The effect of gut microbiota and insecticide resistance on host plant preferences and voracity of the cotton fly, *Spodoptera littoralis*

Vaida Dzemedzionaite

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Swedish University of Agricultural Sciences, SLU
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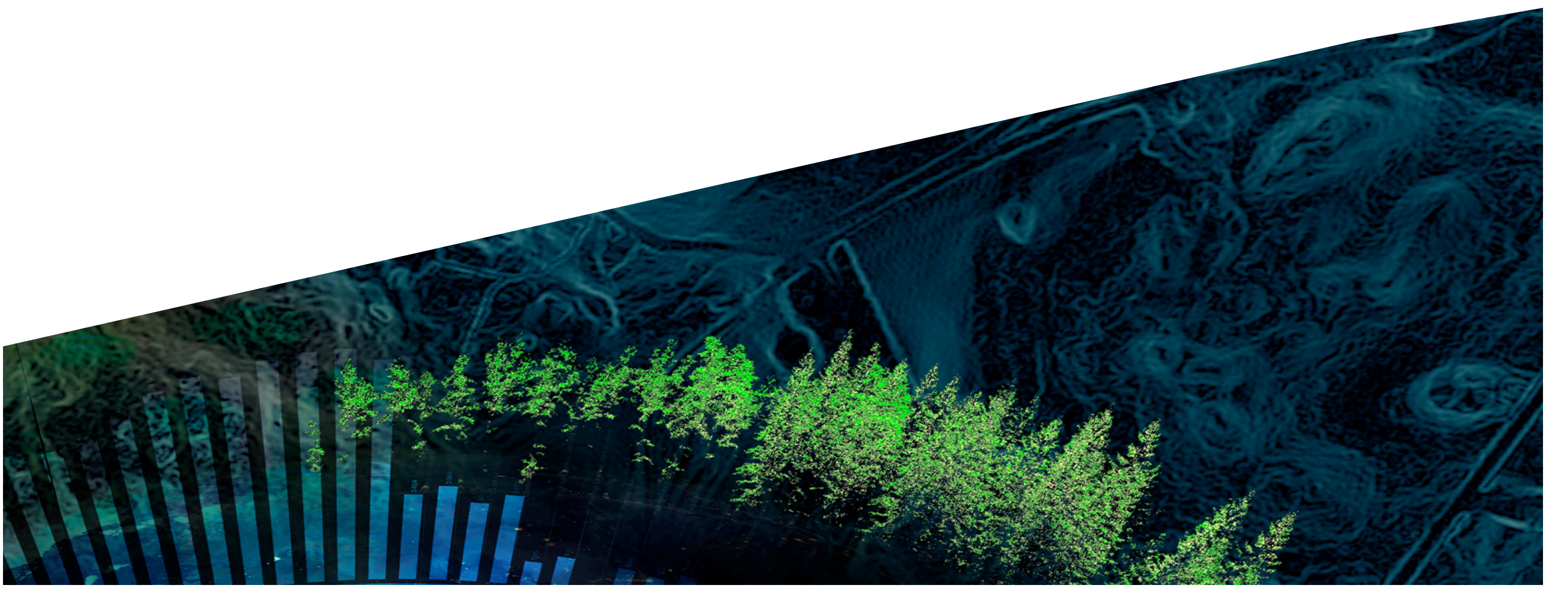

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Foreword

After completing my Bachelor's in Forestry, I realized I wanted to learn more about sustainable food production since food is essential for all the living organisms. The tricky question was if I could incorporate gained knowledge about plants, animals and nature in food production studies. I was lucky to find Pablo Tittonell's speech about agroecology - a term I'd never heard about before. The phrase 'Agroecology works with nature and not against it' gave me an insight to how closely related nature, the environment, people and food we eat are. Therefore, I was very excited once I got accepted to study s master´s program in Agroecology at SLU.

During these two years, I've learned that agroecology covers many aspects. People from different cultures and experiences can interpret it in various ways and create sustainable food production where practice, social movements, and science meet. Farms visits, interviews with people working for food security, discussions with classmates from all over the world and inspiring lecturers sharing their knowledge gave me new perspectives and introduced me to methods that can improve agricultural practices.

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Firstly, I would like to thank my supervisors Kristina Karlsson Green and Audrey Bras for their support, advice, corrections, many hours spent on discussions and shared knowledge during this challenging and exciting period of performing the experiments and writing the thesis.

For the help with the farmers' questionnaires, I want to thank Aneth David for Tanzanian farmers and extension services contacts. Further, Agneta Sundgren from Lantbrukarnas Riksförbund, who took time and gave useful advices on the Swedish questionnaire and Agne Zegleviciute, who shared the questionnaire among Lithuanian farmers. Without their help, making social science part of this thesis would have been very challenging. For the help in the microbiology part I want to thank Paul Becher.

Lastly, I want to say a huge thank you to my family, my partner and my friends for their support, comfort, and encouragement.

Abstract

To counteract pesticide resistance development, it is crucial to understand why and when it evolves. Additionally, to inform the farmers about this term and provide advices on efficient pest management strategies to prevent pesticide resistance. An agroecological approach may be suitable since it applies science, practice and social movements to improve more sustainable food systems by helping developing pest management strategies.

The main aims of this project were gaining better knowledge about the possible connection between insecticide resistance development, host plant range and the effect of gut microbiota and surveying the knowledge among farmers on pesticide resistance development. The experimentations on the pest, *Spodoptera littoralis,* were performed to study if host plant preference and performance with insects that were either pesticide resistant or susceptible and where the gut microbiota was damaged while using the antibiotics or intact.

The preference experiments did not show any results of changed host plant preference or survival rate due to resistance level or gut microbiota status. It was found that larvae's initial host plant choice differs from the final choice indicating that larvae need to feed on the host plant longer to make a choice. However, while treated with the antibiotics, larvae ate significantly more of the leaves than unexposed larvae. Just as for the preference experiment, the efficiency of conversion experiment did not show significant differences in terms of resistance level or gut microbiota. A significant difference in efficiency of conversion was, however, found between the host plants where cotton presented higher indices compared to maize. In addition, larvae gained more weight consuming cotton than on maize, which indicates that maize is a poor host plant and that the host plant plays an important role in larval metabolism.

The social science results indicated that farmers from Sweden, Tanzania and Lithuania are aware of pesticides detrimental effects but still use pesticides very actively, mainly because of their effectiveness. Tanzanian respondents were the only ones who have never heard about pesticide resistance while Swedish farmers presented high knowledge about this process. Better knowledge of pesticide use and pesticide resistance development need to be introduced to Tanzanian farmers to prevent the health problems caused by pesticide application. More sustainable pest management strategies in all the targeted countries are crucial to reduce pesticide use and pesticide resistance development. To do so, communication between researchers and farmers – practitioners is needed.

Keywords: Insecticide resistance, gut microbiota, insect pest, host plant preference, sustainable agriculture

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IPM: Integrated Pest Management

ECI: Efficiency of Conversion of Ingested food

ECD: Efficiency of Conversion of Digested food

AD: Assimilation efficiency (Approximate Digestibility)

1. Introduction

1.1 The role of agroecology in food systems and sustainable pest management

Hunger and poverty still persist in many countries even with the high level of external inputs like machinery or pesticides and intensive agricultural development. An integrated sustainable food production system needs to be developed to combat these global issues (Altieri, 2015). The agroecological approach connects practice, research and social movement concepts to ensure that the food system is less harmful for the environment and public health, it does not negatively affect plants or animals, and is based on fair trade and easy access (FAO, 2018b; Barrios et al, 2020, Altieri, 2018; Gliessman, 2015). Agroecology is based on an interdisciplinary and holistic systems thinking where there are no single solutions (Francis et at, 2003). It is necessary to know how to combine different methods and elements to make this system efficient and achievable. The framework of 10 principles of agroecology plays an important role in the agroecological system and is based on a sustainable way of working with nature. The 10 principles of agroecology are: *Diversity, Co-creation and sharing of knowledge, Synergies, Recycling, Efficiency, Resilience, Human and social values, Culture and food traditions, Responsible governance and finally, Circular and solidarity economy.* With this framework, agroecology aims to resemble ecological functions and processes while reducing equity and justice issues worldwide (Barrios et al, 2020). On a field level, agroecological practices deal with resource-interactions with the environment benefitting production within farms and promoting an alternative sustainable form of agriculture.

Agroecology applies many different methods of reducing pest damage more sustainably and efficiently while harnessing ecosystem services (Gliessman, 2015). Integrated pest management (IPM) is one of the agroecological strategies, promoting a bouquet of effective and sustainable solutions for controlling or eliminating pests while using preventive and curative methods, such as cultural, physical, biological and chemical (Ehler, 2006) (Figure 1). Within this system, pesticides are used at the smallest possible degree to avoid the potential negative effects and resistance development (Flint & Van den Bosch, 2012; Elliott et al, 1995). IPM supports pesticide uses only when biological, physical or cultural treatments fail to control pest populations and we reach the risk of critical yield losses (Barzman et al, 2015; Altieri, 1985). Nonetheless, in many cases, chemical-based pest management is known and used more compared to the alternative plant protection methods. When thinking about sustainable agroecosystems with regards to pest control, a question arises on how to improve pest management while taking into consideration all the potential indirect effects pesticides can cause.

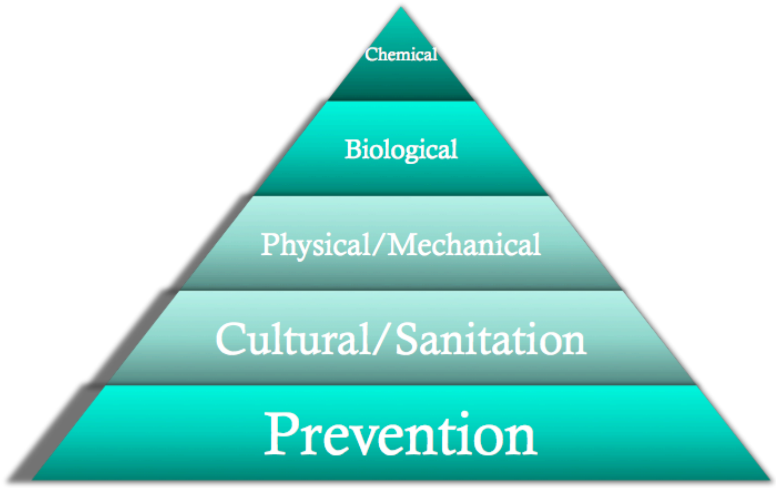

Figure 1. The IPM pyramid presenting sustainable pest management strategies. The most common method appears as a base of the pyramid (Prevention) and the least preferable comes at the top (Chemical) *(Adapted from: Laura Grenville-Briggs Didymus lectures, 2021).*

1.2 The use of pesticides

Since the beginning of agriculture, farmers have been experiencing yield loss partly caused by pests, weeds and diseases. To fight these issues, natural methods such as fire ash or seawater were used, however, they are time consuming and not always effective (Unsworth, 2010; Khan et al. 2000). Pest control plays an important role in agriculture, especially in developing countries that suffer from food deficiency since it minimizes yield loss and prevents starvation (Costa, 2008; World Health Organization, 2018). Additionally, in developed countries pesticides also bring benefits, such as improvement of fruit and vegetable appearances, freshness and easy access.

The introduction of pesticides changed agricultural practices by significantly reducing postharvest losses (Al-Saleh, 1994). Pesticides are defined as a chemical substance or a mixture of substances that aim to control and eliminate pest populations, including weeds, insects, fungi and rodents (Mahmood et al, 2016). Pesticides are known as an effective product to kill or repel pests, help to reduce global hunger and prevent harmful diseases from spreading. Moreover, they are known to be widely used and well adopted in both big and small-scale farms (Cooper & Dobson, 2007). However, being advantageous on the one hand, pesticides are responsible for a vast number of adverse environmental and health effects on the other hand (Matowo et al, 2020).

Ideally, pesticides are supposed to affect only targeted pest groups and have no harmful effect on other organisms (Aktar et at, 2009). Unfortunately, in many cases, they could threaten non-target organisms, since they might be toxic to plants, beneficial insects or humans (World Health Organization, 2020). Pesticide use has a strong linkage to poisoning, whether through the direct contact with the chemical compounds in pesticides or via indirect contact while using contaminated products (Devine & Furlong, 2007). Additionally, pesticides bring ecological consequences, for instance, they enhance agricultural intensification processes that could lead to soil and water contamination, harm several natural populations, and affect agroecosystems (Geiger et al, 2010; Hedlund et al, 2020; Devine & Furlong, 2007). Finally, another concern of pesticide use is their efficiency loss due to the development of resistance in pests, leading to yield loss and food insecurity.

1.3 Pesticide resistance caused by evolutionary processes

Resistance to pesticides is a worldwide issue for food security (Tabashnik & Johnson, 1999). Pesticide resistance evolves when pests are exposed intensively over several generations to a certain type of pesticide, resulting in reduced pesticide efficiency (Devine & Furlong, 2007). This process leads to continuous failures of reaching the expected control level of the pest populations in the long term. Pesticide resistance develops faster if there is either a high dose, frequent or incorrect use (Maino et al, 2018). This concern takes place in the agricultural sector and affects farmers practitioners directly while causing production loss leading to financial instability. Moreover, it is crucial to use pesticides to a minimum dose and build more efficackient resistance management to avoid pesticide resistance (Gardner et al, 1998). To set more effective and sustainable pest management strategies as well as reducing pesticide resistance development, a better understanding of evolution of pesticide resistance is then needed (Desprès et al, 2007; Karlsson Green et al, 2020).

Over time, insects evolved the mechanisms to cope with naturally produced plant defensive chemicals called allelochemicals to survive and feed on (Dermauw et al, 2013). Insects therefore are pre-adapted to handle toxins and could potentially use a similar mechanisms to evolve insecticide resistance. This process is called pre-adaptation hypothesis (Desprès et al, 2007; Hardy et al, 2018). Moreover, Rosenheim et al. (1996) predicted that if insects feed on diverse plants, they would evolve more diverse defensive mechanisms, leading to a higher resistance to insecticides. This suggests that generalists which correspond to species feeding on different plants have higher chance of evolving resistance faster than specialists which feed only on a restricted number of plants.

Similarly, recent research has shown that pesticide resistance could evolve through cross-resistance during the species' adaptation to a new host (Dermauw et al, 2013). The cross-resistance could potentially work in both directions, where a larger host plant range leads to pesticide resistance as well as pesticide resistance leading to a larger host plant range (Bras et al in prep, 2021). However, there is not much known about the cross-resistance connection from an ecological perspective (Bras et al in prep, 2021). A better understanding of ecological and environmental parameters (Figure 2) might be important to develop pesticide resistance management and use this gained knowledge to advise farmers practitioners on chemical pest control strategies (Desprès et al, 2007; Barrios et al, 2020; Karlsson Green et al, 2020).

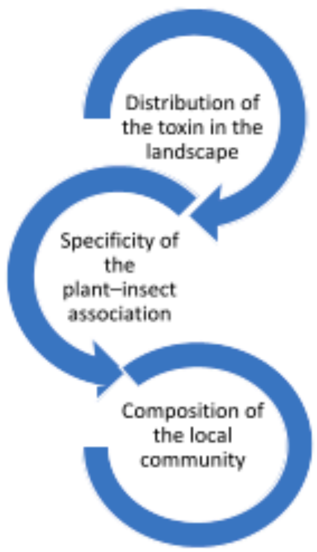

Figure 2. Three main parameters causing uneven insecticide spreading.
(Adapted from: Desprès et al, 2007).

One link behind the cross-resistance might be if gut microbiota is involved in both pesticide resistance and host plant tolerance. Indeed, insects gut microbiota is constituted of different microorganisms, which can play a role in insect's feeding, digestion, development, survival rate, protection from pathogens, resistance to parasitism or even insecticide resistance (Xia et al, 2020; Gadad & Vastrad, 2016; Ugwu et al, 2020). The loss of microorganisms can indicate the absence of natural development or even cause a decrease of insect's survival (Thakur et al, 2016; Fukatsu & Hosokawa, 2002). Previous research studies also discuss the importance of gut microbiota for the host plant performance in Lepidoptera species and whether the gut microbiota influences their host plant preferences (Hammer et al, 2017). According to Gadad and Vastrad (2016), susceptibility to insecticides of Lepidoptera species increases when larvae are treated with antibiotics, which damage the insect's gut microbiota. Following that, presenting an intact gut microbiota could improve insecticide resistance development. However, more research is needed to ensure how gut microbiota is connected with insecticide resistance.

1.3 Pesticide resistance and its socio-economic aspects

Pesticides present two main problems – harmful side effects and resistance development. It is therefore important to decrease their use and simultaneously, improve the correct pesticide usage. A key to this is to understand farmers' perspectives on pesticide use and to ensure that they are applying them in the right and most efficient way. However, some socio-economic angles play a role in farmers' decisions. It is known that modern agriculture is driven by pesticides, big scale production and continuous profit (Gould et at, 2004; Hedlund et al, 2020; Gliessman, 2015; Bakker et al, 2020). Therefore, farmers are obliged to buy new pesticides to secure their yield and sustain high production. If they decide to switch to more sustainable and less harmful methods against the pests, it's hard to move from the treadmill of production (you need to use more pesticides to ensure the efficiency but if you use high amounts, they build resistance). Hence, the development of knowledge that targets alternative ways for pest management is crucial to achieve sustainable agriculture.

However, to make this knowledge useful in practice, it is crucial to know how familiar practitioners are – in this case farmers, with their own role in contributing to pesticide resistance, its development, harmful side effects of chemical plant protection methods and how this knowledge can be helpful for building an effective and more sustainable pest management strategy. Sadly, a lack of farmers' knowledge of pesticides risks and the evolution of pesticide resistance are one of the main problems while integrating more sustainable pest management methods (Petrescu-Mag et al, 2019).

In addition, the knowledge on how plant protection methods vary according to different social contexts and cultures and if farmers are aware of alternative ways can play a role in better communication between researchers and practitioners. Previous studies have shown that the level of economic development in different countries could potentially impact pesticide use. According to Hedlund et al (2020), wealthy countries are more cautious about the hazard chemical compounds and are more likely to control pesticide use compared to less-wealthy countries. Additionally, agricultural knowledge, experience and attitudes on pesticide use could differ between different cultures and traditions (Aktar et al, 2009).

1.5 Project aims and research questions

The overall aims of this project were to better understand the potential links between insecticide resistance development, host plant range and the effect of gut microbiota as well as surveying the knowledge among farmers on pesticide resistance development. The natural science part was performed by experimentally investigating the interaction between resistance level, gut microbiota and insecticide resistance of a polyphagous crop pest, *Spodoptera littoralis,* on its larval host plant preference and performance.

S. littoralis moth is known for its wide range of host plants, feeding on leaves and fruits of more than 44 different plant families. This pest damages crops and causes huge yield loss mostly in African countries but also appears in South Europe (Khan et al. 2010; 2021; Ugwu et al, 2020). Moreover, this species is used as a model species because of its agricultural importance and very flexible host plant preference and performance (Tang et al, 2012). *S. littoralis* has developed a high resistance rate to different insecticides, such as organophosphates, carbamates, and pyrethroids (Mosallanejad et al, 2009). It is then crucial to find control methods of *S. littoralis* to reduce its damage while enhancing food security and sovereignty.

Figure 3. S. *littoralis* larvae on the host plant stem

The social science part of this project included an internet-based survey sent to farmers from three different countries: Sweden, Lithuania and Tanzania. These countries were chosen of interest to compare farmers' pesticide use and pesticide resistance knowledge in developing and developed countries. Additionally, Tanzanian farmers face problems with the pest we have studied (*S. littoralis*) in their fields and must fight this generalist species (Robertson, 1973; Nyambo, 1988).

For decreasing pesticide resistance, it is important to implement management strategies that consider evolutionary processes but also considers farmers' knowledge, experience and needs. Since the agroecology master's program is interdisciplinary and includes both natural and social science aspects, research questions were formulated accordingly.

Natural science:

To understand the role of both the gut microbiota and the development of insecticide resistance, the generalist pest moth *S. littoralis* was used as a model species to answer the following research questions:

- Does larval host plant preference change depending on whether the parents developed or not resistance to a pyrethroid insecticide and whether larvae have an intact gut microbiota or not?
- Is there a difference between pesticide resistant and pesticide susceptible larvae in their ability to detect and feed on plants treated with an insecticide? If so, does the gut microbiota play a role?
- Does the larval efficiency of conversion differ on different host plants depending on whether larvae are resistant or susceptible to pesticides and whether they have an intact gut microbiota or not?

Social science:

The comparisons between Swedish, Lithuanian and Tanzanian farmers' responses were made to define whether different educational background, farming experience or knowledge on evolution affects farmers pest management decisions and their use of pesticides. A questionnaire was sent out to answer the following research questions:

- What are the main plant protection methods that farmers use and do they differ between targeted countries?

- What is farmers' knowledge about pesticide resistance development and how do they handle it? Does it differ between countries?

2. Materials and methods

2.1 Insects

All the experiments were performed with the laboratory strains of *S. littoralis* moths collected in Alexandria, Egypt, in 2008. Egg batches (Figure 4) from a susceptible strain and a resistant strain (reared by a supervisor) of *S. littoralis* exposed for 6 generations to Cypermethrin, a pyrethroid insecticide, were taken from the rearing facility at the Department of Plant Protection Biology at SLU, Alnarp.

The neonate larvae from the egg batches were assembled and grown on a potato-based artificial diet (Appendix n°1). Insect rearing was performed under controlled conditions of 26 ± 1 °C, 65% ± 5% relative humidity (RH), and a photoperiod of 14: 10 h (L:D).

2.2 Antibiotics treatment

To study the impact of gut microbiota we aimed to reduce the bacterial community of *S. littoralis*. After hatching, larvae were fed an artificial diet (where antibiotics were either added or not) on three occasions before the dissection (100 larvae per box with 15g of food). Thus, we had four different experimental conditions: susceptible strain with antibiotics, susceptible strain control, resistant strain with antibiotics and resistant strain control (Figure 5).

Figure 4. S. littoralis egg batches with artificial diet

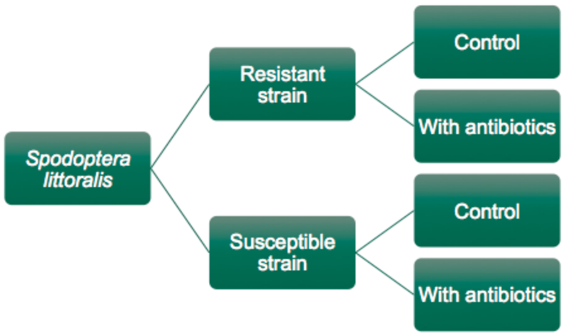

Figure 5. Four different larval treatments with two main factors (the strains and antibiotics treatment) that were used to perform the experiments

The antibiotic mixture consisted of 35 ml of 200 mg Streptomycin diluted in 50 ml of sterilized water and 35 ml of 200 mg Ampicillin diluted in 50 ml of water. The mixture was then mixed into a 1 l of standard *Spodoptera* food. The optimal antibiotic concentration and the effects of the cocktail on larval survival were determined in trial experiments (Heithausen in prep, 2021).

2.3 Insects' gut dissection

To ensure that the antibiotics damaged the larval gut microbiota, we dissected the guts to extract the bacterial communities from it. The guts of a subsample of 3rd instar larvae from four different treatments (six larvae per treatment) were use for the dissection prior to the experiments. Larvae were starved for 2 hours and weighed before the dissection. The dissections were performed under sterile conditions below a laminar hood. Two replicates with dilutions 1:10, 1:100, 1:1000 were made to be able to count the colonies that have grown in the petri dishes. For each replicate, the guts of four larvae were pooled together into 100µl of sterilized milliQ water and 10µl of the solution was homogeneously spread on the petri dishes (8.5cm diameter) (Appendix n°2). LB agar medium was used to grow the bacteria. The petri dishes were placed at 30°C in the incubator and colonies were left to grow for 72 hours. Since the experiment on the efficiency of conversion was performed with a new generation (G=7), dissection was made again following the same protocol to ensure that the gut microbiota was damaged using antibiotics in the larvae food. If the antibiotics method worked, it was expected to get no bacterial colonies growing in the petri dishes after applying the antibiotics.

2.4 Plants

To perform the host plant preference and performance experiments, 4 plant species were chosen: cotton (*Gossypium hirsutum*, Malvaceae), cabbage (*Brassica oleracea v. capitata*, Brassicaceae), maize (*Zea mays*, Poaceae) and giant lily (*Crinum asiaticum,* Amaryllidaceae). Cotton and maize plants are known as preferable ones by *S.littoralis* to feed on (Thöming et at, 2013) while cabbage is not preferred but larvae perform well on this plant (Personal communication, Karlsson Green, 2021) while giant lily was chosen as a novel host plant. All the plants were cultivated for about 5 weeks in the greenhouse until they were used for the feeding preference and performance experiments. Only non-flowering stage plants were used.

2.5 Larval host plant preference and performance: 4-choice experiment.

Standardized choice test was carried out to investigate changes in preference and feeding behaviour of *S. littoralis* between four different plants depending on *S. littoralis'* susceptibility to insecticide and the presence of its gut microbiota (Figure 6). The 3rd instar larvae were used since this stage has been exposed to Cypermethrin during the selection of the strains. To increase larval feeding activity, larvae were starved for 30 min. before being exposed to the four different host plants. In each petri dish (8.5cm diameter), leaf disc pieces (2.5 cm diameter) of the four different host plants were placed upside down with a moistened filter paper (2.5 cm x 2.5 cm) at the bottom to prevent the leaf from drying. At the start of the experiment, each larva was placed in the center of a petri dish and left for 24 hours to feed on leaf discs. In total, 120 larvae were tested, corresponding respectively to 30 larvae per treatment.

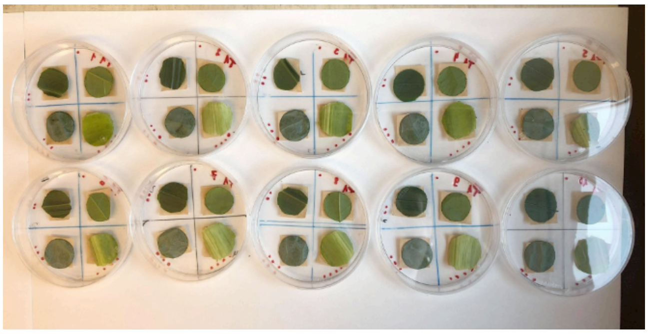

Figure 6. Experimental setup with the four different leaf discs of the host plants

To determine larval first choice of the host plant species, larvae were observed for the first 30 minutes and the host plant on which they went to first was recorded. After that time, observations were performed after 1h, 3h, 6h and 24h. Each time, the percentage of eaten leaf was recorded and at the end of the experiment, the plant that larvae ate the highest percentage from was noted. The data was analysed after 24h to ensure larvae had enough time to make a choice between the host plants.

2.6 Larval preference and survival after insecticide application: 2- choice experiment.

To understand if the resistant larvae have evolved the ability to recognize a pesticide treatment and if the gut microbiota plays a role for this recognition, an experiment where larvae were given a choice between an insecticide treated and an untreated cotton leaf was performed. Additionally, the effect of resistance level and gut microbiota on larvae survival was examined. The observations consisted of 2 cotton disc leaves (3.8 cm diameter) - control one (without insecticide application) and treated with insecticide (ad - cypermethrin + acetone + water, concentration 260 ng/µL) (Figure 7). 50µL of insecticide solution was spread equally while pipetting on one of the leaf discs (Figure 8). Insecticide spreading and the following observations were performed under the fume hood with proper protection for safety. Like the previous experiment, larvae with 4 different treatments (susceptible strain with antibiotics, susceptible strain control, resistant strain with antibiotics, resistant strain control) were used and starved for 30 min before the experiment. After the leaf discs had absorbed insecticide solution, each larva was placed at the center of the petri dish (8.5cm diameter). The first larvae's feeding choice was recorded after 30min, 1h, 3h, 6h and 24h. Same as in 4-choice experiment, data was analyzed after 24h to ensure larvae had enough time to perform feeding choice. The percentage of leave consumption was ranked as in the 4-choice experiment (see paragraph 2.4) and the same number of larvae per treatment (n=30) were used and in total (n=120).

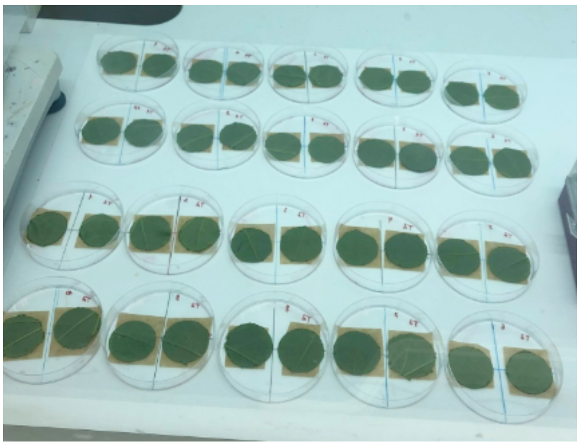

Figure 7. Experimental setup with cotton leaves in the fume hood

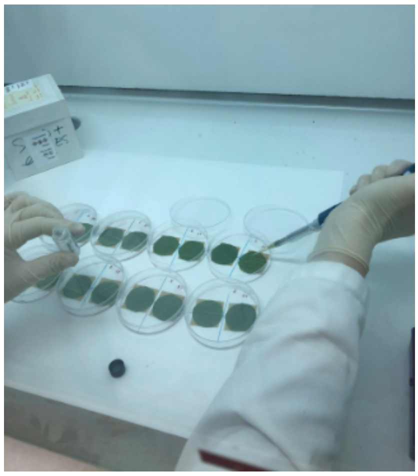

Figure 8. Cypermethrin application on the right side of the leaves in the petri dish

2.7 The Efficiency of conversion

To examine if resistance level and gut microbiota plays a role in *S. littoralis* abilities to utilize its host plant diet and turn it to growth, an efficiency of conversion experiment was performed with maize and cotton disc leaves (3.8 cm diameter) placed upside down with moisture filter paper (3 cm x 3 cm) underneath to prevent the leaf from drying. Cotton and maize were used since both are host plants for *S.littoralis* but present different leaf structures.

Before starting the experiment, larvae were starved for 30min and weighed. Larvae were placed in the center of a plastic cup (5,5 cm x 4 cm x 7 cm) and left to feed on either cotton or maize leaves for 72h. Each day, new leaves were weighed and then given to the larvae to ensure that they had fresh food (Figure 9). After 72h, larvae were weighed again to record their weight gain. At the end, the remaining leaves, the filter paper and larval frass were dried at 80°C for 24h for each larva to avoid measuring water loss (Figure 10). In total, 160 larvae were tested (80 larvae per plant within 4 treatments).

Figure 9. Experimental setup for the efficiency of conversion experiment. Larvae fed on cotton leaves for 72h.

Figure 10. Drying process at the end of the efficiency of conversion experiment.

To calculate the efficiency of conversion indices for each plant, 15 intact leaf discs, their filter paper and larval frass were weighed before and after drying. From this, linear regressions were performed to address the relationship between fresh and dry weight for cotton and maize leaves, filter papers and frass, respectively. The equations for regression lines were used to calculate dry leaf relation to the fresh leaves. That was then used to estimate the different efficiency of conversion nutritional indices calculated as following (Blackford, 1996; Waldbauer, 1968):

- *Efficiency of conversion of ingested food (ECI)*

= larval mass gained/mass of food ingested

- *Efficiency of conversion of digested food (ECD)*

= larval mass gained/(mass of food ingested-mass of frass)

- *Assimilation efficiency (approximate digestibility) (AD)*

= (mass of food ingested-mass of frass)/mass of food ingested

- *Mass of food ingested*

= dry leaf weight - dry leaf leftovers weight

Larval weight (mg) after experiment was subtracted from larval weight (mg) before the experiment to see how larvae weight gain varies between different treatments and host plants.

2.8 Countries and farmers questionnaire

To answer if the targeted countries have different knowledge about pesticide resistance, the questionnaire was designed accordingly. It consisted of information about farmers' education, farming experience, pest management strategies, evolution and the linkage these factors have on knowledge about pesticide resistance. The questionnaire consisted of 23 questions in total and was divided into the following sections: background information (3 questions), information about the farm (4 questions), plant protection problems (2 questions), chemical plant protection (n=4), resistance of chemical plant protection products (n=6), knowledge about evolution (3 questions) and environmental issues/sustainability (1 question). In the questionnaire, 21 questions had multiple choices or checkboxes and were followed by 2 open questions and an additional part with voluntary comments from the respondents.

The quantitative semi-structured questionnaire was designed and shared on the online survey platform '*Google*' forms in Swedish, English and Lithuanian. In Sweden, it was sent to 30 farmers, 70 land surveyor students who own farms and it was additionally shared on the social media '*Facebook*' *via* the group for farmers called '*Spannmålsbönderna*'. In addition, 30 Tanzanian farmers were contacted *via* extension services for farmers or directly through social media and 40 Lithuanian farmers were contacted through the Center for Precision Farming Services and Competencies, Lithuanian Agricultural Advisory Service.

The full Swedish version of the questionnaire can be found in the Appendix n°3

In parallel, we performed a short survey on the main information about agriculture in the targeted countries, which is presented in Table 1.

Table 1. Agricultural information about the targeted countries (Tanzania, Sweden and Lithuania).
(Adapted from: Lerna et al, 2014; Ngowi et al, 2007; Julien et al, 2019; Matowo, 2020; International Trade Administration, 2021; jordbruksverket, 2021; Food and Agriculture Organization of the United Nations, 2021).

	TANZANIA 	SWEDEN 	LITHUANIA 
Climate conditions	Extreme rainfall, dry land	Cold, rainy winters, warm summers, differs between north and south part	Cold winters and rainy warm summers
Main crop production	Corn, wheat, rice, sugar, maize, cassava, sweet potatoes (ref)	Barley, oats, wheat, rye, sugar beets, potatoes	Barley, winter wheat, winter rye, spring wheat, potatoes, oats and pulses.
Average farm size	3 ha	36 ha	12 ha
Farming challenges	Sporadic bans on export-import, extreme climatic conditions lead to land degradation and limits productivity, hard to afford proper machinery	High food waste, seasonally dry land, less people want to continue farming	Hard to protect yields because of banned pesticides
Extra information	Applied pesticides in mixtures missing exact use instructions from the labels or extension workers, poor pesticide use knowledge and side effects	Intensive tillage, increasing pesticide use, heavy machinery	Some banned pesticides are still used, transported from neighboring countries

2.9 Data analysis

Statistical data analysis was performed using RStudio program (Version 1.4.1106, © 2009-2021 RStudio, PBC.) and Microsoft Excel (Version 14.5.5). RStudio was used for the host plant preference experiments' statistical analysis while Microsoft Excel was used for calculating indices and making a linear regression for the efficiency of conversion.

2.9.1 Larval host plant preference and performance: 4-choice experiment.

Initial and final larval preference:

To see if resistance level and the antibiotics treatment have any effect on larval preference, we analyzed if the treatment groups differed in which leaf they chose to feed on initially, as well as which leaf they chose to eat the most at the end of the experiment. The main factors in statistical analysis were 'Strain' and 'Treatment' with four different groups that are combinations of the resistance level and antibiotics treatment (susceptible strain with the antibiotics, susceptible strain control, resistant strain with the antibiotics and resistant strain control). Pearson's Chi-square test was used to determine if there was a statistically significant difference between the susceptible and resistant larvae strains treated with and without the antibiotics in the larval initial and final choice preference of the host plants.

Average consumption between the different treatments and host plants:

A two-way ANOVA test was performed to analyze whether the different treatments had an impact on the average consumption of the host plants in total and the average consumption for each host plant. The main factors in statistical analysis were 'Strain' as resistant and susceptible with 'Treatment' as antibiotics and control. An extra factor was the interaction between the strains and treatments.

2.9.2 Larval preference and survival after insecticide application: 2- choice experiment.

Initial and final larval preference:

As previously mentioned in 2.9.1. paragraph, statistical analysis was performed on 'Strain' and 'Treatment' with 4 different groups that are combinations of the resistance level and antibiotics treatment. Pearson's Chi-square test was used to determine if there was a statistically significant difference between susceptible and resistant larvae strains treated with and without the antibiotics in their initial and final choice preference of the host plants.

Average consumption between different treatments:

A two-way ANOVA test was made to analyze how different treatments affect larvae feeding preference and performance with the control cotton leaf and cotton leaf treated with Cypermethrin. The main factors in statistical analysis were 'Strain' as resistant and susceptible with 'Treatment' as antibiotics and control. An extra factor was interaction between the strains and treatments.

Larval mortality:

Pearson's Chi-square test was used to identify whether larval mortality was affected by different treatments within control and insecticide treated leaf.

2.9.3 Efficiency of conversion

Two-way ANOVA tests were made to analyze the efficiency of conversion of ingested food, efficiency of conversion of digested food and assimilation efficiency (approximate digestibility) between the different treatments and the host plants (cotton and maize). Additionally, larval weight gain and consumption was calculated. The main factors in statistical analysis were 'Strain' as resistant and susceptible with 'Treatment' as antibiotics and control. Also the interaction between strains and antibiotics treatment was included.

2.9.4 Questionnaire

Descriptive statistics were used to analyze farmers' responses about the use of pesticides, the knowledge on evolution and pesticide resistance development between Sweden, Lithuania and Tanzania. The responses from the questionnaires from the targeted countries were firstly translated into English, then transcribed into Excel and afterwards, different calculations such as the number of respondents, the answers choice frequency (%) and analysis were carried out.

The IPA (interpretive phenomenological analysis) was used to represent the open answers with the 'tag clouds' visualization method. The most relevant keywords appearing in the answers were written in the blank sheet and copied to the tag cloud generator as many times as they were mentioned in the answers. Then the tag cloud generator counted the words and situated them into a figure that represents the frequency of the words using different sizes (Heimerl, 2014).

3. Results

3.1 Dissection of insects' gut microbiota

To confirm that the antibiotics treatment worked during the assays, colonies were grown from 3rd instar larvae' gut. We found none or a very little number of bacterial colonies for the larvae treated with the antibiotics diet (Table 2, CFU mean after 78h), whereas many bacterial colonies were found for untreated larvae. Our results indicated that larvae gut microbiota was damaged while using the antibiotics.

Table 2. CFU (colony forming unit) showing the estimation of microorganisms' concentration in the petri dish with and without the antibiotics in larvae food between the resistant and susceptible strains.

Exposition to antibiotics	**Strain**	**Hatching date**	**Dissection date**	**CFU mean after 78h**
No	Susceptible	20.02.21	25.02.21	490
Yes	Susceptible	21.02.21	25.02.21	0
No	Resistant	22.02.21	25.02.21	790000
Yes	Resistant	21.02.21	25.02.21	15
No	Susceptible	24.02.21	01.03.21	TNTC
Yes	Susceptible	23.02.21	01.03.21	7
No	Resistant	23.02.21	01.03.21	3140000
Yes	Resistant	23.02.21	01.03.21	100
No	Susceptible	18.03.21	26.03.21	1000
Yes	Susceptible	18.03.21	26.03.21	10
No	Resistant	18.03.21	26.03.21	40
Yes	Resistant	18.03.21	26.03.21	70
No	Susceptible	25.03.21	02.04.21	20
Yes	Susceptible	25.03.21	02.04.21	0
No	Resistant	25.03.21	02.04.21	30
Yes	Resistant	25.03.21	02.04.21	1

3.2 Larval host plant preference and performance: 4-choice experiment.

Choice test:

The different treatments (resistant strain with antibiotics, resistant strain control, susceptible strain with antibiotics, susceptible strain control) did not affect larvae *initial* choice preference (Chi2: df = 9, χ^2 = 4.880, *p.value* = 0.845). From all 120 individuals, the highest number of larvae preferred maize plant as their initial choice (n=49), followed by cabbage (n=28), cotton (n= 23) and finally giant lily, the new host plant (n=20).

The *final choice* was recorded to detect if larvae 's initial choice differs from the final choice and whether the larvae chose their preferred plant from the beginning or changed their choice after feeding on the plants. Just as the initial choice, the different treatments didn't affect larvae's final choice (Chi2: df = 9, χ^2 = 14.141, *p.value* = 0.117). In contrast to the initial choice preference, most of the larvae preferred to eat cabbage (n=58), cotton (n=48) and maize (n=13). Although giant lily had higher number for the initial choice (n=20), the number decreased drastically for the most eaten leaf (final) choice (n=1).

Average consumption:

In the analyses of whether the different strains and antibiotics treatment influenced larvae consumption, it was found that larvae treated with antibiotics ate significantly more (mean=55%) than unexposed larvae (mean=38.7%) (Figure 11, $F_{1,116}$ = 9.915, *p.value* = 0.002). However, no significant difference was found between the susceptible and resistant strains ($F_{1,116}$= 1.499, *p.value* = 0.223) as well as no interaction between the antibiotics treatment and the strains ($F_{1,116}$ = 0.739, *p.value* = 0.391).

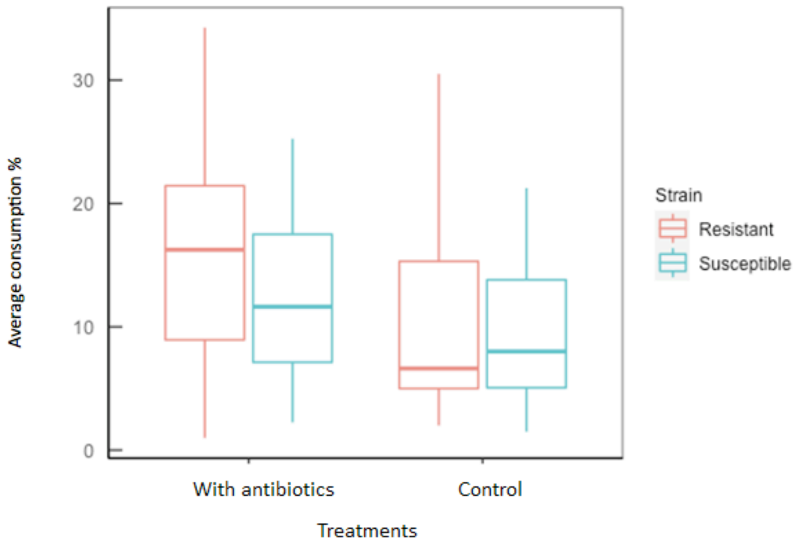

Figure 11. Percentage of average consumption for the two strains treated with the antibiotics and control in *Spodoptera littoralis* including all the host plants

Average consumption between the different treatments and host plants:

The average consumption of each host plant was estimated: cotton 22.8%, cabbage 16.7%, maize 6.6% and lily 0.75% (Figure 12).

Cotton plant. Larvae treated with the antibiotics were found to eat significantly more cotton leaves (29.6%) compared to the control (16.1%) ($F_{1,116}$= 6.754, *p.value* = 0.011). Additionally, a significant interaction effect between the antibiotics treatment and the strains was found ($F_{1,116}$ = 4.264, *p.value* = 0.041) (Figure 12). The resistant strain with the antibiotics had a higher eaten amount (38.1%) compared to the susceptible strain with the antibiotics (21%) while the resistant strain control was recorded to have a lower eaten amount (13.9%) compared to the susceptible strain control (18,3%). However, no significant difference was found between resistant and susceptible strains ($F_{1,116}$= 1.510, *p.value* = 0.222).

Maize plant. Susceptible larvae ate significantly more (11.5%) than the resistant strain (1,7%) (Figure 12) ($F_{1,116}$= 11.859, *p.value* = 0.001). Besides that, larvae had a tendency of eating more while treated with the antibiotics (9,3%) compared to the control (3.8%) ($F_{1,116}$ = 3.700, *p.value* = 0.057). Nevertheless, the interactions between the antibiotics treatment and the different strains had no significant difference ($F_{1,116}$= 3.566, *p.value* = 0.061).

Cabbage plant. A significant difference was found between the resistant and susceptible strains ($F_{1,116}$ = 4.682, *p.value* = 0.033). The larvae from the resistant strain consumed more leaves (21.2%) compared to the susceptible strain (12.2%) (Figure 12). However, neither antibiotics treatment ($F_{1,116}$= 0.377, *p.value* = 0.540) nor antibiotics treatment with different strains ($F_{1,116}$ = 0.039, *p.value* = 0.844) had significant differences.

Lily plant. No significant differences were found in giant lily consumption in terms of the antibiotics treatment ($F_{1,116}$= 0.058, *p.value* = 0.809), different strains ($F_{1,116}$= 0.935, *p.value* = 0.336) or interaction between the antibiotics treatment and the strains ($F_{1,116}$= 0.033, *p.value* = 0.856) (Figure 12).

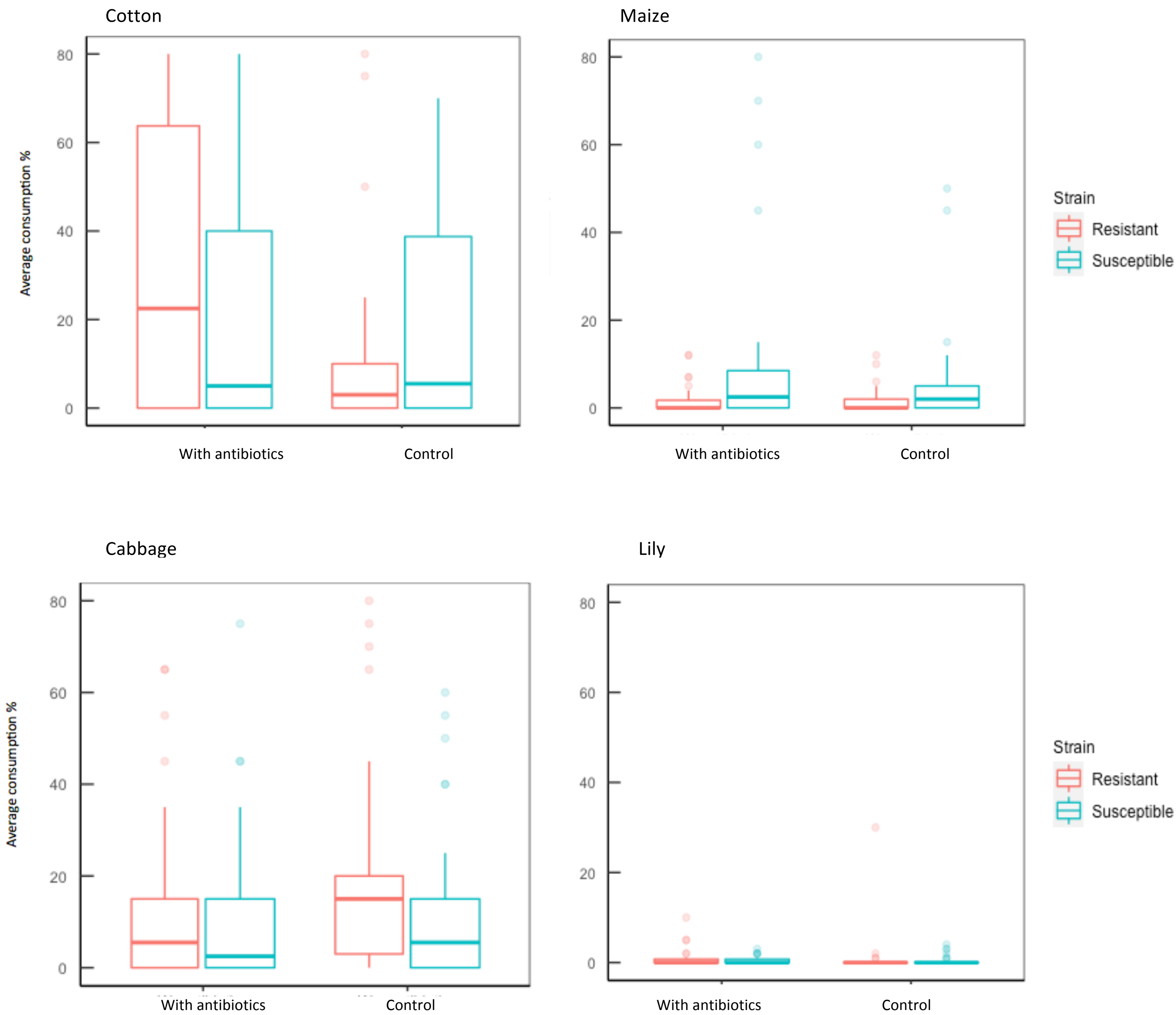

Figure 12. 4-choice experiment. The comparison of the average consumption of all the host plants (cotton, maize, cabbage and lily) between different treatments (resistant strain with antibiotics, resistant strain control, susceptible strain with antibiotics and susceptible strain control).

3.3 Larval preference and survival after insecticide application: 2- choice experiment.

Preference test:

No significant difference between the four treatments between the control cotton leaf and the leaf treated with Cypermethrin was found for both *initial* (Chi2: df = 3, χ^2 = 1.4337, *p.value* = 0.698) and the *final* larval preference (Chi2: df = 3, χ^2 = 1.993, *p.value* = 0.574). Preference for the initial choice between control leaf (n=61) and treated with Cypermethrin (n=59) slightly changed at the final choice most eaten leaf: control leaf (n=67) and treated with Cypermethrin (n=53).

Average consumption:

The average consumption test was performed to analyze if the different treatments affected larval preference when given a choice between a control cotton leaf and a Cypermethrin treated cotton leaf (Figure 13.) No significant differences between the antibiotics treatment ($F_{1,236}$ = 0.648, *p.value* = 0.422), the different strains ($F_{1,236}$ = 0.029, *p.value* = 0.864) or the interaction between the antibiotics treatments and strains ($F_{1,236}$= 0.162, *p.value* = 0.688) were found.

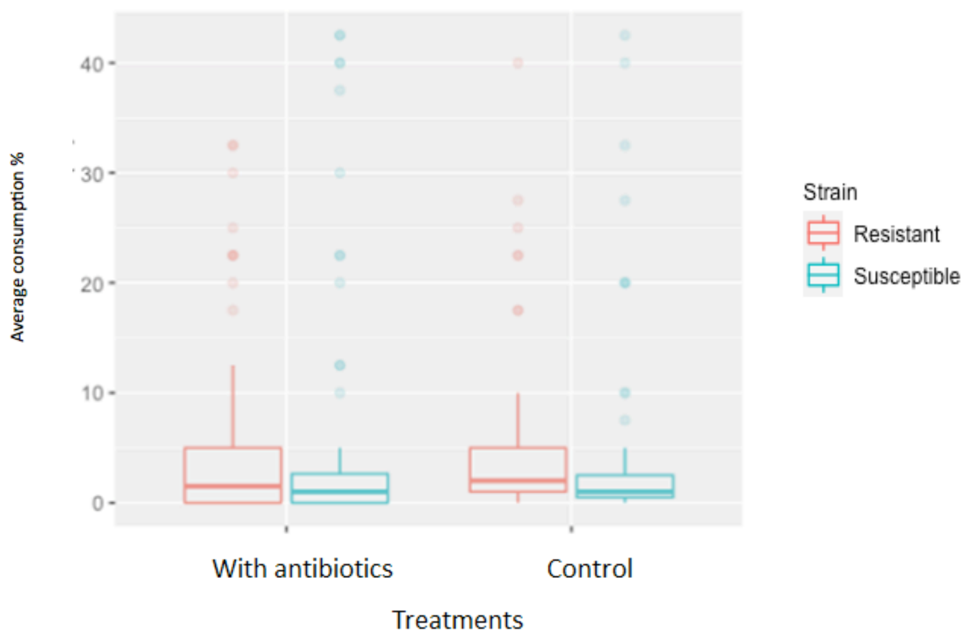

Figure 13. The average consumption of cotton control leaf and treated with Cypermethrin

Average consumption with and without the insecticide:

The control cotton leaf was eaten more (75.7%) compared to the leaf treated with Cypermethrin (10.36%).

Control leaf. No significant differences were observed between the antibiotics treatment ($F_{1,116}$ = 0.866, *p.value* = 0.717), the different strains ($F_{1,116}$ = 0.132, *p.value* = 0.717) or the interaction between treatments and strains ($F_{1,116}$ = 0.184, *p.value* = 0.669) (Figure 14).

Insecticide treated leaf. An almost significant difference was found between the resistant and susceptible strains ($F_{1,116}$ = 3.860, *p.value* = 0.052.), with resistant strain larvae eating slightly more (5.93%) than susceptible strain (4.43%, Figure 14). However, the antibiotics treatment ($F_{1,116}$ = 0.241, *p.value* = 0.624) or the interaction between the antibiotics treatments and strains ($F_{1,116}$ = 0.027, *p.value* = 0.870) had no statistical significance.

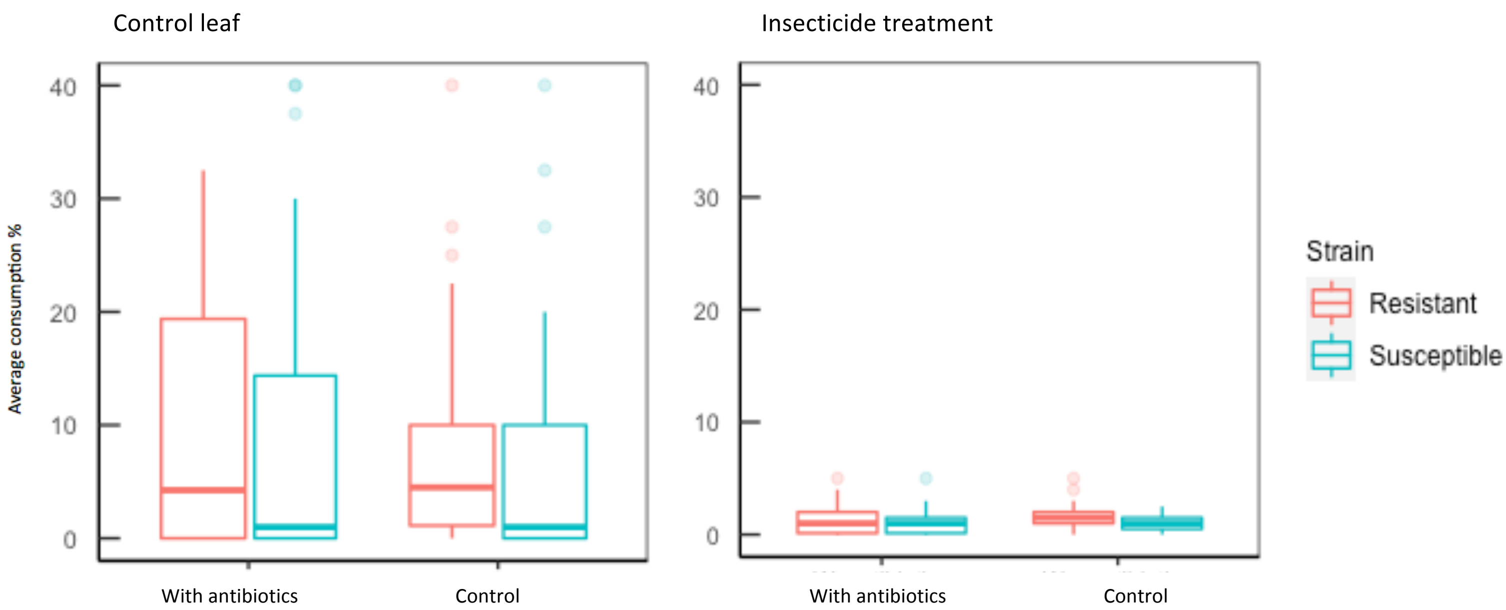

Figure 14. The average consumption (%) of control leaf and treated with the insecticide.

Death rate:

Susceptible strain larvae had a higher number of deaths (n=27) compared to the resistant strain (n=22). However, there was no statistically significant difference found between the treatments (resistant strain with antibiotics, resistant strain control, susceptible strain with antibiotics and susceptible strain control) in cotton leaf after Cypermethrin application (*p.value* = 0.108). Resistant strain with antibiotics had a higher death rate (n=14) compared to the control (n=8) while susceptible strain larvae died more on control leaf (n=15) compared to the treated with antibiotics leaf (n=12).

3.4 Efficiency of conversion experiment

Experiments were performed to analyze whether larvae from different treatments (resistant strain with antibiotics, resistant strain control, susceptible strain with antibiotics, susceptible strain control) have different efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD) and assimilation efficiency (AD) (approximate digestibility) and if that differ between the different host plants (cotton and maize).

Larval weight gain:

Results of larval average weight gain during the experiment showed that there was a significant difference between the resistant and susceptible strains ($F_{1,152}$ = 4.571, *p.value* = 0.034) and a high significant difference between the host plants ($F_{1,152}$ = 258.792, *p.value* = < 2e-16). Susceptible strain larvae gained more weight (mean=4.36 mg) compared to resistant strain (mean=3.78 mg). Moreover, larvae gained more weight while feeding on the cotton plant (mean=3.15 mg) compared to the maize plant (mean=0.92 mg) (Figure 15).

However, no significant differences were found between the antibiotics treatment ($F_{1,152}$ = 0.146, *p.value* = 0.703).

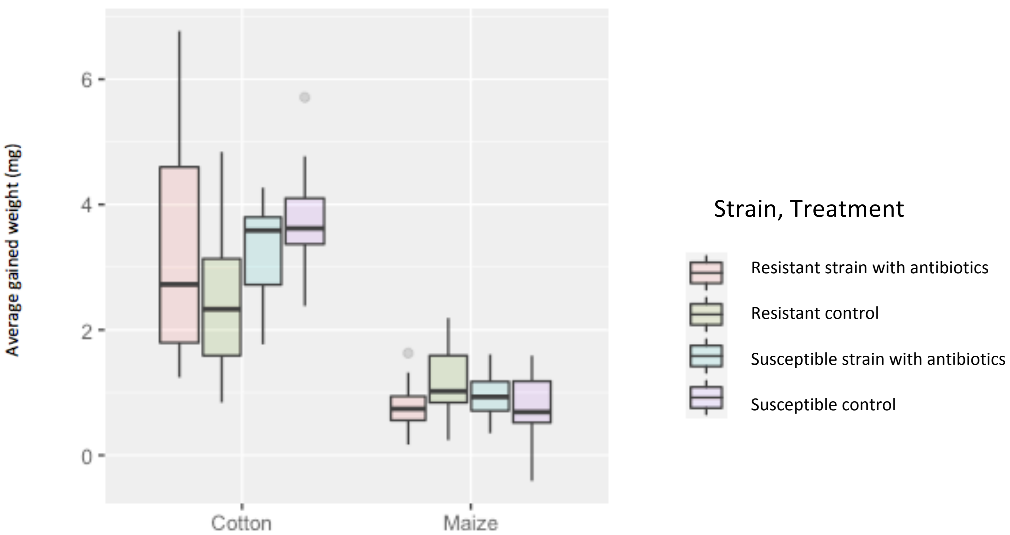

Figure 15. The average gained weight (mg) of cotton and maize

Efficiency of conversion of ingested food:

ECI (Efficiency of conversion of ingested food) was calculated to observe how larvae from different treatments (resistant strain with antibiotics, resistant strain control, susceptible strain with antibiotics, susceptible strain control) and feeding on different host plants (cotton and maize) perform. A high significant difference was found between cotton and maize host plants ($F_{1,152}$ = 213.914, *p.value* = < 2e-16) with cotton presenting a higher ECI (3.48) compared to maize (0.84) (Figure 16).

For the cotton diet, susceptible strain (ECI=4.08) had a highly significant difference compared to resistant strain (ECI=2.87) ($F_{1,76}$ = 11.809, *p.value* = 0.001). However, no significant differences were found between the antibiotics treatment ($F_{1,76}$ = 1.646, *p.value* = 0.203) or interaction between antibiotics treatment and strains ($F_{1,76}$ = 2.518, *p.value* = 0.117).

For the maize diet (Figure 16), there was a significant difference between antibiotics treatment and strains interaction ($F_{1,76}$ = 11.613, *p.value* = 0.001). Susceptible strain larvae fed with antibiotics had a higher ECI (n=0.96) compared to resistant strain control (n=0.76) while larvae without antibiotics treatment had higher ECI on resistant strain (n=0.98) than susceptible strain (n=0.65). However, no significant differences were found between antibiotics treatment ($F_{1,76}$ = 0.382, *p.value* = 0.538) or between the different strains ($F_{1,76}$ = 0.792, *p.value* = 0.376).

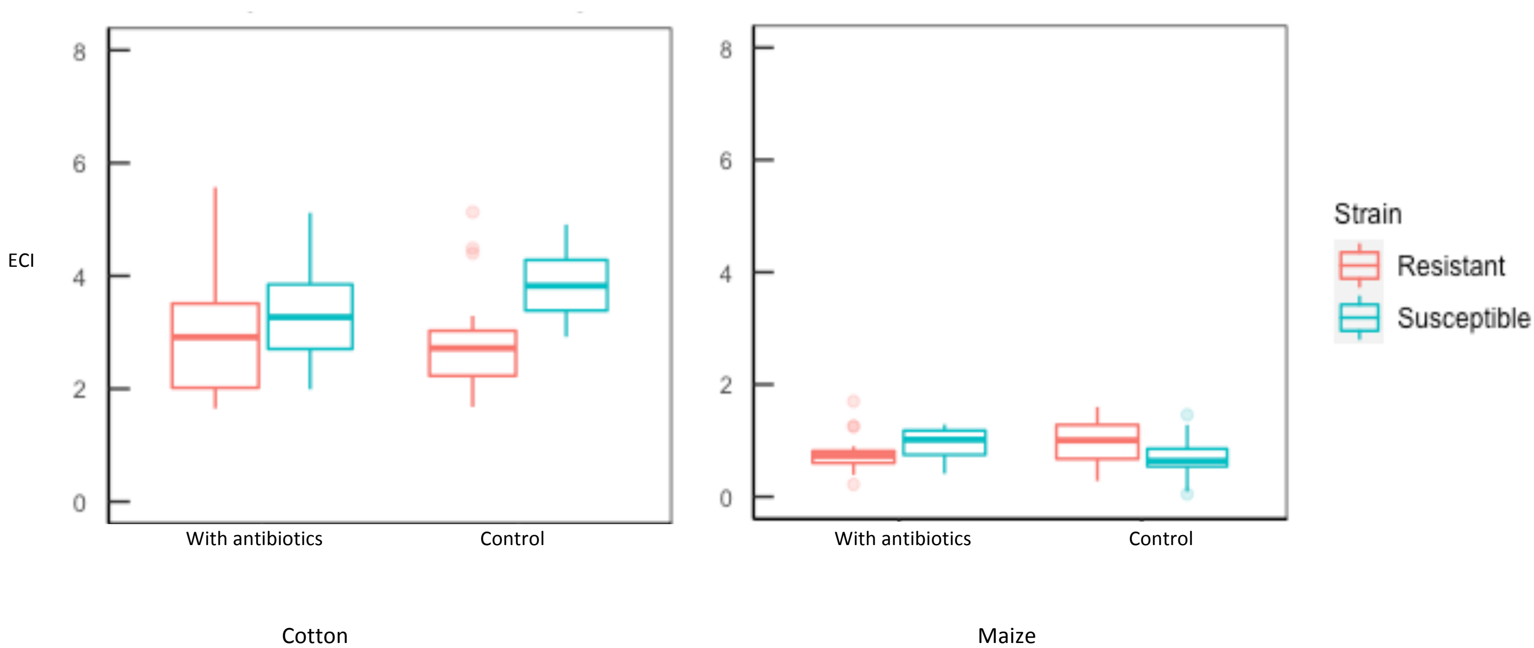

Figure 16. The ECI of cotton and maize

Efficiency of conversion of digested food:

A significant difference of ECD was found between the different host plants ($F_{1,145}$ = 17.483, *p.value* = < 4.99e-05) with higher ECD for cotton (8.96) compared to maize (1.12).

For the cotton diet, no significant differences were found between antibiotics treatment ($F_{1,69}$ = 1.507, *p.value* = 0.224), between the different strains ($F_{1,69}$ = 0.767, *p.value* = 0.384) or interaction between antibiotics treatment and strains ($F_{1,69}$ = 0.646, *p.value* = 0.424) (Figure 17).

For the maize diet, however, there was a significant interaction effect between the antibiotics treatment and the different strains ($F_{1,76}$ = 6.214, *p.value* = 0.015). Resistant strain with the antibiotics treatment had lower ECD (1.04) compared to resistant strain control (1.31) while susceptible strain with the antibiotics had a higher ECD (1.23) than susceptible strain control (ECD=0.88). As for the main factors, no significant differences were found between the antibiotics treatment ($F_{1,76}$ = 0.111, *p.value* = 0.740) or the different strains ($F_{1,76}$ = 0.908, *p.value* = 0.343) (Figure 17).

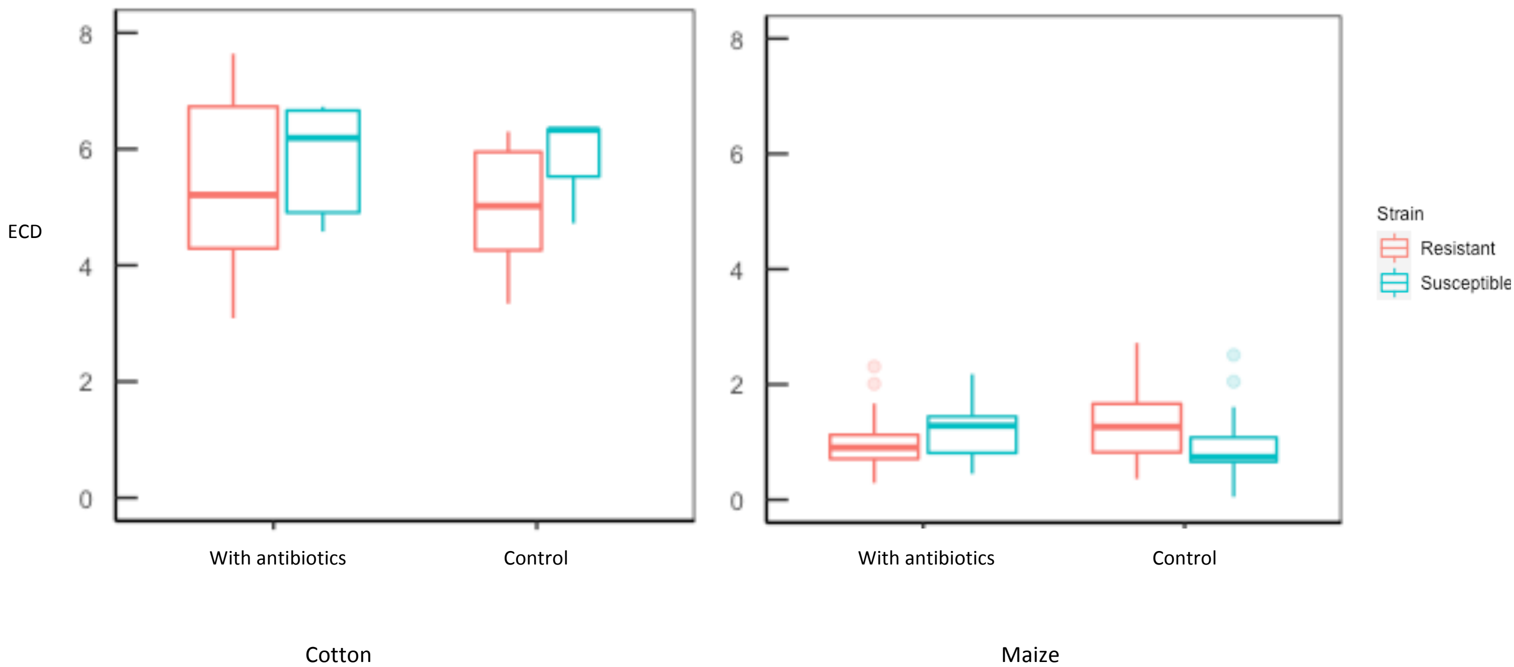

Figure 17. The ECD of cotton and maize

Assimilation efficiency (approximate digestibility):

A significant difference of assimilation efficiency (approximate digestibility) was found between the two host plants ($F_{1,152}$ = 133.582, *p.value* = < 2e-16). Maize had a higher assimilation efficiency (approximate digestibility) (0.79) compared to cotton (0.12) (Figure 18).

For the cotton diet, no significant differences were found between the antibiotics treatment ($F_{1,76}$ = 1.586, *p.value* = 0.446), the different strains ($F_{1,76}$ = 1.874, *p.value* = 0.175) or interaction between antibiotics treatment and strains ($F_{1,76}$ = 0.452, *p.value* = 0.503) (Figure 18).

Same as for the cotton diet, no significant differences were found between the antibiotics treatment ($F_{1,76}$ = 0.619, *p.value* = 0.434), the different strains ($F_{1,76}$ = 1.464, *p.value* = 0.230) or interaction between antibiotics treatment and strains ($F_{1,76}$ = 0.132, *p.value* = 0.718) for the maize diet (Figure 18).

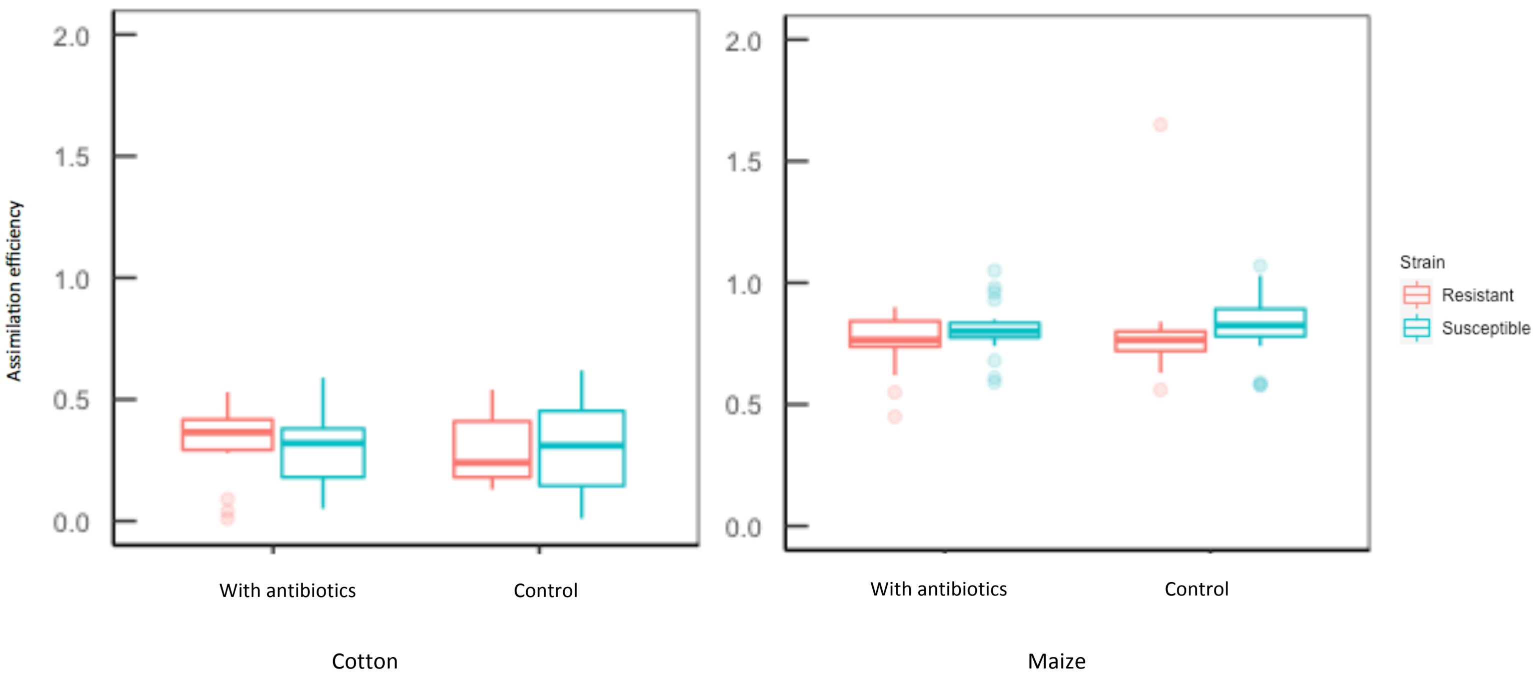

Figure 18. The Assimilation efficiency (approximate digestibility) of cotton and maize. *Please note that the scale of the Y-axis differs.*

Average consumption:

Larvae average consumption was significantly higher on maize (mean=46.96 mg) compared to cotton (mean=40.06 mg) ($F_{1,152}$ = 15.227, *p.value* = 0.0001) (Figure 19). No significant difference was found between the different strains ($F_{1,152}$ = 0.506, *p.value* = 0.478) or antibiotics treatment ($F_{1,152}$= 1.204, *p.value* = 0.274).

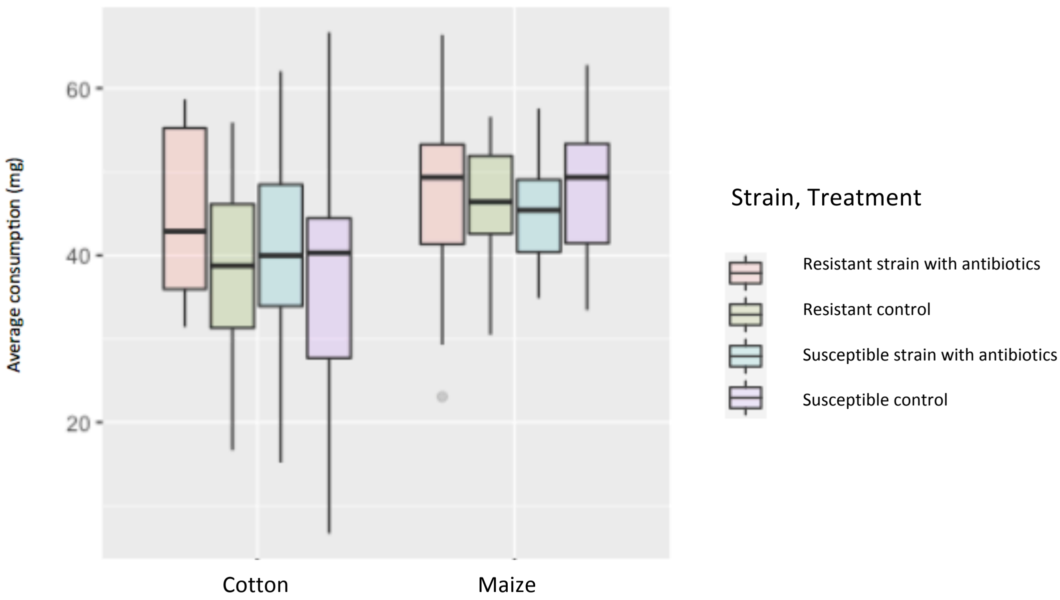

Figure 19. The average consumption of cotton and maize

3.5 Farmers knowledge on chemical plant protection methods, pesticide resistance and evolution

The respondents from all the targeted countries claimed to have problems with insect pests, weeds or fungal diseases. In terms of insect pests, Swedish and Tanzanian respondents had the least problems with it. In contrast, Lithuanian respondents had the biggest problems with the insect pests (Figure 20).

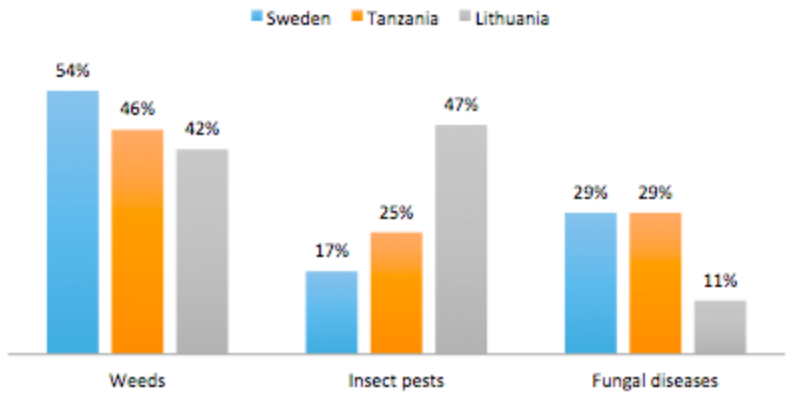

Figure 20. The biggest plant protection problems between the targeted countries

All the targeted countries mostly preferred to use chemical products to protect their yields, followed by crop rotation, which was also highly preferable. However, the difference was found in intercropping and biological plant protection methods as Tanzanian farmers chose to use these methods while Swedish or Lithuanian respondents had very low or none choice (Figure 21). Moreover, Lithuanian respondents highly preferred mechanical plant protection methods.

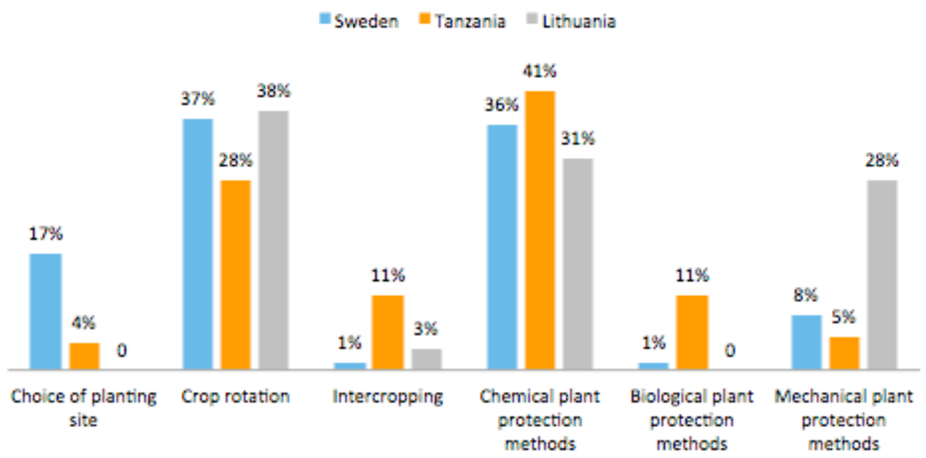

Figure 21. Most common plant protection methods between the targeted countries

In terms of pesticides use, all the targeted countries highly prefer to use pesticides (Swedish and Tanzanian respondents 92% and Lithuanian 82%). The questionnaire indicates that the respondents mostly choose to use pesticides because of their effectiveness (Figure 22). However, 27% of Swedish respondents claimed that lack of other effective methods leads them to this choice.

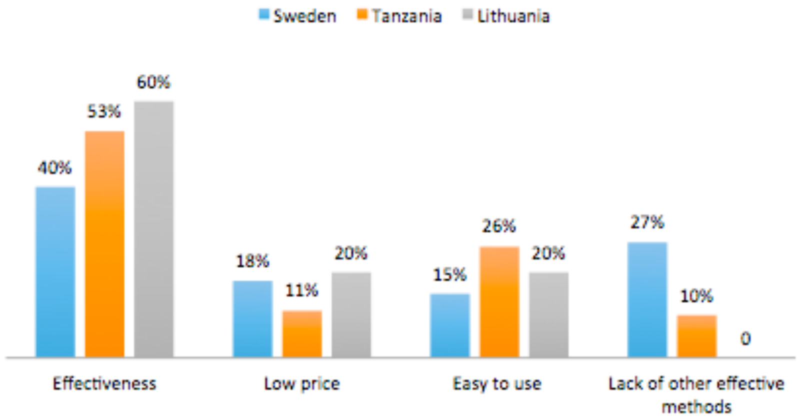

Figure 22. The main reason of using pesticides between the targeted countries

The majority of respondents are aware of pesticide resistance, however, Swedish respondents had the highest rating (Figure 23). It was recorded that Tanzanian respondents were the only ones who have never heard about pesticide resistance before.

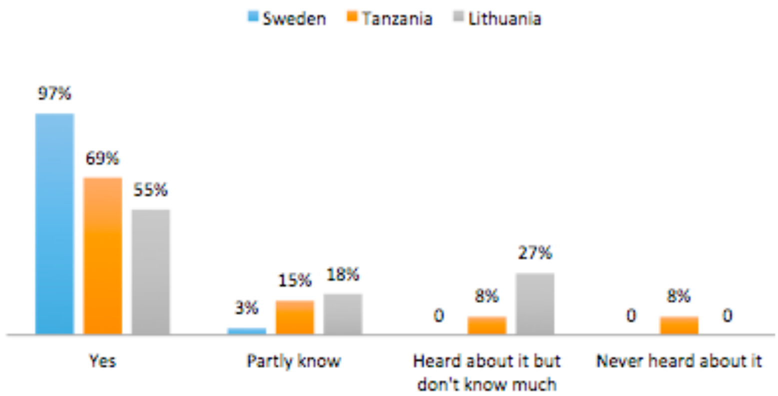

Figure 23. Familiarity with pesticide resistance between the targeted countries

The information sources that farmers use to educate themselves about pesticide resistance varies between targeted countries. ¼ of Swedish farmers respondents use agricultural advisory services followed by the National Board of Agriculture provided information. The majority of Tanzanian farmers rely on agricultural advisory services. In contrast, Lithuanian farmers prefer to check product instructions the same as search for information on the internet, while ¼ of the respondents count on agricultural advisory services (Figure 24).

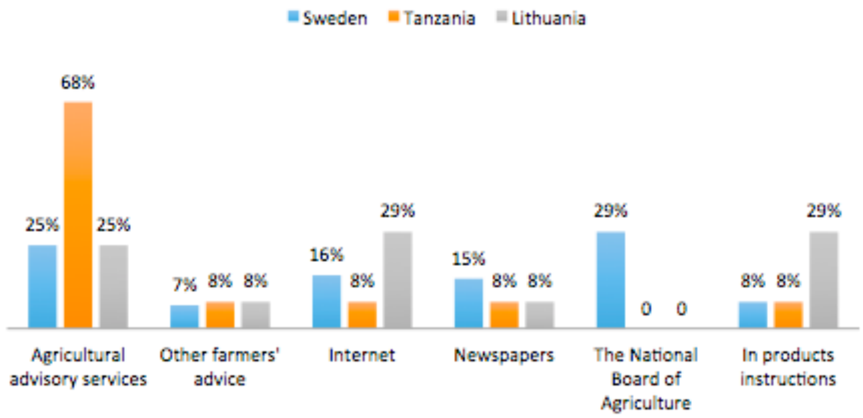

Figure 24. Used information sources about pesticide resistance between the targeted countries

Only a small number of respondents had noticed arising pesticide resistance in connection with pesticide use. The majority of Swedish respondents did not see the connection at all. However, many Lithuanian and Tanzanian respondents claimed they don't know if there's a connection (Figure 25). That could possibly be caused by the lack of knowledge on pesticide resistance development.

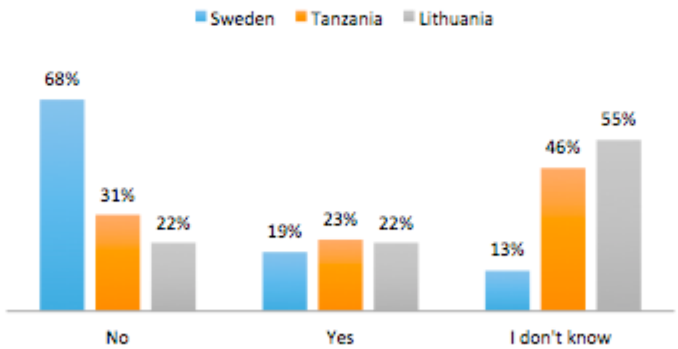

Figure 25. Noticed pesticide resistance in connection with pesticide use

From the respondents who answered ‘yes’ in the previous question about arising pesticide resistance, we asked them to describe how they noticed it and what actions they took. Since this question was not mandatory, only a few respondents answered (Swedish n=5, Tanzanian n=3, Lithuanian n=2) (Table 3).

Table 3. Actions after noticed pesticide resistance in connection with pesticide use. Open answers from respondents. Some answers with the same keywords were merged and presented as the same category.

What actions were taken?	**Sweden**	**Tanzania**	**Lithuania**
Preparation change	2	2	1
Redid the crop rotation	1		
Other strategies use	1		
Advisory services	1	1	
How was it noticed?	**Sweden**	**Tanzania**	**Lithuania**
No results from pesticides	1	3	1
Through the regular control	1		

The respondents from different countries presented various perceptions on why pesticide resistance develops. According to Swedish farmers, the type of preparation, in this case pesticide, plays a role in pesticide resistance development. Moreover, another issue Swedish farmers noted is too many preparations to choose from. However, half of the Lithuanian farmers claimed that the reason pesticide resistance appears is because of too much use of pesticides and this was the highest rate from all three countries (Figure 26). However, most of Tanzanian farmers responded that pesticide resistance development depends on the pest.

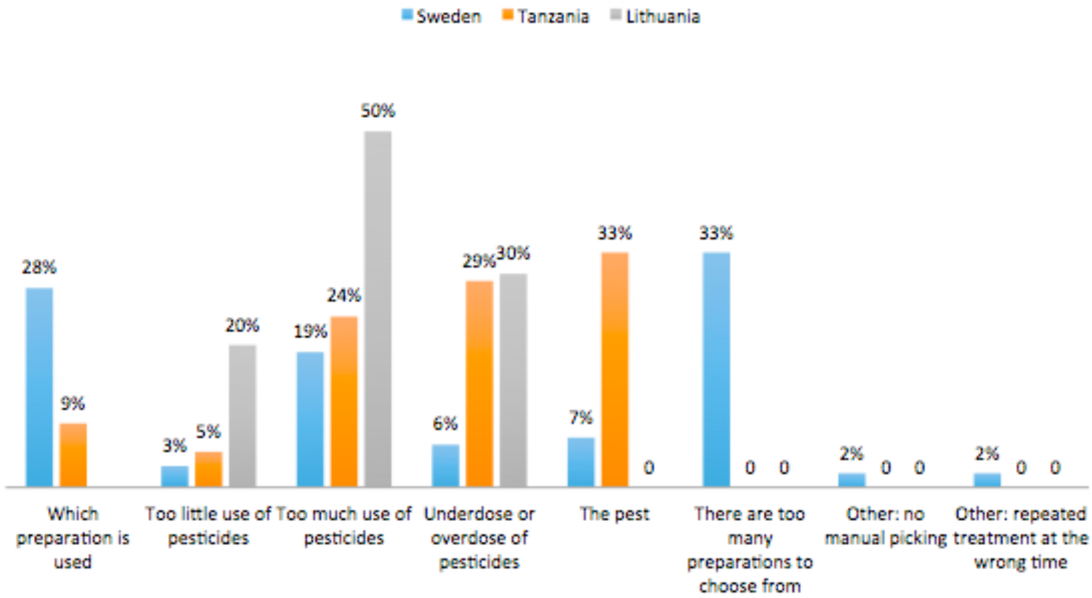

Figure 26. The main reasons for pesticide resistance development between the targeted countries. 'Other' represents farmers typed answers. Some answers with the same keywords were merged and presented as the same category.

Swedish respondents presented the highest knowledge about evolution between the targeted countries while almost half of Tanzanian farmers are not familiar with evolution at all (Figure 27). Most of Lithuanian respondents were familiar with this term, however, some had only party knowledge.

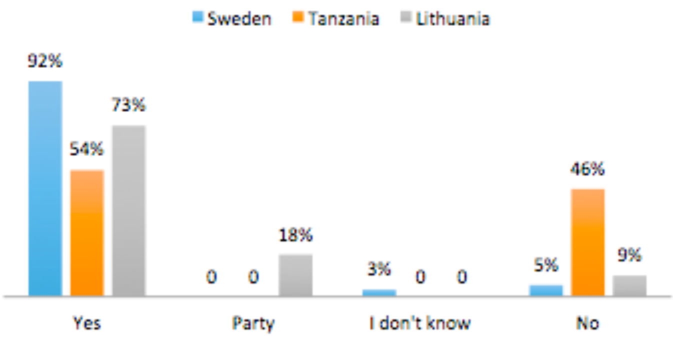

Figure 27. Familiarity with evolution between the targeted countries

All three countries are aware of the negative effect of pesticides use. Swedish and Lithuanian farmers see the development of pesticide resistance as a main negative effect while Tanzanian farmers see negative effects on humans as the main issue. Only a small percentage of Swedish respondents claimed they don't see any negative effects of pesticide use (Figure 28).

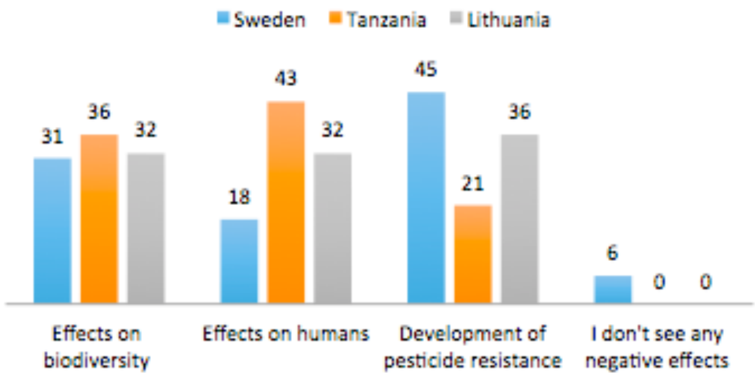

Figure 28. The negative effects of pesticides between the targeted countries

The tag clouds show that between the targeted countries, measures taken to avoid the development of pesticide resistance are different. 25 Swedish farmers, 8 Tanzanian and 8 Lithuanian answered this question since it was not mandatory.

Swedish respondents mostly repeated keywords 'different', 'varied', 'preparations', while Tanzanian respondents had 'chemical rotation' keywords mentioned. Lithuanian respondents mostly used the keywords 'reduce pesticides'. However, Tanzanian and Lithuanian farmers had mentioned the word 'chemical' more times compared to the Swedish farmers, who appeared to describe more diverse measures (Figure 29).

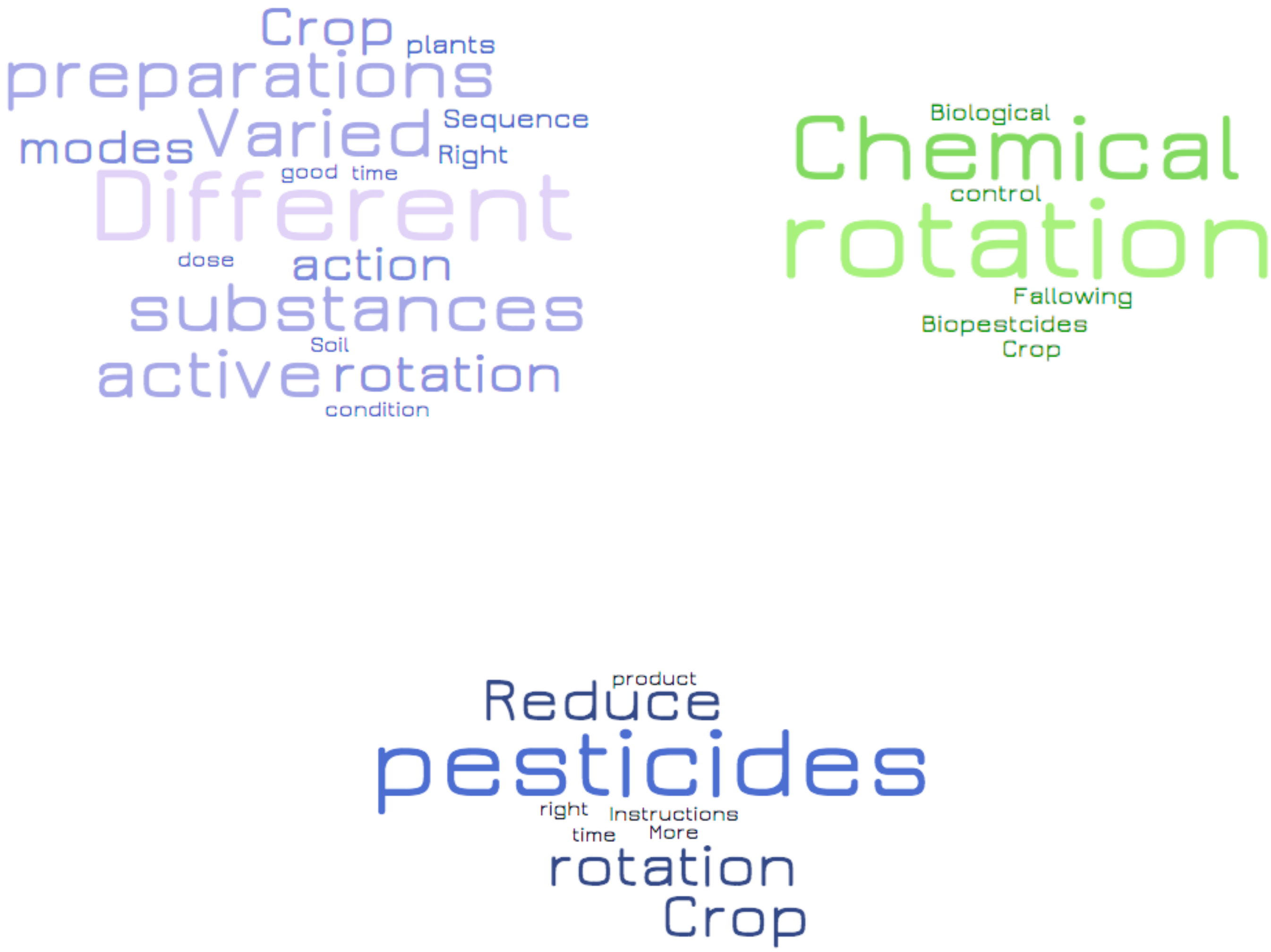

Figure 29. The Tag clouds visualizing the frequency of keywords of taken measures to avoid pesticide resistance development. Swedish (up left), Tanzanian (up right) and Lithuanian (bottom) respondents. 25 Swedish, 8 Tanzanian and 8 Lithuanian farmers' answers.

4. Discussion

In this study, I have tried to answer whether resistance levels and gut microbiota affect host plant preference and performance simultaneously to gain better understanding about cross-resistance and pre-adaptation hypothesis. Additionally, farmers' perceptions on pest management and pesticide resistance development were gathered via questionnaire to understand farmers knowledge between the three targeted countries (Sweden, Lithuania and Tanzania) and to be able to recognize the potential gaps between research findings and farmers' practices.

4.1. Influence of resistance level and gut microbiota on the host plant preference and feeding performance of S. littoralis after applying an insecticide

The 2-choice experiment was performed between an insecticide treated and untreated cotton leaf to examine whether resistant larvae have evolved the ability to recognize pesticide treatment and if the gut microbiota could play a role in their preference. Our results showed that neither initial nor final larvae's host plant choice was affected by the strain between choosing the control leaf and leaf treated with Cypermethrin, which indicated larvae's inability to recognize insecticide treated leaf or react to it accordingly. Moreover, larvae feeding performance (consumption) showed no effects on resistance level or gut microbiota. However, since larvae ate more control leaf than insecticide treated leaf, this suggests that larvae couldn't make a choice from a distance and their initial food choice is random but after they have tasted the treated and untreated leaf, they could make a choice which one to eat the most.

Surprisingly, no affects by the strains were found in terms of the larval death rate. We could have expected to get statistically significance with a higher survival rate for the resistant strain compared to the susceptible. However, this could be explained by the fact that larvae were given a choice which leaf to eat (treated and untreated with Cypermethrin) so they could feed less on the treated leaf and once they start to feel insecticide effect, move on the untreated while in the selection experiment the exposed larvae had no such choice and were provided an artificial diet instead of cotton (Bras, 2021, unpublished data). However, despite that, the larval survival rate was not higher on resistant strain, therefore, it remains unclear what happens later on when larvae reach the adulthood and if larval development and growth differs between susceptible and resistant strains.

Additionally, there were no affects by the gut microbiota found in larvae feeding performance. This contrasts with an earlier study on a related species, *S. litura* where larvae with an intact gut microbiota were more resistant against the insecticides compared to larvae that were treated with antibiotics, which were found to be more susceptible (Gadad & Vastrad, 2016). Even though no significant differences were found between the different larval treatments in our study, a previous research have shown that the reduction of microorganisms in the gut could cause negative effects and abnormalities in larval development and enhance death rate (Fukatsu & Hosokawa, 2002; Xia et al, 2020; Thakur et al, 2016;). Nevertheless, the findings from our project could give new insights about the effect of antibiotics combined with the insecticide, leading to better understanding of insecticide effect (Madhusudhan, 2015).

4.2. Influence of resistance level and gut microbiota on the host plant preference and feeding performance S. littoralis between different host plants

The 4-choice experiment was done to investigate if pesticide resistance development leads to changes in host plant preference and if that could be affected by the gut microbiota, which is a part of the cross-resistance hypothesis. Our results showed that neither initial nor final larvae host plant choice was affected by the strain resistance or the presence of gut microbiota. This shows that the resistant strain has not expanded its host plant range (no cross-resistance) and that the gut microbiota might not affect host plant preference choice. However, previous study has shown that resistance could emerge when a polyphagous pest adapts to another host plant (Dermauw et al, 2013). Nevertheless, more research needs to be carried out to conclude on the previous findings.

Although larvae overall ate more cotton, our results presented that their feeding behavior of a specific plant could differed between the strains and antibiotics treatment. For instance, while feeding on cotton and cabbage the resistant strain treated with the antibiotics ate a higher eaten amount of leaf compared to the susceptible strain with the antibiotics. In contrast, susceptible strain larvae ate in general highly significant more than the resistant strain. Therefore, there may not be any cross-resistance effects of resistance level and antibiotics treatment on preference, but on feeding performance instead.

Concerning the effects of the gut microbiota, we found a significant difference between larvae treated with antibiotics and unexposed larvae. Indeed, their overall consumption was higher compared to unexposed larvae. This leads to the hypothesis that if larvae present a damaged gut microbiota they might need to consume more food to perform better and so to ensure their normal functionality. Nevertheless, previous studies based on Lepidoptera species have shown both positive and negative effects on larval growth after antibiotics treatment, for instance, Thakur (2016) study shows that *S. litura* grew faster while treated with Streptomycin sulphate antibiotics compared to a normal diet, while Xia et al (2020) have observed that the growth and feeding performance for the same species was reduced if treated with an antibiotics cocktail (Ciprofloxacin, Levofloxacin and Metronidazole). This indicates that gut microbiota plays an important role in larval growth, feeding and the lack of gut bacteria could potentially cause larval abnormalities or death. However, even being closely related, Lepidoptera species have different microbiota with diverse bacterial communities leading to different performance with and without antibiotics (Xia et al, 2020). Hence, more research is needed to understand the effect of antibiotics in larval performance experiment throughout the entire life of the insect. That could give more information about how gut microbiota, resistance level and host plants could affect insect's performance.

Our results have shown that larvae mainly chose maize as their initial host plant choice and mainly cotton as their final host plant choice, which was presented in the highest consumption. Meanwhile, the lowest consumption was observed for the new host plant, lily, which could potentially have led to poor feeding performance since the larvae had to go through the adaption process (Simon et al., 2015). The initial and final choice findings indicate that larvae could not make the optimal choice right away, however, after feeding on maize plant, they changed to cotton. Additionally, as it was observed in our experiments, larvae needed to eat the plant first to decide whether it was suitable for continued or long-term consumption. This could be caused by different smell, structure or minerals that the maize provides (Reynolds et al, 2016). As recent studies have shown, maize is advanced silicon-accumulator plant that can have a defensive system against abiotic and biotic factors, such as insect herbivores (Reynolds et al, 2016; Kaya et al, 2006) while cotton was recorded as an intermediate silicon-accumulator plant (De Souza Junior et al, 2021). It was also recorded that when feeding on silicon plants, larvae struggle to gain weight and develop accordingly (Acevedo et al, 2021). This leads to hypothesis that although cotton and maize are about equally preferred by *S.littoralis* (Thöming et at, 2013; Conchou et at, 2017) they perform worse on maize since they can't develop and gain weight as much as on cotton plant, as our results about the larval efficiency of conversion have shown.

4.3. Influence of resistance level and gut microbiota on the efficiency of conversion of S. littoralis larvae

Since previous studies have shown that *S. littoralis* have a high preference for both cotton and maize (Thöming et at, 2013; Conchou et at, 2017) and our 4-choice experiment showed that the cotton was eaten more but maize was preferred for the initial choice, we investigated nutritional status of the plant for the larvae to see whether they differ. The efficiency of conversion ingested food (ECI), digested (ECD) food and assimilation efficiency (approximate digestibility) (AD) are used for distinguishing the consumed food quality (Khedr et al, 2015). We found that the resistant level or gut microbiota did not affect ECI and ECD or AD. However, both ECI and ECD presented significant difference in cotton compared to maize, where larvae gained more weight after feeding on the cotton plant compared to the maize plant. These results show that maize seems to be a poor host plant to feed on (Rösvik et al, 2020). The higher ECI and ECD in cotton mean that larvae were able to use consumed food for body biomass and development (Nathan et al, 2005) and they were able to grow faster on cotton. This could indicate that because of nutritional status, cotton is preferable by larvae and potentially gives them better survival rate and fitness. Our results predict that within our experimental conditions, plant characteristics can play more important role compared to the resistance level or gut microbiota. Moreover, we didn't obtain the effects of the strains or gut microbiota on performance, which suggest no connection to cross-resistance. However, it's hard to predict how this could change in longer observations and more studies need to be done. Controversially, maize presented higher AD, which shows insects ability to digest the food (Devi et at, 2002). This leads to a better consumption performance on maize compared to cotton. As our results have shown, the larval characters in terms of resistance and gut microbiota mostly didn't affect larvae eating behavior, instead the plant characteristics seem to have a larger effect. Maize is not as efficient food source as cotton because of presented low ECI & EDI, in that case larvae need to eat more to get the nutritional benefits from this plant and increase their growth.

4.4. The farmers perceptions of pesticide use among the three targeted countries

Our questionnaire on farmer's use of pesticide has shown that they try to incorporate crop rotation method into their plant protection strategies. However, farmers from the three targeted countries highly prefer pesticide use and refer to the chemical plant protection method as the most effective one. Nevertheless, previous studies have highlighted that Tanzanian farmers have a poor knowledge with regards to appropriate chemical pest control use and rely on pesticide suppliers' recommendations on product dose or estimates the dose according to their farm size or previous experience (Matowo, 2020). Another big issue presented in earlier studies (Ngowi, 2007) is that Tanzanian farmers use of pesticide mixtures without knowing which exact pesticides are included in them. This, apparently, can lead to insecticide resistance development (Metacalf, 1980).

In terms of pesticide resistance awareness, our study showed that while Swedish respondents claimed to be well informed (97%), some Tanzanian respondents had never heard of this term (8%) while some Lithuanian respondents had heard but claimed to not knowing much about it (27%). In connection to that, Swedish respondents responded as having a high knowledge on evolution while almost half of Tanzanian farmers were only familiar with this term. This could be caused by a limited access to agricultural extension officers in Tanzania, since they lack transport for visiting farmers, have poor working conditions, and are not well supported financially (CUTS, 2011; Elifadhili, 2013). These circumstances could then lead to limitations of providing the farmers proper agricultural advisory services.

Our results together with previous literature show that farmers from different countries possibly do not have the same access to knowledge about pests and pesticides. Moreover, they don't perceive pesticide resistance in the same way (Ngowi, 2007) as highlighted in the respondents' answers. Indeed, they had different answers on what could cause pesticide resistance development. Most of the Swedish farmers claimed that the type of pesticide plays an important role together with too many preparations to choose from in causing pesticide resistance development. However, many Lithuanian respondents claimed that the reason for pesticide resistance to occur is because of excessive pesticide use, which is known as one of the main reasons for pesticide resistance development (Maino et al, 2018; Gardner et al, 1998). Controversially, most of the Tanzanian farmers responded that the pest itself mostly enhance pesticide resistance development. Since small-scale Tanzanian farmers face huge yield loss caused by the fall armyworms *S. exempta* and the invasive, *S. frugiperda,* which recently arrived in Africa*,* this could potentially lead to the answers we obtained (Sisay et al, 2018; Mushobozi et al, 2005). Our results from the measures taken by farmers to avoid pesticide resistance development showed that Swedish and Lithuanian farmers use more diverse and sustainable measures, for instance, crop rotation, varied preparations, different substances or plant sequence, while Tanzanian farmers refer to chemical rotation mostly.

Despite of high use of pesticides, all the targeted countries were familiar with the detrimental effects of chemical plant protection products, such as negative environmental effects, negative effects on health or pesticide resistance development in pest species. From all the targeted countries, the concern about negative pesticide effects on humans was highest in Tanzania. This could lead to the hypothesis that Tanzanian farmers experience health problems related to pesticide use more than Swedish or Lithuanian and they might lack access to protective equipment. As Ngowi et al (2007) showed from 61 interviewed of Tanzanian farmers, more than half (68%) claimed experiencing health problems such as headache, dizziness, nausea or skin related problems after a pesticide application. Additionally, two Tanzanian respondents in our performed questionnaire 'comments' section share their opinion that *'more education about chemical products is needed for better and safe plant protection'* and *'I really like organic farming, but I don't know where can I learn about it more and reduce my farming issues'*. Although all the targeted countries prefer to use pesticides instead of other plant protection methods mostly because of its effectiveness, the perception of the effectiveness between the countries differ. For instance, Swedish farmers were less convinced on the effectiveness, which could indicate that they have high expectations on what is "effective" compared to Tanzanian or Lithuanian farmers. Nevertheless, it is crucial to find a way of introducing farmers from different countries to other effective methods without harmful side effects, for instance integrated pest management (IPM) strategy.

Farmers' knowledge and perception on pesticide use and resistance development between the 3 targeted countries can be useful in order to introduce more sustainable and effective pest management strategies and improve the communication between researchers and farmers-practitioners (Devine & Furlong, 2007). Indeed, our results have revealed that with a better knowledge about other effective pest management methods, farmers could make decisions on how to improve plant protection based on their needs and reduced the potential negative impact of their farming practice. However, the changes towards sustainable agriculture must be gradual and based on existing agricultural pest control strategies while integrating more sustainable, less harmful methods, since they have social and economic implications that can differ between the countries (Aniah et al, 2021).

5. Conclusions

In the current study, the resistant strain larvae did not change its host plant preference toward a new host and the gut microbiota did not affect the host plant preference choice meaning that evidence for cross-resistance was not found since. On the contrary, larval feeding performance was influenced by the gut microbiota since larvae ate more while treated with antibiotics. The results from the initial (maize) and final (cabbage) host plant choice showed that *S. littoralis* makes a choice from a distance but once it starts feeding on a host plant, it can change its preference. We concluded that larvae feeding choice doesn't depend on strains or antibiotics treatment but depends on the host plant characteristics.

We tested if pesticide resistant and pesticide susceptible larvae can detect when their host plant is treated with an insecticide and what role could play the gut microbiota. We didn't see any effects of resistance level and gut microbiota that shows no evidence of involvement in cross-resistance. Additionally, no effects were found on larvae survival rate where resistant strain didn't have a better survival than the susceptible strain. Our findings show no correlation between the resistance level or gut microbiota in larval efficiency of conversion, however, the efficiency of conversion of ingested and digested food were higher in cotton while maize presented higher approximate digestibility with higher consumption, showing that larval metabolism is mostly affected by the host plants.

In a second part, we aimed at identifying whether different countries perceive pest management, evolution process and pesticide resistance development differently and how do they react to appeared pesticide resistance in their farm. We found that between the targeted countries, the most commonly used plant protection method are pesticides and farmers perceive this method as the most effective one. However, farmers knowledge about pesticides, pesticide resistance development and evolution varied between the countries, with Tanzanian farmers respondents having shown the greatest absence of knowledge, that potentially could lead to more appeared health problems after pesticide application. There appears to be a gap between the farmers and agricultural advisory officers, indicated from both our questionnaire answers and previous studies (CUTS, 2011; Elifadhili, 2013).

To further understand and improve the knowledge about pesticides and the development of resistance, better communication between researchers and farmers practitioners thus needs to be implemented. Not only would that bring benefits to the farmers in terms of protecting their yields and using more sustainable pest management methods, but simultaneously for researchers, who can apply their findings in practices.

6. Critical reflections and study limitations

For the natural science part, it was impossible to ensure that all the microbes in the larval guts were killed since we used a specific mixture of antibiotics and the exact characteristics of the bacterial colonies stayed unknown. When many insect generations have been reared artificially, it can have an effect on their characteristics, e.g. insects might fly less and the microbial community in their guts might differ (Staudacher et al. 2016). Moreover, the experiments were performed with laboratory strains and additional experiments with the field populations could have brought more applicable results. Additionally, we used one kind of insecticide - Cypermethrin, so the results might not be applicable while using another type of insecticides. In terms of experimental setup for the choice experiments, we used only one side of the leaf (downside) so that could also be a limiting factor because *S. littoralis* might have different feeding preference between the upper and down side of the leaf. In terms of efficiency of conversion experiment, we received some negative values that were unexpected, therefore the methods of this experiment could be improved accordingly.

Concerning the social science part, for instance, a higher number of respondents from different countries would have made broader analysis and more effective comparisons between the different countries. Moreover, the phrasing of the questions and translated answers from different languages could be not exactly what farmers have meant and could have brought miscommunication. For instance, the question about the evolution was very broad and could have involved more following questions to verify farmers knowledge. Finally, more precise questions on how farmers are handling pesticides and information about the equipment they use while applying pesticides could implement a future study to better understand farmers' use of pesticide.

7. Appendix n°1

Spodoptera littoralis artificial diet

Ingredients:

400 g Wheat – germ

240 g Dried yeast Flakes

20 g Methyl – 1- 4- hydroxybenzoate

20 g Sorbic acid

22 g Ascorbic acid

8g Cholesterol

These ingredients make a **yeast-mixture**

3.6 l Distilled water

1700 g Peeled potatoes

DL-a-Tocopherol acetate;

4 ml Vit E (in the fridge)

10 ml Oil

100 ml 96% Ethanol

65 g Plant agar (powder)

24 g Vitamin-mixture (in the fridge)

6 g Sodium benzoate

Instructions:

- Put the water to boil on the stove
- Peel the potatoes
- Slice and mash the potatoes in a mixer
- Add oil, Vit E and ethanol and stir
- Add the yeas-mixture and stir
- Whip the Agar powder into the boiling water
- Pour the Water-agar mixture into the potato-yeast mixture little by little while stirring
- When the mixture reaches 50-60 add the vitamin-mixture and the sodium benzoate and stir well
- Pour hot mixture into the containers for storage and let them cool
- Put the containers into the freezer

Appendix n°2

Dissection protocol (Adapted from: Heithausen in prep, 2021)

STARVING LARVAE

2 hours before dissection the larvae have to be transferred from the boxes they are reared in into small cups without food in order to be starved before the dissection. Take 8 larvae from each of the 4 treatments (resistant, resistant with antibiotic, susceptible, susceptible with antibiotic) and put them into cups. One cup per treatment.

Weigh the larvae and record the collective weight for the eight larvae for each of the 4 treatments.

Remember that the **tools for the dissection** (forceps and beakers and Milli Q water) **should be autoclaved**.

PREPARE the dissection

List of items you need:

- Styrofoam box with ice
- 1 beaker with ethanol to sterilize forceps in
- 1 beaker with ethanol and 1 beaker with autoclaved Milli Q water to sterilize outside of larvae and rinse them
- Forceps
- Small petri dishes to dissect on
- Petri dishes with LB agar
- Plastic spreaders
- Eppendorf tubes + holder
- Pen to mark Eppendorf tubes and petri dishes
- Pipette (100-1000 µl) and pipette tips (blue)
- Waste bag (to be thrown away afterwards in trash container outside the building)

HOW TO DO THE DISECTION, DILUTION AND PLATING

Before dissection, after the two-hour starvation period, the cups with the larvae are put on ice for a while.

While the larvae are on ice, waiting time can be used to mark Eppendorf tubes and to pipette water into the Eppendorf tubes. 1000 µl in the 10^0 tubes and 900 µl in the other tubes for the dilution series (10^{-1}, 10^{-2}, 10^{-3}) (Figure 1).

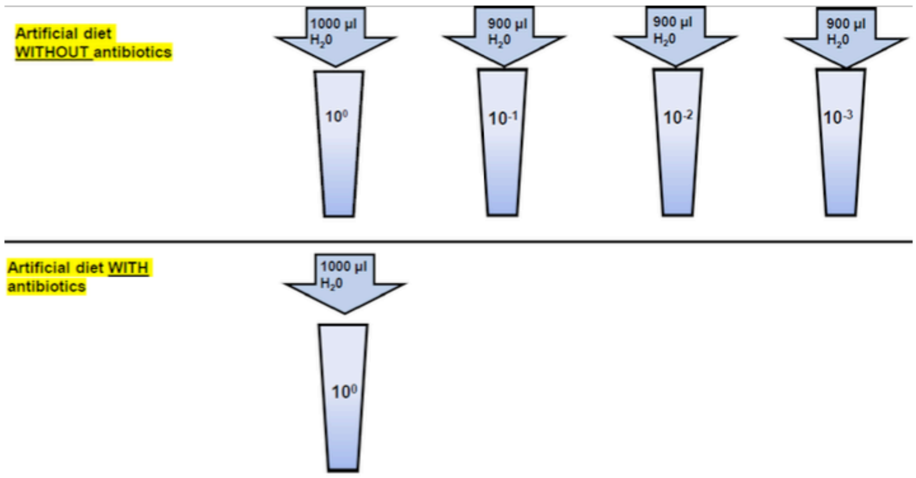

Figure 1 Preparing Eppendorf tubes with water

Each individual larva is taken from the cup with a forceps and dipped into the beaker with 95% ethanol and rinsed with water. The larvae are dissected with two forceps that are sterilized with 95% ethanol between dissection of the different larvae. The two forceps that are used for dissection don't leave the lamina hood; the forceps that is used to take the larvae from the cup outside the hood is a third one.

For each treatment and replicate 4 larvae are dissected and their guts are put into an 1,5 ml Eppendorf tube filled with 1000 µl (1ml) of autoclaved Milli-Q water (10^0) (Figure 2).

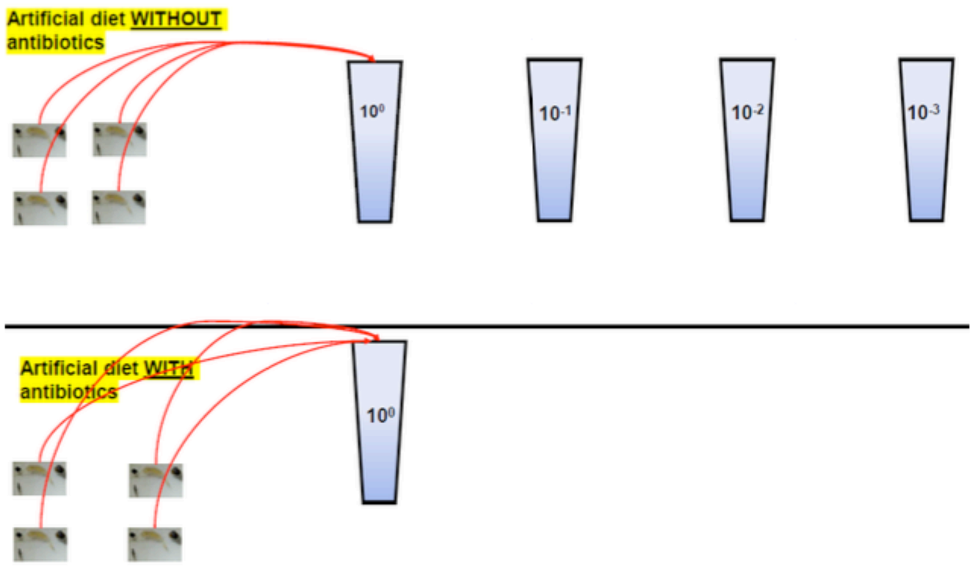

Figure 2 Larval guts are put into Eppendorf tubes

For the treatments <u>without</u> antibiotic diet, the 10^0 Eppendorf tube is vortexed for approximately 30 seconds and then 100 µl from the solution is transferred to another 1,5 ml Eppendorf tube filled with 900 µl autoclaved Milli-Q water (10^{-1}). This dilution series is continued until dilution 3 (10^{-3}) (Figure 3). Vortex between dilution steps.

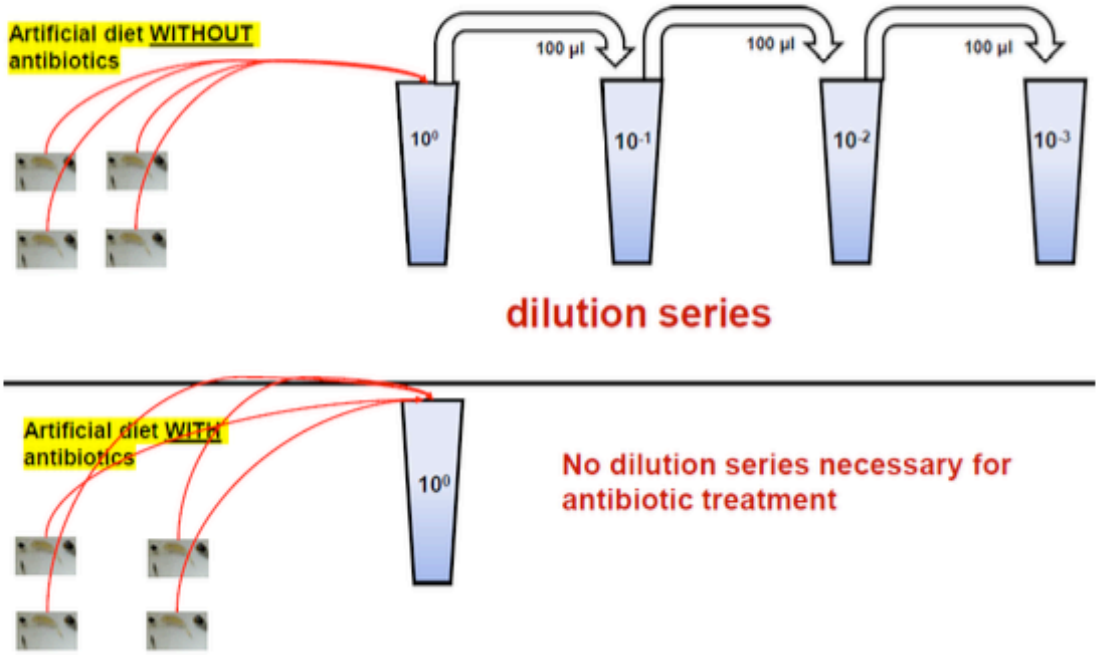

Figure 3 Dilution series

For the treatments <u>with</u> antibiotic diet, 100 µl from the 10^0 Eppendorf tube is spread on a petri dish with LB Agar using a sterile, plastic spreader. (Change spreader for each petri dish!)

For the treatments <u>without</u> antibiotic diet, 3 petri dishes are prepared, using 100 µl each from 10^0 and the dilution steps 2 (10^{-2}) and 3 (10^{-3}) (Figure 4).

Remember to change pipette tips and spreaders between each dilution step and petri dish!

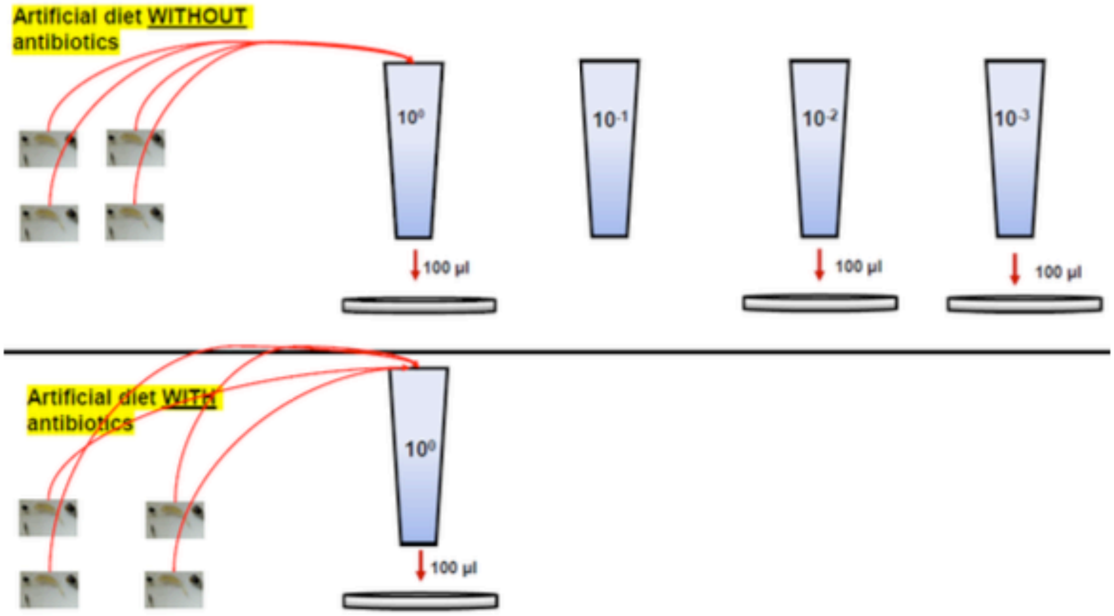

Figure 4 Spreading dilutions on petri dishes

For each of the two replicates 8 petri dishes are needed.
That means in the end 16 petri dishes should have been prepared

- 2x 3 petri dishes (10^0, 10^{-2} and 10^{-3}) for the 2x 4 guts from susceptible without antibiotic diet
- 2x 3 petri dishes (10^0, 10^{-2} and 10^{-3}) for the 2x 4 guts from resistant without antibiotic diet
- 2x 1 petri dish (10^0) for the 2x 4 guts from susceptible with antibiotic diet
- 2x 1 petri dish (10^0) for the 2x 4 guts from resistant with antibiotic diet

The petri dishes are placed in the incubator for 72 hours at 30°C.

Appendix n°3

Questionnaire

Frågor	**Svaralternativ**
Vilket kön har du?	Man
	Kvinna
	Annan
Hur gammal är du?	20-30
	31-40
	41-50
	51-60
	61-70
	71-80
Vad är din utbildningsbakgrund?	Grundskola
	Teoretiskt gymnasieprogram
	Lantbruksutbildning
	Högskoleutbildning
	Annan
Inom vilket län bedriver du din verksamhet?	Blekinge
	Gotland
	Halland
	Jönköping
	Kalmar
	Kronoberg
	Östergötland
	Skåne
	Västra Götaland
Hur stor är din gård (ha)?	<1 ha
	1-2 ha
	3-5 ha
	6-10 ha
	11-30 ha
	31-50 ha
	51-100 ha
	>100 ha

Hur många års erfarenhet av jordbruk har du?	<1 år
	1-2 år
	3-5 år
	6-10 år
	11-15 år
	16-20 år
	>20 år
Är verksamheten konventionell eller ekologisk (certifierad eller icke-certifierad)?	Den är helt konventionell (100%)
	Den är huvudsakligen konventionell (>65%)
	Ungefär hälften är konventionell och hälften ekologisk (certifierad eller icke-certifierad) (35-65%)
	Den är huvudsakligen ekologisk (certifierad eller icke-certifierad)(>65%)
	Den är helt ekologisk (certifierad eller icke-certifierad) (100%)
Vilka är de viktigaste grödorna på din gård?(Kan vara flera val)	Spannmål
	Frukt
	Grönsaker
	Bär
	Fodergrödor
Vilka är de största växtskyddsproblem du möter på din gård? (Kan vara flera val)	Insekter
	Ogräs
	Svampsjukdomar
	Annan (var vänlig specificera)
Vilka av följande metoder använder du i ditt växtskydd? (Kan vara flera val)	Val av växtplats
	Växtföljd
	Biologiska växtskyddsmedel
	Samodling

	Kemiska växtskyddsmedel
	Annan (var vänlig specificera)
Använder du kemiska växtskyddsmedel på din gård?	Ja
	Nej
Om du använder kemiska växtskyddsmedel, vad är den främsta anledningen till det? (Kan vara flera val)	Det är mer effektivt
	Det är billigare
	Det är lättare att använda
	Det finns inga andra effektiva metoder
	Annan (var vänlig specificera)
Om du använder kemiska växtskyddsmedel, vilka källor föredrar du att använda för att välja kemiska växtskyddsmedel? (Kan vara flera val)	Rådgivningstjänster (t.eg hushållningssällskapet)
	Jordbruksverket
	Andra jordbrukares råd
	Internet
	Branschtidningar
	Egen erfarenhet av effektiviteten
	Annan (var vänlig specificera)
Om problemet finns kvar när du använt ett kemiska växtskyddsmedel så som det kan användas, vad gör du då? (Kan vara flera val)	Fortsätter spruta med samma preparat
	Byter till ett annat växtskyddsmedel
	Använder ingen kontrollåtgärd resten av säsongen
	Annan (var vänlig specificera)
Känner du till västskyddsmedelsresistens?	Ja
	Delvis bekant
	Har hört om det men vet inte mycket
	Har aldrig hört talas om det

Om du är bekant med resistensutveckling, tittar du på de resistensstrategier som Jordbruksverket tar fram?	Ja
	Nej
	Jag visste inte det fanns information om detta
Om du är bekant med resistens, var får du information om det? (Kan vara flera val)	Rådgivningstjänster (t.ex. hushållningssällskapet)
	Jordbruksverket
	Andra jordbrukares råd
	I bruksanvisningen till preparatet
	Internet
	Tidningar
	Länsstyrelsens kurs i användande av växtskyddsmedel
	Annan (var vänlig specificera)
Har du uppfattat att resistens uppstått i samband med bekämpning?	Ja
	Nej
	Jag vet inte
Om ja på föregående fråga, hur märkte du det och vad vidtog du för åtgärd?	Öppet svar
Vad tror du är de främsta orsakerna till utveckling av resistens? (Kan vara flera val)	Skadegöraren
	Vilket preparat som används
	För liten användning av växtskyddsmedel
	För mycket användning av växtskyddsmedel
	Annan (var vänlig specificera)
Vilka åtgärder vidtar du för att undvika resistensutveckling?	Öppet svar
Känner du till evolution?	Ja
	Nej

Var har du fått informationen om evolution?	Gymnasium
	Högre utbildning
	Rådgivningstjänster
	Självstudier
	Annan (specificera)
	Jag har ingen information
Betyg dina kunskaper om evolution (5-hög, 3-genomsnitt, 1-låg)	5
	3
	1
Vad anser du vara den största negativa effekten av bekämpningsmedel? (Kan vara flera val)	Negativa effekter på biologisk mångfald
	Negativa effekter på människor
	Utveckling av växtskyddsmedelsresistens
	Annat (specificera)

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