weight, placed in a desiccator for 12 h, and then weighed (Singh and Sinha, 1977). Each sample was done in triplicate.

2.4. LF-NMR measurements

Transverse relaxation measurements were proceeded on NMI20 Benchtop Pulsed NMR Analyzer (Shanghai Niumag Corporation Ltd., Shanghai, China) operating at a resonance frequency for protons of 22.6 MHz. Spin–spin relaxation time (T_2) was measured using the Carr–Purcell–Meiboom–Gill (CPMG) sequence, a common and stable sequence for T_2 measurement (Carr and Purcell, 1954; Meiboom and Gill, 1958). Pests (0.2 g) or infested wheat (2 g) were placed in a 15-mm diameter glass test tube. The T_2 measurements were made with τ value (time between 90 and 180° pulses) of 100 μs. Data from 10,000 echoes were acquired as 32-scan repetitions at 32 °C. The repetition time between subsequent scans was 1.5 s. Relaxation data were calculated by MultiExp Inv Analysis software (Shanghai Niumag Corporation Ltd., Shanghai, China) and the CONTIN algorithm was used for the multi-exponential fitting analysis.

2.5. LF-NMR image measurements

Magnetic resonance imaging was done on NMI20 Benchtop Pulsed NMR Analyzer. For these measurements, the following specifications were used: equipment field of view = 80×80 mm; matrix size = 192×256; echo time = 5.9 ms; repetition time = 160.0 ms, number of scans = 256; and slice width = 10.0 mm. Using these setting, proton density images were measured.

2.6. Statistical analysis

Statistical analyses were done with SPSS software (Version 16.0, Inc., Chicago, IL) and graphical data were generated with Origin Pro 8.5. One-way analysis of variance (ANOVA) followed by Duncan's

multiple comparisons test (at 0.05) was carried out. Values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Moisture content of wheat and S. oryzae

Figure 2 shows the moisture content of wheat and *S. oryzae* at four growth stages. From the larval stage to adult stage, the moisture content decreased from 66 to 44%, and moisture content of the wheat kept at about 13.5%. Moisture content of *S. oryzae* at every growth stage was much higher than that of the wheat kernel. In storage, insects can obtain water through three ways, consuming food, absorbing from unsaturated air, and from metabolic activities, such as oxidation of carbohydrates and fats (Arlian, 1979; Devine, 1978; Yaowaluk et al., 2008). Since the moisture content of wheat was low, the insects must rely on water obtained from unsaturated air or through metabolic activity. It is the basis to get different signals from grains and insects according to their moisture differences in subsequent experiment.

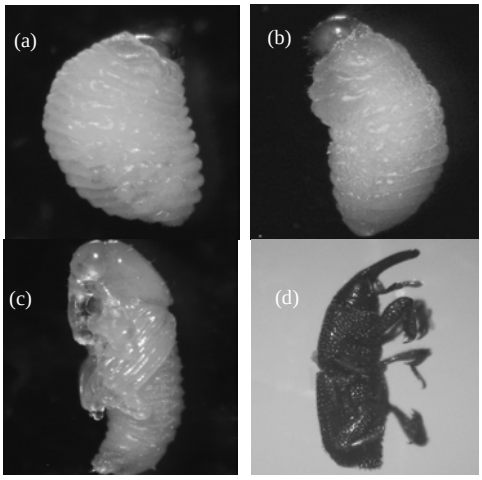

Figure 1 The life cycle of the *Sitophilus oryzae* (rice weevil): (a) small larva, (b) large pupa, (c) pupa, and (d) adult. Photographs are at about ×20 magnification.

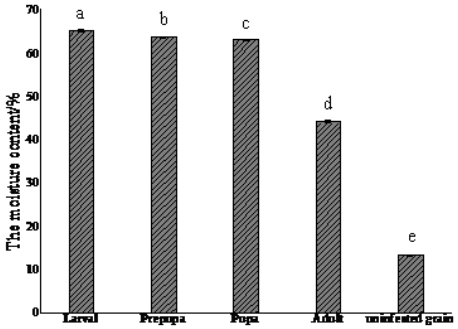

Figure 2 Moisture content of the four *Sitophilus oryzae* growth stages and uninfested grain.

3.2. Transverse relaxation signal analysis for wheat and S. oryzae

Figure 3 shows the distribution of transverse relaxation times T_2 for *S. oryzae* adults and wheat kernels. Two peaks were overlapping for a single wheat kernel (Figure 3a), whereas two separate peaks were observed for multiple wheat kernels (Figure 3b). Two separated peaks for multiple kernels was sharper and narrower than for single kernel. In Figure 3c, 1 peak was observed on single adult, but 3 peaks were obtained on about 90 adults. The peak height was directly related to the proton content and therefore low proton content in the sample reduced the detection accuracy. Accuracy of LF-NMR can be improved by amplifying the magnetic field, increasing the sampling time or by using a larger number of samples. However, amplification of magnetic field and increase sampling times, result in increased equipment costs and longer detection time. Therefore, a certain number of samples were used in the following experiment. As shown in Figure 4, the representative continuous distribution of transverse relaxation times for wheat and *S. oryzae* at the four growth stages. Two T_2 components were detected in both samples, but with different distributions for wheat kernels and insects. The intensity of the signal per unit mass of *S. oryzae* was higher than that of uninfested wheat as the higher moisture content of the insect (Figure 2). The position of the insect

peaks moved leftward as they grew up, indicating that molecular mobility in insect bodies decreases with maturity.

Table 1 shows transverse relaxation times (T_2) and percentages of water component (P_2) in four different growth stages and wheat. In wheat samples, T_{2b} was the short relaxation time at 0.76 ms that was associated with strong boundary water trapped within macromolecular structures, such as starch and protein (Bertram et al., 2001; Li et al., 2014; Shao et al., 2016a; Shao et al., 2016b). The long relaxation time was T_{22} at 96.02 ms that could be the superimposed signal from water and lipid molecules, although the lipid level was very low in wheat. For *S. oryzae*, the short relaxation time T_{21}, ranging from 1.6 to 2.8 ms, was related to the protons integrated with organized protein structures and associated with weakly bound water (Bertram et al., 2001; Li et al., 2014; Shao et al., 2016a; Shao et al., 2016b). The long relaxation time T_{22}, ranging from 37 to 58 ms, could be attributed to immobilized water or capillary water located in the intercellular space or capillaries, and was significantly lower in insects compared to wheat ($p < 0.05$). The P_{22} percentages for the insects at the 4 different insect growth stages ranged from 89.53 to 91.64%, implying that the main water component was immobilized water, irrespective of the *S. oryzae* growth stage. In contrast, the main water component in wheat was strong boundary water, accounting for about 90% water. Therefore, molecular mobility in insects when compared to that of wheat kernels was significantly different as water under low moisture conditions (i.e. in case of wheat) was less mobile. This observation was particularly useful in identifying insect infestations.

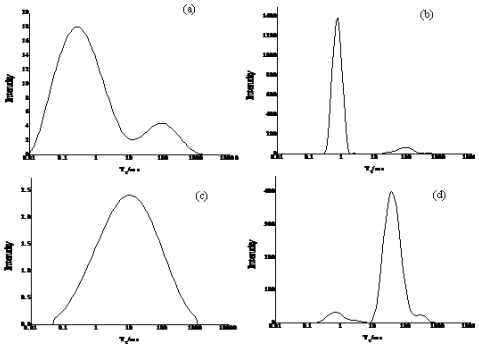

Figure 3 Typical distribution of T_2 relaxation times for *Sitophilus oryzae* adults and wheat kernels: (a) single uninfested wheat kernel; (b) multiple uninfested wheat kernels (Approx. number: 38); (c) single adult; (d) multiple adults (Approx. number: 91).

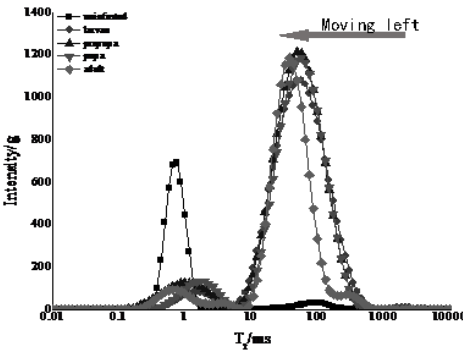

Figure 4 Typical distribution of T_2 relaxation times for *Sitophilus oryzae* at different growth stages and for uninfested grain.

Table 1. T_2 and percentages for each water component obtained by LF-NMR for *Sitophilus oryzae* at the four different growth stages and wheat

Growth stages	T_{2b}/ms	T_{21}/ms	T_{22}/ms	P_{2b} / %	P_{21} / %	P_{22} / %
Small larva	—	1.93 ± 0.47^{a}	57.22 ± 0.00^{b}	—	8.78 ± 0.58^{a}	90.75 ± 1.47^{a}
Large larva	—	1.60 ± 0.26^{a}	51.01 ± 2.78^{b}	—	7.76 ± 1.74^{a}	91.64 ± 1.62^{a}
Pupa	—	2.25 ± 0.42^{ab}	57.22 ± 0.00^{b}	—	7.58 ± 0.35^{a}	90.63 ± 1.06^{a}
Adult	—	2.77±0.75^{b}	37.65 ± 0.00^{a}	—	9.69 ± 0.72^{a}	89.53 ± 0.57^{a}
Wheat	0.76 ± 0.00	—	96.02 ± 12.33^{c}	90.97 ± 0.28	—	5.01 ± 1.28^{b}

Note: $^{a–c}$ means within a column with different letters are significantly different ($P < 0.05$).

Table 2. Moisture content of the infested wheat kernels varied with *S. oryzae* growing up.

Sitophilus oryzae growing days (d)	Moisture content (%)
0	13.44% ± 0.12[a]
5	13.94% ± 0.06[b]
10	14.95 ± 0.11[d]
15	15.19 ± 0.08[e]
20	19.24 ± 0.08[f]
25	20.04 ± 0.03[g]
30	19.09 ± 0.08[f]
Control group of wheat at 30 d	14.18 ± 0.02[c]

Note: [a-h] means within a column with different letters are significantly different ($p < 0.05$).

3.3. Transverse relaxation signal analysis for infested wheat with S. oryzae inside

Table 2 shows that there was a significant increase in the moisture content of infested wheat when insects were developing inside the kernels. This is obvious, as a living organism inside the kernel would add moisture to its microclimate due to respiration and metabolic activity. There was a sharp rise between 15 and 20 d, probably due to metabolic water produced by the insects increased to adapt to higher growth rates. Table 3 shows the transverse relaxation time values for insects that developed inside wheat. T_{2b} ranged from 0.76 to 1.52 ms over 30 d, indicating that the water mobility in wheat changed over time. This occurred because the macromolecular structures of wheat were destroyed by *S. oryzae* to free water from the macromolecular structures and new water component was accumulated in the pest body. It can be confirmed by that the water component P_{2b} decreased after 5 d and P_{22} increased after 10 d as shown in Figure 5. These results suggest that insect infestation not only affected the total moisture content of the stored grain, but also changed the mobility characteristics inside the kernel. However, there was a decrease in T_{22} (96.02 ms to 43.77 ms) as the insects grew up. This was similar to independently detecting *S. oryzae* at each growth stages, although the correlation of this data needs further investigation in future. Especially, T_{22} with 56.75 ms at 5 d was significant from that with 96.02 ms at 0 d ($p < 0.05$), suggesting that the early growth stages of the insect could be detected by LF-NMR. The changes between water component P_{2b} and P_{22} were the opposite of each other in Figure 5. The continued increase in P_{22} from 5 to 25 d showed that the intensity of the *S. oryzae* signal increased gradually, in agreement with the total water content in the infested wheat kernels in Table 2. This is probably due to developed larger insects that contain more water in their bodies. Unexpectedly, P_{2b} increased while P_{22} decreased at 30 d. The P_2 changed in non-monotonic way whereas T_2 values were monotonously changing (seen in Table 3), varied with *S. oryzae* growing up. Only at 30 d, we found that some adult *S. oryzae* climbed out and left empty grain. All indicated that transverse relaxation signal by LF-NMR could accurately measure the whole growth of rice weevil *S. oryzae* in kernels.

Table 3. T_2 values of the infested wheat kernels varied with *S. oryzae* growing up

Infested days (d)	0	5	10	15	20	25	30
T_{2b}/ms	0.76 ± 0[a]	0.89 ± 0.08[ab]	0.92 ± 0.06[b]	1.02 ± 0.05[c]	1.28 ± 0.07[d]	1.32 ± 0[d]	1.52 ± 0[e]
T_{22}/ms	96.02 ± 12.33[d]	56.75 ± 9.28[c]	59.99 ± 9.99[bc]	51.27 ± 7.37[abc]	52.33 ± 4.48[ab]	50.84 ± 2.61[ab]	43.77 ± 6.23[a]

Note: [a-e] means within a row with different letters are significantly different ($p < 0.05$).

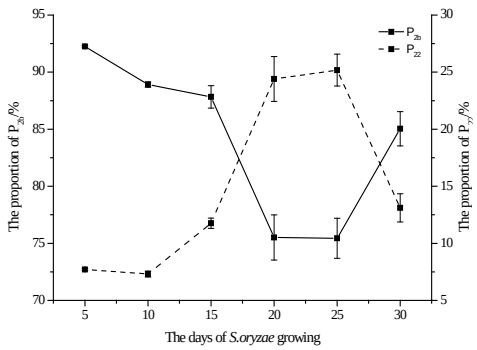

Figure 5 P_{2b} and P_{22} water component percentages of the infested wheat kernels varied with *S. oryzae* growing up.

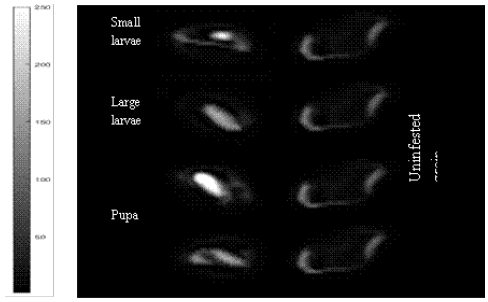

Figure 6 Low field NMR images of an infested wheat kernel at the four growth stages (left column) and for uninfested grain (right column). Sidebar stands for the grey-scale intensity of the images.

3.4. LF-NMR image analysis for infested wheat

Figure 6 shows the LF-NMR images of internally infested grain for the four growth stages and for the uninfested grain. The intensity of the signal is directly related to the sample's proton density (water or oil content); therefore, brighter zones contain larger amounts of water (Alessandra et al., 2010). Once an insect infested a wheat kernel, a bright spot appeared in the endosperm and this became enlarged as the insect grew up. This area was much brighter than the rest of the wheat kernel, suggesting that the moisture content of the insect was higher than that of wheat. This agreed with Figure 2. In addition, a bright tunnel could be seen at the adult stage because the adult had consumed the inside of the grain and was moving around freely within the grain. Therefore, LF-NMR image can visually detect insect infestation.

Conclusions

In summary, we measured and analysed LF-NMR transverse relaxation signal and image differences in wheat and wheat infested by *S. oryzae*. Our findings show that LF-NMR signal measurement or imaging could have potential to detect infested grain with hidden pests.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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IPM guidelines as fundament for sustainability in plant protection: The case for stored product protection

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Extended Abstract

According the EU Framework Directive 2009/128/EC of Sustainable Use of Pesticides (EU, 2009), member states shall implement into practice, amongst others, crop or sector-specific guidelines for integrated pest management (IPM) on a voluntary basis. In this context, article 14 demands that *"member states shall take all necessary measures to promote low pesticide-input pest management, giving wherever possible priority to non-chemical methods"*. Furthermore *"Public authorities and/or organisations representing particular professional users may draw up such guidelines. Member states shall refer to those guidelines that they consider relevant and appropriate in their National Action Plans"*.

For these aims, the EU has defined eight general principles of IPM in Annex III.

These general principles comprise:

- Principle 1: Prevention and/or suppression of stored product pests where ever possible.
- Principle 2: Monitoring of pests with adequate methods and tools.
- Principle 3: Decision-making in collaboration with profesional advisors to apply appropiate plant protection measures.
- Principle 4: Practicable non-chemical measures should be preferred.
- Principle 5: Pest–specific chemical products with the lowest detectable side effects for humans, target-organisms and environment should be preferred.
- Principle 6: Where appropriate, all control measures but mainly the use of chemical products should be restricted to the minimum extent, e.g. by reducing authorized doses, and frequency of application or by partial application.
- Principle 7: Implementation of resistance strategies to maintain the efficacy of the chemical products.
- Principle 8: Documentation of all plant protection measures and evaluation of their success for future decisions.

Barzman et al. (2015) have reviewed in detail application of these eight general principles of IPM from the perspective of what their implementation means for research, advisory services and farmers. There is no doubt that practicable measures of "prevention and suppression" and "monitoring" are of utmost importance to restrict all kinds of direct interventions on a minimum and thus, to keep low risks for human health, the natural environment and groundwater. The sequence of these eight general principles can be considered as a repetitive decision-making tree where misjudgements can be adjusted in the next vegetation period(s). But, Barzman et al. (2015) conclude that there is a need for flexible, locally adapted and practical IPM strategies.

In Germany, the general principles of IPM became binding for farmers and advisors with the entry into force of the revised Plant Protection Act in February 2012. Thereby these general principles are part of the mandatory good plant protection practice. However, due to this practice and several action plans since 2003, high standards of plant protection have already been implemented in Germany for years (Hommel et al., 2013).

Because of the uncontrolled environment, plant protection in arable and horticulture farms is more in focus of critical public debates than stored product protection under controlled environments in stockkeeping or processing companies. These debates and – in many cases - farmers' green attitude have the potential to support development and implementation of innovations to improve plant protection strategies in the context of IPM. In contrast, stored product protection takes place in fully controlled environments (e.g. warehouses, silos, containers, packagings) that are clearly separated from the natural environment. Environmentally-based driving forces to change or improve things are therefore less influential. Only economically-based preconditions, e.g. declined availability of chemical products, storage losses, avoidable discounts in the trade chain, and hygienic reasons are responsible for investments in better trained workers, extension services or storage conditions. It has been shown that protection goals, public goods or dependencies of trading partners are extremely crucial to implement profitably IPM guidelines into practice.

The National Action Plan on Sustainable Use of Plant Protection Products (NAP), issued by the Federal Government in 2013 for 10 years, focuses on all these aspects to strengthen voluntary activities of farmers and all stakeholders to reduce risks to humans, animals and the environment as demanded by the directive 2009/128/EC (https://www.nap-pflanzenschutz.de/en/).

The first IPM guideline for the sector "stored product protection" in Germany was developed in co-operation of different stakeholders (led by the Julius Kühn-Institut, JKI), financially supported by the Federal Ministry of Food and Agriculture (BMEL) and submitted to the BMEL in 2017. After its assessment done by The Scientific Advisory Board of the NAP, it is expected that this guideline will be added to Annex 1 of the NAP in 2018.

The guideline contains a general part with explanations of the eight principles in the context of stored product protection.

This specification of the principles comprises:

Principle 1: Appropriate storage, discharge and conveying, adequate sanitation, sealed chambers or silos, facilities for cleaning, cooling, drying;
Principle 2: Intake control, monitoring, claim advice;
Principle 3: Decision-making for the current problem, solutions to prevent the problem in future, claim advice;
Principle 4: Relocation, cooling, thermic and biological measures;
Principle 5: Choose low-risk products, think about the need for resistance strategies;
Principle 6: Appropriate equipment, dose and frequency of application;
Principle 7: Change of active compounds or alternative measures, monitoring;
Principle 8: Documentation of measures and their success, adjustment of measures for future stored product protection.

In addition, a more detailed table of measures is given for grain and bulk storage in the IPM guideline. Other stored products will be added later. The table shows the sequence of measures to be considered for individual pest problems. There is a clear priority to preventive and non-chemical measures. All measures are evaluated according to their practicability. To this end, the criteria effective, economically viable and proven are considered individually.

The IPM guideline for the sector "stored product protection" will be evaluated regularly and an adoption of advances in IPM is possible. In Germany's NAP, the aim is that 50 % of stock keepers shall apply the guideline 5 years after its publication, i.e. in 2023. To achieve this ambitious goal, a network will start in 2018 under the acronym VSnet for IPM demonstration in on-farm and off-farm commercial grain storage facilities.

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Capability and limitation of anoxic treatments in museum collections protection

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Without precaution, insects may cause serious damage to museum collections. Quarantine of potentially infested objects can be logistically challenging. Anoxia under controlled nitrogen atmosphere is a most compatible but also time-consuming method to eradicate insect pests in all kinds of different materials. Treatment results are usually effected by duration, temperature, humidity and residual oxygen content. During a two-year research project, 34 relevant pest insect species of all developmental stages were tested in several different materials (wood, paper, wool) to monitor treatment success and to determine optimum treatment parameters. Duration of treatment ranged from one to three weeks at temperatures of 20 - 27 °C. As expected, results showed significant differences in mortality among tested species. Highest tolerance of hypoxic conditions was found in older larvae of *Hylotrupes bajulus*. However, this species is an unlikely museum pest. Anobiids and other wood boring beetles are more often an issue related to cultural heritage. Tested imbedding materials in general had no mortality lowering influence. A combination of three weeks exposure time at up to 0.5 % residual oxygen and at 24 °C and 50 % RH is recommended for infested artefacts.

Susceptibility of phosphine-resistant cigarette beetles to various insecticides

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Abstract

Management of phosphine resistance in the cigarette beetle *Lasioderma serricorne* (F.) has become a topic of great interest to the tobacco industry in recent years. Effective use of contact insecticides with modes of action different from that of phosphine can be a key element in preventing or delaying the evolution of phosphine resistance. This study was conducted to ascertain whether five insecticides selected from three mode-of-action classes (fenitrothion, pirimiphos-methyl, permethrin, bifenthrin, and spinosad) can be incorporated into a phosphine-resistance management strategy. Specifically, we examined the contact efficacy of the insecticides to a phosphine-susceptible strain and six resistant strains (38–184-fold in resistance ratio based on LC_{50}). Susceptibility to organophosphates (fenitrothion, pirimiphos-methyl) and spinosad was not significantly different between phosphine-susceptible and phosphine-resistant strains (within 2.3-fold resistance ratio). The absence of the cross-resistance between these insecticides and phosphine makes them ideal for resistance-management programmes. However, high resistance to synthetic pyrethroids (>145-fold for permethrin and >1697-fold for bifenthrin) was found in three of six phosphine-resistant strains. Based on these results, synthetic pyrethroids cannot be recommended as insecticides of primary choice.

Keywords: *Lasioderma serricorne*, resistance management, contact insecticides, pyrethroid resistance

1. Introduction

The cigarette beetle, *Lasioderma serricorne* (F.) is the most important pest of stored tobacco. Fumigation by phosphine, the most important method for disinfestation of stored tobacco, has been used for post-harvest management of insect pests since the 1970s. Phosphine resistance in *L. serricorne*, although first recorded in India and the United States in the 1990s (Zettler, 1990;

RAJENDRAN AND NARASIMHAN, 1994; ZETTLER AND KEEVER, 1994), has spread globally along with international tobacco distribution (HORI AND KASAISHI, 2005; CORESTA, 2013). The tobacco industry had successfully managed the resistance problem by revising the industrial fumigation protocol (CORESTA, 2013). Nevertheless, highly resistant populations able to survive the present fumigation protocol, i.e., 6-d exposure with 600-ppm at 25°C, have emerged recently in the United States (SAĞLAM et al., 2015). The sustainable use of phosphine will be threatened if such high resistance becomes widespread. Therefore, countermeasures capable of preventing or delaying the elevation of resistance are eagerly sought. The emergence and spread of resistance are mainly attributed to heavy reliance on a single insecticide or single mode-of-action insecticides. Therefore, enforcement of an integrated approach incorporating as many different control measures as possible is a principle for successful management of phosphine resistance. The use of insecticides with different modes of action is one such measure. Various contact insecticides, including organophosphates and pyrethroids, have been used as space-spray or surface-spray applications in tobacco warehouses in practice (RYAN, 1999), although their efficacy against phosphine-resistant *L. serricorne* has not been ascertained. The present study assesses the effects of five contact insecticides selected from three mode-of-action classes (fenitrothion, pirimiphos-methyl, permethrin, bifenthrin, and spinosad) (IRAC, 2017), to phosphine-susceptible and phosphine-resistant *L. serricorne*.

2. Materials and Methods

a. Insects

Seven laboratory strains of *L. serricorne*, one phosphine-susceptible and six phosphine-resistant strains, were used for this study (Table 1). The phosphine-susceptible strain TSC has been maintained in the laboratory for decades without exposure to any insecticide. The other six strains (THR, C87, NGY, IWT, SKG, and MLY) were established from phosphine-reistant field populations collected from tobacco warehouses at different times and locations. They underwent laboratory selection with phosphine for at least 13 generations before the study so that uniformity of phosphine susceptibility in individuals in each strain was promoted. They had never exposed to insecticides, except for phosphine, since their collection from the field. All insects were maintained on cornmeal containing yeast (10% mass fraction) at 27°C and 60% r.h.

Table 1 *Lasioderma serricorne* strains used for the study

Strain	Initiation of culture	Origin	Phosphine-susceptibility, LC_{50}[a] (95% confidence interval), ppm	
TSC	Unknown	Unknown	6.6	(3.0–10.3)
THR	1999	Tobacco warehouse in Tokyo, Japan	250.4	(227.2–272.6)
C87	2011	Food and Environment Research Agency (York, UK)	284.5	(248.3–318.9)
NGY	1997	Tobacco warehouse in Aichi, Japan	298.1	(264.9–331.0)
IWT	1999	Tobacco warehouse in Shizuoka, Japan	340.2	(305.7–375.1)
SKG	2010	Tobacco warehouse in Fukushima, Japan	412.6	(333.1–474.1)
MLY	2012	Tobacco warehouse in Shah Alam, Malaysia	1215.5	(1051.3–1351.6)

a Phosphine concentrations required to achieve 50% lethality (LC_{50}) for eggs at 72-h exposure, 25°C, and 75% r.h.

b. Insecticides

The efficacy of five commercial formulations of insecticide from three mode-of-action classes (IRAC, 2017) was evaluated: fenitrothion (Sumithion EC; Sumitomo Chemical Co., Ltd., Tokyo, Japan) and pirimiphos-methyl (Actellic EC; Nihon Nohyaku Co., Ltd., Tokyo, Japan) belonging to IRAC group 1B (organophosphates: acetylcholinesterase inhibitors); permethrin (Adion EC; Sumitomo Chemical

Co., Ltd., Tokyo, Japan) and bifenthrin (Talstar FL; Ishihara Sangyo Kaisha, Ltd., Osaka, Japan) belonging to the group 3A (synthetic pyrethroids: sodium channel modulators); and spinosad (Spinoace SC; Dow AgroSciences Japan, Ltd., Tokyo, Japan) belonging to the group 5 (nicotinic acetylcholine receptor allosteric modulators), all of which belong to different classes from phosphine (group 24A: mitochondrial complex IV electron transport inhibitors).

c. Insecticide susceptibility testing

Insecticide susceptibility was evaluated using dipping method. Thirty adults (collected within 3 d of emergence) that had been anesthetized with CO_2 were put into a glass tube (⌀21 mm, height 25 mm); then both ends of the tube were closed with polyester gauze. The insects within the glass tube were dipped for 10 s in the insecticide solution, which had been adjusted to the appropriate concentration (up to 10000 ppm) with water containing Triton X-100 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 0.02%. After removing free drops of the solution using filter paper, the insects were transferred into a polystyrene vial (⌀25 mm, 50 mm depth) and were maintained at 27°C and 60% r.h.

The mortality was detemined at 24 h after exposure for fentrothion, pirimiphos-methyl, bifenthrin, and spinosad or at 48 h for permethrin. The viability was assessed as a measure of locomotion (i.e., paralyzed adults were regarded as dead).

Data were subjected to a probit analysis using the PriProbit (ver. 1.63) computer program developed by SAKUMA (1998), which was downloaded from https://www.ars.usda.gov/pacific-west-area/parlier/sjvasc/cpq/docs/priprobit-download/. The concentrations to achieve 50% lethality (LC_{50}) and 99% lethality (LC_{99}) were determined.

3. Results

Organophosphates fenitrothion and pirimiphos-methyl exhibited excellent performance at lower concentrations (< 20 ppm in LC_{99}). The susceptibilities were almost equal between the strains, irrespective of phosphine resistance (within 2.2 fold in resistance ratios calculated based on LC_{50}) (Table 2). The spinosad effect was constant among strains, although it was inferior to the two organophosphates tested. However, results show great differences in susceptibility to the two pyrethroids among strains. Three strains C87, SKG, and MLY exhibited high resistance to both permethrin and bifenthrin: most insects survived exposure, even at the highest concentration tested: 10000 ppm. To the other strains, TSC, THR, NGY, and IWT, bifenthrin exhibited high efficacy (≤ 25 ppm in LC_{99}), although permethrin was less effective (235–384 ppm in LC_{99}).

Table 2 Insecticide concentrations necessary to achieve 50% lethality (LC_{50}) and 99% lethality (LC_{99}) and resistance ratios of adults of *Lasioderma serricorne* strains

Insecticide	Strain	LC_{50} (95% CI[a]), ppm	LC_{99} (95% CI[a]), ppm	Resistance ratio[b]
Fenitrothion	TSC	5.9 (5.4–6.3)	11.6 (10.1–14.3)	1
	THR	6.0 (5.6–6.5)	12.5 (10.8–15.6)	1.0
	C87	6.6 (6.1–7.0)	13.0 (11.3–16.1)	1.1
	NGY	7.6 (6.5–9.2)	17.0 (12.7–35.3)	1.3
	IWT	6.2 (5.8–6.6)	11.2 (9.8–13.6)	1.1
	SKG	7.0 (6.6–7.5)	12.7 (11.2–15.3)	1.2
	MLY	5.9 (5.4–6.3)	13.4 (11.4–17.1)	1.0
Pirimiphos-methyl	TSC	4.2 (3.6–4.8)	19.2 (13.8–32.7)	1.9
	THR	2.3 (2.0–2.6)	5.9 (4.9–8.0)	1.1
	C87	2.5 (2.2–2.8)	8.7 (6.9–12.4)	1.2
	NGY	2.4 (2.0–2.8)	7.3 (5.8–10.7)	1.1
	IWT	2.1 (1.8–2.4)	5.5 (4.5–7.8)	1
	SKG	3.8 (3.4–4.1)	9.6 (7.9–13.2)	1.8
	MLY	4.7 (4.2–5.2)	12.2 (9.8–18.0)	2.2
Permethrin	TSC	120.8 (107.4–136.8)	384.0 (294.1–592.7)	1.7
	THR	97.2 (87.8–107.4)	235.3 (191.1–334.8)	1.4

	C87	>10000[c]		>10000[c]		>144.8
	NGY	79.3	(67.5–91.0)	340.2	(249.3–577.2)	1.1
	IWT	69.0	(58.6–78.8)	258.0	(195.6–415.1)	1
	SKG	>10000[c]		>10000[c]		>144.8
	MLY	>10000[c]		>10000[c]		>144.8
Bifenthrin	TSC	6.2	(5.6–6.9)	14.7	(12.2–20.2)	1.1
	THR	5.9	(5.2–6.6)	15.3	(12.5–21.6)	1
	C87	>10000[c]		>10000[c]		>1696.5
	NGY	7.0	(6.1–7.9)	25.2	(19.9–36.5)	1.2
	IWT	6.3	(5.4–7.2)	22.9	(18.0–33.7)	1.1
	SKG	>10000[c]		>10000[c]		>1696.5
	MLY	>10000[c]		>10000[c]		>1696.5
Spinosad	TSC	130.2	(111.4–152.6)	731.9	(511.6–1278.3)	1.9
	THR	152.8	(130.9–180.3)	856.2	(589.1–1543.6)	2.3
	C87	117.9	(101.4–136.8)	581.2	(421.4–952.5)	1.8
	NGY	115.3	(98.1–135.4)	682.1	(476.2–1190.9)	1.7
	IWT	100.8	(85.0–118.6)	722.6	(490.4–1322.4)	1.5
	SKG	125.2	(104.5–150.4)	1007.1	(646.1–2052.0)	1.9
	MLY	67.0	(50.7–83.4)	904.5	(532.3–2263.9)	1

[a] 95% confidence interval.

[b] Resistance ratios were calculated using the respective LC_{50} values relative to the strain which exhibited the smallest LC_{50} value for the respective insecticides.

[c] Probit analyses were not applied because most insects survived exposure even at the highest concentration: 10000 ppm.

4. Discussion

Higher contact toxicities of fenitrothion and pirimiphos-methyl against the same phosphine-susceptible *L. serricorne* strain as that examined in this experiment have been reported already (Orui, 2004). They have been used practically as space-sprays and surface-sprays in tobacco warehouses in the past, but the position as tobacco protectants of organophosphates has been replaced completely by pyrethroids. The absence of cross-resistance with phosphine, as shown in this experiment, makes them ideal for resistance-management. The time might come to review their usefulness against these pests.

Spinosad, derived by fermentation of soil actinomycete, has attracted attention as a grain protectant for its reduced risk properties (Hertlein et al., 2011). However, it has not been applied to tobacco in practice because it requires higher doses to achieve sufficient effects (Branc et al., 2004; Hertlein et al., 2011; Flingelli, 2014). Lower insecticidal activity against *L. serricorne* adults was found using a dipping method in this study (581–1007 ppm in LC_{99}). The cost of spinosad is higher than those of general synthetic insecticides at present. Therefore, it is not practical for the control of *L. serricorne* for economic reasons, although no cross-resistance with phosphine is apparent.

Synthetic pyrethroids are widely used today as surface-spray agents in tobacco warehouses around the world. This study revealed the existence of permethrin resistance and bifenthrin resistance in three of six phosphine-resistant strains which have mutually different origins. Coexistence of deltamethrin-resistance and phosphine-resistance in *L. serricorne* was found also in a population collected in a tobacco warehouse in Germany (Flingelli, 2014). Apart from tobacco pests, the widespread development of pyrethroid resistance has come to pose an immense practical difficulty in many parts of the world (Liu, 2012). These findings suggest that resistance to synthetic pyrethroids has developed in many field populations of *L. serricorne* through long-term use. Therefore, they cannot be recommended today as insecticides of primary choice for *L. serricorne*.

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Rapid detection of phosphine resistance in the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrychidae) from China using ARMS-PCR

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Abstract

The lesser grain borer, *Rhyzopertha dominica* is one of the serious cosmopolitan stored grain pests worldwide. High phosphine resistant *R. dominica* has been reported in several countries. The evolution of strong phosphine resistance is a major challenge for continuous application of the fumigant. Rapid detection of phosphine resistance level is a prime key to implement an appropriate strategy for control the stored-product pests. Dihydrolipoamide dehydrogenase (DLD) is a key metabolic enzyme mediating the phosphine resistance in population of *R. dominica*, *Tribolium castaneum* and *Caenorhabditis elegans*. Analysis of the DLD sequences deposited in GenBank revealed that the P45/49S mutation was the most common one in many PH3-resistant stored-product pest insects. This information now enables direct detection of resistance using molecular diagnosis in field populations. We herein propose a method for rapid detection of phosphine resistance in *R. dominica* according to P49S point mutation of the DLD gene. Our data provides evidence that the ARMS-PCR method can be used for early warning of phosphine resistance in *R. dominica i*n field conditions.

Tetra-primer amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method, in which two pairs of specific primers are applied was carried out based on the single nucleotide mutation in dihydrolipoamide dehydrogenase (DLD) gene. An ARMS-PCR assay was designed for diagnosing CCT to TCT (=P49S in amino acid sequence) mutation in the DLD gene sequences of *R. dominica*. The method employs four primers to amplify a common band from DNA containing the SNP and amplification representing each of the two allelic forms. Primers were designed to amplify fragments of differing sizes for each allele band in order to resolve using agarose gel electrophoresis. Primers were designed using primer1 software (http://cedar.genetics.soton.ac.uk/public_html/primer1.html). To increase the specificity of the reaction, a mismatch is introduced at the 3'end of each of the two allele-specific primers. The results of Taqman@ MGB-probe real-time PCR showed that there were no false-positive results in ARMS-PCR. In addition, the Food and Agriculture Organization (FAO) standard discriminating dose phosphine bioassay for eight *R. dominica* populations collected from China validated that the tetra-primer ARMS-PCR method was an accurate and sensitive method to diagnose *R. dominica* phosphine resistance level. The phosphine concentrations of 20 ppm phosphine for identification of weak resistance in *R. dominica* and 300 ppm for strong resistance according to FAO method were used. The sequences of the DLD gene were isolated from eight geographic populations of *R. dominica* collected from China. Further analysis of the DNA sequences revealed that the single amino acid mutated from proline to serine at the position #49 in the DLD enzyme, which are corresponding to CCC and TCC in nucleotide sequences of laboratory susceptible strain and strong phosphine resistant population of *R. dominica,* respectively. Genomic DNA was extracted from sixteen individuals from each of resistant *R. dominica* population and used as templates for PCR to generate 283-bp common band, 208-bp resistance phenotype and/or 130-bp susceptible phenotype band respectively. The discriminating fragments were a 130-bp band for susceptible (codon CCC) allele and a 208-bp band for mutant (codon TCC) alleles respectively. Therefore, genotype scoring was made according to the basis of presence/absence of 130bp and 208bp fragments, when the individual beetle was either heterozygous for the resistance allele or homozygous susceptible or homozygous resistant. A TaqMan real-time reaction was performed in parallel for P49S allele. In each reaction, a substantial increase in HEX fluorescence indicated a homozygous susceptible genotype, a substantial increase in FAM fluorescence indicated a homozygous resistant genotype and an increase in both signals indicated a heterozygote. The observed and expected ARMS results were conducted from eight populations previously discriminated by the phosphine discrimination dose. DLD allele frequencies in eight *R. dominica* populations were examined by the ARMS-PCR method to test all individuals in susceptibility bioassay. Overall, P49S allele frequency ranged from 9% to 94% in populations collected in this study. Chi-square analysis was carried to determine whether the genotype ratios at each population deviated from Hardy–Weinberg equilibrium. Significant changes in the allele frequencies across genotype frequencies within populations were used to identify phosphine resistance associated with the P49S resistance mutation in *R. dominica*. The phosphine resistance frequency of different geographical populations of *R. dominica* were diagnosed by FAO recommended method with discrimination 20ppm for weak resistance and 300ppm for strong resistance. The phosphine resistance frequency of different geographical populations of *R. dominica* were diagnosed by FAO recommended method with discrimination 20ppm for weak resistance and 300ppm for strong resistance. The results indicated that ARMS-PCR assay was easy to use, more sensitive and specific to detect the P49S mutation in phosphine resistant samples of *R. dominica* than previously used bioassay methods. Previous techniques for gene mutation detection are based on the polymerase chain reaction; many of them require post-PCR manipulations, such as isotopes irradiation, restriction enzymes, are required to two PCRs rounds. For example, using the (RFLP) typing method involved restriction and endonuclease digestion of PCR products, while some mutation sites were hard to find by appropriate restriction enzyme. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) analysis (Campbell 2008), mass spectrometry (MALDI-TOF-MS) assays (Park et al. 2008) and direct DNA sequencing methods are a sensitive, accurate and elegant diagnostic method, but they require expensive equipment and

have complex processing. Fast and economical assays that can be performed with standard PCR instruments are highly desirable for diagnostic analyses and for scientific studies of large numbers of pests. Individual *R. dominica* from distinct geographic populations can be discriminated into resistant homozygote, resistant heterozygote and susceptible homozygote from electrophoretogram after ARMS-PCR assay. Our TaqMan@ MGB probe assay could discriminate P49S mutation in DLD gene according to fluorescence labeling intensity variation and confirmed ARMS-PCR result as well. Our results show that the rapid detection of phosphine resistance in *R. dominica* populations in China provides important information to grain industries for decision-making in pest management strategies. In addition, our results suggest that this method could be applied for the detection of phosphine resistance in other grain pests, such as *T. castaneum* and *Sitophilus oryzae*, whose DLD genes have been sequenced. Our methods could be conducted on dead insects or insect fragments. Indeed, we evaluated consumable, running and capital cost for each method. The ability to quickly diagnose the resistance of these strains would be of great benefit. Furthermore, ARMS-PCR method for identifying the resistance locus mutation provides an opportunity to valuate level of phosphine resistance in other key pest species such as *Cryptolestes ferrugineus*, *S. oryzae* and *Sitophilus zeamais*. In addition, this technology could be extended to solve other pesticides resistance. The development of ARMS-PCR does not require generation of phosphine gas in the laboratory; also does not need collection and culture of field populations. Furthermore, the results are easier to assess with naked eye.

Keywords: Phosphine resistance, Lesser grain borer, Taqman@ probe, ARMS-PCR, Dihydrolipoamide dehydrogenase

Determınatıon of toxıcıty of gaseous ozone agaınst adult stages of German Cockroach (*Blatella Germanıca* L.)

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In this study, the effects of two different concentrations of ozone gas (16.7 and 33.3 mg / L) against *Blatella germanica* adults at different exposure times (10, 20, 30, 40 and 50 minutes) were investigated under laboratory conditions. It was determined that the ozone gas had a noticeable effect on mortality of *B. germanica* adults. In general, ozone gas caused higher paralyisis-mortality rates of *B. germanica* adults than mortality rates of *B.germanica* adults at both concentrations and all exposure times. A concentration of 33.3 mg / L of ozone gas with 40 and 50 minute exposure times killed all cockroach adults after 24 hours. On the other hand, 16.7 mg / L concentration of ozone gas with 50 minute exposure time killed 90% of the *B. germanica* adults after 24 hours. When ozone gas is evaluated in terms of exposure time to *B. germanica* adults, the concentration of 33.3 mg / L of ozone gas with 10-20 minute exposure times caused 65 % adult mortality, with 30 minute exposure time caused 90% adult mortality and with 50 minute exposure times caused 100 % adult mortality after 24 hours. At a concentration of 16.7 mg / L of ozone gas, as the exposure times increased, the adult mortalities gradually increased after 24 hours and the adult mortality reached 90% with 50 minute exposure times. All these results show that ozone gas (33.3 mg / L) with 40-50 minute exposure times can successfully control *B.germanica* adults.

Keywords: Ozone gas, *Blatella germanica*, mortality, biological efficacy.

1. Introduction

Cockroaches are insect species that have remained unchanged since ancient times (Appel, 1995). There are approximately 3,500 species of cockroach in the world (Atkinson et al, 1991). Most types of cockroaches are insect species that live in outdoor environments. However, a few cockroach species are found in the living areas of insects. One of the cockraoch species found in people's

habitats is the German cockroach, *Blatella germanica* (L.), which is one of the most common cockroach species all over the world. It is easily distributed when indoor and outdoor temperature and humidity are suitable for this species.

In addition to the psychological effects to humans, this species has a considerable medical importance, as it harbors bacteria, fungi, helminths, protozoa, viruses that can cause diseases in humans (Mullen et al., 2002). Cockroaches also cause asthma in many people with allergies (Roberts, 1996). The control of German cockroaches is traditionally made with inorganic and synthetic organic insecticides (Rust et al., 1993). Due to adverse effects of the use of intensive chemical insectides in control of the German cockroach, an alternative control method which is not harmful to the environment, humans and animals is needed.

Ozone is a form of three atomic oxygen (O3) molecule. Ozone is produced as a bluish or colorless gas characterized by fresh clean odor in the air following the thunder storm. Ozone is an unbalanced gas and quickly converts oxygen to temperatures above 35 ° C. Therefore, it must be produced during use and can not be stored after it is produced. There is a striking characteristic odor that many people in ozone can notice even at very low concentrations (0.02 ppm by volume) (Kim et al., 2003). Commercially, mostly ozone is produced with pure oxygen or airborne corona current generators (Kim et al., 2003). The control of insects with ozone gas has started with stored product pests. Isikber and Öztekin (2009) tested ozone gas on some stored product pests. These are the application of ozone gas to the insects with products and without product environment. The toxicity data obtained from studies with ozone gas in unfilled environment (empty volume) revealed significant differences between ozone gas sensitivities of developmental periods of *Ephestia cautella* and *Plodia interpunctella*. Ozone application in unfilled environment (empty volume) caused 100% death of *E. cautella* and *P. interpunctella* in all life stages, with the exception of the egg. The toxicity results obtained in study showed that *Tribolium confusum* is generally more resistant to ozone gas than *Ephestia kuehniella.*

To our knowledge, there are no studies on the effect of ozone against *B. germanica*. For this reason, in this study, we determine the optimum ozone concentration and ozonation duration by using the osmotic gas in the adult control of German cockroach.

2. Materials and Methods

Insect

Colonies of *B. germanica* were reared in plastic containers (60 liter) and maintained at room temperature. The cockroaches were provided with water in glass tubes with cotton stoppers and dry dog food. Each container was provided with paper egg cartons as shelter. The adult cockroaches (5-10 days old) were tested for each bioassays at 25 (± 2) ºC and 50 (±5) % relatıve humidity.

Ozone gas fumigation chamber

In empty volume applications, 3 liters of glass jars with a metal cover of 9 cm in diameter were used in all of the biological tests. These hatches have 2 entry pipes, 0.5 cm in diameter and 3 cm in length. A 5 cm long silicone hose is connected to the end of each record (one of the hoses is connected to the vacuum pump and the other to the ozone generator). One of the hoses was provided with ozone gas from the other hole while the air in the glass jar where the biological tests were conducted was discharged. Thus, the ozone gas was periodically circulated in the glass jar. The adjustment of the ozone gas concentration is adjusted according to the flow rate of the pure oxygen gas. The flow rate of the oxygen gas is controlled by the flowmeter placed between the oxygen tube and the ozone generator.

Biological tests and Empty volume applications

For empty volume applications, biological tests were carried out in 3 l metal-lined glass jars (fumigation chamber) at a temperature of 26 ± 1 and 65 ± 5% relative humidity. In all tests, *B. germanica* adults were used. The adults used in the biological tests were placed in 3 l jars where ozone fumigation was carried out and a small amount of food was added to the bottles. The solution was prepared by adding 10 ml of purified water to 100 g of MgNO2 (Magnesium Nitrate) to keep the media noodle in the jars constant at 65 ± 5%. This solution was soaked in a jar until wetted to a drying paper size of 5 x 2 cm. As the individuals exposed to ozone gas were placed in glass jars, the air in the jars used in the tests was evacuated to 760 mm Hg by low pressure pump (KNF, Germany). As the air in the jars is evacuated, the hoses on the covers of the jars are closed with the help of plastic clips to prevent gas in and out of the jars. After taking the air in the jars used in the biological tests, ozone gas is delivered to the ozone generator of the oxygen gas with the help of the flowmeter and ozone gas is produced. In order to produce ozone gas at different concentrations, the oxygen gas flow was set at 5 and 10 l / h and the flow rate was monitored from the flowmeter screen. When the desired flow rate is reached, the clips in the lids of the jars are opened and the produced ozone gas is directed to the fumigation chamber and the pressure inside the jar is filled with ozone gas until reaching normal pressure conditions. Since ozone gas is not a stable gas, it quickly transforms into oxygen form due to the effect of temperature and relative huminity. Therefore, ozone gas application has been completely applied to biological tests. In empty volume applications, application times were determined as 10, 20, 30, 40 and 50 minutes and the air in the jars where the tests were carried out once every 30 minutes in 40 and 50 minute applications was evacuated by vacuum pump and ozone gas was applied again. Experiments were carried out in 3 replicates of 10 individuals each time, leaving 3 controls for each trial. Upon completion of the application period, the ozone gas applied jars were ventilated and the insects were removed from the jars. The adults exposed to ozone gas were placed in 1 l glass jars and a small amount of food that was not exposed to ozone gas were added to the bottles. Dead and alive individuals were counted 1 hour, 6 hours and 24 hours after the termination of the experiments.

3. Conclusion

In this study, the toxicity of ozone gas against adults of *B. germanica* was demonstrated at two different application concentrations and different application times. In this study, as a result of the biological tests, ozone gas was generally observed in both the ozone gas concentration and the paralysis-mortality rate in all the application periods in the adults of *B. germanica*, was higher than the death rate. This has shown that ozone gas is a knockdown feature on this insect which leads to death. When the concentration of 33.3 mg / L of ozone gas was applied to adults of *B. germanica* with 40 and 50 minutes, respectively, it killed all cockroaches after 24 hours of application. On the other hand, when a concentration of 16.7 mg / L of ozone gas was applied to *B. germanica* adults for 50 minutes, it killed 90% of the adults of *B. germanica* after 24 hours of application. When the ozone gas is evaluated in terms of exposure time to *B. germanica* adults, the concentration of 33.3 mg / L of ozone gas with 10-20 minute exposure times caused 65 % adult mortality, with 30 minute exposure time caused 90% adult mortality and with 50 minute exposure times caused 100 % adult mortality after 24 hours. At a concentration of 16.7 mg / L of ozone gas, as the exposure times increased, the adult mortalities gradually increased after 24 hours and the adult mortality reached 90% with 50 minute exposure times. At a concentration of 16.7 mg / L of ozone gas, as the exposure times increased, the adult mortalities gradually increased after 24 hours and the adult mortality reached 90% with 50 minute exposure times. All these results have shown that ozone gas has potential for controlling *B. germanica* and may be an alternative to the synthetic chemicals used in the control of this insect. However, a comprehensive study of the ozone gas applicability in the natural habitat of the German cockroach and the determination of its effect on different factors when applied in natural conditions is necessary.

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Does the lower concentration of anticoagulants affect the efficacy of rodenticide baits?

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Extended abstract

Rodents belong to dominant synanthropic pests in agriculture environment, where cause wide range of damages by feeding on crops, gnawing of materials and faecal/urine contamination (Frankova et al. 2016, Stejskal et al. 2016). Rodents are predominatly controlled by anticoagulant-based rodenticides (AR) with the chronic mode of action (e.g. Frankova et al. 2017). Their delayed efficacy prevents rodents to connect the consumption of the bait with subsequent toxic effects and thus, favours them over other chemical rodenticides. On the other hand, application of ARs is permitted under strict regulation (Regulation (EU) No 528/2012) as ARs are considered as PBT (i.e. persistency, bioaccumulativity and toxicity) substances which pose environmental risks.

In addition, EU Commission recently adopted reclassification of ARs products (Commission Regulation (EU) 2016/1179; shall apply from 1 March 2018) - rodenticides with anticoagulant of 30 ppm or more must be labelled as "toxic to reproduction" and will be available to professional use only. Currently, it concerns seven of the eight approved anticoagulants, which contain 50 ppm of active substance. This Regulation leads manufacturer to produce rodenticide baits with a decreased concentration of anticoagulants to avoid a reclassification of products.

We focused on the testing efficacy of standard (50 ppm) and lower (25 ppm) concentration of anticoagulant in two brodifacoum-based baits in wild house mouse (Mus musculus). The laboratory no-choice feeding tests showed 100% mortality (mean survival time was 5.3 ± 2.1 days) for both concentrations. The consequent field experiments confirmed the previous laboratory results for the new baits with the lowered concentration (i.e. 25 ppm): during the three-week application period we found a significant decrease of both the tested bait and monitoring non-toxic bait consumption. Our study shows promising efficacy of products with the lowered concentration of brodifacoum. Nevertheless, there is a work ahead rodent scientists to illuminate the new baits efficacy in rodent populations with the decreased physiological sensitivity or increased resistance to anticoagulants.

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Session 10
Microbiology, Food Safety, Quarantine, and Regulatory Aspects

Australia's Grains Farm Biosecurity Program – a national initiative in plant biosecurity awareness, education and best management practice.

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Abstract

Sound biosecurity systems contribute to achieving sustainable agricultural and environmental systems, reducing the threat of introducing unwanted pests and supporting food safety and product integrity. Within Australia, the Grains Farm Biosecurity Program (GFBP) is a national initiative to assist in the development and implementation of improved biosecurity practice within its grain industry. Initiated in 2007, the extension focused program contributes to the industry's risk mitigation activities, supports continued market access and promotes a partnership approach involving governments, industry and community. The program is funded through grower levies in partnership with state government agencies and Plant Health Australia.

Using a variety of community engagement strategies, the GFBP has developed a wide range of tools to improve the management of and preparedness for, biosecurity risks in the Australian grains industry at the farm and industry level by highlighting risk pathways and activities throughout the supply chain and encouraging adoption of practices and strategies to mitigate risks. The GFPB also promotes and conducts surveillance for high priority pests especially in on-farm storage. Evaluations indicate an increased awareness of biosecurity risks, industry capacity and voluntary adoption of biosecurity best practices throughout the sector.

The GFBP focus on biosecurity best practice through industry engagement has seen it contribute to safeguarding and maintaining Australia's export reputation, with the program recently winning a national biosecurity award.

Keywords: Grains biosecurity, farm biosecurity, market access, Grains Farm Biosecurity Program, industry engagement

1. Introduction

The Grains Farm Biosecurity Program (GFBP), initiated in 2007, contributes to the Australian grains industry's risk mitigation activities, and promotes a shared responsibility involving governments, industry and community. The program aims to promote and improve the management of, and preparedness for, biosecurity risks in the grains industry at the farm and industry level.

Australia's geographical isolation and strong biosecurity system has ensured that many overseas pests of crop production and post-harvest storage are not present in Australia. Freedom from exotic plant pests provides both a yield advantage as well as market access benefits (especially in relation

to stored product pests such as khapra beetle) for Australian crop production industries such as grains.

Along with exotic pests, endemic pests can also impact upon production and market access. There is a nil tolerance for live insects in delivered grain in Australia, and with increasing on farm storage, prevention and control of stored grain pests is a critical function undertaken by growers. Management of stored grain pests is just one example of how farm biosecurity and hygiene practices are a key component of integrated pest management and form a vital role in minimising the establishment and contamination of pests in stored grain.

Importantly, national and state biosecurity systems are complemented and supported by measures carried out at the industry and regional level. As risks of new pests entering Australia can never be totally eliminated, industry biosecurity is regarded as a shared responsibility where all links in the production and supply chain engage and take responsibility for minimising biosecurity risks that are within their control. Growers implementing farm biosecurity practices, agronomists, researchers and other service providers including contractors can all play an important role in safeguarding the industry at a farm, regional and national level. An aware and trained grains industry has the capacity to minimise the risks posed by new and established pests, and respond effectively to any pest threats that would impact on the future sustainability and viability of the industry (Taylor-Hukins et al. 2015).

2. Materials and Methods

The GFBP has appointed specialised Grains Biosecurity Officers in five key grain growing states of Australia. The program is funded by Grain Producers Australia through grower levies in partnership with state government agencies, and is managed by Plant Health Australia (Bellati et al. 2015).

Yearly, over 100 activities are undertaken using a variety of community engagement strategies. The GFBP has developed a wide range of tools to improve the management of, and preparedness for, biosecurity risks in the Australian grains industry throughout the supply chain.

Core activities undertaken by the Grains Biosecurity Officers include:

- education and training to increase awareness of high priority exotic pests
- facilitating surveillance of high priority exotic pests for early detection and proof of absence
- development of practical resources and training materials
- demonstrating simple and effective methods for implementing farm biosecurity
- building collaborative networks and alliances across industry
- promotion of industry advocates
- emergency response and preparedness

3. Results and Discussion

Monitoring throughout the program indicates an increased awareness of biosecurity risks and voluntary adoption of biosecurity best practices throughout the sector. Table 1 provides a snapshot of results from surveys conducted by the Farm Biosecurity Program (initiative of Animal Health Australia and Plant Health Australia) between 2010 and 2017, measuring the percentage of respondents (grain producers) implementing suggested biosecurity practices. A general trend of uptake can be observed, with particular mention to monitoring stored products.

The national GFBP has aligned key awareness messages and education objectives to current grains industry extension programs, in order to deliver grains biosecurity training and education seamlessly. A critical element to the success of these strategic alliances is value adding to existing program content, where biosecurity messages and exotic pest identification information is embedded within industry training programs such as Pulse Australia best management practice accredited workshops (Bellati et al. 2010). This continues to be a strength of the program, with new linkages and alliances formed in particular for surveillance.

Tab. 1 An extract from surveys conducted by the Farm Biosecurity Program measuring practice change within the grains industry at the farm level

Practice	2010	2013	2017
Keep records	59%	82%	89%
Monitor stored products	52%	80%	93%
Clean machinery/equipment coming in property	57%	73%	81%
Control visitor movement on property	34%	47%	54%
Report anything unusual	31%	42%	49%

The latest initiative from the program is a pilot Sentinel Silo project, using both targeted and general surveiilance to monitor grain storages, 'ag pantries' and other risk sites and pathways for exotic stored product pests. The surveillance is aimed at strengthening evidence of absence, improving industry participation and knowledge of stored grain pests and surveillance and promoting best management practices.

4. Conclusion

The GFBP is Australia's flagship program for promoting farm biosecurity, with its success encouraging other industries to implement similar extension programs. The focus on biosecurity best practice through industry engagement has seen the GFBP contribute to the safeguarding of grains production and helping to maintain Australia's grain export reputation.

The GFBP is celebrating 10 years of success raising awareness of biosecurity among grain growers and helping the industry respond to serious pest incursions. In March 2018, the program was awarded an Australian Biosecurity Award for its ongoing contribution to Australia's biosecurity integrity.

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A commercial method of controlling bedbugs (*Cimex lectularius*) using CO_2 in dwellings

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Abstract

As a result of withdrawing residual insecticides such as organophosphates and carbamates throughout the world, infestation in *Cimex lectularius* has been dramatically increased in recent years. The ability of this pest to

starve for three months and its flattened shape of body enables it to hide in tiny holes/cracks or folds, making it difficult to control. Therefore, as a complementary action, fumigating products like textile products and furniture within the dwellings became a practice in Israel. After the conventional treatment of stripping the room, vacuuming, steaming the mattresses and then spraying with a Pyrethroid – a novel method was developed; instead of steaming the mattress and dry cleaning all textile clothes and products, fumigating all movable furniture and textile products with CO_2 inside the dwellings. Based on several scientific papers on the efficacy of CO_2 on the bedbugs, this method has been successfully implemented in Israel. All textile products are inserted into a sealed, low permeability fumigation cube for three days of exposure time at room temperature to reach a calculated concentration of 100% CO_2. Since textile products absorb some of the CO_2, the concentrations quickly drops to about 80%. During the summer of 2017, numerous treatments have been carried out with a 100% success and repeated treatments were not required. This treatment has proved to be a promising method of controlling *C. lectularius* with no need for evacuation of the residents and saving money and efforts in dry cleaning cloths and textile products. It is highly effective against all life stages of the pest.

Key words: *Cimex lectularius*, CO_2 fumigation, bedbug, Modified Atmospheres.

1. Introduction

From ancient history the Greeks and Romans times, to early Jewish and Christian writings bedbugs appear in the literature and folklore of many cultures and countries (Usinger, 1966). Although there are several species of blood-feeding bugs which belong to Hemiptera: Cimicidae which have been persistent pests of humans throughout recorded history, only two species are truly associated with humans. The common bed bug, *Cimex lectularius* L. and the tropical bed bug, *Cimex hemipterus* (Fabricius). Their biology is strikingly similar and most life history traits and behavioral aspects of their biology are overlapping. *C. lectularius* can be found in most parts of the world although is less abundant in tropical regions, while *C. hemipterus* is mostly found in tropical to sub-tropical areas mainly within the 30° latitudes (Usinger, 1966).

Bedbugs consume only blood, usually feeding on a mammal (e.g., human, bat) or bird. They need at least one blood meal of adequate volume in each active life stage (instar) to develop to the next stage and to reproduce. There are five nymph stages, and each one may feed multiple times if hosts are readily available. The nymph stage has to feed once to be able to molt into the next stage. The length of the life cycle decreases with temperature up to an optimum of 30°C where it takes 24.2 days to complete (Usinger, 1966). Bedbugs can survive long periods without food and this is also affected by temperature. At 10, 18, 27 and 37°C bedbugs can survive on average for 401.9, 175.6, 43.4 and 17.4 days respectively, if fed once (Usinger, 1966). At low temperature (<10°C) adult bedbugs can survive for a long time without a blood meal. First instars nymphs, when newly hatched, can live up to three months without blood (Usinger, 1966). A fed female lays on average one egg per day (Polanco et al., 2011). It is estimated that bedbugs, at least in theory and under optimal conditions, can lay up to 200 to 500 eggs in a life time (Usinger, 1966).

Adult bedbugs may feed every three to five days throughout their estimated six to 12 month life span. The act of biting a host can cause both physical and psychological discomfort, and can result in local allergic skin reactions to injected salivary proteins (Feingold et al., 1968). Yet, there is no solid evidence bedbugs are disease vectors (Goddard et al., 2009)

When bedbugs have completely engorged, they immediately seek a harborage to hide. They are concealed most of the time; they mate, molt and lay eggs in this cryptic location until they require a blood meal or are disturbed (Usinger, 1966). They prefer harborages that contain aggregation pheromones, which they detect with the pedicel of the antenna (Olson et al., 2009; Siljander et al., 2007), and they prefer the company of conspecifics within their refuges. This aggregation behavior protects the insect from detection by their host, increases the chance of mating and helps reduce the loss of water (Benoit et al., 2007). Their favorite sites are wooden frames in box-spring mattresses, behind skirting, wall sockets and cracks in the wall.

The insect prefers to be active at night time, when the host is most likely to be asleep (Romero et al., 2010) and the risk of detection is minimal, but they are also activated by host cues at daytime (Aak et al., 2014). A host seeking bedbug shows positive thermotaxis, the movement towards an up or

down gradient of temperature, but when engorged show negative thermotaxis (Reinhardt and Siva-Jothy, 2007; Reis and Miller, 2011). This results in the bedbugs spending as little time as possible close to their host where the risk of harm and detection is the greatest. Therefore, their biology and cryptic behavior make it difficult to control.

After World War II, widespread use of synthetic insecticides led to sharp declines in bed bug populations in most industrialized countries. By 1997, they were so scarce in the U.S., Canada and Europe that it was difficult to find fresh specimens to use in teaching college entomology classes (Snetsinger, 1997). Many contemporary Pest Management Professionals (PMPs) with years of experience have never seen an active bed bug infestation. During the past 18-20 years, a resurgence of bedbugs has been reported in the U.S., Canada and European countries, Australia and parts of Africa. Infestations have occurred in homes, hotels, hostels, cruise ships, trains, and long-term care facilities (Cooper and Harlan, 2004; Doggett et al., 2004; Hwang et al., 2005; Johnson, 2005).

The increase in trade, the change in pesticides use from residual and more violent once such as organophosphates and organo-clorides, development of resistance to pesticides, the use of second hand furniture and products and the lack of public awareness has led to re-establishment of the pest worldwide, especially in developed countries (Anders et al., 2010; Akhtar and Isman, 2013).

Each country takes its own measures of treating those bedbugs; in Greece, heat treatment for the whole dwelling is applied. Although heat treatment provides no residual effect, there is a potential physical distortion of structures or their contents, as well as flammability risks associated with some kinds of heat sources, may be a concern in particular situations (Usinger, 1966). Moreover, in order to reach the target temperature of more than 47°C, the residents must evacuate their dwellings since the treatment is a 24 h.

In Israel, the conventional treatment consists of several steps; stripping the room, vacuuming, steaming the mattresses and then spraying with a Pyrethroid. After the treatment all cloths, blankets and pillows are either dry cleaned or being washed at a temperature above 60°C. These actions of vacuuming and steaming textile products such as sofas and mattresses requires a lot of working hours which are not always successful. When applying steam treatments, this technique requires practice and care. If the tip is too far away, the steam may not be hot enough to kill all the bedbugs and eggs that it contacts. If the tip is too close, excess moisture may be injected into the treated material, which may lead to other problems (e.g., facilitating dust mite population survival and increase; growth of surface molds). Sometimes the strength of the steam causes the pest to be spread to other hiding places. This time consuming technique of steaming (10-30 seconds) causes mortality of only 84-94% of all stages, if applied properly (Puckett et al., 2013). Also, if the textile cloths are dried in a home dryer, temperatures in the center do not always reach the target temperature of 50°C. Within the army bases, cold treatment is applied. Yet, each of these measures has its own limitations and disadvantages. For example, exposure to low temperatures can kill bedbugs if they are kept cold enough long enough. Bedbugs can tolerate -15°C for short periods and, if acclimated, they can survive at or below 0°C continuously for several days (Usinger, 1966).

Therefore, as a complementary treatment, in order to eradicate the pest from dwellings, a novel method was developed; instead of steaming the mattress and dry cleaning all textile clothes and products, fumigation of all mobile furniture and textile products with CO_2 inside the dwellings is applied. This novel approach is described in this paper.

2. Materials and methods

During the summer of 2017 several commercial treatments were done in several dwellings in Israel.

All textile products were inserted into sealed flexible low permeability fumigation cubes for three days exposure time at room temperature to reach a calculated concentration of 100% CO_2. The surrounding temperatures were 25-30°C. One 13 m^3 and three 7.8 m^3 flexible fumigation cubes were used to reach, when shrinked, 8, 7.7 and two 5.5 m^3 in accordance. The volume of each flexible

fumigation cube was lowered by using a shrink tape around it. After placing the products inside the fumigation cubes a half life-time pressure decay test was applied by negative pressure to ensure gas-tightness of the cubes. Pressure was reduced from 6 mm H_2O to 3 mm H_2O column. Before connecting the pressure hose, an expansion space was left using a perforated plenum. After connecting the pressure hose to the CO_2 cylinder, the cylinder was placed on a scale to measure the amount of the calculated gas at a dosage of 2 kg CO_2/m^3. While introducing CO_2, at the top end of the opposite gas introduction side, approximately 30 cm diameter area was left open to enable air to exhaust. Measurements were taken from the top during the introduction of the gas.

3. Results

The results shown in Fig. 1 are measurements of CO_2 taken from the top end of the four flexible fumigation cubes each at a volume of 8, 7.7, 5.5 and 5.5 m^3. On average 33 min were needed for the gas purging phase. The amount of CO_2 correlates with its concentration; at the 7.7 m^3 fumigation cube 10.3 kg of CO_2 were purged and 85% CO_2 was obtained. The 5.5 m^3 fumigation cube which reached 85% CO_2 was flushed with 10.3 kg and the second 5.5 m^3 fumigation cube was flushed with 9.5 kg CO_2 to reach 75% CO_2 (Table 1). In the case of the 8 m^3 fumigation cube the cylinder was not placed on a scale.

Since the air is being flushed out, measurements were taken from the top end, indicating the weakest point of concentration. Some measurements were taken from the middle (data not shown) indicating an intermediate concentration.

Since all these fumigation cubes were of small volume, at the end of the treatment, after spraying the dwellings, measurements were taken once again, indicating an even distribution of CO_2.

After exposure time of 72 h, measurements were taken again from the top, middle and bottom (only at the 8 m3 cube) of the fumigation cubes (Table 1). Although calculated dosage was of 100% CO_2, it did not reach it due to sorption by the treated textile products.

Table 1: CO_2 concentration (%) in four flexible fumigation cubes at the beginning of exposure time and at the end, after 72 h, the amount of CO_2 (kg) and the time (min) of half life-time pressure decay test.

Volume of the fumigation cube		8 m^3			7.7 m^3		room # 1 5.5 m^3		room # 2 5.5 m^3	
Location of measurment		Bottom	Middle	Top	Middle	Top	Middle	Top	Middle	Top
[CO2] (%)	T_0	-	82	86	-	85	72	75		85
	T_{72}	82.7	73	75.5	84	81.5	80.9	81	81	77
CO2 (kg)					10.3		9.5		10.3	
Time (min) of half time pressure decay test		6			26		24		23	

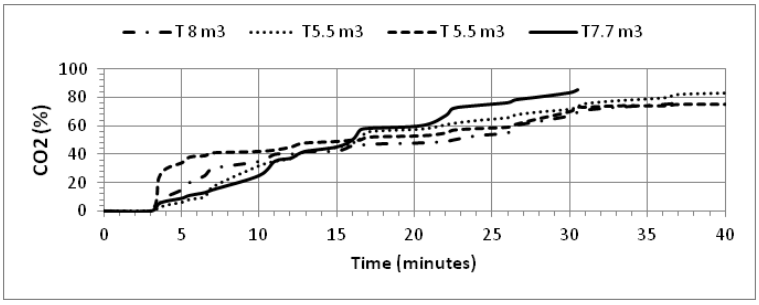

Figure 1: Increase in CO_2 concentration (%) during purging in four flexible fumigation cubes having the volumes of 8, 7.7, 5.5 and 5.5 m^3**.**

Picture 1: *C. lectularius* aggregation in between sofas' folding; adults, nymphs, eggs (left), and a closer view of eggs that hatched (right)-.

Picture 2: Well shrinked fumigation cube (left), and badly placed shrink tape (right).

4. Discussion

Bedbugs (*C. lectularius* L. and *C. hemipterus* F.) are very difficult pests to manage, in part, because of their widespread resistance to insecticides and mostly because of their cryptic behavior (Romero et al., 2007; Zhu et al., 2010). Bedbugs are not limited to sleeping and resting areas such as beds and sofas, instead, literally, anything is susceptible to infestation; from air conditioners outlets, electronics, books, pictures in between sofas (Picture 1) and other household fabrics and equipment. Eliminating bedbugs safely and effectively from these types of items is often more challenging than eliminating bedbugs hiding in cracks and holes in furniture or the structure itself. Therefore either a gaseous treatment is required or physical methods such as heat or cold.

In preliminary laboratory tests by the German Federal Environmental Agency, all life stages of common bedbugs were reportedly killed by constant exposure to very high concentrations of carbon dioxide, at ambient atmospheric pressure, within 24 hours or less (Herrmann et al., 2001). According to Wang et al. (2012), CO_2 fumigation lasting 24-48 h was sufficient to kill all stages of bedbugs at room temperature, depending on the quantity of materials placed in each bag and whether CO_2 was introduced one or two times at the onset.

In these fumigations which were held inside dwellings, in order to ensure both successful treatment and residents' safety, the half life-time pressure decay test is a fundamental step. According to Navarro (1998), in his attempts to correlate gas loss to pressure decay tests, recommended a 5 minutes pressure decay time which were compared with daily CO_2 decay rates of >1% CO_2 daily to obtain successful insect controls (Navarro, 1998). In all of these fumigations, more than 5 minutes were obtained indicating appropriate gastightness for successful treatments (Table 1). Even though pressure tests are not capable of measuring the degree of gas losses through the flexible liner, it still serves as a good indicative measure to predict the degree of gas tightness of the chamber has and whether the fumigation would be successful. It can be understood from Table 1 the significance of this test; there was a decline in gas concentration of 10.5% at the 8 m^3 fumigation cube which obtaind only 6 minutes at the half time pressure decay test. Eventhough that, the results show successful treatment with an acceptable decline in gas concentration (Table 1).

The target concentration of 100 % CO_2 was not achieved due to sorption. Also Wang et al., (2012) could not achieve 100% CO_2 when CO_2 was applied from a cylinder and the bags were filled with fabric materials. In previous cases (data not shown) when purging the gas into the flexible fumigation cube, when the pathway was blocked with a textile products such as duvet it absorbed most of the gas and only after its adsorption target concentration was achieved. Therefore, an expansion space must be kept from the purging point of at least 40-50 cm long with a width of 40 cm. Nevertheless, when placing the shrink tape, it is important to wrap the fumigation cube from the very bottom of it to enable air to be flushed out easily (Picture 2). When placing it not from the very bottom, more gas is needed as it was obtained at the 5.5 m^3 fumigation cube where 10.3 kg of

CO_2 were needed to achieve concentration of 77% CO_2 at the end of exposure time instead of 81% CO_2 with only 9.5 kg of CO_2 (Table 1).

Compared with other insects studied, bedbugs are more sensitive to CO_2 fumigation than other urban insects such as oriental cockroach (*Blatta orientalis* L.) (Gannon et al., 2001), and stored product pests (Navarro, 2006). The relatively short exposure time (2 d) makes CO_2 fumigation a promising technique for eliminating bedbugs from infested household items. In commercial use, the recommended exposure time is of 72 h to ensure mortality. After the conventional treatment of stripping the house, vacuuming and spraying with a pyrethroid the residents may return home and stay at home with no risk.

5. Conclusions

CO_2 is an effective alternative of all life stages of bedbugs compared to conventional fumigants for eliminating bedbugs hiding in infested household items such as clothing, shoes, books, electronics, sofas, and other household items. The CO_2 fumigation may be peformed in one of the empty rooms of the house. There is no need to evacuate the residents from neither their dwellings nor the materials to other places in order to fumigate with poisonous fumigation products, to freezing or heating the chambers.

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Mycotoxin prevalence in stored animal feeds and ingredients in Rwanda

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Abstract

Aflatoxins and fumonisins are fungi metabolites produced when climate conditions are favorable. They contaminate feed ingredients when storage conditions are unfavorable. Aflatoxins and fumonisins have a negative impact on animal health and productivity. Humans are indirectly exposed to mycotoxins when they consume contaminated animal source foods from livestock fed contaminated feeds. A total of 3328 feed samples were collected in all 30 district of Rwanda between March and October 2017. Four categories of participants participated in the study (dairy farmers, poultry farmers, feed processors/grain millers, and feed vendors). Feed samples were highly contaminated with aflatoxins but not fumonisins. Average aflatoxin levels were highest in dairy feeds (108.3 μg/kg) followed by poultry feed (103.81 μg/kg). Average aflatoxin levels were lowest in samples from feed vendors (88.64 μg/kg) compared to samples from feed processors (94.95 μg/kg). This study documents high levels of aflatoxin contamination in feed samples, and recommends year-round surveillance of feed ingredients and mixed feeds for mycotoxin presence. Additionally, more awareness through communication and education needs to be raised among stakeholders in the evolving feed value chain in Rwanda to mitigate the consequences of mycotoxin contamination on public health and animal produtivity.

Keywords: aflatoxins, fumonisins, ELISA, value chain

Introduction

Mycotoxins (e.g., aflatoxins and fumonisins) are secondary metabolites produced by fungi. Aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus* while fumonisins are produced by *Fusarium verticillioides* and *F. proliferatum* in favorable conditions. They contaminate crops especially maize, peanuts and cottonseed throughout sub-Saharan Africa (Binder, Tan, Chin, Handl, & Richard, 2007; Perrone & Gallo, 2017; Richard, 2007). Aflatoxins and fumonisins have a negative impact on human and animal health. Human exposure to these mycotoxins is the result of ingestion of contaminated foods (e.g., maize flour, peanut butter), or indirectly from consumption of animal source foods (e.g., dairy products, eggs) derived from animals previously exposed to aflatoxins in feeds. Aflatoxins are classified as carcinogenic susbtances (IARC, 2002). Fumonisins are associated with neural tube defects, disrupt sphingolipid metabolism and folate transport (Marasas et al., 2004). Fumonisins are also associated with different animal diseases such as Equine Leukoencephalomalacia (ELEM) in horses and Porcine Pulmonary Edema (PPE) in pigs. They are

reported to be nephrotoxic, hepatotoxic and hepatocarcinogenic in a number of livestock and poultry species (Wan Norhas et al., 2009). Mycotoxin feed contamination has attracted worldwide concern due to losses in animal productivity and feed safety (Bryden, 2012; Placinta, D'Mello, & Macdonald, 1999). Different factors contribute to high risk of feed contamination in Africa. Environmental conditions with high relative humidities and temperatures favor fungal growth. Socio-economic status and food production system result in high contamination of feeds (Wagacha & Muthomi, 2008). Feed contamination will not only lead to reduction in animal productivity but will also contribute to milk contamination due to aflatoxin M1 which is the result of aflatoxin B1 metabolism and excreted in milk. A number of studies have reported feed contamination in different African countries (Kang'Ethe & Lang'A, 2009; Mohammed, Munissi, & Nyandoro, 2016; Nyangi et al., 2016). Thus, mycotoxins are a significant risk to animal productivity and food safety in East Africa (Atherstone, Grace, Lindahl, Kang'ethe, & Nelson, 2016). The main objective of this study was to assess the prevalence of aflatoxins and fumonisins in stored animal feeds and ingredients in Rwanda.

Materials and Methods

a. Sample collection

A countrywide survey in all 30 districts of Rwanda was carried out between March and October 2017 targetting four categories of participants: dairy farmers, poultry farmers, feed vendors and feed processors/grain millers. Samples were collected in six rounds by taking approximately 2 kg of feed from each participant who agreed to participate in the study.

b. Sample analysis

All collected feed samples were analyzed using competitive Enzyme-Linked Immunosorbent Assay (ELISA) technique (Catalog #941AFL01M-96 and Catalog #951FUMO01C-96 for Total Aflatoxin Assay and Fumonison ELISA Assay, respectively, Helica Biosystems, Santa Ana, CA, USA).

Results

Table 1. Aflatoxin and fumonisin levels in feed samples collected at different points in the feed value chain.

	Aflatoxins (µg/kg)			Fumonisins (mg/kg)		
	Mean	**SD**	**Median**	**Mean**	**SD**	**Median**
Dairy farmers	109	145	44	1.5	1.8	0.7
Poultry farmers	104	136	48	1.2	1.5	0.6
Feed Vendors	89	129	31	1.5	1.7	0.8
Feed Processors/ Grain Millers	95	103	71	1.0	1.3	0.5

Discussion

Rwanda, a tropical country, offers favorable conditions for mycotoxigenic fungi growth. At all points in the feed value chain high aflatoxin contamination and less fumonisin contamination was documented in samples collected. In this study, the averaged fumonisin contamination was well below the guidance level by the U.S. Food and Drug Administration (USFDA) of 5 mg/kg in maize and maize by-products intended for equids and rabbits.

However, more than 85% of dairy feed samples exceeded the aflatoxin standard of 5 µg/kg for aflatoxin B1 established by Rwanda Standards Board (RSB) standard for feed ingredients. It confirms the concern over aflatoxin contamination in a few feed samples collected from different vendors in Kigali (Rwanda) during a previous study (Nishimwe, Wanjuki, Karangwa, Darnell, & Harvey, 2017). Lack of knowledge and awareness about aflatoxin contamination in grain and feed samples remains a concern. Aflatoxin and fumonisin contamination in animal feeds were also reported in different East African countries (Kang'Ethe & Lang'A, 2009; Mohammed et al., 2016; Nyangi et al., 2016;

Senerwa, 2016). Year-round surveillance and creation of mycotoxin awareness through communication and education along the feed value chain are needed for mitigating mycotoxin contamination in feed value chain of Rwanda.

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Development of sensitive polyclonal antibodies against dominant stored wheat grain fungus for its immunological detection

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Abstract

Fungal infestation causes deterioration of stored food grains. Most fungal species produce secondary metabolites like aflatoxins which are highly toxic to animals and humans. *Aspergillus flavus* has been found to be the predominant contaminant in stored wheat grains collected from the godowns of Food Corporation of India, West Bengal. The present study focuses on the development of sensitive polyclonal antibodies (PAbs) for molecular immunological detection of dominant toxigenic fungus. Pure *A. flavus* isolate was cultured on

coconut agar media and its spores were harvested and inactivated by 4% formaldehyde. The inactivated spores were injected into a rabbit along with Freund's complete/incomplete adjuvant for the development of PAbs. Specificity of the raised antibodies in rabbit serum was examined by enzyme-linked immunosorbent assay (ELISA) using spore proteins as antigen obtained by bead beating method. Out of several proteins (ranging from 10 to 200 kDa present in spore, only two prominent proteins of around 76 kDa and 100 kDa were detected by western blot analysis using raised polyclonal antiserum. The PAbs were purified with protein A column followed by spore proteins conjugated CNBr activated sepharose column for its use in the detection of fungal antigens. This highly purified raised antibody can be used for the development of rapid, sensitive, and accurate techniques (such as dot blot/ELISA) for the detection of toxigenic fungi present in stored wheat grains.

Keywords: Spore Protein, Polyclonal Antibody, Fungus Detection

Introduction

In India, wheat is the third most produced and consumed cereal grain. It has economic importance as a staple food all over the world. It is a seasonal crop and hence needs to be stored safely as buffer stock for year-round consumption. It is estimated that around 30% of the total grain produced in the country is supplied to government storage house like food corporation of India (FCI) for keeping as buffer stock. Sometimes improper storage and handling can cause huge economical loss. Estimated loss of staple food grains during storage due to different biotic and abiotic factors varies widely. It may account to 10% worldwide but can reach 50% in tropical regions (Magan et al., 2007). The biotic factors of storage loss mainly include fungal infestation. Fungal infestation may lead to loss of seed germination capacity, viability, decoloration, foul smell and change in nutritional value (Birck et al., 2006). The most common fungal species causing grain spoilage in storage are *Eurotium* and *Aspergillus* (Tournas et al., 2018). *Aspergillus* species like *A. flavus* are particularly important because they are able to colonise even at very low moisture content on a range of food matrices, resulting in spoilage, and produce varied group of mycotoxins which may lead to refusal of stored food grains (Sohbatzadeh et al., 2016; Aldars-García et al.,2018). *Aspergillus flavus* can also cause aspergillosis in immune-compromised individuals (Amaike and Keller, 2011). Therefore, determination of the mycological contamination of stored grains is a very important issue as it is destined to be used as food and animal feeds. During the last decade, several direct methods like dilution plating, measurement of volatile compounds, evaluation of ergosterol or chitin level and next gen sequencing (NGS), and indirect methods like randomly amplified polymorphic DNA (RAPD) analysis, amplified ribosomal DNA restriction analysis (ARDRA) and denaturing gradient gel electrophoresis (DGGE) have been developed for the detection of fungi. However, numerous disadvantages are associated with these techniques. The direct methods are time consuming, labor intensive, and require mycological expertise; and are not completely accurate (Gourama and Bullerman, 1995). In indirect methods: it is impossible to distinguish among species or to yield quantitative data (Darling and Blum, 2007). As a consequence, there is a clear and urgent need to develop a new reliable method that will be highly specific, relatively rapid, inexpensive, and replicative. Immunological and molecular techniques offer such an alternative. The immunological method is based on antigen and antibody (either monoclonal /MAb/ or polyclonal /PAb/) interaction. Different sources of fungal material like surface washes from the growth medium, mycelial homogenate and extracellular polysaccharide have been used as antigens for the production of antibody but no study has been done by directly using fungal spore. In current study, we have isolated *A. flavus* fungus from stored wheat grain, developed polyclonal antibodies by directly using inactivated *A. flavus* spore and specificity of antibodies was analysed using ELISA and western blot method.

Materials and Methods

Isolation and characterization of *A. flavus* fungus from stored wheat grain

Stored wheat grains were collected from Food Corporation of India (FCI) godown of West Bengal. The grains were plated on aspergillus differentiation agar (AFPA) media for isolation of *A. flavus*. The fungal isolate was identified by analysing morphological characteristic of colony and sequencing

internal transcribed spacer (ITS) region of ribosomal DNA gene. The ITS region was amplified using universal (ITS1 and ITS4) primer set by PCR, then cloned in pTZ57R/T vector (Fermentas Life Sciences, EU) and sequenced in DNA sequencer (ABI 3500, USA). Homology search of nucleotide sequence was performed against the Genbank database for identication of fungal species.

Spores collection and inactivation

The identified *A. flavus* isolate was cultured on coconut agar media [100ml coconut milk and 2% agar] for 7 days at 28°C. The spores were collected in 1xPBS containing 0.1% tween 80. It was then inactivated by keeping in 4% formaldehyde solution, for 5 days at 4°C and followed by several wash with PBS. The inactivated spores were cultured on PDA media for 7 days at 28°C for analysis of inactivation efficiency. The spore density was calculated on haemocytometer under microscope.

Generation of polycolonal antiserum against inactivated spore

Polyclonal antiserum against inactivated *A. flavus* spore proteins was raised by immunization of rabbit (New Zealand) using the standard 6-month antibody production protocol. 10^8 spore per injection in Freund's complete adjuvant was subcutaneously injected for the first time and then in Freund's incomplete adjuvant for subsequent injections (at an interval of four weeks). Blood was collected 10 days after every injection. Sera were harvested by centrifugation at 3000 g for 15 minutes after allowing blood to clot for one hour at 37°C. Sera were stored at −20°C till use. Immunization and blood harvest protocols were followed as per IAEC guideline approval.

Preparation of immunoglobulin fraction from rabbit serum

Sera were first subjected to overnight precipitation at 4°C with ammonium sulphate at 50% saturation and then centrifugation at 5000g for 30 minutes at 4°C. The pellet was resuspended in appropriate volume in binding buffer (0.1 M Tris-Cl pH 8.0) and dialysed using 10 kDa cut off membrane against 1 L of binding buffer for 16 hours at 4°C to remove ammonium sulphate.

Protein-A affinity chromatography

Immunoglobulin rich fraction obtained after dialysis with binding buffer was loaded into a column containing protein A sepharose. Non-specifically bound contaminants were removed by washing with 5 column volumes of binding buffer and then bound IgG were eluted in 1 mL fractions with elution buffer (0.1 M Tris-glycine pH 2.4) into 1.5 mL tubes containing 200 µL of 1 M Tris-Cl pH 9.0 for neutralization. Fractions containing the IgG were checked by measuring OD at 280 nm, then pooled together and concentrated by using centicon YM-50.

Extraction of *A. flavus* spore protein

Spores proteins were extracted by bead beating method (Jenkinson et al., 1981). In brief, about 10^{10} spores were homogenised in 1 mL of extraction buffer [20 mM Tris-Cl (pH 8.0), 1 mM EDTA (pH 8.0) and 2% SDS] with 0.3 mg of glass bead (300 nm size), and then lysed in bead beater for 5 min and then incubated for 5 min in boiling water. Spores debris was pelleted by centrifugation, and the supernatant were analysed in 10% SDS PAGE.

Purification of anti spore protein specific immunoglobulin

Aspergillus flavus spore protein lysate was coupled to cyanogen bromide (CNBr) activated sepharose 4B (GE healthcare) as per manufacturer's protocol. In brief, CNBr activated sepharose CL 4B freeze dried powder (0.5 g) was suspended and subsequently washed with 150 mL of 1 mM HCl. The isolated spore proteins were dialyed in buffer (0.1 M $NaHCO_3$, pH 8.3 with 0.5 M NaCl). 5 mL of dialyed proteins (10 mg/mL) was coupled to activate CNBr sepharose beads by keeping on rotor for overnight at 4°C. The entire material was then transferred to a small glass column, washed and remaining active sites on the beads were blocked by suspending beads in 0.1 M Tris-Cl buffer (pH

8.3) for 2 h at room temperature. The loosely bound antigen was removed by washing with three cycles of alternating pH; each cycle consisting of a wash with acetate buffer (0.1 M, pH 4.0) containing 0.5 M NaCl, followed by wash with Tris-Cl buffer (0.1 M, pH 8.0) containing 0.5 M NaCl. The cupling of antigen in the bead was checked by analyzing an aliquot of antigen coupled beads on 10% SDS-PAGE. Prior to affinity purification, the column was equilibrated with binding buffer (0.1 M Tris-Cl, pH 8.0). Protein A purified immunoglobulin fraction was loaded into the column and unbound antibodies were allowed to pass through and the column was washed with binding buffer until the A 280 of the flow through became negligible. The specific antibody boundto column was eluted with 0.1 M glycine, pH 2.4 and fractions (1ml) were collected in microfuge tubes containing 200 µL of 1 M Tris-Cl pH 9.0 for neutralization. The fractions with A 280 greater than 0.2 (measured using Nanodrop) were pooled together and concentrated by passing through centricon YM- 50 (Millipore) and was checked by 10% SDS PAGE.

Western blot analysis

Western blot analysis was carried out based on protocol suggested by Mahmood and Yang, (2012). *Aspergillus flavus* spore protein lysate was run on 10% SDS PAGE. The proteins were blotted onto nitrocellulose membrane using Mini Trans-Blot ® Cell (Biorad) operated at 20 V for 14 h at room temperature. After the transfer, membrane was blocked with blocking buffer (1XPBS containing 0.05% Tween-20 (PBST) and 3% BSA) for 3 h at room temperature. Membrane was then washed three times using PBST and treated with anti *A. flavus* spore proteins specific affinity purified antibody 1 µg/mL (1:500 dilution) in PBST containing 1% BSA for 3 h at room temperature. Following three times wash with wash buffer; secondary reagent (Protein-A HRP conjugate, GE lifesciences) was added at 1:3000 dilutions in PBST containing 1% BSA and incubated for 2 h at room temperature. After three times wash with wash buffer; colour was developed using Opti-4CN substrate (Biorad) until signal with sufficient intensity was obtained. Reaction was stopped by rinsing the membrane thoroughly with deionised water and then photographed.

Enzyme linked immunosorbent assay (ELISA)

Wells of 96 well ELISA plates (Tarsons, U bottom well) were coated with 100 µL/mL of antigen (spore protein lysate) suspended in coating buffer (0.1 M bicarbonate buffer pH 9.6) for overnight at 4°C. Wells were washed thrice with PBST to remove unbound antigen. Wells were then blocked with 200 µL of blocking buffer (PBST with 3% BSA) for 3 h at room temperature. Wells were washed thrice with washing buffer and 100 µl of 1 µg/mL anti *A. flavus* spore proteins specific affinity purified antibody (1:500 dilutions) in PBST containing 1% BSA was added and incubated at room temperature for 2 h. Following three times wash with wash buffer, 100 µL each of secondary reagents (Protein-A HRP conjugate, GE lifesciences) was added at 1:1000 dilutions and incubated at room temperature for 2 h. Following washing, colour was developed using 100 µL of ABTS substrate (Sigma) along with 1 µL of 30% H_2O_2. Absorbance of the wells was read at 415 nm using microplate ELISA reader (Multiskan spectrum, Thermoscientific).

Results

Identification *A. flavus* fungus

Aspergillus differentiation agar (AFPA) media is a selective media for the enumeration and identification of *A. flavus* and *A. parasiticus* fungus *(*Pitt et al., 1983). The morphological analysis of *A. flavus* showed orange-yellow reverse colony pigmentation on *AFPA* media, colony appeared with globose to sub-globose vesicles and spore showed biseriate seriations, which is a characteristic colony features of *A. flavus* species as described by Klich (2002) (Fig.1). Further, ITS sequence analysis showed 100% query coverage and identity with the repoted ITS sequence for *A. flavus* and confirming its correct identification.

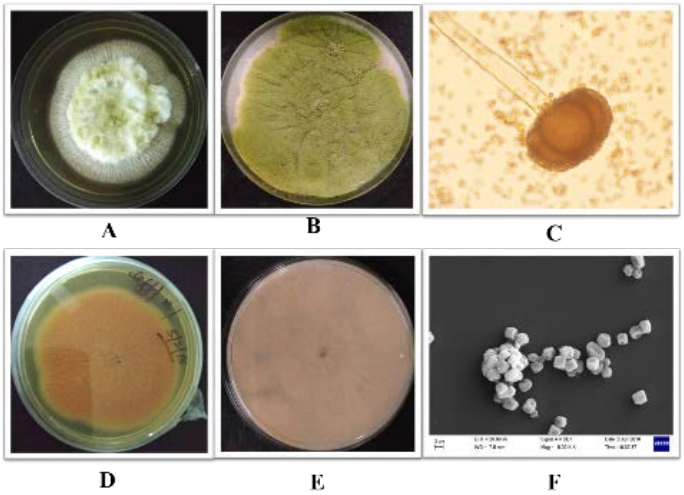

Fig.1. Analysis of *A. flavus* colony characteristics on culture media. A & D' front and reverse view on AFPA. B & E, front and reverse view on coconut media. C, microscopic characteristics of conidial ornamentation on hyphae. F, Spore morphology analysis in SEM.

Purification of anti spore protein Immunoglobulin

SDS PAGE analysis of Immunoglobulin protein fractions obtain after purification through protein A affinity column and through antigen affinity (spore protein) conjugated CNBr column also showed two sharp bands at 50 kDa and 25 kDa corresponding to the heavy and light chains of Immunoglobulin respectively indicating the successful purification of anti spore protein immunolo antibodies (Fig.2). The crude serum protein percipited using 50% ammonium sulphate solution. It showed smear of protein band through out the lane which indicated precipitation of several other proteins along with immunoglobulin. Futher purification through potein A column discarded other proteins and allowed purification of immunoglobulin proteins. The eluted immunoglobulin fraction was passed though the antigen conjugated CNBr activated sepharose column and purification of anti spore proteins antibodies was done.

Analysis of anti spore specific antibody for detection of A. *flavus*

The lysate of spore proteins run on 10% SDS PAGE showed several protein bands ranging from 10 kDa to 200 kDa (Fig.3A). The same amount of proteins was run in 10% SDS PAGE and trans bloted on nitrocellulose membrane. The blot showed two prominent proteins bands of around 76 kDa and 100 kDa (Fig.3B.).

Detection of *A. flavus* fungus by ELISA

The sensitivity of antibody for detection of spore protein was determined by indirect ELISA method. The protein concentration ranging form 10 µg /mL to 200 µg /mL in carbonte buffer were used for detection of sensitivity of antibody. The minimal detectable concentration of antigen was 20 µg /mL.

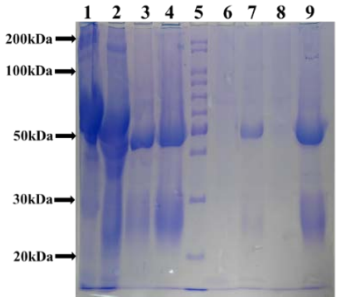

Fig.2. SDS PAGE analysis of IgG purifed from protein A sepharose column. Lane 1, Serum proteins; lane 2, Ammonium sulphate precipitated IgG; lane 3, IgG elute from column; lane 4, Concentrated IgG; lane 5, Protein marker; lane 6, filtrate collected from 50 kDa centricon; lane 7, antigen affinity purified concentrated IgG; lane 8, filtrate collected from 50 kDa centricon of affinity purified IgG; lane 9, unbound IgG from antigen affinty column.

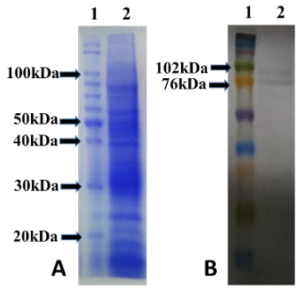

Fig.3. Western blot of *A. flavus* spore protein. (A) comassiae blue stained gel, lane 1, protein ladder, lane 2, spore protein lysate. (B) corresponding western blot profiles of lanes 1-2.

Discussion

India falls in tropical region, where fungal infestation is widely prevelent in pre and post harvested crops. It is estimated that approximately 25–50% of harvested food and feed gets contaminated with mycotoxins (Abdin et al., 2010). In present study, *A. flavus* species was isolated from stored wheat grains, and characterized by morphological and molecular method. Zulkifli and Zakaria, (2017) have suggested that to identify a fungus at species level, both morphological and molecular identification should be applied because for some species, morphological characteristics may be similar. Internal transcribed spacer (ITS) region of ribosomal gene is a universal barcode for molecular identification of fungal species (Pryce et al., 2003). Combination of morphological identification and sequencing of ITS can reliably identify Aspergillus isolates to species level.

Aspergillus flavus can produce aflatoxins, which is a toxic and carcinogenic secondary metabolite, and their contamination in food grains can adversly affect its quality and usability (Sun et al., 2016). Fungus produces sexual/asexual spores, in order to survive for long term in adverse physiological and environmental conditions. It is a key attribute for fungal reproduction, persistence, and dispersal. Therefore, spore would be a suitable antigen for immunological detection of fungal contaminants in stored grains. Polyclonal antibodies were raised against whole inactivated *A. flavus* spore in rabbit and purified through protein-A column followed by antigen affinity chromatography. Resulting antibody showed high specificity against *A. flavus* fungus as seen from the results of western blot where strong positive reaction of antibodies against two spore proteins were visualized. Further, these antibodies were again tested for sensitive ELISA for detection of *A. flavus*. These polyclonal antibodies can be used for the development of a specific and sensitive technique like dot blot ELISA for monitoring fungal contamination level in stored grains.

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Smallholder farmers' perceptions of aflatoxins in maize in kamuli district, Uganda

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Extended Abstract

Keywords: postharvest, harmful effects, mold, outreach, education

Aflatoxins are a family of highly toxic and carcinogenic compounds produced by fungi commonly found on maize. Aflatoxins have been estimated to be widespread in maize in Africa (Wagacha and Muthomi, 2008). Consumption of aflatoxins in foods is associated with liver cancer in adults and stunted growth and development in children. Studies have shown that over 50% of maize in Ugandan markets contain over 10 ppb aflatoxins, the safety limit set by Uganda National Bureau of Standards. Present in maize fields, these fungi (molds) continue to grow on maize when it is insufficiently dried and then stored, thereby increasing aflatoxin levels. Maize is an important staple crop for smallholder farmers. They often have difficulty properly drying and storing maize and thus face the risk of aflatoxin consumption. Previous published studies revealed that many smallholder farmers do not know what aflatoxin is nor the risks they face from it (e.g., Magembe et al., 2016).

Iowa State University Uganda Program (ISU-UP) works in smallholder farmer communities in the Kamuli district of Uganda to improve peoples' health, nutrition and rural livelihoods (www.csrl.cals.iastate.edu). It operates eight Nutrition Education Centers (NECs) where pregnant women and mothers of nutritionally challenged children within their first 1000 days of life can

receive nutritious meals. Mothers are instructed about nutrition and health issues and how to grow and serve nutritious food to their families. Once their children are no longer at nutritional risk, they "graduate" from the program and no longer receive assistance. Nearly all these women or their spouses/partners are smallholder farmers. Additionally, ISU-UP works with other smallholder farmers in the community to improve their livelihood through livestock and improved agricultural practices. ISU-UP will be starting outreach programs in postharvest techniques and practices, providing education in how to reduce postharvest losses and protect themselves against spoilage and the risk of aflatoxin.

This study sought to understand the perceptions and knowledge of smallholder farmers in the Kamuli District of Uganda about their postharvest practices and specifically about aflatoxin to establish a baseline for evaluating future postharvest outreach programs.

During the summer of 2017, 109 face-to-face interviews were conducted with smallholder farmers in the Kamuli District of Uganda with the use of an interpreter. Interviewees were chosen by stratified sampling methods and interviewees shared the characteristic of growing maize. 60 smallholder farmers were chosen at random and 49 were members of an ISU-UP Nutrition Education Center. At least ten farmers were interviewed in each of seven parishes in the Butansi and Namasagali sub-counties. 102 interviews resulted in complete data for analyses. The demographic distribution of the interviewees is shown in Table 1.

Interviewees were asked 37 open-ended questions that took an average of 30 minutes. Responses were transcribed into English. Transcripts were analyzed using grounded theory methodology to identify emergent themes in farmer perception and knowledge of aflatoxins, maize drying, and maize storage practices. Statistical analysis of associations between demographic characteristics and response frequencies used the chi-square test of independence with statistical significance declared at $p < 0.05$.

Table 1. Number of study interviewees by category (n = 102)

Gender	**Female**	**Male**		
	83	19		
Age Group	**18 to 29**	**30 to 39**	**40 to 49**	**50 and over**
	29	30	18	25
Education	**None**	**Primary School**	**Secondary School**	
	23	56	23	
NEC Membership	**Current**	**Graduate**	**None**	
	31	14	57	

Table 2 summarizes the results of the survey relevant to interviewees knowledge of aflatoxin. There were no significant differences in responses due to gender, age, education, or NEC membership for the questions "have you heard of the word aflatoxin," and "are you aware of aflatoxin contamination in crops." Nearly all participants had heard of aflatoxin before, which is different than previous published studies that showed smallholder farmers generally unaware of aflatoxin. When asked if they were "aware of harmful effects of aflatoxin in humans" there was a significant difference between male and female responses – all males said they were aware and only 71% of the females said they were aware. There were no differences in responses due to age, education of NEC membership.

Table 2. Interviewees awareness of aflatoxin

Question	**Female (n=83)**	**Male (n=19)**
Have you heard of the word aflatoxin?	Yes: 92%	Yes: 100%
Are you aware of harmful effects of aflatoxin in humans?	Yes: 71%	Yes: 100%

When asked what the harmful effects on humans were, it was difficult for farmers to correctly articulate those effects (Table 3). There were several misperceptions about the effect of mycotoxins

on human health. While 36% correctly identified "disease," they could not describe the kind of disease. Only 1% identified cancer.

Table 3. Harmful effects perceived by those aware of harmful effects in humans (n=78).

Disease	Stomach Ache	Don't Know	Diarrhea	Bad Smell	Malaria	Loss of Taste	Cancer
36%	25%	8%	6%	4%	3%	2%	1%

The farmers in this study were also asked about postharvest practices that might affect their exposure to aflatoxin. Although the visual presence or absence of mold does not determine the presence or absence of aflatoxin, moldy maize has a higher probability of the presence of aflatoxin. When asked if they "check for moldy maize before feeding to your family," 79% indicated yes and 21% indicated no, with no significant difference between gender, age, education or NEC membership. When asked what they do with moldy maize, a variety of responses were obtained (Table 4). Many of these actions do not reduce the risk of exposure of people or animals to aflatoxin.

Table 4. What interviewees that check for moldy maize do with it when they find it (n=96)

Discard	Animal Feed	Human Food	Mill it	Sell it	Dry Before Use	Blend w/ non-moldy maize
44%	18%	15%	9%	7%	5%	2%

92 % of the farmers indicated that they try to avoid moldy maize. These farmers were asked what they do to avoid moldy maize (Table 5). Most farmers said they repeatedly dried the maize.

Table 5. Practices used to avoid mold in maize (n=94)

Repeat Drying	Nothing	Avoid long-term storage	Dry before storage	Add Red Pepper
71%	11%	10%	2%	1%

From these results, it is clear that there were numerous misunderstandings about aflatoxin, its effect on humans and practices that could be adopted to limit the risk of exposure to aflatoxin. Women smallholder farmers were significantly less aware (only 71%) that aflatoxin had harmful effects on humans, yet they are often the ones that are feeding their families. There exists a clear and important need to educate smallholder farmers in the Kamuli District of Uganda about the dangers of aflatoxin and the postharvest measures that could be taken to prevent exposure. These results provide further impetus for ISU-UP to implement outreach education programs. The Nutrition Education Centers are a good first place to start. Future surveys will follow up with these interviewees to determine the effectiveness of these programs.

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The mycoflora of bulk stored cocoa

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In modern times, agricultural commodities are handled in ever-growing volumes. Nowadays not only cereals but further soft commodities like raw coffee and cocoa are transported as bulk cargo in containers or directly stowed into a ship's hold and stored in silos or bulk stores. This change of environmental conditions impacts upon the development of stored product pests. A typically encountered implication is the incidental occurence en masse of fungivorous beetle species, which becomes especially conspiciuous when the lot is moved. At that point, the initial area of infestation is hardly traceable.

The mycoflora of bulk stored cocoa in the Port of Hamburg was investigated from winter 2013/2014 till late autumn 2014. The survey was conducted as part of a research project, which aimed at developing a integrated concept for protection of bulk stored commodities and was funded by the State Ministry of Economic Affairs, Transport and Innovation of the City of Hamburg. It was shown that growth of mycotoxin producing Aspergilli and other spoilage causing fungal species is not only a problem of storage in the producing countries. The specific characteristics of bulk stored commodities can form a variety of different microhabitats within a lot. Furthermore, frequently found species like Aspergillus ruber can act as a door opener for more fastidious fungi and insects.

Borderline Cases between Biocidal Products Regulation and Plant Protection Products Regulation

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Extended abstract

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Introduction - background

Legislation on the Single Market for goods aims to ensure that products placed on the EU market meet high health, safety and environmental requirements and that products allowed to be sold in the EU can circulate without barriers to trade, and with a minimum of administrative burden.

Plant protection products and biocidal products are used by professionals and non-professionals on harmful organisms, to destroy, deter or render them harmless. Before they can be placed on the market, the authorities are responsible among others for assessing the effectiveness of these products and the risks associated with their use.

Plant protection products are 'pesticides' that protect crops or desirable or useful plants. They will primarily be used in the agricultural sector but also in forestry, horticulture, amenity areas and in home gardens. Relevant function from a fumigation company point of view: protect plants or plant products against pests after harvest.

Regulation (EC) No 1107/2009 is the legislation concerning the placing of plant protection products (PPPs) on the market in the European Union.

Biocidal products used to control unwanted organisms that are harmful to human or animal health, or that cause damage to materials (e.g. dams and dikes). These harmful organisms include pests (e.g. insects, rats or mice) and microorganisms (e.g. moulds or bacteria).

Regulation (EC) No 528/2012 lays down the rules and procedures for authorization of biocidal products.

Both plant protection products and biocidal products contain at least one active substance. Before an active substance can be used within these kind of product in the European Union it must be officially approved.

Borderline cases

The determination of clear borderlines between the Plant Protection Products Regulation (EC) 1107/2009 and the Biocidal Products Regulation (EU) No 528/2012 is determined as a crucial issue for a proper implementation of both legislations. Sometimes difficulties may arise to decide which Directive applies to a given product and use.

The following criteria could be help to examine regarding the area of application:

- The intended purpose of the product.

- The target organism
  - If it is harmful to plant products, then the product wil be considered as a plant protection product.
  - If it is harmful to humans or material (e.g. dams and dikes), then the product can be considered as a biocidal product.
- The place where the product will be applied to achieve the principal intended action.
  - Example: Products applied to the soil before sowing or planting of plants, are intended to destroy plant pests, and should considered as plant protection products (e.g. soil fumigants).
  - In cases where products will be used for a general hygiene purpose (health protection), it is agreed, to consider these products as biocidal products (e.g. fumigants used in storage rooms for food).

Several documents to provide guidance to Member States on borderline cases are available. On the other hand, they are not legally binding since only the Court of Justice can give an authoritative interpretation of existing Community law. For instance, under the biocides legislation where the scope of the application is unclear, issues will be discussed at EU level.

In former times, when the EU Commission finalised decisions on scope issues, these were included in the Biocidal Manual of Decisions. Numerous borderline situations were settled in this document. Since 2015, this guidance provided in the Manual of Decision is obsolete. Now all information with regard to the submission process can be found on the European Chemicals Agency (ECHA) website (responsible for biocides).

Nevertheless, the Manual of Decision is still always a helpful tool for the industry to identify the correct regulatory scope. Again, this guidance is not law: it is not binding.

Practical example

Recently we had a question regarding the regulatory differentiation of the treatment of tobacco (storage protection, fumigation with phosphine-based products).

Status

In the beforehand mentioned biocidal Manual of Decision it was mentioned under 2.1.1.2.:

"According to the Guidance Document 2 on the borderline between biocides and plant protection products, products in the unprocessed state or having undergone only simple preparation such as milling, drying or pressing, derived from plants, but excluding plants themselves are plant products in the meaning of the Plant Protection Products Directive.

If the target organism is detrimental to plant or plant products then the product used is considered as a plant protection product either if applied directly on plants or plants products or applied indirectly on empty structures to control pests of plant or plants products exclusively."

Reversal conclusion would mean that in case further processing steps are required it falls under the scope of the Biocides Regulation (EC) No 528/2012.

Tobacco

All tobacco are different, especially when it comes to curing the leaves. At first harvesting tobacco must be dried and cured. For instance, Virginia tobacco will dried by `flue-curing` where the leaves are hung for four to seven days in a drying barn and cured with warm air from a system of pipes. This drying process fixes the characteristic orange-yellow colour.

After tobacco cured, it will moved from the curing barn into a storage area for processing (e.g. involving several weeks of fermentation). The curing and fermentation processes establish the quality differences between tobaccos. While `drying` may seem like a basic process, the end result is open to infinite variety, reflecting the weather and nutrients in the soil during growing, individual skill and expertise, as well as the type of drying process used.

Conclusions

Due to the fact there are definitely several steps within this treatment needed, it should fall into the above-mentioned Biocide Regulation. For fumigation purposes, only biocide products for product type 18 should be used, which have an approval.

Official feedback given by the German Authority BAuA (Federal Institute for Occupational Safety and Health):

"The storage protection of processed tobacco and tobacco products falls into the regulatory scope of the Regulation (EC) No 528/2012 and it needs a biocide approval. "Processed tobacco" correlates to tobacco after the pass of fermentation, which is in our opinion no simple pass."

ECHA confirmed this opinion too:

"The German competent authority has the possibility to consult with the other national competent authorities and/or to raise formally the issue to the European Commission by requesting a decision according to Article 3 (3) of the Biocidal Products Regulation. The fact that the German competent authority has not made a request for an Article 3 (3) decision indicate that they are confident in the validity of the answer they have provided you with."

Future prospects

However, a same product can be used in several situations and fall under both legislations. Dual authorizations would mean two dossiers, two contacts with different rapporteur Member States respectively evaluating Competent Authorities and two fees. Objective should be a better coordination at EU level and experience sharing between the Member States within the European Union.

A distinct answer by the legislator is required, under which regulatory scope the registration process should be started.

We need a pragmatic solution for the fumigation industry to reduce regulatory workload and costs.

To avoid any confusion the industry would welcome a solution again, consisting of a document or database compiling all the answers relating to possible borderline cases. We need clear regulatory guidance.

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Customer complaints about insect contaminated ready meals

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Abstract

More than one-hundred food complaints about ready meals, coming from mass catering, were analyzed from 2003 to 2017. Even if insects in meals have an enormous negative impact on customers, the percentage relevance, considering the long period and the number of meals served, is negligible. Coleoptera (34%) was the most represented order, followed by Lepidoptera (27%), and Diptera (23%). Coleoptera insects were mainly field

pests, found in salads and spinach, moths were represented by species infesting vegetables (58%) and by stored product pests (42%). Species of hygienic concern were found in Diptera. Few cases of cockroach contamination were reported on different food, but it is important to underline their presence in the meal, as it indicates a heavy environmental infestation and a high hygienic risk.

Keywords: mass catering, food serving, canteen, stored product pests, field pests.

1. Introduction

In Italy, mass catering is worth 6.6 billion euros and each day more than 5 million citizens lunch in schools, hospitals, and companies canteens (Anonymous, 2016). In 2016, Italian mass catering companies amount to 3117 (FIPE, 2017) and provide lunch formed by one portion of pasta or rice, one portion of meat, eggs or fish with vegetables, a piece of bread and fruit.

Complaints about ready meals from customers concern sanitary and hygienic aspects, including insects (Balzaretti and Locatelli, 1993). Insects that contaminate processed food can originate in crops, food industries, and stores (Trematerra and Fleurat-Lessard, 2015). The presence of insect in food repulses consumers and moreover, pests can cause hygienic problems, e.g. cockroaches, domestic flies, and rodents can contaminate food with pathogens (Gorham, 1991). Furthermore, the detection of pests in meals served to children in school canteens is often reported on TV and local press.

Complaints about insects in ready meals served in companies and school canteens, and delivered to Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano over the last years, were analyzed.

2. Materials and Methods

Contaminated ready meals, coming from mass catering, were analyzed in the entomological laboratory of University of Milan from 2003 to 2017. Samples delivered by companies following customer complaints amount to 107. The insects were cooked with the food and most of the times the specimens were damaged and lacked features important for identification to species.

3. Results

The insects present in the contaminated samples were mainly Coleoptera (33.6%), followed by Lepidoptera (27.1%), and Diptera (23.4%). Coleoptera were principally field pests, found in salads and spinach, moths were represented by species infesting vegetables (57.7%) and by stored product pests (42.3%).

In table 1, complaints are grouped according to the environment infested. Almost half complaints concern field pests, stored product pests correspond to 23.4%. Occasional pests are predators or parasitoid insects, that are not directly linked to the food. In sanitary concern insects, flies and cockroaches are grouped. In one school canteen, a student found a head-louse in one serving of pasta. Evidently, it came not from the kitchen, where workers wear caps, but from a student's head, as in the primary schools, head-louse problems are seasonally common.

Tab. 1 Percentage of insects detected in ready meals, grouped according to the colonized environment (n=107).

Origin of the insect	Percentage (%)
Field	49.5
Stored product	23.4
Occasional	7.5
Sanitary concern	19.6
Total	100

All food courses were contaminated by insects, but vegetables constitute 40.2% complaints (table 2). Coleoptera adults and moth larvae were detected in salad and spinach, as they can hide among the leaves. Larva of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was recorded in a tomato

salad. In pasta and rice, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) larvae were mainly found; in a few cases, adults of *Sitophilus oryzae* (L.) (Coleoptera: Dryophthoridae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) were detected.

Tab. 2 Percentage of courses were insects were detected (n=107).

Food type	Percentage (%)
Bread	10.3
Pasta or rice	28.0
Meat, fish	19.6
Vegetables	40.2
Fruit	1.9
Total	100

In pasta with vegetables, field pests were recorded, e.g. wireworm and noctuids larvae. One adult of *Fannia canicularis* (L.) (Diptera: Faniidae) contaminated one serving of pasta with tomato.

In portions of cooked meat or fish, the main contaminants are flesh-flies or blowflies adults. Only in two cases, field pests were found, coming from the vegetables used in the recipe. An adult of *L. serricorne* was detected in one serving of meat cooked with spices. Larvae of *Piophila casei* (L.) (Diptera: Piophilidae) were present in ham plates.

Contaminants of bread belong to different categories. Only three contaminants are flour pests, in three cases insects are of hygienic concern, that is, adults of *Blatta orientalis* L. (Dictyoptera: Blattidae) and *F. canicularis*. Hymenoptera also were recorded, e.g. a parasitoid, an ant and one head of *Polistes* sp. (Hymenoptera: Apocrita: Vespidae).

Only in two cases complaints concerned fruit serving: an adult fruit fly in a fruit salad and one *Periplaneta americana* (L.) (Dictyoptera: Blattidae) adult found by the consumer picking the fruit from a crate.

4. Discussion

Even if the detection of extraneous materials in food rarely happens, this fact causes significant loss of revenue and image to the companies involved. Among foreign matter reported in food, insects are considered one of the most important problems (Lewis, 1993; Edwards and Stringer, 2007). In the cases we reported, insects were detected only in one portion, not in the daily production, and the number of samples, considering the long period of time is negligible.

Insects that contaminate processed food can originate in crops, food industries, and stores (Trematerra and Fleurat-Lessard, 2015, Limonta et al., 2016). The presence of insect in food repulses consumers and moreover, the presence of pests can cause hygienic problems, e.g. cockroaches, domestic flies, and rodents can contaminate food with pathogens (Macovei et al., 2008; Sulaiman et al., 2011; Pava-Ripoll et al., 2012; Wasala et al., 2013). Insects of hygienic concern in this work are mainly represented by Diptera, but also few cases of cockroach contamination were reported on different food. It is important to underline their presence in the meal, as it indicates a heavy environmental infestation and the possibility that good sanitation practices were not strictly followed.

The presence of field insects in vegetables, in particular, if the insect hides inside or in folded leaves, can easily go undected if there is only one specimen. The matter is different when all the servings are contaminated, as it means that the crop wasn't correctly managed and the kitchen assistants didn't properly control and wash the vegetables.

Staff training is essential, as a careful examination of ingredients and of meals by kitchen assistants that serve food will greatly limit the risk of contamination. The adoption of good practices and regular monitoring is mandatory to guarantee safe meals.

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Moulds infesting local and imported rice (*Oryza* spp) in Cameroon

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Abstract

Loss in quality and quantities of rice during storage is an important issue to focus on. Moulds contaminating rice were investigated and their injuries on rice during storage were evaluated. Local and imported rice samples sold in markets and mills were stored for 3 months under laboratory conditions. The contaminated grains were counted and analyzed to characterize storage moulds.

All rice samples evaluated were contaminated by moulds, right from sampling date. The quantity of mouldy grains varied from 1.1% for the rice sample from UNVDA to 4.2% rice brand 'Main dans la Main'. The highest mould infestation in terms of quality and quantity, was recorded on imported rice samples of world rice and 'Main dans la Main' 22.3 and 25.3% respectively; meanwhile 'Tox 3145 parboiled', Uncle Benz and Neima presented 7.5, 8.9 and8.9% respectively.

In general, imported rice samples contained the highest fungal load with a proportion of 65.9% compared to 34.3% for local samples. Among the 67 isolated strains, the genus *Aspergillus* dominated, followed by *Penicillium, Mucor* and *Circinella* with 13.4, 8.9, and 4.4% respectively. Therefore in Cameroon, some locally produced, but mostly some imported rice contain moulds from different genera, which damage rice at different proportions. It is urgent to develop methods to inhibit the growth of potential storage moulds and preserve the quality of rice consumed.

Keys words: Rice, contamination, storage, loss, quality.

1. Introduction

Rice (*Oryza*spp) is the third most widely cultivated cereal in the world after maize and wheat and with an estimate production of 430, 865 and 695mt respectively (FAOSTAT, 2012). Rice constitutes a staple food for half of the world's population. Today, rice is a commodity of strategic significance across many African countries (Hegde and Hegde, 2013), driven by changing food preference in the urban and rural areas and compounded by increased urbanization (Khalil *et al.,* 2009).

About 90% of world's rice is produced in Asia (Food and Agricultural Organization [FAO], 2015. To satisfy their increasing demand with a low production, African countries, particularly west and central Africa import large quantities of rice from Asia (Seck*et al*., 2010; Otsuka and Kijima 2010).

The physical quality of rice is determined by biophysical factors such as the agro-ecological zone of production and production system and by the production practices (Mapiemfu-Lamare 2017). Rice storage problems are very often caused by inadequacies during prior phases, particularly inadequate harvesting and drying (Saunders *et al*., 1978; Barnabas *et al*., 2008; Bala*et al*., 2010). Also, poor rice parboiling can lead to loss at storage (Diop*et al*., 1997; Fofana*et al*., 2011; Ogunbiyi, 2011; Ndindeng *et al*., 2014; 2015). Improper rice storage can lead to both quantitative and qualitative losses caused by pests, insects, rodents, sprouting, discoloration or contamination of grains with unwanted materials or substances. A large amount of rice is lost during storage, 1 - 100% of the total harvest (Hall, 1970; Schulten, 1975; Hopf*et al.,* 1976; Adams and Harman, 1977; De Padua, 1977; FAO and UNECA, 1977; Green, 1977; Harris and Lindblad, 1977; Mushi, 1978; Ren-Yong *et al*., 1990; IRRI, 1997; Appiah *et al*., 2011).

Hermetic storage in airtight bags significantly improves storage by protecting rice from rodents, insects and fungal infestation, but are not currently used in local mills or markets (Jones *et al*., 2011; Gitonga*et al*., 2013).

Grain losses may occur in storage due to moisture losses, rodents, insect infestation and fungal growth and subsequent price discounts for damaged grain (Kaminski and Christiaensen 2014; Kadjo*et al*., 2015; Kadjo*et al*., 2016). In addition, rice sold in markets or mills being it imported or locally produced are usually packaged in jute or plastic bags of 5, 10, 25 or 50 kg stored in inappropriate conditions; open to insects and rodents, high humidity etc. leading to the development of moulds.

This work studies moulds in order to estimate loss of the quality of rice grains locally produced and imported, during storage.

2. Material and Methods

2.1 Rice Samples

Plant material was rice grains: paddy grains or milled rice. Rice samples were collected from mills in Ndop rice development hub (RDH) in Cameroon and in the Mokolo market in Yaounde (a major urban consumption zone and the political capital of Cameroon). RDHs are zones (rice ecologies) where rice research outputs will be integrated across the rice value-chain to achieve the desired development outcomes and impact (Africa Rice, 2011). These samples were paddy and milled rice, imported from Thailand or locally produced and sold in Ndop hub (Table 1).

Rice samples were stored at room temperatures at the Institute of Agricultural Research for Development (IRAD) Yaoundé for 10 weeks. Data on physical quality were evaluated as follows.

The moisture content of rice grains was determined using a Satake Rice Moisture meter (Satake Co. Ltd., Tokyo, Japan) according to manufacturer's instructions and expressed as a percentage. The determination was done in triplicates.

Discolored rice grains were evaluated using a sample of 100 g of rice. Rice grains presenting any yellow, black or purple color, visualized under magnifying glass were manually selected from the normal grains and weighed. The evaluation was done every two weeks till the 10th week of storage

to observe the evolution of discolored rice grains. Discolored rice grains were expressed as a percentage. The rice was held at an average relative humidity and temperature of 80% and 26°C respectively for 10 weeks, and samples taken weekly.

Table 1. Rice samples with respect to their origin and type

Rice variety or brand name	Origin	Type of rice
Main dans la Main (Mm)	Thailand	Milled w hite
Word rice (Wr)	Thailand	Milled w hite
Neima (Ne)	Thailand	Milled w hite
Uncle benz (Ub)	Thailand	Milled parboiled
Nerica L 56 (NE)	Ndop hub-Cameroon	Paddy
Parboiled paddy (Pp)	Ndop hub-Cameroon	Parboiled paddy
Tox 3145 (Tx)	Ndop hub-Cameroon	Paddy
Tox 3145 parboiled (Tx p)	Ndop hub-Cameroon	Parboiled paddy
Bamunka (Ba)	Ndop hub-Cameroon	Milled w hite
Jéhovah (Jé)	Ndop hub-Cameroon	Milled w hite
UNVDA (UN)	Ndop hub-Cameroon	Milled w hite
Ndop rice (Nd)	Ndop hub-Cameroon	Milled w hite

2.2. Characterization of Moulds

To isolate and obtain pure strains, PotatoDextrose Agar and Malt Extract Agar media were used as most of strains grow on semi-solid media.

The Ulster method which is direct and more indicative for the analysis of mould on food was used to detect, isolate and analyze mould present on the rice samples. In the Petri dishes containing moistened filter paper, 20 particles (discolored grains of rice) suspected to be contaminated were placed. The Petri dishes are then placed in a hermetically sealed plastic container in the dark and ventilated for 12 hours. The observation of the strains was carried out only after 5 to 7 days of incubation.

The transfer of strains was done under the laminar flow hood and consisted of aseptically transferring the isolated strain into new PDA and MEA culture media to perform the pure culture. Three (3) repetitions were performed for each strain. From the Petri dish containing the isolated strain, 0.6 cm of mycelium disk is transferred into new dishes containing the culture media. This operation is performed several times in order to obtain pure cultures. The sampling was preferably performed at the growth end of colonies. The Petri dishes were then placed at laboratory temperature (24-28 ° C).

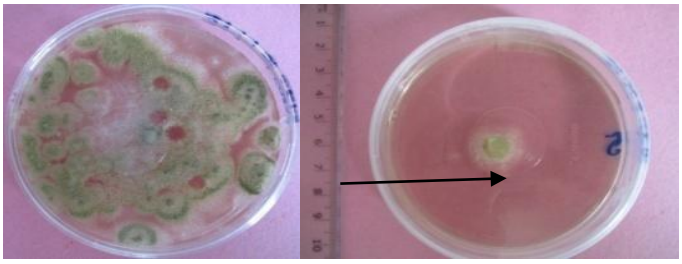

Fig. 1. Obtaining pure strain: a = isolated strain; b = pure strain (Douksouna photos, 2014)

The growth radius of mycelium was evaluated daily (48 hours after incubation) at the same hour. Each diameter is respectively measured on one of the two straight lines forming a right angle passing through the center of the explant (Fig. 2). The following formula was used to calculate the average growth radius.

$$D = \frac{d1 + d2}{2} - d0 \qquad \text{(Singh et } \textit{al.}\text{, 1993)}$$

Where d0 is the diameter of the initial explant; d1 and d2 are the diameters of culture measured in both perpendicular directions.

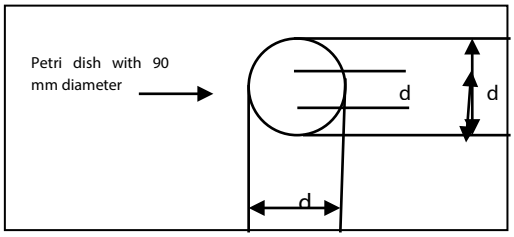

Fig. 2. Principle of measuring the radius growth of mycelium in Petri dish.
d0= diameter of the explant (0.7cm); d1and
d2=diameter perpendicular to pathogens.

The identification of many fungal species that can colonize food and alter the quality or even produce mycotoxins is an essential step in the evaluation of mycotoxic risk. A morphological identification was made based on the macroscopic characters (color, colony aspect, colony relief and the back of the boxes). The observation of the color and texture of the colony on the culture media as well as the microscopic structures made it possible to characterize the genera.

For all cultures obtained after 7 days, an identification key « Toxic Fungi in Food » allowed the characterization of moulds based on the technique of Pitt *et al*., (1997) according to the following characteristics:

- diameters of macroscopic colonies, measured in centimeter on the bottom of the box to evaluate growth;
- characters of colony, the appearance of the colony was observed by the naked eye under day light and in the presence or absence of a diffuse pigment to determine the colors of colonies.

Fungi were examined under the microscope as wet smears. To prepare a wet smear, a needle or an inoculation loop were used to collect a small portion of the colony with conidiogenous structures. The inoculum was taken from the edge of the colony because the fertile structures are young and the number of spores are acceptable. In addition, the structures that can enclose the spores have been taken near the center of the colony where the probability of finding mature spores is greatest.

The sample cut on a slide was first "wet" with a drop of ethanol 70% and a coverslip was laid, the excess liquid was blotted and followed by examination under microscope which is the microscopic study of the nature of differentiated organs. The observation was made at the objective 10 x and 20 x.

2.3. Data Analysis

The data obtained for all the parameters studied, were automated with the Excel software and the analysis were carried out by SPSS version 16.0. The different averages were compared at 5% significance level using Duncan's Multiple Range test.

3. Results and Discussion

3.1. Variation of Moisture Content of Rice Grains

There was a variation in moisture content among rice samples. In general, the level of moisture content was above 13% and could reach 15.6% at sampling. Moisture content (MC) is the weight of water contained in rice expressed in percentage. Moisture content is usually referred to the wet basis, meaning the total weight of grain including the water (IRRI, 2012). Results revealed a high moisture content at sampling date for imported milled rice samples sold at the Mokolo market: Uncle benz, Neima, World rice and Main dans la Main (Fig. 3); suggesting that these rice brands may

be poorly imported and stored by rice traders. Ten weeks after storage at room temperature, the moisture content of all rice samples (except for Tox 3145 whose moisture content was 15.6% at sampling date and remained the same after storage) showed a linear increase; suggesting that storing rice grains at room temperature allows them to re-absorb moisture. On the other hand, moisture content increased only around one unit or even less for rice samples Nerica L56, Tox 3145, Main dans la Main (Fig. 3). These rice samples already had high moisture content at sampling date, around 14%, suggesting that once the moisture content of rice has dropped upon drying, it can increase by some units, but cannot easily increase above 17%. The rice grain is hygroscopic and responds dynamically and physically to moisture and temperature changes in the environment. A dry grain surface re-absorb moisture in a humid environment, while a wet surface desorbs moisture in a relatively dry environment. Moisture adsorption is associated with water reentering the grain. This occurs when the vapor pressure at the surface of a grain is lower than the vapor pressure in the surrounding air (Lan and Kunze, 1996).

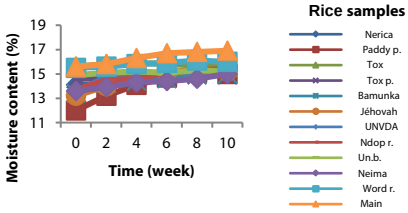

Fig. 3. Variation of moisture content of rice samples as a function of time

3.2. Discolored Rice Grains

Results revealed the presence of discolored rice grains in all the rice samples at the beginning of the experiment. The number of these discolored rice grains increased with time ($p < 0.05$), although at different frequencies within the different rice samples evaluated (Table 2).

All the four imported rice brands showed high number of discolored grains as compared with locally produced rice. Neima, Uncle benz, world rice and Main dans la Main which are the milled rice brands largely found in local markets in Cameroon like the Mokolo market where the sampling was done appeared to have highest number of discolored grains. This suggests that if these rice brands are stored at room temperature and humidity, there will be a large number of discolored grains, which are likely mouldy grain. These imported rice samples showed an increase of their moisture content during storage, meaning that when exposed to the ambient temperature and humidity, rice grains re-absorb moisture, creating favorable conditions for mould development, leading to discolored grains.

The rice brand which had the highest number of discolored grains was Main dans la Main at the beginning of the experimentand after 10 weeks storage. From my personal observations, this rice brand has a poorer appearance in the market and furthermore, its price is always lower than other imported rice brands sold in the market.

Table 2. Evolution of discolored rice grains in imported and local rice with respect to time

Time (week)	Rice brand											
	Nerica L56	Parboiled paddy	Tox 3145	Tox 3145 parboiled	Bamunka	Jéhovah	UNVDA	Ndop rice	Uncle benz	Neima	World rice	Main dans la Main
0	21a	25a	29a	36a	09a	11a	11a	17a	29a	19a	75a	117a
2	30a	49a	44a	107b	14a	14a	17a	25a	60a	46a	146b	170b
4	41a	53a	53a	163c	41ab	41ab	41a	31a	151b	57ab	203c	231c
6	53ab	63ab	59a	167c	45ab	43ab	43ab	38a	225c	68ab	235d	372d
8	57ab	67ab	63ab	173c	46ab	49ab	46ab	42a	231c	74ab	243d	385d
10	61ab	78ab	81ab	178c	49ab	57ab	49ab	53ab	237c	87ab	257d	389d

Means in a column with the same letter are not significantly different at $p < 0.05$ (Duncan's Multiple Range test).

The evaluation of discolored grains in terms of percentage showed that the mean total percentage of discolored rice grains 10 weeks after storage was high in imported rice brand and inTox 3145 parboiled (Fig. 4). The level of discolored rice was equal or higher than 3% in these rice samples, meaning that theycannot be graded premium or grade 1 rice, according to the Quality Standard for milled rice in the Philippines for instance.

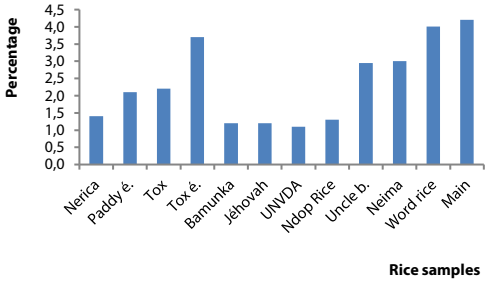

Fig. 4. Percentage of discolored rice grains in per rice brand or samples 10 weeks after storage

Percentages with the same letter are not significantly different at $p < 0.05$ (Duncan's Multiple Range test).

3.3. Characterization of moulds

3.3.1. Description of the isolated strains

The analysis of rice mycoflora revealed several genera distinguished by their morphological characters (Pitt et al., 1997).

The genus *Aspergillus* with seven species isolated: *Aspergillus niger*, *Aspergillus flavus, Apergillus ochraceus, Aspergillus fumigatus, Aspergillus oryzae, Aspergillus japonicas, Aspergillus parasiticus*. The genus *Penicillium,* the genus *Mucor* and the genus *Circinella.*

3.3.2. Characterization of moulds

Isolated colonies were keyed to species using Pitt *et al*., (1997). Results showed different ($p < 0.05$) growth of fungi according to their virulence. The radius growth of all the fungi increased with time from the second day to the fifth day, this at different growth rate (Table 3). The most virulent fungus was *Mucor* sp, whose growth radius was rapid and reached 7.4 cm at day five, followed by *Circinella* sp, *Aspergillus parasiticus* and *A. orchraceus* whose growth was comparable. The slowest growth radius was observed with *A. niger* (Table 3).

Table 3. Radius growth (cm) rate of fungi

An: Aspergillus niger; Af: Aspergillus flavus; Aj: Aspergillus japonicus; Afu: Aspergillus fumigatus; P: Penicillium; Aor: Aspergillus orchraceus;C:Circinella; Ap: Aspergillus parasiticus; M: Mucor.

Time (day)	Fungi									
	An	Ao	Af	Aj	Afu	P	Aor	C	Ap	M
2	2.5c 1	1.9a 1	3.0de 1	2.5c 1	1.3a 1	2.9de 1	2.9de 1	3.0de 1	3.0de 1	3.4f ***1***
3	2.8a 1	2.5a 1	4.0c 12	3.6b 1	2.3a 1	4.0c 1	4.1c 1	4.1c 1	4.2c 1	4.7d ***1***
4	3.1a 1	3.2a 1	5.0cd 2	4.7b 1	3.0a 1	5.4de 2	5.3d 2	5.3d 1	5.4e 1	6.0f ***2***
5	3.4a 1	4.3b 2	6.0de 23	5.8c 2	3.7b 1	6.2de 2	6.5f 2	7.0f 2	6.6f 2	7.4g ***2***

Figures on a line with the same letter are not significantly different at $p < 0.05$; figures in bold and italic represent the comparison in column (Duncan's Multiple Range test).

3.4. Virulence of strains on rice samples

Mycological analysis showed that imported rice samples of world rice and Main dans la Main presented the highest fungal load (Table 4). No strain was isolated on Nerica L56. On world rice and

Main dans la Main rice samples, were isolated respectively eight and five different strains. This result corroborates previous ones obtained in the course of this study, where the rice brands world rice and Main dans la Main presented high moisture content and discolored grains; suggesting that rice grains with high moisture content and discolored grains probably possessed a high fungi load.

Table 4. Identified fungi per rice sample

Fungi	Rice sample												
	NE	Pa	Tx	Tx. P	Ba	Jé	UN	Nd	Ub	Ne	Wr	Mm	
A. flavus	-	-	-	-	x	-	x	-	-	-	x	x	
A.fumigatus	-	-	-	-	-	-	-	-	-	-	x	x	
A. japonicus	-	-	-	-	-	-	-	x	-	-	x	x	
A.niger	-	x	-	-	-	x	-	-	-	x	-	-	
A. ochraceus	-	-	-	-	-	-	-	-	x	-	x	-	
A. oryzae	-	-	-	-	-	-	-	x	-	-	x	x	
A. parasiticus	-	-	-	x	-	-	x	-	-	-	x	x	
Penicillium sp	-	-	-	x	x	-	-	x	x	-	-	-	
Mucor sp	-	-	x	-	-	-	-	-	x	x	-	-	
Circinella sp	-	x	-	-	-	-	-	-	-	-	x	-	
Others	-	-	-	x	-	x	-	-	x	x	x	-	
Total Number (67)	-		2	1	3	2	2	2	3	4	3	8	5

- Means absence; x stands for presence. Main dans la Main (Mm), Word rice (Wr), Neima (Ne), Unclebenz (Ub), Nerica L 56 (NE), Parboiled paddy (Pa), Tox non étuvé (Tx), Tox parboiled (Tx. P), Bamunka (Ba), Jéhovah (Jé), UNVDA (UN), Ndoprice (Nd).

In terms of percentage, the genus *Aspergillus* dominates with a total of 63.8% as compared to the other strains. The genus *Penicillium*, *Mucor*and *Circinella* presented respectively 13.4, 8.9 and 4.4% (Fig. 15).

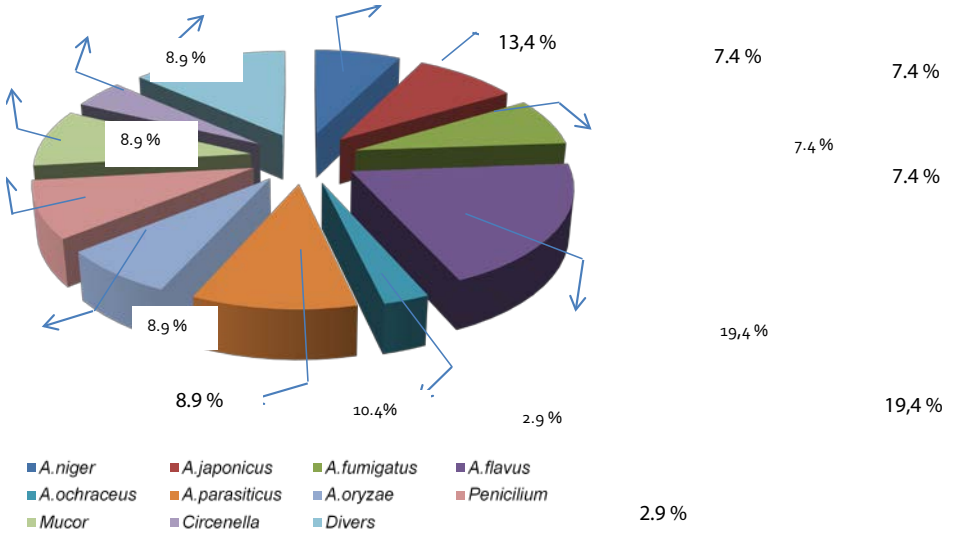

Fig. 5. Proportion (in percentage) of isolated strains infecting rice samples in Cameroon

4. Conclusions

This study evaluated some rice samples in Yaounde (Mokolo market) and Ndop rice development hub, which are respectively the main market of the political capital of Cameroon (Yaounde) and a rice-production division. There were imported milled rice brands sold at the Mokolo market and paddy or milled rice brands produced in Ndop.

It was found that the different rice samples were contaminated by moulds; certainly because of their high level of moisture content. The fungal charge was high in imported rice sample brands as compared to locally produced rice, suggesting that these rice samples may be imported or stored and sold in poor conditions, leading to the development of moulds.

In addition, isolated mycoflora was diverse. Four genera were found: *Aspergillus*, *Penicillium, Mucor*and *Circinella. Aspergillus* species dominate followed by *Penicillium* and *Mucor*.

Further studies can take into consideration all imported rice brands and rice samples produced locally and sold in local markets in Cameroon and assess the presence of toxin like aflatoxin in the rice grains.

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Reduction of fungi and mycotoxin decontamination by ozone gas treatment in three stored rice (*Oryza sativa* L.) varieties

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Abstract

The present work brings together different rice varieties (black, brown and white) evaluated for their differences/ susceptibilities/resistance to ozone (O3) gas treatment for safer storage (mycological and toxicological contamination control). The three rice varieties were separated into two Groups –Control (GC) andtreated Groups (GT) which had O3 gas applied (5 L/min, 40 ppm and 60 min for gas flow). Samples were collected during the storage period to check for the O3 gas effect on fungi reduction (total count and fungi genera identification) and so for the humidity parameters of moisture content (mc) and water activity (aw). It was possible to verify the effectiveness of the O3 application in the samples when compared to Control. It was observed that even at the shortest time of gas exposure, O3 application caused changes to fungi (both growth speed & toxin formation). The grains did not change their organoleptic, physical and biochemical characteristics after O3 application. Recent studies from our Labmico Group indicated that the O3 application in addition to prevention of the biological contaminants, as reported in the current work, also reduces an insecticide (deltamethrin) residues. As O3 treated grain has reduced fungi contamination and toxicity of rice grains in all the varieties studied, it can be considered a potential agent to control fungi spoilage and so for toxigenic strains. Considering that there is a growing concern on the use of agrochemicals and their harmful effects on human health and the environment, O3 application can be a promising to implement decontaminationof highly consumed grains worldwide, such as rice.

Keywords: rice, fungi, storage, ozone, mycotoxins.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most consumed cereals in the world, with 90% of its production coming from Asian countries. Outside of Asia, Brazil is the greatest producer of rice. Asian countries' rice consumption varies from 100 to150 kg/person/year (Sosbai, 2014). There are varieties that express different nutritional characteristics having impacts on the human health. Several consumers around the world prefer rice with translucent appearance, with intact and uniform grains (Castro, et al, 1999). In relation to the grain type, the market indicates a rice consumption migration from type 2 to 1 and parboiled rice in Brazil (Botelho et al, 2010). However, new trends in the food market favor the consumption of other varieties, since the increasingly consumer demand for food-health integration (Aziz et al, 2002).

Due to the demand for quality, it is important toemphasizethe post-harvestinsect protection management control and what they carry during storage: toxigenic fungi (Hoeltz, 2005; Soares et al, 2018). The presence and action of fungi affect the grains physical structures, compromising their quality & safety with deterioration, loss of nutrients and toxins production (Kreibich et al, 2016). Mycotoxins are secondary metabolites, whichare toxic substances able to affect humans and animals, and may be mutagenic, teratogenic or carcinogenic effects (Hoeltz, 2009).

Food contamination can pose risks to consumer health. Grains, such as rice are affected greatly by the presence of fungi and mycotoxins (Hining, 2011). In the field, contamination is influenced by environmental conditions such as air humidity, incomplete drying, product humidity, rainfall at harvest time, insects, soil fungus loads, air and plant health (Fonseca, 2008). The constant movement of insects within an ecosystem contributes to the dispersion of fungal spores, which are carried on the body surface (Bidochka 1997; Saint, 1984; Soares et al, 2018). Water (coming from inside the food or even from the external environment), is the medium that favors microorganisms growth (Pitt, 2009). The main pests present in the grains during storage are beetles and moths. Among the beetles are *Rhyzopertha dominica* F. and *Sitophilus oryzae* L. are some of the most damaging species in rice. Regarding moths, the main ones are *Sitotroga cerealella* and *Ephestia kuehniella* (Lorini, 2010).

Under favorable conditions, fungi develop rapidly during the cultivation, harvesting, transport and storage processes. However, the storage corresponds the main stage in which the grain is susceptible to those types of contamination. (Scussel, 2018). Among the mycotoxins that most affect rice cultivars are aflatoxins, ochratoxin A, zearalenone, citrinin and fumonisins and their main toxigenic fungi are the *Aspergillus, Penicillium* and *Fusarium* species, and the same fungus can produce several different toxins (Aziz and Moussa, 2002).

There are several methods of storage to prevent/control the fungi proliferation, among them temperature reduction and also technologies known as green methods, such as the application of ozone (O_3) gas (Kim and Dave, 1999).O_3 is a colorless gas with a pungentodor, unstable and partly water-soluble, and has high oxidizing power. Within 15 min in contact with the air, it ends up oxidizing and turning O_2. It does not generate residues and is a strong disinfectant agent with action on a great variety of pathogenic organisms, including bacteria, viruses and protozoa. (Botelho da Silva, et al, 2011; Savi et al, 2015). In addition, it is internationally recognized as a GRAS - generally recognized as insurance (Piacentini, 2015; Christ et. al, 2016, 2017).

This work evaluated the susceptibility of three rice varieties to fungi and mycotoxins decontamination byO_3 gas.

2. Materials and Methods

2.1 Materials

Samples: Three rice varieties of (a.1) black, (a.2) brown and (a.3) white with initial mc: 14.41, 13.86 and 13.61%, and aw: 0.060, 0.580 and 0.520, respectively).

Culture media and reagents: Potato dextrose agar (PDA) from Himedia (Curitiba, Parana, Brazil) and chloramphenicol were obtained from Vetec (Duque de Caxias, RJ, Brazil).

Equipment: autoclave, Phoenix (Araraquara, SP, Brazil); microwave oven, Philco (Sao Paulo, SP, Brazil); tweezers, Prolab (São Paulo, SP, Brazil);caliper, Digimatic (Mitutoyo, Tokyo, Japan); drying oven, Olidef-cz (Ribeirao Preto, SP, Brazil); aw meter, Aqua- Lab4TE, Decagon (Sao Jose dos Campos, SP, Brazil), Peagameter, Model Schott-gerate CG 818 (Schott,, Mainz, Germany); laminar flow cabinet, Veco (Campinas, SP, Brazil); fume cabinet, Quimis (Diadema, SP, Brazil); rotary shaker, Marconi (Piracicaba, SP, Brazil) and microbiological incubator, Quimis (Diadema, SP, Brazil); colonies counter, Phoenix (Araraquara, SP, Brazil); sieve system, mesh (2-1mm) Beffer (Caieiras, SP, Brazil); Microscopes - light (LM), CH-Bl45-2, Olympus (Shinjuku, Tokyo, Japan); O_3 gas generator, OP-35-5L, Interozone (Jundiaí, SP, Brazil), thermohigrometer, J-prolab (São José dos Pinhais, PR, Brazil), stereo microscope (SM), Opticam (SP, Brazil).

2.2 Methods

Sample collection: rice varieties were collected (1 kg) in October, 2017, by the Vegetal sanitary defense of Santa Catarina Integrated Development Agricultural company.

O3 application: The storage silos were produced with polyvinyl chloride tubes containing only two openings: one for the O3 gas inlet and one for the O3 gas outlet (25 X 10 cm diameter capacity).The three rice varieties were separated into two Groups –Control (GC) and treated Groups (GT).After grains (300 g)of each rice variety were loaded (50 g) into the O3 chambers, the generated O3 gas was pumped (through the inlet entrance) into the vessels by a compressor (equipped with a filter to prevent the entry of moisture), at continuous flow rate (5 L min-1) (Savi et al, 2016). The gas concentration applied was of 40 ppm for 60 min (Soares et al, 2018). At the end of the O3 gas exposure itseffect on fungi growths (GT) was evaluated and compared to the GC.

Humidity: MC and aw measurement where prior and after O_3 treatment. Each sample (2 g) was submitted (n=2) to drying in oven (105°C+/-5°C) up to constant weight by the gravimetric method of AOAC (2005). To determine a_w, each sample (2 g) was subjected to analysis (n = 2) using the Aqua Lab equipment, 25°C (AOAC 2005).

Mycology: The GT (O_3) and GC (no O_3 treated) rice grains were incubated (5 grains each) on PDA culture medium at 25°C+/1for 7 days. At day 3 and 5 after incubation, the colonies were observed and had their genera identified. After 7 days of incubation, the most representative fungi colony of each plate was evaluated for genus identification, both by analyzing their reverse under ultra violet light and analysis of their hyphae and conidia by SM (x60 and x100) (Ganley, 2006). Identification of fungi: The genera and the species identifications were performed according to Pitt and Hocking (2009).The colony morphology was evaluated by SM analyzes (Scussel et al, 2014).

3. Results

Humidity and fungi: It is known that the mc and a_w are important humidity factors for the development of fungi. Table 1 shows very close values among the varieties evaluated. The variety with the highest mc value had also the highest $a_w$value. Data also reveals that among the three varieties studied, the difference between nutrient availability and other factors in grain composition stands out in relation to a_w difference, since the grain (White) with lower mc (13.61%) &a_w (0.520) is the one that presents more different colonies and also more toxigenic colonies based on the fluorescence in its reverse and genera identification.

The incubated grains did not have their surfaces sterilized, thus providing favorable conditions for epiphytic fungi. These fungi may have a mutualistic relationship (absorbing nutrients and providing defense to the grain) or commensal, where it only removes nutrients (da Silva, 2006). Germination ceased at the time the colonies began to expand through the Petri dish.

Tab. 1 Humidity, fungi genera isolated fluorescence and from different rice (*Oryza sativa*) varieties

Rice Variety	Humidity		Predominant Fungi	
	mc	a_w	Genera	Fluorescence
Black	14.41	0.608	*Aspergillus*	✓
Brown	13.86	0.580	*Mucor*	ND
White	13.61	0.520	*Penicillium*	✓

mc: moisture content; a_w:water activity; ND: not detected; ✓: presence

Fungi and ozone: From the rice samples a.1 (black), a.2 (brown) and a.3 (white) of the image (Fig. 1), that were submitted to O_3 application,it was possible to observe a reduction offungi colonies formation on the grains (GT) when compared to Control (GC).

Mycoflora: By applying stereo microscopy, the reproductive structures of the fungi isolated from rice husk (tegument) surface and endosperm regions it was possible to visualize and identify different stages of reproductive structures of *Aspergillus* genera and also the species identified (*A . niger)* with their characteristic black color (Fig. 2).

Mycotoxins: In addition to the bromatological changes, the development of fungi can harm animal health and the people/workers handling the husk, due to the production of toxins, especially those related to the toxigenic fungi of the genus *Aspergillus*.

From the white rice strains isolated, where toxigenicity tests were applied, after 7 days incubation of the colonies isolated, it was possible to detect fluorescence production at the reverse of the culture medium under UV light - 365nm. That indicates possible presence of mycotoxins (aflatoxins) through the fluorescence compounds produced (Fig. 3).

Proximate composition: From the three rice samples evaluated, the Black rice had the highest lipid and protein contents (3 and 9.8 %) (Tab.2).

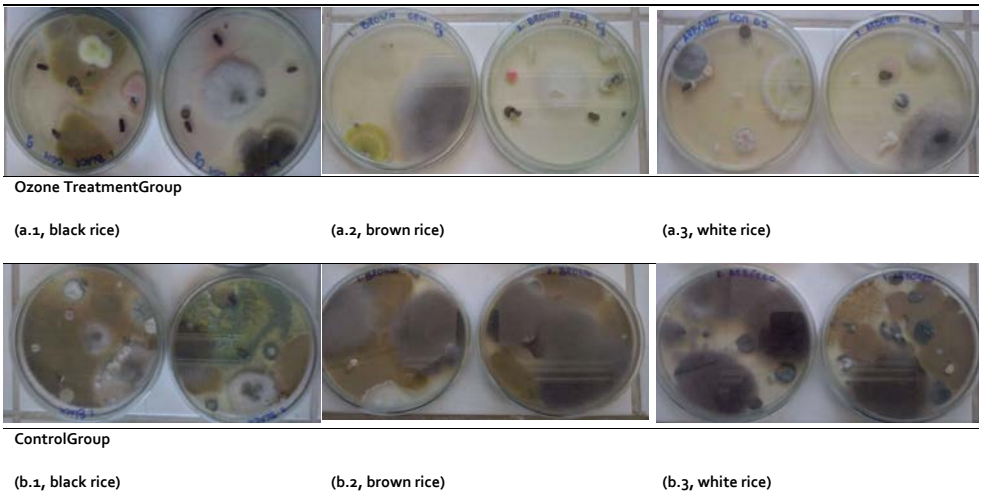

Fig.1 Fungi susceptibility to O_3 gas from three different rice (*Oryza sativa*) varieties: (a) ozone gas treated group and (b) control group.

Fig.2 Stereo micrographs of isolated fungi from rice (*Oryza sativa*) grains varieties (a, b) reproductive structures of *Aspergillus* and (c) species identified of *A. niger* [40, 100and 60x, respectively].

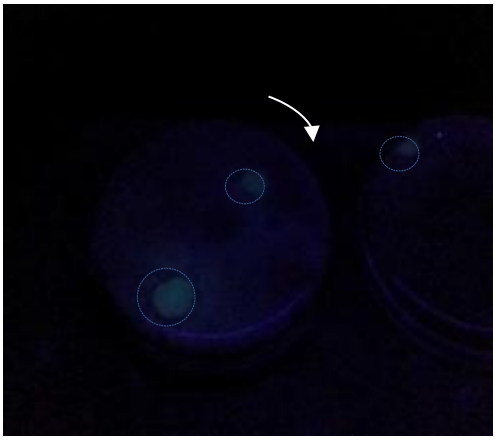

Fig.3 Fungi colonies fluorescence formation seen under ultraviolet light (λ: 365nm) of white rice (*Oryza sativa*) not ozone treated after 7 days of incubation (GC: no gas treatment).

Tab.2 Proximate composition of rice (*Oryza sativa*), varieties -black, brown and white.

Nutritionalvalues*	Proximate composition (per 100 g)	Ricecharacteristics
BLACK		
Fat	3	
Carbohydrates	67.9	
Fiber	4.2	
Protein	9.8	
Salt	0.002	
BROWN		
Fat	1.9	
Carbohydrates	77.5	
Fiber	4.8	
Protein	7.3	
Salt	ND	
WHITE		
Fat	1.3	
Carbohydrates	77	
Fiber	1.9	
Protein	6.9	
Salt	0.002	

*average ND: not detected

4. Discussion

Rice composition versus fungi: Regarding main composition rice presents the highest percentage ofstarch, followed by proteins andsmall amounts of lipids and fibers. In addition, the nutrients are not uniformly distributed in the different fractions of the grain. The (a) outer layers have higher concentrations of proteins, lipids, fibers and vitamins, while (b) the center is starch (Walter, 2008), so non-polishing causes the whole rice grain to have its starch mass protected by a layer of nutrients. Fungi have affinity for media with high concentration of starch and so the grains (Lorini, 2018). This explains the fact that black rice has been the least affected by fungi, its amount of vitamins and other components are higher than that of other varieties, likewise, white rice is polished and therefore facilitates access to fungi, which feed on this starch.After a systematic investigation of rice straw (Chen et al, 2015) verified that the mixed culture of *Trichoderma viride* and *A. niger* had a greater capacity of biodegradation when compared with the pure strains of *T. viride* and *A. niger*.

Ozone gas fungi development control: According to Botelho da Silva (2011), the application of O_3 is efficient however does not rule out the need for good storage conditions. It is noteworthy that due to the complexity of the processing and storage of these grains, O_3 does not dispense need to store under the proper humidity and temperature conditions. In addition, it was realized that even at short O_3 exposure time reduced fungi (only fewer colonies) during the incubation time.

Beber et al, 2015 reported a high reduction in total fungi, especially *Aspergillus* and *Penicillium* fungi genera. O_3 treatment applying concentrations of (10 - 40 mg/L) in silos was shown to be an effective green strategy to reduce the contamination of rice stored in the husk, maintaining safety during storage. Apparently the grains did not have their organoleptic characteristics affected by the application of O_3. Recent studies in our laboratory indicate that the application of O_3, in addition to preventing the presence of biological contaminants as shown in this work, also reduces an insecticide (deltamethrin) residues. (Savi et al, 2015).

5. Conclusions

As O_3 reduced the toxicity and contamination of rice grains in all varieties studied, a potential agent in the treatment of fungi is shown. There is a growing concern about the use of pesticides and their harmful effects on human health and the environment. Its application brings benefits in general and much lower degrees of contamination than the pesticides themselves. Combining its application and safety / control of the amount of gas, O_3 is promising the highly-consumed grain production chain around the world, such as rice.

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Safe Storage Guidelines for Soybeans at Different Temperatures and Moisture Contents

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Abstract

Poor storage capacity of soybean makes it prone to fungal spoilage and heating during storage, resulting in lower quality. Early prediction of the fungal spoilage in stored soybeans is very difficult because fungi are often too small to be seen with the naked eye. Here a new method for fungus to early detection is adopted: it is called counting fungal spores. Soybeans with moisture contents of 11.4, 12.1, 13.0, 13.9, 14.3 and 14.7%, were held at 6 temperatures 10, 15, 20, 25, 30 and 35℃ for180d. Samples were taken at regular intervals and the fungal spores counted. The safe storage conditions (temperature, moisture content, duration) were estimated by means of a curve fitted using the power function fitting. It can predict of soybean spoilage by fungus before there is visible damage.

Keywords: soybean, storage, fungal spoilage, early prediction, spores

1. Introduction

The tolerance of soybean to storage is poor, and the phenomenon of fungal spoilage and caking occurs easily. The storage of fungus is one of the main factors affecting the storage safety of soybean (Shelar and Shaikh, 2008). The study of soybean moisture, storage temperature and fungal growth is an important research direction to solve the early prediction of fungus damage in soybean storage. In recent decades, many reports on soybean storage fungi have been reported, in China and elsewhere in the world. Milner (1946) discovered that fungal infections could lead to a decline in soybean quality, and the increase of respiration and free fatty acids in soybean storage was mainly caused by the growth of harmful fungi. Kennedy (1964) conducted a survey of soybeans in five U.S. states, and found that the main growing fungus in soybeans was Aspergillus glaucus. Dorworth (1968) found that when the soybean moisture was 12.0 to 12.5% mc (moisture content), the storage fungus would slowly infect the soybean. As the moisture content increased, the infection rate increased gradually. Wilson (1993) showed that soybeans at10.5% mc can be stored at any temperature with no fungal growth. There are also some other related research reports about the safety storage and quality of soybeans (Hou et al., 2002; Wilson et al., 1995; Kong et al., 2009).

However, few studies have been reported on the early detection of fungal hazards in soybean storage.

Most of the grain storage fungi have aerobic growth characteristics. During grain storage, under suitable conditions, fungi begin to grow on the grain surface. This paper adopts a new method for early detection of grain storage fungi, which is counting fungal spores, and the prediction of the spoilage of stored soybeans by fungi. By studying the growth of fungi in soybeans with different moisture contents stored at different temperatures, the relationship between soybean moisture and temperature and initial growth time of fungi was preliminarily established, so as to provide safe storage guidelines for soybean storage.

2. Materials and Methods

Samples of soybean harvested from Heilongjiang Province

The soybean moisture was adjusted to 11.4, 11.4, 12.1, 13.0, 13.9, 14.3 and 14.7% respectively by the way of natural drying of water, or spraying water and holding at 4℃ about one month. Then the samples were packed in 1.0L bottle and kept in closed storage in a thermostat at different temperatures (10, 15, 20, 25, 30 and 35℃). Samples were taken every 10d. The moisture content was determined by oven method (105℃ for 3h). The growth of the fungus in the stored grain was determined by counting fungal spores.

Counting fungal spores

Ten g of soybeans were placed in 80 mL test tube, 30 mL of water added, stoppered, shaken for 1 min. The water was filtered through 60-80 um mesh filter cloth, and the filtrate siphoned into the count area of blood cell count board. Fungal spores were counted under a microscope at 600-800 times magnification. This method has been used for repeated experiments on wheat and rice samples with different levels of infection (n = 8), and the relative standard deviation (RSD%) range was 8.2 to 31.4% (Cheng et al. 2011). The fungal spore count correlates well with the plate colony plate counts, the correlation coefficient was $R^2 = 0.8479$ (Cheng et al., 2011). The method is based on the traditional cell counting method. By detecting the concentration limit of fungi spores ($1 \times 10^5.g^{-1}$), it eliminates the interference of fungal spores carried by the sample of no fungal growth, and achieves the purpose of only detecting fungal growth during storage. This method can detect fungal growth on the grain surface before it is seen with the naked eye. If fungi only grow a little, the growth of fungi can be detected presences of spores.

3. Results

The relationship between soybean moisture, storage temperature and the fungal growth was studied by regular sampling. The results showed that soybean storage was safe up to 11.5%mc. About 12.0%mc is the critical moisture for soybean fungus growth, and with the increase of moisture the growth of fungi will accelerate gradually. When the soybean is stored under 15℃, the low temperature inhibits the growth of fungi. Soybean stored over 20℃, might see fungi growth.

Most of the grain storage fungi have aerobic growth characteristics. During soybean storage, under suitable conditions, fungi begin to grow on the grain surface first. According to this feature, the method of counting fungal spores can detect the growth of fungi at the early stages before the infestation can be seen by the naked eye. This allows for early detection and warning of fungal growth.

The concentration of fungi spores 1 to $3 \times 10^5 g^{-1}$was determined as the initial growth limit, and the initial growth time of fungi was recorded by regular detection. The initial growth time was plotted with storage temperature and fitted using a power function curve. The predictive relationship between storage moisture and temperature and initial growth time of fungi was obtained (Fig. 1).

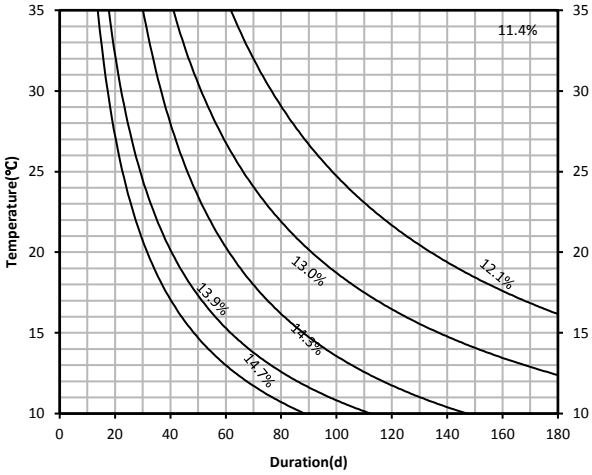

Fig.1 The conditions, temperature, time, and moisture content where there are1 to 3×10^5 g^{-1}fungal spores.

For a given moisture content with a given temperature the duration that soybean can bt safely stored is estimated. This curve is completed under isothermal conditions. Due to the large climate changes in the regional grain storages, the actual situation of local grain storage should be taken into consideration when using this curve.

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Evaluation of aflatoxin contamination of stored maize in the Brong-Ahafo region of Ghana

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Abstract

This study assessed the aflatoxin contamination and the presence of fungi in three maize varieties (*Obatanpa*, *Abontem* and *Aburohemaa*) stored using different storage methods namely storage in hermetic bags, woven polypropylene sacks and local crib in the Nkoranza-South district of the Brong-Ahafo region of Ghana. A factorial design arrangement was laid out in a randomized complete block design (RCBD). The isolation and identification of fungal pathogens associated with maize samples before and after storage were carried out on potato dextrose agar (PDA). Total flatoxin levels in the three maize varieties was determined by the use of enzyme-linked immunosorbent assay (ELISA) at 450 nm wavelength. Six fungi species were identified in the maize namely: *Aspergillus flavus, Penicillium* sp, *Fusarium* sp., *Lasiodiplodia theobromae*, *Colletotrichum gleosporioides* and *Rhizopus*. Before storage, *Abontem* variety recorded significantly higher ($p < 0.05$) total aflatoxin levels (113.56 ppb) compared to *Obatanpa* (2.91 ppb) and Aburohemaa (2.96 ppb). Maize samples stored in the polypropylene sack established significantly higher ($p < 0.05$) total aflatoxin levels of 82.9 ppb compared to hermetic bags (48.9 ppb) and local crib (48.9 ppb) after storage for six months. Aflatoxin levels under the interactive effect of variety and storage method was significant ($p < 0.05$). Overall storage of maize in hermetic bags significantly reduced aflatoxin levels hence the need to encourage maize farmers and traders to adopt hermetic bag storage technology.

Key words: aflatoxin, fungi, maize varieties, *Obatanpa*, *Abontem*, *Aburohemaa*, hermetic bag, polypropylene sack, local crib.

1. Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops grown globally, and it is the third after wheat and rice in total food grain production (Anupama *et al*., 2005). It has a very high adaptability and productivity hence it is produced in most countries of the world (Dlamini *et al*., 2012). Maize is a staple food for an estimated 50% of the population of sub-Saharan Africa (FAOSTAT, 2006). The crop is grown in all the six agro-ecological zones of Ghana and has a cultivated area of 1,023459 ha and an average yield of 1.72 tonne per hectare, making it the major cereal crop (MoFA-SRID, 2015). New varieties with improved quality have been developed in Ghana to increase output. Some improved maize varieties available in Ghana include *Abeleehe, Aburotia, Dobidi, Dorke, Kawanzie, Kwadaso local, Obatanpa, Okomasa, Mamab*a, *Abontem*, and *Aburohema* (Manga, 2010; Tweneboah-Koduah, 2013).

The quality of grain is usually assessed by its germination capacity, weight, microbial contamination, insect infestation and nutritional content. Grain quality is affected by temperature, moisture content, relative humidity, storage period, and several other biological factors (Jayas and White,

2003; Chattha *et al*., 2014). Fungal infestation is the major microbial contamination in stored grains which leads to the production of mycotoxins that subsequently reduces the quality of the grains. Mycotoxins are metabolites of certain fungi. They are toxic to humans and other animal groups even at very low concentrations and are frequently responsible for health-related problems in many countries (Morris *et al.,* 2001). The FAO estimates that, 25% of agricultural crops worldwide is contaminated by mycotoxins especially aflatoxins (Shekhar *et al.,* 2011). Aflatoxins are harmful toxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Omari and Amoah, 2015). It was first isolated in the early 1960s and found to be the most potent naturally formed carcinogenic compounds (Shekhar *et al.,* 2011). The types of aflatoxins produced by these species are B1, B2, G1 and G2. Generally, *A. parasiticus* strains produce B1, B2, G1 and G2 while *A. flavus* produces only B1 and B2 (CAST, 2003; Abbas *et al.,* 2006). The intake of higher doses of aflatoxin can cause acute aflatoxicosis, genotoxicity, hepatocellular carcinoma, suppression of immune system and impaired childhood growth (Fung and Clark, 2004).

Maize is a staple food of most Ghanaian communities and therefore its quality and quantity must not be compromised. Maize has the potential of becoming a non-traditional export commodity of Ghana considering its high productivity but unacceptable aflatoxin levels could be a huge setback. Aflatoxin contamination has been found to occur both at pre- and post-harvest stages of the production chain. Many studies have, however, shown that the bulk of the aflatoxin contamination in Ghana occur at the postharvest stages mainly due to improper food handling and storage practices (Omari and Amoah, 2015). In most developing countries such as Ghana, health issues related to aflatoxin contamination of food stuffs are more problematic since no proper food safety regulations have been established and/or rigorously enforced. The majority of maize produced in Ghana is either used for home consumption or sold in the local markets. Thus, the human health impact will be greatest if there is no monitoring and control mechanisms for aflatoxin contamination of food. For both food safety and economic reasons, there is the need to develop effective ways to mitigate the high and unacceptable levels of aflatoxins in food as it is becoming a serious public health and economic concern throughout the world.

Investigations on aflatoxin contamination of maize have been carried out in different parts of Ghana (Kpodo, 1996; Akrobortu, 2008; Amankwa, 2009; Tweneboah-Koduah, 2013). Most of the investigations focused on the type of storage methods that could be used for managing aflatoxin contamination of maize on cobs. However, most farmers and traders remove the grain from the cob before storage. Maize in this state is generally stored in hermetic bags, polypropylene sacks, jute sacks and mud silos. Tweneboah-Koduah (2013) reported lower concentrations of aflatoxins in *Golden crystal* i.e. yellow maize (19.9 pbb) compared to *Abasa* (24.5 pbb) and *Obatanpa* (27.9 pbb) maize varieties grown and stored in the Central Region of Ghana. To the best of our knowledge, no work has been done on the levels of aflatoxins in maize grown and stored by farmers and traders in the Nkoranza South District of the Brong-Ahafo Region of Ghana.

The Nkoranza-South District is one of the major producers of maize in Ghana. It is the highest maize-producing district in the Brong-Ahafo Region. The main maize varieties cultivated in this district include *Obatanpa*, *Abontem* and *Aburohemaa*. Communities in the district are also predominantly rural with high illiteracy rate, hence, most farmers and traders employ traditional storage methods for their maize. Some of the storage methods coupled with conducive climatic conditions of high rainfall and high temperatures in the region promote fungal growth and the production and accumulation of aflatoxins in maize. Since aflatoxin contamination cannot be assessed visually and also its effect is not immediate, farmers and traders are usually less concerned about it. Furthermore, maize from the district is distributed throughout Ghana hence, high mycotoxin levels in the grains will impact negatively on the human and animal health. Thus, it is very necessary to find ways to reduce aflatoxin levels in this commodity to acceptable levels. The objectives of the study were therefore to identify the presence of aflatoxin-producing fungi species in three maize varieties (*Obatanpa*, *Abontem* and *Aburohemaa*) stored using three storage methods (hermetic bag,

polypropylene sack and local crib), and assess the aflatoxin levels in the three maize varieties stored at ambient conditions under the three storage methods.

2. Materials and methods

Study site

The field work was conducted at four communities (Nkoranza, Bibiani, Akumsa Dumase and Brahoho) in the Nkoranza South District of the Brong-Ahafo Region of Ghana between September 2015 and April 2016. (Figure 1). Nkoranza South District is one of the twenty-two administrative districts in the Brong-Ahafo Region of Ghana. It is located in the middle portion of Brong-Ahafo Region. It lies within longitudes 1° 10'W and 1° 55'W and latitudes 7° 20'N and 7° 55'N covering a total area of about 920 km^2. The district has about 105 settlements, which are mostly rural. It shares boundaries with Nkoranza North District to the North, Techiman Municipality to the West, both in the Brong-Ahafo Region and Offinso North District to the South and Ejura-Sekyeredumase to the South-East in the Ashanti Region (MoFA, 2011). The district lies within the wet semi-equatorial region, having a mean annual rainfall level ranging between 800-1,200 mm. It has its major rainy season from March to June, experiencing her minor rains in September to November. The month of August experiences a short dry season, with the prolonged one in the months of December to March. The district has an average annual temperature of about 26 °C (MoFA, 2011). The dominant occupation of people in the district is agriculture, the proportion of which is about 82% of the district's labour force. It is one of the major producers of maize in the country.

Figure 1: Maps of Brong-Ahafo region (top image) of Ghana and Nkoranza South district (bottom image). Insert in top figure is the map of Ghana.

Experimental setup in the four communities

The experiment was conducted in four communites (Nkoranza, Bibiani, Akumsa Dumase and Brahoho) in the Nkoranza South district of the Brong-Ahafo region from September 2015- April 2016. Three varieties of maize namely *Aburohemaa* (AB), *Abontem* (AN) and *Obatanpa* (OB) commonly grown in the district and three storage methods namely Hermetic bags (HT), Local crib (LC) and Woven polypropylene sack or Poly sack (PS) that are commonly employed by farmers and traders in the district were used for the study. Each maize variety (25 kg) was collected after harvest and stored for six months in each of the storage systems in the four communities. Maize in hermetic bags and woven polypropylene sacks were stored in rooms. Relative humidity and temperature in the storage systems were measured at 30 min intervals using EL-USB LCD 2 thermo-hydrometer data loggers. To determine the impact of storage method on fungal growth and aflatoxin contamination, 1 kg of the maize samples were taken out o the 25 kg sample and analysed for aflatoxin levels and fungal growth prior to storage. The analysis was repeated after six months of storage. A factorial treatment arrangement was used for the study with storage methods (Hermetic, Local crib, Poly sack) and maize varieties (*Aburohemaa*, *Abontem*, *Obatanpa*) being the main factors. The treatment combinations were; (AB×HT, AB×LC, AB×PS, AN×HT, AN×LC, AN×PS, OB×HT, OB×LC, OB×PS). The factorial treatment combination was laid out in a randomized complete block design (RCBD) with the four communities serving as replications.

Isolation and identification of fungi associated with maize samples

Isolation of fungal pathogens associated with maize samples before and after six months storage was carried out on potato dextrose agar (PDA). The PDA was prepared by dissolving 3.9 g of the maize powder in 100 mL of distilled water in a 250 mL conical flask. The conical flask was shaken well together with its content to form a uniform solution. It was covered with aluminium foil and autoclaved at 1.05 kg/cm^2 pressure and 121 °C for 15 min. The PDA was poured into 9 cm petri dishes and allowed to cool. Surface sterilization was done to each sample in 1% w/v sodium hypochlorite for 30 sec and blotted dry with lint-free paper. Five sterilised grains were plated in each Petri dish and incubated at room temperature for five days to induce growth of fungi. Morphological identification of fungus associated with maize grains was done by scraping mycelia plugs advancing from the margins of the grains with a scalpel that is flamed. The mycelia plugs were mounted on slides for microscopic examination using distilled water. Compound microscope at low and high powers was used to examine the prepared slides. Identification of the isolates was based on colour,

morphology of mycelia, conidia and sporulating structures (Agrios, 2005; Barnett and Hunter, 2006). Micrographs were taken using a digital camera.

Determination of levels of total aflatoxins in maize

Enzyme-linked immunosorbent assay (ELISA) was carried out to determine the levels of total aflatoxin in the three maize varieties before and after storage. Celer AFLA ELISA Test Kits (Tecna S.r.l., Trieste, Italy) was used for the ELISA. The kit reagents include premixing microtitre plate (non-coated wells), microtiter plate (coated with anti-aflatoxin antibody), total aflatoxin standards (0, 2, 8, 30 and 80 ppb), enzyme conjugate, washing-buffer 10X, developing solution and stop solution. Sodium chloride and methanol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Aflatoxin analysis

To extract aflatoxins from maize samples, the procedure as reported in the Techna Total Aflatoxin Kit (code MA210/MA211) was followed. Briefly, 50 g of finely ground maize sample and 10 g of NaCl were mixed in 250 mL of 70% v/v methanol. The mixture was vortexed for 10 min and filtered using Whatman Filter Paper No. 1 and the extract (filtrate) was used for the analysis. The assay kit stored in the refrigerator was allowed to thaw to room temperature before analysis. To each premixing well was added 100 μL of enzyme conjugate followed by 50 μL of each standard/sample. The components in the premixing well were thoroughly mixed and 100 μL was transferred into a corresponding anti-aflatoxins antibody coated microwell. The mixture was incubated for 10 minutes at ambient temperature after which the wells were emptied followed by washing with 1X working buffer. A 100 μL of developing solution was added to each well, thoroughly mixed and incubated for 5 minutes followed by addition of 50 μL of stop solution. The absorbance was measured at 450 nm on a Readwell Strip ELISA Analyser (Robonik) plate reader. From a calibration curve of the standard solutions, the concentration of total aflatoxins in maize was determined.

3. Results

Effect of storage method on fungi infestation of maize grains

The results obtained after culturing, isolation and identification of fungi associated with the maize grains before and after storage indicated that the maize samples were infested with different species of storage fungi. Fungi isolated on grains before storage were *Aspergillus flavus, Colletotrichum gleosporioides, Fusarium* sp., *Lasiodiplodia theobromae*, *Penicillium* sp., and *Rhizopus* sp. Six different storage fungi species were again isolated from the maize samples after six months of storage namely, *A. flavus, A. niger, Fusarium* sp., *L. theobromae, Penicillium* sp., and *Rhizopus* sp. No *Colletotrichum gleosporioides* was found in the maize after storage for six months irrespective of the storage method. On the other hand, *Aspergillus niger*, which was not present in the grains before storage was identified in all maize varieties after six months of storage.

Total aflatoxin levels in maize varieties before and after storage

Aflatoxin levels in *Obatanpa*, *Abontem* and *Aburohemaa* maize varieties before storage varied significantly ($p < 0.05$). Aflatoxin levels in *Obatanpa* (2.91 ppb) and *Aburohemaa* (2.96 ppb) were similar compared to *Abontem* (113.56 ppb), which had a significantly high level of aflatoxin ($p < 0.05$) among the three varieties. **S**imilarly, there were significant differences in aflatoxin levels ($p < 0.05$) in all the three maize varieties after six months of storage in three different storage methods. *Obatanpa* and *Aburohemaa* varieties established significantly lower ($p< 0.05$) aflatoxin contamination levels of (5.0 ppb) and (6.6 ppb), respectively compared to *Abontem* (169.3 ppb) as shown in Table 1. Aflatoxin levels among the storage methods also varied significantly. Grains stored in the hermetic bag and local crib had aflatoxin levels of 48.9 ppb each which were significantly lower ($p < 0.05$) than the levels in grains stored in the polypropylene sack (82.9 ppb).

Table 1. Mean aflatoxin levels (ppb) in three maize varieties stored for six months under three storage methods under ambient conditions

Variety (V)	Storage method (SM)			
	Hermetic	Polypropylene sack	Local crib	**Mean**
Obatanpa	4.0	05. Apr	05. Apr	**5.0**
Abontem	138.0	236.1	133.8	**169.3**
Aburohemaa	5.0	7.3	7.6	**6.6**
Mean	**49.0**	**82.9**	**48.9**	
LSD (0.05)	**V**	**SM**	**V*SM**	
	24.88	**24.88**	**43.10**	

Aflatoxin levels under the interactive effect of variety and storage methods was significant ($p < 0.05$). The trend shows that the three maize varieties (*Obatanpa*, *Abontem* and *Aburohemaa*) had low aflatoxin levels when stored in the hermetic bags. The contamination levels increased in poly sack storage but reduced in all the three varieties when stored in the local crib. After storing the three maize varieties in hermetic bags, *Obatanpa* and *Aburohemaa* established contamination levels of 4.1 ppb and 4.7 ppb, respectively which was significantly lower ($p < 0.05$) than contamination levels in *Abontem* variety (137.9 ppb). Aflatoxin levels for the three maize varieties followed the same trend when stored in polypropylene sack and local crib. Contamination levels in *Obatanpa* (5.4 ppb) and *Aburohemaa* (7.3 ppb) when stored in polypropylene sack were similar and significantly ($p < 0.05$) lower than contamination levels in *Abontem* variety (236.1 ppb). Maize varieties stored in the local crib had contamination levels of 5.4 ppb for *Obatanpa* and 7.6 ppb for *Aburohemaa* which were significantly ($p < 0.05$) lower than *Abontem* (133.8 ppb).

4. Discussion

Six different fungi species were isolated on the maize samples before and after storage for six months. The fungal growth could be due to late harvesting of maize by farmers which predisposed the maize grains. It has been shown that maize grains are infested with microorganism right from the field and late harvesting is a contributory factor to field infestation (Widstrom, 1992).

There was varied aflatoxin levels among *Obatanpa*, *Abontem*, and *Aburohemaa* maize varieties before storage. The differences in contamination levels could be due to varied infection levels of the aflatoxin-producing fungus, *A. flavus* in maize grains on the field even before harvest. *Aspergillus flavus* infection can occur at pre-harvest, especially when the crop is in the field (Kuchareck and Raid, 2000; Hurburgh *et al*, 2005). Agriculture in Ghana is rain-fed. Coupled with high temperatures and unavailability of regular rains, the crop is left under stress. Aflatoxin presence at pre-harvest is a common phenomenon when high temperature and drought stress are present during the growth cycle (Cotty and Jaime-Garcia, 2007). This may explain the presence of aflatoxins on maize grains before storage. *Abontem* variety is an extra early maturing maize variety compared to early maturing and intermediate maturing varieties of *Aburohemaa* and *Obatanpa*, respectively. Most farmers do not cultivate one variety and also delay harvest because they want uniform drying of their cobs. Thus, *Abontem* variety will stay on the field for extra days after maturity than *Aburohemaa* and *Obatanpa* before they are harvested together. This might have predisposed that particular variety to moisture and lodging thereby promoting the infection and growth of *Aspergillus*. Different maize varieties have different susceptibilities to microbial attacks (Lokesha *et al*., 1987). Yellow maize is more susceptible to microbial and fungal attack than white maize which could be due to the high nutritive content of the yellow maize variety (Nwogu *et al.,* 1979). This could also explain the high aflatoxin levels in *Abontem* variety before storage.

The high levels of aflatotoxins in *Abontem* variety after six months of storage could be due to the high levels of the mycotoxin in the maize grains prior to storage. Aflatoxin once produced is very stable and storage conditions can only prevent further accumulation but do not reduce aflatoxin concentrations (Bani, 2014).

Maize stored in the woven polypropylene bags had the highest aflatoxin levels after six months of storage, confirming the findings of Udoh *et al.* (2000). Maize stored by traders in Uganda (majority in woven polypropylene bags) for six to seven months had mean aflatoxin levels of 107 ppb which suggests the grains were not suitable for the export and local markets (Kayaa and Warren, 2005). The possible reason for this observation may be the high temperature and high moisture content recorded in the polypropylene sack. Major factors reported for aflatoxin production in maize seeds include moisture content (Manoch *et al*., 1988), relative humidity and temperature in storage (Moreno and Kang, 1999), storage period (Chattha *et al*, 2014) and storage types (Roy and Chourasia, 2001). Grains should therefore be stored at 20 °C, 40-50% relative humidity and 11.5% grain moisture content in order to maintain grain quality (Abba and Lovato, 1999). The most important function of any storage structure is to provide hermetic conditions to the stored product and also give high protection from pests and fungi (Chattha *et al*, 2015). That could have contributed to the low aflatoxin levels in the grains stored in the hermetic bags. The interactive effect of storage method and maize varieties on aflatoxin contamination was significant with the hermetic bag storage giving comparatively lower aflatoxin levels in *Obatanpa* and *Aburohemaa* maize varieties. *Abontem* maize variety, however, had the lowest aflatoxin levels when stored in the local crib compared to the other two storage methods. This could be attributed to the efficiency of the local crib in terms of air flow and circulation which give grains lower moisture contents and subsequently reducing the development of aflatoxins. Low temperatures recorded in the local crib during storage period could also be contributory factor to the low aflatoxin levels observed in *Abontem* variety.

5. Conclusions

Quantitative studies carried out on three maize varieties from the Nkoranza-South District of the Brong-Ahafo Region of Ghana has shown that there are high levels of aflatoxins in the maize. Storage under hermetic conditions drastically reduced the levels of aflatoxins in maize. Improper storage practices such as storage in polypropylene sacks and storage in cribs promote the growth of aflatoxin-producing fungi. There is the need to intensify education for all stakeholders involved in the maize supply chain on aflatoxin contamination in maize and its negative effects on the health of humans and animals and national economy. Most farmers in Ghana are peasant and live below the poverty line. Embracing proper handling and storage practices will reduce post-harvest losses due to fungal infection and improve their income levels. Contaminated grains can be sold to ethanol producers since aflatoxins are eliminated during ethanol production. Farmers should also be educated on timely harvesting of maize and proper drying before storage. Hermetic bags are recommended for the storage of maize since they result in lower aflatoxin levels.

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Effect of Cold Plasma on Storage Toxigenic Fungi - *Aspergillus flavus*

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Abstract

Cold plasma is a novel non-thermal food processing technology that uses energetic, reactive gases to inactivate contaminating microorganisms such as fungi and bacteria. This flexible sanitizing method uses electricity and a carrier gas (air, oxygen, nitrogen, or helium) antimicrobial chemical agents are not required. The primary modes of action are due to UV light and reactive chemical products of the cold plasma ionization process. *Aspergillus flavus* is the predominant species responsible for fungal contamination and subsequent production of aflatoxins mainly in grains during postharvest operations and storage. Due to their relatively high contamination risk, decontamination methods for fungi are of great interest for economic and environmental reasons, as well as in public health. Improved post-harvest processing followed by further prevention of fungal growth is an effective way to restrict aflatoxin contamination and would have major impact on reducing health related risks and on production economics. Thus, the objective is to evaluate the inactivation of *A. flavus* by cold plasma. The experiment was conducted with 3 mm sample *A. flavus*in PDA culture medium. Plasma was applied at different durations (2, 5, 10, 12, 15 and 20 min). After application, the Petri dishes with treated samples were stored at 25°C for 6 days. There was fungal growth after 2 days in the treatments with2 and 5 min durations, 4 days with the treatments with 10 and 12 min durations and there was no fungal growth with the treatments of 15 and 20 min after 6 days. The durationof 15 and 20 min with the plasma parameters tested, were efficient for the inactivation of *A. flavus*. Cold plasma may be a promising green method to be applied in this microorganisms present in grains and other products during storage.

Keywords: cold plasma, fungi, storage, inactivation

1. Introduction

The term "plasma" applies to an ionized gas, containing neutral and electrically charged species, electrons, positive and negative ions, atoms and molecules (Alves, 1995). It is formed from the excitation of a gas or gas mixture by the application of a pressure and energy, which may be the latter mechanical, thermal, nuclear or most common, electric current (Misra et al., 2014).

Cold plasma is a novel non-thermal food processing technology that uses energetic, reactive gases to inactivate contaminating microorganisms such as fungi and bacteria. This flexible sanitizing method uses electricity and a carrier gas (air, oxygen, nitrogen, or helium) antimicrobial chemical agent are not required (Niemira, 2012; Pankaj et al., 2017).

Plasma sterilization can offer an alternative for disinfection methods. This gas presents uniform treatment, can perform the activity at low temperature and without food alteration (taste, odor, structure), and finally, the plasma does not require chemicals, therefore, they do not leave toxic residues (Selcuk; et al. 2008).

Cold plasma has a variety of applications for the food industry, including decontamination of microorganisms in foods such as meats, dairy products, fruits and vegetables, granular and particulate foods (grains, herbs and spices) and germinated seeds. This technology has also been successfully applied for surface sterilization in packaging materials (Mir et al., 2016; Misra et al., 2015, Pankaj et al., 2014, Scholtz et al., 2015).

Cold plasma inactivates microorganisms by three primary mechanisms. The first is the chemical interaction of radicals, reactive species, or charged particles with cell membranes. The second is by damage to membranes and internal cellular components by UV radiation. Finally, DNA strands may be broken by UV generated during recombination of the plasma species. While on a given commodity, one mode of action may be more significant than another, the greatest sanitizing efficacy results from plasma with multiple antimicroorganism's mechanisms (Geyter and Morent, 2012; Fridman et al., 2007; Choi et al., 2006).

Aspergillus flavus is the predominant species responsible for fungal contamination and subsequent production of aflatoxins mainly in grains during postharvest operations and storage (Scussel, 2017). *Aspergillus flavus* is an aflatoxin producer (AFLs) in storage grains in tropical and subtropical climates, especially AFB_1 which is the predominant and most potentially mutagenic, teratogenic and hepatocarcinogenic mycotoxin according to the International Agency for Research on Cancer (IARC, 1993).

Due to their relatively high contamination risk, decontamination methods for fungi are of great interest for economic and environmental reasons, as well as in public health. Improved post-harvest processing followed by further prevention of fungal growth is an effective way to restrict aflatoxin contamination and would have major impact on reducing health related risks and on production economics.

2. Materials and Methods

2.1. Fungi strains

The fungi strains *A. flavus* were obtained from the Food Mycology Laboratory of Mycotoxicology and Food Contaminants (LABMICO) culture collection at the Federal University of Santa Catarina, Florianopolis, SC, Brazil.

2.2.Culture media and chemicals

Culture media - potato dextrose agar (PDA), Kasvi (São Jose dos Pinhais, PR, Brazil) andchloramphenicol, Vetec (Duque de Caxias, RJ, Brazil).

2.3.Equipment

Microbiologicalincubator, Quimis (Diadema, SP, Brazil), autoclave, Phoenix (Araraquara, SP, Brazil), microwaveoven, Philco (Sao Paulo, SP, Brazil); laminar flowcabinet, Veco (Campinas, SP, Brazil). Cold plasma reactor corona discharge type built in borosilicate glass (11.5 x 10.5 cm). The geometry of the reactor is tip-plane in relation to the metal electrodes. The electrical system used consisted of a variac for input voltage adjustments and a 16 kV power supply. For the plasma generation, alternating current was used with no oxygen gas inlet.

2.4. Cold plasma application

A disc (3 mm) with *A. flavus* mycelia material and conidia, taken from the edge of 7-days-old-fungal culture was placed individually inside the reactor. Plasma was applied at different time durations in duplicate (2, 5, 10, 12, 15 and 20 min), whereas the fungi in the control received room air at the same exposure times. Afterward, the Petri dishes containing fungi, including the control, were held in an incubator at 25◦C for 6 days. The efficiency of cold plasma treatment was evaluatedafter 2, 4 and 6 days by measuring the fungi colony diameter (Fraternale et al. 2003; Savi and Scussel, 2014).

2.5. Statistical analysis

The data of fungi colonies growthwere analyzed by analysis of variance (ANOVA).

3. Results and Discussion

The strain of *A. flavus* that received cold plasma exposure for 15 and 20 min did not grow during the 6 days incubation, showing that the plasma parameters used were effectiveat these durationsto inactivate *A. flavus*. For the treatment durationsof 2 to 12 minutes, growth was significantly lower than controls (Figure 1).

After 2 days application of the plasma, there was growth of fungi in the times of 2 and 5 min (10 mm) and control (18mm). In the treatments of 10 and 12 min, the growth was observed after 4 days (16 and 19 mm, respectively).

Allresultscomparedwiththecontroltreatmentdifferedstatistically. In Figure 2, images show the growth of fungi in petri dish containing PDA after 6 days of incubation after treatment.

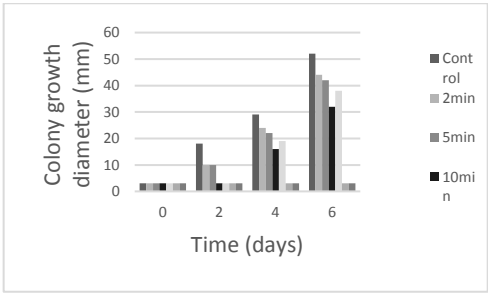

Figure 1. Effect of cold plasma (2, 5, 10, 12, 15, 20 min of exposure) on *A. flavus* growth.

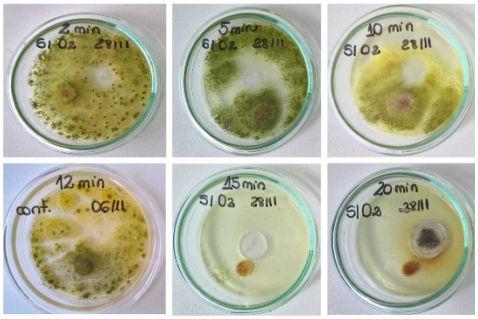

Figure 2. Colony growth of *A. flavus* in Petri dish containing PDA after 6 days of incubation after treatment.

There are fewer studies on the use of cold plasma as a decontaminant agent in fungi in foods than studies involving bacteria. The most studied foods were oilseeds (pistachios, almonds, peanuts and hazelnuts), dates and seeds (tomato, wheat, beans, oats, soya, barley, maize and rye). In relation to fungi, the genus *Aspergillus* and *Penicillium* were the most tested.

No studies were found in the literature to evaluate fungal growth after cold plasma treatment. However, many papers report their efficiency in decontamination and / or reduction of fungi on food surfaces. Dasan et al. (2016) investigated the decontamination of inoculated fungi (*A. flavus* and *Aspergillus parasiticus*) on cold plasma hazelnut surfaces. Significant reductions of 4.50 log (cfu / g) in *A. flavus* and 4.19 log (cfu / g) in *A. parasiticus*were achieved after 5 minutes of 655 W treatments. No growth was observed in *A. flavus* and *A. parasiticus* during storage of plasma-treated hazelnuts, while in the control samples the fungi continued to grow under storage conditions (30 days at 25°C).

In rice cereal bars, Suhem et al. (2013) studied the effect of cold plasma treatment to inhibit the growth of *A. flavus*. The treatment was applied to the surface of the cereal bars with 40W potency and exposure time of 20 min, reducing approximately 4 logcfu/g and also preventing growth of the fungus on the surface of the bars for at least 20 days.

Selcuk et al. (2008) successfully decontaminated the seeds of wheat, bean, chickpea, soybean, barley, oat, rye, lentil, and corn, contaminated with *Aspergillus* and *Penicillium sp.* to less than 1% of initial count depending on treatment times. The treatment times varied from 30 s to 30 min. The results suggested that after plasma treatment food quality of wheat and beans were not affected or only marginally affected. The seeds were found to be viable post plasma processing.

The mechanism by which plasma causes the cell death of microorganisms is due to reactive species of O_2, OH, NO2, etc. which have been extensively associated with direct oxidative effects on the outer surface of microbial cells. Reactive oxygen species are expected to significantly affect membrane lipids due to their location along the cell surface of the microorganisms, allowing them to be bombarded by these strong oxidizing agents (Scholtz et al., 2015; Laroussi, 2005; Mendis, Rosenberg, Azam, 2000). Oxidation of amino acids and nucleic acids can also cause changes that result in death or injury of the microorganism such as bacteria and fungi (Mir; Shah; Mir, 2016; Guo et al., 2015).

4. Conclusions

*Aspergillusflavus*treated was significantly reducedby cold plasma treatment application. The best results of antifungal effect were observed on 15 and 20 min of exposure, that were completely inhibited showing to be an effective method for this storage fungus. Cold plasma may be a promising green method to be appliedto control this microorganism present in grains and other

products during storage. It also could be a promising method of decontamination in industries and storage units, in order to avoid contamination and ensure food security to the consumer.

Acknowledgements

We thank to the laboratory of plasma of the University of Southern Santa Catarina(UNISUL) forsupport of this research and to the National Council of Scientific and Technological Development (CNPq) for the financial support.

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Computer-Aid Molecular Docking Technology in Cereal Mycotoxin Analysis

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Abstract

Computer-aid molecular docking is a simulative process that receptors and ligands recognize each other through energy matching and geometric matching. It is widely used in bioactive compounds simulative screening and preliminary exploring the bioactivity and toxicity of molecular, which plays important guiding role in toxicity and bioactivity study of molecular entities. In our study, we used the computer-aid molecular docking software-discovery studio 3.1 client to test the mechanism of aflatoxins such as aflatoxin B1, B2, M1, M2, G1, G2 and the results of our experiment help to illustrate the pathway of aflatoxin's toxication. We also used this technology to test the preliminary toxicity of zearalenone (ZEN) and its two degradation products: α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL), which indicates that these three products possessed significant estrogenic activity. The order of the estrogenic activity is: α-zearalenol > zearalenone >β-zearalenol.

Key words: computer-aid molecular docking, aflatoxin, zearalenone, toxicity

1. Introduction

Over the last few decades, computer-aid molecular docking technology has grown significantly in the development of new drug molecules. As a powerful technique, it relives the tension in drug discovery such as time-consuming, high-cost and low success rates. Additionally, with rapid development of biological structures and computer technology, this technology is widely used in mycotoxin toxicity pathway research (Shoichet et al., 2002; Powers and Setzer, 2015).

Using direct docking methods or virtual high-throughput screening, affinity of molecules to targets can be estimated based on compounds' conformation and complementarity with residues in binding site. Through analysis of binding free energies, further filtering and optimization of possible molecules subsequently follow, a limited number of lead molecules are selected for *in vitro* bioactivity tests. Automatic docking is aimed at the determination of the optimal position and orientation of molecule in binding pocket of particular protein target (Verdonk et al., 2011; Śledź and Caflisch, 2017). Quality of protein–ligand interactions are usually qualified by ligand efficiency (LE) and average binding energy per non-hydrogen atom of the ligand. While virtual high-throughput screening is performed to evaluate libraries of molecules for binding affinity to the protein target. This HTS strategy can shortlist compounds that are most likely to bind to the selected target with the highest affinity. A plethora of software have been developed for molecular docking including Dock, GOLD, and AutoDock, et. In addition, other docking strategies such as flexible ligand docking, fragment docking and fragment growing have been used in high-throughput docking campaigns (Macalino et al., 2015; Leelanada and Lindert, 2016).

In our research, we used the computer-aid molecular docking software-Discovery Studio 3.1 client (Accelrys, USA) to test the mechanism of aflatoxins and illustrate the pathway of aflatoxin's toxication. We also used this technology to test the preliminary toxicity of zearalenone and its two degradation products: α-zearalenol and β-zearalenol, which indicates that these three products possessed significant estrogenic activity.

2. Materials and Methods

The 2D structure of Aflatoxin B1 and Oltipraz was generated by ChemDraw Ultra 12.0 (CambridgeSoft, Cambridge, MA, USA). A homology model of protein receptor was constructed from crystal structure of PDB: obtained from the RCSB protein Data Bank,

Water molecules were removed and H atoms were added to the structure. 3D structures of the compounds were generated and optimized by the Discovery Studio 2.1 package (Accelrys, San Diego, CA, USA). The receptor-grid files were carried out using a grid-receptor generation program using default settings after ensuring that the ligands and the protein are in correct form. The GOLD program in the Discovery Studio software was used to perform the docking simulations, which allows full flexibility of the ligand.

The structures of the aflatoxins， zearalenone, α-zearalenol and β-zearalenol were drawn in chem3D with standard lengths and angles. The Gasteiger–Huckel charge, with a distance-dependent dielectric function, and AM1 docking calculations were applied for the minimization of

the molecules. To modify the structure of receptor, missing atoms, bonds, and contacts were checked, hydrogen atoms were added to the enzyme structure, and water molecules were removed. Intercalation models were optimized using the CHARMm forcefield with the added parameters. After performing the docking simulation, the scores of the docked conformers were ranked the best binding modes in the cavity was picked out.

3. Results and Discussion

3.1. Aflatoxin toxicity analysis

Aflatoxins are among the most potent natural hepato-carcinogenic products, which are produced mainly by the fungi *Aspergillus flavus* and *A. parasiticus*. Twelve aflatoxins analogues including aflatoxin B1, B2, G1, M1, P1, Q1, H1, GM, B2a and aflatoxicol have been separated and identified. The basic structures of aflatoxins are dihydrofuran, coumarin and aflatoxin B1(AFTB1), which is the analogue of dihydrofuran oxynaphthalene, contains two furan rings (the basic toxic structure) and one cumarin (Eaton and Gallagher, 1994; Koudande, 2013).

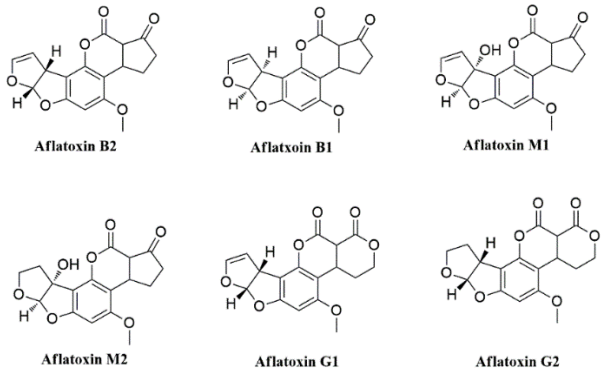

Fig. 1 The chemical structures of aflatoxin's metabolic products

The interaction between AFTB1 and receptor proteins

In our research, we chose some kinds of proteins which play significant roles in cell apoptosis, estrogen metabolism, immunosuppression and digestive system function as the potential targets of the toxic pathway of aflatoxin b1, including Caspase-1, cell division protein kinase 2, serine/threonine protein kinase chk1, progesterone receptor, androgen receptor, estrogen receptor, alpha-thrombin, prostaglandin g/h synthase 2, estradiol 17-beta-dehydrogenase 1, macrophage migration inhibitory factor and estrogen sulfotransferase. We made AFTB1 molecular docked with the above proteins by molecular docking software, the results was shown in **Tab 1.** As the results shown, estrogen sulfotransferase was proved to be the best dock receptor of AFTB1 and the score were 130.22 and -10.9013 by Libdock and CDocker, respectively.

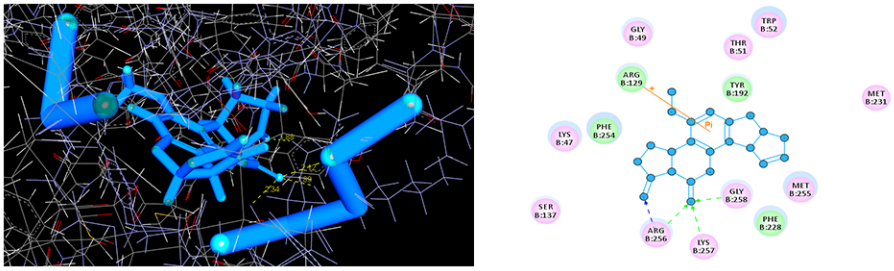

Fig. 2 The interaction between AFTB1 and estrogen sulfotransferase

Tab. 1 AFTB1 targets predicted by Libdock and CDocker

Protein	PDB	Libdock score	CDocker score
caspase-1	3D6F	828.002	/
cell division protein kinase 2	1HCK	972.643	-0.549952
serine/threonine-protein kinase chk1	1IA8	100.060	/
progesterone receptor	1A28	986.966	/
androgen receptor	5DIU	113.528	-209.024
estrogen receptor	1QKM	110.000	-221.885
alpha-thrombin	1ABI	822.093	/
prostaglandin g/h synthase 2	1PD2	117.653	/
estradiol 17-beta-dehydrogenase 1	1BHS	986.731	/
macrophage migration inhibitory factor	1MFI	908.723	-968.253
estrogen sulfotransferase	1HY3	130.022	-109.013
sex hormone-binding globulin	1D2S	111.558	-162.633

The interaction between AFTB1 and estrogen sulfotransferase

We made AFTB1 docked with estrogen sulfotransferase, the docking result was shown in **Fig. 2.** AFTB1 could perfectly docked into the formed cavity of estrogen sulfotransferase protein and there was formed cation-π interaction between the benzene ring and arginine residue (ARG B:129). The carbonyl group in coumarin formed hydrophobic interactions with tyrosine residue（TYR B:192）and the oxygen atom in furan ring formed hydrophobic interactions with arginine residue (ARG B:256).

The interaction between AFTB2 and estrogen sulfotransferase

We made AFTB2 docked with estrogen sulfotransferase, the docking result was shown in **Fig. 3.** AFTB1 could perfectly docked into the formed cavity of estrogen sulfotransferase protein and there was formed cation-π interaction between the benzene ring and arginine residue (ARG B: 129). The carbonyl group in coumarin formed hydrophobic interactions with arginine residue（ARG B:256），allysine residue (LYS B:257) and glycine residue (GLY B:258).

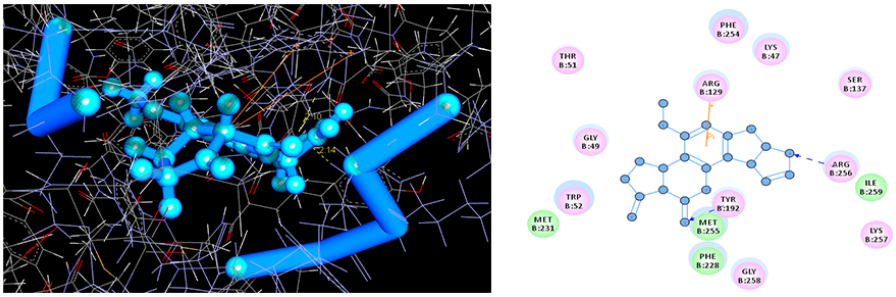

Fig. 3 The interaction between AFTB2 and estrogen sulfotransferase

The interaction between AFTBM1 and estrogen sulfotransferase

We made AFTB M1 docked with estrogen sulfotransferase, the docking result was shown in **Fig. 4.** AFTB1 could perfectly docked into the formed cavity of estrogen sulfotransferase protein and there was formed cation-π interaction between the benzene ring and arginine residue (ARG B:129). The phenolic hydroxy group furan ring hydrophobic interactions with arginine residue（ARG B:256), allysine residue (LYS B:257) and glycine residue (GLY B:258). The oxygen atom in furan ring formed

hydrophobic interactions with arginine residue (ARG B:256).

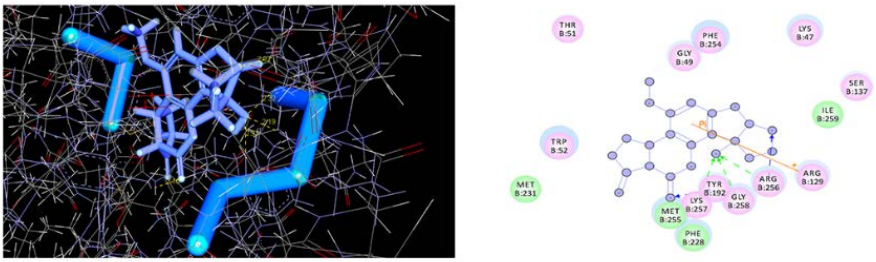

Fig 4. The interaction between AFTB M1 and estrogen sulfotransferase

The interaction between AFTB M2 and estrogen sulfotransferase

We made AFTB M2 docked with estrogen sulfotransferase, the docking result was shown in **Fig. 5.** There was formed cation-π interaction between the benzene ring and arginine residue (ARG B:129). The phenolic hydroxy group furan ring hydrophobic interactions with arginine residue（ARG B:256），lyrosine residue（TYR B:192），and glycine residue (GLY B:258). The oxygen atom in furan ring formed hydrophobic interactions with arginine residue（ARG B:256），the carbonyl group in coumarin formed hydrophobic interactions with tyrosine residue (TYR B:192).

The interaction between AFTB G1 and estrogen sulfotransferase

We made AFTB G1 docked with estrogen sulfotransferase, the docking result was shown in **Fig. 6.** There was formed cation-π interaction between the benzene ring and arginine residue (ARG B:129). The inside carbonyl group in coumarin formed hydrophobic interactions with arginine residue (ARG B:256)， allysine residue (LYS B:257) and glycine residue (GLY B:258). The outside carbonyl group in coumarin formed hydrophobic interactions with allysine residue (LYS B:47), The oxygen atom in furan ring formed hydrophobic interactions with arginine residue(ARG B:256).

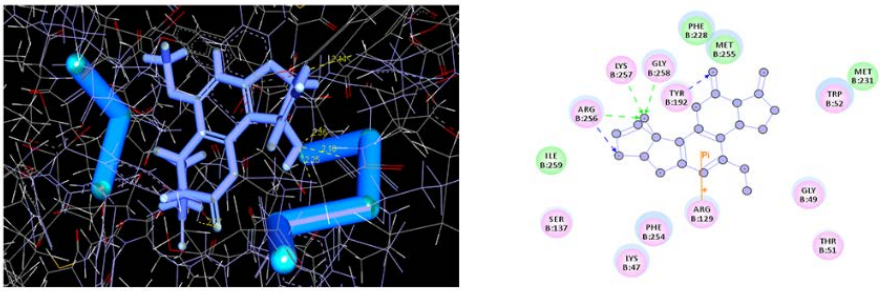

Fig. 5 The interaction between AFTB M2 and estrogen sulfotransferase

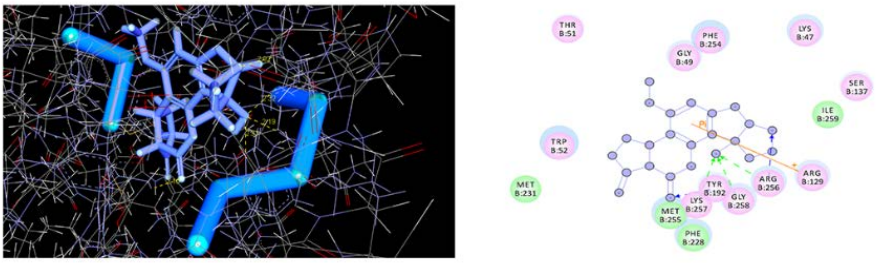

Fig. 6. The interaction between AFTB M2 and estrogen sulfotransferase

The interaction between AFTB G2 and estrogen sulfotransferase

We made AFTB G1 docked with estrogen sulfotransferase, the docking result was shown in **Fig. 7.** There was no formed cation-π interaction between the benzene ring and any residue. The inside carbonyl group in coumarin formed hydrophobic interactions with arginine residue (ARG B:256), allysine residue (LYS B:257) and glycine residue (GLY B:258). The outside carbonyl group in coumarin formed hydrophobic interactions with allysine residue (LYS B:47).

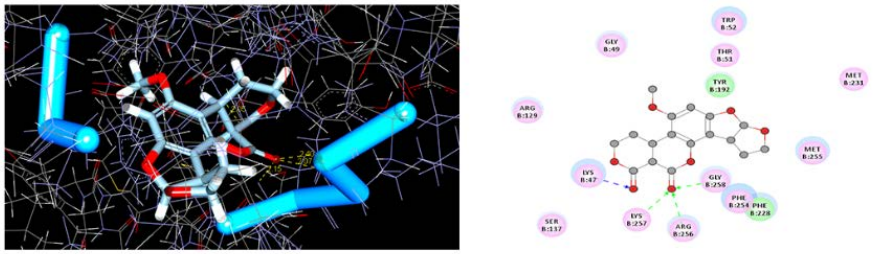

Fig.7 The interaction between AFTB G2 and estrogen sulfotransferase

The Binding energy and docking score between ligand and receptors

The results of binding energy and docking score between aflatoxin B1, B2, M1, M2, G1, G2 and estrogen sulfotransferase were shown in **Tab 2**. The binding energy between AFTB1, AFTB2, AFTB M1, AFTB M2, AFTB G1, AFTB G2 with estrogen sulfotransferase was -10.9013 kcal/mol, -20.2356 kcal/mol, -8.5654 kcal/mol, - 8.2156 kcal/mol, -19.5298 kcal/mol, -14.1768 kcal/mol. AFTB M1 was proved to be the best binding ligand with estrogen sulfotransferase receptor.

Tab 2 the Binding energy and docking score between ligand and receptors

Ligand	Binding energy	Number
Aflatoxin M2	-8.21584	10
Aflatoxin M1	-8.5654	10
Aflatoxin B1	-10.9013	10
Aflatoxin G2	-14.1769	10
Aflatoxin G1	-19.5298	10
Aflatoxin B2	-20.2356	10

3.2. Estrogenic effect in zearalenone

Zearalenone [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-resorcylic acid lactone, abbreviated as ZEN] is a mycotoxin that is produced by *Fusarium roseum* and is usually isolated from moldy corn (Reed et al, 2004). Zeranol a synthetic tetrahydro-derivative of ZEN, has been used as a growth promoter

for food-producing animals (Caldwell et al., 1970). Earlier studies have shown that ZEN and ZOL have strong estrogenic effects, and each of them was reported to have a similar dose–response curve pattern in stimulating uterine weight gains in neonatal rats or immature mice (Urry et al, 1966). The most commonly ZOL are α-ZOL and β-ZOL, which are shown in **Fig. 8.**

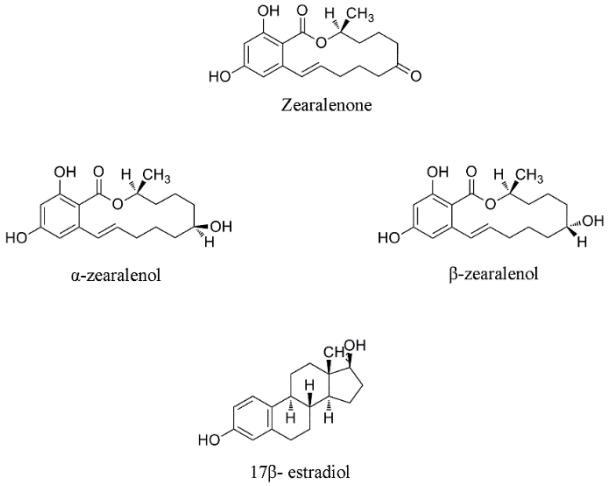

Fig. 8 Chemical structure of ZEN, α-ZOL, β-ZOL and β-estriol

The interaction between ZEN and α-estrogen receptor protein

In order to prove the estrogen effect of ZEN in molecular stage, we used the molecular docking software to simulate the binding situation between ZEN and α-estrogen receptor protein, as **Fig. 9** shown, ZEN can perfectly docked into the cavity of estrogen receptor protein crystal structure, and the hydroxy group of benzene ring formed strong hydrophobic interactions with contiguous alanine residue (ALA350) and glutamic acid residue (GLU353), which strengthen the binding ability between the ligand sand the receptors.

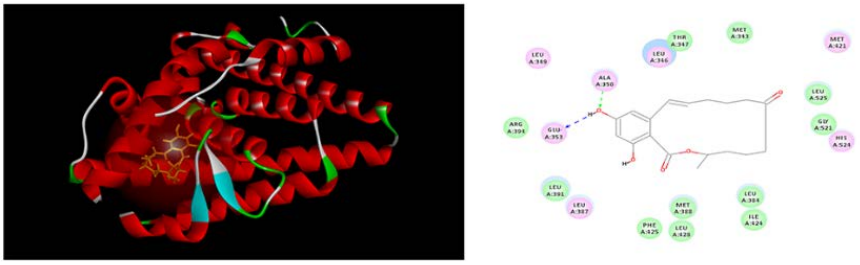

Fig. 9 The interaction between ZEN and α-estrogen receptor protein

The interaction between ZEN and β-estrogen receptor protein

We used the molecular docking software to simulate the binding situation between ZEN and β-estrogen receptor protein, as **Fig 10** shown, the 3-hydroxy group in benzene ring of ZEN formed strong hydrophobic interactions with contiguous histidine residue (HIS475) and glycine residue (GLY472), which strengthen the binding ability between the ligand sand the receptors.

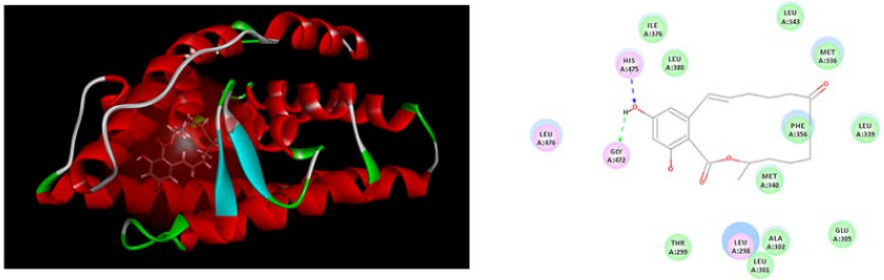

Fig. 10 The interaction between ZEN and β-estrogen receptor protein

The interaction between ZOL and estrogen receptor protein

We have proved that ZEN molecule had favorable binding ability with α-estrogen receptor protein and β-estrogen receptor protein, which demonstrated that ZEN possessed strong estrogen effect. In the following study, we would prove whether ZOL had the similar estrogen effect.

We used the molecular docking software to simulate the binding situation between α-ZOL, β-ZOL and α- estrogen receptor protein, β-estrogen receptor protein. From Fig 11, It was proved that α-ZOL can perfectly docked into the cavity of estrogen receptor protein crystal structure, 3-hydroxy group in benzene ring of α-ZOL formed strong hydrophobic interactions with contiguous arginine residue (ARG394) in α- estrogen receptor protein and glycine residue (GLY472) in β-estrogen receptor protein; aliphatic hydroxyl group in aliphatic chain formed strong hydrophobic interactions with methionine (MET421) in α- estrogen receptor protein and glutamic residue (GLU305) in β-estrogen receptor protein. The binding ability between β-ZOL and estrogen receptor protein was relatively weak and there only one existed hydrophobic interaction according to the docking results (Fig. 12)

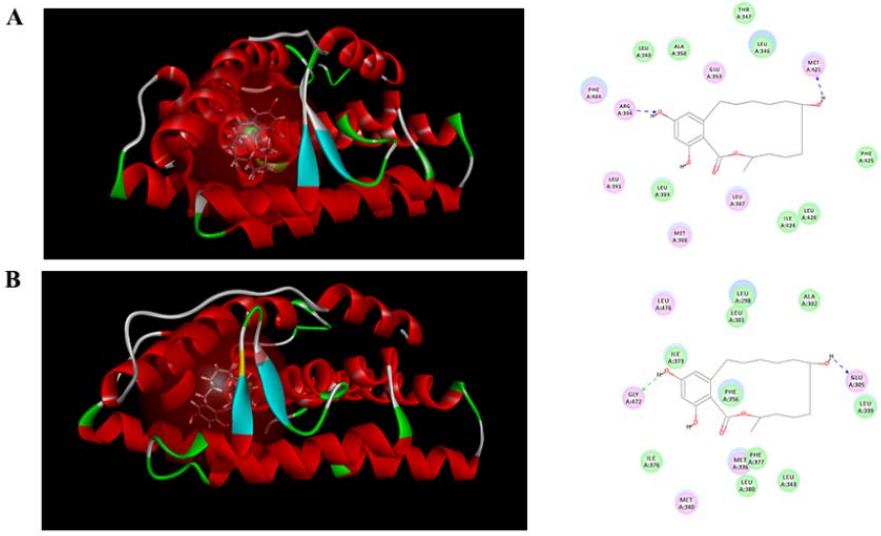

Fig. 11 The interaction between α-ZOL and estrogen receptor protein

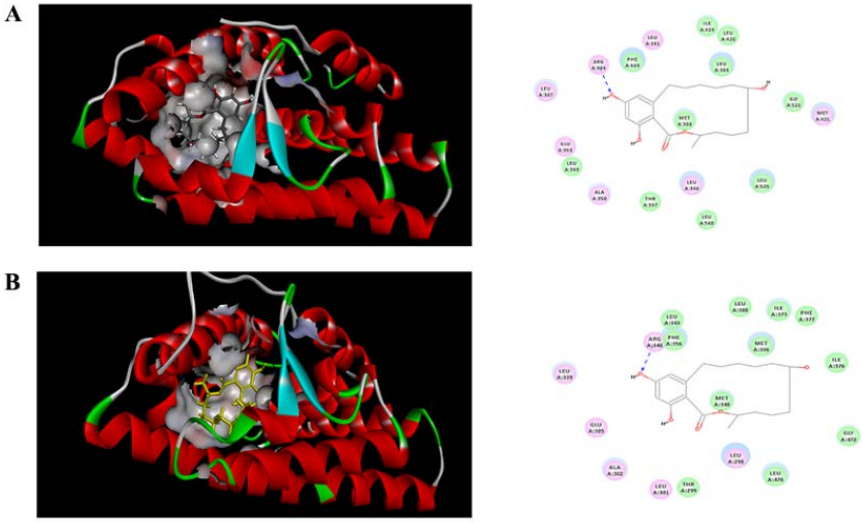

Fig. 12 The interaction between β-ZOL and estrogen receptor protein

The binding ability and docking score between ligands and recepor

The results of binding energy and docking score between 17β- estradiol (positive control), ZEN, α-ZOL, β-ZOL and estrogen receptor protein were shown in Tab 3. The order of the estrogenic activity is: α-zearalenol > zearalenone >β-zearalenol.

Tab3 the Binding energy and docking score between ligand and receptors

Ligand	Estrogen receptor protein	Binding energy （kcal/mol）	Libdocks score
17β- estradiol	α- estrogen receptor protein	-26.8334	98.9602
17β- estradiol	β-estrogen receptor protein	-26.8334	108.235
ZEN	α- estrogen receptor protein	-38.9894	113.57
ZEN	β-estrogen receptor protein	-38.9894	106.956
α-ZOL	α- estrogen receptor protein	-41.937	94.0647
α-ZOL	β-estrogen receptor protein	-41.937	72.6476
β-ZOL	α- estrogen receptor protein	-27.6144	115.28
β-ZOL	β-estrogen receptor protein	-27.6144	107.458

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Insects and mycobiota in *Phaseolus vulgaris* L. grains sold in retail stores

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Abstract

In Brazil beans are an important protein source and the great variety of *Phaseolus* and *Vigna* beans grains are sold in retail markets. The objective of this study was to isolate fungi from insects and *Phaseolus vulgaris* (var. Pinto) from 15 samples of different retail stores in São Paulo. The samples were placed in Petri dishes containing culture medium of potato-dextrose-agar and incubated at 25°C for 7 days. Fungi were identified in several insects: *Callosobruchus maculatus* (yeasts - 50%), *Sitophilus* spp. (*Chaetomium* spp. – 3.1%; *Rhizopus stolonifer*- 3.1%; Non Sporulating Fungi (NSF) – 12.5% and *Eurotium chevalieri* - 9.4%, *Acanthoscelides obtectus* (*Penicillium* spp. – 18.5% and yeasts – 18.5%) and *Zabrotes subfasciatus* (*Alternaria alternata* – 13.6 % and *Penicillium* spp. – 41 %). No fungi were observed in the parasitoid *Dinarmus basalis*. In grain samples, the following fungi were found: *Penicillium* spp. (6%), *E. chevalieri* (5%), *R. stolonifer* (0.3%), *Aspergillus flavus* (3 %), NSF (8 %), Yeasts (2.6%), *Phoma* spp. (1.6%) and *Alternaria alternata* (3.6%).

Keywords: bean, grain, fungi, insects

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most widely consumed legumes in the world (Barros and Prudencio, 2016). Bean growers are looking for new storage options that maintain the quality of seed beans for use in planting and also for beans produced for the retail market. The most frequent causes of losses in storage beans are: insects, fungi and rodents. This causes the decrease in quality, as taste and the appearance of grain (Bragantini, 2005). The stored beans are mainly attacked by *Acanthoscelides obtectus* (Say), *Zabrotes subfasciatus* and *Callosobruchus maculatus* (Botelho, 2002). Insects are vectors for fungi and cause physical damage to the grain. The control of both effects is important in the safety and quality of stored grains (Aquino and Potenza, 2013). This study analyzed the insects and mycobiota in samples of *Phaseolus vulgaris* (pinto beans) purchased in several retail markets in São Paulo.

2. Materials and methods

Fifteen 1-kg samples of *Phaseolus vulgaris* (pinto bean) were purchased in retail markets of São Paulo. Samples were sieved and the insects collected using aspirator. Samples were held at 27 ± 2º C and 70 ± 5% relative humidity for 45 days, sieved and the emerged insects collected. For the fungal isolation, the samples were submitted to direct plating on potato dextrose agar (PDA) and

incubated at 25 ± 1 °C for 7 days (Berjak, 1987). The counting of fungal genera was quantified in percentage and subsequently, the different morphological mycelium was isolated in test tubes containing PDA and submitted to the technique of microculture for the species identification (Riddell, 1950).

3. Results

Fifty percent of the *C. maculatus* individuals - present in the samples - were positive for yeasts. *Sitophilus* spp. was the insect species associated with the greatest variety of fungi: *Chaetomium* spp. (3.1%), *Rhizopus stolanifer* (3.1%), NFS (12.5%) and *Eurotim chevalieri* (9.4%). No fungi were isolated in *D. basalis* individuals. The fungi *Penicillium* spp. and yeasts were isolated for 18.5 of *A. obtectus*. Plated individuals of *Z. subfasciatus* presented 13.6% of *A. alternata* and 41% of *Penicillium* spp. (Table 1).

Table 1 - Frequency (%) of yeasts and fungi isolated from insects collected in samples of *Phaseulos vulgaris*.

Insects	Microorganism isolated	Insects with microrganisms (%)	Number of Insects
Callosobruchus maculatus	Yeasts	50.0	4
***Sitophilus* spp.**	*Chaetomium* spp.	03. Jan	38
	Rhizopus stolanifer	03. Jan	
	NSF	12. Mai	
	Eurotim chevalieri	09. Apr	
Dinarmus basalis	(---)	(---)	2
Acanthoscelides obtectus	*Penicillium* spp.	18. Mai	27
	Yeasts	18. Mai	
Zabrotes subfasciatus	*Alternaria alternata*	13. Jun	22
	Penicillium spp.	41.0	

Non Sporulated Fungi – NSF.

(---) - No fungal grow th.

In the bean samples it was observed 6 % of *Penicillium* spp.; 5% of *E. chevalieri*; 0.3% of *R. stolonifer*, 3% *A. flavus*; 8 % NFS; 2.6% Yeast; 1.6% of *Phoma* spp. and 3.6% of *A. alternate* (Figure 1).

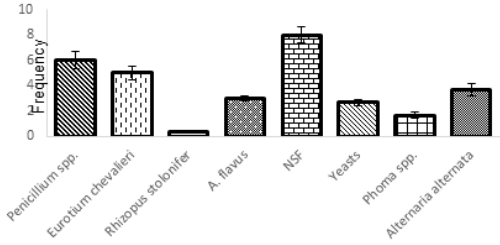

Figure 1 - Frequency of fungi species isolated from *Phaseolus vulgaris*.

4. Discussion

According to Bull (1993), grain quality can be affected even in the field before harvest, where infestation by insects and fungus contamination begins. Storage fungi are always present in high numbers and in all types of materials such as air, dust, water, which are normal constituents of grain and seed film (Lazzari, 1997). (Tseng et al, 1995) identified the fungi genera from grains of *P. vulgaris* collected in Taiwan: *Aspergillus* (48.5%), *Penicillium* (27.6%), *Eurotium* (6.7%), *Rhizopus* (5.3%) and *Curvularia* (2.4%). (Domijan et al, 2005) in a study to identify fungi transmitted by seeds of *Phaseolus vulgaris*: *Cladosporium* spp. (98%) *Alternaria* spp. (75 %), *Aspergillus* spp. (73%), *Rhizopus* spp. (73%), *Penicillium* spp. (69%), *Fusarium* spp. (38%), *Botrytis* spp. (27%), *Trichothecium* spp. (24%) and *Chaetomium* spp. (18%). It was concluded that the storage conditions should be monitored in the retail market to prevent loss of quality caused by insects and fungi.

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Naturally existing *Beauveria* on the surface of stored wheat kernels, and their pathogenicity on *Rhyzopertha dominica* and *Sitophilus oryzae* adults

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Abstract

Entomopathogenic fungi have been investigated to control stored product pests, as an alternative strategy to chemical insecticides. Although many studies evaluated isolates from various sources, few studies surveyed fungi naturally infecting stored product pests, revealing predominantly *Beauveria* isolates. This study aimed to reveal the amount of *Beauveria* carried on the surface of stored wheat kernels, and their pathogenicity against *Rhyzopertha dominica* and *Sitophilus oryzae* adults. Sixteen wheat samples from different storage facilities in four cities were examined for existence of *Beauveria*. One-hundred g of wheat was washed in 100 mL of 2% Tween80 solution. After increasing concentration of possible fungi by centrifugation, the liquid was spread on medium with dodine and monitored at 25±2°C. Nine of the isolates were tested for pathogenicity at 500 ppm (w/w) at 25±2°C, 65±5% r.h. in darkness with five replicates. While only four samples did not have *Beauveria*, others had 17-2992 cfu/100 g wheat. Six samples had 17-50, four samples 150-858, one sample 1625 and one had 2992 cfu/100 g wheat. Mortalities against *R. dominica* adults ranged between 5-86% and 32-100% in 7 and 14 days, respectively. Mortality of *S. oryzae* ranged from 3-45% and 8-83% in 7 and 14 days, respectively. This study demonstrated that wheat kernels can naturally carry *Beauveria* with various levels of pathogenicity. Potential naturally occurring entomopathogenic fungi can be isolated directly from stored commodities to be evaluated as biological control agents for stored product pest control.

Keywords: microbial control, biological control, entomopathogen, survey.

1. Introduction

Cereals are important for human consumption and livestock in the world. After harvesting they are usually stored for various lengths of time. During storage, they need to be protected against insect and mite pests. Unless suppressed, the populations of these pests cause reduction in the weight and value as well as decline of seed germination (Moino et al., 1998; Padin et al., 2002; Haq et al., 2005; Stejskal et al., 2015). The use of synthetic insecticides to suppress pest populations has been commonly practiced (Athanassiou & Palyvos, 2006); however, its negative effects such as pest resistance to the chemicals (Arthur, 1996), residue accumulation in grains (Ferizli et al., 2005), and

detrimental effects on humans and the environment (Michalaki et al., 2007) have directed researchers to seek nontoxic and environmentally friendly methods to suppress stored product pests. One alternative that has been considered is the use of entomopathogenic fungi (Moino et al., 1998; Michalaki et al., 2007; Sewify et al., 2014; Wakil & Schmitt, 2014) due to their organic nature and low hazard to human and the environment (Moore et al., 2000). The potential of entomopathogenic fungi as bioinsecticides against insect pests of stored products has been reported in many literature (Cherry et al., 2005; Wakil & Ghazanfar, 2010; Shams et al., 2011; Barra et al., 2013; Khashaveh & Chelav, 2013; Sewify et al., 2014). The potential of entomopathogenic fungi in combination with diatomaceous earth has also been reported (Athanassiou & Steenberg, 2007; Athanassiou et al., 2008; Wakil et al., 2011; Riasat et al., 2011, 2013; Shafighi et al., 2014). There are few studies investigated the natural occurrence of entomopathogenic fungi in stored product pests (Odour et al., 2000; Wakefield et al., 2005; Wakil et al., 2014; Er et al., 2016). Most commonly, *B. bassiana* has been found and most of the literature evaluated this species as a biocontrol agent. In this study wheat kernels are examined for existence of *Beauveria* as source of inoculum and the pathogenicity of obtained isolates was tested against adults of two coleopteran pests of stored grains, *Rhyzopertha dominica* and *Sitophilus oryzae*.

2. Materials and Methods

Insect cultures

Rhyzopertha dominica and *Sitophilus oryzae* cultures have been maintained in our laboratory. Starting insects had been originally obtained from surrounding storage facilities. Durum wheat with 12% moisture content was used for the cultures. Glass jars of 1 L capacity with 250 g of wheat were used. Adults of mixed sex were placed into the jars and kept for three days for oviposition. After removing the adults, the cultures were incubated for the emergence of new generation adults. One-week old adults were used for the bioassays. All the cultures were maintained at 26±2 ºC and 65±5% relative humidity in darkness.

Wheat samples

Sixteen wheat samples from different storage facilities in four cities in Turkey were taken as described in Er et al. (2016) and examined for existence of *Beauveria*. Eight samples were from Osmaniye, 5 from Hatay, 2 from Adana and one from Kahramanmaraş.

Processing samples and isolation of fungi

Each sample is mixed and 100 g of it was used for fungus isolation. Wheat kernels were washed by placing in 100 mL of 2% Tween 80 solution and agitating. After removing the kernels, particles in the liquid were sedimented in two 50 mL capacity tubes by using a fixed angle centrifuge at 8000 g for 10 mins. Sediments in both tubes were combined and filled to 5 mL. After increasing concentration of possible fungi by centrifugation, 200 µL of the liquid was spread on oat meal agar supplemented with 650 µL/L dodine and 1% streptomycin + penicillin. The experiment was conducted with three replicates. Growth of fungi was monitored at 25±2ºC and number of *Beauveria* colonies in each petri dish was recorded. Selected *Beauveria* samples are subcultured and purified on potato dextrose agar at the same conditions. Nine *Beauveria* isolates were used for spore production following mass production procedure described by Barış (2016). One-hundred g of rice was soaked overnight with tap water and the excess water was drained. The rice supplemented with 1.5 g of $CaSO_4$ and $CaCO_3$ was sterilized in a polyethylene bag (25 cm x 38 cm). After cooling, it was inoculated with 10 mL of spore suspension ($2x10^7$ spores/mL) and sealed. Following fungal growth at 25±2°C, 12/12 photoperiod for 14 days the culture was dried at 25±2°C. Spores were separated from substrate by using a 500 µm sieve.

Pathogenicity tests

Centrifuge tubes of 50 mL capacity each with 40 g of wheat were used for the tests. Wheat in each tube was mixed with 20 mg of spores producing a final concentration of 500 ppm (w/w) by shaking for 5 minutes. Twenty adults were released in each tube and kept at 25±2ºC, 65±5% relative humidity in darkness. Wheat kernels without spores were used as control. The experiment had five replicates.

3. Results

Among the 16 samples, 12 had *Beauveria* colonies in at least one of the Petri plates, while only four samples did not have *Beauveria* colonies. The mean number varied between 17 and 2992 cfu/100 g wheat. Six samples had 17-50, four samples 150-858, one sample 1625 and one had 2992 cfu/100 g wheat. When these fungi were tested, *R. dominica* adult mortalities ranged between 5-86% and 32-100% in 7 and 14 days, respectively. *S. oryzae* mortalities were lower and recorded as 3-45% and 8-83% in 7 and 14 days, respectively.

4. Discussion

This study demonstrated that stored wheat kernels can naturally carry *Beauveria* at various levels, but in rather low concentrations. These results support the findings of previous survey studies for natural fungal infections on stored product pests (Odour et al., 2000; Wakefield et al., 2005; Wakil et al., 2014; Er et al., 2016). In all these studies, a low percentage of insects were found to be naturally infected by *Beauveria*. The reason could be the existence of low *Beauveria* inoculum in their habitats as shown in this study.

Testing the isolates in the study resulted in various levels of mortalities in both insect species. Such variation in the pathogenicity of isolates within the same species was commonly reported (Moino et al., 1998; Kassa et al., 2002; Wakefield et al., 2005; Sewify et al., 2014). The efficacy of the isolates against *S. oryzae* adults was lower than that against *R. dominica* adults. Similarly, Moino et al. (1998) and Sewify et al. (2014) found that *R. dominica* is more susceptible than *S. oryzae*. All the findings together suggest that diversity of *Beauveria* isolates obtained in this study is similar to those reported earlier in terms of their pathogenicities. Therefore, naturally occurring potential entomopathogenic fungi can be isolated directly from stored commodities to be evaluated as biological control agents for stored product pest control.

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Pulses Protein Quality Control at Different Storage Conditions for Further Protein Extraction – A Review

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Abstract

The storage conditions are of extreme importance with regards to grains (cereal & pulses) components (carbohydrates, lipids, proteins) preservation and quality for industry (that may interfere to whole process and quality of the final product). In addition, the vegetarian consumers' interest of protein supplement (capsules)

from pulses such as beans (*Phaseolus vulgaris* L.), chickpeas (*Cicer arietinum L.*), lentils (*Lens culinaris* L.), peas (*Pisum sativum* L.), peanuts (*Arachis hypogaea* L.), also soybeans (*Glycine max* L.) has grown considerably, mainly due to their non-lactose&non-animal-based ingredients and also non-transgenic in some of the pulses. Therefore, there is a need of information regarding pulses storage conditions on their components' quality/quantity and so for safety of the raw material utilized for protein extract purposes. In addition, to get safe pulses raw materials for protein extraction aimed for vegetarian supplements, one needs to take into account (a) quite controlled storage conditions, apart from (b) pesticide residues and mycotoxins contamination control. Therefore, the present reviewgathers and compiles the characterization of six different pulses by evaluating amino acids profile as indicators of protein quality, and compares them with different varieties for further protein extraction.

Key words: pulses, beans, peas, lentils, storage, protein, denaturation, fungi, mycotoxins.

1. Introduction

1.1 Pulses consumption and proteins

Pulses, are dry seeds from Fabaceae or Leguminosae family, which are cultivated throughout the world due to its easy adaptation to different climates. They are highly nutritious and play an important role on human diet (Aykroyd et al, 1982; Ofuya, 2005). They include beans (*Phaseolus vulgaris* L.), chickpeas (*Cicer arietinum L.*), lentils (*Lens culinaris* L.), peas (*Pisum sativum* L.), peanuts (*Arachis hypogaea* L.) and soybeans (*Glycine max* L.). Furthermore, vegetarian protein supplements consumption makes the pulseslikely to grow considerably, since they are lactose-free and non-animal based protein products (FAO, 2016; Aykroyd et al, 1982). Among the different pulses, the high oil content - soybeans followed by peanuts, as well as lentils and beans are the main protein sources, with 42.0, 32.0, 31.0, and 30.0%, respectively (Table 1).

1.2 Storage conditions and protein quality

There are physicochemical and biological changes that occur during pulses storage and affect considerably their components' quality and quantity. Therefore the storage conditions require a special care to maintain the quality and safety of the raw material utilized for protein extraction (Nasar-abbas et al, 2008). Data reported in the literature (especially during hot seasons) lead to protein denaturation. Inactivation starts taking place at temperature around 35°C. The same for humidity, as most proteins have globular shape, low moisture content (mc) and water activity (a_w) lead to their 3D structure alteration (the opposite - high humidity contents - lead to living organisms proliferation). That includes also the storage *length of time*, which, when pulses are kept under adverse conditions for long time, they may add to the other components reactions and so to the proteins (Labuza et al, 1982). Regarding *pH*, its reduction, causes pulses alterations (due to fermentation and oxidative reactions by fungi/bacteria & insects/mites activities to carbohydrates and lipids, respectively) leading to protein denaturation (including enzymes inactivation affecting catalysis) (Klupsaite et al, 2015). Furthermore, the Living Organisms (L.O.) infestation/ infection, such as insects, mites and fungi, can lead to protein reduction (components consumed by (L.O.), thus interfering with quality and quantity. In addition, pesticide residues (from field and storage applications) and mycotoxins (from toxigenic fungi) play a role in contamination of peas/lentils protein extracts (Cegielska et al, 2003). Regarding the impurities that may come from the field (foreign matter & broken/deteriorated/toxin contaminated pulses), despite of their pre-cleaning and cleaning reduction, they can bring also contamination to pulse in storage (Waliyar et al, 2014).

Considering the lack of information regarding specifics on pulses proximate composition, aiming the proteins and amino acids profile, also their alterations under storage conditions: the current work gathered literature data regarding pulses characteristics, storage specifics for protein quality/quantity improvement and extraction methodologies, including contaminants and impurities that may be brought into the protein process.

Tab 1. Protein percentages in the proximate composition of different pulses

Pulses	Proximate composition (%)						
	Protein	Carbohydrate	Lipid	Fiber	Ash	mc[a]	Kcal/100 g
Beans[b]	27.1-29.5	54.3-64.1	0.5-16.3	12.7-24.9	2.70-4.30	9.0-13.4	333-409
Chickpeas[b]	22.9-24.8	60.8	6.2	17.4	2.64	11.5	36
Lentils[b]	26.1-31.3	58.2	2.1	30.5	2.55	10.8	353
Peas[b]	22.8-26.5	60.4-63.2	0.8-1.5	15.0-25.5	2.70-3.45	0.6-10.6	341-343
Peanut[b]	28.8-32.0	1.8	47.0	3.7	3.80	5.8	620
Soybean[b]	38.6-42.0	29.8-33.3	22.2-23.8	5.8	5.24-5.57	5.4	172

Tiwari et al (2012); Vieira et al (1999); Atasie et al (2009); Iqbal et al (2006); Bhatty et al (1976); Radhakrishnan et al (2016); Shelepina et al (2016); [a]moisture content [b]rangefromdifferentvarieties.

2. 2. Pulses *versus* amino acids profile and proteins

2.1 Bean (*P. vulgaris*)

They represent nearly 80% of total pulse production in Latin America (Pachico, 1989), in which Brazil remains the largest grower and consumer of common beans worldwide (Torres et al, 2009; Duranti, 2006).Table 2 presents the amino acids profile and protein range among 6 Northern American (Canadian) beans varieties (Pires et al, 2005). Their protein content ranges from 27.1 to 29.5% (Freya and Ed Hoff varieties, respectively). Amino acids amounts are similar for all bean varieties, however, beans show high levels of Glutamic and Aspartic Acid, followed by Arginine. There is evidence that environmental factors such as geographic location and season may significantly influence the protein content and lipids of beans (Bhatty et al, 1976). Raw beans are also good source of water-soluble B vitamins; however, they are poor sources of lipo-soluble vitamins and vitamin C (Pires et al, 2005).

2.2 Chickpeas (*C. arietinum*)

Chickpeas are one of the oldest and most widely consumed pulses in the world, especially in India (Angulo et al, 2018; McVay et al, 2016). It is a healthy vegetarian food, a good source of fiber, vitamins (thiamine, riboflavin and niacin) and micro-minerals. However the chemical composition of crops changes with their varieties, soil and area climatic conditions (Iqbal et al, 2016). Table 3 shows the amino acid profile of different chickpea varieties grown in Pakistan. The protein content ranges from 22.9 to 24.8% (Nifa-95 and Nifa-88 varieties, respectively). Although amino acid amounts vary among varieties, they are rich in Glutamic, Aspartic Acids and Leucine (Radhakrishnan et al, 2014).

2.6 Lentils (*L. culinaris*)

Lentils are a staple food grown in West Asia, Africa and India, being their primary component of farming systems. Lentil plays a significant role in human and animal nutrition, besides the maintenance and improvement of soil health. They are particularly high in protein and low in fat (Iqbal et al, 2006), an excellent source of both soluble and insoluble fibers, as well as complex carbohydrates, B vitamins and minerals (Adsule et al., 1989; Muehlbauer et al., 1985; Yadavet al, 2007). Table 4 presents the range of protein of lentil varieties grown in Chile and Iraq (27.7 to 31.3% for Pioneer Red and Amasya varieties, respectively). Lentils show high levels of Glutamic and Aspartic Acid, followed by Arginine. ´

2.4 Peas (*P. sativum*)

Peas are widely produced in Russia and China, followed by Canada, Europe, Australia and United States (Henchion et al, 2017). Sevey (2008) showed that peas have by far the highest protein content (21-25%) and total digestible nutrients (86-87%). Pea contains 5 to 20% less Trypsin inhibitors than soybean, that allows it to be fed directly to livestock without having to go through the extrusion heating process (Amarakoon, 2012). Globulins (up to 80-90%), followed by albumins and glutenins are the main pea proteins. Separate groupings of salt-soluble proteins contain unequal amount of essential amino acid, limiting nutritional value (Methionine, Tryptophane and Cysteine). Their low content is characteristic of vicilines and viciline-related protein (Shelepina et al, 2016). Table 5

presents the range of protein (22.1-26.6%) content with the amino acids profile, showing high levels of Glutamic and Aspartic Acids, followed by Arginine.

2.5 Peanuts (*A. hypogaea*)

Also known as groundnut, is originated in South America and it's one of the world's major food pulse (oil seed) (Singh et al, 1992). It became the fourth most important source of edible oil and thethird most important source of vegetable proteins (Hammons, 1994; Savage, 1994). Its concentrate and isolate products have the advantage of removing the insoluble and partly indigestible carbohydrates (one third of the raw material) (Singh, 1991; Lusas, 1985). Protein makes from 12.0 to 36.4% of the peanuts kernel (Sekhom et al, 1970), but can also cause hypersensitivity reactions (angioedema, asthma, abdominal discomfort and anaphylactic shock), (Moneret et al, 1998), being the second food to produce such allergic symptoms, only after milk and eggs (Hammons, 1994; Yu et al, 2007). Table 6 presents Korean peanuts varieties amino acid profile (high in Isoleucine, Glutamic and Aspartic acid) and protein content (from 28.8 to 32.0% for Suwon 88 and Pungan, respectively).

2.6 Soybean (*G.max*)

The oil seed, native of Southeastern Asia, has the highest protein content and the highest gross output of vegetable oil among the cultivated crops in the world (Morse et al, 1949; Singh, 2010), with high expansion in Brazil. (Agroanalysis, 1996; Vieira et al, 1999; Fearnside, 2001). The Food and Drug Administration (FDA) approved in 1999 a health claim on food labels for products containing soy protein about the association between soy protein and a reduced risk of coronary heart disease, and the beneficial effects of isoflavones, saponins and fiber (Barbosa, 2006). Various epidemiological studies have demonstrated a lower incidence of breast, prostate and colon cancer in Asian populations (for which consumption is 20 to 50 times higher than for occidental populations).Based on six varieties aimed to human consumption, it is possible to see the difference in amino acid (Table 7). All varieties present a superior and excellent balance of essential amino acids by the FAO/WHO pattern (protein: 39.5 to 45.0 g protein) and high levels of Glutamic and Aspartic Acids, followed by Phenylalanine and Tyrosine (Vieira et al, 1999).

Tab. 2. Protein amino acids profile from BEANS (*P. vulgaris*) - Canadian varieties

Amino acid	FAO[a]	Bean - Canadian varieties (%)					
		Ed Hoff	Erfordia	Frey a	Fribo	Klein-K.	Maris Bread
ESSENTIAL							
Histidine	1.6	2.7	2.6	2.6	2.6	2.6	2.6
Isoleucine	1.3	4.3	4.2	4.3	4.4	4.3	4.2
Leucine	1.9	8.2	8.1	8.3	8.4	8.5	8.2
Lysine	1.6	6.7	6.6	6.6	6.7	6.6	6.5
Methionine	NI[c]	0.8	0.8	0.7	0.8	0.7	0.7
Cysteine (half)[b]	NI[c]	1.9	1.6	2.0	1.7	1.5	1.7
Phenylalanina	NI[c]	4.4	4.5	4.3	4.5	4.4	4.4
Tyrosine[b]	NI[c]	2.8	2.8	2.9	2.7	2.8	2.9
Threonine	0.9	3.4	3.3	3.3	3.3	3.3	3.2
Tryptophan	0.5	1.1	1.0	1.1	1.1	1.0	1.1
Valine	1.3	3.8	3.8	3.9	4.0	3.9	3.8
NON ESSENTIAL							
Arginine[b]	NI[c]	10.3	11.0	10.7	10.3	9.9	10.6
Alanine	NI[c]	4.2	4.2	4.1	4.2	4.3	4.2
Aspartic Acid	NI[c]	13.0	13.0	1.9	12.8	13.3	12.9
Glutamic Acid	NI[c]	20.2	20.4	20.2	20.2	20.3	20.4
Glycine	NI[c]	4.2	4.2	4.1	4.2	4.3	4.2
Proline	NI[c]	3.9	4.0	4.1	4.0	4.4	4.0
Serine	NI[c]	4.0	4.1	4.0	3.9	4.0	4.0
PROTEIN (%)	NI[c]	27.1	28.3	29.5	28.2	27.9	29.1

Kaldyet al (1974)[a] adults essential amino acid reference pattern from Food and Agriculture Organization, [b]considered essential or non-essential, [c]NI:NotInformed.

Tab. 3. Protein amino acids profile from CHICKPEAS (*C. arietinum*) -Pakistani varieties

Amino acid	FAO[a]	Chickpeas - Pakistan varieties (%)

		Nifa-88	Nifa-95	Kabuli Hassan-2k
ESSENTIAL				
Histidine	1.6	2.9	3.2	3.0
Isoleucine	1.3	4.5	4.8	5.2
Leucine	1.9	8.2	8.1	8.3
Lysine	1.6	6.7	7.0	7.8
Methionine	NI[c]	0.8	1.1	1.3
Cysteine(half)[b]	NI[c]	0.4	0.6	0.8
Phenylalanine	NI[c]	5.0	5.3	6.2
Tyrosine[b]	NI[c]	2.8	2.8	2.9
Threonine	0.9	2.7	3.0	3.5
Tryptophan	0.5	0.6	0.9	1.1
Valine	1.3	4.1	4.0	5.2
NON ESSENTIAL				
Arginine[b]	NI[c]	8.2	8.5	9.5
Alanine	NI[c]	5.0	5.2	4.7
Aspartic Acid	NI[c]	11.3	11.5	10.2
Glutamic Acid	NI[c]	17.6	17.8	16.5
Glycine	NI[c]	3.4	3.6	4.0
Proline	NI[c]	3.9	4.1	3.5
Serine	NI[c]	3.3	5.0	4.2
PROTEIN (%)	NI[c]	22.9	24.1	24.8

Iqbal et al (2006) [a] adults essential amino acid reference pattern from Food and Agriculture Organization, [b]considered essential or non-essential, [c]NI: NotInformed.

Tab. 4. Protein amino acids profile from LENTIL (*L.culinaris*) - Chilean and Persian varieties

Amino acid	FAO[a]	Lentil - Chilean and Persian varieties (%)					
		Tekoa	Morden	Amasaya	Eskiseher	Pioneer Red	Sloven
ESSENTIAL							
Histidine	1.6	2.3	2.0	2.3	2.0	1.9	2.1
Isoleucine	1.3	3.4	3.7	3.5	3.3	3.5	3.7
Leucine	1.9	6.1	6.7	6.4	6.1	6.3	6.7
Lysine	1.6	6.3	6.5	6.5	5.9	6.1	6.3
Methionine	NI[c]	0.6	0.6	0.6	0.5	0.7	0.6
Cysteine(half)[b]	NI[c]	1.4	1.2	1.3	.12	1.4	1.5
Phenylalanine	NI[c]	3.9	4.3	4.1	3.9	4.1	4.3
Tyrosine[b]	NI[c]	2.4	2.4	2.1	2.3	2.4	2.4
Threonine	0.9	3.0	3.2	3.1	3.0	3.2	3.3
Tryptophan	0.5	0.9	0.9	0.9	0.9	1.0	0.8
Valine	1.3	3.9	4.1	4.1	3.8	4.1	4.0
NON ESSENTIAL							
Arginine[b]	NI[c]	6.8	6.4	7.0	7.3	6.6	7.1
Alanine	NI[c]	3.6	3.8	3.6	3.4	3.7	3.9
Aspartic Acid	NI[c]	9.4	9.7	10.0	9.8	10.0	10.3
Glutamic Acid	NI[c]	13.5	14.2	14.1	13.4	14.1	14.8
Glycine	NI[c]	3.5	3.3	3.5	3.3	3.3	3.7
Proline	NI[c]	3.9	4.0	4.1	4.0	4.4	4.0
Serine	NI[c]	3.4	3.4	3.3	3.3	3.5	3.6
PROTEIN (%)	NI[c]	28.3	26.1	30.3	31.3	27.7	28.9

Bhatty et al (1976) [a] adults essential amino acid reference pattern from Food and Agriculture Organization, [b]considered essential or non-essential, [c]NI:NotInformed.

3. Pulses composition

3.1 Protein and nutritional quality

Protein-calorie malnutrition is a big problem that has been affecting mankind, also indicating that the protein gap may increase in the future unless something addresses the issue. Access adequate proteins of animal origin are difficult and expensive (Reach, 2012). An alternative to improve the nutritional status of those countries is to supplement the diet with pulse proteins (Leterme, 2002).

Tab. 5. Protein amino acids profile from PEAS (*P. sativum*) - Russian varieties

Amino acid	FAO[a]	Pea - Russian varieties(%)			
		Express	Highlight	Baroness	Titan
ESSENTIAL					
Isoleucine	1.3	4.7	4.4	4.6	4.6
Leucine	1.9	7.5	7.2	7.1	7.2
Lysine	1.6	7.7	7.6	7.8	7.8
Methionine(half)	NI[c]	2.8	2.6	2.5	2.5
Cysteine(half)	NI[c]	2.5	2.5	2.5	2.5
Phenylalanine	NI[c]	5.2	4.9	5.0	5.0
Tyrosine[b]	NI[c]	3.6	3.6	3.7	3.5
Threonine	0.9	4.3	4.1	3.9	4.0
Valine	1.3	5.1	4.9	5.0	5.0
Histidine	NI[c]	2.6	2.6	2.5	2.5
NON ESSENTIAL					
Arginine[b]	NI[c]	8.5	8.5	8.6	8.6
Alanine	NI[c]	4.6	4.5	4.6	4.6
Aspartic Acid	NI[c]	11.5	11.4	11.9	11.8
Glutamic Acid	NI[c]	16.6	16.5	16.7	16.7
Glycine	NI[c]	4.6	4.7	4.5	4.6
Proline	NI[c]	4.4	4.5	4.5	4.9
Serine	NI[c]	4.9	5.0	4.9	4.9
PROTEIN (%)	NI[c]	25.0	26.5	22.8	25.1

Shelepina et al (2016) [a] adults essential amino acid reference pattern from Food and Agriculture Organization, [b]considered essential or non-essential, [c]NI: NotInformed.

Tab. 6. Protein amino acids profile from PEANUTS (*A. hypogaea*) - Korean varieties

Amino acid	FAO[a]	Peanut - Korean varieties (%)			
		Suwon 88	Daewon	Daekwang	Sonan
ESSENTIAL					
Histidine	1.6	0.6	0.6	0.5	0.6
Isoleucine	1.3	7.8	7.7	6.9	7.8
Leucine	1.9	1.8	1.8	1.6	1.8
Lysine	1.6	0.9	0.9	0.8	0.9
Methionine	NI[c]	2.2	2.5	2.6	2.4
Cysteine(half)[b]	NI[c]	0.2	0.2	0.2	0.1
Phenylalanine	NI[c]	1.4	1.4	1.2	1.4
Tyrosine[b]	NI[c]	1.0	0.9	0.8	1.0
Threonine	0.9	0.7	0.7	0.6	0.7
Valine	1.3	1.0	1.0	0.8	1.0
NON ESSENTIAL					
Arginine[b]	NI[c]	3.0	3.0	2.7	3.0
Alanine	NI[c]	1.1	1.0	0.9	1.0
Aspartic Acid	NI[c]	3.1	3.1	2.7	3.1
Glutamic Acid	NI[c]	5.3	5.3	4.8	5.4
Glycine	NI[c]	1.6	1.6	1.6	1.5
Proline	NI[c]	1.1	1.0	0.9	1.7
Serine	NI[c]	1.4	1.4	1.3	1.4
PROTEIN (%)	NI[c]	32.0	31.4	28.8	31.8

Radhakrishnan et al (2016) [a] adults essential amino acid reference pattern from Food and Agriculture Organization, [b] considered essential or non-essentia, I[c]NI: NotInformed.

Tab. 7. Protein amino acids profile from SOYBEAN (*G. max*) - Brazilian varieties

Amino acid	FAO[a]	Soybean - Brazilian varieties (%)					
		IAS-4	Embrapa-4	Davis	BR-16	Iguaçu	IAS-5

ESSENTIAL							
Histidine	1.6	1.9	1.8	1.9	2.0	2.4	2.4
Isoleucine	1.3	1.4	3.5	3.8	3.7	4.1	4.0
Leucine	1.9	7.8	7.1	7.4	7.3	7.8	7.9
Lysine	1.6	6.4	5.6	5.8	6.0	6.8	6.9
Methionine	NI[c]	1.3	1.2	1.3	1.3	1.4	1.3
Cysteine[b]	NI[c]	2.5	2.0	2.5	2.2	2.4	2.3
Met+Cys	1.7	3.8	3.2	3.8	3.5	3.8	3.6
Phenylalanine	NI[c]	6.2	6.1	6.2	6.0	6.5	6.7
Phe+Tyr	1.9	9.4	9.1	9.5	9.1	9.9	10.2
Threonine	0.9	3.9	3.5	3.9	3.8	4.0	4.1
Tryptophan	0.5	1.7	1.6	1.5	1.6	1.5	1.5
Valine	1.3	4.1	4.1	4.3	4.1	4.6	4.4
NON ESSENTIAL							
Arginine[b]	NI[c]	7.4	7.4	6.8	6.8	7.3	7.2
Alanine	NI[c]	4.3	3.8	4.0	3.9	4.2	4.4
Aspartic Acid	NI[c]	12.8	13.1	14.2	13.7	14.6	14.7
Glutamic Acid	NI[c]	18.3	20.9	22.1	20.7	23.6	23.3
Glycine	NI[c]	3.7	3.6	3.6	3.4	3.8	3.9
Proline	NI[c]	8.3	7.5	7.9	7.6	8.0	8.7
Serine	NI[c]	5.3	4.8	5.2	5.0	5.5	5.0
PROTEIN (%)	NI[c]	9.30	41.95	38.55	38.56	38.57	40.17

Vieira et al (1999) [a] adults essential amino acid reference pattern from Food and Agriculture Organization, [b] considered essential or non-essential, [c]NI: NotInformed.

According to FAO (2011), it is necessary to increase food production by 70%. It should be satisfied by the most promising and valuable crops (low-cost and complete vegetable protein). The main types of protein preparations obtained from pea, for example, are defatted flour (56–59% of protein), concentrated pea protein (65–72% of protein) and isolate (90% of protein). According to Shelepina et al (2016), in terms of amino acid composition and assimilation of pulses protein (isolates and concentrates), they are close to proteins of animal origin.

Pulses have many good qualities and can be considered most suitable for protein extraction. They are mainly storage proteins belonging to the groups of albumins, globulins and glutelins, with the salt-soluble globulins constituting the main proteins found in the seeds. These proteins are part of the defensive mechanism of the seed, but are considered as anti-nutritional factors for the human diet (Tiwari et al, 2011).

Apart from their nutritional properties, pulses proteins also possess functional properties (gelling and emulsifying), playing an important role in food formulation and processing. Intrinsic factors (amino acid composition), extrinsic (pH, temperature, solvent, salt) or environmental factors, besides processing treatments (heating, drying, concentrating) or other intentional modifications (chemical or enzymatic modification) can all contribute to influence the functional properties of these proteins (Klupsaite et al, 2015).

3.2 Pulses anti-nutritional factors

To reduce the ANFs and improve quality, seeds are processed by several physical and chemical methods to remove the undesirable compounds: soaking, cooking, germination, fermentation, selective extraction, membrane filtration, irradiation and enzyme treatments (Vidal-valverde, 1994). Those treatments lead to a significant reduction or total elimination of ANFs. Germination and fermentation processes lead to catabolism of the seed components whereas other processes (cooking) may cause thermal degradation or may involve extraction of non-nutritional components. ANFs found in pulses can be separated into several groups based on their chemical and physical properties (non-protein amino acids, quinolizidine alkaloids, cyanogenic glycosides, pyrimidine glycosides, isoflavones, tannins, oligosaccharides, saponins, phytates, lectins or protease inhibitors) (Bell and Charlwood, 1980). Many ANFs are toxic, unpalatable or indigestible, but can be eliminated

by selection of plant genotypes or postharvest processing (germination, boiling, leaching, fermentation), including protein extraction (Ali et al, 2000).

4 Pulses` contaminants and storage conditions

The quality of post-harvest conditions (cleaning, sorting, drying, storage, transport and marketing) can cause high amount of losses and health risks that conflict with national and international standards regulations. It is important to avoid the microorganism's development, especially due to the fungi contamination, with potential production of micotoxins (Anderson, 1954).

Freshly harvested pulses can be safely stored in silos, with a filling and a proper sealing to prevent its deterioration. During uploading, the quantity of air must be minimized, decreasing pulses and microorganism's respiration (21- 0.02%) and pH (due to fermentation). Immediate treatment is essential to prevent quality and quantity deterioration from mold development, ensuring short and long-term preservation of pulses. That includes sealed storage, chemical treatment, chilling and drying. Otherwise, pulses were not being appropriate to further protein extraction (Brooker, 1992)

4.1 Environmental contaminants

It is becoming increasingly apparent that although the major part of the food supply is both safe and nutritious, some risk is unavoidable. The very nature of the industrial society, which includes a substantial part of the world`s population, has increased the risk that foods may become contaminated by a wide variety of chemicals introduced into the environment by man, intentionally or accidentally (Hathcock, 2012).

The polychlorinated biphenyls, polybrominated biphenyls, aflatoxins, nitrites, nitrates and nitroso compound, and metals such as mercury and lead, havedifferent contaminant properties which make them potential problems in the environment. That includes relatively widespread use or distribution of the chemical, in some cases a long biological half-life persistence in the environment, increased residue levels along the food chain, and the potential of an increased risk for adverse health effects in human and food producing animals (Cordle et al, 1982; Hathcock, 2012).

4.2 Fungal and mycotoxins contamination *versus* storage conditions

A high number of toxic microbial metabolites contaminates agricultural products due to the diversity of fungal and bacterial species that coloniesthem from field to storage (Maciorowskiet al, 2007). One of the most important effects of post harvest decays of seed and feed by fungi is the induction of mycotoxicoses, in animals and humans. This is caused by foods and feed consumption invaded by toxigenic fungi (producing mycotoxins). They are secondary metabolites produced by filamentous fungi which may contaminate food, feed and raw materials used in producing them (Agrios, 1978; Moss, 1989; Amadi et al, 2009; Savi et al, 2015; Scussel et al, 2018). Mycotoxicoses caused by widespread fungi such as *Aspergillus*, *Penicillium*, *Fusarium* and *Stachybotrys* can result in severe illness and death. *Aspergillus* and *Penicillium* produce their toxins mostly in stored seeds, hay or commercially processed foods and feeds although infection of seeds usually takes place in the field. Adams (1977) has reported that storage fungi especially *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* species infect grains after harvest and can grow on them during storage, although other toxigenic fungi are also found on grains, such as *Alternaria*, *Trichoderma*, *Fusarium*, *Paecilomyces*, *Chaetomium* and *Acremonium*. Generally, mycotoxins have been implicated as causative agents of different health disorders (Ciegler and Bennett, 1980). Both, the toxigenic fungi and the mycotoxins they produce, are potential problems economy perspectives wise. Aflatoxinsare the most commonand widespread mycotoxin, and it is known to be produced by different species of *Aspergillus* (*A.flavus, A.oryzae and A.terreus*). The liver is the target organ, but it is also hepatoxic and carcinogenic (Eaton et al, 1994). While more than 25 different species of fungi are known to invade stored grains and pulses (Duan et al., 2007), species of *Aspergillus* and *Penicillum* are responsible for most spoilage and germ damage during storage. They cause reduction on cooking / baking quality, nutritive values, produce undesirable odors and color, also change appearance of stored food grade

grains. In addition, they make products unacceptable for edible purposes or lower their market quality (Embaby et al, 2003).

5. Conclusions

Pulses are widely cultivated, and a good food resource, once it has easy adaptation to different climates and highly nutritious. It is considered most suitable for protein isolates preparation due to their high protein content, low cost and wide acceptability. Several methods are used for protein extraction and every technique has its own advantages. The functionality of pulse proteins is closely related to their physical and chemical properties, such as molecular weight, amino acid composition and sequence, structure, surface electrostatic charge, and effective hydrophobicity. Storage conditions (impaired/ aggressive temperature/humidity) can lead to physical, chemical, enzymatic or genetic proteins modifications thus altering their functionality, interfering also to food industries final products (Klupsaite et al, 2015).

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Mites in aromatic, condiment and medicinal dehydrated plants in bulk sale in the city of São Paulo.

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Mites infest stored goods, especially when the environment is hot and humid. Infested foods may have their taste altered and, in some cases, cause diseases to consumers. In this way, the detection of arthropods in food must be carried out throughout the production chain, as the external and internal markets are increasingly demanding for the quality and health of food. Aromatic, condiment and medicinal dehydrated plants are largely sold in bulk, but little is known about infestations by mites. The objective of this work was to evaluate the diversity of mites in 10 samples of *Coriandrum sativum*, *Pimpinella anisum*, *Petroselinum sativum*, *Chamomila recutita*, *Baccharis trimera*, *Bixa Orellana*, *Cassia angustifolia*, *Origanum vulgare*, *Ocimum basilicum*, *Melissa officinalis*, *Mentha piperita*, *Rosmarinus officinalis*, *Peumusboldus*, *Salvia officinalis*, *Thymus vulgaris*, *Laurus nobilis*, *Hibiscus sabdariffa*, *Myristica fragans*, *Capsicum annum* and *Curcuma longa* acquired in the establishments of bulk sale in the city of São Paulo. A total of 2,589 specimens of mites corresponding to 10 species, *Tyrophagus putrescentiae*, *Glycyphagus destructor*, *Ameroseius* sp., *Blattisocius tarsalis*, *Typhlodromus transvaalensis*, *Tetrabdella* sp., *Cheyletus malaccensis*, *Pronematulus* sp., *Raphignatus* sp. and *Tydeus* sp. The mite *Typhlodromus transvaalensis* was recorded for the first time in Brazil infested stored products.

Key words: Mite, stored products.

Mitochondrial genome organization varies among different groups of the booklouse, *Liposcelis bostrychophila*

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Abstract

The booklouse, *Liposcelis bostrychophila* is an important stored pest worldwide. The mt genome of an asexual strain (Beibei, China) of the booklouse, *L. bostrychophila*, comprises two chromosomes; each chromosome contains approximate half of the 37 genes typically found in animals. The mt genomes of two sexual strains of *L. bostrychophila*, however, comprise five and seven chromosomes respectively; each chromosome contains one to six genes. To understand mt genome evolution in *L. bostrychophila*, we sequenced the mt genomes of six strains of asexual *L. bostrychophila* collected from different locations in China, Croatia and USA. The mt genomes of all of the six asexual strains of *L.* bostrychophila collected in China, Croatia and USA have two chromosomes. Phylogenetic analysis of mt genome sequences divided nine strains of *L. bostrychophila* into four groups. Each group has a distinct mt genome organization and substantial sequence divergence (48.7-87.4%) from other groups. Furthermore, the seven asexual strains of *L.* bostrychophila including the published Beibei strain are more closely related to two other species of booklice, *L. paeta* and *L. sculptilis*, than to the sexual strains of *L. bostrychophila*. Our results revealed highly divergent mt genomes in the booklouse, *L. bostrychophila*, and indicated that *L. bostrychophila* is a cryptic species.

Keywords: Mitochondrial genome, *Liposcelis bostrychophila*, intraspecific variation, cryptic species, evolution

Extended abstract

The booklouse, *Liposcelis bostrychophila* Badonnel is an important pest of stored products around the world (Nayak *et al.* 2014) and has two types of reproductive mode: parthenogenesis and sexual reproduction (Mockford *et al*. 2008). Its interception number at a number of entry points into China is increasing with the development of international trade. DNA barcode was used to identify different species of booklice. However, it cannot distinguish *L. bostrychophila* as different strains for this species differs greatly in *cox1* gene fragment sequences. The *cox1* gene belongs to the mitochondrial (mt) genome which included normally 13 protein coding genes, two ribosome genes and 22 transfer RNA genes. The sequence diversity in cox1 gene of different strains of *L. bostrychophila* implied there may be divergence in mt genome sequences in intra-specific level (Yang *et al.* 2013). The mt genome of *L. bostrychophila* was reported to split into two minichromosomes (Wei *et al.* 2012). Every minichromosome accounted for a half the length and the gene number of regular mt genomes. However, the sexual *L. bostrychophila* collected outdoors was reported to have five or seven minichromosomes in their mitochondrial genomes which added the complexity of this species (Perlman *et al.* 2015; Yang *et al.* 2015). Subsequently, to explore further the mt genome variations in *L. bostrychophila*, we sequenced the mt genomes of six strains of asexual *L. bostrychophila* collected from different locations in China, Croatia and USA.

To reconstruct the mitochondrial genomes of the six strains of *L. bostrychophila*, *cox1*, *rrnS* and *rrnL* gene fragments were chosen as "anchors" to get the mitochondrial genome sequences. We firstly sequenced the *cox1*, *rrnS* and *rrnL* gene fragments by using universal primer pairs (Folmer *et al*. 1994; Kambhampati *et al.* 1995). Then, Long PCR primers were designed to amplify the chromosomes where the gene fragments located. The prepared libraries were then sent to the BGI company for next generation sequencing by using an Illumina sequencer. The mt genomes of all six asexual strains of *L. bostrychophila* collected in China, Croatia and USA have two chromosomes (Figure 1). The six newly sequenced mt genomes could be divided into three groups based on their mt genome rearrangements and sequence similarities. Each group has a distinct mt genome organization and substantial sequence divergence (48.7-87.4%) from other groups. Furthermore, all published mt genomes in *Liposcelis* genus, including one published asexual strain in China (Wei *et al.* 2012) and two published sexual strains of *L. bostrychophila*, *L. entomophila* (Chen *et al.* 2014)*, L. paeta, L. decolor* (Chen *et al.* 2014) and *L. sculptilis* (Shi *et al.* 2016) together with data in this research were included in the phylogenetic analysis. After fundamental bioinformatic analysis and annotation, phylogeny of the genus *Liposcelis* was inferred by using MrBayes (Ronquist *et al.* 2003) and RAxML (Stamatakis *et al.* 2006) softwares with two concatenated datasets. Phylogenetic analysis of mt genome sequences divided nine strains of *L. bostrychophila* into four groups. The seven asexual strains of *L. bostrychophila* are more closely related to *L. paeta* and *L. sculptilis*, than to the sexual strains of *L. bostrychophila*. The two sexual strains formed the monophyly.

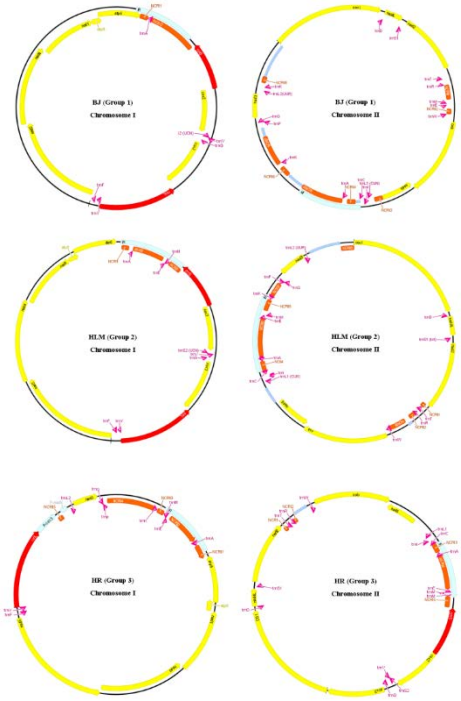

Figure 1. The mitochondrial genome organizations of three groups of *L. bostrychophila*. The transcriptional direction is indicated with arrows. Coding genes are shown in grey, non-coding regions in black, the identical region between the two chromosomes in white. Abbreviations of gene names are: cox1–3 for cytochrome oxidase subunits 1–3, cob for cytochrome b, nad1–6 and nad4L for NADH dehydrogenase subunits 1–6 and 4L, rrnL and rrnS for large and small rRNA subunits, atp6 and atp8 for ATP synthase subunits 6 and 8. tRNA genes are indicated with their one-letter corresponding amino acids.

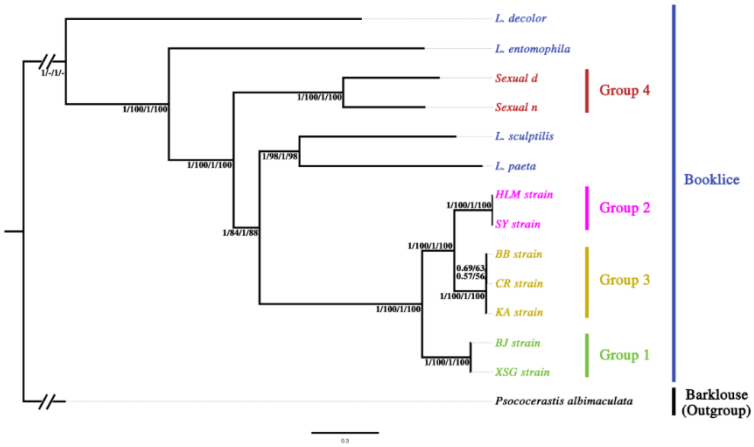

Figure 2. Bayesian inference (BI) and Maximum likehood (ML) phylogenetic trees inferred from mitochondrial genomes of booklice. Numbers above the branches show support for tree nodes from nucleotide sequences of the two datasets: Bayesian posterior probability of PCG12, ML bootstrap support values of PCG123, Bayesian posterior probability of PCG12, ML bootstrap support values of PCG123. Group 1 is in green, Group 2 in pink, Group 3 in brown, Group 4 in red, other species of booklice in blue, the outgroup in black.

Our results revealed highly divergent mt genomes in *L. bostrychophila* and indicated that *L. bostrychophila* is a cryptic species. Cryptic species is a common question in plant quarantine, mt genome sequencing and phylogenetic analysis maybe as one way to resolve it.

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