

Norwegian University
of Life Sciences

Master's Thesis 2018 60 ECTS

Faculty of Chemistry, Biotechnology and Food Science

Multitrophic effects of the relationship between a plant growth-promoting rhizobacteria (*Bacillus amyloliquefaciens*) and arugula (*Eruca sativa*) on the predator *Doru luteipes*, using *Plutella xlyostella* and *Spodoptera frugiperda*.

Kim J. F. Hagbardsland

Biology

Abstract

Background. Plant growth-promoting rhizobacteria (PGPR) provide several benefits to plants, such as faster growth, larger yields and resistance to pests. When attacked by herbivores, plants defend themselves in various ways using both direct and indirect defenses. One example of indirect defense is the attraction of predators using herbivore induced plant volatiles. Specialist herbivores are thought to be better at suppressing the plant defenses of their host-plant compared to a generalist herbivore. This study investigates the predator *Doru luteipes*' choices in a series of olfactometry experiments using arugula (*Eruca sativa*) as the host-plant, *Plutella xylostella* larvae as the specialist herbivore, *Spodoptera frugiperda* larvae as the generalist herbivore and *Bacillus amyloliquefaciens* as the PGPR.

Methodology/Principal findings. Prior to experiments some plants were inoculated with *B. amyloliquefaciens*, larvae were starved for 24 hours and *D. luteipes* were starved for 72 hours. Then, inoculated and non-inoculated plants were subjected to larval herbivory for 12 hours. The olfactometry setup contained a source of clean air, a humidifier, a flow regulator, two glass containers housing a plant/treatment and a Y-tube. A choice was considered to have been made when *D. luteipes* passed the halfway mark of one of the Y-tube branches, individuals that had not done so within five minutes were considered unresponsive. The results indicated a tendency for preference for plants damaged by *S. frugiperda* and inoculated with the *B. amyloliquefaciens* strain GB03. **Conclusions/Significance.** Based on these findings the components used in this study could be used in integrated pest management. However, the data is not conclusive and warrants further investigation.

Sammendrag

Bakgrunn. Det finnes mange jordlevende bakterier som gir fordeler til planter, slik som raskere vekst, større avling og motstandsdyktighet mot skadegjørere. Når planter blir angrepet av herbivorer forsvaret de seg på forskjellige måter ved bruk av både direkte- og indirekte forsvarsmetoder. Et eksempel på indirekte forsvar er tiltrekningen av predatorer ved bruk av flyktige stoffer. Planteetende spesialister antas å være bedre til å minske effekten av forsvaret til vertsplantene deres, sammenlignet med planteetende generalister. I denne studien undersøkes predatoren *Doru luteipes* valg i en rekke olfaktometer eksperimenter, ved bruk av rucola (*Eruca sativa*) som vertsplante, *Plutella xylostella* larver som planteetende spesialist, *Spodoptera frugiperda* larver som planteetende generalist og *Bacillus amyloliquefaciens* som fordelaktig jordlevende bakterie. **Metode/Hovedfunn.** I forkant av eksperimentene ble noen planter inokulert med *B. amyloliquefaciens*, larver ble sultet i 24 timer og *D. luteipes* ble sultet i 72 timer. Deretter ble inokulerte og ikke-inokulerte planter utsatt for skader fra larver i 12 timer. Olfaktometer oppsettet bestod av en kilde med ren luft, en luftfukter, en mengde regulerende ventil, to glass beholdere som hver inneholdt en plante/behandling og en Y-tube. Et valg ble regnet som tatt dersom *D. luteipes* beveget seg lenger enn halvveis i en av armene i Y-tuben, individer som ikke hadde tatt et valg innen fem minutter ble notert som ikke responderende og utelatt fra statistikken. Resultatene indikerer en tendens for preferanse for planter skadet av *S. frugiperda* som også var inokulert med *B. amyloliquefaciens* varianten GB03. **Konklusjon/Viktighet.** Basert på disse funnene er det mulig at komponentene i denne studien kan brukes i integrert skadedyrkontroll. Resultatene fra denne studien er ikke nok til å konkludere noe med, derfor anbefales videre undersøkelser.

Acknowledgements

I would like to thank the following people for playing a part in the making of this thesis, without you this would not have been possible:

Richard Meadow

Geir Kjølberg Knudsen

José Mauricio Simões Bento

Italo Delalibera Jr.

Rafaela Cristina dos Santos

Natalia Naranjo-Guevara

Franciele Santos

Mariana Oliveira Garrigós Leite

Arodi Prado

Mateus Tonelli

Fernando Sujimoto

Vitor Isaias

Ingeborg Klingen

Cathrine Strømø

Georgia Mae Bell

Solange Aparecida Vieira Barros

Table of contents

1. Introduction	1
1.1 Soil bacteria	1
1.2 Plant defenses	1
1.3 Specialist and generalist herbivores	2
1.4 <i>Bacillus amyloliquefaciens</i>	2
1.5 <i>Plutella xylostella</i>	2
1.6 <i>Spodoptera frugiperda</i>	3
1.7 <i>Doru luteipes</i>	3
1.8 <i>Eruca sativa</i>	3
2. Materials and methods.....	4
2.1 Rhizobacteria multiplication.....	4
2.2 Plant cultivation.....	4
2.3 Insect rearing	4
2.4 Treatments.....	5
2.5 Olfactometer Y-tube experiments.....	6
2.6 Statistical analyses.....	7
3. Results.....	8
3.1 Bacteria acquisition	8
3.2 Olfactometry Y-tube experiments	8
4. Discussion	12
4.1 Bacteria acquisition of arugula (<i>Eruca sativa</i>).....	12
4.2 Earwig choice.....	12
4.3 What could have been done differently?	14
4.4 Future work.....	14
5. Literature.....	15

1. Introduction

1.1 Soil bacteria

Soil-borne microbial mutualists, such as rhizobacteria and mycorrhizal fungi can affect plants in several ways. For example, they can promote growth and higher yields when used as a bio fertilizer (Vessey, 2003; Rodriguez & Sanders, 2015; Bender, Wagg & van der Heijden, 2016), as well as provide resistance against arthropod pests (Ramamoorthy, Viswanathan, Raguchander, Praksam & Smayyappan, 2001; Pineda et al., 2013). Plant Growth Promoting Rhizobacteria (PGPR) naturally occur in the soil, inhabiting the area around or on the root surface. They can affect plant growth directly in the following ways: nitrogen fixation (Glick, Patten, Holguin & Penrose, 1999), phosphate solubilization (Rifat, Safdar, Ummay, Rabia & Iftikhar, 2010), siderophore production assisting in iron uptake (Rajkumar, Ae, Prasad & Freitas, 2010), production of the phytohormone indole acetic acid (IAA) which promotes growth (Glick, 2012) and 1-Aminocyclopropane-1-carboxylate (ACC) deaminase which decreases ethylene levels in the soil (Saleem, Arshad, Hussain & Bhatti, 2007). Indirectly they can act as biocontrol agents by producing antifungal metabolites and inducing systemic resistance against pathogens (Lugtenberg & Kamilova, 2009). PGPRs can interact in various ways with aboveground insects, such as inducing resistance against herbivores or attracting predators (van der Putten, Vet, Harvey & Wackers, 2001; Pineda, Zheng, Van Loon, Pieterse & Dicke, 2010). PGPRs potentially hold great importance for agricultural ecosystems as they can contribute to reduced use of agro-chemicals like fertilizers and pesticides (Weyens, van der Lelie, Taghavi, Newman & Vangronsveld, 2009; Yang, Kloepper & Ryu, 2008).

1.2 Plant defenses

Induced defenses are produced by plants after an herbivore attack; direct defenses reduce growth and/or reproduction of herbivores (Schoonhoven, van Loon & Dicke, 2005), and indirect defenses are for example the emission of herbivore induced plant volatiles to attract the pests' natural enemies (Price et al., 1980; Stenberg, Heil, Ahman & Bjorkman, 2015). Natural enemies use these chemical cues to search for prey or hosts and can consequently suppress herbivore populations (Turlings, Tumlinson & Lewis, 1990; de Moraes, Lewis & Paré, 1998; Dicke & van Loon, 2000; Kessler & Baldwin, 2002). Plant-associated microbes can influence indirect plant defenses and recruitment of the pests' natural enemies (Guerrieri, Lingua, Digilio, Massa & Berta, 2004; Godschalx, Schädler, Trisel, Balkan & Ballhorn, 2015). Plant-beneficial, soil-borne bacteria have been known to induce plant defenses (D'Alessandro et al., 2014) and may cause interactions with higher trophic levels (Ryu, Murphy, Mysore & Kloepper, 2004). These bacteria have also been shown to produce volatile organic compounds (VOCs), which while enhancing plant fitness (Blom et al., 2011; Bailly & Weisskopf, 2012) and reducing pathogen infectivity in the soil (Hunziker et al., 2015) might interfere with the attraction of herbivore antagonists such as predators or parasitoids (Pineda et al., 2013).

1.3 Specialist and generalist herbivores

Specialist herbivores are thought to either tolerate the plant defenses of their host plant or evade them better than a generalist predator. The induced plant response from specialist damage will in some cases be distinct compared to a generalist herbivore. This could be due to activation of fewer or different pathways. However, recent studies show that if the herbivores are from the same feeding guild, the differences may be less noticeable (Ali & Agrawal, 2012). For some systems, the interaction between plants and soil-borne beneficial microorganisms can improve resistance to generalist herbivores, but not necessarily to specialists (Pineda, Zheng, van Loon & Dicke, 2012). To understand how a PGPR, herbivores, a predator and a host-plant function as a system, the following components were chosen:

1.4 *Bacillus amyloliquefaciens*

The *Bacillus amyloliquefaciens* strain GB03 (Formerly *Bacillus subtilis*) (Fan, Blom, Klenk & Borriss, 2017) is a commercially produced PGPR used as a biological fungicide (Kodiak®, Bayer CropScience) in the seeds of cotton, peanuts, beans, soybeans, wheat, barley and corn (U.S. EPA, 2005). The GB03 strain's ability to stimulate plant growth through bacterial volatile emission was observed in *Arabidopsis* plants (Ryu et al., 2003). Similarly, the GB03 strain showed developmental improvement in *Arabidopsis* through increased photosynthetic capacity and salinity tolerance (Zhang et al., 2008a; Zhang et al., 2008b). GB03 has also been shown to increase sulfur uptake in *Arabidopsis*, which increased protection against the beet armyworm (Aziz et al., 2016).

1.5 *Plutella xylostella*

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is a crucifer specialist herbivore that is considered highly invasive and is present wherever its host plants can be found (Shelton, 2004). It is estimated that *P. xylostella* causes worldwide damage in the range of US \$4-5 billion annually (Zalucki et al., 2012). The species is mainly wind-dispersed (Chapman et al., 2002 & Coulson et al., 2002), but can also be a hitchhiker found on plants, vehicles and containers to name a few (Centre for Agriculture and Bioscience International (CABI), 2018a). It is a very difficult pest to control as it develops resistance quickly to most insecticides and cultural control is unreliable (Furlong, Wright & Dossall, 2013). There are examples of biological control and integrated pest management (IPM) being effective in controlling the *P. xylostella* populations (Talekar and Shelton, 1993; Furlong, Wright & Dossall, 2013).

1.6 *Spodoptera frugiperda*

The fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a generalist herbivore that primarily feeds on members of the Poaceae family, but it is also found in cruciferous crops. Although native to the tropical and subtropical Americas, it is now widespread over both American continents (CABI, 2018b). It has also spread to many African (Food and Agriculture Organization of the United Nations, 2018) and some Asian countries (CABI, 2018b). In Nicaragua *S. frugiperda* has been recorded to cause up to 73% yield loss in corn *Zea mays* (Hruska & Gould, 1997), while in Africa *S. frugiperda* appears to be more damaging than other *Spodoptera* species (Devi, 2018). Control of this pest is mainly done using insecticides, other viable methods include host-plant resistance (Estruch et al., 1996), destroying overwintering sites, mating disruption (Guerrero, Malo, Coll & Quero, 2014) and attraction of parasitoids (CABI, 2018b).

1.7 *Doru luteipes*

The earwig *Doru luteipes* (Scudder) (Dermaptera: Forficulidae) is a nocturnal omnivore (Alvarenga, Vendramim & Cruz, 1996) that often preys on aphids (Bacci, Picanço, Gusmo, Barreto & Galvan, 2002), lepidopteran eggs and larvae (Reis, Oliveira & Cruz, 1988; Fenoglio & Trumper, 2007). It is present in several South American countries (Steinmann, 1993, p. 533-534) and is considered a potential biological control agent in corn (*Z. mays*) (Waquil, Viana & Cruz, 2002) and brassicas (Bacci et al., 2002). Several studies on insecticide selectivity involving *D. luteipes* and its prey, have found that *D. luteipes* is resistant to many insecticides and can be used as a biological control in conjunction with insecticides (Bacci et al., 2002; Campos, Picanço, Martins, Tomaz & Guedes, 2011); however, there are few studies on how to attract *D. luteipes*.

1.8 *Eruca sativa*

Arugula Eruca sativa Mill. is a member of the Brassicaceae family cultivated in most parts of the world. It's commonly used in Asia to make Eruca seed oil, in Africa as a medicinal plant and in the Americas and Europe as a condiment and salad ingredient (Padulosi & Pignone, 1996). In 2016 arugula production spanned 40 949 hectares, making it the second most commonly grown leafy green in Brazil (Koch, Pacotte, Udsen & Angelini, 2017). Pests of *E. sativa* include aphids (Menéndez, Romero, Folcia & Martínez-Ghersa, 2010), lepidopterans (Ogran et al., 2016) and coleopterans (Ekbom, 1998).

In this study *E. sativa* was used as the host plant, *S. frugiperda* was used as the generalist herbivore, *P. xylostella* was used as the specialist herbivore, *D. luteipes* was used as the predator, and the *B. amyloliquefaciens* strain GB03 was used as the PGPR. The goal was to answer the following question: Are plants that are inoculated with the GB03 strain, and damaged by specialist or generalist herbivores, more attractive to earwigs?

2. Materials and methods

2.1 Rhizobacteria multiplication

The colonies of *B. amyloquefaciens* GB03 were obtained from the collection of microorganisms at Texas Tech University in Lubbock, Texas, USA in March 2014 and are being multiplied and kept in the Laboratory of Chemical Ecology and Behavior of Insects at Luiz de Queiroz College of Agriculture in Piracicaba, São Paulo, Brazil. A flamed platinum loop was used to scoop up bacteria from the colonized Petri dish and spread onto a new Petri dish (9 cm in diameter) using a zig-zag motion. The new Petri dish contained Trypticase Soy Agar (TSA) composed of 15g casein, 5g soy peptone, 5g sodium and 15g agar for each 1L of distilled water. Then the Petri dishes were incubated in a BOD incubator ($25 \pm 3^\circ\text{C}$, $65 \pm 5\%$ RH, 12 L:12 D) for 24 hours and stored in a refrigerator (4°C) until use.

2.2 Plant cultivation

Commercial arugula (*E. sativa*) seeds were sown in Basiplant® potting soil (250 ml) with 2.5g of fertilizer (Osmocote Plus® 15-09-12). To inoculate the plants with the rhizobacteria GB03, Falcon tubes containing 5 mL of liquid TSA and GB03 from the Petri dishes were left in a shaker for 24 hours at 25°C and held at 150 rpm. Seeds given the GB03 treatment were immersed in the inoculum, while the seeds given the control treatment were immersed in liquid TSA culture medium without any bacteria. All seeds were immersed for thirty minutes prior to sowing. 20 plants, 10 inoculated and 10 non-inoculated, at 4 weeks of age were weighed on an analytical balance to obtain their fresh weight. Afterwards, the plants were placed in an oven at 60°C for 72 hours and re-weighed to obtain their dry weight. A difference in weight between inoculated and non-inoculated plants indicates successful inoculation of GB03.

All plants were grown in a screen house under natural light and temperature from November 2017 - March 2018 in Piracicaba, São Paulo, Brazil. They were watered three times a day with an automatic irrigation system until 30 days after emergence, after which they were used in the experiments.

2.3 Insect rearing

The caterpillars used in the experiments were obtained from laboratory rearing and kept under controlled conditions ($25 \pm 3^\circ\text{C}$, $65 \pm 5\%$ RH, 12 L:12 D). *S. frugiperda* caterpillars were fed an artificial diet as described by Parra (2007). *P. xylostella* caterpillars were fed a natural diet based on cabbage leaves (*Brassica oleracea* L).

Females of the earwig *D. luteipes* were collected by hand in maize and sugar cane plantations in Piracicaba, São Paulo, Brazil. The individuals were taken to the laboratory and kept in closed plastic boxes (23 cm x 7 cm x 14 cm). Simulation of *D. luteipes*' natural habitat was adapted from methods described by Pasini, Parra & Lopes (2007), Cruz (2009) and Butnariu, Pasini, Reis & Bessa (2013). The plastic boxes were covered with aluminum foil to reduce light incidence. To supplement oviposition substrate and refuge, pieces of wet cotton were inserted into 3 cm long sections of transparent drinking straws. Corrugated cardboard was placed in the boxes as additional refuge to reduce cannibalism. The earwigs were fed an artificial diet consisting of dry cat food which contains animal and plant protein and fat as well as various vitamins and minerals (35%), wheat bran (27%), brewer's yeast (23%), milk powder (14%), methyl paraben (0,5%) and sorbic acid (0,5%). The cotton was moistened, and food replenished twice weekly. In cases where eggs were laid, both the eggs and the female were transferred to a 14 cm Petri dish. Two days after the eggs hatched, the female was returned to her original box and the nymphs were placed in a box containing other nymphs of similar age and kept until adulthood.

2.4 Treatments

One day prior to experiments, inoculated and non-inoculated plants (30 days old) were transferred from the screenhouse to the laboratory, where they were exposed to supplementary lighting (60-80 μmol , 12 L: 12 D) and the pot was covered with aluminum foil to contain odors from the soil. 12 hours prior to the experiments, five third-instar caterpillars of *S. frugiperda* or *P. xylostella* were placed on arugula plants (*E. sativa*) to inflict herbivore damage. The caterpillars had been starved for 24 hours before being introduced to the plant. Then, both caterpillar-infested and undamaged arugula plants were covered with voile bags (22 x 30 cm) for 12 hours. The following treatments were used for the olfactory experiments:

- (i) Non-inoculated undamaged plant (NU)
- (ii) Inoculated undamaged plant (IU)
- (iii) Trypticase soy agar (TSA)
- (iv) Trypticase soy agar containing GB03 (TSA-GB03)
- (v) Non-inoculated plant damaged with *S. frugiperda* (NDS)
- (vi) Non-inoculated plant damaged with *P. xylostella* (NDP)
- (vii) Inoculated plant damaged with *S. frugiperda* (IDS)
- (viii) Inoculated plant damaged with *P. xylostella* (IDP)
- (ix) Clean air (CA)

2.5 Olfactometer Y-tube experiments

The system used in this experiment was composed of a source of clean, filtered air, two glass containers and a glass Y-tube (Fig. 1). The following combinations of treatments were used:

- | | |
|-----------------------|--------------------|
| (i) NU vs. CA | (vii) IDS vs. IU |
| (ii) NDS vs. NU | (viii) IDP vs. IU |
| (iii) NDP vs. NU | (ix) IDS vs. IDP |
| (iv) NDS vs. NDP | (x) NDS vs. IU |
| (v) IU vs. NU | (xi) NDP vs. IU |
| (vi) TSA vs. TSA-GB03 | (xii) IDS vs. NDS |
| | (xiii) IDP vs. NDP |

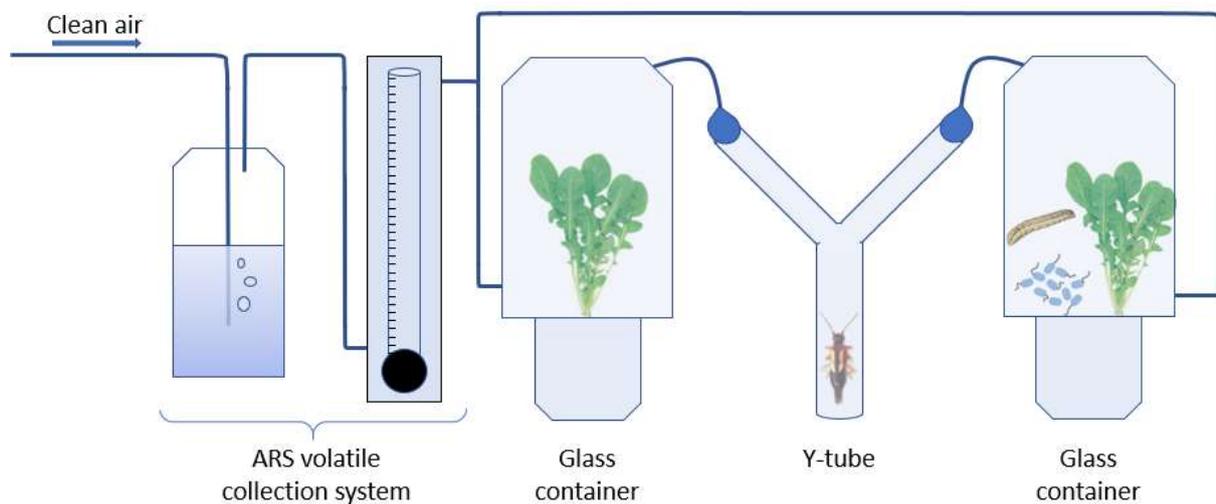


Figure 1. Schematic drawing of the system used in the olfactometry experiment. The illustration shows from left to right: a source of clear air, a humidifier and flow regulator (ARS volatile collection system), two glass chambers containing a plant/treatment and a Y-tube.

The glass Y-tube had one base (25 cm) and two branches (20 cm), and a narrow internal diameter (0.9 cm). This Y-tube was chosen to accommodate for the positively thigmotactic nature of earwigs. Clean air was supplied to the olfactometer system via an ARS Volatile Collection System (Analytical Research Systems, Gainesville, FL, USA), which allowed for flow regulation and humidified the air. From the ARS, air flowed through two glass chambers (10 cm diameter x 5 cm height), each containing a single plant/treatment, into the branches of the Y-tube. Air flow was adjusted to 1 L/min/branch. The experiments were conducted under controlled conditions ($25 \pm 1^\circ\text{C}$, $70 \pm 10\% \text{RH}$) at night (19:00 - 22:00) using only red lights to simulate night time. Earwigs were individually introduced into the long base of the Y-tube and observed for 5 min or until a choice had been made. A choice was defined as an earwig going beyond the halfway point of a branch. The individuals that did not chose a branch within 5 minutes were excluded from the

statistics. Earwigs were used only once. Each earwig was observed in a clean Y-tube. When a clean Y-tube was connected to the system, the treatments swapped branches and the Y-tube was rotated to avoid side bias. After being used once, each Y-tube was washed with acetone (90% v/v) and dried at 170 °C for 2 minutes. Every ten insects, a new pair of plants/treatments were used. At least 30 earwigs were observed for each combination of treatments. Each combination of treatments was observed on at least two different days. To reduce variability, only adult females of *D. luteipes* were used in the olfactometer experiments. Prior to the experiments the earwigs were starved for 72 hours.

2.6 Statistical analyses

The fresh and dry weights of inoculated and non-inoculated plants were compared using the Welch's t-test due to the heteroscedasticity of the data. The exact binomial test of goodness-of-fit was used for analyzing the earwig choice in olfactometer experiments, with the null hypothesis being that the distribution would be 50/50 (McDonald, 2014). Statistical analyses were performed using R version 3.5.1 (<https://www.r-project.org/>).

3. Results

3.1 Bacteria acquisition

A significant difference was observed in fresh weight between inoculated ($M = 1.30\text{g}$, $SD = 0.84$) and non-inoculated ($M = 0.16\text{g}$, $SD = 0.09$) plants; $t(9.19) = 4.287$, $P = 0.002$. There was also a significant difference in dry weight between inoculated ($M = 0.08\text{g}$, $SD = 0.05$) and non-inoculated ($M = 0.01\text{g}$, $SD = 0.01$) plants; $t(9.22) = 4.522$, $P = 0.001$.

3.2 Olfactometry Y-tube experiments

D. luteipes females were responsive in olfactometer tests to plant volatiles of *E. sativa*. First, they were significantly more likely to choose non-inoculated undamaged plants (NU) over clean air (CA) ($P = 0.016$) (Fig. 2).

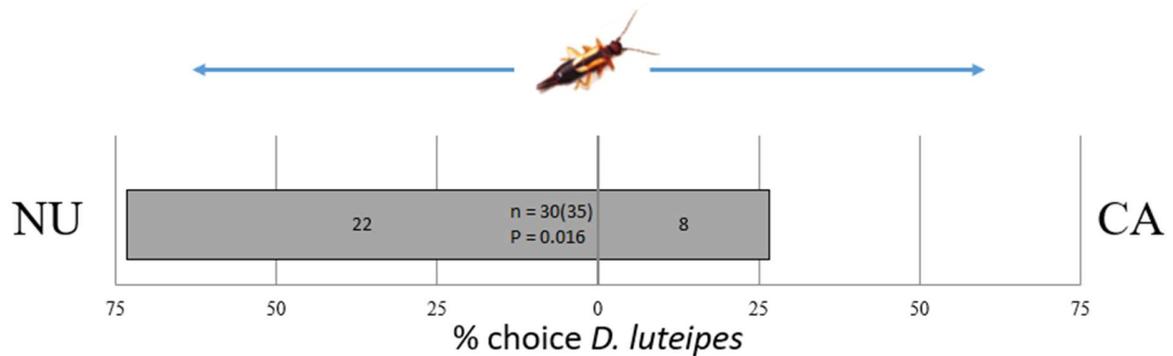


Figure 2. Distribution of choices made by *Doru luteipes* between non-inoculated undamaged plants of *Eruca sativa* (NU) and clean air (CA); total number of individuals tested in parentheses.

Given the choice between non-inoculated undamaged plants and non-inoculated plants of *E. sativa* damaged by *S. frugiperda* (NDS), there was a tendency for preference for NDS over NU ($P = 0.099$). There was no significant difference between the attraction of earwigs to non-inoculated plants damaged by *P. xylostella* (NDP) and NU ($P = 0.36$). When earwigs were exposed to non-inoculated plants damaged with both larvae (NDS vs. NDP), although not significant, more of them chose NDS ($P = 0.2$) (Fig. 3).

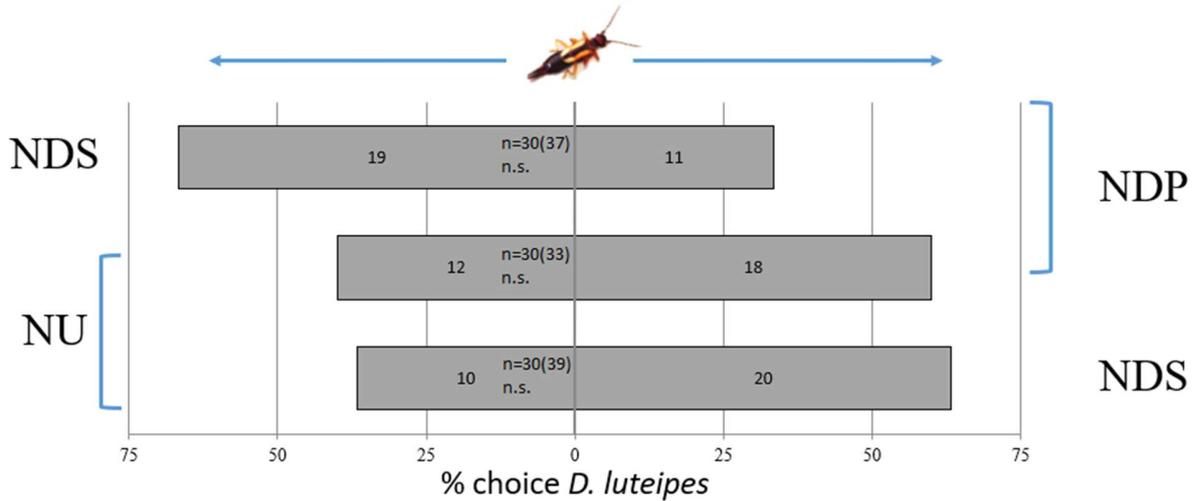


Figure 3. Distribution of choices made by *Doru luteipes* between non-inoculated plants of *Eruca sativa* damaged by *Spodoptera frugiperda* (NDS), damaged by *Plutella xylostella* (NDP) and undamaged (NU); total number of individuals tested in parentheses.

Earwigs did not show any preference to inoculated undamaged plants (IU) over NU ($P = 0.47$). When they were exposed to odors from Petri dishes with TSA agar and TSA containing GB03, there was a tendency for preference for TSA without GB03 ($P = 0.099$) (Fig. 4).

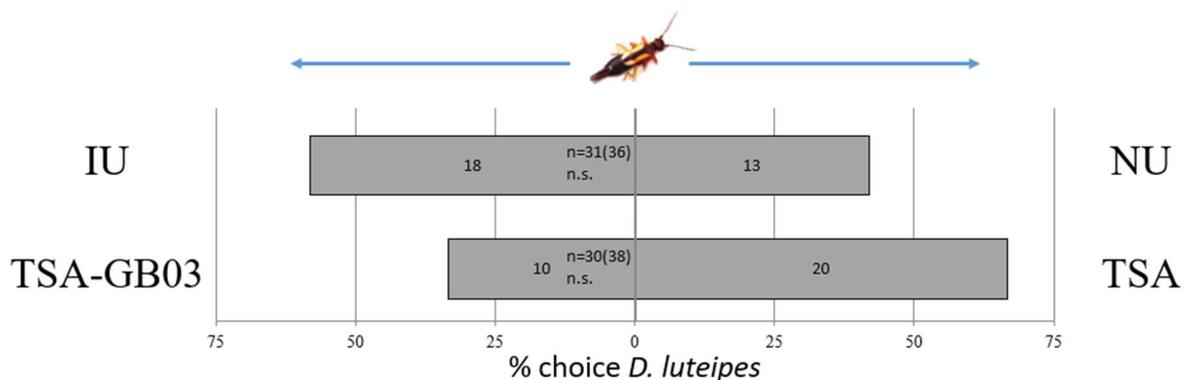


Figure 4. Distribution of choices made by *Doru luteipes* between non-inoculated undamaged plants (NU) and inoculated undamaged plants (IU) of *Eruca sativa*, and TSA agar (TSA) and TSA agar with GB03 (TSA-GB03); total number of individuals tested in parentheses.

When comparing inoculated undamaged plants (IU) to inoculated plants of *E. sativa* damaged by *S. frugiperda* (IDS), there was a weak tendency for preference for IDS ($P = 0.11$). There were no statistically significant differences when comparing inoculated plants damaged by *P. xylostella* (IDP) to IU ($P = 0.85$). When earwigs were exposed to IDS and IDP there was a tendency for preference for IDS ($P = 0.099$) (Fig. 5).

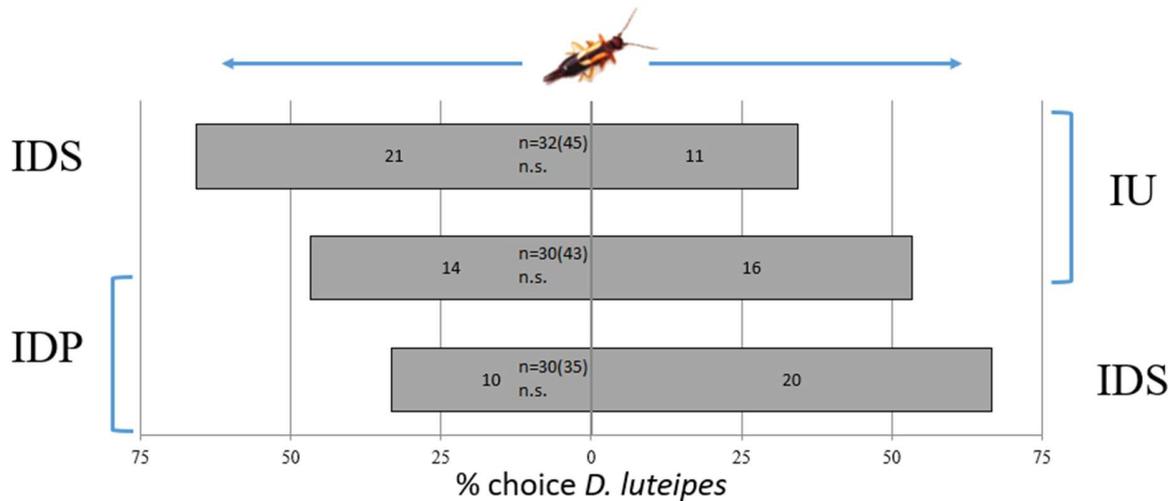


Figure 5. Distribution of choices made by *Doru luteipes* between inoculated plants damaged by *Spodoptera frugiperda* (IDS), damaged by *Plutella xylostella* (IDP) and inoculated undamaged plants (IU) of *Eruca sativa*; total number of individuals tested in parentheses.

There were no statistically significant differences when contrasting NDS and IU ($P = 0.2$) or NDP and IU ($P = 0.28$) (Fig. 6).

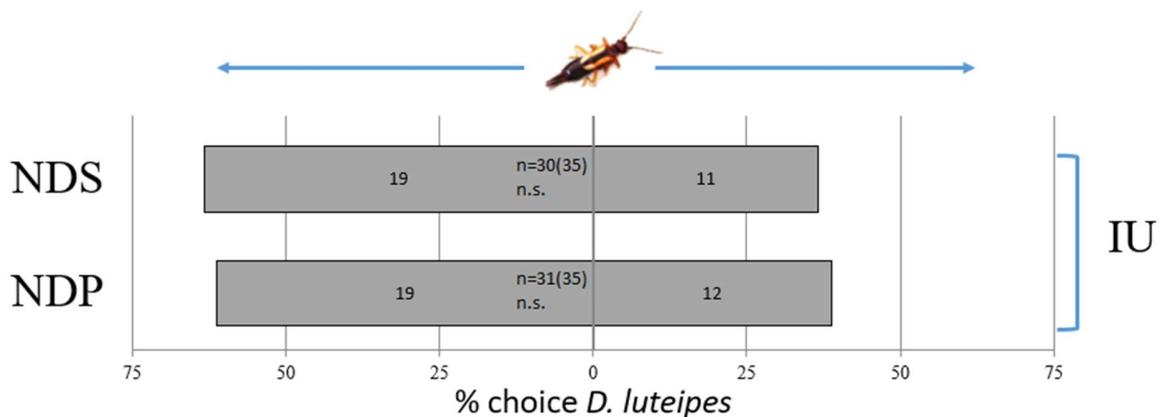


Figure 6. Distribution of choices made by *Doru luteipes* between non-inoculated plants damaged by *Spodoptera frugiperda* (NDS) and *Plutella xylostella* (NDP) and inoculated undamaged plants (IU) of *Eruca sativa*; total number of individuals tested in parentheses.

When comparing plants damaged by *S. frugiperda*, earwigs preferred inoculated plants (IDS) over non-inoculated plants of *E. sativa* (NDS) ($P = 0.043$). There was no statistically significant difference when comparing inoculated (IDP) and non-inoculated plants (NDP) damaged by *P. xylostella* ($P = 0.2$) (Fig. 7).

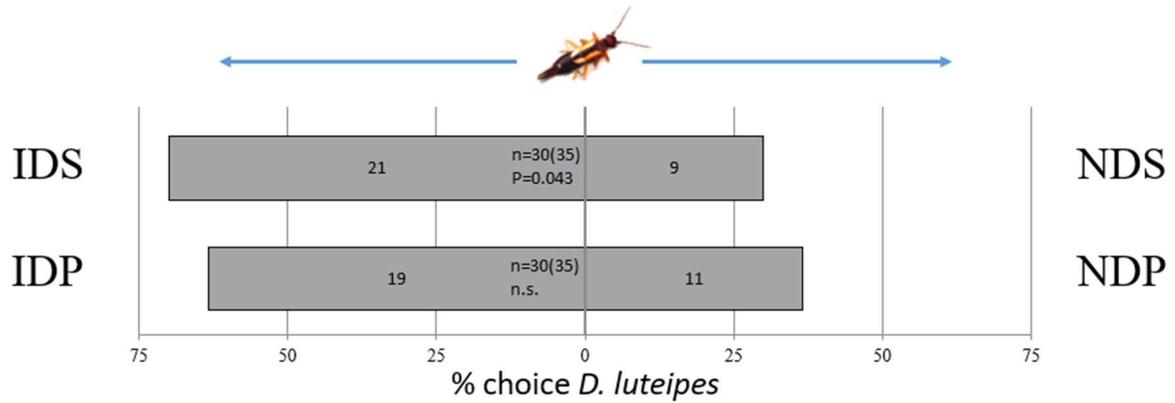


Figure 7. Distribution of choices made by *Doru luteipes* between inoculated plants of *Eruca sativa* damaged by *Spodoptera frugiperda* (IDS) and non-inoculated plants damaged by *S. frugiperda* (NDS) and between inoculated plants of *E. sativa* damaged by *Plutella xylostella* (IDP) and non-inoculated plants damaged by *P. xylostella* (NDP); total number of individuals tested in parentheses.

4. Discussion

4.1 Bacteria acquisition of arugula (*Eruca sativa*)

Previous research has shown that plants inoculated with GB03 grow larger and faster than plants that have not been inoculated with GB03 (Ryu et al., 2003). The results show a significant difference in weight and thus it is safe to assume that the plants were successfully inoculated.

4.2 Earwig choice

The results from two experiments showed statistical significance: comparing clean air to non-inoculated undamaged plants (Fig. 2), and comparing inoculated plants damaged by *S. frugiperda* to non-inoculated plants damaged by *S. frugiperda* (Fig. 7). In the other experiments, the results indicated tendencies that can be ranked based on wins and losses. The treatments, in order of most attractive to least attractive for *D. luteipes* were:

- inoculated plants damaged by *S. frugiperda*
- non-inoculated plants damaged by *S. frugiperda*
- inoculated plants damaged by *P. xylostella*
- non-inoculated plants damaged by *P. xylostella*
- inoculated undamaged plants
- non-inoculated undamaged plants

Looking at this ranking a pattern emerges:

Firstly, plants damaged by *S. frugiperda* were preferred over plants damaged by *P. xylostella*. This could be explained by most of the earwigs used in this study coming from corn and sugar cane plantations, where *S. frugiperda* would have been a big part of their diet. *D. luteipes* is also a well-known predator of *S. frugiperda* (Cruz & Oliveira, 1997) so VOCs associated with it might be more attractive to them. In a study by Vogel, Kroymann & Mitchell-Olds (2007) on *Boechera divaricarpa* they found that *P. xylostella* attacks induced ethylene (ET) and salicylic acid (SA) genes. In the 2012 review by Ali and Agrawal, studies that compared leaf-chewing insects to phloem-feeding insects found that phloem-feeding insects mainly induced SA and ET genes, while leaf-chewing insects mainly induced jasmonate (JA) and ET genes. The earwigs might have interpreted the VOCs from plants damaged by *P. xylostella* to be from a phloem-feeder, such as aphids, and chosen plants damaged by *S. frugiperda* because it is more economical to attack one larva compared to 20 aphids. Another reason might be that *P. xylostella* is considered a crucifer specialist, so it might interact with the plant in a way that produces smaller quantities of VOCs (Sobhy, Miyake, Shinya & Galis, 2017).

Secondly, inoculated plants were preferred over non-inoculated plants. In this study the plants used were all 30 days of age, which means that the plants inoculated by GB03 were significantly larger than the non-inoculated plants. The larger plants should be able to produce larger quantities of defense compounds, which would release larger quantities of VOCs, that in turn would seem more attractive to the earwig. Another reason for this could be a change in plant metabolites, which some soil microbes are known to influence (Sharifi, Lee & Ryu, 2018).

Given the tendencies of preference for *E. sativa* inoculated with GB03, this might indicate that there is a symbiotic effect between GB03 and *E. sativa*. Not only does the arugula plant grow faster, but also when under attack by herbivores it releases volatiles that seem more attractive to the predator *D. luteipes*. In a study on prey handling rates in a laboratory setting by Reis, Oliveira and Cruz (1988), *D. luteipes* was shown to eat 21 *S. frugiperda* larvae a day as adults and 12 larvae a day as nymphs. Nymphs were also shown to eat 13 eggs of *S. frugiperda* a day when larvae were not available. It has been claimed that if 70% of corn plants had earwigs on them, it would be enough to keep the damage from *S. frugiperda* below the economic threshold (Waquil, Viana & Cruz, 2002); this might be true in other systems as well, if it is possible to attract earwigs. However, more research on the topic is needed, as the findings are not conclusive.

When comparing TSA and TSA with GB03, there was a tendency for preference for TSA (Fig. 4). GB03 in a Petri dish smells of bacteria/rot, which might be why it seemed deterring to the earwigs. Since all the plants in these experiments had the pot and soil covered by aluminum foil, VOCs released from the bacteria in the soil may not have made their way into the Y-tube. Earwigs rarely fly so it would be interesting to find out if they are deterred by odors from the soil when seeking out foraging sites --and if the odors produced by GB03 in the soil affects them in any way.

The distribution of earwig choice seems to be two-thirds to one side and one third to the other for all the experiments except for IDP vs IU (Fig. 5). These tendencies are in line with the predictions made prior to the experiments and the method is robust enough to not cause any bias. Regarding how these results would translate to a field setting, inoculating plants with GB03 does increase growth which leads to more resistant plants. However, there is no hard evidence that earwigs would be more attracted to the inoculated plants over other nearby plants.

4.3 What could have been done differently?

Because most results show tendencies while not being statistically significant, a sample size of at least 50 repetitions per experiment would have been preferable. To be able to truly rank the different treatments, the following experiments should have been included: IDS vs NDP, IDP vs NDS.

A control of mechanical damage to the plants should have been included. Only laboratory reared naïve, unmated female earwigs should have been used in the bioassays. However, *Doru lineare* (Eschs.) (Dermaptera: Forficulidae) is parthenogenetic (Cocco, Butnariu, Bessa & Pasini, 2013), if this trait is present in *D. luteipes* it might not matter whether they were mated or not. No males were kept with the females, but there is no guarantee that they had not been mated in the wild as they laid eggs.

Arugula does not provide *D. luteipes* with the optimal foraging environment, as there are no narrow spaces for the earwigs to seek refuge in. Therefore, it would have been better to use a different brassica such as cabbage (*Brassica oleracea*). This would increase the applicability of this study to the real world.

4.4 Future work

To explore these interactions further, it would be beneficial to analyze and compare the volatile organic compounds (VOCs) produced by all the treatments tested in this experiment. A chemical analysis of the VOCs would give insight into the mechanisms of the plant when inoculated by GB03, and how the different herbivores illicit different responses. It could also help identify which compounds are attractive to *D. luteipes*, which in turn can be used to create synthetic blends, made to attract large numbers of predators before an herbivore attack reaches the economic threshold. Due to the recent discovery of *D. lineare* exhibiting parthenogenesis (Cocco et al., 2013), it would be worthwhile to investigate whether *D. luteipes* can be parthenogenetic.

5. Literature

- Ali, J. G. & Agrawal, A. A. (2012). Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, 17(5), 293-302.
- Alvarenga C.D, Vendramim J.D. & Cruz, I. (1996). Efeito do Predador *Doru luteipes* (Scud.) Sobre o Crescimento Populacional de *Schizaphis graminum* (Rond.) em Diferentes Genótipos de Sorgo. *An. Soc. Entomol. Brasil* 25(1), 137-140.
- Aziz, M., Nadipalli, R. K., Xie, X., Sun, Y., Surowiec, K., Zhang, J. & Paré, P. W. (2016). Augmenting Sulfur Metabolism and Herbivore Defense in *Arabidopsis* by Bacterial Volatile Signaling. *Frontiers in plant science*, 7, 458.
- Bacci, L., Picanço, M. C., Gusmo, M. R., Barreto, R. W. & Galvan, T. L. (2002). Selective insecticides to the earwig *Doru luteipes* (Scudder) used for controlling the green peach aphid in Brassicaceae. *Horticultura Brasileira*, 2, 174-179.
- Bailly, A. & Weisskopf, L. (2012). The modulating effect of bacterial volatiles on plant growth: Current knowledge and future challenges. *Plant Signaling & Behavior*, 7(1), 79-85.
- Fan, B., Blom, J., Klenk, H. P. & Borriess, R. (2017). *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* Form an “Operational Group *B. amyloliquefaciens*” within the *B. subtilis* Species. *Complex. Front. Microbiol.* <https://doi.org/10.3389/fmicb.2017.00022>
- Bender, S. F., Wagg, C. & van der Heijden, M.G.A. (2016). An Underground Revolution: Biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology & Evolution*, 31(6), 440-452.
- Blom, D., Fabbri, C., Connor, E. C., Schiestl, F. P., Klauser, D. R., Boller, T., Eberl, L. & Weisskopf L. (2011). Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. *Environ. Microbiol.* 13, 3047–3058. <https://doi.org/10.1111/j.1462-2920.2011.02582.x>
- Butnariu, A., Pasini, A., Reis, F. & Bessa, E. (2013). Maternal Care by the Earwig *Doru lineare* Eschs. (Dermaptera: Forficulidae). *Journal of Insect Behavior*, 26(5), 667-678.
- CABI. (2018a). *Plutella xylostella*. In: *Invasive Species Compendium*. Wallingford, UK: CAB International. www.cabi.org/isc.
- CABI. (2018b). *Spodoptera frugiperda*. In: *Invasive Species Compendium*. Wallingford, UK: CAB International. www.cabi.org/isc.
- Campos, M. R., Picanço, M. C., Martins, J. C., Tomaz, A. C. & Guedes, R.N.C. (2011). Insecticide selectivity and behavioral response of the earwig *Doru luteipes*. *Crop Protection*, 30(12), 1535-1540.

- Chapman, J. W., Reynolds, D. R., Smith, A. D., Riley, J. R., Pedgley, D. E. & Woiwod, I. P. (2002). High-altitude migration of the diamondback moth *Plutella xylostella* to the U.K.: a study using radar, aerial netting, and ground trapping. *Ecological Entomology*, 27(6), 641-650.
- Cocco, J., Butnariu, A., Bessa, E. & Pasini, A. (2013). Sex produces as numerous and long-lived offspring as parthenogenesis in a new parthenogenetic insect. *Canadian Journal of Zoology*, 91(3), 187-190.
- Coulson, S. J., Hodkinson, I. D., Webb, N. R., Mikkola, K., Harrison, J. A. & Pedgley, D. E. (2002). Aerial colonization of high Arctic islands by invertebrates: the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) as a potential indicator species. *Diversity and Distributions*, 8(6), 327-334.
- Cruz, I. (2009). Métodos de criação de agentes entomófagos de *Spodoptera frugiperda* (J.E. Smith). In Bueno, V.H.P. (Ed.), *Controle biológico de pragas: produção massal e controle de qualidade* (p. 111-135). Lavras: Federal University of Lavras.
- D'Alessandro, M., Erb, M., Ton, J., Brandenburg, A., Karlen, D., Zopfi, J. & Turlings, T. C. J. (2014). Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. *Plant, Cell & Environment*, 37(4), 813-826.
- Devi, S. (2018). Fall armyworm threatens food security in southern Africa. *The Lancet*, 391(10122), 727.
- Dicke, M. & van Loon, J.J.A. (2000.) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata*, 97, 237-249.
- Ekbom, B. (1998). Clutch size and larval performance of pollen beetles on different host plants. *Oikos*, 1, 56-64.
- Estruch J. J., Warren G. W., Mulling M. A., Nye G. J., Craig J. A. & Koziel M.G. (1996). Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proceedings of the National Academy of Sciences of the United States of America*, 93(11), 5389-5394.
- Food and Agriculture Organization of the United Nations. 2018. Briefing note on fall armyworm (FAW) in Africa. 16 February 2018, 7 pp. Retrieved from: <http://www.fao.org/3/a-bt415e.pdf>
- Fenoglio, M. S. & Trumper, E. V. (2007). Influence of Weather Conditions and Density of *Doru luteipes* (Dermaptera: Forficulidae) on *Diatraea saccharalis* (Lepidoptera: Crambidae) Egg Mortality. *Environmental entomology*, 36, 1159-1165.
- Furlong, M. J., Wright, D. J. & Dossall, L. M. (2013). Diamondback Moth Ecology and Management: Problems, Progress, and Prospects. *Annual Review of Entomology*, 58, 517-541.
- Glick, B.R., Patten, C.L., Holguin, G. & Penrose, G.M. (1999). *Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria*. London: Imperial College Press.

- Glick, B. R. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications. *Scientifica*.
<http://dx.doi.org/10.6064/2012/963401>
- Godschalx, A.L., Schädler, M., Trisel, J.A., Balkan, M.A. & Ballhorn, D.J. (2015). Ants are less attracted to the extrafloral nectar of plants with symbiotic, nitrogen-fixing rhizobia. *Ecology*, 96(2), 348-354.
- Guerrero, A., Malo, E., Coll, J. & Quero, C. (2014). Semiochemical and natural product-based approaches to control Spodoptera spp. (Lepidoptera: Noctuidae). *Journal of Pest Science*, 87(2), 231-247.
- Guerrieri, E., Lingua, G., Digilio, M.C., Massa, N. & Berta, G. (2004). Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? *Ecol Entomol*, 29, 753-756.
- Hruska, A.J. & Gould, F. (1997) Fall armyworm (Lepidoptera: Noctuidae) and *Diatraea lineolata* (Lepidoptera: Pyralidae): impact of larval population level and temporal occurrence on maize yield in Nicaragua. *Journal of economic entomology*, 2, 611-622.
- Hunziker, L., Bönisch, D., Groenhagen, U., Bailly, A., Schulz, S. & Weisskopf, L. (2015). Pseudomonas strains naturally associated with potato plants produce volatiles with high potential for inhibition of *Phytophthora infestans*. *Applied and environmental microbiology*, 81(3), 821-830.
- Kessler, A. & Baldwin, I.T. (2002) Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, 291 (5511), 2141-2144.
- Koch, P., Pacotte, M., Udsen, S. & Angelini, R. (2017). O mercado de folhosas: Números e tendências, presented at 2º Seminário Nacional Folhosas, Nova Friburgo, RJ.
- Lugtenberg B, Kamilova F. (2009). Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol*, 63, 541-556.
- McDonald, J.H. (2014). *Handbook of Biological Statistics* (3rd ed.). Baltimore, Maryland: Sparky House Publishing.
- Menéndez, A. I., Romero, A. M., Folcia, A. M. & Martínez-Ghersa, M. A. (2010). Aphid and episodic O₃ injury in arugula plants (*Eruca sativa* Mill) grown in open-top field chambers. *Agriculture, ecosystems & environment*, 135(1), 10-14.
- de Moraes, C. M., Lewis, W. J., Pare, P. W., Alborn, H. T. & Tumlinson, J. H. (1998). Herbivore-infested plants selectively attract parasitoids. *Nature*, 394(6685), 570.
- Ogran, A., Landau, N., Hanin, N., Levy, M., Gafni, Y. & Barazani, O. (2016). Intraspecific variation in defense against a generalist lepidopteran herbivore in populations of *Eruca sativa* (Mill.). *Ecology and Evolution*, 6(1), 363-374.

- Padulosi, S. & Pignone D. (1997). *Rocket: a Mediterranean crop for the world*. Report of a workshop, 13-14 December 1996, Legnaro (Padova), Italy. International Plant Genetic Resources Institute, Rome, Italy.
- Parra, J.R.P. (2007). *Técnicas de criação de insetos para programas de controle biológico*. (6th ed.). Piracicaba: FEALQ.
- Pasini, A., Parra, J. R. P. & Lopes, J. M. (2007) Dieta Artificial para criação de Doru luteipes (Scudder) (Dermaptera: Foliculidae), predador da Lagarta-do-Cartucho do Milho, Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae). *Neotropical Entomology*, 36(2), 308-311.
- Pineda, A., Zheng, S.J., Van Loon, J.J., Pieterse, C.M. & Dicke, M. (2010). Helping plants to deal with insects: the role of beneficial soil-borne microbes. *Trends in Plant Science*, 15, 507-514.
- Pineda, A., Zheng, S. J., Van Loon, J. J. A. & Dicke, M. (2012). Rhizobacteria modify plant–aphid interactions: a case of induced systemic susceptibility. *Plant Biology*, 14, 83-90.
- Pineda A., Soler R., Weldegergis B. T., Shimwela M. M., van Loon J. J. A. & Dicke, M. (2013). Non-Pathogenic rhizobacteria interfere with the attraction of parasitoids to aphid-induced plant volatiles via jasmonic acid signalling. *Plant Cell Environ*, 36, 393–404.
- Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N., & Weis, A.E. (1980). Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, 11, 41-65.
- van der Putten, W. H., Vet, L.E.M, Harvey, J.A. & Wackers, F.L. (2001). Linking above and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology & Evolution*, 16, 547-554.
- Rajkumar, M., Ae, N., Prasad, M. N. V. & Freitas, H. (2010). Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnology*, 28(3), 142-149.
- Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V. & Smaiyappan, R. (2001). Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protection*, 20, 1–11.
- Reis, L.L., Oliveira, L.J. & Cruz, I. (1988). Biologia e potencial de Doru luteipes no controle de Spodoptera frugiperda. *Pesquisa Agropecuária Brasileira*, 23(4), 333-342.
- Rifat, H., Safdar, A., Ummay, A., Rabia, K. & Iftikhar, A. (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology*, 60(4), 579-598.
- Rodriguez A. & Sanders, I.R. (2015). The role of community and population ecology in applying mycorrhizal fungi for improved food security. *The IMSE Journal*, 9, 1053-1061.

- Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Wei, H. X., Paré, P. W. & Kloepper, J. W. (2003). Bacterial volatiles promote growth in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, 100(8), 4927.
- Ryu, C. M., Murphy, J. F., Mysore, K. S. & Kloepper, J. W. (2004). Plant growth-promoting rhizobacteria systemically protect Arabidopsis thaliana against Cucumber mosaic virus by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. *Plant Journal*, 39(3), 381-392.
- Saleem, M., Arshad, M., Hussain, S. & Bhatti, A. (2007). Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *Journal of Industrial Microbiology & Biotechnology*, 34(10), 635-648.
- Schoonhoven, L.M., van Loon, J.J.A. & Dicke, M. (2005). *Insect Plant Biology*. Oxford: Oxford University Press.
- Sharifi, R., Lee, S. M. & Ryu, C. M. (2018). Microbe-induced plant volatiles. *New Phytologist*, 220(3), 684-691.
- Sobhy, I., Miyake, A., Shinya, T. & Galis, I. (2017). Oral Secretions Affect HIPVs Induced by Generalist (*Mythimna loreyi*) and Specialist (*Parnara guttata*) Herbivores in Rice. *Journal of Chemical Ecology*, 43(9), 929-943.
- Steinmann H. (1993) *Dermaptera. Eudermaptera II. Das Tierreich, Teilband 108*. Berlin: Walter de Gruyter
- Stenberg, J.A., Heil, M., Ahman, I. & Bjorkman, C. (2015). Optimizing crops for biocontrol of pests and disease. *Trends Plant Science*, 20, 698-712.
- Talekar, N.S. & A.M. Shelton. (1993). Biology, ecology and management of the diamondback moth. *Annu. Rev. Entomol.*, 38, 275-301.
- Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990). Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, 250, 1251-1253.
- U.S. EPA. (2005). Pesticide Product Label, Kodiak Concentrate Biological Fungicide 07/05/2005, Retrieved from https://www3.epa.gov/pesticides/chem_search/ppls/000264-00951-20050705.pdf
- Vessey, J. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255(2), 571-586.
- Vogel, H., Kroymann, J. & Mitchell-Olds, T. (2007). Different Transcript Patterns in Response to Specialist and Generalist Herbivores in the Wild Arabidopsis Relative *Boechera divaricarpa* (Path Analysis of Plant Defense). *PLoS ONE*, 2(10), 1081.
- Waquil, J.M., Viana, P.A. & Cruz, I (2002). *Cultivo do Milho: Manejo integrado de Pragas (MIP)*. Comunicado Técnico 50, Ministério da Agricultura, Pecuária e Abastecimento. Sete Lagoas, MG.

- Weyens, N., Van Der Lelie, D., Taghavi, S., Newman, L. & Vangronsveld, J. (2009). Exploiting plant-microbe partnerships to improve biomass production and remediation. *Trends Biotechnology*, 27, 591-598.
- Yang, J., Kloepper, J.W. & Ryu, C.M (2009) Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Science*, 14, 1-4.
- Zalucki, M. P., Shabbir, A., Silva, R., Adamson, D., Shu-Sheng, L. & Furlong, M. J. (2012). Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella* (Lepidoptera: Plutellidae): just how long is a piece of string? *Journal of economic entomology*, 105(4), 1115-1129.
- Zhang, H., Xie, X., Kim, M. S., Korniyev, D. A., Holaday, S. & Paré, P. W. (2008a). Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. *Plant Journal*, 56(2), 264-273.
- Zhang, H., Kim, M. S., Sun, Y., Dowd, S. E., Shi, H. & Paré, P. W. (2008b). Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. Molecular plant-microbe interactions. *MPMI*, 21(6), 737-744.



Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitenskapelige universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway