Understanding the physical and biological effects of dust-induced insect death

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not been previously submitted for a degree at any tertiary educational institution.

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31 August 2018

A note on formatting and style

This thesis comprises a number of published papers as well as some submitted or ready to submit manuscripts, each of which represents a chapter. In these cases, the formatting style follows the respective journal guidelines.

This thesis has continuous pagination. For published documents, the original journal page numbers are also provided

Yanyu Li

STATEMENT OF CONTRIBUTION

This thesis includes three original papers published in peer reviewed international conference proceedings and four unpublished publications. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the school of veterinary and life sciences under the supervision of Professor Yonglin Ren, Professor Yang Cao, Dr Manjree Agarwal and Dr David Eagling.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

My contribution to the work and those of the co-authors involved the following:

Thesis Chapter	**Publication Title**	**Status**	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution**
Chapter 1	Literature review--New non-chemical technologies (inert dust-SAS powder) to control insect pests in grain during storage and transport	Placed in this thesis	60%, Concept, bibliographic search, and writing manuscript	David Eagling, concept, input into manuscript, 5% Manjree Agarwal, concept, input into manuscript, 10% Yang Cao, concept and input into manuscript, 10% Yonglin Ren, concept, input into manuscript, 15%
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The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work.

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ABSTRACT

Background

Quality and quantity of stored grain is constantly changing due to insect and fungal activity. The efficacy of storage method dictates the quality of grain. Traditional chemical pesticides, though effective, were often criticised for issues like increasing insect resistance, chemical residue, environmental contamination and human health risk. The diatomaceous earth based formulations could reduce chemical pesticides usage at some extent. But the slow insect killing and being non-food grade limited wide application. The high recommended dosage (500 to 3500 ppm) results in several adverse effects on grain, including reduce in the flow ability and bulk density, visible residue, extra dust generation during processing. Synthetic amorphous silica (SAS) consists three types: pyrogenic, precipitated, surface-treated SAS. These dusts can be distinguished from natural amorphous silica such as diatomaceous earth by its high chemical purity, the finely particulate nature and characteristics of particles. All types of SAS have been widely used in topical and oral medicines, food and cosmetics for many decades without evidence of adverse human health risks. Based on extensive physico-chemical, ecotoxicology, human health and epidemiology data, SAS as non-chemical method for pest management is revolutionary and advantageous compared to traditional approaches. However, their insecticidal mechanism is poorly understood. The optimal application protocol is not developed. This study described a comprehensive investigation of insecticidal mechanism of SAS particles and their application as an alternative practical stored grain pest control method.

Results

The first study was aimed to investigate the efficacy of different synthetic amorphous silica (SAS) powders against different insect species at multiple developmental stages compared with diatomaceous earth (DE). The stationary stages, egg and pupa, were more tolerant than that of the mobile stages, larva and adult, upon SAS and DE exposure. The insect infestation

cannot be completely control by all the SAS and DE. A 100% of hatching rate was observed and more than 32% of pupa emerged in all the dust treated groups. Larva stage was most susceptible to the SAS and DE. Newly emerged adults were more susceptible to SASs and DE than older adults. The outcome for larvae was opposite. Among the three insect species adults, when treated by SAS and DE, *T. castaneum* was the most tolerant species and *C. ferrugineus* was the most susceptible. The efficacy of SAS against insects was higher than that of DE. Among of SASs, precipitated SAS performed better than pyrogenic SAS in term of mortality. Hydrophobic SAS powders were more effective against *T. castaneum* adult, while hydrophilic SAS powders were more effective against *T. castaneum* larvae, pupae and *Sitophilus oryzae* adults.

We evaluated the physical property of aforementioned SAS and DE in relation to efficacy. SAS powders have higher specific surface area, total pore volume, oil sorption capacity and smaller particle size than DE. In term of the SAS powders produced by different methods, pyrogenic SAS powders had higher oil sorption capacity but lower total pore volume and specific surface area, and larger particle size than precipitated SAS. Comparing with hydrophilic SAS, the particle size of hydrophobic SAS was smaller while has lower oil sorption capacity. There was a significant relationship between physical property of powder and insecticidal efficacy in SAS without a specific index.

We developed and evaluated a rapid screening protocol to identify electrostatic charge dictates attachment processes during initial contact between SAS and insects. The charge ability of three major stored grain insects, *Sitophilus oryzae*, *Tribolium castaneum* and *Cryptolestes ferrugineus* and four hydrophilic precipitated SAS and one DE was assessed on two insulated surfaces filter paper and glass. After contact with insulation surfaces, synthetic amorphous silica (SAS) and DE carry negative charges due to attaining electrons from insulation surfaces, while stored product insects carry charges of opposite polarity from electron loss. According to Coulomb’s law, the SAS particles would then be passively attracted by insect via the mere effect of electrostatic forces. A linear correlation was observed between electrostatic charge and bioactivity of dust.

After exposure to SAS, the changes in water content and other physiological components of insects led to changes in coloration and gross appearance. The heterogeneous distribution made visual comparisons difficult. Hyperspectral imaging systems with optically tuneable filters can record images at hundreds of contiguous wavelengths (narrow spectral resolution) in the form of a hypercube (three-dimensional hyperspectral data). Hyperspectral imaging coupled with back propagation neural network models was employed to quantify differences in parameters which reflected the response of *T. castaneum* and *S. oryzae* to hydrophobic and hydrophilic precipitated SAS. The presence of SAS on ventral and dorsal cuticle of two insect species caused differential values of relative reflectance in visible and short-wave near-infrared ranges. The control samples of all groups were correctly classified by BPNN model and misclassification occurred only with the two SAS treated. These results suggested that the differences in absorption characteristics of cuticular fat and protein contributed to the varied performance. The recognition rate between two SAS treated was within the acceptable identification range. This suggested that both SASs have similar effect on insect with varied degree.

We investigated how these two hydrophobic and hydrophilic precipitated SASs physically influenced insect in intersegmental membrane and their biological effects. Both SASs rapidly reduced insect locomotion to the limiting value within 3.5 hours and 12 hours for *S. oryzae* and *T. castaneum*, respectively. In addition, we found that there was significant differential decrease in straightness and upward length which were used as parameters to evaluate insect behaviours. Environmental scanning electron microscope (ESEM) images and data of stride length directly exhibited SAS eroded insect intersegmental membrane and absorbed the vital body fluid, eventually caused irreversible structural damage. The hydrophilic SAS was more effective in changing these parameters in *S. oryzae,* while hydrophobic SAS was more effective in *T. castaneum*. Male population was more susceptible than female.

We further evaluated the efficacy of SAS structural treatment combined with a new integrated trap as insect control in the field trial. Insect infestation was monitored by integrated trap utilising insect behaviours. Prior to SAS treatment, five integrated traps captured 1722 g insect inside a warehouse in seven day. Synthetic amorphous silica was aerogelize and dispersed uniformly in different locations of the warehouse. The mortality of five major species of stored grain insect adults reached 100% within three days post exposure.

Conclusion

SAS powders are food-grade, quick, effective, low cost and easy to apply as an insect control method. They don’t have the disadvantages of traditional chemical pesticide regarding to occupational health, environmental and safety concern. Detecting the electrostatic charge is an effective protocol for SAS efficacy evaluation. As an emerging non-destructive and reagent-less analytical technique, hyperspectral imaging proved to be highly efficient in pesticidal effect evaluation. Intersegmental membrane is a promising target site for new inert dust pesticide products.

LIST OF PUBLICATIONS

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ABBREVIATIONS

ABS	Acrylonitrile-Butadiene-Styrene
ANN	Artificial neural network
ANOVA	Analysis of variance
ASAG	Academy of State Administration of Grain, Beijing, China
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
BH-CF	*Cryptolestes ferrugineus* was first collected in Beihai, Guangxi, China
BH-OS	*Oryzaephilus surinamensis* was first collected in Beihai, Guangxi, China
BPNN	Back propagation artificial neural network
CA	Controlled atmosphere
CAS	Chemical Abstracts Service
DE	Diatomaceous earth
df	Degrees of freedom
CL	Confidence interval
D(v, 0.1)	The respective diameters at 10% cumulative volume
D(v, 0.5)	The respective diameters at 50% cumulative volume
D(v, 0.9)	The respective diameters at 90% cumulative volume
EPA	Environmental Protection Agency
ESEM	Environmental scanning electron microscope
F	F value
Fig	Figure
Figs	Figures
GRAS	Generally Recognized as Safe
GZ-SZ	*Sitophilus zeamais* was first collected in Guangzhou, Guangdong, China
Hz	Hertz
IARC	International Agency for Research on Cancer
IPM	Integrated pest management
IR	Infrared radiation
ISO	International Organization for Standardization
KD	Know down
KD_{50}	Knockdown 50% of the population
KD_{95}	Knockdown 95% of the population
Kg	Kilo gram
kW	Kilowatt
LD_{50}	Lethal dose for control 50% of population
LD_{95}	Lethal dose for control 95% of population
LED	Light-emitting diode

MU	Murdoch University
NIR	Near infrared light
OP	Organo phosphorous
PC	Principal component
PCA	Principal component analysis
PCs	Principal components
QH-TC	*Tribolium castaneum was first collected in Qihe, Shandong, China*
RH	Relative humidity
ROI	Region of interest
SAS	Synthetic amorphous silica
SAS1	Hydrophilic precipitated synthetic amorphous silica
SAS2	Hydrophilic precipitated synthetic amorphous silica
SAS3	Hydrophilic precipitated synthetic amorphous silica
SAS4	Hydrophilic precipitated synthetic amorphous silica
SAS5	Hydrophobic precipitated synthetic amorphous silica
SAS6	Hydrophilic precipitated synthetic amorphous silica
SAS7	Hydrophilic precipitated synthetic amorphous silica
SAS8	Hydrophilic pyrogenic synthetic amorphous silica
SAS9	Hydrophobic pyrogenic synthetic amorphous silica
SE	Standard Error
SEM	Scanning electron microscope
Sig	Significance level
SNK	Student-Newman-Kreuls
SNP	Silica nanoparticles
SPSS	Statistical Package for the Social Sciences
TEOS	Tetraethylorthosilicate
2-D	2-dimensional
3D	3-dimensional
TZ-SO	*Sitophilus oryzae* was first collected in Tongzhou, Beijing, China
Vis	Visible light
UV	Ultraviolet light
WH-RD	*Rhizopertha dominica* was first collected in Wuhan, Hubei, China
ZJ-CF	*Cryptolestes ferrugineus* was first collected in Zhanjiang, Guangdong, China

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Chapter 1. Introduction and literature review

1.1 General introduction

Food supply is an urgent global issue due to fast paced population growth and farmland deterioration (Boserup, 2017). The large time gap between harvest and utilization only exacerbates supply difficulties. Transport and process are often time consuming. As a result, both short-term and long-term storage is required post-harvest. The efficacy of storage method dictates the quality of grain and ultimately the maximal market price. A steadily increased demand in high quality grain is evident worldwide. In light of this global trend, we set out to review the management of insect pests in grain storage facilities and the potential and efficacy of inert dust as an alternative insecticide. The scope of this review covers the period from 1935 until July of 2018. A Dialog® computer-based evaluation search was conducted in the CAB Abstracts, 1975-2016; Biosis Previews, 1975-2016; Life Sciences Collection, 1985-2016; Agricola, 1975-2016; Agris International, 1975-2016; European Directory of Agrochemical Products; Oceanic Abstracts, 1975-2016; and Google and internet search accessed until 25th July 2018.

1.1.1 Stored grain

Grains, oilseeds, legumes and their finished products or their by-products are not always consumed immediately after harvesting or processing, thus they frequently must be stored for relatively long periods. Due to their relative durability in storage, their value is not only as a food for mankind, but also it is safe to say, for the successful development of agriculture by man. Hence, the evolution of civilization would have been impossible if stored grain had not existed. We provide grain with the stable conditions in which it can be stored from season to season. Nevertheless, it is a mistake to suppose that stored grain may be regarded as a perfectly stable material which may be treated like sand or bricks and left without any attention in any sort of storage. Grain is energy-rich, oxygen consuming and heat generating. Quality and nutritional changes in grain are unavoidable side effects of the storage process. Grain factors, environmental factors both organic and inorganic, and management methods determine the outcome at the end of the storage period (Jayas et al., 1994). Also, quality and quantity of stored grain are constantly changing (Oxley, 1949) due to fungal and insect pest activity. Insect infestation is deemed one of the most important factors, because it accounts for major loss of volume and the affected grain is automatically rejected by many countries (Emery et al., 2003). Therefore, storage difficulties must be fairly recognized and properly studied.

1.1.2 Stored product insects

Of the very many known insect species there are perhaps 100 which are responsible for damage to stored products and of these about 20 are major pests of cosmopolitan distribution. The stored product insect found in stored cereal grains, grain products and grain legumes as an infesting pest is not a new problem. The earliest records of insects associated with stored food products are those of a flour beetle which was found in an Egyptian tomb dating back to 2500 *B.C.*, and of “beetles and weevils” which were found in the tomb of Tutankhamen (1390-1380 *B.C.*) (Munro, 1966).

Most storage insects are found worldwide occupying various niches, according to commodity and weather conditions, although a few species are not always found in some countries (e.g., *Trogoderma granarium* Everts in Australia). The two main insects in Australia are the grain beetles *Rhyzopertha dominica* (F.) and *Tribolium castaneum* Herbst, presumably because these species can survive on the generally dry grain under dry Australian weather conditions. The rice weevil (*Sitophilus oryzae* (L.)) and the maize weevil (*Sitophilus zeamais* Motschulsky) are often found on rice and maize in China.

Storage insects are largely cosmopolitan. Some species damage whole cereals, legumes and grains, or solid cereal products. Some are cosmopolitan pests of grain products, such as flour, damaged grain and dried fruit. Some are scavengers or mould feeders. Stored product insects not only consume these materials but also contaminate them with insect fragments, faeces, webbing, and a variety of microflora (Snelson, 1987), which reduces commercial value. They can also increase the moisture content in grain locally, giving rise to mould growth. They therefore constitute a major sanitation and quality control problem.

1.1.3 Stored product insect management

Integrated Pest Management (IPM) that reduces infestation and avoids economic damage in raw commodities, food storage facilities, and milling and processing plants, typically involves chemical control methods (Zettler and Arthur, 2000). Since the 1950s, synthetic pesticides have been widely utilized in large grain bulks for their high efficacy, relatively low cost and ease of application. Common fumigants include phosphine, methyl bromide, chloropicrin and dichlorvos, among which methyl bromide and phosphine (PH_3) are the most widely used. Methyl bromide has been phased out of general use since 2005 due to environmental concerns as it depletes atmospheric ozone. Phosphine is the only registered fumigant which is likely to be continuously used in large scale given its favorable characteristics such as low sorption and rapid desorption in commodity fumigation. Yet, phosphine resistance in insects has been frequently reported across the world (Daglish and Collins, 1998) and is threatening its future use

(Benhalima et al., 2004; Collins et al., 2005; Herron, 1990; Lorini and Galley, 1999; Zettler et al., 1989; Zettler and Cuperus, 1990).

Controlled atmosphere (CA) is a method that achieves insect pest control by storing grain in a low oxygen, high nitrogen atmosphere (Shejbal, 1980). Theoretically, this is very efficient, yet in practice, long exposure times are required to ensure the desired result. An air tight facility is mandatory to maintain the nitrogen gradient which is unachievable in most storage settings.

Inert dust is a nonchemical method for management of stored grain insect pests. Commercial products in this category are immune to degradation due to temperature fluctuation, thus they provide protection throughout the storage period. Low environmental requirement makes this method compatible to all types of storage facilities. Inert dust has minimal mammalian toxicity. The oral lethal dose of silicon dioxide for 50% of a population of rats is 3160 mg/kg (Golob, 1997). FAO posts no limitation regarding its use in products for human consumption. The quality of treated grain is also not adversely affected. Through the processing of wheat or rice, 99% of dust can be removed (Subramanyam and Roesli, 2000). Given the unique physical nature of its pesticidal mechanism, insect resistance against inert dust is unlikely. In the last decade, many inert dust formulations have been assessed against stored grain insect pests in grain storage (Table 1.1).

The idea of using inert dust to control grain insect pests is actually an ancient one with historical records dating back to more than a thousand years ago. In ancient China, plant ash and lime powder were used to prevent insect pest infestation in grain barns. In the 1940s, Cotton and Frankenfeld (1949) initiated research on the control of stored grain insects and other types of commodity insects with inorganic powder. Inert dusts are often used as grain protectants (Golob and Webley, 1980), such as diatomaceous earth and silica aerogel (Michalaki et al., 2006; Subramanyam and Roesli, 2000). Inert dust has been reported for structural treatments (Bridgeman, 1994; Cook et al., 2004) and as a direct grain surface application to prevent insect re-infestation from an outside source (Chomchalow, 2003; Naito, 1988).

1.1.4 Discussion

In the last 20 years, the development of safe and environmentally friendly pesticides has been given priority due to the increased concern about safety issues surrounding the traditional chemical products. Methyl bromide, an important stored grain fumigant, was included in the list of controlled materials by United Nations Environment Program in 1992 because of its destructive effect on the atmospheric ozone layer. Its usage was prohibited in all developed countries in 2005 and in developing countries in 2015 according to the *Montreal Protocol* (United Nations Environmental Program, 1995). In addition, strong resistance in stored grain pests to phosphine is spreading globally and threatens its continue use. There is, therefore, an urgent need to develop new fumigants and new methods for stored grain pest control. Inert dust is one of the non-chemical alternatives to replace or reduce application of chemical pesticides.

1.2 Inert dust products for control of stored grain insect pests

1.2.1 Natural dust products

1.2.1.1 Mineral based products

Diatomaceous earth dusts are currently the most widely used inert dusts in the field of grain storage. Diatomaceous earths (or diatomite) are the fossilized remains of diatoms (Fig. 1.1), composed mainly of amorphous hydrated silica with traces mostly of aluminum, iron oxide, magnesium, sodium and lime. Unprocessed diatomaceous earth contains 50% or more water. During processing, the water content is reduced to 2-6% and milling reduces particle size to between 0.5 and 100 μm with the majority falling between 10 and 50 μm (Korunic, 1998). The result of this process is a fine, talc-like powder or dust considered to be non-toxic to mammals. Brief and occasional exposures to diatomaceous earth cause minimal health risk similar to other inert dusts (Clark-Cooper, 1977; International Diatom Producers Association (IDPA), 1990a). According to the Environmental Protection Agency (EPA) in the USA, natural diatomaceous earth is described as amorphous silicon dioxide which is classified as Generally Recognized as Safe (GRAS) as a food additive (Anon, 1991). Because diatomaceous earth can absorb liquids two to three times its own weight and remains free flowing, it is frequently utilized as a pesticide adjuvant (Snetsinger, 1988). Diatomaceous earth shows good insecticidal efficacy. In some countries such as the United States, Canada, China, Australia and Japan, various commercial diatomaceous earth based insecticidal products are available. Common products on the market are: Insecto and Perma Guard D-10® from U.S.A, Protect-It from Canada, Dryacide from Australia and Pu Liang Tai® from China (Liu, 2005) (Table 1.1). The insecticidal action of diatomaceous earth occurs as a result of dusted insects slowly losing body water through cuticle damage caused by the abrasive particles. Diatomaceous earth alone is not usually considered to be an effective insecticide. Many formulations are composed of diatomaceous earth and other insecticides to reduce dust dosage, most frequently pyrethrum (0.1 to 0.2%) and piperonyl butoxide (1.0%) (e.g., Diacide Homeguard, Diatect, Perma Guard D-20 and Perma-Guard D-21), providing a “double barreled effect” and fast killing rate. However, when such physiological poisons are added to the diatomaceous earth compositions, the products became toxic to avian and mammalian species, including humans, and lose their insect specific killing advantage. Due to increased manufactural cost, the relatively high price also makes the products less appealing to customers. Despite the vast potential, diatomaceous earth-based products are mostly limited to home and garden pest control, or research specimens. Application to stored grain is only occasionally done. Also, their efficacy is influenced by various factors like temperature, humidity, insect species and commodities (Alexander et al., 1944; Ebeling, 1971; Fields and Korunic, 2000).

Fig. 1.1. Scanning electron micrograph of diatom remains in the diatomaceous earth, Celite 209 (Fields and Korunic, 2000).

Since the 20th century, many researchers have conducted experiments on diatomaceous earth against numerous stored grain insects. The results suggested that diatomaceous earth was effective at killing *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus* and *Cryptolestes pusillus* (Arthur, 2000a), and a few diatomaceous earth dusts have achieved promising results in stored grain insect control (Debnath et al., 2011; Fam et al., 1975). However, the efficacy of diatomaceous earth from different sources (mines) on insects has not been very consistent (McLaughlin, 1994; Snetsinger, 1988). Diatomaceous earth from salt water is more commonly used, being cheaper but supposedly less efficacious (Snetsinger, 1988), while the results from Korunic (1997) showed that the efficacy of diatomaceous earth against insects depends on different physical and morphological characteristics of the diatoms rather than its origin. Formulations of diatomaceous earth collected from different parts of the world have displayed significant differences in their efficacy against insects despite a similar mode of action, similar physical properties and similar diatom species composition. This is a very intriguing finding. However, there is a current belief that different diatomaceous earth products have the same or similar efficacy against the same targeted insect pest. To register diatomaceous earth today in the USA, there is no need to submit the results of efficacy tests against insects.

Table 1.1. Diatomaceous earth dusts registered in the United States and their components (Subramanyam and Roesli, 2000).

Primary name of diatomaceous earth dust products	EPA Registration NO.[1]	% ingredients[2]	
		SIO: PBO: Pyrethrins	
Ant and Roach Killer	67425-1	5:0:0	
Chem Tech Insecticide	68276-3	88:1.1:0.2	
Crop Guard	7665-1	85:0:0	G[3]
DE Insecticide	65462-4	90:1.1:0.2	
D-E Insecticide	64721-1	85:0:0	
Dia-Fil 610	56910-1	100:0:0	
Diatect D-20	42850-1	88:0.2:1.0	
Diatomic Earth	65460-1	99.9:0:0	H
Diatoms Dust Insect Powder	45220-9	90:0:0	

Diatect Multipurpose Powder	42850-2	88:0.2:1.0	
Diatect pet powder	42850-3	82:0:0	
Dryacide	67595-1	97:0:0	G.H
Dry Purocide Insecticide	1021-1665	60:10:0.1	
Eaton’s K,I,O	55-67	85:0:0	
Eco-fresh Brand DE Insect And Slug Killer	664880-4	85:0:0	
Enforect Insecticide Powder	40849-66	95:0.1:0.1	
Flea Away	68497-1	95:0:0	
Harper Valley Diatomaceous Earth	69261-1	100:0:0	P.W
Harper Valley Diatomaceous Earth Crawling Insect Killer	69261-2	100:0:0	F
Harper Valley Diatomaceous Earth Grain Insecticide	69261-3	100:0:0	G
Insectaside DE	56194-1	100:0:0	
Insectigone (Sure Fire)	59913-1	77.69:0:0	H
Insecolo	66923-1	97:0:0	G
Insecto	48398-1	90:0:0	G.H
Melocide DE-100	65789-1	100:0:0	F.S
Melocide DE-200	65789-2	83.6:0:0	G
Organic Plus CROP Insecticide	65462-6	97.9:1.1:0.2	
Organic Plus DE/Pyrethium Insecticide	65462-7	97.9:1.1:0.2	
Organic Plus Diatomaccous Earth Crawling Insect Killer	65462-1	90:0:0	H
Organic Plus PCO Pyrethium/Diatomaccous Earth	65462-5	97.9:1.1:0.2	H
Organic Plus Pyrethium/DE Insecticide	65462-2	97.9:1.1:0.2	

Organic Plus Pyrethium/Organic Solutions Pyrekill insecticide	67197-4	88.5:1.0:0.1	
Supernatural Plant Protection Insecticide	64721-2	88.5:0.1:1.0	
Organic Resources Multipurpose Insecticide	70126-1	82.9:1.0:0.2	
Perma Guard Commercial Insecticide-D-20	67197-6	88:1.0:0.2	F
Perma Guard Grain or Seed Storage Insecticide-D-10	67197-1	100:0:0	G
Perma Guard Household Insecticide-D-10	67197-2	88:1.0:0.2	H
Perma Guard Kleen Bind-20	67197-7	88:1.0:0.2	G
Perma Guard Pet and Animal Insecticide-D-20	67197-5	88.2:1.0:0.2	
Protect-it	66923-2	90:00:00	G
Satetiworld	68215-1	81.1:0:0	H
shellshock Insecticide	43739-10	66:0:0	

[1]The number after the hyphen refers to the number of products registered chronologically as primary name products. [2]SIO_2=silicon dioxide, PBO=piperonyl butoxide, a synergist typically used with pyrethrins. [3]Labeled uses: F=food processing plants. G=Grain, H=household, P=poultry processing plants. S=stored bird seed. W=warehouse. The products without usage identification can be used to control insect pests of ornamentals, field crops, and vegetables.

Zeolite powder is another kind of mineral based product and can be either alkaline metal based with water content, or aluminum silicate mineral containing alkaline earth metal. There are more than 30 types of zeolites. The mineral is processed to powder form by mechanical milling. Zeolite powder has been tested on *S. Zeamais* in corn storage by Haryadi et al., (1994). In a 4 week experiment, corn was mixed with 5% (w/w) zeolite powder and held under conditions simulating a storage facility. The death rate of *S. zeamais* reached 100%. However, the efficacy of an inert dust is evaluated on the onset time of insecticidal effect more than the final mortality of the exposed insects. Tricalcium phosphate is also a mineral food additive used for its anti-agglomerating effect in maintaining food consistency. Researchers have used tricalcium phosphate as a grain protective agent against *Bruchidae* (Fam et al., 1975). A concentration of 1000-2500 mg/kg effectively controlled a *Bruchidae* infestation. High cost accompanied with the high dosage rate rendered this approach impractical in the food industry. The high dust content in grain also caused concerns.

1.2.1.2 Plant based products

Paddy husk ash with high silicate content, is an effective protectant when mixed with maize at 1% (Mihale et al., 2009). This is a common practice on small farms in developing countries. In practice, desirable insecticidal effect requires the ash make up to 5% of grain weight (Golob and Webley, 1980). Sand and plant ash can effectively protect grain stored on small family farms with a dosage of more than 20% of the targeted grain weight (Golob et al., 1982). A higher dose usually provides better protection. Although the abrasive properties of the materials may play a role in interfering with insect development, it is more likely the physical blockage of inter-grain space prevents insects from performing normal behaviour. Locally-available dusts will continue to be used as an important grain protectant on small financially strained family farms in developing countries because of their low cost and easy access. This method has no potential in large scale application, partly because it was developed for small-scale storage only and also due to the high ash volume involved. It is particularly difficult to remove ash from the grain and high ash content in food is unacceptable by modern industrial standards. The replacements for ash need to either have a much lower effective dosage or require no removal at all.

1.2.2 Synthetic powders products

Diatomaceous earth dusts with high silica content and uniform size distribution present much better insecticidal efficacy (Korunić, 1997). Investigation of the entomotoxicity of synthetic powder products is mostly inspired by this finding.

1.2.2.1 Silica aerogel

Silica aerogel is produced from a distilled silicate solution. It is very light and hydrophilic. It has better efficacy than diatomaceous earth at a low dose. Low effective dose reduces potential inhalation compare to some products (Golob, 1997). Researchers such as Maceljski and Korunic (1972) suggested that silica aerogel possesses superior insecticidal efficacy among the inert dusts and is resistant to the adverse effect of high environmental moist level. Silica aerogel produces a much higher mortality rate in insects than diatomaceous earth, because of its stronger dehydration effect (La Hue, 1965a; McLaughlin, 1994). Silica aerogel breaches the cement and wax sublayers of the insect's epicuticle (Le Patourel et al., 1989; Maceljski and Korunic, 1972). Maceljski and Korunic (1972) conducted research and demonstrated that Dri-Die (95% silica aerogel + 5% ammonium fluorosilicate) could prevent regeneration of the cement and wax sublayers. But silica aerogel alone cannot achieve this without facilitation from ammonium fluorosilicate, especially under high moisture conditions.

Silica aerogel spray provides better insect control in grain storage facilities. Cotton and Frankenfeld (1949) reported different efficacies of silica aerogel with different dosages against *T. confusum*, *S. zeamais* and *S. granariues* in wheat and other grains. In wheat with 12% moisture content, the recommended dosage is 0.25 g/kg, while with 14% moisture content a dosage of 0.5 g/kg is required. In Australia, the inert dust product, Dryacide, was used together with silica aerogel. During a small scale experiment, Desmarchelier and Dines (1987) found that at 25C° and 65% RH, a dosage of 0.1% (w/w) Dryacide achieved 100% mortality in *R. dominica, T. castaneum* and *S. oryzae*. Dryacide and Protect-It, the two products registered in the United States, both contain silica aerogel. Current data support the fact that combined diatomaceous earth and silica is more efficacious than diatomaceous earth alone.

Although the aforementioned hydrophilic materials are quite effective when dry, such materials lose an appreciable portion of their insect killing capacity upon exposure to humid atmosphere or when in direct contact with water. Such “moisturized” silica dusts are ineffective as insecticides. An incidental finding (Ralph, 1964) showed that silica powder coated or impregnated with a proper hydrophobic agent, a liquid organosilicon polymer for instance, preserved most of its insecticidal activity in a high humidity environment. Hydrophobic siliceous insecticidal compositions described in U.S. Patent 3,159,536, issued to Ralph (1964) showed substantial resistance to 100% RH during a twenty-four hour period, while comparison groups consisting of untreated hydrophilic siliceous material suffered loss of efficacy (Knight and Bessette, 1997).

The environment within the grain storage facilities is generally in our favour given the low humidity. Under 30±1°C, 75±5% RH, modified silica nanoparticles (without any surface capping) (Fig. 1.2) and the surface-functionalized silica nanoparticles (Fig. 1.3) act with the same efficacy against *S. oryzae*, indicating that entomotoxicity of silica against insects is not related to the surface groups attachment to the insect cuticle (Debnath et al., 2011).

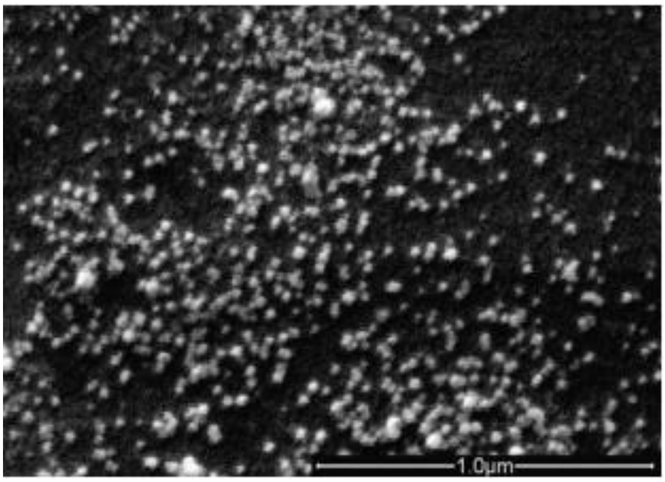


Fig. 1.2. Field emission scanning electron microscopy (FE-SEM) of modified silica (Debnath et al., 2011).

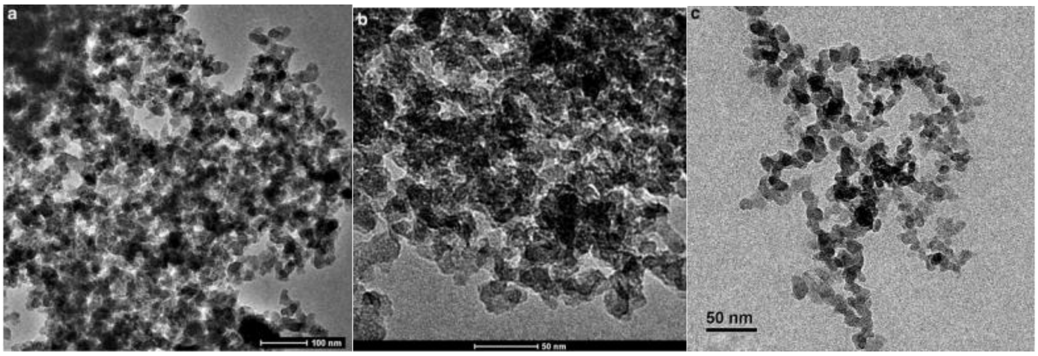


Fig. 1.3. Electron micrographs of three different types of silica nanoparticles. a Transmission electron microscope (TEM) image of hydrophilic silica nanoparticle (SNP), b Transmission electron microscope (TEM) image of hydrophobic SNP, c Transmission electron microscope (TEM) image of lipophilic SNP (Debnath et al., 2011).

1.2.2.2 Synthetic zeolite

Synthetic zeolites consist of microporous crystalline aluminosilicates with the formula, $M_{x/n}\left[\left(AlO_2\right)_x\left(SiO_2\right)_y\right]\cdot wH_2O$, where M is an alkali or alkaline-earth cation (Na, K, Li and/or Ca, Mg, Ba, Sr), n is the cation charge, w is the number of water molecules per unit cell, x and y are the total number of tetrahedral per unit cell, and the ratio y/x usually has values ranging from 1 to ∞ (Smedt et al., 2015). Some of the Al^{3+} replacing Si^{4+} in the silica framework results in a higher bulk density and reduced stability (Celik et al., 2010). The aluminium-rich zeolites with large pores have the capability to absorb liquid approximate to 30% of its own weight without any volume modification. The ideal particle size of synthetic zeolites used against insects is less than 2 µm, possessing the strongest adhesive affinity to insect cuticle. Both characteristics contribute to the insecticidal effect (Smedt et al., 2015).

Several commercial formulations are available for pest control in fruit tree production systems. For better deposition on the leaves and other tree surfaces, synthetic zeolites are applied as a particle film. However, the application of synthetic zeolites in the stored grain environment is limited. Lü et al., (2017) evaluated the effectiveness of a synthetic zeolite against the cowpea weevil, *Callosobruchus macculatus* (Fabricius), on concrete surfaces and cowpea products. On a concrete surface, the synthetic zeolite dose for 100% mortality was 5 g/m^2 after 72 h of exposure. Complete mortality of cowpea weevil with a 1 g/kg dose occurred after 4 days. Both doses were effective in killing cowpea weevils within 4 months. Compared with the control, the treated group showed marked reduction in fertility which is evident by the smaller number of eggs and progeny. Based on this result, the recommended dose is 1 g/kg as a surface treatment for cowpea. The obvious disadvantage is slow speed of kill which makes this choice a less than desirable one. The prolonged time for 100% kill increases the possibility of insect survival. The synthetic zeolites high affinity to water makes relative humidity an important factor regarding their efficacy and gives them a coincidental inhibiting effect on microbial organism activity (Glenn et al., 2001; Smedt et al., 2015).

1.2.2.3 Synthetic nanostructured alumina

Synthetic nanostructured alumina are produced via a combustion synthesis technique by utilizing glycine as fuel and aluminum nitrate as oxidizer (Toniolo et al., 2005). The final products contain more than 99.6% alumina (Yang and Watts, 2005). These purified compounds are relatively dense (0.108 g/cc) (Stadler et al., 2012) small particles (primary particle size,13nm; average aggregate particle size, 201.0 nm) and have many industrial applications such as pesticide encapsulating agents (Chauzat and Faucon, 2007), biocompatible material for medical and dental composites, and abrasives because of their hardness, toughness and wear resistance (Toniolo et al., 2005).

A recent study by Stadler et al., (2012) compared the efficacy of a synthetic nanostructured aluminum and Protect-It, which is considered to be the most effective commercially available diatomaceous earth. The synthetic nanostructured aluminum was more effective at a lower dose than Protect-It. The LT_{95} values for the synthetic nanostructured aluminum against *S. oryzae* and *R. dominica* (controlled F1 progeny) with a dose of 250 ppm at 27±1°C and 75% RH (grain moisture of 14.7%) were 10.9 days and 25 days, respectively. Protect-It was less effective and required 185.8 days and 84.6 days, respectively, at a similar dose (Stadler et al., 2012). The insecticidal mechanism of synthetic nanostructured aluminum is not well documented. Stadler et al., (2012) reported that toxicity of synthetic nanostructured aluminum decreased as the relative humidity increase and they proposed that the mechanism may be similar to that of diatomaceous earth particles. Interestingly, they observed a higher tolerance in *R. dominica* than in *S. oryzae*, which is the opposite to the observed results for diatomaceous earth and silica treatments in the literature (Subramanyam and Roesli, 2000). Among the chemical components, whether it is particle size or agglomerate structure, which contribute to the higher insecticidal activity of synthetic nanostructured alumina is yet to be determined. In addition, the major reported adverse effect of synthetic nanostructured aluminum is plant growth inhibition (Yang and Watts, 2005).

1.2.2.4 Synthetic amorphous silica

Synthetic amorphous silica (SAS) consist of nano-sized primary particles of nano- or micrometer-size aggregates and of agglomerates in the micrometer-size range. Synthetic amorphous silica (SAS) are generated either via a wet processing method (includes silica gel, precipitated silica and colloidal silica) or a thermal processing method (pyrogenic silica). Surface treated, hydrophobic SAS types also belong to this category. Recognized as Generally Regarded AS Safe (GRAS) additives in food and feed, SAS are distinctive from other forms of amorphous silica in terms of their high chemical purity, their finely particulate nature and the characteristics of the particles observable via microscopy, e.g., shape, structure and degree of fusion. None of these intentionally manufactured SAS contain crystalline silica. Because of differences in physic-chemical properties, SAS are used in a variety of products, e.g., fillers in the rubber industry, additives in tyre compounds, free-flow and anti-caking agents in powder materials, and liquid carriers, particularly in the manufacture of animal feed and agrochemicals. Other uses are toothpaste additives, paints, silicon rubber, insulated material, liquid systems in coatings, adhesives, printing inks, plastisol car undercoats and cosmetics.

A series of precipitated and fumed silica products were screened and identified to be lethal to adult *Prostephanus truncatus* (Barbosa et al., 1994). The persistence of two products, Gasil 23D and Aerosil R972, was then assessed over 40 weeks; a period equivalent to a storage season in most of tropical Africa. The results demonstrated the potential use as an insecticide in long-term storage. Very few progeny emerged from all four dosage groups tested. Gasil 23D was also found to be fast acting. It achieved 100%

adult mortality within a 48 hour exposure while Dryacide only resulted in 45% mortality under the same conditions. Dryacide was still much more effective compared to a standard diatomaceous earth product, Kensil F, obtained from Kenya, which produced only 3% adult mortality of *P. truncatus* after 28 d exposure to 0.5% (w/w) of the product (Golob, 1997).

Preliminary simulated field trails have been conducted in Ghana to assess the potential of Gasil 23D and Dryacide as protectants for maize against insect infestation. Batches of 10 kg maize grain or husked maize cob were treated with these two products at 0.1 or 0.2% (w/w) on grain and 0.2 or 0.4% (w/w) on cobs, and examined after 3 and 6 months storage in jute sacks. Weight loss of stored grain after three months was half that of the controlled groups. Gasil-treated grain was less damaged than untreated grain at a six month check point, but there were no differences between Dryacide-treated grain and corresponding controls. The large variation in data may have masked Dryacide's protective effect. Particle size is a key factor in determining efficacy. The products with smaller primary particles, such as pyrogenic silica, were selected for more intensive study. However, precipitated silica products with larger primary particle sizes, such as Gasil 23D, were more effective at different concentrations and different periods of storage (Barbosa et al., 1994). It is expected that SAS require lower protective doses. However, SAS application for stored grain is still at its infancy.

1.2.3 Physical and chemical comparisons of dusts

The variations in the dust properties lead to the different insecticidal efficacies. Faulde et al., (2006) reported that hydrophobic formation of diatomaceous earths was effective under high humidity for the control of German cockroaches, *Blattella germanica* (L.) (Orthoptera: Blattellidae). The efficacy of un-modified diatomaceous earths were reduced, indicating the surface property is strongly linked to insecticidal efficacy (Alexander et al., 1944; Ebeling, 1971). Debnath et al., (2011) also reported lipophilic and hydrophobic silica nanoparticles (SNP) were more effective than hydrophilic SNPs against *S. oryzae* at the dose of 1 g/kg. Melichar and Willomitzer (1965) demonstrated strong correlation between insecticidal efficacy and particle surface properties in 17 silica dioxides against the chicken mite, *Dermanyssus gallinae*. Silica aerogal (AL-1), which has an enormous specific surface (700 m^2/g), caused complete knockdown of adult male German cockroaches in 2 min compared to 39 min by Dri-die (Ebeling, 1960). Given the high oil absorption capacity, modified diatomaceous earths are expected to be highly effective pesticides. Baker et al., (1976) tested the effects of tricalcium phosphate on *T. castaneum* adults and *Tenebrio molitor* L. larvae. They were not able to establish correlation between toxicity and particle size, but suggested that lower bulk density tricalcium phosphate (0.37 g/cm^3) was more effective than high bulk density tricalcium phosphate (0.52-0.9 g/cm^3). Based on their findings, many researchers have initiated investigations on the entomotoxicity of SNPs. Among these follow up studies some results are contradictory. Fumed silica with extremely low bulk density and high surface area was expected to show high efficacy. However, Barbosa et al., (1994) reported the opposite result. In their evaluation study, precipitated silica, Gasil 23D, was more effective than the pyrogenic silica, Aerosil R972, against *P. truncatus*.

Smaller substrate particles provide a larger surface area for effective attachment which is a favorable trait. However, excessively small substrate particle sizes may result in agglomeration and interfere with aeration. Larger particles provide better aeration efficiency (owing to increased interparticle space) but limited effective contact surface (Ashok et al., 2008).

Only the silica gel particles with a diameter smaller than 50 μm maximum can adhere to the surface of the termite's body. The silica gel particles dehydrate the termites through the adhesion site (Miyazaki, 1993). In silica gel with mixed particle sizes, when the proportion of particles with less than 50 μm diameter increases, the mortality of termites increases accordingly (Miyazaki, 1993).

1.2.4 The factors affecting insect mortality

1.2.4.1 Temperature and humidity

Many researchers have investigated the effects of environmental relative humidity and grain moisture content on diatomaceous earth, and concluded that an increase in environmental relative humidity and grain moisture content reduces the insecticidal effect (Alexander et al., 1944; Debnath et al., 2011; Li, 2013; Maceljski and Korunic, 1972). Fields and Korunic (2000) evaluated the impact of grain moisture content and temperature on the insecticidal effect of diatomaceous earth from different sources. The results showed that grain moisture content and temperature both have a great negative impact on the insecticidal effect of diatomaceous earth. Over 15% grain moisture content or 75% RH and above, the insecticidal effect will be significantly reduced. Cao and Li (2001) tested the lethal effect of Protect-It on *Liposcelis entomophila* at 50-75％ RH. The results showed that the death rate of *L. entomophila* reduced gradually with the increase of humidity. Arthur (2000b) measured the influence of temperature on the insecticidal effect of diatomaceous earth. The results showed that different diatomaceous earths reacted differently to temperature changes and higher temperature increased the insecticidal effect for Protect-It. On the contrary, synthetic nanostructured aluminum performed better at low temperature (Stadler et al., 2012). For each diatomaceous earth, the same temperature has different effects on insecticidal efficacy depending on insect species. Compared to grain moisture content and environmental relative humidity, temperature has less effect on the insecticidal effect of inert dusts. High temperature increased the efficacy of diatomaceous earth against *C. ferrugineus*, but had the opposite effect against *T. castaneum*. For *S. oryzae*, the sensitivity to diatomaceous earth shows a positive relation with temperature increase (Michalaki et al., 2006). For *R. dominica* and *S. granarius*, the sensitivity to Dryacide at 30℃° was twice that at 20℃°. However, for *T. confusum*, the sensitivity decreased when temperature increased (Aldryhim, 1990). The level of insect activity may be key to explaining the temperature effect. When placed at the preferred temperature, the insects become more active. Increased roaming, feeding and other activities cause more frequent contact with the dust, and eventually higher mortality.

It was presumed that because high temperature enhances the vaporization effect, abrasive diatomaceous earth or hydrophilic silica would provide greater killing efficiency at low humidity. Because moist air has little evaporative power, diatomaceous earth and hydrophilic silica was even less effective at high humidity (Knight and Bessette, 1997).

Hydrophilic silica control insect pests by reducing environmental humidity, thus creating an environment too dry for household insect pest survival. Environmental humidity can be adjusted to a range of 50% to 100% RH with humidity-controlling hydrophilic silica. The expected survival time for Reticulitermes is only forty hours at a maximum relative humidity of 80%. Also, with Coptotermes, lethal dehydration can be expected within eighty hours at a maximum relative humidity of 60% (Miyazaki, 1993). The insecticidal activity of amorphous silica mixed with grain decreases rapidly when the grain moisture content is above 14% (Le Patourel, 1986). Moisture content of 15% is presumed to be the upper safe limit for wheat storage between 15 and 25℃° (Pixton, 1982). The *Sitophilus* spp. were particularly resistant to amorphous silica in wheat with high moisture content. At 9% moisture content the silica treatment was nearly twice more effective against *S. oryzae* at higher temperatures than at lower temperatures. Under the same conditions, the effectiveness of treatment against *T. confusum* did not vary markedly between 15 and 25C° over a range of grain moisture contents. Deposition of silica particles on insect cuticle largely happens when the insect moves through the treated grain. *T. confusum* was observed to be more active than *S. oryzae* at 15C°, which may be the leading cause of this variation (Shawir et al., 1988).

1.2.4.2 Commodities

Diatomaceous earth showed different efficacy against different pests in different grains. Athanassiou et al., (2003) evaluated the effects of the diatomaceous earth product, SilicoSec, against *S. oryzae* in four grains. Death rates from high to low were obtained in the grains, paddy, barley, rice and corn, respectively, at a dose of 1 g/kg at 26C°, 60± 5% RH for 48 hours (Aldryhim, 1990). Mewis and Ulrichs (2001) evaluated three diatomaceous earth products, Protect-It, PyriSecs and DEBBM, against *S. oryzae* in corn, wheat and barley. The highest death rate was observed in corn and the lowest in barley with a dose of 500 mg/kg at 25C°, 55% RH for 7days. Some grains can also interfere with a diatomaceous earth's insecticidal effectiveness. Chanbang et al., (2007) reported that Insecto and Protect-It performed suboptimally against *R. dominica* in brown rice at the recommended dose rate due to the present of cuticle rice bran. The characteristics of grains should be taken into consideration when inert dusts are utilized as a pest control method. The ranking according to inert dust LD_{50} from high to low is rice > corn > oat > barley > wheat (Athanassiou et al., 2003). Interestingly, Athanassiou et al., (2008) conducted feeding trials on *S. oryzae* with barley, wheat and corn. *S. oryzae* fed on barley showed highest sensitivity to diatomaceous earth formulations, followed by the group on wheat, while those on maize were least sensitive.

1.2.4.3 Insect species and development stage

The efficacy of inert dust or silica against different species of stored-product insect pests is different (Fields and Korunic, 2000). This can be contributed to by the vast differences among species in cuticular permeability, composition of cuticle, mobility, feeding pattern, adaptation to low internal water content, and ability to avoid inert dust. Even different strains of the same species showed different tolerance to inert dust. According to Rigaux et al., (2001), for *T. castaneum*, the lethal dose for 50% mortality (LD_{50}) for the most tolerant strains (413 ppm) is twice that for the most susceptible strains (238 ppm) upon exposure to diatomaceous earth. The tolerant strains lose their water much slower compared to their sensitive counterparts in both treated and untreated groups. Tolerant strains were also less active when forced to move through either treated grain or over filter paper treated with diatomaceous earth, which greatly reduced the exposure and allowed more time to compensate water loss (Rigaux et al., 2001). Water is key for the insects' physiological activities. As with mammals, dehydration leads to death. The lethal dehydration level for *S. oryzae* is 28% body weight (Arlian, 1979), while it is 15 to 18% for *T. castaneum* (Rigaux et al., 2001). Different insects have different inherent water content. Stored-product insects tend to have a lower water content (50% for *S. oryzae*, 52% for *C. ferrugineus* and 52-53% for *T. castaneum* (Fields et al., 1998; Rigaux et al., 2001)) compared to species living in other environments (an average of 69% (Hadley, 1994)). High water content coincides with high susceptibility to inert dusts. Liu (2005) reported that *O. surinamensis* and *C.*

ferrugineus were most susceptible and *T. castaneum* was most tolerant to diatomaceous earth in a structure treatment.

Morphological and physiological differences also contribute to sensitivity to inert dusts. The greater the body surface relative to volume, the more susceptible an insect is to inert dusts. *C. ferrugineus, C. pusillus* and *C. turcicus* are readily susceptible to dusts given their large body surface to volume ratio. Li et al., (2011) confirmed this theory in their tests of four new silica dusts against adults of phosphine resistant strains of *C. ferrugineus*. A 100% of mortality can be obtained within 4 hours at the dosage of 50 mg/kg.

Different development stages of the same insect also show different susceptibility. The time required to kill 100% of adult *T. confusum* and *Tenerbio molitor* in the absence of food at a dose of 2 and 4 g/m^2 of Fossil Shield® at 25±1°C and 62±2% RH was within 14 days (Mewis and Ulrichs, 2001), while a 100% mortality of larvae *T. confusum* was obtained within 4 days, but the larvae *T. molitor* were not affected by the product. Some developmental stages are significantly more sensitive, such as first instar larvae of *Plodia interpunnctella*. Here, 100% mortality can be achieved within 1 day at a dose of 4 g/m^2, while the 3rd instar and 4th instar larvae suffered little mortality. Also, two weeks old *P. interpunnctella* larvae tend to be less susceptible than *T. confusum* larvae at the same age. Vayias and Athanassiou (2004) reported the detrimental effect of SilicoSec®, which is a new diatomaceous earth formulation of freshwater origin from Germany. The *T. confusum* larvae were much more susceptible to SilicoSec® than adults, while the young larvae and young adults were more susceptible than old larvae and old adults, respectively (Vayias and Athanassiou, 2004). This phenomenon can be explained by structural differences and variation in cuticular lipid composition at different developmental stages (Bai et al., 2008, 2007). Noble-Nesbitt (1990) observed that *T. molitor* larvae were able to absorb water from sub-saturated air, while adults were not, and this was due to the structural differences in the rectal complex of these two stages. Hydrophobic silica with sufficient exposure is lethal to all developmental stages, except eggs, of the entire class Insecta (Hexapoda) (Vrba, 1992).

Behavioural avoidance to insecticide treatments can also be used to explain variation in the susceptibility to dusts. The insecticidal activity of dusts and others insecticides decreases rapidly as insect behaviours are modified to avoid an insecticide-treated surface (Prickett and Ratcliffe, 1977). Generally, dust is applied as a 30 cm surface treatment to a grain bulk; a proportion of grain stored beetle pests are likely in time to encounter which acts as a boundary between the exterior and the untreated grain. The survival of these insects is largely dependent on the frequency of contact, the length of exposure time, the insecticide formulation, the physical persistence of the insecticide and the insects' susceptibility to the dust. An avoidance response was observed in *T. castaneum* upon exposure to dusts, but not in *S. granarius* (Gowers and Le Patourel, 1984).

1.2.5 Mode of action

1.2.5.1 Digestive tract obstruction theory

Extensive research has been conducted on the insecticidal mechanism of inert dust since the 20th century. Smith (1969), in his popular article, suggested that colloidal silica particles ingested by larvae of *Coleomegilla maculata lengi* and *Leptinotarsa decemlineata* (Say), and that the particles interfere with digestion. There is very little scientific proof to validate this claim. According to Carlson and Ball (1962), insects feeding on diatomaceous earth was observed in *S. oryzae* and *S. granaries* adults. During necropsy, dyed diatomaceous earth was found in the esophagus, midgut and hindgut, most often in the latter two sites. No abnormality, damage or inert dust particle penetration was found in the digestive tract linings. No clinical signs of disease were present in adjacent internal (fat body, Malpighian tubes and trachea, e.g.) organs. These findings did not support the possibility that feeding on diatomaceous earth was the cause of death.

1.2.5.2 Respiratory dehydration and obstruction theory

As early as 1953, Roeder (1953) reported that dehydration in insects could occur via the tracheal system, if the insects could be induced to keep spiracles open. However, when evaluating the effect of the silica aerogel, Aerosil 380, on the mortality of *T. confusum*, Vrba and Nosal (1983) were not able to secure evidence that silica aerogels caused permanent or long-term opening of the spiracles. In the middle of the 20th century, some researchers proposed that insect stoma and trachea blockage by diatomaceous earth particles was the cause of their death (DeCrosta, 1979). However, in earlier work, after treating *S. granarius* and *Acanthosocelides obtrctus* with crystal SiO_2 and bentonite powder, Chiu (1939) reported no difference in the oxygen consumption of adult insects, which suggested tracheal obstruction was unlikely.

1.2.5.3 Traumatic dehydration theory

Many researchers reported epidermal damage in insects treated by diatomaceous earth. Mewis and Reichmuth (1998) observed epidermal wax layer damage and particle embedment in *T. molitor* adults under electron microscope, after they were treated with diatomaceous earth. The result confirmed absorption of the upper epidermal wax layer by diatomaceous earth particles.

Stored grain insects live in a dry environment. Water is attained through feeding. Insects rely on the epidermal wax layer to preserve water. Some scholars suggested that the insecticidal mechanism of diatomaceous earth was the breach of this protective barrier leading to death by dehydration (Ebeling, 1971). Through a series of investigations, Ebeling (1971) concluded that diatomaceous earth absorbed the epidermal lipid layer, inflicted mild abrasive damage, and eventually cause dehydration in pests. Ebeling (1971) reported that the loss of water holding ability in insect epidermis was induced by friction from diatomaceous earth particles. The insects consequently died of dehydration. Mewis and Reichmuth (1998) treated the pupae of *T. molitor* with diatomaceous earth (Home Shield), and observed weight loss in the treated groups. The most obvious reason was reduced water content, thus dehydration. Mewis and Ulrichs (2001) also observed similar results in *T. confusum* adults treated with 4 g/m^2 diatomaceous earth (Fossil Shield) for 7 days without food. The treated adults lost 20% of their weight while the control group only lost 10%. Carlson and Ball (1962) reported the same finding as well. They placed *T. castaneum, T. confusum, R. dominica, S. granaries* and *S. oryzae* adults treated with diatomaceous earth for 10 seconds in a dry environment (0% RH), weighed the subjects every 24 hours and recorded the mortality. Treated *T. castaneum* and *T. confusum* presented significant weight reduction. The investigations of Rigaux et al., (2001) on *T. castaneum* echoed these findings. The traumatic dehydration theory is well supported given the ample amount of evidence.

Inert dusts cause desiccation in insect cuticle by destroying the epidermal wax layer. The lethal threshold is 60% of body water content or 30% total body weight (Ebeling, 1971). Inert dusts such as silica aerogels possess tremendous lipid absorbability (Subramanyam and Roesli, 2000). Waxes from cuticle are absorbed when in direct contact with inert dusts. The dry environment the insects live in further exacerbate dehydration from the breached physical barrier. Due to the large surface area in relation to their body weight, insects often face more challenges in retaining their body water content than mammals.

Currently, academics are unable to reach consensus on the insecticidal mechanism of inert dusts. Much of the recent results support physic-chemical removal of the epicuticular, lipid-water barrier as proposed by Ebeling (1971) and dehydration as the cause of death. But mortality in *S. oryzae* caused by diatomaceous earth cannot be explained by this theory (Carlson and Ball, 1962), since weight loss (i.e. desiccation) in these beetles is minimal.

The weight loss in insects when exposed to inert dusts is a well documented fact. But whether superficial desiccation can cause such drastic dehydration is still questionable.

1.2.5.4 Cuticular structural

The insecticidal effect of inert dust by destroying the epidermal wax layer, blocking spiracles and tracheae is generally accepted. Due to the time consuming nature of dehydration via superficial damage or spiracles and tracheae blockage, rapid insect death after inert dust application cannot be well explained. Li (2006) studied the ultra-structure of cuticle from six common grain storage pests. Environment scanning electron microscopy was employed. Li (2006) reported that little particles were found in the pronotum and crestal furrow of the shard base of *S. oryzae* and *T. castaneum*. Large amounts of particles accumulated at the femurotibial pleat of *T. castaneum.* In *T. castaneum,* the intersegmental membrane was obviously damaged and the intersegmental membrane in the articular fossa was completely destroyed. This finding supports the theory that the closed shielding of insect somite and the narrowness of internode pleat or crestal furrow were among the key factors influencing the insecticidal efficacy of inert dust.

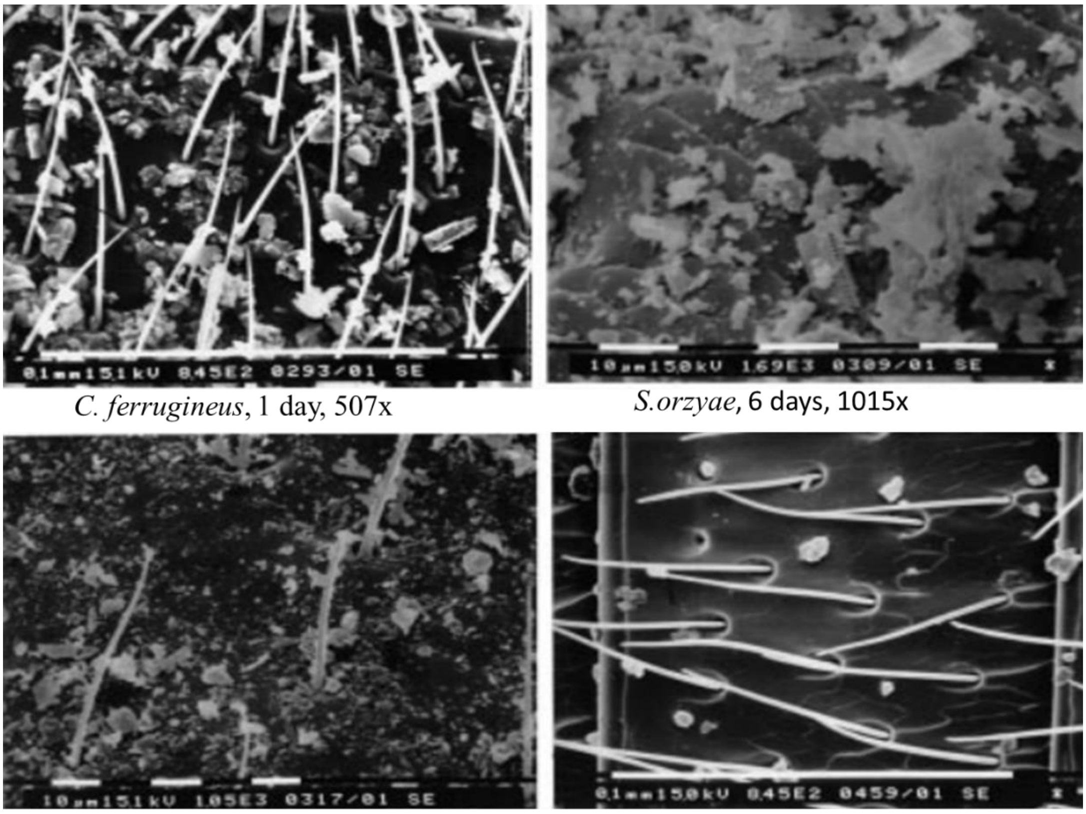
It is widely accepted that silica is both abrasive and lipid sorptive, and both properties can cause surface desiccation by damaging the epicuticle waterproofing barrier (Fig. 1.4). The epidermal wax layer in the insect cuticle consists of a uniform monolayer lipid and determines the permeability of the cuticle which is core to water balance. Cook et al., (2008) stated that a range of *Acarus siro* L. lipids was absorbed by three diatomaceous earth products after 18 and 72 h exposure. These long-chain lipids are believed to be responsible for the waterproofing property of cuticle. Although traditional methods can demonstrate the absorptive action through direct measurement of lipids transferred from the cuticle onto a silica particle, it is time consuming and labour intensive, and requires specialist skills and experience. Most of the research is focused on mode of action of diatomaceous earth. Synthetic amorphous silica received little attention until our ongoing investigation on the change to insect body surface post exposure. No related data were previously available.

Adrien (1968) indicated that insecticidal effect of inert dusts may be stress related, caused by the release of a paralyzing agent from the corpora cardiac. He suggested the possible interaction of inert dusts and bursicon, a hormonal agent, which is involved in melanization, endocuticle decomposition, water regulation and tracheal emptying. Bursicon may also plays a role in cuticular dehydration and plastization. This latter work is particularly interesting since some of our preliminary studies with *T. confusum* have indicated that inert dusts absorb a photosensitive substance secreted by the insect.

1.2.5.5 Exoskeleton junction damage

The efficacy of dust may vary when it encounters different cuticular waxes. Insects with thicker cuticular wax layers have been shown to be less susceptible (Bartlett, 1951). Nair (1957) investigated four species of beetles *S. oryzae*, *T. castaneum*, *R. dominica* and *Bruchus chinensis*. *S. oryzae* has a wax layer that is protected by an additional cement layer; *T. castaneum* has no such structure, but a very hard wax; and *R. dominica* has softer wax than *T. castaneum*. *Bruchus chinensis* has the softest layer of the four species. The rate of desiccation caused by three sorptive dusts was in descending order, *B. chinensis* > *R. dominica* > *T. castaneum* > *S. oryzae*, indicating the extra protective effect from the cement layer against desiccation. Insects with softer waxes were more susceptible than those with harder waxes (Ebeling, 1971).

Wang (2008) discussed the effect of inert dust on the intersegmental membrane in insects. The investigation revealed behaviour changes in inert dust treated *T. castaneum* and *O. surinamensis*. Locomotion capacity was greatly reduced after treatment. Wang (2008) observed the behaviour of *T. castaneum* and *O. surinamensis* when challenged with new inert dust. Moving speed was reduced in treated *O. surinamensis* and eluding behaviour observed. In an attempt to remove attached inert dust and avoid abdominal contact with the dust, the insect stood upright on its tarsi. The paralysis of the hind legs set in next; the insect then lost normal posture and mobility. In the final stage, all the tarsi and antennae were completely paralyzed before death. Behavioural changes, neurological pathology and physical trauma were all observed in *T. molitor* treated with inert dust. These results further our understanding of the insecticidal mechanism of inert dust which provide guidelines for practical applications.



C. ferrugineus, 1 day, 507x *S.orzyae*, 6 days, 1015x

R. dominica, 6 days, 630x *T. castaneum*, 6 days, 507x

Fig. 1.4. Scanning electron micrographs of the ventral surface of four stored grain insect species after released in wheat treated with 600 ppm diatomaceous earth (Celite 209) for various durations (Debnath et al., 2011).

1.2.6 Resistance of stored product insect pests to inert dust

So far, no insect resistance to inert dust has been reported. Silicon dioxide and other trace metal oxides do not participate in insect metabolism like chemical insecticides. The possible resistance can only arise from behavioural changes, such as avoidance behaviour (Korunic, 1998). Tolerance varies among species. For example, *Lepidoglyphus destructor* is more resilient than *Acarus siro* and *Tyrophagus putrescentiae* (Cook and Armitage, 1999), possibly due to the hard chaeta on the body surface preventing inert dust attachment (Collins and Cook, 2006).

Although the presence of insecticide dust provides rapid kill under continuous exposure, the survival rate can be relatively high. Choice box studies confirmed that the repellent effect on insects is negatively related to the dust's efficacy (Knight and Bessette, 1997).

1.2.7 Field trial studies in stored grain

Due to its safety, effectiveness and long-term protection, diatomaceous earths or silica aerogels have been used for eliminating stored grain insects in field trials since the 1960s (La Hue, 1965b; Redlinger and Womack, 1966; Strong and Sbur, 1963). With a dosage of 0.35% (w/w), diatomaceous earths were effective for 12 months (Golob, 1997). However, one unacceptable commercial disadvantage is that they cause wear to handling machinery. This disadvantage can be relieved to some extent by reducing the dose to 0.04-0.1% (w/w). However, users working in these areas worry about their health because these kinds of product are very light, tended to float in the air and have caused respiratory illnesses. The technology for using inert dusts for storage protection have not really been fully developed. The poor distribution of the dust throughout the grain bulk results in unreasonable control, allowing insects to survive in areas of low dust concentration. For example, although excellent control against a series of stored product insects with the product, Insecto, was obtained in the laboratory, there were significant numbers of live adult *R. dominica* and *S. oryzae* continually found in a small field trail (Golob, 1997).

Dryacide is mainly used as a structural treatment in Australia either as a dust at 2 g/m^2 or as a 10% aqueous slurry to provide 6 g/m^2. Dust application is confined to grain-handling machinery, ducts and vertical silos, and slurries are applied to horizontal grain stores. Slurries are particularly useful where there is a need for personnel to avoid exposure to very dusty atmospheres which would be created if the dry dust was applied.

Although Dryacide remains sufficiently active to exert control when applied as an aqueous suspension, other inert dusts lose their efficacy when applied in this way (Maceljski and Korunic, 1972; McLaughlin, 1994). Dryacide is prohibited from use in bulk handling systems because it affects the physical properties of grain, such as bulk density and flow characteristics (Jackson and Webley, 1994). The same problems have been faced with other commercially available inert dusts. Furthermore, removing the dusts in order to process grain for consumption can be tedious. These dusts are therefore being replaced by methods which either require smaller quantities of material or use materials that do not need to be removed before the grain is consumed.

In recent years, inert dust has been used on a very large scale in China, including at a national scale and for small farm stores. This is primarily due to the development of food grade inert dust and application technologies (Chen et al., 2016; Dong et al., 2016; Wang et al., 2016; Zhou et al., 2017). Three state endorsed projects are dedicated to develop application methods of diatomite insecticides that are adaptable to the local conditions, i.e., structure treatment, grain bulk surface treatment, and insect repellant for entrance and ventilation systems. Operators of warehouses, silos and other facilities conduct the sanitation before loading grain. Food grade inert dust can be used for structural treatment to replace or reduce chemical pesticide application. The recommended dosage of food grade inert dust is 1~2 g/m^2, which is significantly lower than the dose that is allowed to be used in grain and does not need to be removed. The dust is very light and can be distributed evenly in different locations of the warehouse by being sprayed with a newly developed powder blower (Wu, 2011; Zhang et al., 2014). Five major stored product insects, *R. dominic*a, *S. zeamais, O. surinamensis, T. castaneum* and *C. ferrugineus*, exhibited 100% mortality when exposed for three days to surfaces treated with 3 g/m^2 food grade inert dust. The 30-50 cm surface layer in a 6 m high grain bulk is an important area for stored product insect activity. Mixing food grade inert dust with grain in this layer is effective at controlling most of an insect population in a grain bulk but also can prevent the insects from outside the warehouse getting into the grain bulk. Mixed with grain, food grade inert dust can effectively prevent and control *R. dominica*, *O. surinamensis, S. zeamais, S. oryzae*, *C. ferrugineus*, *T. castaneum* and other stored product insects (Li, 2006; Wang et al., 2011, 2009a). In addition, it does not influence the quality of the stored product (Wang et al., 2011, 2009a). Food grade inert dust also has been used on gates or windows of warehouses as an insect repellent line to prevent and control insects from getting into the building. Aerosolized food grade inert dust is an innovative insecticide, which has been proven effective in field trials. The dusting can be completed in two hours in a 60 m × 21 m × 11 m warehouse where the height of the grain bulk is 6 m with only two workers. Dust can be distributed evenly to the whole grain bulk and is very effective for insect control. A 100% mortality result for *C. pusillus* can be achieved with a very low dosage of 0.5 mg/kg (Wang et al., 2016).

1.2.8 Disadvantage of current commercially available dust products

The recommended dosage for current commercialized diatomaceous earth is 500 to 3500 ppm. The high dose rate does have some adverse effects on grain, including a reduction in the flow ability and bulk density, visible residue, extra dust generation during processing and interference with grain moisture test.

For mite control, the dose of Dryacide is about 1000 times higher than organo-phosphorous (OP) pesticide. Consequently, the cost is also much higher (Cook and Armitage, 1999). Although a number of commercial formulations have been registered and are widely applied in the field (Wang et al., 2009b; Yang et al., 2011), the complications mentioned above can make this practice difficult (Desmarchelier and Dines, 1987; Jackson and Webley, 1994; Korunić, 1997; Kozak, 1966). The most significant adverse effect is reduced bulk density which is extensively used as a grading criterion. Canadian Western Red Spring wheat requires a minimum bulk density of 750 kg/m^3 to be considered grade No. 1. Application of diatomaceous earth at the recommended dose of 500–3500 ppm would result in downgrading. Twenty five diatomaceous earths of different origin were tested for their effects on wheat bulk density (Fig. 1.5). All diatomaceous earth dusts decreased wheat bulk density with significant variation among them. The most active diatomaceous earth formulations against stored grain insects, such as Protect-It, Dryacide, Insecto, Dicalite, DE Eu and DiaFil, also had the greatest effect on the bulk density.

Natural diatomaceous earth, registered as an insecticide, is predominately amorphous silicon dioxide with less than 1% (freshwater diatomaceous earth) or less than 3% (marine diatomaceous earth) crystalline silicon dioxide. Processing (particularly calcining) introduces contamination such as critobalite (IARC, 1997). Lung damage (silicosis) due to dust inhalation is a concern. Crystalline silica has been associated with silicosis and was classified by the International Agency for Research on Cancer (IARC) as a probable carcinogen. Amorphous silicon dioxide is a mild irritant to the human upper respiratory tract, eyes, and exposed skin, but this is unrelated to silicosis (International Diatom Producers Association (IDPA), 1990b). Developing more potent products are necessary. Minimal effective dosage is advised when inert dusts are used. Extensive data are available regarding the physic-chemical, ecotoxicological and toxicological properties of synthetic amorphous silica. Primary SAS particles usually form aggregates and agglomerates and are not normally found as discrete particles in air or aqueous environments. Both nanostructure synthetic amorphous silica (i.e., the “bulk material") and nano-objects of silica dissolve in aqueous environments. None of the synthetic amorphous silica types was shown to be biopersistent or bioaccumulative. All synthetic amorphous silica products have a short limited time in animals. In animal studies, no relevant differences in the toxicities of the different commercial synthetic amorphous silica types were found (Fruijtier-Pölloth, 2012).

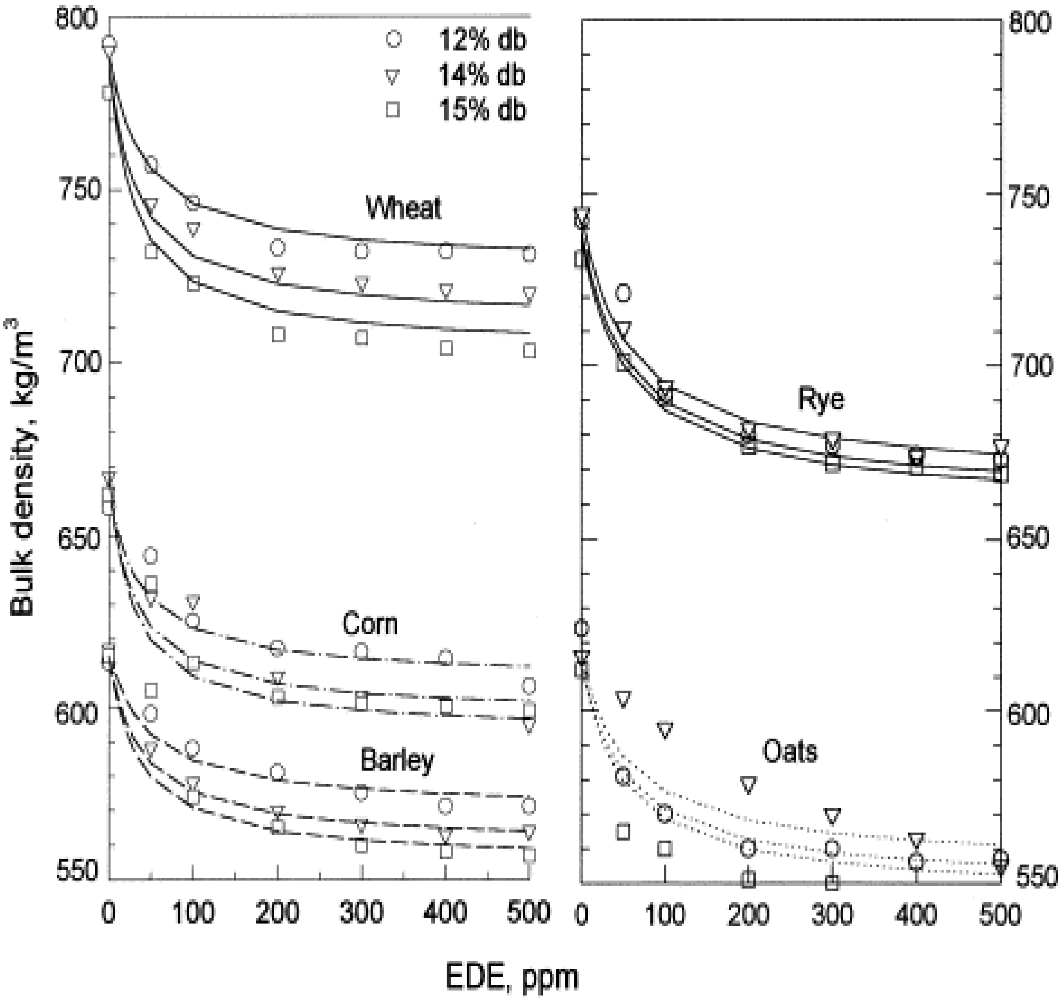


Fig.1.5. The predicted reduction in bulk density among five grains with different moisture contents caused by different dosage of enhanced diatomaceous earth (EDE) (Korunic et al., 1998).

Particle surface characteristics are more relevant to synthetic amorphous silica insecticidal efficacy than the particle size. Synthetic amorphous silica products affect insect membrane structures and integrity. Cellular toxicity is linked to the interactions between outer and inner cell membranes, signaling pathways, cross membrane transport and biomembrane integrity. Inflammatory responses can be induced by the release of cytokines and other proinflammatory agents due to compromised biomembrane. Most of these observations were attained in *vitro*; the only effect demonstrated in animal studies were inflammatory responses after high dose synthetic amorphous silica products were inhaled, or introduced via intratracheal, intraperitoneal, subcutaneous or intravenous methods (Fruijtier-Pölloth, 2012).

Commercial synthetic amorphous silica products (including colloidal silicon dioxide and surface-treated forms) are well-studied materials that have been utilized for decades in oral and topical pharmaceutical and cosmetic products, and as an anti-caking agent in food. There were no reports of adverse reactions regarding human health. They are also considered environmentally friendly. All these properties make synthetic amorphous silica products attractive alternatives from traditional chemical pesticides.

1.3 Study Aim

Based on drawbacks from diatomaceous earth and limited or no information on mode of action for upcoming synthetic amorphous silica powders, my research is focused on the insecticidal mechanism of synthetic amorphous silica powders and their application as an alternative practical stored grain pest control method. Current results support the fact that synthetic amorphous silica powders are effective broad spectrum insecticides. Diatomaceous earth is the most commercially marketed inert dust. Synthetic amorphous silica powders share all its advantages while providing more effective pest control. Yet, very little attention has been given to these inert dusts by the scientific community. I am interested in the properties of synthetic amorphous silica powders and their interaction with grain insect pests, and hope to provide valuable new insights to help reveal their insecticidal mechanism.

Due to the extremely low human health risk, synthetic amorphous silica powders are much safer to both operators and consumers, an advantage not enjoyed by traditional chemical pest control products. The operation cost is also relatively low too.

My investigation will also suggest several new possible approaches to develop future potent inert dust-based insecticide products.

1.4 Research questions and the structure of the thesis

The insecticidal mechanism and biological effect of synthetic amorphous silica (SAS) was investigated, involving aspects of biology, physics, optics, material science and kinematics. Both laboratory and field trial data were evaluated. The thesis is aimed to address the questions set out below.

1.4.1 How does insect activity level affect the efficacy of SAS powders? Are there differences in effectiveness among different SAS powders?

In Chapter 2, ten dusts (nine SAS powders with different polarity and processing procedures compared with one commercial diatomaceous earth (Dryacide)) were evaluated against various developmental stages (stationary stages and moving stages) of *T. castaneum* in a laboratory setting. The more highly effective SAS powders were then selected for mechanism studies.

1.4.2 How do synthetic amorphous silica powders attach to an insect body? What is the key factor contributing to the efficacy of synthetic amorphous silica powders?

Chapter 3 investigated the hypothesis that electrostatic charge affects the attachment processes during initial contact of synthetic amorphous silica. In this chapter, precise measurement of electrostatic charges was carried out on three stored grain insect species, four selected synthetic amorphous silica powders and one diatomaceous earth. The Chapter described a comprehensive evaluation of dusting effectiveness in relation to electrostatic charge and proposed new protocols for dusting.

1.4.3 How does synthetic amorphous silica affect the whole insect cuticle?

In Chapter 4, the efficacy of two synthetic amorphous silica powders was investigated against two insect species and cuticle change in both species was measured with hyperspectral imaging techniques. The hyperspectral data were analyzed using a modified version of artificial neural network (ANN) to accurately demonstrate how synthetic amorphous silica impact the insect cuticle.

1.4.4 What is the main target site of synthetic amorphous silica?

In Chapter 5, the physical action and biological effects of synthetic amorphous silica were assessed by high speed photography and a locomotion compensator was used to further elucidate the effect of synthetic amorphous silica on *T. castaneum* and *S. oryzae* of both genders. Intersegmental frictional devices were chosen as the interesting areas based on Young’s modulus.

1.4.5 How can dust application be optimized in a field trial? A case study of synthetic amorphous silica application

From Chapter 2 to Chapter 5 it was evident that synthetic amorphous silica would be a promising non-chemical pesticide. Therefore, Chapter 6 set out to develop a new insect detecting technology to identify the insect species in a grain bulk and evaluate the efficacy of one SAS as a structural treatment in a field trail.

1.4.6 What are the key questions that should be addressed in future research?

In the conclusion to the six chapters, the main results from this thesis are discussed and some unanswered questions are advocated as candidates for further study.

Chapter 2. Evaluation of efficacy of different synthetic amorphous silica powders and a diatomaceous earth against different developmental stages of *Tribolium Castaneum*

2.1 Abstract

The present study was performed to investigate the efficacy of different synthetic amorphous silica (SAS) powders against *Tribolium castaneum* at multiple developmental stages and compare this with the efficacy of the commercial diatomaceous earth, Dryacide. *T. castaneum* was selected as the bioassay target for its feeding habit. As an external feeder, it was easy to collect specimens of different developmental stages. Synthetic amorphous silica powders have a higher specific surface area, total pore volume, oil sorption capacity and smaller particle size compared with diatomaceous earth. Among SAS, precipitated SAS powders have better value for these parameters, except oil sorption capacity than pyrogenic SAS powders. After surface modified, the oil sorption capacity decreases and the particle size becomes small.

Bioassay studies suggested that eggs and pupas were the tolerance stages compared with larvae and adult, which implied that the stationary stages were less influenced by the above products. The efficacy of synthetic amorphous silica powders and diatomaceous earth against larvae (LD_{50} = 14.980-76.202 g h/m^2; LD_{95} = 28.476-153.478 g h/m^2) was nearly two to three folds higher than adults (LD_{50} = 38.876-119.246 g h/m^2; LD_{95} = 55.694–164.302 g h/m^2). Larvae and newly emerged adults were more sensitive to all the synthetic amorphous silica powders and diatomaceous earth than newly hatched larvae and adults, respectively. Two and five times more diatomaceous earth (> 4 g/m^2) were required than the best synthetic amorphous silica to achieve a similar efficacy for larvae and adults, among which most of the precipitated SAS powders showed better effectiveness than the pyrogenic SAS powders (SAS8 and SAS9). In conclusion from the above results, hydrophobic SAS powders were more

effective against adults and hydrophilic powders were more effective against larvae. For larvae, the LD_{50} values of the hydrophilic synthetic silica powders, SAS4 and SAS8, were 15.586 g h/m^2 and 20.394 g h/m^2 at 25±2°C and 55±5% RH, respectively, while the value for the hydrophobic synthetic silica powders, SAS5 and SAS9, were 38.876 g h/m^2 and 51.928 g h/m^2 at 25±2°C and 55±5% RH respectively. However, LD_{50} values of adults were 47.298 and 119.246 g h/m^2 for the hydrophilic synthetic silica powders, SAS4 and SAS8, respectively, while for the hydrophobic synthetic silica powders, SAS5 and SAS9, they were only 38.876 and 66.632 g h/m^2, respectively. Particle size and oil sorption capacity had less impact on efficacy for larvae and adult, while specific surface area and total pore volume were negatively related to the larvicidal efficacy and only total pore volume was negatively related to the efficacy against adults.

2.2 Introduction

With increasing awareness of health and environmental risks caused by synthetic pesticides, many countries reject grain fumigated by phosphine or mixed with chemical pesticides. Insect resistance increases significantly with improper application of pesticides. Inherent biological factors also contribute to resistance. Although methyl bromide is an effective synthetic insecticide, its destructive effect on the ozone atmospheric ozone layer makes it unfit for future use. Developing an alternative grain pest control method is therefore unavoidable.

Inert dusts are a promising nonchemical alternative. These products are persistent and stable at either high temperature or low temperature. Numbers of inert dust formulations are currently commercially available. Effective application for grain storage has been reported (Subramanyam and Roesli, 2000), although there are many factors that impact efficacy, including insects species, grain moisture content, relative humidity, temperature and method of application (Subramanyam and Roesli, 2000). Many formulations are composed of diatomaceous earth and other insecticides, most frequently pyrethrum (0.1 to 0.2%) (e.g., PyriSec®) (Athanassiou et al., 2007) and piperonyl butoxide (1.0%) (e.g., Diacide Homeguard, Diatect, Perma Guard D-20, and Perma-Guard D-21), providing a "double barreled effect" and fast killing rate. However, when such chemicals are added to the diatomaceous earth compositions, the products became toxic to avian and mammalian species including humans, and therefore lose their insect specific killing advantage. The insecticidal efficacy of silica base is mainly relate to high amorphous silicon dioxide content with a uniform particle size (Korunić, 1997). In addition, diatomaceous earth is a natural product and its relatively low efficacy can partly be explained by the possibility that insects have had previous contact with similar material in the environment and become tolerant. Synthetic amorphous silica (SAS) consist of nano-sized primary particles, nano- or micrometer-size aggregates and agglomerates in the micrometer- size range. They have high chemical purity and contain no detectable crystalline silica (Fruijtier-Pölloth, 2012). Synthetic amorphous silica powders are promising alternatives for insect management.

Tribolium castaneum is the most tolerant species when exposed to inert dust, either from structure treatment or when dust is mixed with grain. Increase in temperature generally improves the effectiveness of inert dust against insects due to accelerated insect movement and increased rate of water loss via the spiracles. However, the opposite is true for *T. castaneum* (Arthur, 2000b).

We investigate the potential of nine synthetic amorphous silica powders against four developmental stages of *T. castaneum* as a structure treatment in comparison with Dryacid (a commercially available diatomaceous earth product). *T. castaneum* was selected as the bioassay target for its feeding habit. As an external feeder, it was easy to collect specimens of different developmental stage. The results also provide dosage guideline for other species as *T. castaneum* is the most tolerant insect pests to inert dusts.

2.3 Materials and Methods

2.3.1 Insects

T. Castaneum was obtained from the Academy of State Administration of Grain (ASAG), Beijing, China. To ensure consistency within the experiment, adults were kept for one week after emergence. About 200 first-generation adults were mixed with medium comprising 1 part yeast and 10 parts whole-meal flour milled from Chinese hard wheat, and then kept for 4 days in a growth chamber at 30˚C and 70% RH. The adults were then sieved out to obtain media that contained only insect eggs, which was then also kept at 30°C and 70% RH. As soon as the adult insects emerged from these eggs, they were transferred to new medium and kept at the same temperature and relative humidity conditions for another 20 days before testing.

For easy egg separation, the mated adults were reared on the flour with particle size smaller than 80 meshes, while *T. Castaneum* eggs are bigger than 60 meshes. After ovipositing for 2 days, the adults were removed from the media and the eggs were collected from the media by sieving with an 80 meshes screen. The eggs were then examined under a binocular microscope and, if visibly healthy, transferred into a watch glass. Visual examination immediately afterwards confirmed that the specimens were undamaged and active.

T. Castaneum larvae were 2-4 instars old when used and were separated from adults by sieving gently with 3500 μm mesh sieve. These larvae were then tipped gently onto a

15 cm diameter Whatman No.1 filter paper and left for sufficient time for the larvae to cling to the paper. This paper was then inverted at a 45º angle above a second paper and gently tapped so that media was removed from the larvae.

Tests were performed with pupae (2-4 days old) at laboratory temperatures ranging from 26 to 30ºC and relative humidities from 65 to 75%. Adults and pupae were left behind on a 4000 µm mesh sieve when sieved from the medium. These adults and pupae were then put on the top of a “Pyramid” composed of three different sizes of petri dishes which left the adults to climb down to the bottom. The remaining pupae were examined microscopically to ensure only healthy specimens were used for testing.

2.3.2 Dusts

We evaluated the ten silica-based materials listed in Table 2.1. SAS5 and SAS9 were hydrophobic and the others were hydrophilic. Diatomaceous earth is a natural amorphous silica powder derived from fossilised diatoms, while the others are synthetic amorphous silica. They were prepared by different methods. Precipitated silica is produced by a wet process, and pyrogenic silica is produced by a thermal process.

2.3.3 Measurement of dusts’ physical properties

Pore-size distributions and Brunauer-Emmett-Teller (BET) surface areas were determined from N_2 adsorption/desorption isotherms at 200°C for 2 hours (BK200C, JWGB SCI. & TECH., China), using the Barrett-Joyner-Halenda (BJH) and multipoint BET methods respectively. A Malvern laser diffraction analyzer (Mastersizer2000) measured the particle size distribution of the dusts in a dry analysis. The oil absorption capacity test followed the procedure described by Kim et al., (2015).

2.3.4 Bioassays

Prior to bioassay, 9 cm (inner diameter) glass petri dishes were washed and disinfected by heating at 100°C for 2 hours. The same size of filter paper was pasted to the internal bottom of the petri dishes without any gaps as the cultures would otherwise escape under the paper which would affect population eradication. Also, using filter paper helped to distribute the dust evenly and prevent it to agglomerate. A total of 13.2 mg (2 g/m^2) of the SAS or 26.4 mg (4 g/m^2) of diatomaceous earth, measured with a balance (0.1 mg, ML204/02, Mettler Toledo, Shanghai, China), was applied to each dish. Then, the dishes were shaken manually several times to achieve even distribution of dust on the surface of the filter paper. However, all the dusts flocculated easily even with gentle shaking. To avoid the variation among replicates and samples, we broke all the agglomerations with dissecting needles. For each dose, there were three replicates. An additional set was left untreated as control. All bioassays were performed at 30±1°C and 65±5% RH. To each petri dish 30 *T. Castaneum* of a given development stage were added.

To determine the ovicidal and pupicidal activity of the tested products, eggs and pupae were rolling on the dust-treated surface of the petri dish to ensure contact with the dust. This was achieved by the moving the petri dishes to and fro in different directions, before placing in a climate chamber as described for experiments above. Then, hatched larvae and emerged adults, whether dead or alive, were counted every 24 hours after treatment using a stereomicroscope.

For determination of the larvicidal activity, the efficacy was measured as the mean percentage of dead larvae of the three replicates every hour after treatment. A larva was

counted as dead when no motion was visible after touching with a brush and further became black due to dehydration which was observed using a stereomicroscope.
Every 2 h, the number of adults that were active (walking normally) or affected were recorded using a stereomicroscope. Affected adults included individuals that were walking abnormally, were immobile but with legs or antennae moving, or not moving and unresponsive to being touched with a probe. For calculation of mortality rate, adults that had not recovered by the end of the 24 h post-treatment observation period were assumed to have died or to be unable to recover.

2.3.5 Statistical evaluation

Efficacy was determined for each product by comparing LD_{50}-values and LD_{95}-values calculated by Probit analysis. Difference in the average survival time was estimated using the Log-Rank test.
A two-factor analysis of variance (ANOVA) (Montgomery, 1959; Underwood, 1997) was used to test whether variation was significant between different times and between different dusts. Dust and time were treated as fixed factors that were orthogonal to each other. Although mortality, hatching and emergence values did not follow a Gaussian distribution, the major effects pointed to by the ANOVA were reliable because the method was particularly robust to such divergence in well replicated assays, and clear trends were pointed to by very high F values (Khan and Rayner, 2004). The analysis was performed on SPSS.
Three replications were taken. A Student-Newman-Kreuls (SNK) test and Duncan test were used to perform post hoc multiple comparisons.
To examine correlations between physical property and efficacy, an inter-item correlation matrix analysis was conducted with the LD_{50} value for larvae and adults as

dependent variable and the values of particle size, pore-size distributions, BET surface areas and oil sorption capacity as explanatory variables.

2.4 Results

2.4.1 Physical property of synthetic amorphous silica powders and diatomaceous earth

In Table 2.1, the particle size of the nine SAS powders and diatomaceous earth are displayed. Regarding the particle size distribution, the hydrophilic and hydrophobic precipitated SAS showed particle size in the volume weighted mean range 3.33–8.04 µm. In the case of the surface treated hydrophobic precipitated SAS (SAS5), the particle size was 3.33±0.04 µm and smaller than its bare precipitated SAS (SAS4). Compared with the precipitated SAS, thermal process on both hydrophilic and hydrophobic pyrogenic SAS resulted in a larger agglomerate size, increasing to 20.76±0.31 and 7.32±0.42 µm for SAS8 and SAS9, respectively.

We measured the specific surface areas of the SAS powders and diatomaceous earth and their porosity values using nitrogen isotherm experiments (Table 2.1, BET and BJH analyses). The SAS powders showed a specific surface area above 100 m^2/g except for SAS7 which was 57.45 m^2/g, whereas that of diatomaceous earth was only 37.59 m^2/g. These particles showed the presence of surface coating with a specific surface area of 123.44 and 101.33 m^2/g for hydrophobic precipitated SAS (SAS5) and hydrophobic pyrogenic SAS (SAS9), respectively. Most of the precipitated SAS showed a larger specific surface area than that of the pyrogenic SAS. We also estimated the porosity of the SAS and diatomaceous earth. As summarized in Table 2.1, all of the samples showed an extremely high porosity. In particular, the single point adsorption total pore volume of the precipitated SAS was 0.40-1.05 cm^3/g, which was higher than that of pyrogenic SAS. Regarding the treated surface, the coating process improved the porosity values. The single point adsorption total pore volume of the diatomaceous earth was 0.25 cm^3/g, which was significantly lower than that of the SAS powders. The oil sorption capacity of various SAS powders and diatomaceous earth was determined by gelation with linseed oil, as shown in Table 2.1. It should be noted that the SAS powders showed oil sorption capacity from 209.30±1.69 to 642.37±2.84 mL oil/100g of dust, which was much higher than that of the diatomaceous earth (178.88±1.23 mL oil/100g of dust). By using two different SAS process samples, we clearly showed that pyrogenic SAS powders with thermal process demonstrated a superior absorption capacity of oil compared to that of the precipitated SAS powders. A surface coating of hydrophobic precipitated SAS5 and pyrogenic SAS9 was less efficient at absorbing oil than bare hydrophilic precipitated SAS4 and pyrogenic SAS8.

Table 2.1. Results of the laboratory analysis of nine SAS and one DE differentiated according to their properties and process methods.

SAS and DE powder	Processing methods	Polarity	Volume weighted mean D[4, 3] (µm)	D(v, 0.1)[a] (µm)	D(v, 0.5)[a] (µm)	D(v, 0.9)[a] (µm)	Specific surface area (BET) (m^2/g)	Total pore Volume (cm^3/g)	Oil sorption capacity (mL oil/100 g of dust)
SAS1	precipitated	hydrophilic	3.72±0.06	1.60±0.03	3.34±0.04	6.39±1.24	230.71	1.32	382.67±1.09
SAS2	precipitated	hydrophilic	8.04±1.08	1.63±0.01	4.92±0.11	15.39±2.76	180.91	1.03	319.74±1.12
SAS3	precipitated	hydrophilic	4.46±0.02	1.82±0.01	4.02±0.02	7.78±1.50	201.82	1.05	406.29±0.50
SAS4	precipitated	hydrophilic	7.09±0.02	2.14±0.01	5.78±0.03	14.12±2.62	207.07	0.94	446.36±1.18
SAS5	precipitated	hydrophobic	3.33±0.04	1.45±0.02	2.98±0.04	2.75±1.10	123.44	1.05	241.50±1.23
SAS6	precipitated	hydrophilic	26.30±0.66	2.62±0.03	15.86±0.61	65.10±12.07	162.17	0.93	305.32±0.95
SAS7	precipitated	hydrophilic	3.73±0.14	1.00±0.00	3.03±0.10	7.53±1.43	57.45	0.40	209.30±1.69
SAS8	pyrogenic	hydrophilic	7.32±0.42	1.73±0.05	5.91±0.27	15.02±2.90	101.33	0.29	334.99±0.89
SAS9	pyrogenic	hydrophobic	20.76±0.31	6.39±0.15	15.57±0.28	40.80±7.43	178.52	0.44	642.37±2.84
DE	milled	hydrophilic	11.62±0.10	1.96±0.01	7.09±0.03	28.81±5.20	37.59	0.25	178.88±1.23

[a] Where D(v, 0.1), D(v, 0.5) and D(v, 0.9) were the respective diameters at 10, 50 and 90% cumulative volume.

2.4.2 Hatchability of egg hatching and mortality of newly hatched larvae exposed to different dusts

Figure 2.1 illustrates the effect on the hatchability of eggs and the survival of newly hatched larvae by different dusts. The ANOVA analysis for the hatchability of eggs revealed significant differences at each exposure interval ($F = 1175.973$; $df = 5$; $P < 0.001$), but not for various treated and untreated groups ($F = 1.476$; $df = 10$). The associated interaction exposure interval × dust ($F = 0.780$; $df = 50$) (Table 2.2) was not significant.

All the eggs in each group hatched in six days. Except for diatomaceous earth and SAS8, the lethal rate for newly hatched larvae experienced a significant increase above 80% on the seventh day. On the eighth day, the mortality of newly hatched larvae reached a maximum of 100%. The ANOVA analysis indicated that newly hatched larval mortality was significantly affected by different dusts ($F = 115.639$; $df = 10$; $P < 0.001$) and exposure times ($F = 3036.023$; $df = 5$; $P < 0.001$). The significant interaction between dust and time ($F = 42.460$; $df = 50$; $P < 0.001$) can be explained almost entirely by the large variance of the factor 'time', shown by its extremely high sum of squares value in comparison to the other term (Table 2.3). Mortality for all the synthetic amorphous silica except SAS8, was significantly different to that of diatomaceous earth (Fig. 1). Among the SAS powders most of the precipitated SAS powders showed better effectiveness than that of the pyrogenic SAS powders (SAS8 and SAS9). Mortality for all dusts was significantly different to the control (Fig. 1). For newly hatched larvae, hydrophobic SAS5 and 9 were more effective than hydrophilic SAS4 and 8 (Fig. 1).

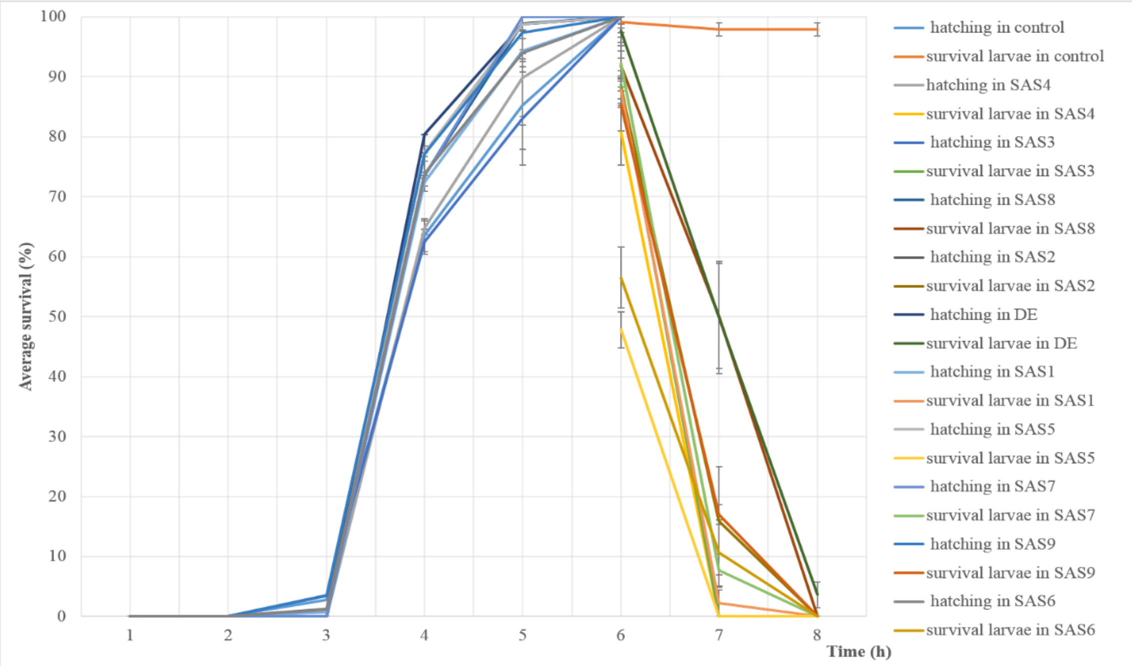


Fig. 2.1. Mean percentage of hatchability of eggs and survivability of newly hatched larvae in *T. castaneum* eggs exposed to 2 g/m^2 of various SAS powders and 4 g/m^2 of diatomaceous earth (30±1°C and 65±5% RH).

Table 2.2. ANOVA parameters for main effects and interactions for hatchability of *T. castaneum* eggs (30±1°C and 65±5% RH).

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	627.501	10	62.750	1.476	0.155
Time	250014.645	5	50002.929	1175.973	0.000
Dust × Time	1657.287	50	33.146	0.780	0.842
Error	5612.705	132	42.520		
Total	1465081.121	198			

Table 2.3. ANOVA parameters for main effects and interactions for newly hatched *T. castaneum* larval mortality (30±1°C and 65±5% RH).

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	22092.465	10	2209.246	115.639	0.000
Time	290011.176	5	58002.235	3036.023	0.000
Dust × Time	40559.142	50	811.183	42.460	0.000
Error	2521.818	132	19.105		
Total	542816.119	198			

2.4.3 Mortality of larvae exposed to different dusts

Using the Log-Rank test (Mantel, 1966) the average survival times were estimated (Fig. 2.2) to compare the speed of action of the ten dusts at LD_{95} (Table 2.4). The LD_{95} values of the larvae treated with SAS1, SAS2, SAS3, SAS4, SAS5, SAS6, SAS7, SAS8, SAS9 and diatomaceous earth were 28.476 g h/m^2 (95% fiducial limits, 26.226-31.254 g h/m^2), 37.052 g h/m^2 (95% fiducial limits, 34.252-40.600 g h/m^2), 37.956 g h/m^2 (95% fiducial limits, 36.174-40.020 g h/m^2), 31.750 g h/m^2 (95% fiducial limits, 28.758-35.598 g h/m^2), 55.696 g h/m^2 (95% fiducial limits, 52.452-60.188 g h/m^2), 34.018 g h/m^2 (95% fiducial limits, 31.706-36.844 g h/m^2), 92.072 g h/m^2 (95% fiducial limits, 78.816-114.21 g h/m^2), 35.626 g h/m^2 (95% fiducial limits, 33.626-37.976 g h/m^2), 112.884 g h/m^2 (95% fiducial limits, 96.338-139.986 g h/m^2) and 153.478 g h/m^2 (95% fiducial limits, 132.510-185.972 g h/m^2), respectively.

For larval survival, the ANOVA analysis showed significant differences of the main effect of dusts ((F = 103.466; df = 9; P < 0.001), and exposure interval (F = 470.641; df = 10; P < 0.001) (Table 2.5). Results from the Log-Rank test showed that survival times for the larvae treated with different dusts for 3, 14 and 17.5 hours were significantly different (Table 2.5). Synthetic amorphous silica powders significantly reduced the survival of larvae of *T. castaneum* compared to diatomaceous earth (Fig. 2.2). Interestingly, hydrophilic SAS (SAS4 and SAS8) were more effective than hydrophobic SAS (SAS5 and SAS9) against larvae (Fig. 2.2). The lowest survival of larvae was from the precipitated silica treated groups (SAS1, SAS2, SAS4 and SAS6).

Table 2.4. Lethal dose values and regression curve parameters for 2 g/m^2 of various SAS powders and 4 g/m^2 of diatomaceous earth tested against *T. castaneum* larva (30±1°C and 65±5% RH).

dust	Mean ± SE	LD (95% CL) (g h/m^2)	
	Intercept	LD_{50}	LD_{95}
SAS1	-1.826±0.061	14.980(13.668-16.412)	28.476(26.226-31.254
SAS2	-2.184±0.074	21.136(19.098-23.156)	37.052(34.252-40.600)
SAS3	-2.335±0.080	22.268(20.912-23.584)	37.956(36.174-40.020)
SAS4	-1.586±0.055	15.586(13.618-17.624)	31.750(28.758-35.598)
SAS5	-1.151±0.045	38.876(36.556-41.076)	55.696(52.452-60.188)
SAS6	-2.290±0.078	19.796(18.180-21.446)	34.018(31.706-36.844)
SAS7	-1.620±0.048	45.692(39.232-53.696)	92.072(78.816-114.21)
SAS8	-2.202±0.073	20.394(18.896-21.868)	35.626(33.626-37.976)
SAS9	-1.401±0.043	51.928(44.890-61.024)	112.884(96.338-139.986)
DE	-1.622±0.047	76.202(67.690-88.184)	153.478(132.510-185.972)

Table 2.5. ANOVA parameters of main effects and interactions for *T. castaneum* larval survival (30±1°C and 65±5% RH).

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	65677.203	9	7297.467	103.466	0.000
Time	331942.105	10	33194.210	470.641	0.000
Dust × Time	46786.021	75	623.814	8.845	0.000
Error	13400.667	190	70.530		
Total	1016441.0	285			

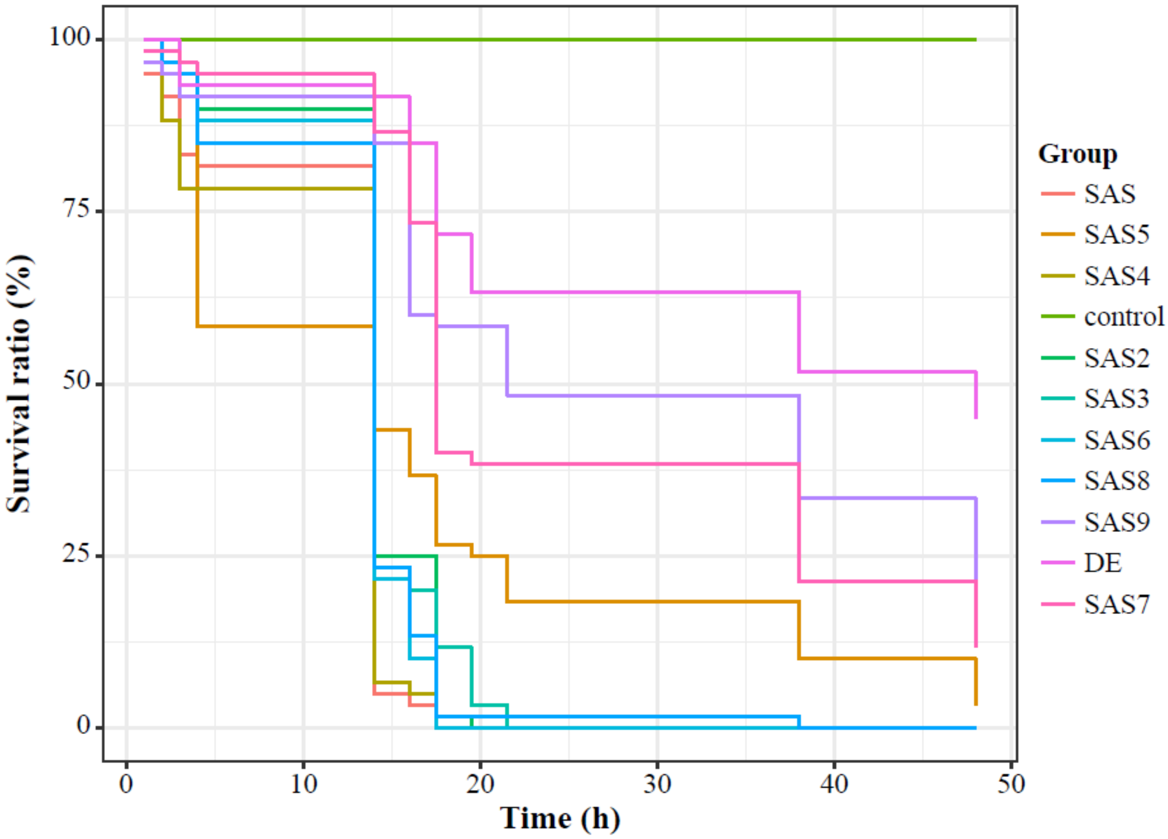


Fig. 2.2. Survival curve of the *T. Castaneum* larvae exposed to 2 g/m^2 of various SAS powders and 4 g/m^2 of diatomaceous earth at 30±1°C and 65±5% RH.

2.4.4 Rate of pupal emergence and mortality of newly emerged adults after exposure to different dusts

As shown in Fig. 2.3, the pupae, like eggs, were more tolerant than the other developmental stages when exposed to dusts and cannot be completely eliminated. However, all the newly emerged adults were completely killed by all the dusts within 24 hours, while the pupae required 92 hours of exposure (Fig. 2.3). No mortality was observed in the young adult controls.

There was significant difference in the emergence rate of *T. Castaneum* pupae among the treated and untreated groups, but not for the SAS4 treated group ($F = 13.487$; df = 10; $P < 0.001$) (Table 2.6). One of the hydrophilic powders, SAS1, was most effective in terms of eliminating pupae. Diatomaceous earth was ineffective against pupae in contrast to synthetic amorphous silica powders. The ANOVA analysis for pupal emergence revealed significant differences for exposure interval ($F = 151.729$; $df = 10$; $P < 0.001$) and interaction for exposure interval × dust ($F = 1.889$; $df = 10$; $P < 0.001$) (Table 2.6).

For newly emerged adult mortality, the ANOVA analysis showed significant differences for the main effect of dust ($F = 6.711$; $df = 9$; $P < 0.001$) and exposure interval ($F = 155.371$; $df = 10$; $P < 0.001$), but not for interactions (Table 2.7). According to exposure interval analysis, the total mortality was significantly different among groups at 16.5, 42 and 90 hours. Although no significant differences were found for newly emerged adult mortality between diatomaceous earth and most synthetic amorphous silica powders, highest mortality was obtained with the precipitated silica treated groups (SAS1, SAS3 and SAS4) from the beginning of pupa emergence (12 h) to 4.5 hours later (16.5 h). There was no significant difference for newly emerged adult mortality between hydrophilic SAS and hydrophobic SAS (Fig. 2.3).

Table 2.6. ANOVA parameters of main effects and interactions for *T. castaneum* pupal emergence (30±1°C and 65±5% RH).

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	11453.168	10	1145.317	13.487	0.000
Time	128844.077	10	12884.408	151.729	0.000
Dust × Time	16043.802	100	160.438	1.889	0.000
Error	20550.0	242	84.917		
Total	467450.0	363			

Table 2.7. ANOVA parameters of main effects and interactions for newly emerged *T. castaneum* adult mortality (30±1°C and 65±5% RH).

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	21496.803	9	2388.534	6.711	0.000
Time	553010.074	10	55301.007	155.371	0.000
Dust × Time	42794.767	90	475.497	1.336	0.046
Error	73320.982	220	333.277		
Total	2088767.873	330			

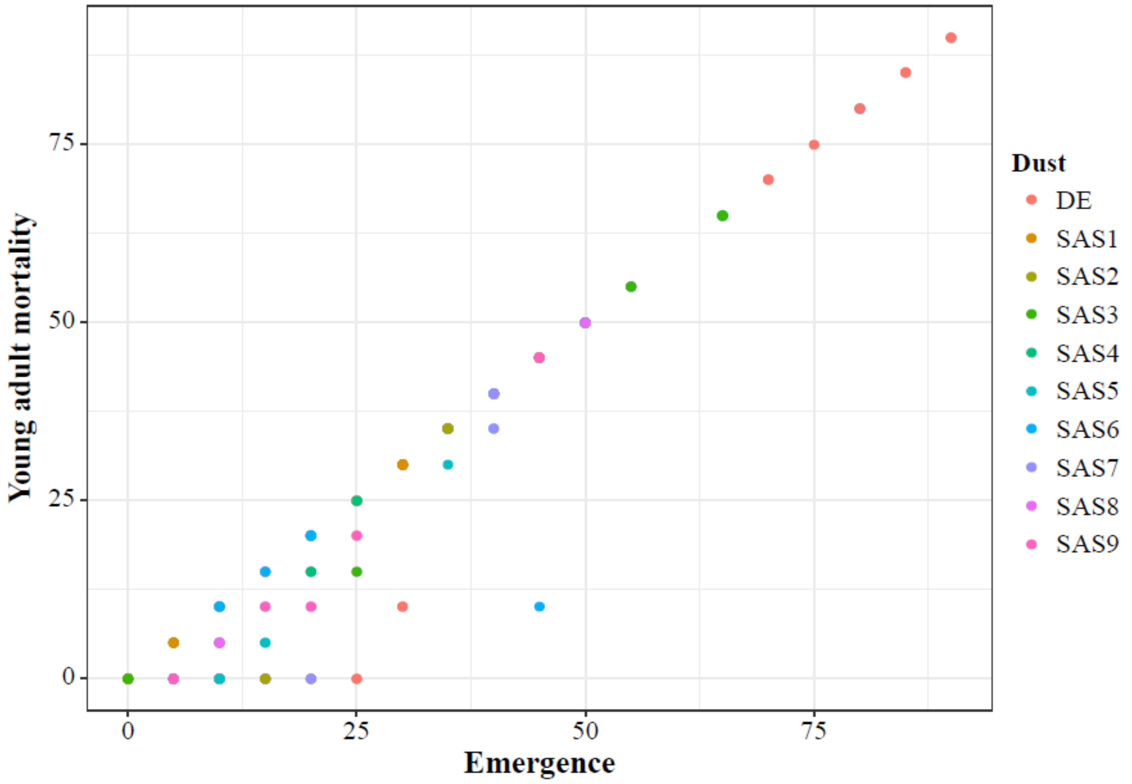


Fig. 2.3. Mean percentage of Pupal emergence and newly emerged adult mortality of *T. castaneum* exposed during the pupal stage to 2 g/m^2 of various SAS powders and 4 g/m^2 of diatomaceous earth (30±1°C and 65±5% RH).

2.4.5 Mortality of adults exposed to different dusts

Bioassay results were used to determine the lethal dose for *T. Castaneum* adults (Table 2.8). These suggested that the lethal dose at LD_{95} for the most effective synthetic amorphous silica, SAS5, was 38.876 g h/m^2 with a 95% confidence interval of 37.232-40.458. SAS1 was the second more effective powder against *T. Castaneum* adults with a lethal dose at LD_{95} being 42.764 g h/m^2 with a 95% confidence interval of 41.246-44.258. This was followed by SAS3, SAS4, SAS2, SAS9, SAS7, diatomaceous earth, SAS6 and SAS8. No mortality was observed in the control adults.

ANOVA statistical analysis showed that adult mortality among dusts ($F = 1130.431$; df = 9; $P < 0.001$), exposure period ($F = 336.855$; df = 78; $P < 0.001$), and interaction between dust and time ($F = 9.938$; df = 368; $P < 0.001$) were significantly different (Table 2.9). Hydrophobic SAS powders (SAS5 and SAS9) were more effective than hydrophilic ones (SAS4 and SAS8) for adult elimination (Fig. 2.4). The survival time among synthetic amorphous silica treated adults was significantly lower than that for diatomaceous earth except for SAS6, SAS7 and SAS8 (Fig. 2.4).

Table 2.8. Lethal dose values and regression curve parameters for 2 g/m^2 of various SAS powders and 4 g/m^2 of diatomaceous earth tested against *T. castaneum* adults (30±1°C and 65±5% RH).

dust	Mean ± SE	LD (95% CL) (g h/m^2)	
	Intercept	LD_{50}	LD_{95}
SAS1	-3.910±0.087	42.764(41.246-44.258)	60.760(58.290-63.854)
SAS2	-3.485±0.061	56.872(55.832-57.898)	83.710(81.972-85.632)
SAS3	-3.219±0.069	42.596(40.846-44.274)	64.362(61.678-67.658)
SAS4	-3.600±0.071	47.298(45.718-48.846)	68.906(66.418-71.892)
SAS5	-3.802±0.085	38.876(37.232-40.458)	55.694(53.270-58.756)
SAS6	-3.374±0.040	95.126(94.106-96.132)	141.506(139.796-143.39)
SAS7	-3.426±0.052	73.214(72.090-74.318)	108.368(106.470-110.440)
SAS8	-4.353±0.053	19.246(118.126-120.370)	164.302(161.990-166.814)
SAS9	-2.959±0.047	66.632(65.740-67.512)	103.668(102.146-105.294)
DE	-2.461±0.035	71.226(69.508-72.880)	118.828(116.392-121.482)

Table 2.9. ANOVA parameters of main effects and interactions for *T. castaneum* adult mortality (30±1°C and 65±5% RH).

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	577902.761	9	64211.418	1130.431	0.000
Time	1492471.522	78	19134.250	336.855	0.000
Dust × Time	2077461.170	368	564.528	9.938	0.000
Error	51803.962	912	56.803		
Total	6281226.676	1368			

Table 2.10. Inter-item correlation matrix analysis with the LD_{50} values of larvae and adults as the dependent variable and the values of particle size, pore-size distributions, BET surface areas and oil sorption capacity as explanatory variables.

Parameters	Coefficient of impact of parameters on LD_{50} values	
	Larvae	Adult
Particle size	0.154	0.453
Pore-size distributions	-0.673	-0.660
BET surface areas	-0.723	-0.473
Oil sorption capacity	-0.248	-0.144

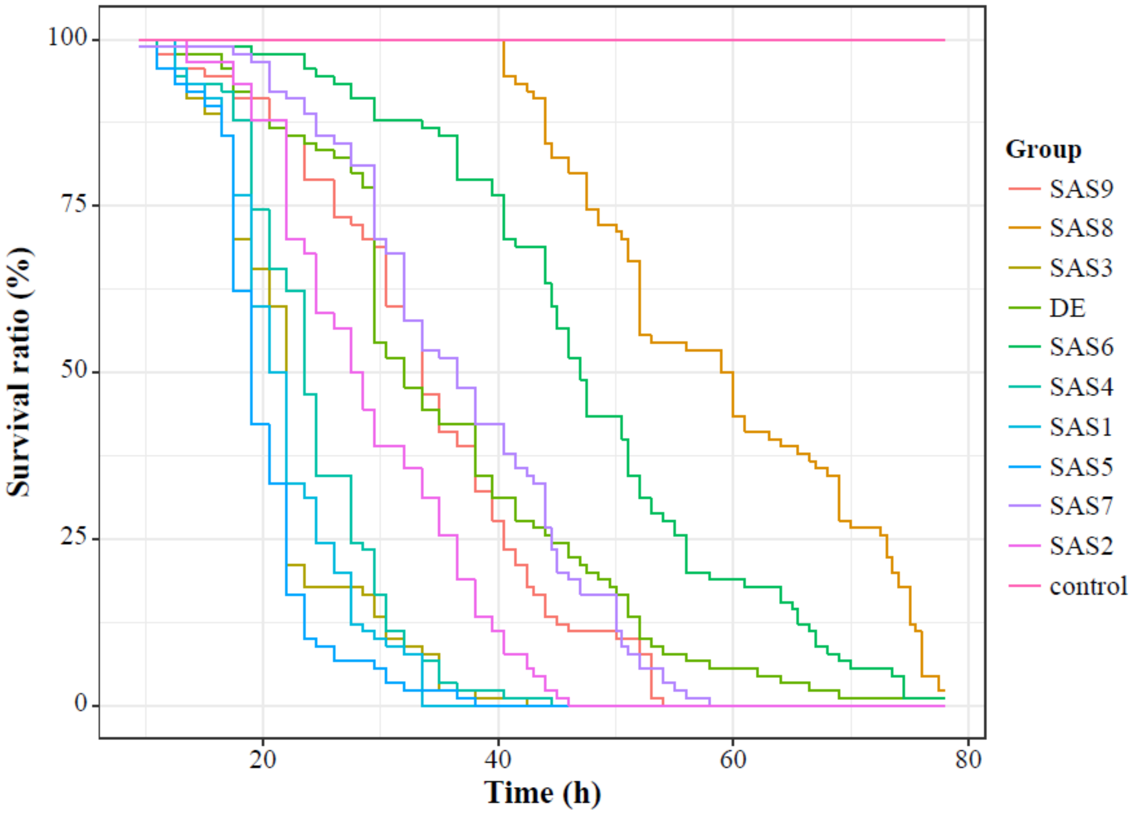


Fig. 2.4. Survival curve of *T. Castaneum* adults exposed to 2 g/m^2 of various SAS powders and 4 g/m^2 of diatomaceous earth at 30±1°C and 65±5% RH.

2.4.6 Examination of correlations between physical properties of dust and efficacy

Inter-item correlation matrix analysis of the parameters particle size, pore-size distribution, BET surface area, and oil sorption capacity, and the LD_{50} values showed that the pore size distribution and BET surface area are the main effect on speed of action against larvae, while only the pore size distribution is the main effect on speed of action against adults (Table 2.10). These parameters have a negative impact on LD_{50} values which means they are related to a fast mode of action. In contrast, oil sorption capacity and particle size have less influence on speed of action than the other two parameters against larvae and adults.

2.5 Discussion

2.5.1 Effect of the physical properties of dust on efficacy against insects

We evaluated the efficacy of nine SAS powders against *T. Castaneum* at different developmental stages and compared this with one commercial diatomaceous earth. Diatomaceous earth achieved 100% mortality slower than the SAS powders. This can be explained by the different patterns of physical properties between diatomaceous earth and SAS powders (Korunić, 1997) which have impacts on efficacy. Small particles (SAS) offer larger specific contact surface area compared to larger particles (diatomaceous earth). However, the hypothesis that the smaller the particle the better the efficacy is not proven. Pyrogenic silica (SAS8 and SAS9) have smaller primary particle sizes (5–50 nm) compared to precipitated silica (SAS1 to SAS7) (5-100 nm). These properties, especially the density, will influent the impingement of the dusts on the insects. Several independent studies also showed that smaller particles of aerosols couldn't be impinging on an insect surface (Arthur et al., 2014, 2017, 2018; Teske et al., 2000). Korunic (1997) has reported that adherence positively correlates with the insecticidal activity of a given diatomaceous earth. Higher oil absorption capacity of SAS suggests cuticular lipid absorption in insects (Ebeling, 1971; Korunić, 1997). We did not observe this effect, thus doubt its relevance with insecticidal efficacy.

2.5.2 Effect of morphological structure and physiology on mortality of insects

Vayias and Athanassiou (2004) studied the mortality of adults and larvae of *T. Castaneum* when exposed to a diatomaceous earth formulation, SilicoSec, and concluded that larvae were more sensitive to diatomaceous earth than adults. In the same work, the sensitivity order in *T. Castaneum* larvae and adults to another diatomaceous earth formulation, Fossil Shield, was similar (Mewis and Ulrichs, 2001). In our experiment, we observed a similar variation in insecticidal efficacy against different developmental stages for all the dusts. Mortality was higher in larvae and newly emerged adults, but not in newly hatched larvae and adults. There was fluid present in the broken eggshells at hatching, which is possibly related to the low mortality in new hatching larvae. In our study, after the newly hatched larvae emerged, they were placed in the treated arenas. This treatment produced a high individual mortality. For larval control, the hydrophilic SAS are more effective than the hydrophobic ones. In contrast, the adult stage was more susceptible to the hydrophobic SAS than to hydrophilic SAS.

One of the most interesting results from this study was that two stationary stages, egg and pupae, were far more tolerant to all the dusts than the two active stages, larvae and adults. At the egg stage, there was little or no mortality regardless of the exposure time and high absorption ability of SAS. A 100% hatching rate was achieved for all the SAS powders and diatomaceous earth. The retention rate of dust on the surface indicated by visual observation was poor. In our later trail, the hatch rate of eggs was still 100% even when buried in the dust. Due to the absent of movement, there was not fresh dust exchange. Compared with the egg stage, pupae were not completely stationary and rolling occurs at intervals. Older pupal stages just prior to emergence were less active and showed better dust attachment on the surface, and better dust exchange. More than half of pupae could not emerge.

Apart from these factors, insect activity level may also be responsible for these variations. Several studies have been carried out on the dust mechanisms of stored product insect pests, mostly on the cuticle damage caused by lipid absorption. However, the biological effect of dust was not well documented. In our study, we observed that different stages (eggs, young and old larvae, pupae, and adults) shared similar variation in mortality due to dust and respiration rates at 30°C in normal atmospheric air. Respiration is a good index of the physiological responses of insects to the environment (Emekci et al., 2002). Detailed information on insect activity level and mortality during dust applications is lacking, but would be useful to re-evaluate the current findings or for predicting efficacy in the field.

Chapter 3. Evaluation of the effect of electrostatic charge between stored grain insects and synthetic amorphous silica (SAS) on insect mortality

3.1 Abstract

This chapter describes a comprehensive evaluation of dusting effectiveness and proposed new protocols for dusting. In this study, three major stored grain insects, *Sitophilus oryzae*, *Tribolium castaneum* and *Cryptolestes ferrugineus* carried positive electrostatic charge by contact with filter paper and glass. Statistical analysis showed that the insects could be grouped on the basis of charge-to-mass ratio. The properties and amount of the charge differed significantly among the electronic characteristics of the insulation surface and insect species. Charge accumulated by dusts in similar condition was negative and can be assigned to different groups statistically in relation to charge-to-mass ratio. The dosage of the dust also interacted with the dust charge-to-mass ratio. During the investigation of the effect of electrostatic charge on the treatment of stored grain pests by dust, we observed a linear correlation between charge-to-mass ratio and bioactivity of dust. This discovery solves for the first time, the problem of how to apply dusting and provides an effective non-chemical pesticide alternative for pest management.

3.2 Introduction

Synthetic pesticides are widely used to control stored grain insect pests because of their high efficacy and relatively low cost. However, with increased consumer awareness of the potential abuse of chemical pesticides and concerns over insects developing pesticide resistance, there is an increasing interest in the development of alternatives to conventional pesticides. The high cost of developing new chemical products is another reason for this direction.

Inert dusts offer such an alternative method with successful precedents for commercial applications in stored grain pest control (Banks and Fields, 1995; Ebeling, 1971; Golob, 1997; Korunic, 1998; Mewis and Ulrichs, 2001; Subramanyam and Roesli, 2000). The most recent research and application was largely focused on synthetic amorphous silica (SAS). Synthetic amorphous silicon is a distinct, manufactured form of silicon dioxide as a pyrogenic (fumed), precipitated, gel or colloidal SAS. It consists of nano-sized primary particles, nano- or micrometre-sized aggregates and micrometre-size agglomerates (Fruijtier-Pölloth, 2012). The vast majority of commercial SAS powders with the exception of colloidal SAS and some nanoscaled aggregates, are a mix of complex aciniform (grape-like) particle aggregates of dimensions no less than 100 nm (Fruijtier-pölloth, 2012). Analysis indicates that pyrogenic, precipitated and gel particles do not fit the classical definition of nanoparticles due to size (ISO, 2010). Therefore, only the precipitated SAS was involved in this study. No known incident has been reported regarding human health issues or environmental risks with these materials. Synthetic amorphous silica powders have been widely used as anti-caking agents, adsorbents, fillers, thickening agents, and free-flow agents in pharmaceuticals, cosmetics, and food and feed products. Particle shape and surface characteristics are directly related to the biological activity of SAS (Fruijtier-pölloth, 2012).

The action of inert dust varies greatly depending on the composition of the products, type of formulation, insect species, and environmental conditions (Alexander et al., 1944; Vayias and Stephou, 2009; Wigglesworth, 1944). However, no comprehensive investigation of its efficacy has been conducted. Like other contact insecticides, SAS particles have to establish attachment to the insect’s surface before insecticidal action. However, the attachment processes during initial contact of SAS with the insect is poorly understood.

It has been suspected electrostatic charge plays a significant role in dusting efficacy. A resting electric charge exists on the surface of any insulate body and establishes a surrounding electrostatic field. The polarity and strength of the electrostatic field differs based on the materials, surface roughness, temperature, insect species and other properties. However, there has been no comprehensive study that can confirm this theory and there is a critical need for a quantified protocol for applying dusting.

Insects accumulate charge when in contact with an insulate substance (Davies, 1969; Edwards, 1962; McGonigle et al., 2002; McGonigle and Jackson, 2002). The quantity of the charge depends on the interaction between the insect and the substance (Takle and Lackie, 1985). The electrostatic charge in insects was first explored in 1929 (Law, 2001) yet its important role in electrostatic spray technology has been largely neglected until recently (Moon et al., 2003; Warnke, 1976; Zhao et al., 2008). In Moon et al., (2003) the link between bio-efficacy and the electrostatic deposits of charged insecticide on electrically isolated insects was discovered. Electrostatic charge can affect the adhesion of entomopathogenic fungi to host insect cuticle (Boucias et al., 1988; Colin et al., 1992; Hajek, 1994; Lord and Howard, 2004; Shah et al., 2007) and is involved in the interaction between the parasite Varroa jacobsoni and its host Apis mellifera (Castner and Nation, 1984).

We have for the first time comprehensively investigated the correlation of the insecticidal efficacy and charge-mass-ratio in three stored grain insect pest populations with five dusts (four different hydrophilic precipitated SAS powders and Protect-it, which is one of the most effective commercial diatomaceous earth) on two dielectric surfaces. Our findings provide an explanation why poor dusts adhesion to some species has been observed, such as to *Tribolium castaneum*, and *T. confusum*, we therefore propose a new mechanism of action and new guidelines for dusting.

3.3 Materials and Methods

3.3.1 Dusts

We selected four hydrophilic precipitated SAS powders and one natural diatomaceous earth (DE) which were provided by Murdoch University (MU), Western Australia and Academy of State Administration of Grain (ASAG), China in this study. Synthetic amorphous silica powders exclusively contain amorphous silicon dioxide while natural products, based on diatomaceous earth (DE), contain a small amount (<1%) of crystalline silicon dioxide. All these products are classified as food additives (E551).

3.3.2 Insects

The insect species tested are shown in Table 3.1. They were cultured at 30±1℃, 75±5% RH at the Academy of State Administration of Grain, Beijing, China. The insect culturing and handing follows the protocols described by Winks (1982) for secondary feeders and primary feeders. *Sitophilus oryzae* was reared on wheat. *Cryptolestes ferrugineus* and *T. castaneum* were reared on a medium containing 10 parts wheat-meal flour milled from Chinese hard wheat, 10 parts oat and 1part yeast. Prior to grinding for rearing, the wheat was washed, disinfected in oven at 80℃ for 4 hours, and then conditioned to 13.5% moisture content. Twenty-day old adults were used in the study.

Table 3.1. Contents of each insect sample.

Insect species	Geographical strains
Sitophilus oryzae（L.）	Tongzhou (TZ-SO)
Cryptolestes ferrugineus (Stephens)	Zhanjiang (ZJ-CF)
Tribolium castaneum (Herbst)	Qihe (QH-TC)

3.3.3 Measurement of temperature and relative humidity

During the electrostatic charge measurement and bioassay assessment, the whole Faraday system and all replicates were kept in climate controlled laboratories at the desired temperature with a tolerance limit of ±0.2°C. Temperature and humidity were automatically monitored with a temperature and humidity control system (CD901, Guangzhou Shenzhen Dongdahengfeng Automotive Parts Co. Ltd). The individual bioassay experiments were performed at 28±1°C, 65±5% RH, while others were placed at 24.5±0.5°C, 28.5±0.8% RH.

3.3.4 Measurement of electrostatic charge

Two types of dielectric carriers were used: 9 cm diameter glass petri dishes and Whatman No.1 filter papers.

The net charge of insects and dusts was measured by dropping them into a Faraday cup (ESD-China.com) connected to a Static Charge Coulomb meter (ES111B, www.ESDEMC.com) (Fig. 3.1). Both Faraday cup and the surface to be tested were housed within an earthed Faraday cage. The operator was also earthed. Prior to experimentation, each dielectric surface was cleaned with 100% ethyl alcohol to remove traces of dirt and allowed to dry. The Static Charge Coulomb meter showed that this was also effective in removing any residual charge.

3.3.5 Triboelectric series of surface materials with relation to different insect species

All the insects were placed on an earthed surface to remove charge before being allowed in contact with surface material. This discharging procedure was necessary to establish a baseline for the charging capacity of different insect species and was checked at regular intervals by placing the discharging insects into the Faraday cup.

Fig.3.1. Photograph of a faraday cup connected to a Static Charge Coulomb meter.

Each group of fifty insects gained or lost electrons from/to the contact surfaces by gently shaking them in a lidded Petri dish for 1 minute before tipping into the Faraday cup. Any deflection of the Static Charge Coulomb meter was recorded. In the filter paper surface test, we used Whatman No.1 filter paper to cover all the inside surfaces of a seal container. The mass of each replica was measured, and the charge-to-mass ratio calculated. There were fifteen replications.

3.3.6 Variation contributed by surface materials and categories on charge accumulation by dusts

We evaluated the accumulated charge on four SAS powders and one DE after contact with different surfaces (glass and filter paper) at different dosages (1 and 2 g/m^2). The dose of 2 g/m^2 corresponds to that recommended for surface treatment to control *S. oryzae* and *T. castaneum*, while the low dose of 1 g/m^2 was barely enough to produce a deflection on the indicator, yet still effective for sensitive species, such as *C. ferrugineus*.

The amount of each dust calculated for a 1 and 2 g/m^2 dosage on a 9 cm diameter surface was added to the substrate surface and evenly spread by gently shaking it in a lidded petri dish for a period of 1 min before transferring the dusts into the Faraday cup. Any deflection of the electrometer indicator was recorded. In the filter paper surface test, we used Whatman No.1 filter paper to cover all of the inside surface of a lidded petri dish. The mass of the replica was measured, and the charge-to-mass ratio calculated. In each trial nine plates were used and residual charges removal was always ensured.

3.3.7 Variation in dust efficacy contributed to by the different dusts, insect species and surface materials

The effects of electrostatic charge on the efficacy of the five dusts were determined by exposing charged *C. ferrugineus, S. oryzae* and *T. castaneum* to the deposits of charged dusts and testing the outcome at 28±1℃, 65±5% RH. Each of the five dusts was added to the substrate surface at an estimated concentration of 0.2 g/m^2 for *C. ferrugineus*, 2 g/m^2 for *S. oryzae* and 2 g/m^2 for *T. castaneum,* and evenly charged by gently shaking backwards and forwards for 1 min. Replicated groups of 20 adults of each species achieved electrostatic charge by contact with the insulated surfaces before being transferred to the dust deposits. The onset of toxic action was determined by knockdown (KD) and paralysis. In the case of bioassays using *S. oryzae*, the sides of petri dishes were treated with “Fluon” to prevent escape. The time for irreversible KD to occur (KT) was determined by periodic observation. The insects were considered KD when they were dorsally recumbent or could regain sternal posture but lost proprioceptive coordination within at least two minutes. An additional set was left untreated as control. KD_{50} and KD_{95} values (time for 50% and 95% KD, respectively) were calculated by interpolation of KD between times when data were collected; average KD values were obtained from the individual KD data.

3.3.8 Data analysis

Work function of static charge of three insect pests contacted with two dielectric surfaces was calculated as a charge-to-mass ratio. The data of charge-to-mass were transformed to a $ln\ x$ scale. A two-way analysis of variance (ANOVA) was used to test whether the charge-to-mass ratios induced on three stored grain insect species by two different materials were significantly different and whether significant differences existed between filter paper and glass dielectric surfaces. *Post hoc* tests were performed using Student-Newman-Kreuls (SNK) and Tukey's HSD test ($p < 0.05$) to compare both the surface and insect species (IBM SPSS Statistics ver. 20).

The work function of static charge of dusts in contact with filter paper and glass was calculated as charge-to-mass ratios. The data of charge-to-mass ratios were transformed to a $ln\ x$ scale and were subjected to three-way ANOVA to determine differences among the dusts on both surfaces. Tukey's HSD test ($p < 0.05$) was performed to detect significant differences between treatments.

Insecticidal efficacy of each product against three insect species was calculated as a percentage. Adult mortality on untreated surfaces (control) across all exposure times after treatment was 0%. Therefore, mortality data in dust treatment were not corrected for mortality in the control treatment (Abbott, 1925). Time-mortality data for three insect species against various dusts were subjected to probit analysis (Abbott, 1925) for determination of the time for 50% (KD_{50}) and 95% (KD_{95}) rates of KD and associated statistics.

In addition, we used a three-way ANOVA with partial interactions between factors to evaluate the effects of insect species (*C. ferrugineus*, *S. oryzae* and *T. castaneum*), dust categories (four hydrophilic precipitated SAS powders and one natural diatomaceous earth) and dielectric surfaces (filter paper and glass). To comply with ANOVA requirements (homoscedasticity for response variables and normal distribution of the residuals), the KD_{95} value was analysed after $ln\ x$ transformation. Although KD_{95} values did not strictly follow a normal distribution, ANOVA results are still reliable because ANOVA is fairly robust to such divergence in well replicated assays. Very high F values confirmed this. Means per experimental group were compared using the multiple comparison Tukey test at 5% when applicable.

To examine correlations between electrostatic charge and efficacy, hierarchical clustering analysis using complete distance was conducted on the KD_{95} values of the three replicates as dependent variable and the values of insect, dust and dielectric surface as explanatory variables. Cluster robust standard error terms were calculated, considering the dependency of the three replicates.

The relationship between electrostatic charge and efficacy was evaluated by multiple linear regression.

3.4 Results

Precise measurement of the electrostatic charges carried by stored grain insects and SAS powders allows us to investigate the role of this abiotic factor in pest control. The grain insects and SAS powders obtain positive and negative electrostatic charge respectively when in contact with the same surface. The polarity and the amount of charge is significantly different among insect species, dust categories and dielectric surfaces. Electrostatic charge was a key component in efficacy of SAS powders against insect species.

3.4.1 Triboelectric series of surface materials with relation to different insect species

Three insect species were all positively charged on two types of surface. Filter paper surface charging different species were arranged in the order of charge-to-mass as follow: *C. ferrugineus*, then *S. oryzae*, then *T. castaneum* [3.457 (±0.317) × 10^{-6} C/kg, 2.371 (±0.089) × 10^{-6} C/kg, 0.100 (±0.023) ×10^{-6} C/kg], respectively (Fig. 3.2). In *C. ferrugineus* and *S. oryzae*, the charge was higher on filter paper than on glass. Interestingly, in *T. castaneum*, we observed a totally opposite outcome where the glass surface generated a higher charge. The reduction in the amount of charge acquired by different species on glass varied. The charge-to-mass ratio of *S. oryzae* experienced a significant drop from filter paper carrier to glass carrier (Table 3.2). The charge-to-mass ratio of *C. ferrugineus* fell gradually from one medium to the other and the change was much less dramatic (Table 3.2). Thus, with glass surface the order of charge-to-mass were as follows: *C. ferrugineus,* then *S. oryzae*, then *T. castaneum* [3.419 (±0. 249) ×10^{-6} C/kg, 1.947 (±0.130) ×10^{-6} C/kg, 1.066 (±0.075) ×10^{-6} C/kg, respectively] (Fig. 3.2). The significant interaction between insect × surface ($F_{1.548, 1.264} = 7.346$; $P < 0.008$) (Table 3.3) can be explained almost entirely by the large variance of the factor ‘insect’, shown by its extremely high sum of squares value in comparison to the other factor ‘surface’ (Table 3.3).

3.4.2 Variation contributed by surface material, dose and dust type on electrostatic charge of dust

When fraction occurred between dusts and two insulated materials, dusts tended to obtain electrons from both glass and filter paper carriers and were negatively charged, as shown in Fig. 3.3.

The difference between the charging of glass carrier and filter paper ($F_{2.311,\ 0.454}$ = 198.399; P < 0.000) was most significant (Table 3.4). All the different dusts got a higher charge-to-mass on the glass carrier than on the filter paper carrier, which ranged from 1.38 times (2 g/m^2 DE) to 9.89 times greater (1 g/m^2 SAS4).

Concentrations of 1 g/m^2 and 2 g/m^2 are recommended dosages of dust for pest control. Variation in capacity in gaining electrons from dust between low and high dose was significant ($F_{29.617,\ 0.454}$ = 2542.903; P < 0.000). A large increase in electrostatic charge from 1.70 times (SAS1) to 4.88 times greater (DE) was observed when the lower dose was used on the glass carrier (Fig. 3.3). With the lower dose, SAS2 and SAS4 on the filter paper actually generated a much higher electrostatic charge compare to the high dose under the same condition. (Fig. 3.3). Over all, on filter paper, SAS2 and SAS4 performed better under the lower dose, SAS1, SAS3 and DE performed better under the high dose; on glass carrier, all dusts attained a higher charge under the low dose.

All the dusts behaved very differently from each other ($F_{13.598,\ 0.454}$ = 194.596; P < 0.000). The charge-to-mass ratio of all the SAS was higher than that of DE (Fig. 3.3). SAS1 was the best one in getting static charge among the five dusts, followed by SAS3, SAS2 and SAS4 at the 2 g/m^2 dosage on both carriers and at the 1 g/m^2 dose on the filter paper carrier. The range of charge-to-mass ratio fluctuated slightly among SAS3, SAS2 and SAS4 at the 1 g/m^2 dose on the glass carrier.

The significant interaction between dust and dose ($F_{2.158,\ 0.454}$ = 46.322; P < 0.000), and between dust and surface ($F_{1.393,\ 0.454}$ = 29.907; P < 0.000) can be explained by the large variance of the factors ‘dose’, ‘dust’ and ‘surface,’ shown by their extremely high sum of squares value (Table 3.4). This interaction term is significant because the rank order of dust was inconsistent from surface to surface and different dose.

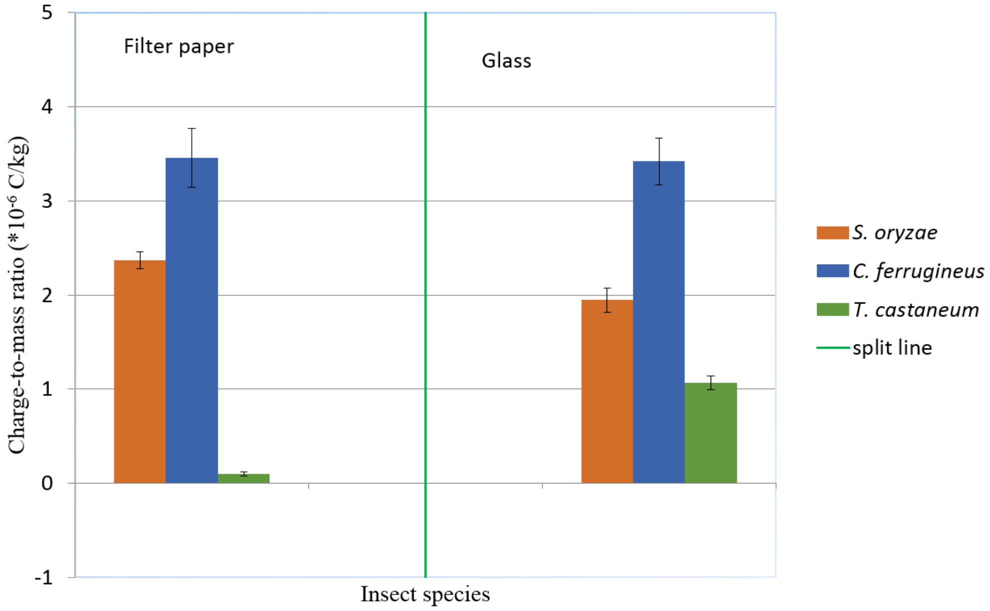


Fig. 3.2. Charge-to mass ratio acquired by three species of stored grain insect adults after shaking on two insulated surfaces for 1 min at 24.5±0.5°C and 28.5±0.8% RH.

Table 3.2. ANOVA parameters showing the significance of variation of charge-to-mass ratio of three insect species on two insulated surfaces at 24.5±0.5°C and 28.5±0.8% RH.

Insect species	Source	Type III Sum of Squares	df	Mean Square	F	*P*
T. castaneum	Surface	7.017	1	7.017	152.561	0.000
	Error	1.288	28	0.046		
	Total	18.482	30			
C. ferrugineus	Surface	0.011	1	0.011	0.017	0.897
	Error	18.057	28	0.645		
	Total	372.690	30			
S.oryzae	Surface	0.809	1	0.809	7.261	
	Error	1.782	16	0.111		
	Total	86.506	18			

Table 3.3. ANOVA parameters showing the significance of variation between insect and surface based on the same weight of insect at 24.5±0.5°C and 28.5±0.8% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Insect	24.553	2	12.276	116.533	0.000
Surface	0.127	1	0.127	1.210	0.293
Insect × Surface	1.548	2	0.774	7.346	0.008
Error	1.264	12	0.105		
Total	103.868	18			

Table 3.4. ANOVA parameters showing the significance of variation between dust, dose and surface on the same weight of dust at 24.5±0.5°C and 28.5±0.8% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	13.598	6	2.266	194.596	0.000
Dose	29.617	1	29.617	2542.903	0.000
surface	2.311	1	2.311	198.399	0.000
Dust × Dose	2.158	4	0.540	46.322	0.000
Dust × Surface	1.393	4	0.348	29.907	0.000
Dose × Surface	4.439	1	4.439	381.157	0.000
Dose × Surface × Dust	0.298	3	0.099	8.526	0.000
Error	0.454	39	0.012		
Total	343.863	60			

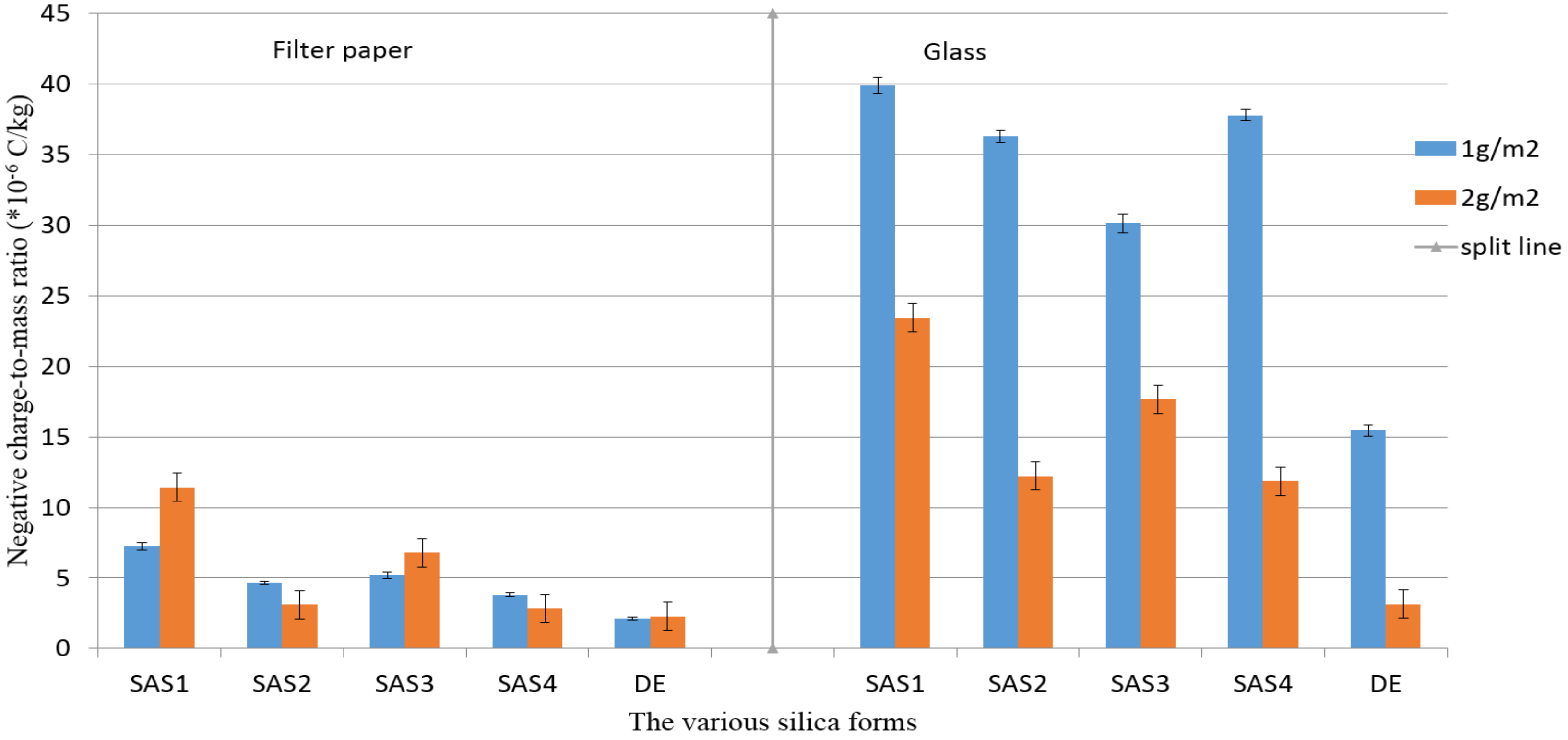


Fig. 3.3. Negative charge-to-mass ratio accumulation by two dosages of different inert dust products after shaking on each of two surfaces for 1 min at 24.5±0.5°C and 28.5±1%.

3.4.3 Comparison of efficacy

For the bioassays, the following three species with different charge-to-mass were used: (i) *T.castaneum*, which lost the smallest number of electrons to two insulated surfaces and was the most tolerant species to diatomaceous earth; (ii) *C. ferrugineus*, which lost the largest number of electrons to two insulated surfaces and was the most sensitive species to diatomaceous earth; (iii) *S.oryzae*, which ranked second in charging capacity on two insulated surfaces and was more resistant to phosphine than its sibling species, *S. zeamais*.

The results of the comparison of dust efficacy against the three insect species on two insulated surfaces are given in Tables 3.5, 3.7 and 3.9. Insecticidal efficacy was demonstrated for all tested dust products, with significant differences in speed onset of action (KD_{50} and KD_{95} values)（Tables 3.6, 3.8 and 3.10). The SAS powders were more effective against the three insect species than the DE on both surfaces. In addition, the SAS powders showed a significant ranking in insecticidal activity. The SAS1 was found to have the quickest onset of action with the lowest KD_{50} and KD_{95} values in all the tests except that against *T.castaneum* on filter paper.

Among the three species, when treated by dust, *T. castaneum* was the most tolerant species and *C. ferrugineus* was the most sensitive. The duration of onset of action were 1.3 and 19 times longer for *T. castaneum* compared to *S.oryzae* and *C. ferrugineus*. Also, a 0.2 g/m^2 low dose of dusts provided very rapid knockdown of adult *C. ferrugineus* which is 10% of that for the other two species. The cumulative mortality was plotted against knockdown time in minutes. On filter paper, it took nearly 4 days for 95% knockdown of *T. castaneum* on SAS1, but less than 2.7 hours for knockdown of *C. ferrugineus*. This rapid action suggests that the characteristic high positive charge-to-mass ratio is likely to positively contribute to this phenomenon.

The differences in KD_{95} on the two insulated surfaces were small for *S.oryzae* and *C. ferrugineus* (Tables 3.8 and 3.10). However, appreciable differences between the two insulated surfaces were observed for *T.castaneum*. For *T.castaneum* the values of KD_{50} and KD_{95} on glass were smaller than on filter paper by 1.1 and 1.7 times, respectively.

Table 3.5. Probit regression estimates (mean ± SE) for *T. castaneum* adult exposed to two different surfaces treated with 2 g/m^2 of various SAS powders and 4 g/m^2 of diatomaceous earth at 28±1℃, 65±5% RH.

Dielectric surface	Dust	Mean ± SE Intercept	KD (95% CL)（h）	
			KD_{50}	KD_{95}
Filter paper	SAS1	-1.793±0.115	38.286(31.914-42.748)	73.414(66.493-85.316)
	SAS2	-3.008±0.150	47.435(44.668-49.786)	73.372(69.944-77.917)
	SAS3	-2.058±0.122	38.033(32.850-41.864)	68.426(62.776-77.491)
	SAS4	-2.415±0.103	47.015(44.460-49.329)	79.041(75.296-83.761)
	DE	-2.057±0.070	58.223(55.306-60.987)	104.771(99.888-110.707)
Glass	SAS1	-4.342±0.233	31.083(28.635-33.425)	42.856(39.250-49.923)
	SAS2	-3.141±0.118	42.027(39.623-44.332)	64.035(60.304-69.069)
	SAS3	-3.196±0.155	32.633(29.211-35.574)	49.430(45.035-57.146)
	SAS4	-4.024±0.148	41.098(39.454-42.717)	57.897(55.348-61.141)
	DE	-3.461±0.142	46.082(43.296-48.646)	67.982(63.893-73.819)

Table 3.6. ANOVA parameters showing the significance of variation between dust and surface for *T. castaneum* at 28±1℃, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	0.972	4	0.243	5.497	0.004
Surface	0.547	1	0.547	12.376	0.002
Dust × Surface	0.080	4	0.020	0.451	0.771
Error	0.884	20	0.044		
Total	526.165	30			

Table 3.7. Probit regression estimates (mean ± SE) for *C. ferrugineus* adult exposed to two different surfaces treated with 2 g/m^2 of various SAS powders and 4 g/m^2 of diatomaceous earth at 28±1℃, 65±5% RH.

Dielectric surface	Dust	Mean ± SE Intercept	KD (95% CL)（h）	
			KD_{50}	KD_{95}
Filter paper	SAS1	-4.942±0.246	2.031(1.915-2.143)	2.706(2.536-2.979)
	SAS2	-3.907±0.243	2.261(2.096-2.390)	3.213(3.025-3.510)
	SAS3	-3.567±0.164	2.217(2.049-2.368)	3.239(3.009-3.596)
	SAS4	-3.253±0.152	2.717(2.359-2.987)	4.090(3.712-4.813)
	DE	-3.478±0.122	3.476(3.135-3.784)	5.120(4.672-5.897)
Glass	SAS1	-3.277±0.207	1.409(1.323-1.485)	2.117(1.998-2.281)
	SAS2	-3.067±0.148	2.419(2.257-2.564)	3.717(3.478-4.065)
	SAS3	-4.271±0.203	2.162(2.044-2.272)	2.994(2.823-3.246)
	SAS4	-4.414±0.188	2.373(2.284-2.460)	3.258(3.119-3.438)
	DE	-3.241±0.135	2.874(2.534-3.149)	4.333(3.954-4.990)

Table 3.8. ANOVA parameters showing the significance of variation between between dust and surface for *C. ferrugineus* at 28±1℃, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	16280.0	4	4070.000	37.0	0.000
Surface	367.5	1	367.500	3.341	0.083
Dust × Surface	286.667	4	71.667	0.652	0.632
Error	2200.0	20	110.0		
Total	131375.0	30			

Table 3.9. Probit regression estimates (mean ± SE) for *S.oryzae* adult exposed to two different surfaces treated with 2 g/m^2 of various SAS powders and 4 g/m^2 of diatomaceous earth at 28±1℃, 65±5% RH.

Dielectric surface	Dust	Mean ± SE Intercept	KD (95% CL)（h）	
			KD_{50}	KD_{95}
Filter paper	SAS1	-5.517±0.260	28.335(26.861-29.552)	36.783(34.734-40.434)
	SAS2	-5.361±0.240	30.852(29.443-32.272)	40.319(37.734-45.053)
	SAS3	-5.001±0.233	29.850(28.069-31.455)	39.668(36.843-45.362)
	SAS4	-4.295±0.166	30.509(28.171-32.962)	42.194(38.358-50.170)
	DE	-3.167±0.089	41.162(38.864-43.639)	62.539(58.285-68.425)
Glass	SAS1	-5.431±0.256	28.634(27.951-29.258)	37.306(36.132-38.861)
	SAS2	-4.320±0.190	32.022(31.254-32.818)	44.214(42.415-46.586)
	SAS3	-4.346±0.201	30.905(29.370-32.428)	42.602(39.629-47.861)
	SAS4	-3.868±0.165	32.735(31.679-33.833)	46.656(44.268-49.973)
	DE	-4.967±0.193	33.791(32.033-35.895)	44.980(41.531-51.345)

Table 3.10. ANOVA parameters showing the significance of variation between between dust and surface for *S. oryzae* at 28±1℃, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	1.589	4	0.397	22.083	0.000
Surface	0.006	1	0.006	0.332	0.571
Dust × Surface	0.592	4	0.148	8.231	0.000
Error	0.360	20	0.018		
Total	532.892	30			

3.4.4 Variation contributed by dust and insect species on the efficacy of the different dusts against insects on two insulated surfaces

Some basic exploratory analysis was conducted to assess the variance of contribution from all factors, including dust variety, insulated surface type and insect species. The hierarchical clustering analysis on top variable was in accordance with the insect mortality results. Figure 3.4 presents the hierarchical clustering of the samples. Samples were marked with different colours based on: dust varieties, insulated surfaces and insect species. As shown, samples were clustered quite well according to variety and dust treatment. The difference between *C. ferrugineus* and the other two insect species appeared to be the main contributor of the data variance. The second major source of the data variance appeared to be the dust treatments. SAS1 behaved significantly differently from the rest of the dusts.

There was a linear correlation between charge-to-mass ratio and bioactivity of dust. The multiple linear regression model was developed through multiple regression analysis of the KD_{50}’s for each insect species to describe the relationship.

$f(\chi,y) = a + b\chi - cy$

where $f(\chi,y)$ is the time for KD_{50} for insect species, χ is insect charge-to-mass ratio, y is dust charge-to-mass ratio (Coefficients with 95% confidence bounds, a = 50.32

(45.58 to 55.05), b = -6.062 (-8.496 to -3.629), c = -0.5232 (-0.8353 to -0.211); Goodness of fit: SSE: 340.4, R^2: 0.7221, Adjusted R^2: 0.6894, Root mean square error (RMSE): 4.475). Charge-to-mass ratio effects were similar for each insect species and could be made linear with a reciprocal transformation of the KD_{50} plotted against time. The effect of charge-to-mass ratio of insect on the KD_{50} was more obvious than that of dust.

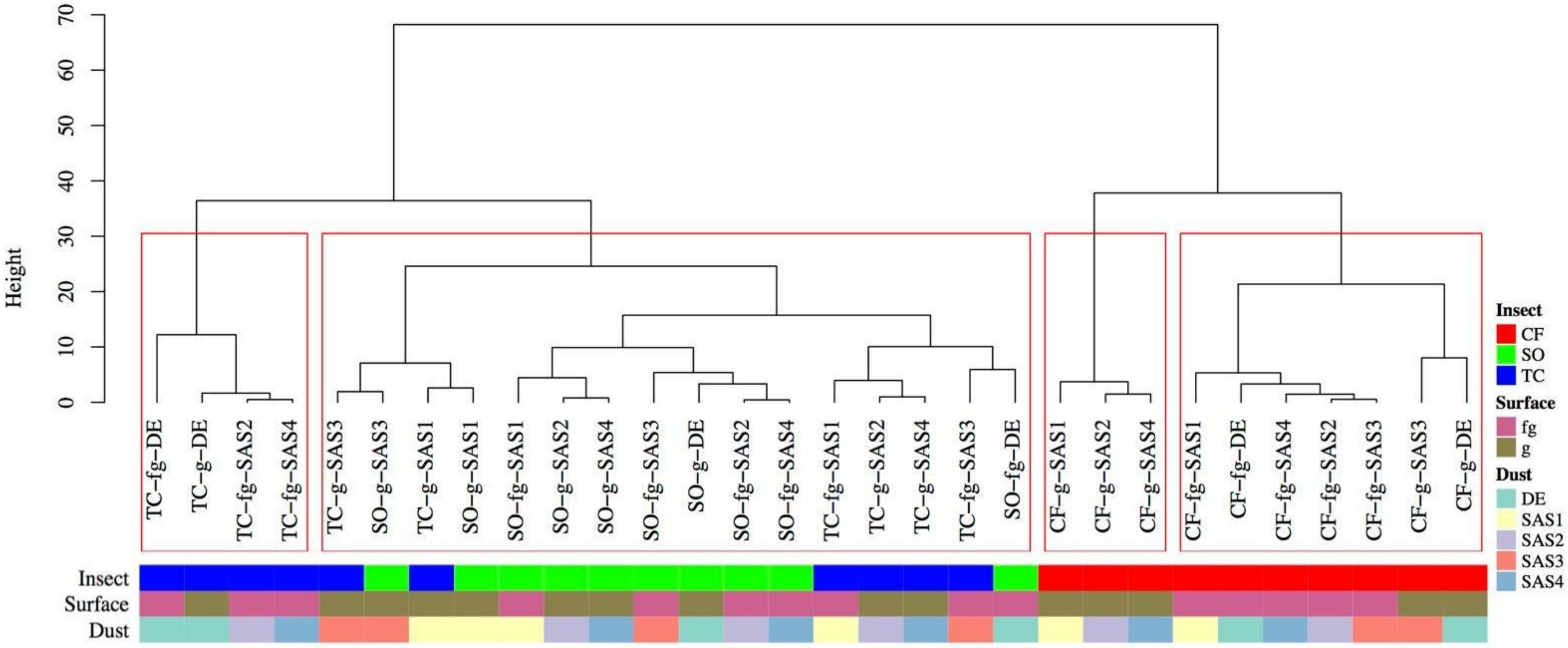


Fig. 3.4. The hierarchical clustering analysis of mortality data. Samples were marked with different colours based on the factors: dust variety as "Dust", surface material as "Surface" and insect type as "Insect". Abbreviations used in the table: **CF,** *C. ferrugineus*; **SO,** *S.oryzae*; **TC,** *T.castaneum*; **fg,** filter paper; **g,** glass.

3.5 Discussion

The results presented here are part of a research program to develop new non-chemical technology for controlling stored grain insects and other insects. Although insecticidal efficacy of silica products has been observed in various studies (Banks and Fields, 1995; Ebeling, 1971; Fruijtier-Pölloth, 2012; Golob, 1997; Korunic, 1998; Mewis and Ulrichs, 2001; Subramanyam and Roesli, 2000), there is urgent need for precise protocols and guidelines in applying the dusts to obtain optimal results. Mewis and Ulrichs (2001) observed differences in insecticidal efficacy of different silica products among different insect species and even between developmental stages within species. However, what determines efficacy against stored grain insect pests is currently unknown. For different silica products, main variations in intrinsic toxicity and reactivity of the material, surface area and surface chemistry in contact with the cell, and morphology (size, shape, state of aggregation) (Zhang et al., 2012) explains differences in efficacy. However, all of these studies have a serious limitation since they only evaluate efficacy on one specific aspect. On the other hand, we have performed a comprehensive study taking all electrostatic factors between insects and insecticidal dusts into consideration, and thoroughly explored the relationship between efficacy and electrostatic field in terms of both insects and dusts. The experiments conducted showed that the polarity of electrostatic charge acquired by insects and dusts from dielectric surfaces is opposite and the amount varies with insect species, dust categories and dielectric surfaces, and we demonstrated the insecticidal efficacy was strongly correlated with electrostatic charge.

The charge measurements on insects reported here varied significantly for different species. This can be explained by different levels of insect activity. The charge on the insect increased with the level of activity and distance moved. This is in agreement with McGonigle's (2002) study. He theorised that the amount of charge acquired by insects was directly proportional to the distance they crawled. Preliminary experiments revealed that species had inherent differences in behaviour. *T. castaneum* tended to congregate, while it was observed that, when transferred from filter paper to glass, the moving ability of *S. oryzae* was distinctly decreased as was that of *C. ferrugineus* to a lesser extent. On glass carrier, *S. oryzae* were prone to lose balance and took 54 (±11) seconds to regain proper posture (Gowers and Le Patourel, 1984). Their locomotion capacity was greatly compromised when the surface could not provide sufficient friction. However, due to high flexibility, the average time taken for *C. ferrugineus* to regain normal posture on glass was only 2 (±1) sec and their movement was less hindered, resulting a slight decrease of the charge quantity. Different charging rates of different insect species can also be explained by the different work function in contact with two dissimilar materials, meaning the substances with positive charge had a high work function (Davies, 1969). The outer layer of insect, which theoretically contains the determinants for work function of the cuticle, varies with the type of insect. These surface components, such as sialic acid from glycoconjugates, carboxyl groups of amino acids, phosphate groups from phospholids, and sulphated polysacchaies associated with the plasma membrane, were involved in surface charge (Burry and Wood, 1979). Verities of roughness of insect cuticle also play an important role in charging the insects. In preliminary experiments, insects with distinguishable surface topography were investigated. A rough surface provides larger contact surface and more contact opportunities than smooth surface (Federle et al., 2000; Gorb and Gorb, 2009). In all cases, the rate of dust picking up was directly proportional to the difference between the charge of adhering dust and insect's charge. In field trial (Zhang et al., 2014), the dose of 2 g/m^2 examined in the present study is effective for controlling *T. castaneum*, while at the same time, has satisfactory efficacy against *S. oryzae*. However, in bulk grain, where *C. ferrugineus* inhabit, the dose can likely be reduced. The presence of electrostatic charge on the insect could contribute to this reduction. Comparison between SAS and DE showed significantly larger amounts of SAS adhesion to and retention on insects throughout the experimental period because of their different electrostatic charge. This led to better effectiveness of SAS than DE. In theory, it is possible to device empirical methods for rapid screening of high effective SAS powders based on an electrostatic charge evaluation of siliceous particles, such as agitation test in a glass graduated cylinder. If a partially to completely surface-treated siliceous material has a specific electrical conductivity of 10^{-5} mho per centimetre or less, the particles will cling to the walls of the container and agitate. This property, presently referred to as “cingability”, persists for several hours. Siliceous materials with specific electrical conductivity less than 10^{-7} mho per centimetre and is likely to be effectively insecticidal (Ralph, 1964). The more effective dry hydrophobic siliceous insecticides are characterized by being finely divided, have a low bulk density with low unit weight per unit volume, and low electrical conductivity, or differently stated, electrical insulating properties. The particles that fall into this category can readily pick up electrostatic charged and tend to repel each other. For ideal insecticidal effect, the aggregate particles should have a size below 15 microns, a surface area between 100-250 square meters per gram and retain the electrical conductivity of not more than 10^{-7} gram per centimetre at a bulk density of 0.2 gram per cubic centimeter (Ralph, 1964). David and Gardiner (1950) listed several studies showing that fine particles adhere more readily to insects than coarse powders, and manufactured forms of amorphous silica (agglomerate: 1-250 µm) had smaller particles than milled natural DE (1-50 microns) which increased their electrostatic charge. Both observations may explain why the efficacy of SAS was higher than that of DE. We believe that integration of the particle's electrostatic property into a rational design is highly beneficial in terms of improving efficacy of the products.

According to Davies (1969), the key element that determines the size and the sign of electrostatic charge is the work function of two contact materials. Lewis and Hughes (1957) demonstrated that retention of dust by flies varied with exposure to different surfaces, which is in accordance with our results that show efficacy of dust against three stored grain insect pests is significantly different on different surfaces. Based on findings by different independent investigators and our own experiments, we formed a conclusion that efficacy of dust is directly linked to electrostatic charge of the powder and target pests. This is also supported by Gowers and Patourel's (1984) discovery that toxicity of a deposit applied to grain and sacking was considerably lower than that applied to three other substrates (glass, tile and concrete). The outer layer of grain kernels could be classified as a good dielectric material, thus contact with grain kernel surface is sufficient to charge a crawling insect and leads to an increase in the insecticidal effect of the SAS powders. However, the surfaces of kernels vary. Some are smooth, like those of maize and bean, and some are rough, such as rice and barley. Hence, applying dust is a very complex procedure. Apart from insects and dust products, characteristics of grain kernels should also be considered in our new protocol.

In summary, electrostatic charge is a key component in determining efficacy of SAS. Based on this information, we are developing a module in our stored grain management system to provide a precise and personalized SAS application guideline for applying SAS to suit different conditions. The system can thus facilitate farmers and managers to design an optimal SAS strategy accommodating different commodities at ease. Our findings have unraveled the factors contributing to the efficacy of SAS application, but also have the potential to revolutionize methods of industrial stored grain pest control.

Chapter 4. Effect of synthetic amorphous silica (SAS) powder on the cuticle of *Tribolium castaneum* and *Sitophilus oryzae*

4.1 Abstract

Insect cuticle is the first layer of protection from environment damage. Therefore, understanding the effect of synthetic amorphous silica (SAS) on insect cuticlular properties is essential to contribute to the mode of action and thereby, the development of highly efficient SAS products for the management of insect pests. A comparison of insect mortalities between hydrophobic and hydrophilic SAS against *Tribolium castaneum* and *Sitophilus oryzae*, showed there were significant differences in the LD_{50} values 47.298 (45.716-48.846) and 38.998 (37.432-40.504) g h/m^{2}for *T. castaneum* exposure to hydrophilic SAS and hydrophobic SAS, respectively; 47.220 (46.250-48.182) and 57.636 (56.964-58.306) g h/m^{2}for *S. oryzae* exposure to hydrophilic SAS and hydrophobic SAS, respectively) and in the LD_{95} values (68.908 (66.418-70.588) and 56.110 (53.782-59.008) g h/m^{2}for *T. castaneum* exposure to hydrophilic SAS and hydrophobic SAS, respectively; 67.124 (65.464-69.012) and 78.888 (77.742-80.128) g h/m^2 for *S. oryzae* exposure to hydrophilic SAS and hydrophobic SAS respectively. *T. castaneum* was more susceptible to hydrophobic SAS while *S. oryzae* was more susceptible to hydrophilic SAS.

A hyperspectral reflectance imaging approach was used to directly indicate the impact of SAS on the insect cuticle. The results showed that ventral reflectance from control groups were higher than that of SAS-treated groups, both in visible and short-wave near-infrared wavelength ranges (400-1000 nm). In contrast, the SAS-treated groups showed much higher dorsal reflectance. Evidence suggested that the differences in absorption characteristics of cuticular fat and protein (866, 870, 927, 935,991, 993, 994, 997, 998, 1000, 1003, 1007 and 1008 nm) may contribute to the varied performance. The overall recognition rates of the back propagation neural network models for control

and SAS-treated groups were 100% for the calibration and predictions sets, respectively, which indicated that the effects of both SAS on insect cuticles was significant. Also, consistent with the assumption that the efficacy was different between the SAS products, the lowest rates of the model for two treatment groups were 90.63 and 76.92% in the calibration and prediction sets, respectively.

4.2 Introduction

Two types of synthetic amorphous silica (SAS), hydrophilic and hydrophobic SAS are described by different Chemical Abstracts Service (CAS) numbers. Hydrophilic SAS types can be further divided into two groups by process methods. One is produced in a wet process and is described by CAS number 112926-00-8 (includes silica gel, precipitated silica and colloidal silica), and the other is produced in a thermal process that is described by CAS number 112945-52-5 (pyrogenic silica). All forms of SAS can be surface modified and become hydrophobic, i.e., silica dimethicone silylate, silica dimethyl silylate and silica silylate (CAS 67762-90-7, 68611-44-9 and 68909-20-6). The SAS is used in a variety of products, such as free-flow and anti-caking agents in powder materials, and as liquid carriers, particularly in the manufacture of animal feed and agrochemicals, and no associated human health issue or environment risks have been reported (Fruijtier-Pölloth, 2012).

Insect cuticle is the part of insect body most directly exposed and in contact to SAS. The efficacy of SAS powders varies against different species of insect (Chapter 3). These differences can be attributed to differences in the permeability of insect cuticle to SAS and the resultant loss of internal water content. Different insects have different body water contents. For example, stored-product insects generally have a relatively lower water content (50% for *S. oryzae* (Fields et al., 1998) and 52-53% for *T.*

castaneum (Rigaux et al., 2001)) compare to insects living in other environments which have an average of 69% (Hadley, 1994). Water is essential for maintaining insect life, e.g., the loss of 56% and 30-36% moisture is lethal to *S. oryzae* (Arlian, 1979) and *T. castaneum* (Rigaux et al., 2001), respectively. Previous studies have indicated several different insecticidal mechanisms of diatomaceous earth (DE) that include some factors which effect insect cuticle. These are abrasion (David and Gardiner, 1950; Kalmus, 1944; Wigglesworth, 1944), absorption (Alexander et al., 1944; Ebeling and Wagner, 1959; Hunt, 1947), waterproofing components of the epicuticle that cause desiccation, damage to the digestive tract (Boyce, 1932; Richardson and Glover, 1932), blockage of the spiracles and tracheae (Webb, 1946), and interference to insect hormone (Adrien, 1968). The effectiveness of these insecticides is often related to their specific surface characteristics and decreases when humidity increases (Ebeling and Wagner, 1961; Fields and Korunic, 2000).

Studies on DE demonstrated that there was a direct relationship between efficacy against insects and high silica content with a uniform size distribution (Korunić, 1997). Following this logic, SAS possesses the potential to be an effective insecticide. Diatomaceous earth is considered suitable for the storage environment, especially for products that cater to organic markets. Synthetic amorphous silicon could be a viable alternative to DE based on their shared properties and is likely to be superior in terms of safety and effectiveness. Preliminary studies on SAS showed insecticidal activity on *Prostephanus truncaus* (Horn), a major pest of stored maize in Africa (Barbosa et al., 1994). Mortality due to SAS was observed at rates comparable to those recommended for DE. The mode of action of SAS has not yet been established. Detailed mortality studies are needed to understand mechanisms and to determine whether it is a reliable product for insect pest control. Our study investigated changes to cuticular properties of two insect species treated with two different SAS products using the hyperspectral system. This technique records images at hundreds of contiguous wavelengths (narrow spectral resolution) in the form of a hypercube (three-dimensional hyperspectral data). The principals of the technique have been detailed in Maftoonazad and Ramaswamy (2006). This technique has the ability to rapidly and simultaneously measure multiple parameters, including internal structure characteristics, morphology information, and chemical composition, and it is superior to single machine vision technology or spectroscopy analysis technology. Visible/near-infrared hyperspectral imaging techniques were used to successfully identify different species and geographical strains of *S. oryzae* and *Sitophilus zeamais* (Cao et al., 2015). Consequently, we chose to utilize hyperspectral imaging for this study.

The objectives of this study were: (1) to compare the efficacy of hydrophilic SAS and hydrophobic SAS against two different insect species, *S. oryzae* and *T. castaneum*; (2) based on mortality results from (1), to understand hyperspectral imaging changes of insect cuticle and subsequently to elucidate the effects of SAS on the cuticle of insect; and then (3) to accurately establish principal parameters for the two SAS products on *T. castaneum* and *S. oryzae*.

4.3 Experimental procedures

4.3.1 Synthetic amorphous silica

We chose one hydrophobic and one hydrophilic SAS provided by Murdoch University (MU), Western Australia. The hydrophobic SAS was modified from the hydrophilic one. Both were precipitated SAS. Their specific surface areas were, respectively, 123.44 and 207.07 m^2/g (BET) for hydrophobic and hydrophilic SAS, determined from N_2

adsorption/desorption isotherms at 200°C for 2 hours (BK200C, JWGB SCI. & TECH., China) (Fig. 4.1), using multipoint Brunauer-Emmett-Teller (BET) methods.

Fig. 4.1. Photograph of surface area and pore size analyzer.

4.3.2 Insects

T. castaneum and *S. oryzae* were acquired from the academy of state administration of grain, Beijing, China, and cultured at 27±0.5°C (mean ± SE) and 65±0.8% RH as described by Winks (1982). *T. castaneum* were maintained on a feed medium (1 kg) comprised of 1 part yeast and 12 parts whole meal flour milled from Chinese hard wheat. *S. oryzae* were cultured on whole wheat. All experiments were carried out on 5-15 day old adults. The entire process was completed at the Academy of State Administration of Grain, Beijing, China.

Adults were separated according to sex. Prior to the bioassay and imaging experiment, the insects were cleaned with purified water and the water on the surface of the insect was removed with water-absorbing paper. After the image acquisition had been completed, all insects were dissected to find the genitalia to confirm gender.

4.3.3 Hyperspectral imaging system

A hyperspectral imaging system developed for acquiring reflectance images from insect samples was used (Cao et al., 2015) (Fig. 4.2). The system was composed of a hyperspectral imager (including a high performance CCD camera, model HSI- VNIR-B1621, Isuzu Optics, Taiwan, China) coupled with a long-focus lens (model OL23; Schneider, Bad Kreuznach， Germany), a short-focus lens (model OL50; Schneider, Bad Kreuznach， Germany), a linear motorized slide (model IRCP0076-1COMB; Isuzu Optics, Taiwan, China), a light Source (for reflection) (model 3900-ER; IT, New York， USA), a light source (for Transmission) (model 9135-HT; IT, New York， USA), a black box (model IRCP0075-1COMB; Isuzu Optics, Taiwan, China), a white calibration tile (300 mm×25 mm×10 mm; Speicm， Oulu， Finland) and a computer system for image analysis. The reflectance light source structure was composed of a 150 W halogen light source, which was connected to a dual fiber optic line light.

The linear motorized slide was set to run at a speed of 0.17 and 0.19 mm/s to move *T. castaneum* and *S. oryzae*, respectively, perpendicularly to the imaging area. The slide was set to operate in continuous acquisition mode and each insect was line scanned to obtain 3D hyperspectral reflectance.

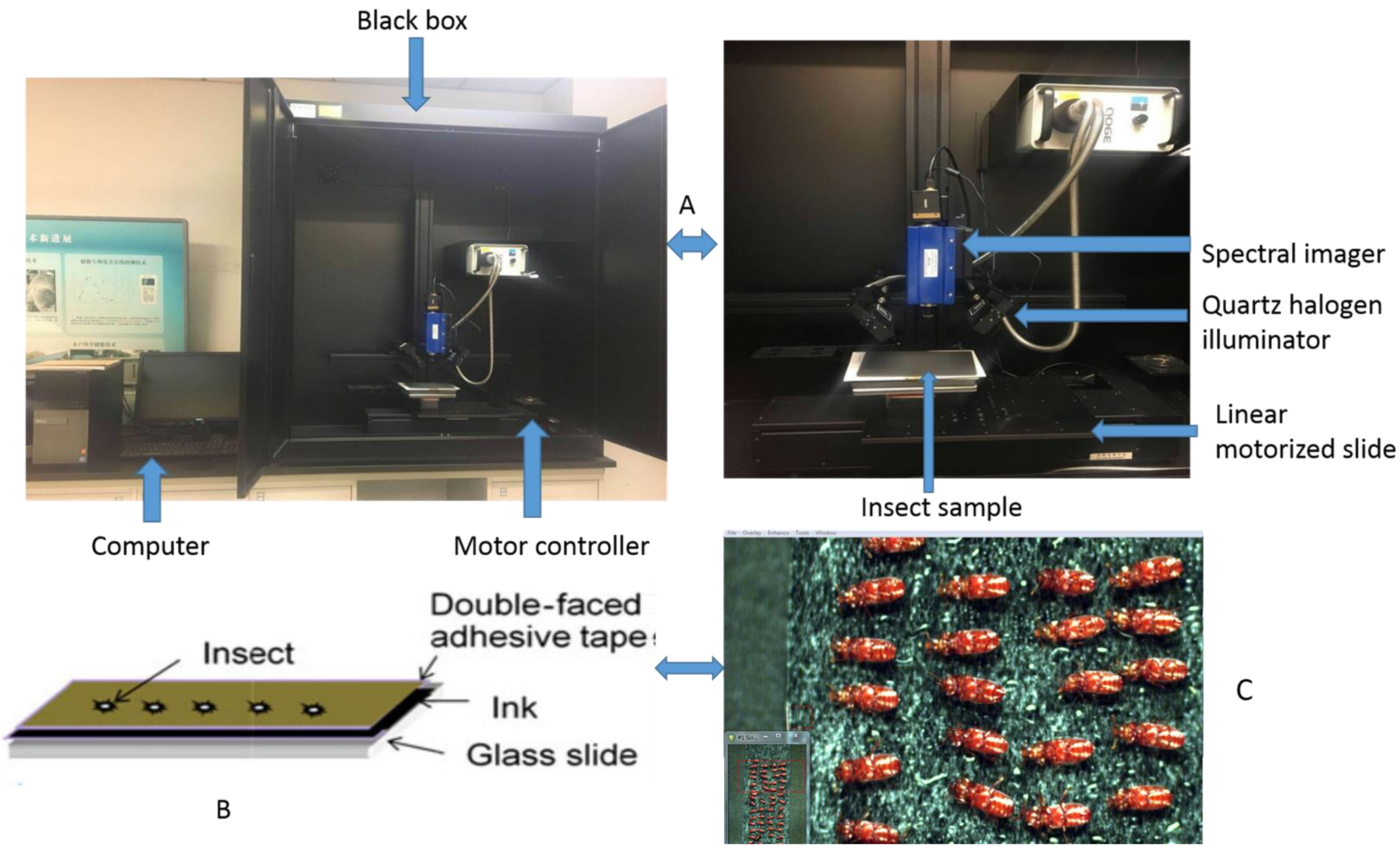


Fig. 4.2. Schematic of the hyperspectral imaging system with details of the specimen stand: A-photograph of hyperspectral imaging system; B-sketch map of stand; C-photograph of insect specimens on stand.

4.3.4 Bioassays

Prior to use for bioassays, 16 cm diameter glass petri dishes were washed and disinfected by heating at 100°C for 2 hours. A Whatman No.1 filter paper of the same diameter was pasted to the internal base of a petri dish without any gaps in order to prevent insects crawling beneath the paper. The filter paper helped to distribute the SAS evenly and prevent agglomeration. A dose of SAS at 2 g/m^2 was added into each dish. Dishes were shaken manually several times to achieve an even distribution of SAS on the filter paper. However, both of the powders flocculated easily even when shaken gently. Therefore, to avoid variation between replicates and samples, agglomerations were broken using dissecting needles. For each dosage, there were three replicates. An additional set was left untreated as the control. Replicates were at a 1:1 sex ratio with groups of 50 adults of each species. All bioassays were performed inside a growth chamber at 28±1°C, 65±5% RH. Every 2 h, mortality was measured and dead insects were collected in new clean petri dishes. In all cases, insects were counted as dead if they showed no movement over a period of one minute. Each SAS treatment was replicated three times with both insect species. In the case of bioassays using *S. oryzae,* the insides of the petri dishes were treated with a band of fluon to stop the insects from escaping. For this study, a total of 1800 insects were tested, with 900 insects of each species used.

4.3.5 Spectral sample preparation

Insect samples were collected after 100% mortality was achieved. After exposure, the content of each petri dish was transferred onto a 100 µm stainless steel mesh and SAS was blown from the insect body using clean air. The mesh retained the insects while allowing the SAS particles to pass through. The mesh was then placed into a clean petri dish. After that, the insects were checked using a low-powered binocular microscope (model EZ4, Leica) to ensure that little, if any, of the SAS remained. Note: it was not possible to remove 100% of SAS from the insect body.

The untreated control insects were subjected to the same cleaning procedure for comparison. To obtain the untreated control insect image, insects were frozen to keep them immobile temporarily. First, insects were put on a frozen cold gel pack and movement ceased immediately. Then they were picked up gently with a brush and fixed on a ready-made stand with the dorsum or venter downward. To avoid water condensation on the insects' body, filter paper was used to separate the stands and cold gel pack. The procedure was followed as described by Cao et al., (2015).

Using the steps described above, 50 insects were pasted in the same orientation onto a single stand. For the control groups, each stand sample was scanned before the insects recovered from freezing. During the image acquirement period, stands were put on a black flat cooled iron cube with a cold gel pack inside. In total, there were twelve stands prepared for the experiment. The experiment in total consisted of 600 insect samples with each sample placed dorsal-ventral or ventral-dorsal and subjected to a hyper-spectrometer. When the platform moved, a few insects changed positions, and so their images were discarded.

4.3.6 Image acquisition and preprocessing

A total of 854 images were acquired for each test sample at an exposure time of 7 mins for each hyperspectral image. The hyperspectral imaging system had a 0.64 nm/pixel spectral resolution covering the spectral region of 400 to 1000 nm using a 1608 pixels camera. The resulting hyperspectral reflectance images had 6.4 nm-spectral resolution per pixel and 854 wavelengths after 10 spectral binning operations. Thus, a special block of a 1608×854×854 reflectance image was created; that is, one representing a 2-D image with x axis and y axis coordinate information and the other one representing the spectral information. This information was stored for subsequent analyses.

The calibration of hyperspectral images was conducted as done previously (Cao et al., 2015). Starting from the spectral image of each sample, the reflectance spectrum was obtained by averaging the reflectance values of the pixels located inside a region of interest (ROI). Spectral reflectance data were converted into apparent absorbance units: log (1/R).

Due to the small size of the insects, it was difficult to achieve identical positions of each specimen regarding the placement of legs and head. To combat this problem, a morphological open algorithm was previously developed. By choosing the pixels from the processed data along the edge of insect body, focusing on the tergum of the thorax and abdomen, the algorithm generated perfect results by estimating the background accurately (Cao et al., 2015). Threshold segmentation was then selected manually to cut the image into two parts (target area and background area).

Single-insect images from 50 non-touching insects in the original images were obtained and labeled. A 50×50 median filter was applied to the sub-images to reduce the artifacts. A two-way array, in which all the pixels reflected intensities of a sample rearranged into a column at each of the 854 wavelengths, was extracted by ENVI 4.7 (Research System, Inc., USA) software. This resulted in k×854 size two-dimensional arrays, where k is the total number of pixels in a labeled insect and given as the input to the discriminated classifier.

4.3.7 Statistical analysis

4.3.7.1 Bioassay data analysis

Adult mortality of *T. castaneum* and *S. oryzae* at different exposure times to surface treatments by both SAS products were calculated as a percentage. Adult mortality in control groups across all exposure times after treatment was 0%. Therefore, mortality data were not corrected for mortality in the control treatment using Abbott's formula. Time-mortality data were subjected to probit analysis (SPSS, 2014) for determining 50% (LD_{50}) and 95% (LD_{95}) mortality of *T. castaneum* and *S. oryzae* with associated statistics.

4.3.7.2 Exploratory analysis

All the spectral data collected from the ENVI platform were in TXT data format and were converted into a complete Excel sheet. All data were loaded into MATLAB (Math works, matlabR2009b, Inc., USA) for quality control, exploratory analysis, and all downstream statistical analysis.

Exploratory analysis was performed on (1) the whole data which were merged using in-house scripts and (2) the mean data, which were obtained by averaging the replicate measurements. Considering that hyperspectral data are highly interrelated (collinear), principal component analysis (PCA) was used to eliminate the problem. PCA can generate a set of uncorrelated variables from a set of correlated variables. These newly-generated variables were called principal components (PCs). The goal of PCA is to reduce the dimensionality of the data set to an optimal level and thus, introduce a new set of meaning variables (Abdi and Williams, 2010).

4.3.7.3 Characteristic wavelength selection

The images acquired by using the hyperspectral imaging system were particularly informative because images at 854 wavelength bands were obtained for each insect sample. The extracted spectral data from hyperspectral images possess variables (wavelengths) of dimensionality with redundancy among contiguous variables (wavelengths) (Cao et al., 2015) as the wavelength variables correlated with the nearby variables were considered redundant. Researchers tend to focus on a few vital wavelengths that are most influential on the quality evaluation of the product and wavelengths having no discrimination power are eliminated. The selected wavelengths reduce the data dimensionality while preserving the most important information contained in the lower dimensional data space.

Principal components analysis was applied. The wavelengths that corresponded to the highest reflectance values were considered optimal wavelengths. These optimal wavelengths that carry maximum spectral information could be implemented in multispectral imaging in further studies. Wavelengths which correspond to the lowest reflectance values were ignored for they had little value in prediction.

Only the selected optimal wavelengths were used to establish identification models instead of using the whole spectral range.

4.3.7.4 Model development

The corrected spectra for both species were used for classification of insect samples into three categories: control, hydrophilic SAS treatment, and hydrophobic SAS treatment. The linear classification method cannot provide a complete solution to the insect damage classification problem, thus the non-linear approach of an artificial neural network (ANN) was used. It is a powerful data-modelling tool that is capable of capturing and representing complex relationships between inputs and outputs (Bachtiar et al., 2011; Kaya and Kayci, 2014). The ANN computational strategy applied in this study was similar to that developed by Cao et al., (2015). Data were randomly divided into a learning (training) set, a verification set and a test set. Each set consisted of a number of samples (insect damaged by SAS specimens) characterized by input variables (characters) and identified by cuticle changed by SAS (output). There are several different types of supervised ANN which can be used for classification problems. Preliminary experiments on the data set with some of these types (radial basis function, linear, probabilistic and multilayer perceptron networks) suggested that the back propagation artificial neural network (BPNN) would be the most efficient for the purpose. The BPNN is generally one of the most commonly used types of ANN and can model functions of almost any arbitrary complexity. The BPNN conventionally consists of neurons arranged in layers (an input layer, one or more hidden layers and an output layer). Each layer might have a different number of nodes. The input layer receives the information about the system (the nodes of this layer are simple distributive nodes, which do not alter the input value at all). The hidden layer processes the information initiated at the input, while the output layer is the observable response or behaviour. By running the data on specimens from the training set, including the output variable (the identification), through the network and comparing the actual output generate with the desired or target outputs, the network automatically adjusts the weights and thresholds in order to minimize the overall error. This process is equivalent to fitting the model represented by the network to the training data available. Reaching the desired value indicates the best moment to stop the training procedure and is helpful in the search for the optimal network architecture. The latter largely consists of the estimation of an appropriate number of nodes in the hidden layer(s), which is one of the most critical tasks in ANN design. Unlike the input and output layers, the number and size of hidden layers are not predictable and will vary according to the complexity of the data.

One cycle through all the training patterns is defined as an epoch. Before the optimal accordance of the network output errors is achieved for all training patterns, many epochs are required for the back propagation.

In this study, the learning rate factor and momentum factor were set to 0.1; the initial weights were 0.3; the scale function used was the 'tan h' function. The permitted error was set at 0.002 and the epoch was set to 1000.

The hyperspecTM-M image capture software platform was used during the entire experiment to acquire the hyperspectral reflection image from insects. Image processing and data analysis were implemented in ENVI 4.7 (Research System, Inc., USA) and MATLAB (Math works, matlabR2009b, Inc., USA), and ANN computation was performed using a NeuroShell 2.

4.4 Results

4.4.1 Lethal dose values

The lethal dose regression slopes were significantly different between the two SAS products tested (Table 4.1). For *T. castaneum*, hydrophobic SAS was significantly more effective than the hydrophilic one. The LD_{95} for hydrophobic SAS was 56.110 g h/m^2, while it was 68.908 g h/m^2 for hydrophilic SAS. Interestingly, for *S. oryzae*, hydrophobic SAS was slower than hydrophilic SAS in killing 95% of the test population. The LD_{95} for hydrophobic and hydrophilic SAS against *S. oryzae* were 78.88 g h/m^2 and 67.12 g h/m^2, respectively. Analysis of variance revealed that the difference between responses of the two insect species after exposure to hydrophilic SAS was numerically close but statistically different. *T. castaneum* was more susceptible than *S. oryzae* to hydrophobic SAS. The LD_{95} values were 1.4 times higher than the corresponding LD_{50} values.

The insects tested were of the same age and from similar geographical strains, so therefore they had similar visual appearance. After exposure to SAS, the changes in moisture and other physiological components of the insects led to changes in coloration and gross appearance between specimens. These differences in visual appearance between individuals of the same species indicated heterogeneous distribution of moisture and physiological components, making visual comparisons difficult. Therefore, hyperspectral imaging was used to quantify difference in parameters between insect species in response to the two SAS products.

4.4.2 Spectral analysis

Figure 4.3 shows the average relative reflectance spectra of dorsal and ventral regions of *T. castaneum* and *S. oryzae* before and after treatment with the two SAS products tested. The spectra are derived from the surface features of the insects and differences are based on their reflected spectral characteristics. Mean reflectance changed after the insects were treated with SAS. The untreated and SAS-treated dorsal and ventral regions of insects showed distinct differences in spectra obtained across the wavelength range. As shown (Fig. 4.3), values of the relative reflectance for the 400-700 nm range were much lower than those of 700-1000 nm range. These results suggest that near-infrared regions have advantages over the visible wavelengths in distinguishing insect species and in detecting the effect of SAS treatment. In the 900-1000 nm range, values of reflectance of all *T. castaneum* groups were much higher than those of *S. oryzae*. Treated individuals of both insect species had significantly higher values than the control groups for the ventral side on the 600–1100 nm range. Values were lower for the dorsal side. The differences between the values of the hydrophobic SAS treated groups and the control groups were greater than that of the hydrophilic SAS treated groups and their controls (except for the dorsal sides of *S. oryzae*). The identification criteria by reflectance spectroscopy are based on qualitative differences in the spectra. In our studies, different data pre-treatment combinations were employed to optimize the performance of classification and quantification.

The characteristic wavelengths for SAS treated individuals and controls were selected based on multivariate image analysis and loading weights. Figure 4.4 shows the maximal coefficient in the first three PC loadings. Eight variable effective wavelength groups were selected. These were 489, 866 and 1008 nm for hydrophilic SAS treatments of *T. castaneum*, ventral surface; 456, 870 and 1008 nm for hydrophobic SAS treatments of *T. castaneum*, ventral surface; 423, 993 and 993 nm for hydrophilic SAS treatments of *T. castaneum*, dorsal surface; 994, 997 and 1003 nm for hydrophobic SAS treatments of *T. castaneum*, dorsal surface; 935, 1007 and 1007 nm for hydrophilic SAS treatments of *S. oryzae*, ventral surface; 553, 927 and 1000 nm for hydrophobic SAS treatments of *S. oryzae*, ventral surface; 449, 991 and 998 nm for hydrophilic SAS treatments of *S. oryzae*, dorsal surface; 456, 997 and 1003 nm for hydrophobic SAS treatments of *S. oryzae*, dorsal surface. Peaks at 866, 870, 927, 935,991, 993, 994, 997, 998, 1000, 1003, 1007 and 1008 nm were due to C-H and O-H stretching overtones and combinations related to protein and fat contents (Nortvedt et al., 1998; Šašić and Ozaki, 2001; Wold et al., 1996; Wu et al., 2008). Peaks at 423, 456, 449, 489 and 553 nm were due to the slight colour change after exposure to SAS. Interestingly, the selected optical wavelength here did not include the broad feature around 968 nm due to 2v1+v3 (v1: symmetric stretching; V3: anti symmetric stretching) water vibration (Wu et al., 2008).

4.4.3 Principal component analysis (PCA)

Figure 4.5 shows a 3-dimensional (3D) principal component score plot of the dorsal and ventral regions of *T. castaneum* and *S. oryzae* before and after treatment. The scoring system consisted of the top three PCs. These factors covered most of the whole spectral information, thus providing the best parameter for a clear discrimination. On the dorsum of the insect, PC1 interpreted 98.64 and 93.58% variances, PC2 interpreted 1.06 and 5.53% variances, and PC3 interpreted 0.24 and 0.79% variances, for *T. castaneum* and *S. oryzae*, respectively. Through PCA, the accumulated variance contribution rated from these three factors was up to 99.94 and 99.9% for the venter regions of *T. castaneum* and *S. oryzae*, respectively. Similarly, these first three PCs accounted for 99.8 and 99.85 % of the overall variance for the venter of *T. castaneum* and *S. oryzae*, respectively (for *T. castaneum*, PC1 = 96.72%, PC2 = 2.29%, PC3 = 0.79%; for *S. oryzae*, PC1 = 97.99%, PC2 = 1.36%, PC3 = 0.5%).

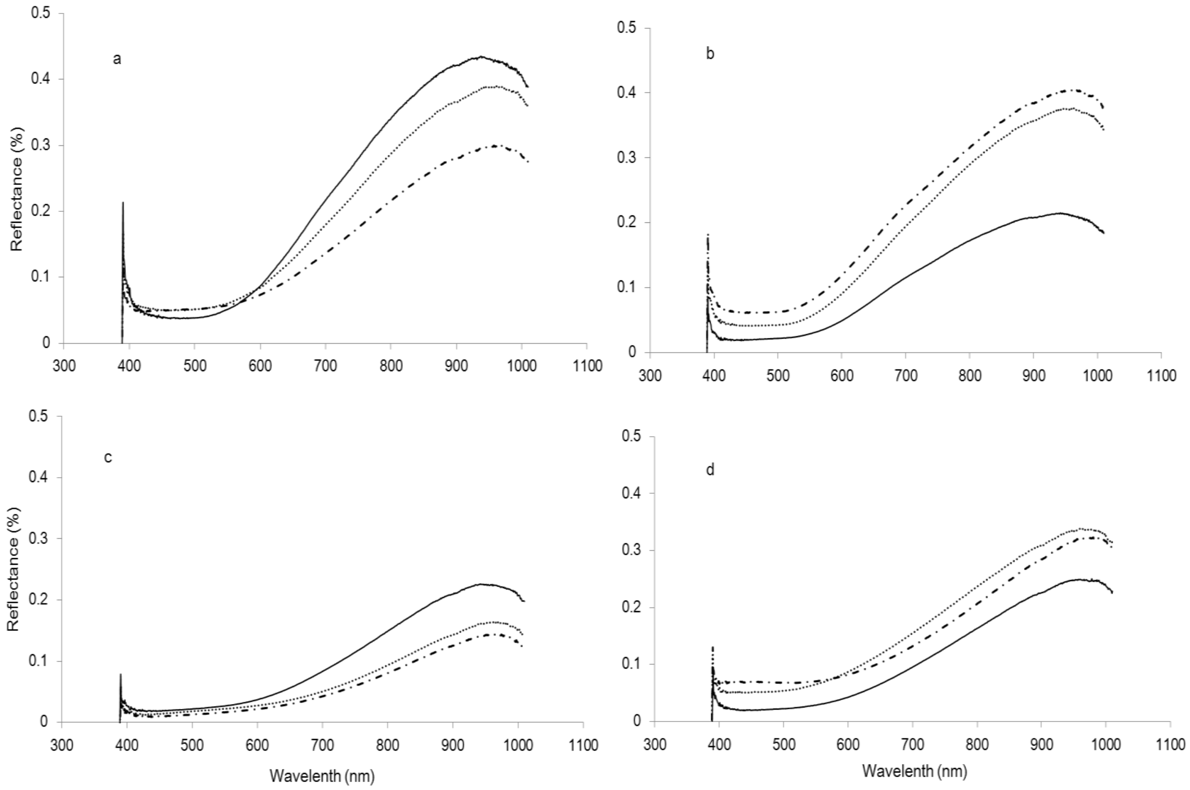


Fig. 4.3. Spectral curve wavelengths from the effects of two types of SAS on the dorsal and ventral surfaces of *T. castaneum* and *S. oryzae*. a and b are for *T. castaneum* (a. venter, b. dorsum. – control; – · – · hydrophilic SAS;....... hydrophobic SAS); c and d are for *S. oryzae* (c. venter, d. dorsum. – control; – · – · hydrophilic SAS; .... hydrophobic SAS).

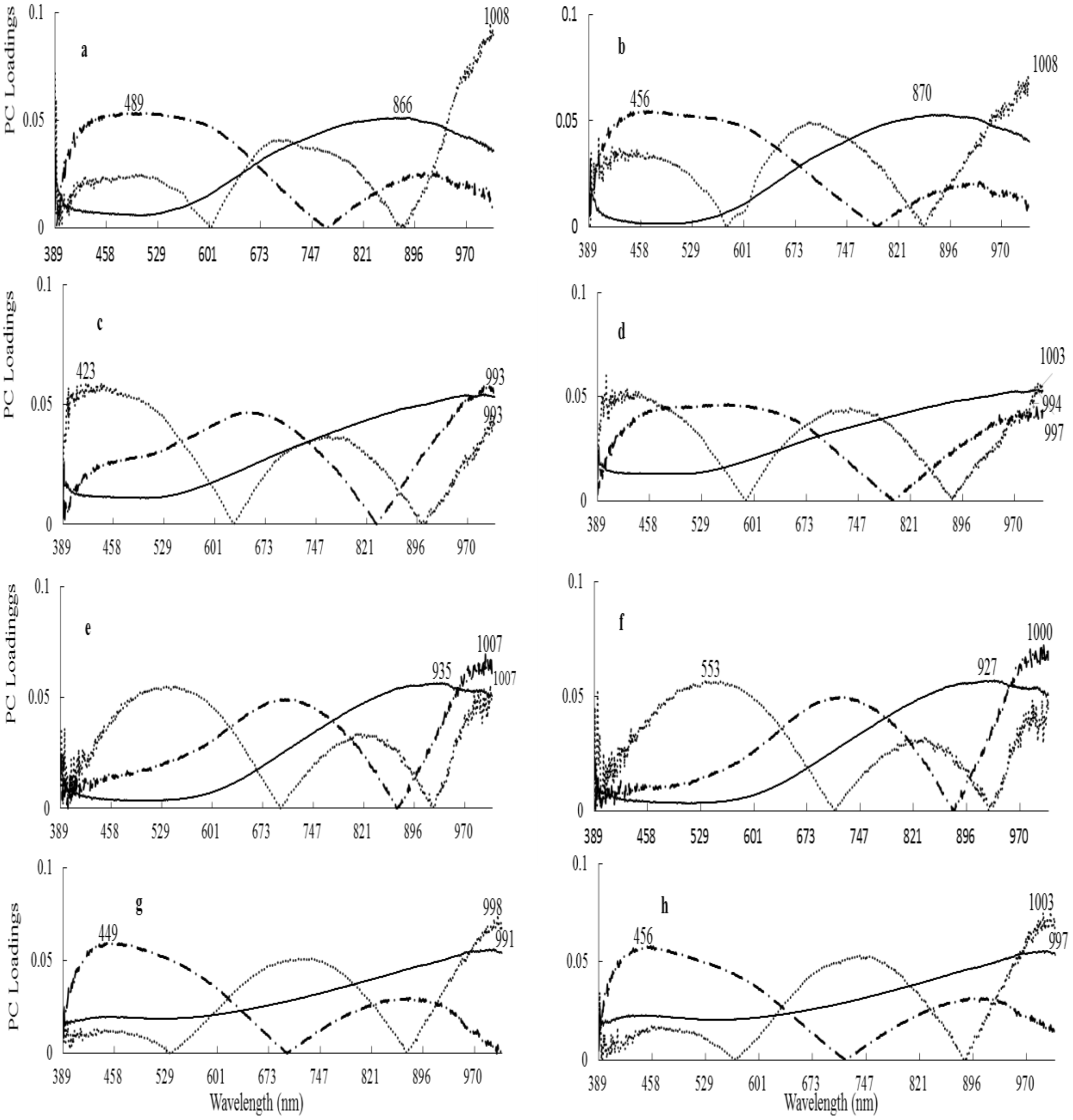


Fig. 4.4. The first three PC mean factors (absolute values) of dorsal and ventral surface properties for *T. castaneum* and *S. oryzae* changed by hydrophilic SAS treatments and hydrophobic SAS treatments (– First PC factor loadings; - · - · Second PC factor loadings and .... Third PC factor loadings).

a. *T. castaneum* ventral surface: hydrophilic SAS and control
b. *T. castaneum* ventral surface: hydrophobic SAS and control
c. *T. castaneum* dorsal surface: hydrophilic SAS and control
d. *T. castaneum* dorsal surface: hydrophobic SAS and control
e. *S. oryzae* ventral surface: hydrophilic SAS and control
f. *S. oryzae* ventral surface: hydrophobic SAS and control
g. *S. oryzae* dorsal surface: hydrophilic SAS and control
h. *S. oryzae* dorsal surface: hydrophobic SAS and control

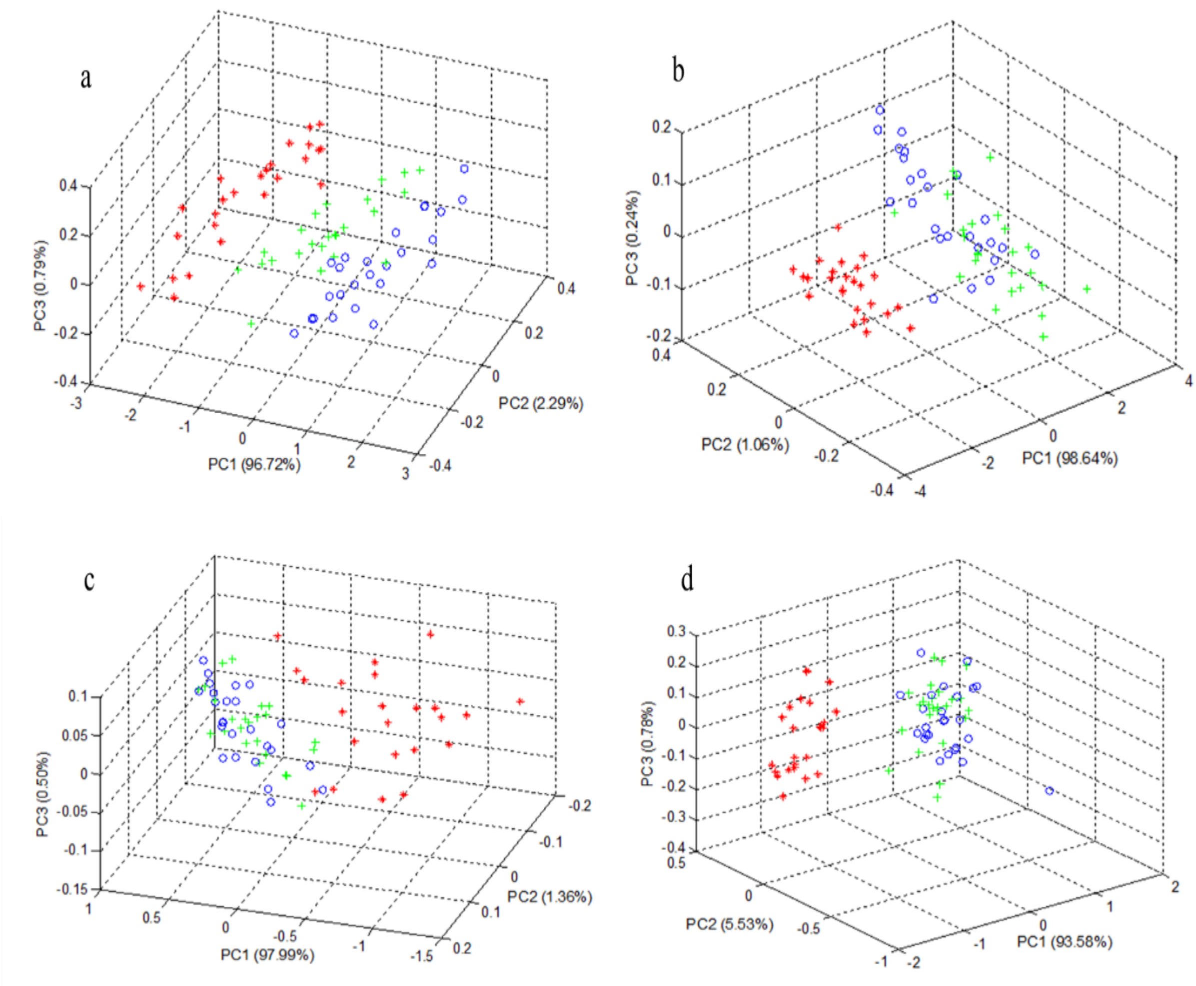


Fig. 4.5. PCA three-dimensional score plot for discriminating dorsal and ventral surface properties of *T. castaneum* and *S. oryzae* pre- and post-treatment by hydrophilic and hydrophobic SAS. a and b are *T. castaneum* (a. venter, b. dorsum. * control; + hydrophilic SAS; o hydrophobic SAS). c and d are *S. oryzae* (c. venter, d. dorsum. * control; + hydrophilic SAS; o hydrophobic SAS).

As shown in Fig. 4.5, distribution patterns were clearly identified without overlapping between treatment groups and controls, however, the patterns were not well defined for groups treated with different SAS products within the same insect species and some overlap was observed. This may suggest that both SAS have similar effect on the insects even though their inherent compositional characteristics are very different. This demonstrates that geometrical exploration based on PCA score plots can generate a cluster trend in a 3-dimension space, but it is not the best tool for discriminating between insect groups. In addition, the PCA cannot provide a definite index to describe the exact differences and will reduce the reliability of the results. Based on this finding, different recognition model methods were utilized in following studies.

4.4.4 BPNN

The insects were separately processed according to positions and species to evaluate surface property changes on the insects' dorsum and venter after exposure to two SAS products. All samples were randomly divided into a calibration set (two-thirds of the insect samples) and a validation set (one-third of the insect samples). The calibration set was used to compute the parameters of the models, while the validation set was used to validate the network generalization capability.

The optimum models were obtained by the use of the principal components (PCs) after PCA was performed to eliminate multidimensionality. The optimal number of PCs was determined by the discriminating rate from the calibration set. Diagnostic results showed that all of the SAS treatment groups, regardless of species, were noticeably different from those of the control groups, which is consistent with the above results from PCA. In Table 4.2, the highest recognition models for the ventral and dorsal regions of *T. castaneum* were acquired with 6 and 8 PCs, respectively. For the dorsum, the percent correct classifications of the calibration and the validation data from varied origins were 93.88 and 99%. For the venter, the optimal model classified hydrophilic SAS treated groups, hydrophobic SAS treated groups and the controls with 95.74 and 96.88% accuracy in both the calibration set and prediction set as can be seen in Table 4.2. Table 4.2 showed that the highest recognition BPNN model for ventral and dorsal regions of *S. oryzae* were acquired with nine PC inputs. With this, the highest recognition rate was 90.48 and 95.29% in the calibration and prediction sets for the *S. oryzae* dorsum. Also, for the *S. oryzae* venter, the percentage of correctly identified varieties was 97.67 and 95.4% for the calibration and prediction sample sets as can be seen in Table 4.2. Data presented in Tables 4.3 and 4.4 illustrated the detailed recognition results of the optimal BPNN model. The control samples of all groups were correctly classified by the model and misclassification occurred only with the two groups treated by the two different SAS products. One and two samples were misclassified between both SAS treated *T. castaneum* groups. Only one and two samples of the *S. oryzae* dorsum treated by hydrophilic SAS were misclassified respectively as that control and treated by hydrophobic SAS, resulting in the lowest recognition rate of 76.92%, yet still within the acceptable identification range. The detectable difference between hydrophobic and hydrophilic SAS treatment groups does exist. It is indisputable that the difference in characteristics between the SAS treated groups and controls was larger than that between groups treated by different SAS.

Table 4.1. Lethal dose values and regression curve parameters for hydrophilic and hydrophobic SAS tested on *T. castaneum* and *S. oryzae*.

Insect species	Polarity of SAS	Mean ± SE	LD (95% CL) (g h/m^2)		χ^2 (df)	*P*
		Intercept	LD_{50}	LD_{95}		
T. castaneum	hydrophilic	-3.600±0.071	47.298(45.716-48.846)	68.908(66.418-70.588)	805.793(88)	0.000
	hydrophobic	-3.748±0.084	38.998(37.432-40.504)	56.110(53.782-59.008)	668.650(64)	0.000
S. oryzae	hydrophilic	-3.902±0.080	47.220(23.125-24.091)	67.124(65.464-69.012)	269.706(70)	0.000
	hydrophobic	-4.461±0.082	57.636(56.964-58.306)	78.888(77.742-80.128)	160.928(88)	0.000

Table 4.2. Recognition results of the BPNN model with a different number of PCs in distinguishing hydrophilic and hydrophobic SAS affected dorsal and ventral surface property of *T. castaneum* and *S. oryzae*.

Number of PCs	Dorsum				Venter			
	T. castaneum		*S. oryzae*		*T. castaneum*		*S. oryzae*	
	Calibration set	Prediction set	Calibration set	Prediction set	Calibration set	Prediction set	Calibration set	Prediction set
1	85.71	70.00	30.95	37.65	25.53	37.50	41.86	47.12
2	83.67	90.00	85.71	85.88	93.62	85.42	86.04	81.61
3	87.76	90.00	83.33	84.71	95.74	90.63	90.70	90.80
4	89.80	92.00	85.71	88.24	95.74	93.75	93.02	89.66
5	89.80	93.00	85.71	83.53	95.74	94.79	93.02	93.10
6	89.80	98.00	90.48	91.67	95.74	96.88	93.02	89.66
7	93.88	96.00	90.48	89.41	95.74	94.79	93.02	94.25
8	93.88	99.00	92.86	88.24	95.74	94.79	93.02	91.95
9	85.71	98.00	90.48	95.29	95.74	94.79	97.67	95.40
10	91.84	96.00	88.10	95.29	95.74	95.83	90.70	94.25

Table 4.3. Classification accuracies for controls, and SAS treated ventral and dorsal surfaces of *T. castaneum*, using the optimal BPNN model.

Regions	Actual strain		Recognized strain			Recognition rate (%)
			control	hydrophilic SAS treated	hydrophobic SAS treated	
Venter	Calibration set	control	25	0	0	100
		hydrophilic SAS treated	0	35	1	97.22
		hydrophobic SAS treated	0	2	33	94.29
	Prediction set	control	21	0	0	100
		hydrophilic SAS treated	0	12	0	100
		hydrophobic SAS treated	0	2	12	85.71
Dorsum	Calibration set	control	26	0	0	100
		hydrophilic SAS treated	0	38	1	97.44
		hydrophobic SAS treated	0	0	35	100
	Prediction set	control	22	0	0	100
		hydrophilic SAS treated	0	11	1	91.67
		hydrophobic SAS treated	0	2	13	86.67

Table 4.4. Classification accuracies for controls, and SAS treated ventral and dorsal surfaces of *S. oryzae*, using the optimal BPNN model.

Regions	Actual strain		Recognized strain			Recognition rate (%)
			control	hydrophilic SAS treated	hydrophobic SAS treated	
Venter	Calibration set	control	22	0	0	100
		hydrophilic SAS treated	0	32	2	96.97
		hydrophobic SAS treated	0	0	32	100
	Prediction set	control	16	0	0	100
		hydrophilic SAS treated	0	8	2	80
		hydrophobic SAS treated	0	1	16	94.12
Dorsum	Calibration set	control	20	0	0	100
		hydrophilic SAS treated	0	29	3	90.63
		hydrophobic SAS treated	0	1	32	96.97
	Prediction set	control	14	0	0	100
		hydrophilic SAS treated	1	10	2	76.92
		hydrophobic SAS treated	0	1	14	93.33

4.5 Discussion

Being the interface between a living animal and the environment, the cuticle surface of an insect serves many functions, such as defense against a variety of external factors (camouflage against predators, mechanical stresses and water loss), transport of epidermal secretions and as a chemical reservoir for the storage of metabolic waste products. It is also involved in communication between individuals. This makes the cuticle surface an ideal target for the development of new insecticides. Many existing insecticides exploit the cuticle surface characteristics to achieve control of the insect. When insects are in contact with hydrophilic SAS, they lose weight rapidly and it has been postulated that death is due to desiccation (Ebeling, 1971). The mode of action of desiccation is based on the hydrophilic nature of SAS. Hydrophilic particles compete for water in the insect body and absorb it. There are 2-6 silanol (Si-OH) numbers per square nanometre on a hydrophilic SAS surface (Fruijtier-Pölloth, 2012) resulting in a water layer being coated on particles upon exposure to humid atmospheric conditions or in direct contact with water. Such "moisturized" hydrophilic SAS materials are ineffective as insecticides under conditions of high moisture and humidity. Hydrophobic SAS is developed to circumvent this issue (Ralph, 1964). Hydrophobic siliceous insecticidal compositions described in U.S. Patent3,159,536, issued to Ralph (1964) were substantially unaffected when exposed to 100% relative humidity for 24 h. However, modified SAS (with surface-functionalized SAS) showed lower toxicity compare to hydrophilic SAS on *S. oryzae*. This indicates that this surface group of hydrophobic SAS are not superior in effectiveness. Further study is needed to establish the detailed mechanism of action of SAS.

Two hypothetical mechanisms against insects are most widely accepted for SAS. One is that of removing the waterproof layer and causing desiccation of the insect (Ebeling, 1971). The other is destruction of the insect's joint structure combined with absorption of body fluid. Both lead to discoloration upon death (Miyazaki, 1993). The results of this study demonstrated genuine cuticle damage caused by SAS. Although electron scanning microscopy can be applied to evaluate the sample's surface topography and composition, its pretreatment and process requires high vacuum conditions, which have the potential to cause extra damage to insect soft tissue. Hyperspectral imaging techniques require no special pretreatment because it is undertaken under regular temperature and pressure conditions. After data collection, the control cultures are still alive and unaffected, thus, they can be used in the future while providing continuance between trials.

Different SAS products cause differential changes to insect cuticles. Spectral readings supported this finding. In previous study (Cook et al., 2008), there was no evidence of abrasion caused by SAS that was detectable with scanning electron microscopy. The BPNN model is far superior in identifying change in surface structural parameters after exposure to SAS. It is possible that the changes in epicuticle are subtle and unevenly distributed, which lead to non-liner hyperspectral data. There is a high nonlinearity of the modeled relationship in ANN. Therefore, the hyperspectral imaging technique coupled with ANN models are better suited for classification of the cuticle parameters for the treatment and control of stored grain insect pests by SAS. These results are in agreement with those reported (Cao et al., 2015).

Adult beetle cuticles are composed mainly of catecholes, protein, chitin, pigment melanin and water, and small amounts of lipids (Kramer et al., 1995; Vincent and Wegst, 2004) Chemical composition varies, even within the same species. Near-infrared spectroscopy coupled with neural networks were employed previously(Dowell et al., 1999). Changes in chemical composition during SAS treatment of *T. castaneum* and *S. oryzae* were determined using hyperspectral imaging techniques. During the selection of effective wavelength regions on classification accuracies, multivariate image analysis results showed that both the visible and NIR region generated useful data. Further analysis showed that some functional groups were responsible for peak values. The absorbance probably corresponds with the third overtone of a C-H stretching vibration of fat and protein, or the second overtone of an O-H stretching of fat or protein (Šašić and Ozaki, 2001; Wu et al., 2008). Although these short-wave NIR investigations are not exclusively used for insect studies, the proposed wavelengths are similar. The peak at around 968 nm due to 2v1 + v3 (v1: symmetric stretching; v3: anti-symmetric stretching) water vibration was weak with unclear indications, which suggested the influence of water was not strong. It is possible that 968 nm is only one of the feature peaks of water vibration and it is not the main factor. In addition, there is loss of body water by exposure to SAS and hence this is not obvious in short-wave NIR. Further investigation is needed to confirm desiccation of the powder model utilizing IR.

Our trials showed that hyperspectral imaging techniques provided good accuracy in detecting powder damage to *T. castaneum* and *S. oryzae*. The BPNN classifiers give relatively good classification accuracy (100% for the powder-damaged samples). The results were achieved from the average spectral data for the dorsum and venter of insects, which were adequate for detection of insect surface changes caused by SAS. For further understanding of the mechanism of SAS damage against the insect, the frictional system of the insect is recommended as a region of interest.

Chapter 5. Evaluation of the physical effects on insect intersegment frictional devices and associated biological impacts of two synthetic amorphous silica (SAS) powders

5.1 Abstract

Synthetic amorphous silicon (SAS) is a food-grade, fast acting, effective and low cost product in contrast to diatomaceous earth, and has become a promising nonchemical pesticide alternative to replace chemical products. The mechanism of its physical effects which generate no resistance was not well understood. This directly limits its practical application. To offer a better understanding of the mode of action of SAS, the current study qualitatively measures movement of and biological effects on insects affected by SAS based on computerized video tracking systems. New systems were developed for recording the biological effects on the anatomical structures of *T. castaneum* and *S. oryzae* by hydrophilic and hydrophobic SAS and the impact these have on the insects' behaviour. Contact with SAS caused loss of locomotion and behavioural changes in adult insects, suggesting destruction of the intersegment frictional devices. The effect to the insects' locomotor and stride length at various times during exposure to dried deposits of SAS was determined and in most cases was found to drop to a limiting value within about 3.5 h and 12 h for *S. oryzae* and *T. castaneum*, respectively. The effect of SAS on insect locomotion and behaviour varies with insect species, gender and powder categories. *S. oryzae*'s locomotion and behaviour were more sensitive to SAS than *T. castaneum*'s and males were more affected. *S. oryzae*'s locomotion and behaviour were sensitive to hydrophilic SAS, and *T. castaneum*'s locomotion and behaviour to hydrophobic SAS. Synthetic amorphous silica invaded the intersegmental frictional devices and absorbed the vital body fluids. Lethal effect is not instantaneous, but structural damage is irreversible. The insects' locomotion and behaviour was severely affected, which made activities necessary for survival and reproduction difficult or impossible, including feeding and mating. Resistance is unlikely to raise given the physical nature of the

causes. These findings further support the possibility that non-chemical pesticide SAS is superior to chemical pesticides.

5.2 Introduction

With regard to mode of action of inert dust, insect mortality could be attributed to the impairment of the digestive tract (Smith, 1969). Ingestion is the primary method of delivery for conventional pesticides. However, pests will only ingest certain substances and in small amounts. This fact limits the degree of tissue damage and insecticidal effectiveness. Observation has suggested that insects generally will not ingest fatal amounts of dehydrating pesticide. After exposure to the SAS, Aerosil 380, the rates, levels and concavity of mortality curves of *T. castaneum* had significant differences between the “fed” and “unfed” groups. With feeding, the mortality never attained 100% within the test periods, while under food deprivation condition, 100% mortality always occured (Vrba et al., 1983). Many recent results can be explained by the physic-chemical removal of the epicuticular, lipid-water barrier as proposed by Ebeling (1971), with mortality resulting from desiccation. However, the basis for his theory cannot explain nor account for mortality in *S. oryzae* caused by diatomaceous earth as noted by Carlson and Ball (1962), since weight loss (i.e. desiccation) in these beetles was insignificant. Upon exposure to inert dust, insects lose weight rapidly. Death by desiccation has been postulated. But the link between desiccation and death cannot be demonstrated. There is no explanation that inert dust could cause insects to lose weight or to be desiccated unless the hydrophilic particles compete with the water in the body of the insect and absorb a large amount of body fluid. Many previous investigations were aimed to establish the mode of action for the inert dust insecticidal characteristic.

Yet several important questions remain unanswered, especially regarding the precise regional and temporal regulation of the various steps in the process.

The external skeleton in arthropods is the main advantage and constraint in the evolution of their extremities. The anatomical structure of arthropod extremities is completely different from that of vertebrates. In arthropods, the leg is a cuticular tube containing muscles that control parts of the tube, called segments, and the physical properties of various parts of the tube are vastly different. Based on mechanical properties, the cuticle can be classified into two main types. One is present in each segment, relatively hard and stiff, which is typical of sclerites, and thus imparts skeletal rigidity. The other is commonly encountered in the arthrodial membrane area, and is relatively soft and flexible. These two types of cuticle can be related directly to function. The former chiefly serves as a protection device, while the latter often subserves locomotion. Usually, surfaces of both segments are smooth within the joint which decreases friction during segment movements. Also, microtrichia deformations enable epidermal secretions, which may be surface-active and aid in the lubrication of contacting microtrichia (Gorb, 2001). Previous studies suggested that damage caused by inert dust occurs to the insects' protective wax coat on the cuticle of segments (Ebeling, 1971; Subramanyam and Roesli, 2000). The exoskeleton provides absolute protection against most foreign agents such as pesticidal liquids and powders. Insects will try to remove the residue from their body surface. The inert dust on the hard, stable and dry segments is much easier to remove than it is on the soft, flexible and moist intersegmental membranes. Preliminary work has shown that the mortality rates were not significantly different in *T. confusum* that were "cleaned" and "not cleaned" after exposure to the SAS, Aerosil 380 (Vrba et al., 1983). It is therefore proposed that synthetic amorphous silica works against insects by destroying insect joint systems.

Building upon this, in the present study, specific nontoxic SAS are used as substitutes for the poisonous and environmental unfriendly chemical agents to prevent stored grain insect pests from rapidly developing large populations. Mobility analysis by high speed photography and locomotion compensator has been used to further elucidate the effects of SAS on the friction systems of *T. castaneum* and *S. oryzae.* In addition, a secondary aim of the study has been to propose a new delivery method of a pesticide via insect intersegment frictional devices.

5.3 Methods and materials

5.3.1 Insects

S. oryzae and *T. castaneum* adults were collected from stored grain farms in Tongzhou country, Beijing and Qihe city, Shandong, China from June to September, 2015. A total of 2000 adults (1100 males and 900 females) were collected and reared in the laboratory of the Academy of State Administration of Grain, Beijing, China, under the following environmental conditions: 30±1℃, 75±5% RH. Healthy *S. oryzae* and *T. castaneum* were selected from the field collection. *S. oryzae* were reared on whole wheat. *T. castaneum* were reared were reared on 10 parts wheat-meal flour and 1 part yeast (Winks, 1982). Upon emergence, 20 day old adults were selected for use in the study. These unmated adults were sexed using their distinctive abdominal and leg characteristics.

5.3.2 Dusts

In our investigation, we used a custom-made hydrophilic SAS (SAS4) and a surface-functional hydrophobic SAS (SAS5) from Murdoch University, Perth, Australia. These were prepared by the precipitate method and have a D (v, 0.9) aggregated particle size of 14.12±2.62 µm and 2.75±1.10 µm respectively (Fig. 5.1).

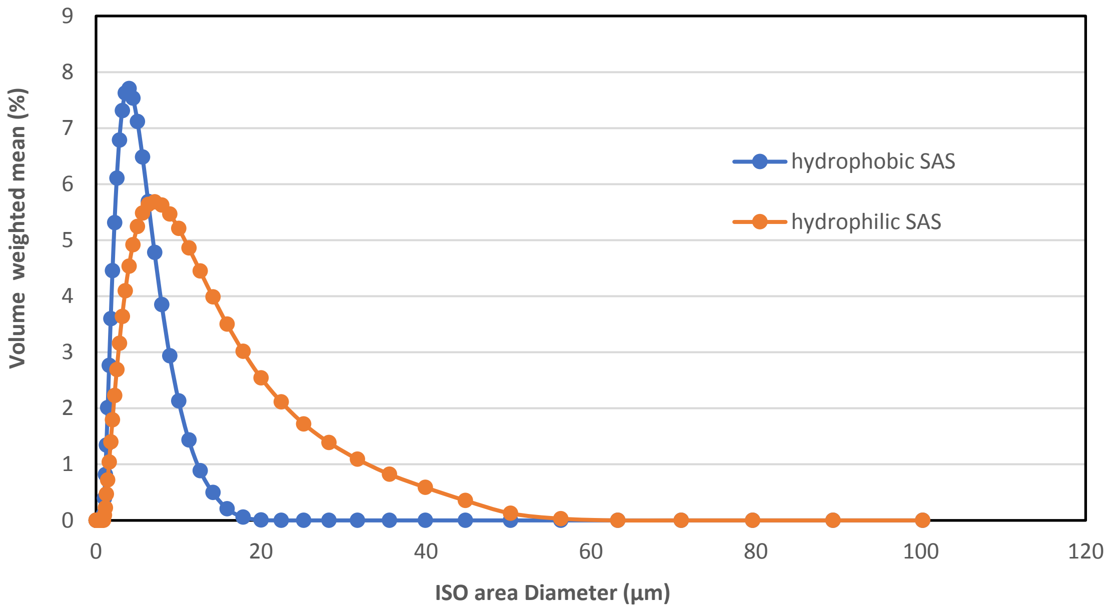


Fig. 5.1. Size distribution by volume weighted mean of hydrophilic and hydrophobic SAS.

5.3.3 Continuous exposure tests

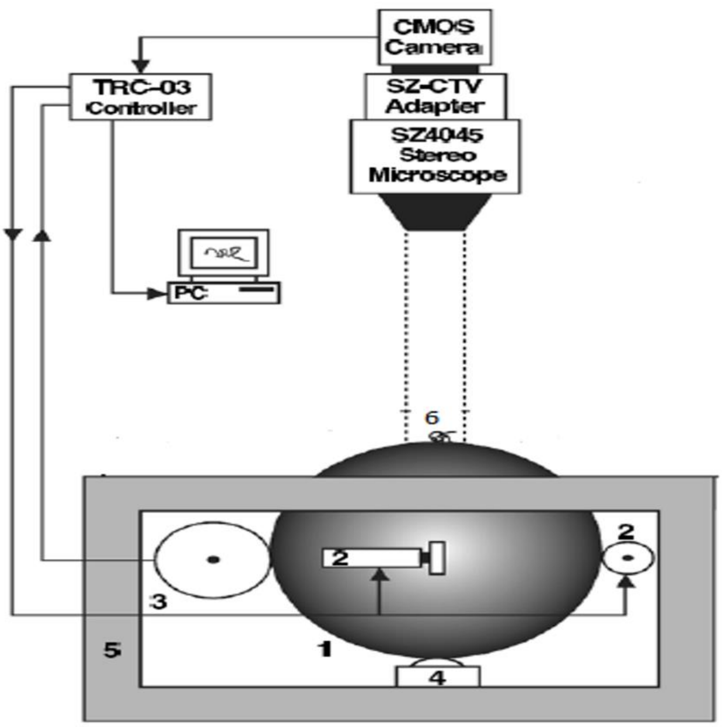
The intrinsic insecticidal activity of both SAS powders was determined by exposing *S. oryzae* and *T. castaneum* to a powder deposition. The adults (13 male and 13 female adult replicates of each species per treatment) were dumped individually onto lightweight deposits of the hydrophilic and hydrophobic SAS (2 g/m^2) spread evenly over the surface of filter papers waxed into the floor of 7 cm diameter glass petri dishes and maintained at a 28±1°C, 65±5% RH. Their speed of action in terms of knockdown (KD) and paralysis was determined from periodic observation. For *S. oryzae*, the locomotory capacity was observed every 30 minutes for a total 3.5 hours; for *T. castaneum*, we monitored the movement every 2 hours for a total 12 hours. An untreated set of papers served as a control series.

5.3.4 Locomotion recording

To compare the damage to the frictional systems of the leg joints by the two SAS powders, we prepared two types of experimental evaluation, creeping speed and stride length. The creeping speed evaluation was performed with a locomotion compensator, TrackSpehere LC-100 (Syntech, Hilversum, Netherlands) (Fig. 5.2). This instrument has often been used for real-time tracking of the orientation behavior of mico-arthropods (Van Tilborg et al., 2003). When the motion of the sphere ran stably, we placed a test insect on the top of the sphere. The optical and visual conditions of the test insect were controlled by a video camera inserted in the light path of a stereomicroscope. Prior to each recording session, each insect was allowed to walk freely on the top of the sphere for 1 min. The recording session was 1 min and was displayed on a computer screen under the control of the tracking software TrackSphereTM V3.1 (Syntech, Hilversum, Netherlands). For each replicate, a new insect was transferred with a fine camel brush to the top of the sphere.

5.3.5 Stride length measurement

The measurement of stride length of each insect was filmed using a high-speed video camera (TroubleShooter TS500ME, Fastec Imaging Corporation) mounted on the video tube of the microscope at 500 frames per second and the images were digitized and analyzed using Midas motion analysis software. The reflectance light source structure was composed of a 150 W halogen light source, which was connected to a dual fiber optic line light. To ensure accurate recording of minute insect footprints, the images were amplified by 1.5 times, so targets were visible only within a small region. A cold light source was used in the present study to avoid heating, which was connected to a dual fiber optic line light. We prepared a wall-enclosed circular surface with a graph paper bottom (25×25×20 mm). The graph paper was used as a measuring scale. We place a single stored grain insect on the bottom left hand side of the paper and tracked the free walking behaviour for more than ten steps or 3 minutes, at which stage the insects were considered to be KD (Knight and Bessette, 1997).



1. Sphere;
2. Servo-motors
3. Rotation encoder
4. Ball bearing
5. Aluminum frame
6. Insect

Fig. 5.2. Experimental setup of the locomotion compensator (LC-100 prototype).

5.3.6 Observations on the structure of the insect joints

For microstructural images, an environmental scanning electron microscope (ESEM) (LEO435P, London, UK) was used. The insect body became very fragile after exposure to SAS. Thus, their natural state cannot be maintained very well if processed under the conventional SEM. The advantage of ESEM was that the insects could be imaged under low pressure (up to around 10 torr) without a conductive coating. A gaseous environment in the specimen chamber can keep the specimen in the “wet” condition.

5.3.7 Track preprocessing and data analysis

The following parameters were used to quantify the tracks in the locomotion compensator: (1) walking speed (mm/sec), is the distance (in mm) walked during one second. These values were averaged for each track; (2) straightness of walking (ranging from 0 to 1, straight line), i.e., the quotient of vector length (from the origin to the final point of the track) and total track length. It describes how straight or tortuous is the track of the insect. In general, the more tortuous the track the lower the walking speed will be as insects slow down when turning; (3) angles (x, y) (ranges from -1 to +1) shows preference for a particular angle, and should be interpreted in combination with the vector length; (4) upward length (mm) is the summed projection of the displacements made by the insect on the axis parallel to a particular direction.
Experiments on the servo-sphere are frequently designed to study the responses to a stimulus, so the odours are coming from a particular direction, but kinetic responses can be studied as well. A positive or negative sign indicates that the insect moves toward or away from the source. For each experimental condition the mean angles by Rayleigh Z tests using the procedure described by Batschelet (1981) (U tests) were tested. Rayleigh Z tests is employed to analysis the distribution of the data. The data were distributed uniformly under null hypothesis. If not, it suggests that there is a direction insect prefers. Calculations and statistical tests on circular data were done using the Oriana software (Kovach Computing Services, Anglesey, UK).
Each recording session was loaded into MATLAB (Math works, matlabR2009b, Inc., USA) to separate images by frame. From this huge image data set, the important frames, which were the continuous points when the insect pulvillus of the mesothoracic leg touched the graph paper, were selected. The selected points were marked to form a

moving track. The stride length was calculated by two different pixel locations. Prior to measuring the stride length for further analyses, we calibrated the measurement scale on the mirs software.

SPSS® 24.0 software was used for the statistical analysis. We calculated the average value of each set of observed data as the mean ± SE. The differences in each biological and physiological character were compared using a one-way analysis of variance (ANOVA) among all experimental groups. Although angle, speed, straightness and stride length values did not follow a Gaussian distribution very well, the major effects pointed to by the ANOVA were reliable because the method was particularly robust to such divergence in well replicated assays, and clear trends were pointed to by very high F values (Khan and Rayner, 2004). A Student-Newman-Kreuls (SNK) test and Duncan test were used to perform post hoc multiple comparisons for the exposure time periods. Levene's test was used to determine the variances in the upward length of the two insect species groups when treated by two SAS powders.

5.4 Results

Hydrophilic and hydrophobic SAS at the dose of 2 g/m^2 were shown to cause disorientation with subsequent knock-down in a high proportion of *S. oryzae* and *T. castaneum* within 3.5 h and 12 h, respectively. The particles attached to the insect joints, induced severe reduced ranges of motion and sensation that can lead to behavioural changes, such as abnormal walk, and upwards length. There were significant differences between the two SAS powders, genders, exposure time periods and species.

5.4.1 Locomotion impairment

In order to analyze the tracks, we first examined the angle of the tracks (Tables 5.1 and 5.2) for which no significant difference was not found between *S. oryzae* and *T. castaneum* when subjected to both SAS powders at several time periods (*S. oryzae*: $F = 0.932$, $df = 7$; *T. castaneum*: $F = 0.751$, $df = 6$). The angle of the two insect species was not significantly different between hydrophilic and hydrophobic SAS (*S. oryzae*: $F = 1.082$, $df = 1$; *T. castaneum*: $F = 1.776$, $df = 1$), and between male and female populations (*S. oryzae*: $F = 1.308$, $df = 1$; *T. castaneum*: $F = 0.650$, $df = 1$). In addition, no significant difference was observed in all the interactions for exposure time × dust (*S. oryzae*: $F = 2.416$, $df = 7$; *T. castaneum*: $F = 0.842$, $df = 6$), dust × gender (*S. oryzae*: $F = 0.277$, $df = 1$; *T. castaneum*: $F = 0.152$, $df = 1$), time × gender (*S. oryzae*: $F = 0.666$, $df = 7$; *T. castaneum*: $F = 0.569$, $df = 6$), and dust × time × gender (*S. oryzae*: $F = 0.722$, $df = 7$; *T. castaneum*: $F = 0.557$, $df = 6$).

All the control groups walked continuously and vigorously on the top of the sphere, as soon as they were placed on it. We observed only a few occasional stops during head lifting. Although both of hydrophilic and hydrophobic SAS significantly influenced the friction systems of *T. castaneum* and *S. oryzae*, the locomotion ability of *S. oryzae* could be more affected by SAS powders according to specific test times (Fig. 5.3). In general, hydrophobic SAS caused more locomotion impairment in *T. castaneum* than hydrophilic SAS. The observation was the opposite for *S. oryzae*, which was consistent with our previous results. The ANOVA analysis (Tables 5.3 and 5.4) indicated the length of exposure time had significant effect on the reduction of average walking speed in both species (*S. oryzae*: $F = 250.295$, $df = 7$, $P < 0.001$; *T. castaneum*: $F = 72.241$, $df = 6$, $P < 0.001$) were significant. Most of the insects could not walked continuously for one minute at the end of the experimental session. Walking speed of both insect species was by far the main variable between hydrophobic and hydrophilic SAS (*S. oryzae*: $F =$

54.995, df = 1, $P < 0.001$; *T. castaneum*: F = 16.383, df = 1, $P < 0.001$). Gender dictated the average walking speed of *S. oryzae* which was evident in control group and treated group (1.5 hours after exposure) (F = 38.189, df = 1, $P < 0.001$). However, there was no significant difference in the speed of female and male *T. castaneum* (F = 0.039, df = 1, $P < 0.001$). The associated interactions for exposure time × dust (*S. oryzae*: F = 1.745, df = 7; *T. castaneum*: F = 0.871, df = 6), dust × gender (*S. oryzae*: F = 1.732, df = 1; *T. castaneum*: F = 0.411, df = 1), time × gender (*S. oryzae*: F = 1.999, df = 7; *T. castaneum*: F = 2.543, df = 6), and dust × time × gender (*S. oryzae*: F = 1.693, df = 7; *T. castaneum*: F = 0.535, df = 6) were not significant.

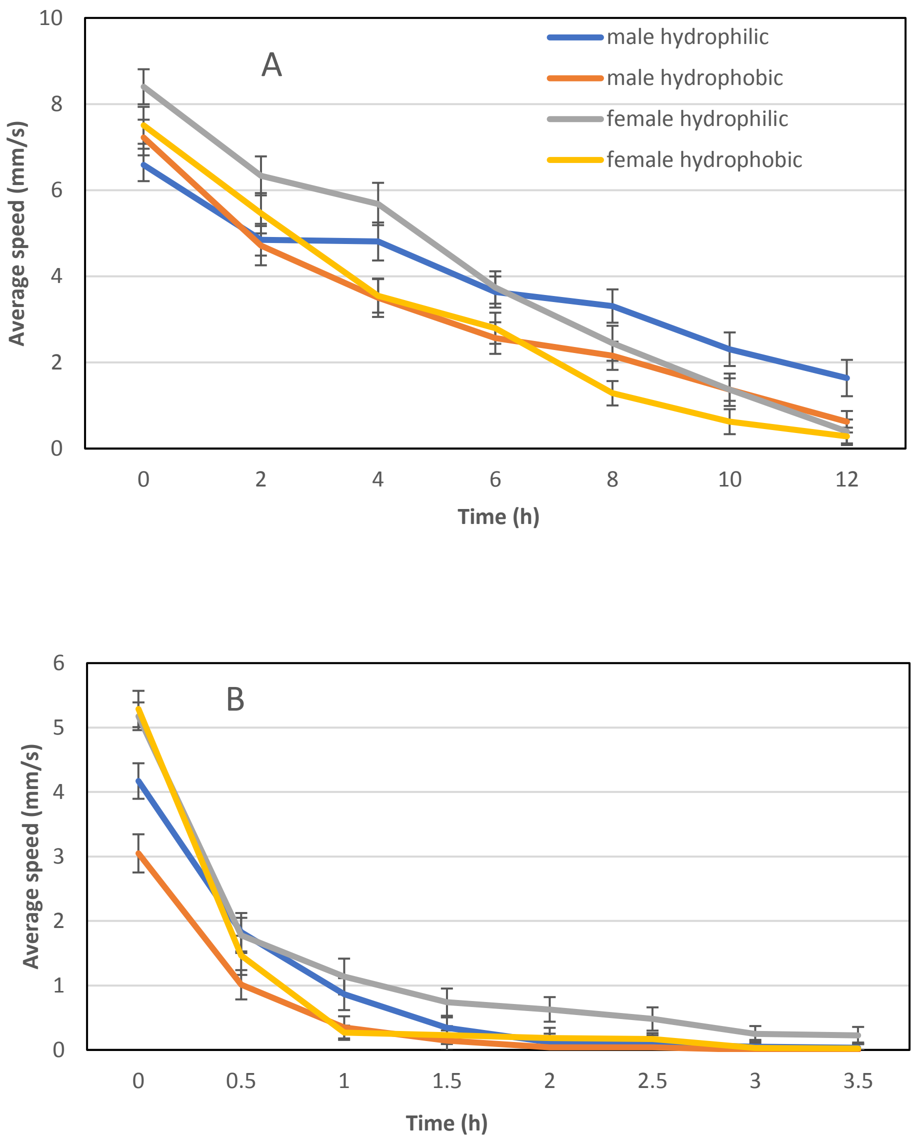


Fig. 5.3. Average walking speed of female and male *T. castaneum* and *S. oryzae* on the locomotion compensator after exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different durations at 28±1°C, 65±5% RH. A. *T. castaneum*; B. *S. oryzae*.

Table 5.1. Results of one-way ANOVA for the significance of variation between dust, gender, and exposure time on the angle of *S. oryzae* exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different periods at 28±1°C, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	0.351	1	0.351	1.082	0.299
Gender	0.424	1	0.424	1.308	0.254
Time	2.114	7	0.302	0.932	0.482
Dust × Time	5.481	7	0.783	2.416	0.020
Dust × Gender	0.090	1	0.090	0.277	0.599
Time × Gender	1.511	7	0.216	0.666	0.701
Dust × Time × Gender	1.638	7	0.234	0.722	0.653
Error	124.131	383	0.324		
Total	136.878	415			

Table 5.2. Results of one-way ANOVA for the significance of variation between dust, gender, and exposure time on the angle of *T. castaneum* exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different durations at 28±1°C, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	0.610	1	0.610	1.776	0.183
Gender	0.223	1	0.223	0.650	0.421
Time	1.548	6	0.258	0.751	0.609
Dust × Time	1.735	6	0.289	0.842	0.538
Dust × Gender	0.052	1	0.052	0.152	0.697
Time × Gender	1.173	6	0.196	0.569	0.755
Dust × Time × Gender	1.148	6	0.191	0.557	0.764
Error	115.098	335	0.344		
Total	126.167	363			

Table 5.3. Results of one-way ANOVA for the significance of variation between dust, gender, and exposure time on the speed of *S. oryzae* exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different periods at 28±1°C, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	3.415	1	3.415	54.995	0.000
Gender	2.371	1	2.371	38.189	0.000
Time	108.798	7	15.543	250.295	0.000
Dust × Time	0.759	7	0.108	1.745	0.097
Dust × Gender	0.108	1	0.108	1.732	0.189
Time × Gender	0.869	7	0.124	1.999	0.054
Dust × Time × Gender	0.736	7	0.105	1.693	0.109
Error	23.783	383	0.062		
Total	227.640	415			

Table 5.4. Results of one-way ANOVA for the significance of variation between dust, gender, and exposure time on the speed of *T. castaneum* exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different durations at 28±1°C, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	65.095	1	65.095	16.383	0.000
Gender	0.157	1	0.157	0.039	0.843
Time	1722.227	6	287.038	72.241	0.000
Dust × Time	20.762	6	3.460	0.871	0.516
Dust × Gender	1.634	1	1.634	0.411	0.522
Time × Gender	60.617	6	10.103	2.543	0.020
Dust × Time × Gender	12.756	6	2.126	0.535	0.782
Error	1335.039	336	3.973		
Total	7782.409	364			

Table 5.5. Results of one-way ANOVA for the significance of variation between dust, gender, and exposure time on the straightness of *S. oryzae* exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different durations at 28±1°C, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	0.878	1	0.878	18.791	0.000
Gender	0.051	1	0.051	1.082	0.299
Time	3.943	7	0.563	12.062	0.000
Dust × Time	0.630	7	0.090	1.928	0.064
Dust × Gender	5.601E-6	1	5.601E-6	0.000	0.991
Time × Gender	0.382	7	0.055	1.169	0.319
Dust × Time × Gender	0.265	7	0.038	0.811	0.578
Error	17.887	383	0.047		
Total	251.979	415			

Table 5.6. Results of one-way ANOVA for the significance of variation between dust, gender, and exposure time on the straightness of *T. castaneum* exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different durations at 28±1°C, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	0.731	1	0.731	8.758	0.003
Gender	0.056	1	0.056	0.670	0.414
Time	10.895	6	1.816	21.762	0.000
Dust × Time	0.317	6	0.053	0.633	0.704
Dust × Gender	0.006	1	0.006	0.071	0.790
Time × Gender	1.816	6	0.303	3.627	0.002
Dust × Time × Gender	0.199	6	0.033	0.397	0.881
Error	28.036	336	0.083		
Total	173.901	364			

5.4.2 Behavioral change

The tracking pattern (straightness) was then analyzed (Fig. 5.4). Both synthetic amorphous silica powders significantly decreased the straightness of the paths compared to the control for different exposure time periods (*S. oryzae*: $F = 12.062$, $df = 7$, $P < 0.001$; *T. castaneum*: $F = 21.762$, $df = 6$, $P < 0.001$) (Tables 5.5 and 5.6). The straightness value of *S. oryzae* under hydrophobic SAS was significantly lower than in the presence of hydrophilic SAS at 1 h, 1.5 h and 2 h exposure time ($F = 18.791$, $df = 1$, $P < 0.001$). In *T. castaneum*, both hydrophobic and hydrophilic SAS significantly reduced the straightness of path 2 h and 8 h post exposure ($F = 8.758$, $df = 1$, $P < 0.001$). Straightness was not affected by gender factor for both insect species (*S. oryzae*: $F = 1.082$, $df = 1$; *T. castaneum*: $F = 0.670$, $df = 1$). No significant difference in straightness was observed for the two insect species in all the interactions for exposure time × dust (*S. oryzae*: $F = 1.928$, $df = 7$; *T. castaneum*: $F = 0.633$, $df = 6$), dust × gender (*S. oryzae*: $F = 0.000$, $df = 1$; *T. castaneum*: $F = 0.071$, $df = 1$), time × gender (*S. oryzae*: $F = 1.169$, $df = 7$; *T. castaneum*: $F = 3.627$, $df = 6$) and dust × time × gender (*S. oryzae*: $F = 0.811$, $df = 7$; *T. castaneum*: $F = 0.397$, $df = 6$) (Tables 5.5 and 5.6).

Upward length (UL) of *S. oryzae* and *T. castaneum* was affected in the same way and to the same degree as straightness by both of the SAS powders. In the control groups, UL was high for both *S. oryzae* and *T. castaneum*. This changed in the presence of SAS (Fig. 5.5). Since no difference in the mean upward length existed between exposure periods, powder types, and male and female populations in either species, statistical comparisons of upward length variances can be made. Comparison of these variances by Levene’s Test showed the variance in upward length for *S. oryzae* and *T. castaneum* to be significantly reduced with increasing exposure time (*S. oryzae*: $F = 74.518$, $df = 7$, P < 0.001; *T. castaneum*: F = 20.100, df = 6, P < 0.001). In turn, a weak direct effect of the types of SAS on the two insect species was observed (*S. oryzae*: F = 3.151, df = 1, P > 0.05; *T. castaneum*: F = 0.003, df = 1, P > 0.05). In addition, the average of UL value was similar for both genders of *S. oryzae* and *T. castaneum* (*S. oryzae*: F = 3.609, df = 1, P > 0.05; *T. castaneum*: F = 2.139, df = 1, P > 0.05).

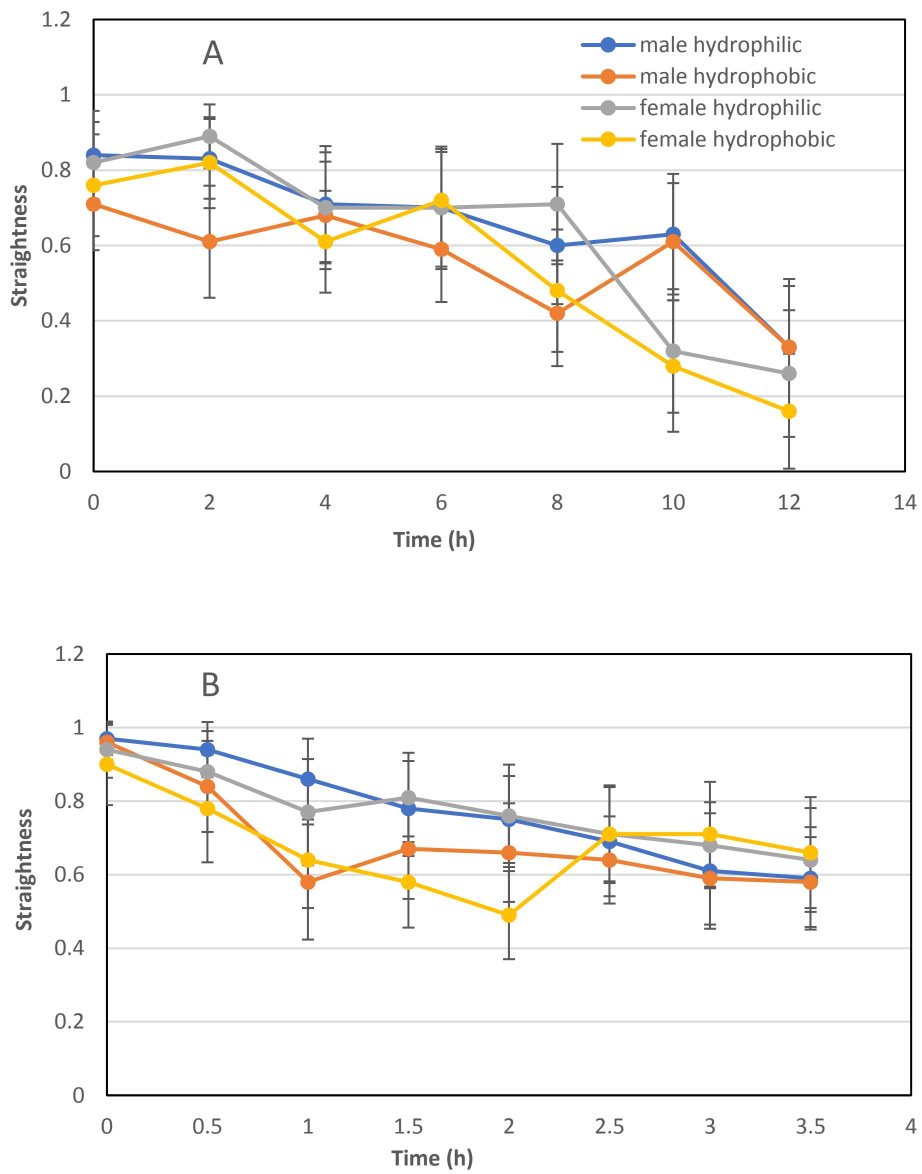


Fig. 5.4. Straightness of female and male *T. castaneum* and *S. oryzae* on the locomotion compensator after exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different durations at 28±1°C, 65±5% RH. A. *T. castaneum*; B. *S. oryzae*.

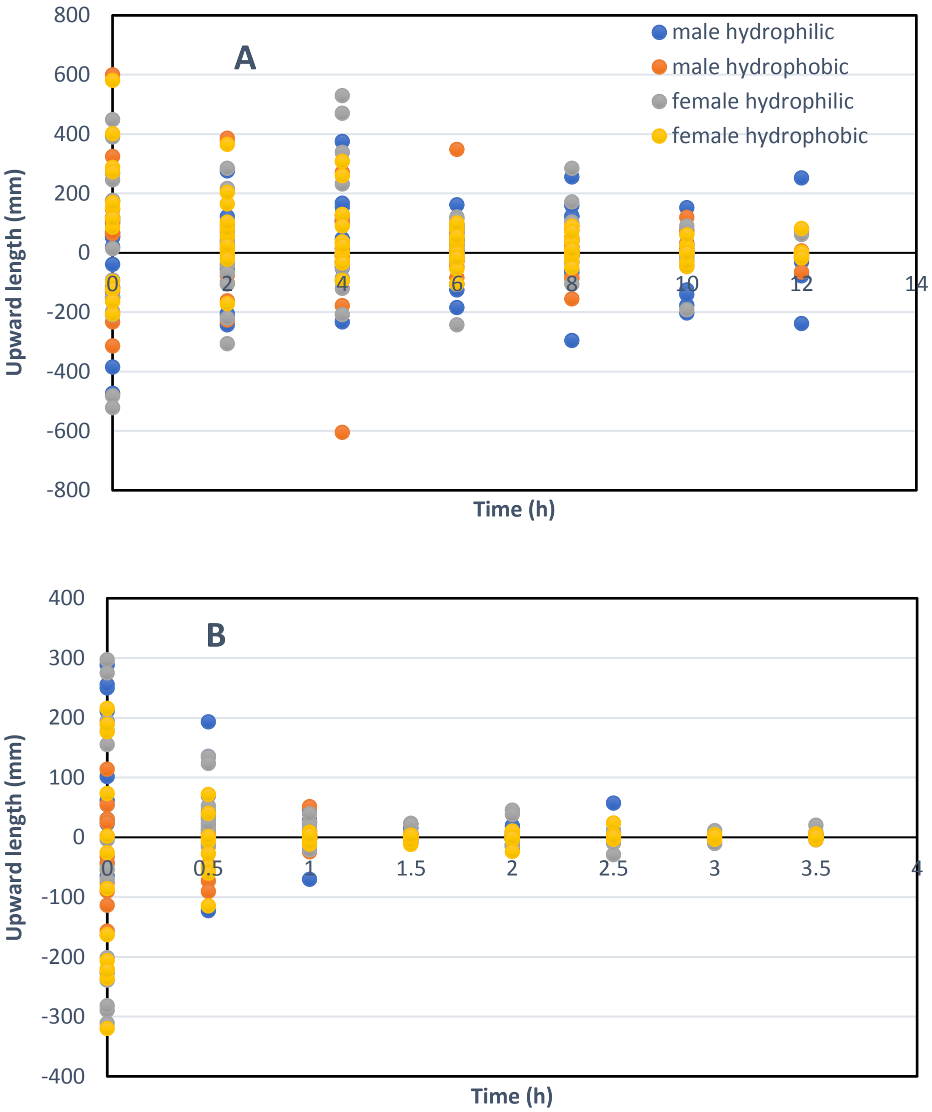


Fig. 5.5. Upward length of female and male *T. castaneum* and *S. oryzae* on the locomotion compensator after exposure to 2 g/m2 of hydrophilic and hydrophobic SAS respectively for different duration at 28±1°C, 65±5% RH. A. *T. castaneum*; B. *S. oryzae*.

5.4.3 Structure damage

The results of the stride length of female and male *S. oryzae* and *T. castaneum* affected by hydrophilic and hydrophobic SAS are summarized in Fig. 5.6. The data were subjected to a one way ANOVA to determine any significant differences in the deviations of gender, dusts and times (Tables 5.7 and 5.8). *S. oryzae* was more sensitive than *T. castaneum* to deposits of SAS, with the stride length dropped rapidly by more than 37.27% and 30.01% during the observation period for *S. oryzae* and *T. castaneum*, respectively (*S. oryzae*: $F = 57.483$, $df = 5$, $P < 0.001$; *T. castaneum*: $F = 57.480$, $df = 6$, $P < 0.001$). Appreciable differences between genders and between the two SAS powders were observed for *S. oryzae* (Fig. 5.6). The decreased rate of stride length for *S. oryzae* on hydrophilic SAS was larger than on hydrophobic SAS throughout the experimental period ($F = 23.245$, $df = 1$, $P < 0.001$), while it was not significant difference for *T. castaneum* exposed to the two SAS powders ($F = 6.044$, $df = 1$). Both SAS powders were noticeably more effective on the stride length of the male population than on the female population for *S. oryzae* ($F = 52.289$, $df = 1$, $P < 0.001$) but not for *T. castaneum* ($F = 4.408$, $df = 1$). No significant differences for two insect species were found in all the interactions, exposure time × dust (*S. oryzae*: $F = 1.885$, $df = 5$; *T. castaneum*: $F = 1.535$, $df = 6$), dust × gender (*S. oryzae*: $F = 9.992$, $df = 1$; *T. castaneum*: $F = 0.985$, $df = 1$), time × gender (*S. oryzae*: $F = 2.000$, $df = 5$; *T. castaneum*: $F = 1.985$, $df = 6$) and dust × time × gender (*S. oryzae*: $F = 0.290$, $df = 5$; *T. castaneum*: $F = 0.891$, $df = 6$) (Tables 5.7 and 5.8).

Figure 5.7 is an enlarged view of leg joints of *S. oryzae* and *T. castaneum* invaded by SAS particles. The SAS particles are minute in relation to insect legs. The particles possess extremely sharp edges and thus are abrasive by nature. When insects move through the particles, they penetrate the protective body plates and tend to pierce the joints. Sharp SAS particles pierce and penetrate the insect intersegment frictional devices and wear off the setae during joint movement.

Each particle can absorb liquid up to four times its own weight. Once the friction systems have been breached, the particles begin to absorb the vital body fluids. The combined effects of both haemorrhage and particle adsorption quickly led to lethal dehydration (Figs. 5.7A, 5.7B, 5.7D, and 5.7E). The invading particles can also migrate further into the body cavities, and interfere with breathing, digestion, reproduction and/ or body movements.

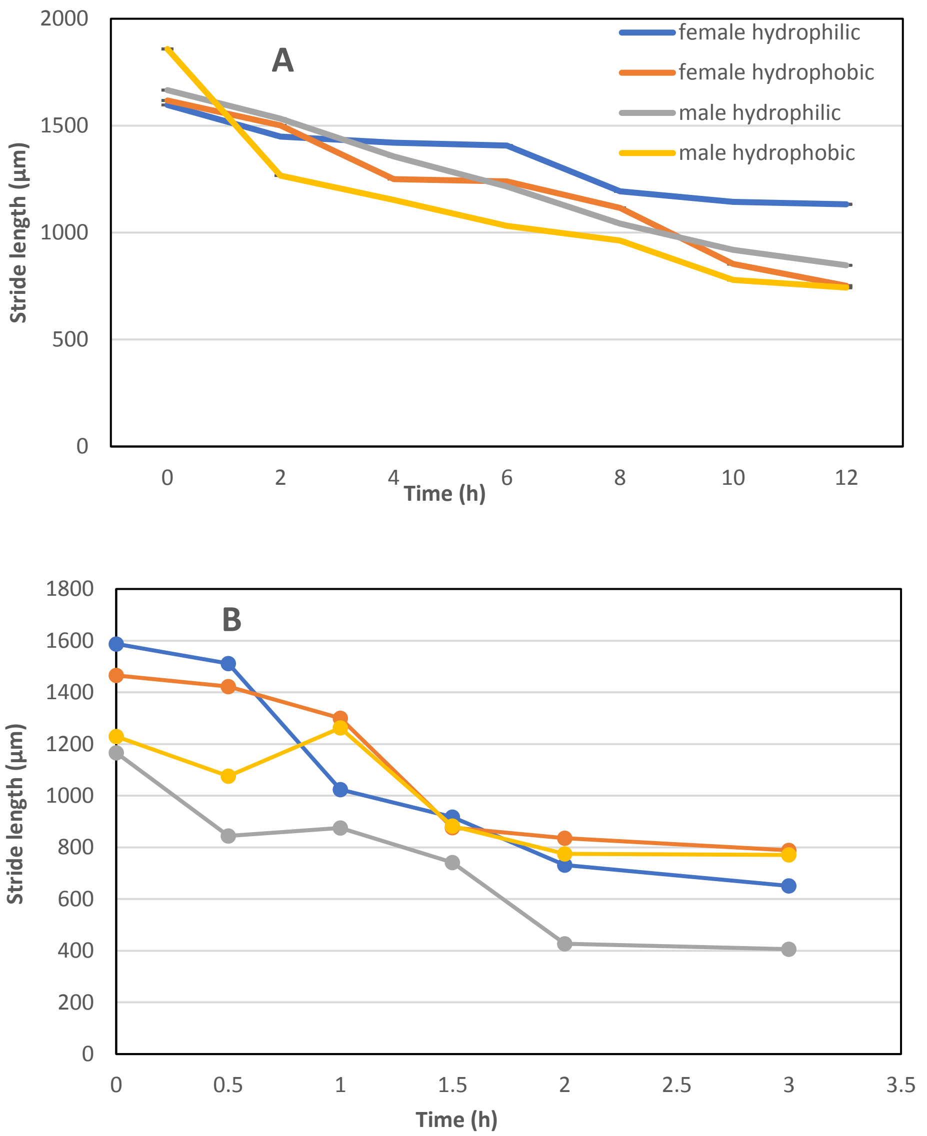


Fig. 5.6. Stride length of female and male *T. castaneum* and *S. oryzae* exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different durations at 28±1°C, 65±5% RH. A. *T. castaneum*; B. *S. oryzae*.

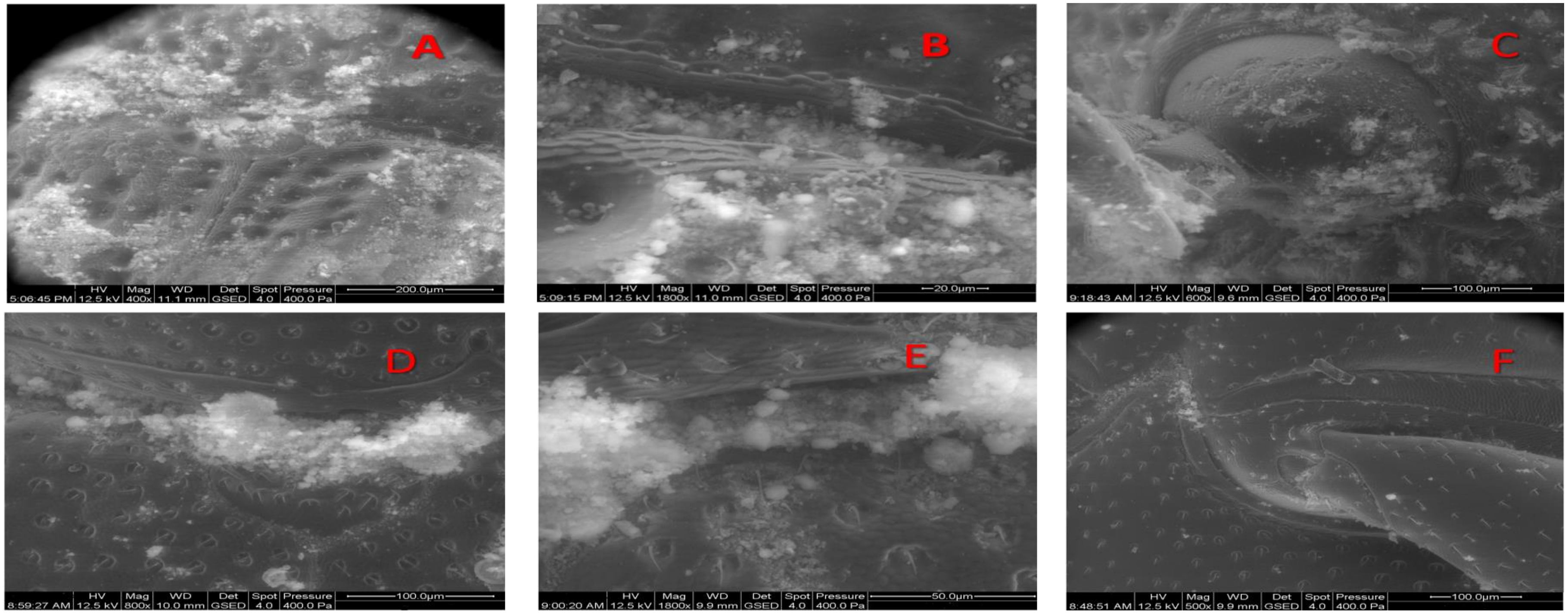


Fig. 5.7. A and B: ESEM images of the friction system of *T. castaneum* and *S. oryzae* invaded by SAS. C: Thoraco-abdominal intersegmental frictional devices of *S. oryzae*, dorsal aspect. D: Cuticle of coxa of media leg and joint with body of *S. oryzae*. E: Crano-thoracic intersegmental frictional devices of *T. castaneum*, dorsal aspect. F: Thoraco-abdominal intersegmental frictional devices of *T. castaneum*, ventral aspect. Cuticle of coxa of proleg and joint with body of *T. castaneum.*

Table 5.7. Results of one-way ANOVA for the significance of variation between dust, gender, and exposure time on the stride length of *S. oryzae* exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different durations at 28±1°C, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	2383285.050	1	2383285.050	23.245	0.000
Gender	5361090.790	1	5361090.790	52.289	0.001
Time	29467783.600	5	5893556.719	57.483	0.000
Dust × Time	966194.764	5	193238.953	1.885	0.097
Dust × Gender	1024461.086	1	1024461.086	9.992	0.002
Time × Gender	1025317.056	5	205063.411	2.000	0.079
Dust × Time × Gender	148539.483	5	29707.897	0.290	0.918
Error	29015252.830	283	102527.395		
Total	355181674.300	307			

Table 5.8. Results of one-way ANOVA for the significance of variation between dust, gender, and exposure time on the stride length of *T. castaneum* exposure to 2 g/m^2 of hydrophilic and hydrophobic SAS respectively for different durations at 28±1°C, 65±5% RH.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Dust	1061971.136	1	1061971.136	6.044	0.014
Gender	774537.024	1	774537.024	4.408	0.037
Time	60600275.330	6	10100045.890	57.480	0.000
Dust × Time	1618530.057	6	269755.010	1.535	0.166
Dust × Gender	172998.894	1	172998.894	0.985	0.322
Time × Gender	2092763.162	6	348793.860	1.985	0.067
Dust × Time × Gender	939801.177	6	156633.530	0.891	0.501
Error	58688260.760	334	175713.356		
Total	590964203.800	362			

5.5 Discussion

The most obvious advantage of SAS powders is the physical nature of its insecticidal mechanism, which has been thoroughly examined in our investigation. The results suggest that the insect intersegmental frictional devices were damaged rapidly by the two SAS powders in a few hours, resulting in reduced locomotion and coordination, and additional behavioral changes. The locomotion capacity decreased after exposure to SAS for *S. oryzae* (0.5 h) and *T. castaneum* (2 h), respectively. The change in upward length and straightness reflected the behavioral changes. The male populations in the two species were more susceptible than the females to both SAS powders. Hydrophilic SAS was more effective against *S. oryzae* than hydrophobic SAS. Interestingly, it was the opposite for *T. castaneum*. This was consistent with our previous results. Based on these results, we propose a new pesticide delivery method via insect intersegment frictional devices. It is highly unlikely for insects to develop immunity to SAS powder. In principle, these SAS do not interrupt basic physiological activities by blocking a specific biochemical event, instead they cause non-specific adverse effects to multiple insect activities in a short period of time.

5.5.1 Disruptionure of insect locomotion and coordination

Insects were considered knocked down if they were unable to co-ordinate their locomotory movements and regain a normal stance (Prickett and Ratcliffe, 1977). Large quantities of SAS accumulate on the legs, thorax and elytra (Gowers and Le Patourel, 1984), especially covering different intersegmental frictional devices (Ebeling and Wagner, 1961). This research provides the first detailed examination of the structural destruction hypothesis by describing the kinematics of the leg in insects. Our kinematics data have described insect movability levels in relation to the degree of damage, thus allowing us to generate a preliminary hypothesis regarding the physical mechanisms of friction devices damage. Locomotion capacity is a prerequisite for many insect activities such as feeding and mating. Insect management can be achieved by crippling the target insects. Without an initial incursion into the grain bulk, an infestation is unlikely to occur.

5.5.2 Behavioral charges

The arthrodial membranes exist in the movable joints of legs, antennae, organs of copulation, ovipositor, mouthparts and between segments of the body. During movement, the cuticular membranes exhibit great elasticity. The attachment of synthetic amorphous silica (SAS) particles induces a tactile deterrence that can lead to disruption of the insect’s behaviour to such a degree that it is unable to feed and eventually starves (Glenn et al., 2001).

These results confirmed and extended previous studies on the orientation of insects and range of movement (Lang, 2009) upon inert dust exposure. However, nearly all of these studies used different methods ranging from simple racetrack tubes to artificial dispersal. The link between insect avoidance behaviour and resistance to SAS cannot be well established. The diverse nature of insect behaviour makes it difficult for cross species comparisons and repetition of certain experiments. The great advantage of a locomotion compensator over other approaches is that it allows exploration of the biological effect with direct parameters (straightness and upward length in relation to olfactory sensation). We can obtain more precise information about the relationship between insect signal transduction pathways and intermediary membrane. The disruption in the signal transduction pathways affects insect feeding, development, locomotion and reproduction.

5.5.3 Structural damage

In arthropods, their body is constituted by different segments with intermediary membranous connections. The segments are, more or less, hard and rigid (Hepburn and Chandler, 1976), and often serve as the fixation site for other structure. The intermediary membranous area is flexible and/or elastic (Hackman and Goldberg, 1987), and responsible for movability. The intersegmental membrane usually consists of very thin plates, with minor supportive function to increase the overall stability. In these areas cuticle is commonly less tanned and less rigid (Vincent and Wegst, 2004). This feature is reflected by the much lower value of Young's modulus in intersegmental membrane comparing to deep tanned, dry cuticle on other parts of insect body (Vincent and Wegst, 2004). Synthetic amorphous silica (SAS) cause severe and extensive damage in the joints. The segments are mostly unaffected. Some joints are completely disabled. This further confirmed our previous results (Li, 2006).

After the exposure of insects to the SAS, the exoskeleton is covered with the SAS particles. Differences in effect of SAS cannot be explained solely by the amount of powder deposit. It is necessary to consider the replenishment of dislodged powder (Gowers and Le Patourel, 1984). Results show that on intermediary membranous surfaces deposition of SAS is more rapid and these SAS aggregations are readily dislodged as the insects climb and rub over one another, resulting in fresh powder accumulating on the joints. Exchange of powder in this manner would ensure that there was always unsaturated powder available for adsorption, while any vigorous attempt to remove SAS may result in a more rapid loss of water. High toughness with a relatively low stiffness gives the insect leg an exceptional ability to tolerate defects such as cracks and damage. However, toughness of cuticle in insect legs decreases with desiccation (Dirks and Taylor, 2012).

We observed a relationship between high mortality and severe structural damage. The leg of arthropods is a cuticular tube. Many insect joints contain surfaces that are covered by cuticular protuberances or depressions. This thin shell is the primary barrier isolating body fluids from the external environment. A 10% body fluid loss is fatal to insects (Knight and Bessette, 1997). The joint types include simple polyaxial or monoaxial joints and complex joints with defined rotation axes. All the joints are open to the adjacent segments and allows free fluid communication. Thus, a structural breach can lead to severe systemic haemorrhage, not just limited to the fluid within the affected joint. This structure weakness is not only present in leg joints but also in antennal joints, and the craniothoracic joint (Gorb, 2001). For this reason, intersegmental membranes are the primary target areas for SAS and other pesticides.

5.5.4 Difference in species and gender

Our results showed that the movability and behaviour of two insect species affected by SAS were significantly different between species and gender. *T. castaneum* is the most tolerant stored product insect species to SAS, however, the male is more susceptible than the female. This can be explained by the effect of an electrical field generated between SAS and insect. The amount of electrostatic charge *T. castaneum* carries is significantly lower than that of *S. oryzae*, resulting in a lower attachment of SAS and a longer time to achieve the same biological effect (Chapter 3).

Both species and gender have different morphological properties of the epicuticle (Kuitunen and Gorb, 2011). Scanning electron microscope (SEM) analysis showed that *T. castaneum* has smoother, smaller and tougher intersegmental frictional devices than *S. oryzae*. There is also a circle of hair in the crano-thoracic and thoraco-abdominal intersegmental frictional devices of *T. castaneum* (Li, 2006), which is likely to play an important role in preventing foreign material, such as SAS, entering these areas. The different sensitivity between male and female populations can be contribute to the differences in structure. Since the sexual signaling is based colour, the male colouration is often based on interference in the structure and wax coverage (Kuitunen and Gorb, 2011). Wax coverage plays an important role in the colour tuning of the male cuticle. In addition, the roughness of the epicuticle surface can cause the reflected light to scatter (Hooper et al., 2006; Parker et al., 1998). All these factors can cause the male to be more sensitive to SAS than the female.

According to Whittier et al., (1992), changing the ratio of gender in a medfly population will result in unsuccessful mating. The SAS damage to the insect friction system may, at least in part, effect interactions between males and females during courtship, resulting in mating failure (Barton et al., 2006). This would be a very effective strategy for controlling the insect population. *S. oryzae* is a primary feeding insect and lives mostly inside the grain kernel. For attached pesticides, the main factor in terms of their effectiveness is the speed at which the insect moves when it emerges from the grain kernel. The effect on movability of *S. oryzae* appears within half an hour when exposed to SAS. These effects suggest SAS is a very promising method in stored grain insect control.

5.5.5 Variation between hydrophobic and hydrophilic SAS

The terms hydrophobic and hydrophilic describe the apparent repulsion and attraction between water and surfaces. When one material repels or attracts water from its surface, it shows hydrophobicity or hydrophilicity, respectively. Given the large specific surface area, hydrophobic and hydrophilic SAS are used as absorbents and drug carriers in industry. Hydrophobic SAS were found to be effective and efficient absorbents of oils and organic liquids (Dowell et al., 1999). Hydrophilic aerogels can especially be used as carrier materials for oral delivery of drugs whose immediate release is desirable (Dowell et al., 1999).

Insect cuticular lipids are composed mainly of fatty acids, alcohols, esters, glycerides, sterols, aldehydes, ketones and hydrocarbons (Lockey, 1988). Long-chain hydrocarbons are one of the main components of cuticular lipids in insects, but their concentration can vary widely, from 3 to nearly 95% of the total lipid (Dowell et al., 1999). *T. castaneum* was more susceptible to hydrophobic SAS while *S. oryzae* was more susceptible to hydrophilic SAS. This difference can be explained by the lack of polar –OH groups on the surface of hydrophobic aerogels, which would interact with a relatively low hydrocarbon content of the total surface lipid in *S. oryzae* (Baker et al., 1984).

Chapter 6. Evaluation of synesthetic amorphous silica for structural treatment of empty grain storage

6.1 Abstract

The distribution of application of synthetic amorphous silica, dispensed from an SAS applicator or blower was characterize inside an empty farm scale warehouse using measurements of concentration and assessments of effect on adults of five stored grain insect species in bioassay arenas. The infestations were monitored by a new integrated trap based on light, hormone, food luring, and insect behaviour in the grain storage system. The conventional screening method was used as the control. A new mobile duster was used to disperse the powder into aerosols. Petri dishes were prepared with *Rhyzopertha dominica* (Fabricius), *Sitophilus zeamais* (Motschulsky), *Oryzaephilus surinamensis* (Linne), *Tribolium castaneum* (Herbst), and *Cryptolestes ferrugineus* (Stephens) adults, using 20 specimens each. Twenty-four hours after insecticide exposure, the dishes were brought to the laboratory, and placed in a growth chamber and held for a 3 days moribund (knockdown) assessment. One gram of flour or 3 g of wheat kernels were added as feed. The assessment trend for both integrated traps and artificial screening methods was similar. The capture ability of the integrated trap was highly effective. At a *C. ferrugineus* population density of 45 per kg (grain), the manual screening detection method using 5 integrated traps captured 1722 g of mainly *C. ferrugineus* /warehouse in seven days. One gram of *C. ferrugineus* contains approximately 6791~7142 individuals. Food grade synthetic amorphous silica powder was used as a structural treatment in an empty warehouse. The duster dispersed the powder as an aerogel uniformly in several locations of the warehouse. The mortality rate of the adults of the five major species of stored grain pest reached 100% on the third day. Combing SAS structural treatments with new integrated trapping is a safe,

effective and time-saving method for insect control, and is easy to automate to reduce labour costs.

6.2 Introduction

Economic losses caused by pests include direct damage caused by their activities and indirect damage as a consequence of market rejection due to infestation and the cost of chemical treatment. Since the 1950s, synthetic pesticides have been widely utilized in large grain bulks for their high efficacy, relatively low cost and ease of application. Common fumigants include phosphine, methyl bromide, chloropicrin and dichlorvos. Methyl bromide and phosphine (PH_3) are the most widely used. Methyl bromide was phased out of general use after 2005 due to environmental concerns as it depletes the atmospheric ozone. Phosphine is the only registered fumigant which is likely to be continuously used in a large scale given its favorable characteristics such as low sorption and rapid desorption in commodity fumigation. Yet, insect resistance to phosphine has been frequently reported across the world (Daglish and Collins, 1998) and is threatening the future use of phosphine (Benhalima et al., 2004; Collins et al., 2005; Herron, 1990; Lorini and Galley, 1999). These events have placed an increasing demand on the development of low-cost, low chemical-input, integrated pest management storage strategies which must be able to guarantee the same degree of effectiveness as chemical strategies.

Detection of insect populations is essential to limit damage to stored grain and is important for guiding the conduct of pest management and evaluating the efficacy of control. To detect insect infestation, grain is usually sampled using mechanical or manual methods, with the sampled grain then sieved to estimate insect numbers and the level of infestation. This is a commonly used laboratory method (Lippert and Hagstrum,

1987) and also conventionally used by elevator operators, grain buyers and farmers under most grain-storage conditions (Jian et al., 2012; Perez-Mendoza et al., 2004). An alternative method of detecting insects is using visual or pheromone lure traps. This method has been used for several decades to monitor the presence of insects and determine patterns of pest distribution (Neethirajan et al., 2007; Wu et al., 2009; Yao et al., 2005). Stored grain insect behaviour is influenced by food odours and aggregation pheromones (Phillips et al., 1993; Seifelnasr et al., 1982). However, these traps are too specific and catch only one or a few pest species, thus they are unable to provide an accurate overall estimation. This limitation has prevented wide usage of the trapping method. Different species are attracted only to certain wavelengths. Grain storage insect pests share many behavioural treats, such as, strongly phototaxic, a preference for flying and inhabiting the top layer of a grain bulk. An integrated trap is developed based on these facts.

Sanitization of the structures before loading grain into a warehouse is an important procedure of integrated pest management (IPM) programs. Historically, pest management professionals utilized dichlorvos as a space or a structural treatment, but resistance to this insecticide has been reported (Li et al., 2016). The grain industry demands alternative control strategies. Due to their high safety, high effectiveness and long-term protection, diatomaceous earths or silica aerogels have been used for eliminating stored grain insects in field trials since the 1960s (Chen et al., 2016; Dong et al., 2016; La Hue, 1965a; Redlinger and Womack, 1966; Wang et al., 2016; Zhou et al., 2017). However, several adverse factors have limited their use such as high dosage, visible residue and slow “speed of kill”. Also, the technologies that utilize synthetic amorphous silica powders for storage protection have not been fully developed. The poor distribution of the powder throughout the grain bulk results in poor control by allowing insects to survive in areas of low powder concentration. Food grade synthetic amorphous silica powder is a food additive and a new physical protective agent that contains no crystalline silica (Wu, 2011). It was developed by the Academy of State Administration of Grain, China. Compared to traditional diatomaceous earth, it has a good insecticidal effect, a fast acting speed, a low dosage rate, and a low cost. Food grade synthetic amorphous silica powder can kill *R. dominica*, *T. castaneum, S. zeamais, O. surinamensis* and *C. ferrugineus* (Li, 2006).

In this study, the efficacy of food grade synthetic amorphous silica powder was directly assessed against five stored grain insect species using the same controlled environment (tightly sealed pilot scale warehouse. The insects were exposed to the powder in confined petri dishes that could be removed after treatment. Interpretation of insect captures obtained through a new light trap was used to evaluate the efficacy of food grade synthetic amorphous silica powder in a structural treatment.

6.3 Methods and materials

6.3.1 Structure of integrate trap

The trap is mainly comprised of the following components (Fig. 6.1): lampshade, pest-attracting modulator tube, light guide ball, fan, pest collecting bag, and chassis. The integrated light, colour, food and pheromone are all in one device.

Three 15W fluorescent lamps were selected as the attracting light source. A yellow plastic ball was positioned in the middle of the annular modulator tube for the purpose of increasing illumination, providing a yellow colour and dispersing the light farther. A 3W negative pressure fan was installed inside an insect sample collecting bag. A food lure, consisting of roasted bean flour, wheat flour, roasted peanut flour and 2 drops of sex pheromone, was also used in this study and placed in a dish under the light balls. A sex pheromone lure was also arranged inside the light guide ball. The scent was released from the bottom of the ball and spread by the airflow from the fan.

Four wavelengths (365nm (UV), 450nm (Vis), 550nm (Vis) and 720nm (NIR)) of LEDs were chosen to evaluate the wavelength effect.

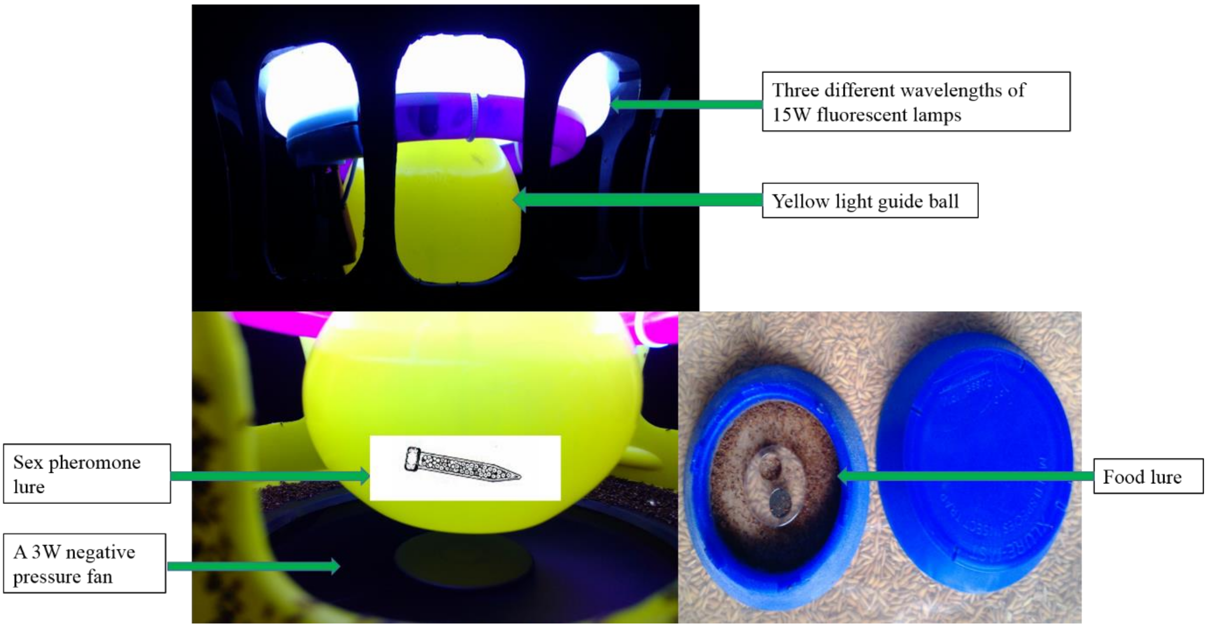


Fig. 6.1. Photograph of the integrate trap.

6.3.2 Synthetic amorphous silica powder

Food grade synthetic amorphous silica powder is a hydrophilic precipitated silica. It is compliant to the standards of food additive (E551) and is developed as a nonchemical pesticide by the Academy of State Administration of Grain, China. The D (v, 0.1), D (v, 0.5), and D (v, 0.9) (The respective diameters at 10, 50, and 90% cumulative volume.) aggregated particle sizes were 1.78±0.04 µm, 4.31±0.03 µm and 9.42±1.75 µm, and aerosolized by the duster.

6.3.3 Insect

The insect species tested are shown in Table 6.1. They were cultured at 30±1℃, 75±5% RH in the Academy of State Administration of Grain, Beijing, China. The techniques of insect culturing and handling generally follow those described by Winks (1982) for primary and secondary feeders. *R. dominica, S. oryzae* and *S. zeamais* were reared on wheat. *O. surinamensis* was reared on oats. *C. ferrugineus, T. castaneum* and *T. confusum* were reared on a medium containing 10 parts wheat-meal flour milled from Chinese hard wheat, 10 parts oats and 1part yeast. Prior to use for insect rearing, the wheat was wash and disinfested in the oven at 80℃ for 4 hours, and then conditioned to 13.5% moisture content. For this experiment, adults 20 days old were used.

6.3.4 Mobile duster

The mobile duster used was manufactured by Tenghui Machinery Co., Ltd. and is shown in Fig. 6.2. The main technical parameters were, a 220 V (50 Hz) power supply, a motor with a speed of 5000 r/min, an 8 kg powder capacity, a 12 m horizontal spraying distance in static wind, and a 0.6 kg/min spraying volume. The power of this duster was 1.5 kW. The total weight was 39 kg. The principle of operation was the same as with a venturi gas jet pump. Airflow was supplied by a vortex air pump. When the working fluid flew through the venturi, the velocity increased and pressure reduced to form a vacuum in the throat of pipe. In the absorption process, the powder was evenly mixed with the airflow. Then the mixture of aerogel was ejected into the warehouse from the nozzle of an air hose.

6.3.5 Warehouses and grain storage

6.3.5.1 Conditions for testing integrate traps

The grain surface trapping tests were conducted in warehouses numbered, No.22, 46, 47, and 51 in late September, 2014. Warehouse No.9 was empty and was 30 m in length, 18 m in width, and 7 meters in height for evaluating new trapping capacity in the empty warehouse. Details of the stored grain in each warehouse are shown in table 6.2.

Table 6.1. Contents of each insect sample.

Insect species	Geographical strains
Rhizopertha dominica (Fabricius)	Wuhan (WH-RD)
Sitophilus zeamais （Motschulsky）	Guangzhou (GZ-SZ)
Oryzaephilus surinamensis (Linné)	Beihai (BH-OS)
Cryptolestes ferrugineus (Stephens)	Beihai (BH-CF)
Tribolium castaneum (Herbst)	Qihe (QH-TC)

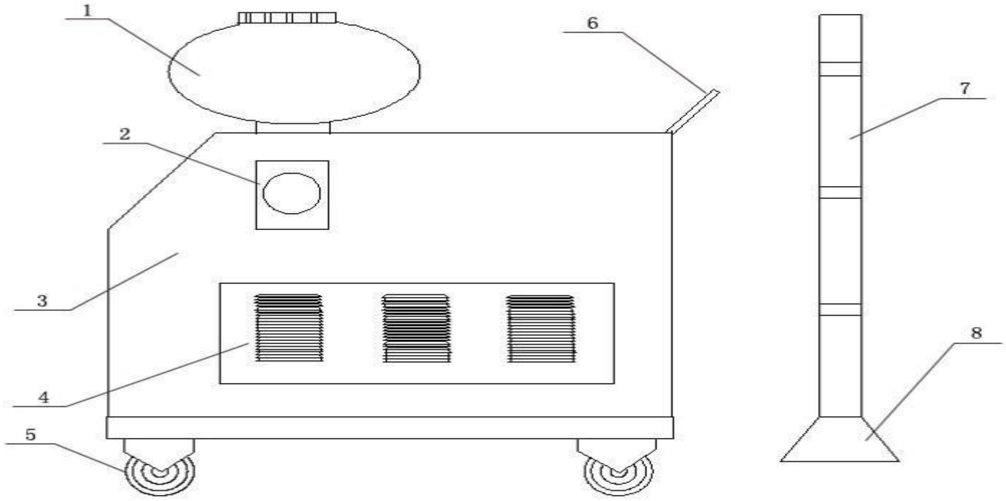


Fig. 6.2. Blower for the application of synthetic amorphous silica powder (1. Powder container, 2. ABS hard powder pipe connector, 3. Host, 4. Fan, 5. Moving wheels, 6. Handles, 7. ABS powder blowing host, 8. Powder blowing nozzle).

6.3.5.2 Conditions for testing structural treatment

The total area of the empty warehouse used for testing structural treatment was 4885 m^2, 60 m in length, 27 m in width, 8 m vertical distance from the storehouse floor to platform, and 2.5 m vertical height from platform beam to ridge.

6.3.6 Observation spots and sample collection spots

6.3.6.1 Set-up of sample collection spots for manual method

The manual screening method was used as the control and carried out at twenty two spots (Fig. 6.3), where one kilogram of grain was sampled. Each spot covered one square meter.

Table 6.2. Basic indices of the grain in storage used in the trapping trials.

Warehouse No.	Variety	Quantity (ton)	Moisture content (%)	Brown rice yield of paddy or test weight (%)
22	Early indica rice	1474	12.2	77.9
51	Early indica rice	1617	12.3	78.0
46	Australian wheat	1678	10.4	782
47	Australian wheat	1927	9.6	812
9	empty			

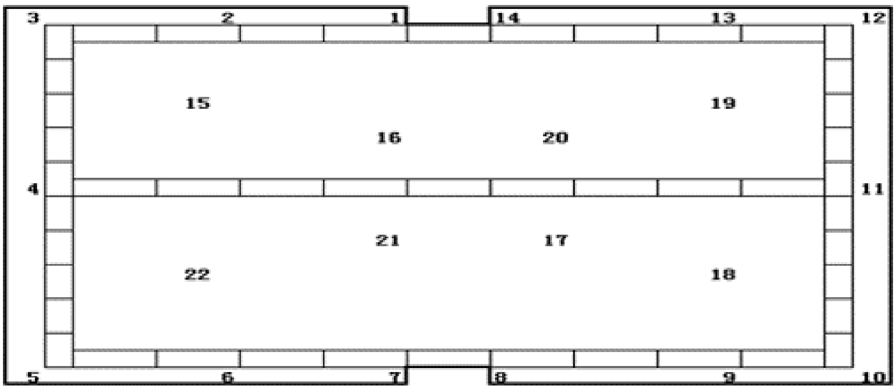


Fig. 6.3. The diagram of manual screening method Spots in a test warehouse.

6.3.6.2 Evaluation of effect of SAS as structural treatment in empty grain storehouse

Before used as a container, a clean petri dish (70 mm inner diameter base, and 75 mm diameter cover) was disinfected in a 100℃ oven for 2 hours. A Whatman No.1 filter paper of the same diameter was pasted onto the base of the petri dish to simulate the rough surface of the warehouse. Then a film of fluon was brushed onto the inner wall to prevent insects from escape. Twenty adult insects (<20 days old) of each species were added to each individual petri dish with small quantities of flour or wheat kernels to simulate spilled grain. During dusting, a complete set of petri dishes (five dishes for each insect species and three empty dishes for powder accumulation to evaluate the concentration) was located in each position of the warehouse illustrated in Fig. 6.4. Therefore, there were a total of 60 uncovered petri dishes in each warehouse.

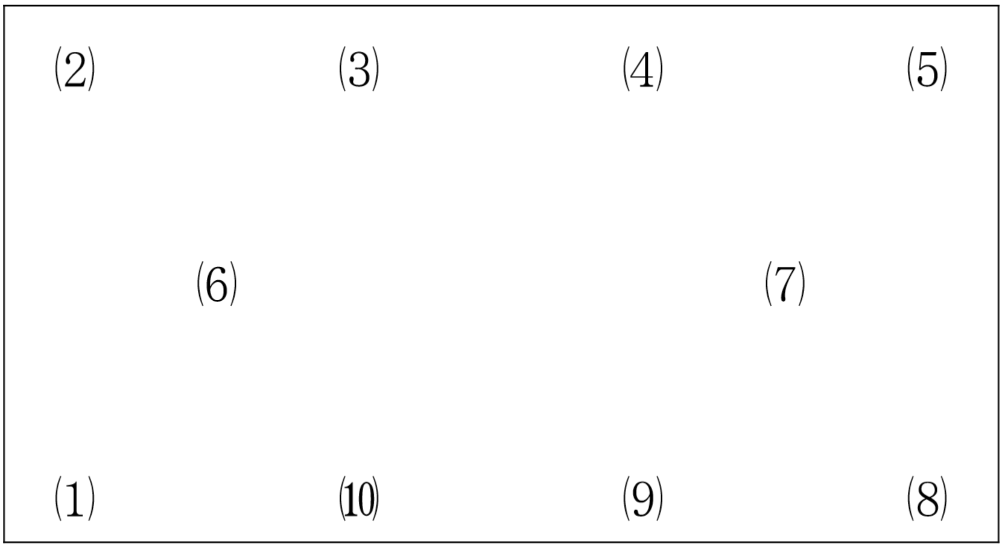


Fig. 6.4. Layout of test petri dishes in a test warehouse for structural treatment.

6.3.7 Detecting methods and dusting

6.3.7.1 Trapping procedures

For interior of empty warehouse trial and exterior area of the warehouse, light traps were hung approximately 3 m above the ground. For bulk grain trials, light traps were hung at approximately 1 to 1.5 m above the grain surface. There was a 1 m^2 anti-fire blanket under the light traps for safety reasons and for the collection of the trapped insects.

The insect population density was very high in No. 22 according to our pre-testing survey; thus 5 light traps were placed here. One was set up in the center of the warehouse and four were located four meters away from the four corners (Fig. 6.5). For the other warehouses, there was only one trap suspended in the middle of the building. Insects predominantly inhabit the top 30 cm layer of the grain bulk. We stirred the surface of the grain with a rake every two to three days to promote insect activity.

The number of light-up hours for the traps were designed for two different purposes. When the population density was low, the purpose was to estimate the density. Thus, the light-up hours were from 17:00 – 24:00; the period when insects were most active. When the population density in grain bulk was high, the purpose was to control the insects, so the lights were switched on all the time. Leakage of light through doors and windows was eliminated to avoid affecting the effectiveness of light trapping.

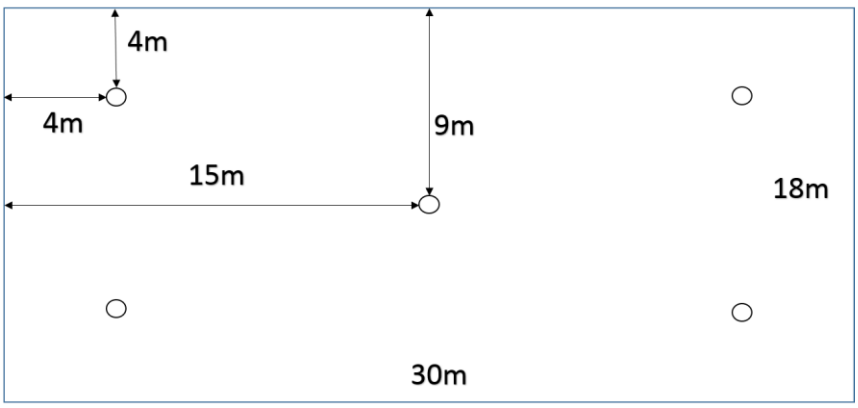


Fig. 6.5. Layout diagram of the five light traps in warehouse 22.

6.3.7.2 Method of dispersal from the duster

Air outlet and hard tube were connected to a hose. The joints were fixed by clamps. Several hard tubes were designed according to the height of the grain loading line. The quantity of product in the duster reservoir was weighed before and after application to ensure accurate dose measurement. All the windows and doors were sealed before dusting to avoid powder leakage. The ratio of air velocity and powder was adjusted according to the size of the warehouse to achieve uniform powder distribution. Three operators were required, a nozzle holder, a driver and a door keeper. Retrograde application was used to reduce aerosol-operator contact time. The amount of powder used in this study was 15 kg. The calculation was as followed:

Amount of powder = dosage × total inner surface area of warehouse

The dosage was 3 g/m^2. Total area was 4885 m^2, including wall and ceiling surfaces. The total amount of powder was 14.7 kg.

6.3.8 Capturing and mortality assessment

The pest-collecting bags were collected at 3:00 pm every day and replaced by a new one. Due to the enormous quantity of attracted insects, it was impossible to count in individual with routine methods. Therefore, a microbalance was used to estimate the number of insect per gram.

Following powder application, the warehouses remained sealed for 24 hours to allow the powder particles to settle. Following the settling period, petri dishes covered with lids were brought to the laboratory where they were placed in an environmental chamber maintained at 28℃ and 60% RH. Insects in dishes that did not receive any flour prior to powder application received 1.0 g of clean flour or wheat kernels as feed.

Individual adults in each dish were scored as live or moribund (knocked down or not capable of walking) at 3 days post application. For powder distribution, the amount of powder in each position was calculated by subtracting the weight of the empty dish prior to the powder application with the use of a microbalance.

6.3.9 Statistical analysis

Statistical comparison between the manual screening method and integrated traps was conducted using a generalized linear model approach to evaluate capture capacity. In addition, effects of temperature on insect population were further characterized using linear trend analyses.

After structural treatment by food grade synthetic amorphous silica powder, adult mortality of *T. castaneum* and *S. oryzae* at different exposure times were calculated as a percentage. Adult mortality in control groups across all exposure times after treatment was 0%. Therefore, mortality data were not corrected for mortality in the control treatment using Abbott’s formula (Abbott, 1925).

6.4 Results

6.4.1 Improvement of collection means

Instead of electric shock and adhesion, a 3W negative pressure fan was used to form a negative pressure zone under the light trap for insect collection. Besides the collecting function, it also prevented the captured insects from escaping. The airflow from the fan also contributed to pheromone dispersion.

The number of trapped *C. ferrugineus* caught differed significantly among the four wavelengths. The most effective wavelength for *C. ferrugineus* was 365 nm, while *C.*

ferrugineus were equally attracted by 550 nm and 720 nm. The wavelength of 450 nm showed no attracting effect.

Figure 6.6 shows that the capture capacity of the new integrated trap was positively correlated with that of manual screening methods. The integrated trapping method could replace the manual screening method. It was more reliable and less labour intensive.

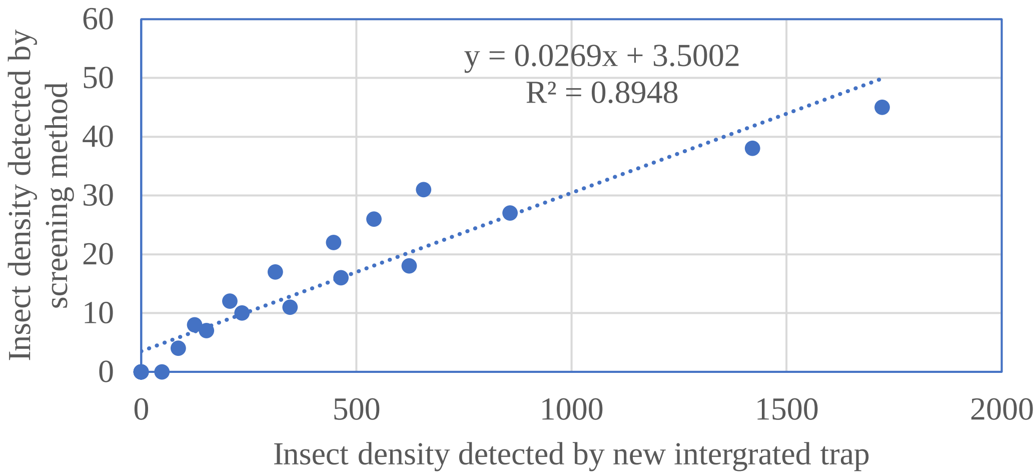


Fig. 6.6. Relationship between the new integrated trap and manual screening method.

6.4.2 Detection in the insect population density

The new integrated trap was highly effective for low insect population densities (Table 6.3). In warehouse No.47, the manual screening method detected no insects, while 48 *C. ferrugineus* were captured by the new trap from 5 pm to 0 am on 17th September. After seven days, this number dropped to zero.

For the empty warehouse (No. 9), the light trap was switched on for 24 hours. The manual detection method detected nothing, while 252 *C. ferrugineus* and 6 *S. zeamais* were captured by the new trap. Similarly, the number dropped to zero five days later.

For exterior area of the warehouse, during the 12 h of trapping from 7 pm on 22nd September to 7am on 23rd September, an integrated trap captured six insect species, including 124 *C. ferrugineus*, 5 *R. dominica*, 2 *S. zeamais*, 1 *S. paniceum*, 1 *T. castaneum*, 1 *L. oryzae*, and numerous flies and mosquitoes. Similar insect species were found inside and outside the warehouse (Table 6.4). This new trap is very useful to prevent insects entering the storage facility, especially when the windows were open for cooling at night.

The insect species captured both inside and outside warehouse were *C. ferrugineus*, *R. dominica*, *S. zeamais*, *T. castaneum*, *S. cerealella* and *Liposcelididae* (Table 6.4). The predominant insect population was *C. ferrugineus*.

There were 8 kg of insects captured on the four grain surfaces. Each gram of *C. ferrugineus* represents 6791 to 7142 individuals, so 8 kg of *C. ferrugineus* equaled 57 million individuals.

These numbers correlated with the temperature inside the warehouse. Largest numbers of insects of different species was captured above 26℃. When the temperature was between 20℃ and 26℃, the number dropped by half. Few insects were captured under the 20℃.

Table 6.3. Estimates of correlations of the quantity of pests among catching periods by screening method and new trapping method under four warehouse conditions.

Check time	Warehouse No.	Temperature in space of warehouse (℃)	Temperature in the upper layer of grain bulk (℃)	Insect density (pests/kg)	Number of lights	Light up time (h)	Insect trapped by new trap (g)
17th -23rd September	22	28.7	24.9	45	5	7×24	1722
	51	28.3	24.7	31	1	7×24	656
	46	28.8	24.3	22	1	7×24	447
	47	29.6	31.5	None	1	7×7	48
24th -30th September	22	27.2	24.3	38	5	7×24	1421
	51	26.8	23.9	26	1	7×24	541
	46	26.3	23.5	16	1	7×24	464
	47	27.8	31.2	None	1	7×7	0
1st -7th October	22	24.3	23.0	27	5	7×24	857
	51	23.7	21.8	17	1	7×24	312
	46	25.3	22.4	12	1	7×24	206
	47	26.8	29.3	None	1	7×7	0
8th -14th October	22	22.6	23.0	18	5	7×24	623
	51	22.1	21.4	10	1	7×24	234
	46	22.2	22.0	7	1	7×24	152
	47	26.8	28.4	None	1	7×7	0
15th -21st October	22	23.2	22.4	11	5	7×24	346
	51	22.8	21.1	8	1	7×24	124
	46	22.7	21.2	4	1	7×24	86
	47	25.2	27.9	None	1	7×7	0

Table 6.4. Compare the captured insect species between inside and outside of the warehouse.

types	Inside of warehouse	Outside of warehouse
1	*R. dominica*	*R. dominica*
2	*C. ferrugineus*	*C. ferrugineus*
3	*S. zeamais*	*S. zeamais*
4	*T. castaneum*	*T. castaneum*
5	*S. cerealella*	*S. cerealella and C. cautella*
6	*L. lididae*	*L. oryzae*
7		*L. serricone*
8		flies and mosquitoes

6.4.3 SAS Powder distribution within the empty warehouse

Powder concentration was an estimate of the mass of spray particles that landed on surfaces. The concentration decreased with increasing distance from the dispersion point. The graphs in Fig. 6.7 illustrated this relationship. The concentration of powder particles peaked primarily at the start (1 and 2) and end (9 and 10) of dusting. Even after 24 h, certain concentrations of powder aerosol were still being registered in the air, but the data also indicate minimal particle settling during the last period of the treatment (5 and 8) in the center of the warehouse (6 and 7). The powder concentration estimation and calculation were our first attempts to quantify this parameter. Potential validation testing and additions to the dispersion methods are being considered for future research efforts.

6.4.4 Evaluation of the insecticidal effect of food grade synthetic amorphous silica powder against adults of five stored grain insect species in an empty warehouse

Although the powder deposition varied in different locations of the warehouse, it was still highly effective against the adults of five stored grain insect species and 100% mortality was observed within three days (Table 6.5). *R. dominica*, *O. surinamensis* and *C. ferrugineus* were the most sensitive species to the structural treatment, with above 95% of the population eliminated within 24 hour in the presence of food. After a 24 hour exposure, the mortality rate of *S. zeamais* and *T. castaneum* dropped. However, *S. zeamais* and *T. castaneum* had lost their locomotion and feeding ability, and rendered functionally incapable.

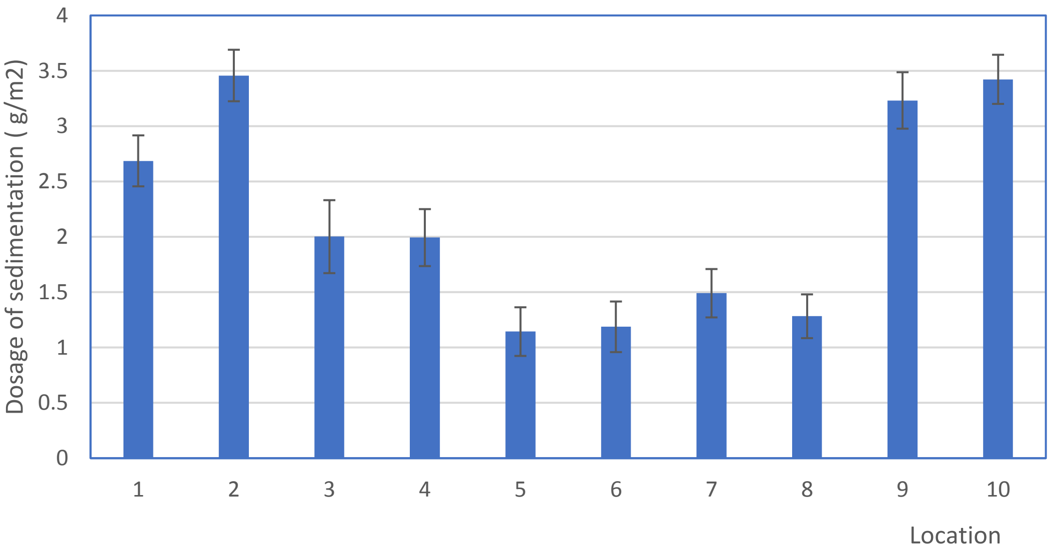


Fig 6.7. The dosage of powder distributed at each sampling point in an empty warehouse 24 hours after application.

Table 6.5. Percentage mortality of adult five stored grain insect species at 24-60 hours check points after SAS powder application in an empty warehouse.

Type of test insect	Average mortality of test group ± SE (%)		
	24 h	36 h	60 h
R. dominica	100.0±0.0	100.0±0.0	100.0±0.0
S. zeamais	7.8±2.2	42.7±3.2	100.0±0.0
O. surinamensis	98.4±1.0	100.0±0.0	100.0±0.0
T. castneum	31.9±6.4	92.7±2.9	100.0±0.0
C. ferrugineus	100.0±0.0	100.0±0.0	100.0±0.0

6.5 Discussion

This is the first trial to evaluate the combined integrated trap and food grade synthetic amorphous silica powder method in insect pest control. The results demonstrated the effectiveness of the method of trapping in both high and low insect population density situations. Early detection of insects by trapping facilitates the management strategy design. The traditional detection methods in stored grain facilities, such as manual inspection, sieving, cracking-floatation and Berlese funnels, are less sensitive and labour intensive (Neethirajan et al., 2007). Several newly developed techniques, like acoustic detection, carbon dioxide measurement, uric acid measurement, near infrared spectroscopy and soft X-ray, to some extent increase the detection accuracy, but the high cost and complex operating procedures prohibited wide application (Neethirajan et al., 2007). Some of these detection techniques require removal of detection devices prior to fumigation procedure. In addition, all these techniques only focus on detection, but not on the pest control aspect. In our trial, the integrated trap positively reflected the manual screening method, such that it could replace the manual screening method for routine inspections. The scientific community and grain industry both showed great interest in this new combined pest control method, mainly for its high effectiveness, low cost, and integration of detection and pest elimination.

The integrated trap was effective in attracting and sampling stored product insects. The insect species captured cover most of the stored product insects, certain flies and mosquitos. The effectiveness can be attributed to the fact that this trap integrates light, colour, food, and pheromone. The light sources used as traps include incandescent, fluorescent and ultraviolet. Different insect species respond uniquely to the visible and invisible spectral areas (Neethirajan et al., 2007), so four fluorescent bulbs of different wavelengths have been selected in this study. Previous studies suggested that yellow colour is most effective for attracting stored grain insects (Qi, 2015). Farmers and stored grain pest managers have used yellow stick boards to monitor pest insects. The attractiveness of coloured objects is a combination of their specific reflectance and shapes or silhouettes that stand out against a contrasting background. We placed a yellow plastic ball in the center of the annular fluorescent tubes to add colour attraction in the dark or dimly lit areas. For those insects that were less active and could not fly, the base was the major capture site. Collection of the captured insect is an indispensable part for trapping. Most traps or other devices mainly rely on electrocutors (Neethirajan et al., 2007). But this technology is unsafe when applied at the grain surface. Also, the smell from the burning insects interferes with food and pheromone lures. Therefore, the 3 W negative pressure fan suction collection method was proposed. It was effective at collecting insects and improved the dispersion of food scent and pheromone. In areas of high pest population density, the anti-fire blanket and food attracting base under the main trap also function as trapped insect collectors.

A comparison of aerosol concentrations at ten locations in the empty warehouse showed that the deposition was generally less at the beginning of powder dispersion and increased at the end of dispersion. The high concentration points suggested that the airflow from the powder blower is not very stable. The operators usually apply strong air volume at the beginning and the end of dispersion. During the application period, the air volume is switch to low or intermediate. We suspected that the lower concentration points (No. 6 and No. 7) were related to the long distance from these points to the aerosol dispersion outlet, which is in accordance with others reports on the utilization of aerosols for insect control inside storage facilities (Arthur et al., 2018). The deposition in most of the locations in the warehouse was lower than the desired value (3 g/m^2). Our previous laboratory study showed that D (v, 0.5) of food grade synthetic amorphous silica powder is 4.31±0.03 µm. In chapter 3, it was shown that the electrostatic charge was low at low dosage, so the smaller droplets may not be settling on the floor surface and continue to float in the air 24 h after application (Arthur et al., 2014, 2017). However, under this test condition, 100% mortality of adults of five major stored grain insect species was observed after 3 days and the order of the insect sensitivity to SAS was similar with our previous results (Li, 2006). Future research is needed to measure the aerosol particle size in the air and on the floor, and also to provide a means to help facilitate aerosol dispersal to mitigate the limitations that have been discussed.

The trial examined a storage strategy that combined integrated pest detection with physical control instead of chemical application. The basic strategy is to prevent pest infestation by the application of food grade synthetic amorphous silica powder as a structural treatment and monitor the process with integrated traps. The number of trapped insects reflected the insect population change in the warehouses. The principle of this integrated trap was based on the insects' ability to fly or climb. The food grade synthetic amorphous silica powder destroys the insect joint system, incapacitates locomotion and causes behavioural changes. In situations of low pest population density, it is only necessary to monitor the trends in insect population change with a trap. However, in situations of high population density, integrated traps and a food grade synthetic amorphous silica powder treatment should be combined. The aim of the strategy is to prevent insect infestation. The experiment showed that the strategy can also be effective in disinfesting grain. In addition to protection from insects, food grade synthetic amorphous silica powder does not require removal from the grain bulk, has no half-life period and provides long term protection, which no other insecticide can match.

Chapter 7. General discussion and summary

Synthetic amorphous silica (SAS) powders, including pyrogenic, precipitated and their surface-treated SAS, have been widely used in industrial and consumer applications including food, cosmetics and pharmaceutical products for many decades without documented incidents regarding environment and human health. These is little information about SAS's application in insect pest control or the mechanism of their insecticidal effect. In our study, SAS powder with different polarity and processing procedure against different stored grain insect species at multiple developmental stages were tested to evaluate their insecticidal efficacy. A diatomaceous earth was also used as comparison. Their mode of action against insects was comprehensively investigated from biology, physical, optical, material science and kinematic perspectives. These laboratory data provided an application guideline and were further proved in the field tests. The efficacy of SAS is higher than that of DE against stored product insect species due to distinct physical property. The different efficacy of SAS did not depend on one specific physical parameter but it rather depended on an integration of multiple factors. The stationary insect stages were buried into SAS with high absorption capacity, no significant difference in hatching and emergence rate between treated and control groups were detected. The activity of insects was a key factor in SAS bioactivity. A significant linear correlation between electrostatic charge and bioactivity of dust was observed accounting the surrounding electrostatic field of both insect and SAS particles. The stored grain insects and SAS obtained positive and negative electrostatic charges, respectively, when in contact with an insulate surface. Among the SAS, the precipitated SAS and their surface-treated form were significantly more effective against insects. The presence of these SAS powders on the insect cuticle resulted in changes in water content and other physiological features. The differences in absorption characteristics of cuticular fat and protein may contribute to the varied hyperspectral performance.

Hyperspectral imaging coupled with back propagation artificial neural network (BPNN) correctly classified the control samples of all groups from the hydrophilic and hydrophobic SAS treated and misclassified between two SAS treated groups resulting in low recognition rate but still within the acceptable identification range. These suggested that hydrophilic and hydrophobic SAS against insects shared similar mode of action with some variation. Different properties of hydrophilic and hydrophobic SAS were correlated with efficacy against different insect species, for example *T. castaneum* was more susceptible to hydrophobic SAS while *S. oryzae* was more susceptible to hydrophilic SAS. Insect cuticle was the most damaged part. But the insects were most severely affected by the SAS induced damage in the intersegmental membranes. The intersegmental membranes with irreversible structural damages was associated with locomotion impairment and biological effect within 3.5 and 12 h for *S. oryzae* and *T. castaneum* respectively. In the field trial, the mortality rate of adults from five major species of stored grain insect reached 100% by the third day with the SAS deposition in different locations of the warehouse.

7.1 Development and evaluation of highly effective SAS

These findings have important practical applications. Diatomaceous earth with a uniform size distribution of high amorphous silica content was more effective against insects (Korunić, 1997). This finding led to several investigations of the nanoparticle as an alternative to insecticide for its ultra-fine particle size (Debnath et al., 2011). Based on the results of current studies, the efficacy would be very different if insects were exposed to different primary particle sizes (pyrogenic silica and precipitated silica). The effectiveness of pyrogenic silica on insects is much lower than precipitated silica. Pyrogenic and precipitated SAS forms are composed of more than 100 nm diameter aggregates and agglomerates. There were not primary particles outside of the reaction zone (Fruijtier-Pölloth, 2012). The effects of SAS in insect depend on the characteristics of their aggregates and agglomerates, as well as on the size of their primary particles. Our result showed that the size of aggregates and agglomerates of SAS together with their morphology and their charge, their coating and the reactivity of their surface were shown to influence their interactions with insects.

Precipitated silica have a larger particle size and contain more bound water than pyrogenic silica (Fruijtier-Pölloth, 2012). The particle size distribution of both hydrophilic and hydrophobic pyrogenic silica was wider than the precipitated silica in either solid or liquid phase by Laser particle size analyser. Larger particles provide better aeration efficiency (owing to increased interparticle space) but limited surface for effective attachment (Ashok et al., 2008). Also the density of pyrogenic silica was lower than that of pyrogenic silica in a packaged product.

Particle size is an important factor in determining deposition, distribution, and effectiveness of SAS powders and concentrated sprays. The failure to control insects with SAS is often due to low deposition and poor adherence, rather than to under dosing. Even with high dose pyrogenic SAS structural treatment, when the number of particles per square millimeter of surface exceed thousands, insect infestation can remain uncontrolled. Large and light agglomerates can be blocked by the insect's hair and unable to breach the intersegmental membrane or cuticle. These SAS particles settle on insect surface and can be dislodged easily resulting in a low mortality rate (Mewis and Ulrichs, 2001). Under field conditions these extremely minute individual particles might be carried away by the air current. The heavy particles in precipitated silica, deposit better. However, heavier particles decreased powders picked up due to their weight. Based on our results, there was a linear relationship between electrostatic charge and efficacy of SAS against stored product insect species. Synthetic amorphous silica (SAS) and stored product insects carry electrostatic charges of opposite polarity in contact with insulation surfaces. The electrical fields generated between SAS and insects affected the amount of SAS attachment on insect cuticle. According to Coulomb's law, the SAS particles would then be passively attracted to insects via the mere effect of electrostatic forces. Therefore, the agglomerates deposit weight that should be smaller than electrostatic forces for optimal attachment.

In our study, the results demonstrated that insecticidal effects of SAS were correlated with surface area and porosity. A large specific surface area and total pore volume may promote the adsorption of body fluid. Due to their surface characteristics, silica particles adsorb macromolecules, such as peptides and proteins from the body fluid onto their surface. We observed protein absorption by SAS in the experiment. The adsorption process was influenced by an affinity between specific biomolecules and pore size of particles. Precipitated silica is typically associated with meso/macroporous pore structures, while pyrogenic silica is generally associated more with microporous structures (Fruijtier-Pölloth, 2012). The large protein molecules were adsorbed rapidly onto the amorphous silica particles with large pores (Diao et al., 2010). The high specific area due to mesopores or micropores did not offer a higher coefficient of effective use of surface area (Katiyar et al., 2005). The morphology of these materials is also important for commercialization in protein purification. The synthesis procedures can be tuned to sharpen these distributions further. For example, the synthesis of amorphous mesoporous silica went through tetraethylorthosilicate (TEOS) with a template of surfactant molecules, typically amphiphilic polymers, under either alkaline or acidic conditions. The products have uniformed pores in the size range between 1.5 and 50 nm (Fruijtier-Pölloth, 2012).

By treating with a reactive silane, the important surface parameters of hydrophilic SAS can be adjusted to produce a range of different hydrophobic types with water repellent characteristics. Hydrophilicity or hydrophobicity is distinguished from the value of effect by non-polar, resulting in hydrophobic surfaces as oil were removed from water and chemical separation processes to separate non-polar and polar compounds. The efficacy of hydrophobic SAS against insect species is less influenced by high humidity condition. However, there was an event showed that the surface silanol groups (Si-O-H) played an important role in absorbing the proteins from the cell membrane, causing loss of membrane flexibility and resiliency (Pandurangi et al., 1990).

The primary particles of precipitated silica and pyrogenic silica tend to agglomerate. These agglomerates assemble by weak forces, such as van der Waals forces and simple physical adhesion forces (Gray and Muranko, 2006). After powder is expelled from a blower, these groups of particles tend to break up without liberation of primary particles (Fruijtier-Pölloth, 2012). The end agglomerate size depended on several parameters of the blower, such as airflow pressure, chamber temperature, and ultrasonication. Proportionately more powder is deposited from a cloud of heavy powder expelled from a blower at a low velocity than from a cloud of light powder expelled at a high velocity and with a large volume of air (Arthur et al., 2018, 2017, 2014). Factors worth further investigation include the size, concentration, and distribution of silica particles within treated arenas during silica applications, particularly the spatial and temporal deposition patterns.

Under field conditions, very light and large particles or agglomerates did not deposited on insect surface very well. An electric field around the insect may possibly cause an abstraction conditions due to differences in temperature, humidity, insect species and commodities. It would be interesting to know if an insect in different stored bulk commodities shows a similar or different pattern of electric field. There are numerous gaps in our knowledge regarding the relationships between efficacy and the parameters of SAS. For example, do insects always carry a positive electrostatic charge when contact with different types of stored products? What is the amount and polarity of different types of stored products? Are the stored products surrounded by a film, or field of resistance? What kinds of dust particles can penetrate the film of resistance and make contact with the surface of the stored product or other objects? What is the optimal primary particle size to form an effective agglomerate size against insects? What is the mostly weight range of protein molecular in a stored product? Which pore size of SAS is the best for absorbing body fluid, microporous, mesoporous or macroporous? What is the standpoint of particle density? The Faraday cup method of measuring electrostatic charge is a well-established, easy and cheap method and was used in our study to examine the relevance of electrostatic charge to the efficacy of different SAS against different insect species. For more exact analyses of insecticidal mechanism in an ecophysiological context, newer hyperspectral imaging systems are often used, allowing precise results to be obtained on hyperspectral changes of insect cuticle. With these methods, we were able to study the efficacy of SAS with different parameters at different environmental conditions and focus on the evaluation and development of effective SAS for insect control.

7.2 Insecticidal mechanism of SAS

The insecticidal mechanism of inert dusts, mainly DE, have been extensively researched by a wide variety of researchers over the last century (Alexander et al., 1944; Beament, 1945; Ebeling, 1964; Ebeling and Wagner, 1959; Li, 2006; Malia et al., 2016; Smith, 1969; Webb, 1946; Wigglesworth, 1942; Zacker and Kunike, 1931). Despite being used in a wide range of industrial and consumer applications including food, cosmetics and pharmaceutical products for many decades, very little research has been carried out to establish the insecticidal mechanism of SAS. As summarized earlier, the most acceptable DE insecticidal mode of action is desiccation. Water loss occurs via evaporation through the cuticle and via respiration. Cuticular water loss may often represent more than 80% of the total water loss in many taxa under normal conditions (Chown, 2002). While epicuticular waxes (mainly hydrocarbons) limit water exchange, a considerable percentage of wax must be removed to enable water to pass through an insect's the protective wax layers. The exoskeleton of insects shows expansive specializations across an individual, across the developmental stages, and across the class Insecta. The mechanical properties of cuticle displays a fare wider range that can vary over several orders of magnitude depending on the type of cuticle. Where is the target site on cuticle that is involved during the penetration of the insect epidermis by SAS? Our research has answered this question and shown that the structural damages to intersegmental membranes are crucial, leading to the loss of locomotion ability within 12 hours for the most tolerant store product insect species, *T. castaneum*. This study has also found that insect behaviour substantially changes in response to the effects of SAS. Intersegmental membranes exist in attachment systems adapted to connect different structures together, such as leg-joints, antennae and large spines. Cuticle is acellular and is not vascularized. Insects have an open circulatory system in which their organs are "bathed" in hemolymph (the blood of the insect) which flows freely around them, not being contained in vessels like mammals (Chapman, 2013). Injury to intersegmental membranes of an insect caused by SAS will induce haemorrhage, and affect organ functions. Intersegmental injury also significantly hinder the antennae movement. If the intersegmental membranous damage cause change in water content of an insect body, then it is possible that more severe damage occurs in other parts cuticle, such as the sclerotized cuticle. Several previous studies showed that very small changes in water content can strongly affect the static and dynamic biomechanical properties of cuticle (Klocke and Schmitz, 2011). It is unclear if this phenomena did occur, but it would definitely be worth investigate further in the future. This would imply some active modelling and possibly some remodeling within the cuticle, which up to now has been undiscovered.

The slow "speed of kill" can give insects enough time in healing micro-damage induced by DE, resulting in low mortality of stored product insect exposure to DE in present of food (Mewis and Ulrichs, 2001). Thus the tissue repair process after dust treatment should be assessed in an efficacy evaluation of dusts. However, based on the mechanism in our study, the efficacy of a SAS is evaluated on the onset time of insecticidal effect more than the final mortality of the exposure insects. When the segments were sustained major damage, such as legs, the values of stiffness and load energies will decline (Parle and Taylor, 2013), especially in adults, who lack the repair capacity (Parle et al., 2017). To what the injury excesses the capabilities of repair? How much powder deposit in individual insect can induce this degree of damage?

7.3 Adaptations and potential use

In summary, SAS powders are food-grade, fast killing, low cost and easy to apply. Because they are highly effective, one advantage of their use is the avoidance of chemical insecticides. Another one is the reduction in cost due to the extremely low effective dosage and long term protection. Also the food grade synthetic amorphous silica application complies to current hygiene standards and application recommendations, thus they do not require removal from grain bulks. They also no half-life period, which no other insecticide can remedy.

The SAS powders are capable of directly damaging the exoskeleton in most insects by penetration. There are over one million species of arthropods including common pests such as ants, cockroaches, fleas, termites, and spiders. All are potential targets. Investigation of these possibilities could open a whole new chapter on the search of this non-chemical methods for pest control.

References:

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267. https://doi.org/10.1093/jee/18.2.265a

Abdi, H., Williams, L.J., 2010. Principal component analysis. Wiley Comput. Stat. 2, 433–459. https://doi.org/10.1002/wics.101

Adrien, A., 1968. Selective toxicity and related topics. London, UK.

Aldryhim, Y.N., 1990. Efficacy of the amorphous silica Dust , Dryacide, against *Tribolium confusum* DUV and *Sitophilus Granarius* (L .) (Coleoptera : Tenebrionidae and Curculionidae). J. Stored Prod. Res. 26, 207–210.

Alexander, P., Kitchener, J.A., Briscoe, H.V.A., 1944. Inert dust insecticides: Part I. Mechanism of action. Ann. Appl. Biol. 31, 143–149. https://doi.org/10.1111/j.1744-7348.1944.tb06225.x

Anon, 1991. Facts: silicon dioxide and Silica Gel, 21T-1021.

Arlian, L.G., 1979. Significance of passive sorption of atmospheric water vapor and feeding water balance the rice weevil, *Sitophilus Oryzae*. Comp. Biochem. Physiol. -- Part A Physiol. 62, 725–733. https://doi.org/10.1016/0300-9629(79)90131-2

Arthur, F.H., 2000a. Immediate and delayed mortality of *Oryzaephilus surinamensis* (L.) exposed on wheat treated with diatomaceous earth: effects of temperature, relative humidity, and exposure interval. J. Stored Prod. Res. 37, 13–21. https://doi.org/10.1016/S0022-474X(99)00058-2

Arthur, F.H., 2000b. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae): Effects of temperature and relative humidity. J. Econ. Entomol. 93, 526–532. https://doi.org/10.1603/0022-0493-93.2.526

Arthur, F.H., Campbell, J.F., Brabec, D.L., Ducatte, G.R., Donaldson, J.E., 2018.

Aerosol insecticide distribution inside a flour mill: Assessment using droplet measurements and bioassays. J. Stored Prod. Res. 77, 26–33. https://doi.org/10.1016/j.jspr.2017.12.004

Arthur, F.H., Campbell, J.F., Donaldson, J.E., 2017. Laboratory evaluation of particle size, food contamination, and residual efficacy of pyrethrin + methoprene aerosol. J. Stored Prod. Res. 72, 100–110. https://doi.org/10.1016/j.jspr.2017.04.003

Arthur, F.H., Campbell, J.F., Ducatte, G.R., 2014. Susceptibility of Tribolium confusum (Coleoptera: Tenebrionidae) to pyrethrin aerosol: Effects of aerosol particle size, concentration, and exposure conditions. J. Econ. Entomol. 107, 2239–2251. https://doi.org/10.1603/EC14296

Ashok, P., Larroche, C., Soccol, C.R., 2008. Introduction, in: Ashok, P., Larroche, C., Soccol, C.R. (Eds.), Current Developments in Solid-State Fermentation. Springer, New York, pp. 3–12.

Athanassiou, C.G., Kavallieratos, N.G., Meletsis, C.M., 2007. Insecticidal effect of three diatomaceous earth formulations, applied alone or in combination, against three stored-product beetle species on wheat and maize. J. Stored Prod. Res. 43, 330–334. https://doi.org/10.1016/j.jspr.2006.08.004

Athanassiou, C.G., Kavallieratos, N.G., Tsaganou, F.C., Vayias, B.J., Dimizas, C.B., Buchelos, C.T., 2003. Effect of grain type on the insecticidal efficacy of SilicoSec against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Prot. 22, 1141–1147. https://doi.org/10.1016/S0261-2194(03)00155-8

Athanassiou, C.G., Kavallieratos, N.G., Vayias, B.J., Panoussakis, E.C., 2008. Influence of grain type on the susceptibility of different *Sitophilus oryzae* (L.) populations, obtained from different rearing media, to three diatomaceous earth formulations. J. Stored Prod. Res. 44, 279–284. https://doi.org/10.1016/j.jspr.2008.02.006

Bachtiar, L.R., Unsworth, C.P., Newcomb, R.D., Crampin, E.J., 2011. Using artificial neural networks to classify unknown volatile chemicals from the firings of insect olfactory sensory neurons. Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. EMBS 2752–2755. https://doi.org/10.1109/IEMBS.2011.6090754

Bai, C., Cao, Y., Li, Y., Shang, Y., Guo, Q., 2008. Lipids components of cuticular layer of *Liposcelis paeta* (Psocopotera: Liposcelididae). J. Chinese Cereal. Oils Assoc. 23, 128–132.

Bai, C., Cao, Y., Shang, Y., Guo, Q., Li, Y., 2007. Distinguishing *Sitophilus oryzae* (Linnaeus) and *Sitophilus zeamais* (Motschulsky) (Coleoptera :Curculionidae) by the integumental cuticular componnents. Grain Storage 36, 3–6.

Baker, J.E., Highland, H.A., Engle, G.C., 1976. Bulk density of tricalcium phosphate as a significant variable in the suppression of insect populations in flour and wheat soy blend. Environ. Entomol. 5, 909–919. https://doi.org/10.1093/ee/5.5.909

Baker, J.E., Woo, S.M., Nelson, D.R., Fatland, C.L., 1984. Olefins as major components of epicuticular lipids of three *Sitophilus* weevils. Comp. Biochem. Physiol. Part B Comp. Biochem. 77, 877–884. https://doi.org/10.1016/0305-0491(84)90327-4

Banks, J.H., Fields, P.G., 1995. Physical Methods for Insect Control in Stored-Grain Ecosystems, in: Jayas, D.S., White, N.D.G., Muir, W.E. (Eds.), Stored Grain Ecosystems. Marcel Dekker, INC., New York, pp. 353–409.

Barbosa, A., Golob, P., Jenkins, N., 1994. Silica aerogels as alternative protectants of maize against *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) infestations, in: Proceedings of the 6th International Working Conference on Stored-Product Protection. Canberra, Australia, pp. 623–627.

Bartlett, B.R., 1951. The action of certain “inert” dust materials on parasitic hymenoptera. J. Econ. Entomol. 44, 891–896. https://doi.org/10.1093/jee/44.6.891

Barton, L., Armsworth, C., Baxter, I., Poppy, G., Gaunt, L., Nansen, C., 2006. Adhesive powder uptake and transfer by Mediterranean fruit flies, *Ceratitis capitata* (Dipt., Tephritidae). J. Appl. Entomol. 130, 257–262. https://doi.org/10.1111/j.1439-0418.2006.01061.x

Batschelet, E., 1981. Circular statistics in biology. Academic Press, New York, UK.

Beament, J.W.L., 1945. The cuticular lipids of insects. J. Exp. Biol. 21, 115–131.

Benhalima, H., Chaudhry, M.Q., Mills, K.A., Price, N.R., 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J. Stored Prod. Res. 40, 241–249. https://doi.org/10.1016/S0022-474X(03)00012-2

Boserup, E., 2017. The conditions of agricultural growth: the economics of agraian change under popolation pressure, 1st Editio. ed. Routledge, New York, UK.

Boucias, D.G., Pendland, J.C., Latge, J.P., 1988. Nonspecific factors involved in attachment of entomopathogenic deuteromycetes to host insect cuticle. Appl. Environ. Microbiol. 54, 1795–1805.

Boyce, A.M., 1932. Mortality of *Rhagoletis Completa* Cress. (Diptera: Trypetidae) through ingestion of certain solid materials. J. Econ. Entomol. 25, 1053–1059. https://doi.org/10.1093/jee/25.5.1053

Bridgeman, B.W., 1994. Structural treatment with amorphous silica slurry: an integral component of GRAINCO's IPM strategy, in: Proceedings of the 6th International Working Conference on Stored-Product Protection. Canberra, Australia, pp. 628–630.

Burry, R.W., Wood, J.G., 1979. Contributions of lipids and proteins to the surface charge of membranes. An electron microscopy study with cationized and anionized ferritin. J. Cell Biol. 82, 726–741. https://doi.org/10.1083/jcb.82.3.726

Cao, Y., Li, G., 2001. Lethal effect of diatomite to the imago of *Liposcelis entomophila* at different humidity. Grain Storage 30, 9–12.

Cao, Y., Zhang, C., Chen, Q., Li, Y., Qi, S., Tian, L., Ren, Y., 2015. Identification of species and geographical strains of *Sitophilus oryzae* and *Sitophilus zeamais* using the visible/near-infrared hyperspectral imaging technique. Pest Manag. Sci. 71, 1113–1121. https://doi.org/10.1002/ps.3893

Carlson, S.D., Ball, H.J., 1962. Mode of action and insecticidal value of a diatomaceous earth as a grain protectant. J. Econ. Entomol. 55, 964–970. https://doi.org/10.1093/jee/55.6.964

Castner, J.L., Nation, J.L., 1984. Cuticular Lipids for Species Recognition of Mole Crickets (Orthoptera : Gryllotalpidae) I . *Scapteriscus didactylus* , *Scapteriscus imitatus* , and *Scapteriscus vicinus*. Florida Entomol. 67, 155–160. https://doi.org/10.2307/3494115

Celik, F.E., Kim, T.J., Bell, A.T., 2010. Effect of zeolite framework type and Si/Al ratio on dimethoxymethane carbonylation. J. Catal. 270, 185–195. https://doi.org/10.1016/j.jcat.2009.12.021

Chanbang, Y., Arthur, F.H., Wilde, G.E., Throne, J.E., 2007. Efficacy of diatomaceous earth to control *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) in rough rice: Impacts of temperature and relative humidity. Crop Prot. 26, 923–929. https://doi.org/10.1016/j.cropro.2006.08.009

Chapman, R.F., 2013. The insects: structure and function. Cambridge University Press.

Chauzat, M.P., Faucon, J.P., 2007. Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera* L .) in France. Pest Manag. Sci. 63, 1100–1106. https://doi.org/10.1002/ps.1451

Chen, J., Yang, C., Zheng, S., Zhang, Z., 2016. Research on the bidirection application of the insect dust in brick silo. Grain Oil Food Sci. 24, 95–97.

Chiu, S.F., 1939. Toxicity studies of so-called “inert” materials with the bean weevil, Acanthoscelides obtectus (Say). J. Econ. Entomol. 32, 240–248.

Chomchalow, N., 2003. Protection of stored products with special reference to Thailand. Assumpt. Univ. J. Technol. 7, 31–47.

Chown, S.L., 2002. Respiratory water loss in insects. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 133, 791–804. https://doi.org/10.1016/S1095-6433(02)00200-3

Clark-Cooper, W., 1977. Effects of diatomaceous earth on human health. Ad Hoc Technical Committee Diatomaceous Earth Producers, no. 1.

Colin, M.E., Richard, D., Fourcassie, V., Belzunces, L.P., 1992. Attraction of *Varroa jacobsoni*, parasite of *Apis mellifera* by electrical charges. J. Insect Physiol. 38, 111–117. https://doi.org/10.1016/0022-1910(92)90039-G

Collins, D.A., Cook, D.A., 2006. Laboratory studies evaluating the efficacy of diatomaceous earths, on treated surfaces, against stored-product insect and mite pests. J. Stored Prod. Res. 42, 51–60. https://doi.org/10.1016/j.jspr.2004.09.002

Collins, P.J., Daglish, G.J., Pavic, H., Kopittke, R.A., 2005. Response of mixed-age cultures of phosphine-resistant and susceptible strains of lesser grain borer, *Rhyzopertha dominica*, to phosphine at a range of concentrations and exposure periods. J. Stored Prod. Res. 41, 373–385. https://doi.org/10.1016/j.jspr.2004.05.002

Cook, D.A., Armitage, D.M., 1999. The efficacy of Dryacide, an inert dust, against two species of Astigmatid mites, *Glycyphagus destructor* and *Acarus siro*, at nine temperature and moisture content combinations on stored grain. Exp. Appl. Acarol. 23, 51–63. https://doi.org/10.1023/A:1006148711827

Cook, D.A., Collins, D.A., Collins, L.E., 2004. Efficacy of diatomaceous earths, applied as structural treatments, against stored product insects and mites.

Cook, D.A., Wakefield, M.E., Bryning, G.P., 2008. The physical action of three diatomaceous earths against the cuticle of the flour mite *Acarus siro* L. (Acari: Acaridae). Pest Manag. Sci. 64, 141–146. https://doi.org/10.1002/ps.1484

Cotton, R.T., Frankenfeld, J.C., 1949. Silica aerogel for protecting stored seed or milled cereal products from insects. J. Econ. Entomol. 42, 553–553. https://doi.org/10.1093/jee/42.3.553

Daglish, G.J., Collins, P.J., 1998. Improving the relevance of assays for phosphine resistance, in: Proceedings of the 7th International Working Conference on Stored-Product Protection - Volume 1. Beijing, China, pp. 584–593.

David, W.A.L., Gardiner, B.O.C., 1950. Factors influencing the action of dust insecticides. Bull. Entomol. Res. 41, 1–61. https://doi.org/10.1017/S0007485300027474

Davies, D.K., 1969. Charge generation on dielectric surfaces. J. Phys. D. Appl. Phys. 2, 1533–1537.

Debnath, N., Das, S., Seth, D., Chandra, R., Bhattacharya, S.C., Goswami, A., 2011. Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). J. Pest Sci. (2004). 84, 99–105. https://doi.org/10.1007/s10340-010-0332-3

DeCrosta, A., 1979. Mother Nature’s bug-killer [Diatomaceous earth], Organic Gardening (USA).

Desmarchelier, J.M., Dines, J.C., 1987. Dryacide treatment of stored wheat : Its efficacy against insects , and after processing. Aust. J. Exp. Agric. 27, 309–312.

Diao, X., Wang, Y., Zhao, J., Zhu, S., 2010. Effect of pore-size of mesoporous SBA-15 on adsorption of bovine serum albumin and lysozyme protein. Chinese J. Chem.

Eng. 18, 493–499. https://doi.org/10.1016/S1004-9541(10)60248-0

Dirks, J.H., Taylor, D., 2012. Fracture toughness of locust cuticle. J. Exp. Biol. 215, 1502–1508. https://doi.org/10.1242/jeb.068221

Dong, Z., Wang, X., Shen, B., Wang, F., Wang, H., Song, W., Guo, B., Shao, W., He, F., Zhang, Z., Cao, Y., Gao, B., 2016. Comparative studies of pest control of food grade inert dust by transverse circulation and direct exhaust. Grain Storage 35, 35–38.

Dowell, F.E., Throne, J.E., Wang, D., Baker, J.E., 1999. Identifying stored-grain insects using near-infrared spectroscopy. J. Econ. Entomol. 92, 165–169. https://doi.org/10.1093/jee/92.1.165

Ebeling, W., 1971. Sorptive dusts for pest control. Annu. Rev. Entomol. 16, 123–58. https://doi.org/10.1146/annurev.en.16.010171.001011

Ebeling, W., 1964. Permeability of insect cuticle, in: Rockstein, M. (Ed.), The Physiology of Insecta. Academic Press, New York, pp. 508–523.

Ebeling, W., 1960. Control of the tropical rat mite. J. Econ. Entomol. 53, 475–6. https://doi.org/10.1093/jee/53.3.475

Ebeling, W., Wagner, R.E., 1961. Physiochemical mechanisms for the removal of insect wax by means of finely divided powders. Hilgardia 30, 531–564. https://doi.org/10.1017/CBO9781107415324.004

Ebeling, W., Wagner, R.E., 1959. Rapid desiccation of drywood termites with inert sorptive dusts and other substances. J. Econ. Entomol. 52, 190–207. https://doi.org/10.1093/jee/52.2.190

Edwards, D.K., 1962. Electrostatic charges on insects due to contact with different substrates. Can. J. Zool. 40, 579–584. https://doi.org/10.1139/z62-051

Emekci, M., Navarro, S., Donahaye, E., Rindner, M., Azrieli, A., 2002. Respiration of

Tribolium castaneum (Herbst) at reduced oxygen concentrations. J. Stored Prod. Res 38, 413–425. https://doi.org/10.1016/S0022-474X(01)00045-5

Emery, R.N., Collins, P.J., Wallbank, B.E., 2003. Monitoring and managing phosphine resistance in Australia, in: Proceedings of the Australian Postharvest Technical Conference, Canberra. New York, UK, pp. 142–151.

Fam, E.Z., El-Nahal, A.K.M., Fahmy, S.M.H., 1975. Influence of grain moisture on the efficiency of silica aerogel and katelsous used as grain protectants. Bull. Econ. Ser.

Faulde, M.K., Scharninghausen, J.J., Cavaljuga, S., 2006. Toxic and behavioural effects of different modified diatomaceous earths on the German cockroach, *Blattella germanica* (L.) (Orthoptera : Blattellidae) under simulated field conditions. J. Stored Prod. Res 42, 253–263. https://doi.org/10.1016/j.jspr.2005.03.001

Federle, W., Rohrseitz, K., Hölldobler, B., 2000. Attachment forces of ants measured with a centrifuge: Better “wax-runners” have a poorer attachment to a smooth surface. J. Exp. Biol. 203, 505–512.

Fields, P., Korunic, Z., 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. J. Stored Prod. Res. 36, 1–13. https://doi.org/10.1016/S0022-474X(99)00021-1

Fields, P.G., Fleurat-Lessard, F., Lavenseau, L., Febvay, G., Peypelut, L., Bonnot, G., 1998. The effect of cold acclimation and deacclimation on cold tolerance, trehalose and free amino acid levels in *Sitophilus granarius* and *Cryptolestes ferrugineus* (Coleoptera). J. Insect Physiol. 44, 955–965. https://doi.org/10.1016/S0022-1910(98)00055-9

Fruijtier-Pölloth, C., 2012. The toxicological mode of action and the safety of synthetic amorphous silica-A nanostructured material. Toxicology 294, 61–79.

https://doi.org/10.1016/j.tox.2012.02.001

Glenn, D.M., Puterka, G.J., Drake, S.R., Unruh, T.R., Knight, A.L., Baherle, P., Prado, E., Baugher, T.A., 2001. Particle film aplication influences apple leaf physiology, fruit yield, and fruit quality. J. Am. Soc. Hortic. Sci. 126, 175–181. https://doi.org/10.1007/s10584-010-9936-0

Golob, P., 1997. Current status and future perspectives for inert dusts for control of stored product insects. J. Stored Prod. Res. 33, 69–79. https://doi.org/10.1016/S0022-474X(96)00031-8

Golob, P., Mwambula, J., Mhango, V., Ngulube, F., 1982. The use of locally available materials as protectants of maize grain against insect infestation during storage in Malawi. J. Stored Prod. Res. 18, 67–74. https://doi.org/10.1016/0022-474X(82)90004-2

Golob, P., Webley, D.J., 1980. The use of plants and minerals as traditional protectants of stored products. Tropical Products Institute Publication, London, UK.

Gorb, E., Gorb, S., 2009. Effects of surface topography and chemistry of Rumex obtusifolius leaves on the attachment of the beetle *Gastrophysa viridula*. Entomol. Exp. Appl. 130, 222–228. https://doi.org/10.1111/j.1570-7458.2008.00806.x

Gorb, S.N., 2001. Attachment devices of insect cuticle. Kluwer academic publishers, London, UK. https://doi.org/10.1023/A:1021758915911

Gowers, S.L., Le Patourel, G.N.J., 1984. Toxicity of deposits of an amorphous silica dust on different surfaces and their pick-up by *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). J. Stored Prod. Res. 20, 25–29. https://doi.org/10.1016/0022-474X(84)90032-8

Gray, C.A., Muranko, H., 2006. Studies of robustness of industrial aciniform aggregates and agglomerates - carbon black and amorphous silicas: A review amplified by new data. J. Occup. Environ. Med. 48, 1279–1290. https://doi.org/10.1097/01.jom.0000251477.40643.2a

Hackman, R.H., Goldberg, M., 1987. Comparative study of some expanding arthropod cuticles: The relation between composition, structure and function. J. Insect Physiol. 33, 39–50. https://doi.org/10.1016/0022-1910(87)90102-8

Hadley, N.F., 1994. Water relations of terrestrial arthropods. Academy Press.

Hajek, A., 1994. Interactions between fungal pathogens and insect hosts. Annu. Rev. Entomol. 39, 293–322. https://doi.org/10.1146/annurev.ento.39.1.293

Haryadi, Y., Syarief, R., Hubeis, M., Herawati, I., 1994. Effect of zeolite on the development of *Sitophilus zeamais* Motsch, in: Proceedings of the 6th International Working Conference on Stored Product Protection. Canberra, Australia, pp. 633–634.

Hepburn, H.R., Chandler, H.D., 1976. Material properties of arthropod cuticles-arthrodial membranes. J. Comp. Physiol. 109, 177–198. https://doi.org/10.1007/BF00689417

Herron, G.A., 1990. Resistance to grain protectants and phosphine in *Coleopterous* pests of grain stored on farms in New South Wales. J. Aust. Entomol. Soc. 29, 183–189. https://doi.org/10.1111/j.1440-6055.1990.tb00345.x

Hooper, I.R., Vukusic, P., Wootton, R.J., 2006. Detailed optical study of the transparent wing membranes of the dragonfly *Aeshna cyanea*. Opt. Express 14, 4891–4897.

Hunt, C.R., 1947. Toxicity of insecticide dust diluents and carriers to larvae of the Mexican bean beetle. J. Econ. Entomol. 40, 215–219. https://doi.org/10.1093/jee/40.2.215

IARC, 1997. Silica, some silicates, coal dust and para-aramid fibrils. Iarc Monographs On The Evaluation Of Carcinogenic Risks To Humans.

International Diatom Producers Association (IDPA), 1990a. Heath and safety aspects of diatomaceous earth. Information on: Occupational exposure limits, recommended work practices, respiratory protection, hazard communnication standard, Medical Program, IARC, Glossary of Terms.

International Diatom Producers Association (IDPA), 1990b. The nature and safe handling of diatomaceous earth. Comments by W. Clark Copper.

ISO, 2014. The international organization for standardization 14046. ISO/TS 80004-1.

Jackson, K., Webley, D., 1994. Effects of Dryacide on the physical properties of grains, pulses and oilseeds, in: Proceedings of the 6th International Working Conference on Stored Product Protection. Canberra, Australia, pp. 635–637.

Jayas, D.S., White, N.D.G., Muir, W.E., 1994. Stored-grain ecosystems. CRC Press.

Jian, F., Larson, R., Jayas, D.S., White, N.D.G., 2012. Three dimensional temporal and spatial distribution of adult *Oryzaephilus surinamensis* and *Sitophilus oryzae* in stored wheat under different temperatures, moisture contents, and adult densities. J. Stored Prod. Res. 49, 155–165. https://doi.org/10.1016/j.jspr.2012.01.001

Kalmus, H., 1944. Action of inert dust on insects. Nature 153, 714–715.

Katiyar, A., Ji, L., Smirniotis, P., Pinto, N.G., 2005. Protein adsorption on the mesoporous molecular sieve silicate SBA-15: Effects of pH and pore size. J. Chromatogr. A 1069, 119–126. https://doi.org/10.1016/j.chroma.2004.10.077

Kaya, Y., Kayci, L., 2014. Application of artificial neural network for automatic detection of butterfly species using color and texture features. Vis. Comput. 30, 71–79. https://doi.org/10.1007/s00371-013-0782-8

Khan, A., Rayner, G.D., 2004. Robustness to non-normality of common tests for the many-sample location problem. J. Appl. Math. Decis. Sci. 7, 187–206. https://doi.org/10.1155/S1173912603000178

Kim, K.D., Cho, Y.K., Han, S.W., Park, E.J., Kim, D.H., Jeong, M.G., Kim, Y.D., Moon, J., Hong, E., Lim, D.C., 2015. Porous silica particles as oil absorbents: Comparison of meso-, macro-, and meso/macro-structures. Bull. Korean Chem. Soc. 36, 1751–1757. https://doi.org/10.1002/bkcs.10330

Klocke, D., Schmitz, H., 2011. Water as a major modulator of the mechanical properties of insect cuticle. Acta Biomater. 7, 2935–2942. https://doi.org/10.1016/j.actbio.2011.04.004

Knight, A.M., Bessette, S.M., 1997. Non-hazardous pest control United States Patent 5,693,344.

Korunic, Z., 1998. Diatomaceous earths, a group of natural insecticides. J. Stored Prod. Res. 34, 87–97. https://doi.org/10.1016/S0022-474X(97)00039-8

Korunić, Z., 1997. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. J. Stored Prod. Res. 33, 219–229. https://doi.org/10.1016/S0022-474X(97)00004-0

Korunic, Z., Cenkowski, S., Fields, P., 1998. Grain bulk density as affected by diatomaceous earth and application method. Postharvest Biol. Technol. 13, 81–89. https://doi.org/10.1016/S0925-5214(97)00076-8

Kozak, S., 1966. Correction for the effect of diatomaceous earth on moisture content of wheat as determined by capacitance measurements. Agron. J. 58, 135–137. https://doi.org/10.2134/agronj1966.00021962005800020001x

Kramer, K.J., Hopkins, T.L., Schaefer, J., 1995. Applications of solids NMR to the analysis of insect sclerotized structures. Insect Biochem. Mol. Biol. 25, 1067–1080. https://doi.org/10.1016/0965-1748(95)00053-4

Kuitunen, K., Gorb, S.N., 2011. Effects of cuticle structure and crystalline wax coverage on the coloration in young and old males of *Calopteryx splendens* and

Calopteryx virgo. Zoology 114, 129–139. https://doi.org/10.1016/j.zool.2011.01.003

La Hue, D.W., 1965a. Evaluation of malathion, synergised pyrethrins, and a diatomaceous earth as wheat protectants in small bins. USDA, Agricultural Research Service, Marketing Research Report, 726.

La Hue, D.W., 1965b. Evaluation of malathion, diazinon, a silica aerogel, and a diatomaceous earth as protectants on wheat against lesser grain borer attack in small bins.

Lang, T., 2009. Research in abrasion effect of inert dust to stored grain insects, master thesis. Henan University of Technology.

Law, S.E., 2001. Agricultural electrostatic spray application: A review of significant research and development during the 20th century. J. Electrostat. 51–52, 25–42. https://doi.org/10.1016/S0304-3886(01)00040-7

Le Patourel, G.N.J., 1986. The effect of grain moisture content on the toxicity of a sorptive silica dust to four species of grain beetle. J. Stored Prod. Res. 22, 63–69. https://doi.org/10.1016/0022-474X(86)90020-2

Le Patourel, G.N.J., Shawir, M., Moustafa, F.I., 1989. Accumulation of mineral dusts from wheat by *Sitophilus oryzae* (L.) (Coleoptera: curculionidae). J. Stored Prod. Res. 25, 65–72. https://doi.org/10.1016/0022-474X(89)90012-X

Lewis, C.T., Hughes, J.C., 1957. Studies concerning the uptake of contact insecticides. Bull. Entomol. Res. 48, 755. https://doi.org/10.1017/S000748530000287X

Li, J., 2013. The evaluation studies on insecticidal effect of food-grade inert dust, master thesis. Henan University of Technology.

Li, Y., 2006. Study on diatomite insecticide mechanism, master thesis. Henan University of Technology.

Li, Y., Agarwal, M., Cao, Y., Li, F., Ren, Y., 2016. Field trials using ethyl formate as grain surface and empty silo treatments, in: Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016). New Delhi, India, pp. 244–251.

Li, Y., Cao, Y., Li, G., 2011. Research on the inert dust against phosphine resistance of *Cryptolestes ferrugineus*. J. Life Sci. 5, 400–404.

Lippert, G.E., Hagstrum, D.W., 1987. Detection or estimation of insect populations in bulk-stored wheat with probe traps. J. Econ. Entomol. 80, 601–604. https://doi.org/10.1093/jee/80.3.601

Liu, X., 2005. Study on the screening and mechanism for inert powder insecticide, master thesis. Henan University of Technology.

Lockey, K.H., 1988. Lipids of the insect cuticle: origin, composition and function. Comp. Biochem. Physiol. Part B Comp. Biochem. 89, 595–645. https://doi.org/10.1016/0305-0491(88)90305-7

Lord, J.C., Howard, R.W., 2004. A proposed role for the cuticular fatty amides of *Liposcelis bostrychophila* (Psocoptera: Liposcelidae) in preventing adhesion of entomopathogenic fungi with Dry-conidia. Mycopathologia 158, 211–217. https://doi.org/10.1023/B:MYCO.0000041837.29478.78

Lorini, I., Galley, D.J., 1999. Deltamethrin resistance in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), a pest of stored grain in Brazil. J. Stored Prod. Res. 35, 37–45. https://doi.org/10.1016/S0022-474X(98)00028-9

Lü, J., Sehgal, B., Subramanyam, B., 2017. Insecticidal potential of a synthetic zeolite against the cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). J. Stored Prod. Res. 72, 28–34. https://doi.org/10.1016/j.jspr.2017.03.001

Maceljski, M., Korunic, Z., 1972. Trials of inert dusts in water suspension for controlling stored-product pests. Plant Prot. Beogr. Yugosl. 22, 119–128.

Maftoonazad, N., Ramaswamy, H.S., 2006. Spectral measurements in fruits and vegetables. Stewart Postharvest Rev. 2, 1–7. https://doi.org/10.2212/spr.2006.5.12

Malia, H.A.E., Rosi-Denadai, C.A., Guedes, N.M.P., Martins, G.F., Guedes, R.N.C., 2016. Diatomaceous earth impairment of water balance in the maize weevil, *Sitophilus zeamais*. J. Pest Sci. (2004). 89, 945–954. https://doi.org/10.1007/s10340-016-0732-0

Mantel, N., 1966. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother. Reports 50, 163–170.

McGonigle, D.F., Jackson, C.W., 2002. Effect of surface material on electrostatic charging of houseflies (*Musca domestica* L). Pest Manag. Sci. 58, 374–380. https://doi.org/10.1002/ps.463

McGonigle, D.F., Jackson, C.W., Davidson, J.L., 2002. Triboelectrification of houseflies (*Musca domestica* L.) walking on synthetic dielectric surfaces. J. Electrostat. 54, 167–177. https://doi.org/10.1016/S0304-3886(01)00177-2

McLaughlin, A., 1994. Laboratory trials on desiccant dust insecticides, in: Proceedings of the 6th International Working Conference on Stored-Product Protection. Canberra, Australia, pp. 638–645.

Melichar, B., Willomitzer, J., 1965. Bewerting der physikalischen insectizide. Sci. Paraceut. 2: 589–597, in: Proc. 25th Congr. Pharmaceut. Sei. Prague.

Mewis, I., Reichmuth, C., 1998. Diatomaceous earths against the coleoptera granary weevil *Sitophilus granarius* (Curculionide), the confused flour beetle *Tribolium confusum* (Tenebrionidae), the Mealworm *Tenebrio molitor* (Tennebrionidae), in: Jin.Zuxun, Liang, Q., Liang, Y., Tan, X., Guo, L. (Eds.), Proceedings of the 7th

International Working Conference on Stored-Product Protection. Beijing, China, pp. 14–19.

Mewis, I., Ulrichs, C., 2001. Action of amorphous diatomaceous earth against different stages of the stored product pests *Tribolium confusum*, *Tenebrio molitor*, *Sitophilus granarius* and *Plodia interpunctella*. J. Stored Prod. Res. 37, 153–164. https://doi.org/10.1016/S0022-474X(00)00016-3

Michalaki, M.P., Athanassiou, C.G., Kavallieratos, N.G., Batta, Y.A., Balotis, G.N., 2006. Effectiveness of *Metarhizium anisopliae* (Metschinkoff) Sorokin applied alone or in combination with diatomaceous earth against *Tribolium confusum* Du Val larvae: Influence of temperature, relative humidity and type of commodity. Crop Prot. 25, 418–425. https://doi.org/10.1016/j.cropro.2005.07.003

Mihale, M.J., Deng, A.L., Selemani, H.O., Mugisha-Kamatenesi, M., Kidukuli, A.W., Ogendo, J.O., 2009. Use of indigenous knowledge in the management of field and storage pests around Lake Victoria basin in Tanzania. African J. Environ. Sci. Technol. 3, 251–259. https://doi.org/10.5897/AJEST09.043

Miyazaki, Y.N., 1993. Silica gels for controlling insect pests, United Stateds Patent 5,192,545.

Montgomery, D.J., 1959. Static Electrification of Solids. Solid State Phys. 9, 139–197. https://doi.org/10.1016/S0081-1947(08)60565-2

Moon, J.D., Lee, D.H., Kang, T.G., Yon, K.S., 2003. A capacitive type of electrostatic spraying nozzle. J. Electrostat. 57, 363–379. https://doi.org/10.1016/S0304-3886(02)00173-0

Munro, J.W., 1966. Pest of stored products. Hutchinson & Co. Ltd., London, UK.

Nair, M.R.G.K., 1957. Structure of waterproofing epicuticular layers in insects in relation to inert dust action. Indian J. Entomol. 10, 37–49.

Naito, A., 1988. Low-cost technology for controlling soybean insect pests in Indonesia, in: Food and Fertilizer Technology Center. pp. 1–14.

Neethirajan, S., Karunakaran, C., Jayas, D.S., White, N.D.G., 2007. Detection techniques for stored-product insects in grain. Food Control 18, 157–162. https://doi.org/10.1016/j.foodcont.2005.09.008

Noble-Nesbitt, J., 1990. Cellular differentiation in relation to water vapour absorption in the rectal complex of the mealworm, *Tenebrio molitor*. Tissue Cell 22, 925–940. https://doi.org/10.1016/0040-8166(90)90053-C

Nortvedt, R., Torrissen, O.J., Tuene, S., 1998. Application of near-infrared transmittance spectroscopy in the determination of fat, protein and dry matter in Atlantic halibut fillet. Chemom. Intell. Lab. Syst. 42, 199–207. https://doi.org/10.1016/S0169-7439(98)00012-4

Oxley, T.A., 1949. The scientific principles of grain storage. Nature 163, 784.

Pandurangi, R.S., Seehra, M.S., Razzaboni, B.L., Bolsaitis, P., 1990. Surface and bulk infrared modes of crystalline and amorphous silica particles: A study of the relation of surface structure to cytotoxicity of respirable silica. Environ. Health Perspect. 86, 327–336. https://doi.org/10.1289/ehp.9086327

Parker, A.R., Mckenzie, D.R., Large, M.C.J., 1998. Multilayer reflectors in animals using green and gold beetles as contrasting examples. J. Exp. Biol. 201, 1307–1313.

Parle, E., Dirks, J.H., Taylor, D., 2017. Damage, repair and regeneration in insect cuticle: The story so far, and possibilities for the future. Arthropod Struct. Dev. 46, 49–55. https://doi.org/10.1016/j.asd.2016.11.008

Parle, E., Taylor, D., 2013. The Self-healing properties of insect cuticle. J. Postgrad. Res. XII, 90–111.

Perez-Mendoza, J., Flinn, P.W., Campbell, J.F., Hagstrum, D.W., Throne, J.E., 2004. Detection of stored-grain insect infestation in wheat transported in railroad hopper-cars. J. Econ. Entomol. 97, 1474–1483. https://doi.org/10.1093/jee/97.4.1474

Phillips, T.W., Jiang, X.L., Burkholder, W.E., Phillips, J.K., Tran, H.Q., 1993. Behavioral responses to food volatiles by two species of stored-product coleoptera, *Sitophilus oryzae* (curculionidae) and *Tribolium castaneum* (tenebrionidae). J. Chem. Ecol. 19, 723–734.

Pixton, S.W., 1982. The importance of moisture and equilibrium relative humidity in strored products. Trop. Stored Prod. Inf. 43, 16–29.

Prickett, A.J., Ratcliffe, C.A., 1977. The behaviour of *Tribolium castaneum* (Herbst) and *Sitophilus granarius* (L.) the presence of insecticide-treated surfaces. J. Stored Prod. Res. 13, 145–148. https://doi.org/10.1016/0022-474X(77)90013-3

Qi, Y., 2015. Studies on the application of colour preferences and sticky coloured card technolgy for main grain storage pest, Master thesis. Henan University of Technology.

Ralph, M., 1964. Hydrophobic siliceous insecticidal compositions, United States Patent 3,159,536.

Redlinger, L.M., Womack, H., 1966. Evaluation of four inert dusts for the protection of shelled corn in Georgia from insect attack. USDA, Agricultural Research Service Report, ARS 51-6.

Richardson, C.H., Glover, L.H., 1932. Some effects of certain “Inert” and toxic substances upon the twelve-spotted cucumber beetle, *Diabrotica Duodecimpunctata* (Fab.). J. Econ. Entomol. 25, 1176–1181. https://doi.org/10.1093/jee/25.6.1176

Rigaux, M., Haubruge, E., Fields, P.G., 2001. Mechanisms for tolerance to diatomaceous earth between strains of *Tribolium castaneum*. Entomol. Exp. Appl. 101, 33–39. https://doi.org/10.1023/A:1019273506494

Roeder, K.D., 1953. Insect physiology, J. Wiley &. ed. J. Wiley & Sons, Inc., New York.

Šašić, S., Ozaki, Y., 2001. Short-wave near-infrared spectroscopy of biological fluids. 1. Quantitative analysis of fat, protein, and lactose in raw milk by partial least-squares regression and band assignment. Anal. Chem. 73, 64–71. https://doi.org/10.1021/ac000469c

Seifelnasr, Y.E., Hopkins, T.L., Mills, R.B., 1982. Olfactory responses of adult *Tribolium castaneum* (Herbst), to volatiles of wheat and millet kernels, milled fractions, and extracts. J. Chem. Ecol. 8, 1463–1472. https://doi.org/10.1007/BF00989103

Shah, F.A., Allen, N., Wright, C.J., Butt, T.M., 2007. Repeated in vitro subculturing alters spore surface properties and virulence of *Metarhizium anisopliae*. FEMS Microbiol. Lett. 276, 60–66. https://doi.org/10.1111/j.1574-6968.2007.00927.x

Shawir, M., Le Patourel, G.N.J., Moustafa, F.I., 1988. Amorphous silica as an additive to dust formulations of insecticides for stored grain pest control. J. Stored Prod. Res. 24, 123–130. https://doi.org/10.1016/0022-474X(88)90009-4

Shejbal, J., 1980. Controlled atmosphere storage of grains. Elsevier scientific publishing companny, New York.

Smedt, C.D., Someus, E., Spanoghe, P., 2015. Potential and actual uses of zeolites in crop protection. Pest Manag. Sci. 71, 1355–1367. https://doi.org/10.1002/ps.3999

Smith, B.C., 1969. Effects of silica on the survival of *Coleomegilia maculata lengi* (Coleoptera: Coccenellidae) and *Leptmotarsa decemlineata* (Coleoptera: Chrysomelidae). Can. Entomol. 101, 460–462. https://doi.org/10.4039/Ent101460-

5

Snelson, J.T., 1987. Grain protectants. Australian Centre for International Agricultural Research, Canberra, Australia.

Snetsinger, R., 1988. Report on Shellshock insecticide. Report of Department of Entomology, Pennsylvania State University.

Stadler, T., Buteler, M., Weaver, D.K., Sofie, S., 2012. Comparative toxicity of nanostructured alumina and a commercial inert dust for *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) at varying ambient humidity levels. J. Stored Prod. Res. 48, 81–90. https://doi.org/10.1016/j.jspr.2011.09.004

Strong, R.G., Sbur, D.E., 1963. Protection of wheat seed with diatomaceous earth. J. Econ. Entomol. 56, 372–374. https://doi.org/10.1093/jee/56.3.372

Subramanyam, B., Roesli, R., 2000. Inert Dusts, in: Subramanyam, B., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Springer US, pp. 321–380. https://doi.org/978-1-4615-4353-4

Takle, G., Lackie, A.M., 1985. Surface charge of insect haemocytes, examined using cell electrophoresis and cationized ferritin-binding. J. Cell Sci. 75, 207–214.

Teske, M.E., Thistle, H.W., Mickle, R.E., 2000. Modeling finer droplet aerial spray drift and deposition. Appl. Eng. Agric. 16, 351–357. https://doi.org/10.13031/2013.5216

Toniolo, J.C., Lima, M.D., Takimi, A.S., Bergmann, C.P., 2005. Synthesis of alumina powders by the glycine-nitrate combustion process. Mater. Res. Bull. 40, 561–571. https://doi.org/10.1016/j.materresbull.2004.07.019

Underwood, A.J., 1997. Ecological experiments: their logical design and interpretation using analysis of variance. Cambridge University Press, Melbourne, Australia. https://doi.org/10.1017/CBO9780511806407

United Nations Environmental Program, U.N.E.P., 1995. Report of the seventh meeting of the parties to the Montreal Protocol on substances that deplete the ozone layer. Geneva.

Van Tilborg, M., Van der Pers, J.N.C., Roessingh, P., Sabelis, M.W., 2003. State-dependent and odor-mediated anemotactic responses of a micro-arthropod on a novel type of locomotion compensator. Behav. Res. Methods, Instruments, Comput. 35, 478–482. https://doi.org/10.3758/BF03195526

Vayias, B.J., Athanassiou, C.G., 2004. Factors affecting the insecticidal efficacy of the diatomaceous earth formulation SilicoSec against adults and larvae of the confused flour beetle, *Tribolium confusum* DuVal (Coleoptera: Tenebrionidae). Crop Prot. 23, 565–573. https://doi.org/10.1016/j.cropro.2003.11.006

Vayias, B.J., Stephou, V.K., 2009. Factors affecting the insecticidal efficacy of an enhanced diatomaceous earth formulation against three stored-product insect species. J. Stored Prod. Res. 45, 226–231. https://doi.org/10.1016/j.jspr.2009.03.002

Vincent, J.F. V., Wegst, U.G.K., 2004. Design and mechanical properties of insect cuticle. Arthropod Struct. Dev. 33, 187–199. https://doi.org/10.1016/j.asd.2004.05.006

Vrba, C.H., 1992. Insecicíedes, United States Patent, 5,122,518.

Vrba, C.H., Arai, H.P., Nosal, M., 1983. The effect of silica aerogel on the mortality of *Tribolium confusum* (Duval) as a function of exposure time and food deprivation. Can. J. Zool. 61, 1481–1486.

Wang, J., Xu, W., Cao, Y., Li, Y., 2011. Control effects of inert dust against stored grain insect pests in different types of storages. Grain Storage 40, 34–41.

Wang, P., 2008. Inert powder insecticidal mechanism, master thesis. Henan University of Technology.

Wang, X., Cao, Y., Zhang, Z., Qi, Y., Shi, T., Zhang, T., Ji, Z., Yang, X., Zhang, H., Li, Y., 2016. Application of Food grade inert dust aerosol control insects in grain stored houses with tranverse ventilation. J. Chinese Cereal. Oils Assoc. 31, 90–91.

Wang, X., Li, Y., Zhang, T., Shi, Z., Xin, L., 2009a. Application of inert dust for insects disinfestations of cereal grain in north China. Technol. Cereal. Oils 37–39.

Wang, X., Li, Y., Zhang, T., Shi, Z., Xin, L., 2009b. Research on the insect prevention effect of new inert powder protective agent in the warehouse of north of China. Grain Oil Storage Sci. Technol. Commun. 25, 37–39.

Warnke, U., 1976. Effects of electric charges on honeybees. Bee World 57, 50–56. https://doi.org/10.1080/0005772X.1976.11097592

Webb, J.E., 1946. The penetration of derris through the spiracles and cuticle of melophagus ovinus, L. Bull. Entomol. Res. 36, 15–22. https://doi.org/10.1017/S0007485300023889

Whittier, T.S., Kaneshiro, K.Y., Prescott, L.D., 1992. Mating behavior of Mediterranean fruit flies (Diptera: Tephritidae) in a natural environment. Ann. Entomol. Soc. Am. 96822, 214–218. https://doi.org/10.1093/aesa/85.2.214

Wigglesworth, V.B., 1944. Action of inert dust on insects. Nature 153, 493–494. https://doi.org/10.1038/153746a0

Wigglesworth, V.B., 1942. Some notes on the integument of insects in relation to the entry of contact insecticides. Bull. Entomol. Res. 33, 205–218. https://doi.org/10.1017/S0007485300026493

Winks, R.G., 1982. The toxicity of phosphine to adults of *Tribolium castaneum* (Herbst): Time as a response factor. J. Stored Prod. Res. 18, 159–169. https://doi.org/10.1016/0022-474X(82)90026-1

Wold, J.P., Jakobsen, T., Krane, L., 1996. Atlantic salmon average fat content estimated by near-infrared transmittance spectroscopy. J. Food Sci. 61, 74–77. https://doi.org/10.1111/j.1365-2621.1996.tb14728.x

Wu, D., He, Y., Feng, S., 2008. Short-wave near-infrared spectroscopy analysis of major compounds in milk powder and wavelength assignment. Anal. Chim. Acta 610, 232–242. https://doi.org/10.1016/j.aca.2008.01.056

Wu, Y., Duan, Y., Jiang, Y., 2009. Review on lighting for insect pests control. J. Henan Agric. Sci. 127–130.

Wu, Z., 2011. Green ecological technology for grain storage. Science and Technology of China Press, Beijing, China.

Yang, L., Liu, T., Li, J., Wang, J., Cao, Y., Li, Y., 2011. High and big horizontal warehouse inert powder insect prevention grain storage test. Grain Oil Storage Sci. Technol. Commun. 19, 65–67.

Yang, L., Watts, D.J., 2005. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. Toxicol. Lett. 158, 122–132. https://doi.org/10.1016/j.toxlet.2005.03.003

Yao, W., Xue, M., Du, Y., 2005. The dectection of phototaxis of the 8 species stored product insects. Grain Storage 34, 3–5.

Zacker, F., Kunike, G., 1931. Untersuchungen uber die insektizide wirkung von oxyden und karbonaten. Arboric. Aust. Biol. Reichsans 18, 201–231.

Zettler, J.L., Arthur, F.H., 2000. Chemical control of stored product insects with fumigants and residual treatments. Crop Prot. 19, 577–582. https://doi.org/10.1016/S0261-2194(00)00075-2

Zettler, J.L., Cuperus, G.W., 1990. Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. J. Econ. Entomol. 83, 1677–1681. https://doi.org/10.1093/jee/83.5.1677

Zettler, J.L., Halliday, W.R., Arthur, F.H., 1989. Phosphine resistance in insects infesting stored peanuts in the Southeastern United States. J. Econ. Entomol. 82, 1508–1511. https://doi.org/10.1093/jee/82.6.1508

Zhang, H., Dunphy, D.R., Jiang, X., Meng, H., Sun, B., Tarn, D., Xue, M., Wang, X., Lin, S., Ji, Z., Li, R., Garcia, F.L., Yang, J., Kirk, M.L., Xia, T., Zink, J.I., Nel, A., Brinker, C.J., 2012. Processing pathway dependence of amorphous silica nanoparticle toxicity: Colloidal vs pyrolytic. J. Am. Chem. Soc. 134, 15790–15804. https://doi.org/10.1021/ja304907c

Zhang, T., Cao, Y., Li, Y., Gao, Y., Feng, J., 2014. Food-grade inert dust as structural treatment against insect pests, in: Arthur, F.H., Kengkanpanich, R., Chayaprasert, W., Suthisut, D. (Eds.), Proceeding of the 11th International Working Conference on Stored Product Protection. Chiang Mai, Thailand, pp. 883–884. https://doi.org/10.14455/DOA.res.2014.41

Zhao, S., Castle, G.S.P., Adamiak, K., 2008. Factors affecting deposition in electrostatic pesticide spraying. J. Electrostat. 66, 594–601. https://doi.org/10.1016/j.elstat.2008.06.009

Zhou, G., Li, N., Wu, Y., Li, H., Zhang, Z., Cao, Y., Zhang, T., 2017. Effect evalaation of food grade inert dust against stored grain insect pests in rice warehouse. Grain Storage 46, 36–39.