Effectiveness of Entomopathogenic Nematode-Induced Systemic Resistance against Pests and Pathogens of Tobacco and Soybeans in Tennessee

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Julia Grace Ferguson
May 2016

Dedication

This thesis is dedicated to my mom and dad, Mary and Frank Ferguson, for always supporting me in my endeavors and encouraging my love of science. I also dedicate this thesis to Trevor for always remembering how much I love cheese and for embracing my love for what I do. Thank you all so much!

Acknowledgements

I would like to thank Dr. Jerome Grant, Dr. Darrell Hensley, Dr. Frank Hale, and Dr. Seong-Hoon Cho for staying with me for the last two years and my many ups and downs with my project. Thank you all for your patience and kindness. Many thanks especially to my major advisor, Dr. Grant, for going out of your way to help me during a very busy time, I truly appreciate all you have done. I would like to thank Dr. Parwinder Grewal and Dr. Ruisheng An for their consideration and generosity on my project and providing guidance along the way as well as helping to provide funding. Thanks to the kind folks at the Greeneville Research and Education Center for providing the field for my study as well as always providing ice cream for those extremely hot summer days. A special thank you to Beth Long for taking a chance on me as an undergrad and I can honestly say I would not be where I am without you. I am grateful for my friends I have made in the department, you know who you are, who helped me weigh all those worms or drive to the middle of nowhere to check traps in tomato fields. You guys are awesome and I will miss you all so much! I would like to extend a giant "thank you" to the University of Tennessee for being my home for the last 7 years as well as to the Department of Entomology and Plant Pathology for being my home for the last 2 years. Thank you for all the support, guidance, and good memories! GO VOLS!

Abstract

Entomopathogenic nematodes (EPNs) *Steinernema* and *Heterorhabditis* are currently used as biological control agents in multiple cropping systems. EPNs also have been shown to induce resistance in some treated plant species, such as tomatoes and hostas. Recent studies where EPNs are applied to the soil have shown reduction in the number of plant parasitic nematodes and foliar nematodes. Other studies have also shown reduction in development of above-ground chewing and sucking insect pests as well as a pathogen. However, the mechanisms that cause these reductions are poorly understood. This research hypothesized that EPNs could induce resistance in tobacco and soybean plants. This hypothesis was tested by conducting a field study on tobacco against naturally-occurring pests and pathogens and a greenhouse study on tobacco and soybean to determine the development of the chewing pest *Helicoverpa zea* (corn earworm). In both studies larval cadavers of greater wax moth (*Galleria mellonella*) infected by *Steinernema carpocapsae* were applied to the soil around plant roots while control treatments of freeze-killed wax moth cadavers were applied in the same manner. Actigard 50WG, with a mode of action that induces resistance in plants, was used as a soil treatment in both studies as a positive control. In the field study, incidences of pest and disease damage on selected leaves and the number of insects on each soil-treated plant were recorded over a five-week period before the tobacco was topped. In the greenhouse study, larvae were exposed to plants 5, 10, or 15 days after treatment (DAT) and then weighed every 48 hrs. Results of both studies showed no evidence of an EPN-induced resistance effect against a chewing pest in the greenhouse setting or against pest damage in the field. EPN soil treatments also did not differ from control treatments in the tobacco

field in controlling plant disease or the number of insects on the plants. More research is necessary to better understand the mechanisms involved in the phenomenon of EPN-induced resistance and its effect on different plant species.

Table of Contents

Chapter I- Literature Review and Introduction....1

Chapter II- Field Assessment of the Effectiveness of Entomopathogenic Nematode-Induced Systemic Resistance on Tobacco....9

i. Introduction....9

ii. Materials and Methods....10

iii. Results and Discussion....14

iv. Summary....20

Chapter III- Laboratory and Greenhouse Assessment of the Effectiveness of Entomopathogenic Nematode-Induced Systemic Resistance on Soybean and Tobacco....21

i. Introduction....21

ii. Materials and Methods....22

iii. Results and Discussion....25

iv. Summary....29

Chapter IV- Conclusions....41

Literature Cited....45

Vita....49

List of Tables

Table 1. List of insect pests currently controlled by applications of nematodes. Excerpt, Grewal 2012. 4

Table 2. Insect damage scale used to assess percent leaf damage attributed to insects.....13

Table 3. Horsfall-Barratt plant disease rating used to assess percent damage of leaf attributed to plant disease.....15

Table 4. Comparison of mean larval weights of *Helicoverpa zea* among soil treatments (Actigard, Control, and Entompathogenic Nematodes [EPNs]) on tobacco in the greenhouse, 2015 27

Table 5. Comparison of mean larval weights of *Helicoverpa zea* among soil treatments (Actigard, Control, and Entompathogenic Nematodes [EPNs]) at 5, 10, and 15 days after treatment (DAT) on tobacco in the greenhouse, 2015 27

Table 6. Comparison of mean larval weights of *Helicoverpa zea* among soil treatments (Actigard, Control, and Entompathogenic Nematodes [EPNs]) on soybean in the greenhouse, 2015 30

Table 7. Comparison of mean larval weights of *Helicoverpa zea* among soil treatments (Actigard, Control, and Entompathogenic Nematodes [EPNs]) at 5, 10, and 15 days after treatment (DAT) on soybean in the greenhouse, 2015 30

List of Figures

Figure 1. Panoramic view of tobacco field. The tobacco has started to bloom, Greeneville, TN, 2015.....11

Figure 2. Mean disease rating (Horsfall-Barratt, scale 1-12) on tobacco in the four soil treatments over the observational time, Greeneville, TN, 2015. Observation time represents biweekly observations from, July 17-August 20, 2015 17

Figure 3. Mean insect density per tobacco plant over the observational time between the four soil treatments, Greeneville, TN, 2015. Observation time represents biweekly observations from July 17-August 20, 2015 18

Figure 4. Mean insect density per tobacco plant throughout the observational period, Greeneville, TN, 2015. Observation time represents biweekly observations from July 17-August 20, 2015. Error bars show minimum and maximum insect density on each date.....19

Figure 5. A clip cage on a tobacco plant.....24

Figure 6. Mean larval weight of *Helicoverpa zea* on tobacco in the greenhouse over the observation time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period. 31

Figure 7. Mean larval weight of *Helicoverpa zea* on tobacco 5 DAT in greenhouse over the observational time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period. 32

Figure 8. Mean larval weight of *Helicoverpa zea* on tobacco 10 DAT in the greenhouse over the observational time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.....33

Figure 9. Mean larval weight of *Helicoverpa zea* on tobacco 15 DAT in the greenhouse over the observational time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.....34

Figure 10. Mean larval weight of *Helicoverpa zea* on tobacco in the greenhouse over the observation time between soil treatments and separated by sub-treatments (DAT). Observation time represents larval weight observed every 48 hrs over a 30 day period...35

Figure 11. Mean larval weight of *Helicoverpa zea* on soybean in the greenhouse over the observation time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period. 36

Figure 12. Mean larval weight of *Helicoverpa zea* on soybean 5 DAT in the greenhouse over the observation time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.....37

Figure 13. Mean larval weight of *Helicoverpa zea* on soybean 10 DAT in the greenhouse over the observational time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.....38

Figure 14. Mean larval weight of *Helicoverpa zea* on soybean 15 DAT in the greenhouse over the observation time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.....39

Figure 15. Mean larval weight of *Helicoverpa zea* on soybean in the greenhouse over the observation time between soil treatments among sub-treatments (DAT). Observation time represents larval weight observed every 48 hrs over a 30 day period. 40

Chapter I

Literature Review and Introduction

The global human population currently exceeds seven billion and continues to increase (U.S. Population Reference Bureau 2015). According to the Food and Agricultural Organization (FAO), by 2050 the world's human population will exceed nine billion, which is a 34% increase from today's population (FAO 2011). The increase in population thereby creates the need for an increase in production of food and other economically important crops (Abrol and Shankar 2012). Many global issues, such as land availability, fertilizers, water availability, insects, nematodes, bacteria, fungi, viruses, birds, rodents, as well as other competitors of plant products, limit this needed increase in productivity (Arbrol and Shankar 2012). Insects and plant diseases are a universal issue that affects economic yields and productivity of all plant growers (Arbol and Shankar 2012). Yield loss from different kinds of pests has been estimated to be around USD $500 billion worldwide (Liebhold and Tobin 2008).

Since the development and widespread use of synthetic pesticides, great results have been achieved in controlling problematic pests. These pest reductions, which provide tremendous benefits to farmers, land managers, and others, serve as a catalyst for the use of more pesticides. Because of this increased demand, the global synthetic pesticide market in 2009 was valued at USD$41 billion and increasing (Rizvi *et al*. 2012). But, along with the effective control of pests, many adverse consequences regarding the use of these chemicals have been documented (Arbol and Shankar 2012).

Dependence on chemicals has led to severe problems in insecticidal resistance in more than 500 insect pest species (Georghiou 1990). In addition, severe problems with pollution to the environment, pest resurgence, and health problems in humans and animals have been recognized. The publication of Rachel Carson's *Silent Spring* in 1962 was an eye-opener to society and led to awareness of the negative effects of pesticides (Carson 1962, Murphy 2005). The concepts of integrated pest management (IPM) to decrease the harmful effects of pesticides and to reduce the amount of chemicals used for controlling crop pests originated from this awareness (Arbol and Shankar 2012). Effective and sustainable pest management is essential to meet the rising population needs as well as the health and wellbeing of future generations.

One management option that continues to grow in research activities and commercial use is biological control. Biological control can be defined as "the use of parasitoid, pathogen, predator, antagonist, or competitor populations to suppress a pest population, making the pest less abundant and less damaging than it would be in the absence of the biological control agents" (Norris *et al.* 2003). There are many advantages to biological control and its use in agricultural pest management systems. These include: 1) less air pollution, 2) targeting specific or a small number of related pests (whereas broad-spectrum chemical pesticides may affect beneficial insects or other animals in addition to the pest), 3) ease of use, and 4) ease of incorporation into IPM programs (Rizvi *et al.* 2012). One possible biological control tool as a component used in an IPM program is entomopathogenic nematodes (EPN) (Phylum: Nematoda, Order: Rhabiditida).

EPNs are soft bodied, non-segmented roundworms that naturally occur in soil environments, infect and kill insects (Kaya and Gaugler 1993). Steinernematidae and Heterorhabditidae families are the most common EPNs used as biological control agents of insect pests (Grewal *et al*. 2005). EPNs are mutualistically associated with bacteria in the family Enterobacteriacae: Steinernematids are associated with the genus *Xenorhabdus* and Heterorhabditids are associated with the genus *Photorhabdus*. Infective juveniles, the only stage of the nematode that can survive outside the host in the soil environment, carry the bacteria and penetrate a suitable insect host by means of natural openings or by breaking the cuticle (Miles *et al*. 2012). Once inside the hemocoel, the infective juveniles release the bacteria (Kaya and Gaugler 1993) that proliferate rapidly, killing the insect by septicemia within 1 to 3 days. Feeding on the bacteria and host tissues, the nematodes will produce two or three generations (Kaya and Gaugler 1993). As the food source depletes, new infective juveniles are produced that emerge from the host cadaver, and search for another host to infect. Only one bacterium species is specifically associated with each nematode species (Kaya and Gaugler 1993). The nematode relies on the bacteria to kill the host and break down the host tissues, as well as serve as a food source; the bacteria depend upon the nematode for transmission from one insect host to another (Kaya and Gaugler 1993).

Nematodes have been known to be parasites of insects since the 17th century, but it was not until the 1930s when serious consideration was given to nematodes to be used to control insect pests (Smart 1995). In 1929, Glaser and Fox found a nematode infecting grubs of Japanese beetle, *Popillia japonica*, on a golf course in New Jersey and did experimental trials using nematodes for its control (Glaser and Fox 1930, Glaser and

Farrell 1935). Successful results in Glaser and Fox's trials found that 81% of the Japanese beetle grubs examined in some plots were infected by these nematodes.

EPNs are an appealing alternative to chemical insecticides because they are easy to mass-produce, safe, well suited for IPM systems, can easily be applied, and are compatible with a wide array of agrochemicals (Grewal 2012; Elhers and Sharpiro-Ilan 2005). Today, EPNs are most commonly used to control citrus root weevil (*Diaprepes abbreviates)* (Coleoptera: Curculionidae) in Florida, fungus gnats (Diptera: Cecidomyiidae) in Europe, and white grubs (Coleoptera: Scarabaeidae) in turfgrass in both North America and Europe. EPNs have even out ranked an insecticide, aldrin, as the most effective control method for black vine weevil (*Otiorhynchus sulcatus*) (Coleoptera: Curculionidae) in greenhouse and nurseries in Europe (Grewal 2012). EPNs have a broad host range of insects that they can infect and are currently mostly used for controlling soil-dwelling insects (Table 1).

Table 1. List of insect pests currently controlled by applications of nematodes. Excerpt, Grewal 2012.

Sector	Nematode	Target Pest
Citrus	*S. riobravae* *H. bacteriophora*	Citrus root weevil
Turfgrass	*S. carpocapsae* *S. scapterisci* *H. bacteriophora*	Hunting billbug, Black cutworm, Sod webworm, Fall armyworm Mole crickets White grubs
Greenhouse	*S. feltiae*	Fungus gnats, Black vine weevil
Mushroom	*S. feltiae*	Sciarid flies
Cranberries	*S. carpocapsae*	Cranberry girdler
Nursery	*H. bacteriophora* *H. megidis*	Black vine weevil
Pet vet	*S. carpocapsae*	Dog flea

In more recent studies, EPNs have shown to have additional benefits in plant production. EPNs that are applied to the soil have resulted in some non-target effects that are positive in protection of the plant. These studies have shown reduction in plant parasitic nematode populations after EPNs were applied to the soil (reviewed in Lewis and Grewal 2005). Some examples include *Heterorhabdtis bacteriophora* lowering populations of plant parasitic nematodes in turfgrass (Somasekhar *et al*. 2002) and *Steinernema carpocapsae* reducing populations of the plant parasitic nematode *Globodera rostochiensis*, more commonly known as the potato cyst nematode, in potato plants (Perry *et al*. 1998). Also, interestingly, the application of dead EPN infective juveniles has also been shown to reduce populations of multiple species of plant parasitic nematodes (Jagdale *et al.* 2002; Molina *et al*. 2007). Many more examples of this phenomenon in soil applications of EPNs effecting and lowering populations of plant parasitic nematodes have been reported (see Lewis and Grewal 2005 for a review).

Researchers reported that *S. carpocapse* infected host larval cadavers of greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), applied to the soil reduced plant parasitic nematodes and also reduced populations of a foliar nematode (*Aphelenchoides fragariae*) in the above-ground parts of hosta (*Hosta* spp.) plants suggesting possible induced resistance (Jagdale and Grewal 2008). Other studies revealed that soil applications of *S. carpocasae* infective juveniles and their corresponding symbiotic bacteria *Xenorhabdus nematophilus* stimulated the production of key defense enzymes in hosta and *Arabidopsis thaliana* leaves as well as induced the expression of a plant defense protein promoter *PR1* gene in *A. thaliana* (Jagdale *et al*. 2009). It was proposed

that the antagonistic effect of EPNs on plant parasitic nematodes may be partially attributed to this EPN-induced plant defense response (Jagdale *et al*. 2009).

Induced resistance is a common plant defense response to pathogens, pests and can be activated by a range of biotic and abiotic factors (Panda and Khush 1995). A network of signal transduction pathways regulate induced defense responses in plants of which hormonal signals salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play a major role (Heil and Bostock 2002; Walters 2011). Exposure to pathogenic and non-pathogenic organisms, as well as certain chemicals and herbivore damage can stimulate the SA, JA, and ET pathways. This stimulation can lead to the activation of systemic expression that provides broad-spectrum, long-lasting disease resistance that has been shown to be efficient against plant pathogenic bacteria, fungi, and viruses (Heil and Bostock 2002; Pieterse *et al*. 2014). This widespread phenomenon has been and is currently being studied further for its potential use in plant protection.

A recent study that further examines EPN-induced resistance on tomato (*Solanum lycopersicum*) plants was performed by An *et al.* (2016). Their study examined the influence of EPN-induced resistance on the development of a bacterial pathogen (*Pseudomonas syringae*), a generalist sucking pest (silverleaf whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae)), and a generalist chewing pest ((beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae)). In this study, they found that the two targeted pests and pathogen had slower development on EPN-treated tomato plants compared to those on control plants (i.e., those not treated with EPNs). This study shows the possible effectiveness of EPNs to induce and maintain resistance in plants against a bacterial pathogen and pests with multiple eating habits.

Marketing on this phenomenon of "helping the plant help itself" or inducing resistance in the plant contributed to the development of products such as Bioyeild, Messenger, and Actigard 50WG. Actigard 50WG (50% wettable granule) is commercially sold and marketed to induce resistance in plants for the control of several fungal, bacterial, and viral plant diseases. The active ingredient is Acibenzolar-S-methyl or 1,2,3-benzothiadiazole-7-thiocarboxylic acid-S-methyl-ester, which stimulates induced resistance in plants (Syngenta US 2014). Acibenzolar-S-methyl is an analog of SA, a plant hormone that is involved in signaling and mediating plant defense against pathogens and induced resistance (Myresiotis *et al*. 2014).

Actigard 50WG, which can be easily applied foliarly or to the soil along with other pesticides in a tank mix or as a wettable granule (WG) form, may be used in IPM programs. However, the Actigard 50WG label states that the pesticide is toxic to fish and aquatic invertebrates, thus it can pose an environmental hazard if not used according to label. The label also states that the product causes harm or irritation when in contact with skin and that PPE (Personal Protective Equipment) should be worn to protect exposed skin when applying the product. Because of these concerns, chemical products such as Actigard may pose potential risks to the environment and to the user. Therefore, biological products that can boost plant immunity against a range of pests and pathogens and that are safe to the environment and humans are advantageous to use in an IPM program.

In this study, the hypothesis was that EPN-induced resistance is broad spectrum with activity in tobacco (*Nicotiana tabacum*) and soybean (*Glycine max*). Two studies performed to test this hypothesis were a field study on tobacco and a greenhouse study on

tobacco and soybean. The objective of the field study was to determine if EPNs could induce resistance in a field setting with naturally-occurring pests and pathogens on burley tobacco. In the field study, soil treatments of EPNs and Actigard 50WG were compared with a control for effects of induced resistance on the number of insects per plant, plant disease damage, and insect damage over the observational period. The objective of the greenhouse study was to determine if EPNs could induce resistance in both tobacco and soybean plants against a generalist-chewing pest (corn earworm, *Helicoverpa zea*). Soil treatments of EPNs and Actigard 50WG were compared to a control to assess their effects on the larval development of *H. zea* on tobacco and soybean plants at 5, 10, and 15 days after treatment (DAT).

Presently, little is known concerning the mechanisms and possibilities of EPN-induced resistance. This research was conducted to gain a better understanding of EPN-induced resistance in two plant species, tobacco and soybean, against a model chewing insect pest in a greenhouse chamber setting as well as in a field setting with naturally-occurring insect pests and plant diseases. A long-term goal of this project was to develop EPN-induced resistance as a strategy for integrated pest and disease management in economically important crops and to expand the potential use and awareness of biological control.

Chapter II

Field Assessment of the Effectiveness of Entomopathogenic Nematode-Induced Systemic Resistance on Tobacco

i. Introduction

Entomopathogenic nematodes (EPNs) are found naturally in soil (Kaya and Gaugler 1995), but a closer examination of their effects and relationship with plants is needed. A non-laboratory setting is essential to fully assess how effective soil applications of EPNs are at inducing resistance and therefore controlling pests and diseases in a natural setting. A study was conducted to assess the effects of the soil treatments of EPNs as well as Actigard 50WG in a field setting on burley tobacco. Actigard 50 WG is a commercially sold product that is an analog to salicylic acid whose mode of action induces resistance in plants (Myresiotis *et al.* 2014), which is the predicted mode of action of EPNs applied to the soil. Four soil treatments were evaluated: EPN infected *Galleria mellonella* cadavers; aqueous solution of EPNs; Actigard 50WG; and non-infected *G. mellonella* cadavers. The three null hypotheses of this research were that soil treatments did not have an effect on foliar insect damage, soil treatments did not have an effect on insect density, and soil treatments also did not have an effect on foliar plant disease over the observational time.

ii. Materials and Methods

Field Location and Experimental Design

This study was conducted at the one of the UT AGResearch Centers, the Research and Education Center located in Greeneville, Tennessee. The experiment lasted five weeks starting July 17, 2015, when soil treatments were applied and ending August 20, 2015 after the burley tobacco had been topped. The field, surrounded by grass, was located in a relatively flat area. A water treatment facility was located within 50 m of the field. The soil types in the field were Ooltewah, Elk, and Dewey. Size of the field was slightly under 0.4 ha with 42 rows, averaging 125 plants per row, with ca. 53 cm between plants and ca. 106cm between rows. The field was arranged into four plots, and only two plots received soil treatments. Each plot had two rows (ca. 55 plants per row) on either side as well as two plants at the beginning and end of each row as "buffer" zones. Four soil treatments were each applied to two different rows; thus eight rows were selected to receive treatments with no treated rows directly beside one another. Each row had 55 plants and in that row 11 plants were selected to receive the treatment designated to that row. No treated plants were directly beside one another in the rows. Plants that received soil treatments had two healthy leaves at the eighth or ninth leaf selected and marked on the underside using a Sharpie® permanent marker. Healthy leaves were selected to have all the plants begin around the same levels of "damage" to be compared over the time period of this study.

Tobacco

The burley tobacco variety in the field was KT 206 LC (Figure 1). The seeds were sown on March 18, 2015 in a greenhouse and then transplanted into the field using a

carousel mechanical transplanter on June 5, 2015. Fungicides consisted of Ridomil Gold SL (Mefenoxam 479.36 g ai per L) applied at 29.57 ml per 4046.86 m^2 on June 4, 2015 prior to transplanting for black shank disease (caused by the fungus *Phytophthora parasitica*) control and Quadris (Azoxystrobin 249.27 g ai per L) applied foliarly at 236.59 ml per 4046.86 m^2 on June 11, 2015 applied for blue mold (caused by the pathogen *Peronospora tabacina*) prevention. Herbicides consisted of Spartan Charge (Sulfentrazone 479.36 g ai per L) applied at 354.88 ml per 4046.86 m^2 and Command 3ME (Clomazone 359.52 g ai per L) applied at 946.35 ml per 4046.86 m^2, Herbicide applications were made on June 4, 2015 before transplanting. An insecticide, Sevin (Carbaryl 479.36 g ai per L), at the rate of 0.907 kg per 4046.86 m^2 was also applied on June 17, 2015 for the control of flea beetles (Coleoptera: Chrysomelidae).



Figure 1. Panoramic view of tobacco field. The tobacco has started to bloom, Greeneville, TN, 2015.

Soil Treatments

Four soil treatments were used in this study: EPN aqueous suspension, EPN-infected cadavers, Actigard 50WG, and non-infected cadavers as the control. Before the

study began soil samples were taken and analyzed for EPN content. Soil treatments were applied at the base of the above-ground plant. The control treatment consisted of three freeze-killed last-instar greater wax moth (*Galleria mellonella*) cadavers that were buried about 4 cm in the soil at the base of the selected plants close to the roots. EPN-infected cadavers (EPNC) were obtained by infecting live last-instars of *G. mellonella* with EPN infective juveniles. To infect the larvae, infective juvenile EPNs *Steinernema carpocapsae* carrying the symbiotic bacteria *Xenorhabdus nematophila* were placed in a petri dish (100 mm x 15 mm) with filter paper as aqueous solution. In each dish, approximately 10 larvae were placed and exposed to 3 ml of a 500-nematode/ml solution. Larvae of *G. mellonella* were then left for 48-72 hrs, which is the average amount of time for the infective juvenile EPNs to infect and kill the host larvae (Kaya and Gaugler 1993). Once a larva is infected and EPNs begin to reproduce it will produce, on average, 200,000 infective juvenile nematodes (Grewal *et al*. 1994). Three infected cadavers were then buried around 4 cm in the soil at the base of the plant close to the roots. Another soil treatment used was EPNs in an aqueous suspension (EPNL). Infective juveniles of the *S. carpocapsae* species and its symbiotic bacteria *X. nematophila* in a solution with a concentration of 7,000 nematodes/ml were applied to the soil at the base of selected plants at a rate of 25 mL per plant. The last soil treatment was Actigard 50WG, of which the active ingredient is Acibenzolar-S-methyl, which stimulates systematic acquired resistance in plants (Syngenta US 2014). Acibenzolar-S-methyl is an analog of salicylic acid, a plant hormone that is involved in signaling and mediating plant defense against pathogens and systematic acquired resistance (Myresiotis et al. 2014). A solution with 10

mg of Actigard 50WG was mixed with 25 ml of water and applied to the soil at the base of the selected plant close to the roots.

Data Collection

Three main categories of data were documented on select treated plants: insect density, insect damage rating, and plant disease rating. Insect density included all stages from egg to adult of all insects found and was conducted by starting at the top of the plant looking above and below each leaf and at the stem on the entire above-ground portion of the plant and counting all insect types. This number was then recorded.

Insect damage was assessed on both the leaves that were selected and previously marked on the plant. Each chosen plant was given two insect damage ratings that corresponded to the two different leaves. The ratings were determined on a 1-5 scale which corresponded to different percentage ranges of damage to the entire leaf selected that is attributed to insects, as seen in Table 2.

Table 2. Insect damage scale used to assess percent leaf damage attributed to insects.

Rating	% Leaf Damage
1	0-10
2	11-40
3	41-60
4	61-80
5	81-100

[1]Rating scale values correspond to ranges in percent leaf damage that is attributed to insects.

The plant disease rating was assessed using the Horsfall-Barratt scale, which is used to describe the visual assessment of plant diseases. The scale is intended to interpret the percentage of foliage infected (Table 3) (Horsfall and Barratt 1945). As with the insect damage assessment, each plant was given two ratings evaluating disease infection on each of the two leaves chosen on the selected plants.

Data Analysis

To determine if there were any differences in four soil treatments over the observational period in insect density, insect damage, and plant disease, a mixed model analysis at $\alpha = 0.05$ was used. Data were analyzed in JMP Pro 12 (SAS Institute Inc. Cary, NC). No transformations were needed or used in the analysis.

iii. Results and Discussion

In the overall data collected on insect damage to the leaves, 98% of the ratings over the observational period were classified as '1', which is associated with 0-10% leaf damage. Because of the lack of variation in the data, no p-value could be produced. Evidence of high percent leaf damage by insects, especially tobacco hornworm (*Manduca sexta*) (Lepidoptera: Sphingidae), was observed in the field, but was not present on the leaves chosen for examination in this study. Insect damage that occurred in the field as the tobacco matured was mainly observed in higher and newer leaves. The scale was given a small percentage range for the lowest percent damage to illustrate small differences from essentially no damage to some damage.

Plant disease was rated using the Horsfall-Barratt scale on the two leaves chosen on the plants receiving soil treatments. No significant differences in plant disease rating

Table 3. Horsfall-Barratt plant disease rating used to assess percent damage of leaf attributed to plant disease.

Rating	% Leaf Damage
1	0
2	0-3
3	3-6
4	6-12
5	12-25
6	25-50
7	50-75
8	75-87
9	87-94
10	94-97
11	97-100
12	100

[1]Rating scale corresponds to ranges in percent leaf damage or infection attributed to plant disease.

($p=0.305$) over the observational time were observed between soil treatments resulting in plant disease damage. Accepting the null hypothesis, the soil treatments did not have an effect on plant diseases nor their spread in the field. The two main diseases that were observed were blue mold (*Peronospora tabacina*) and target leaf spot (*Rhizoctonia solani*), which both can occur in tobacco production across Tennessee. However, a statistically significant difference ($p<0.001$) in plant disease rating was determined between time and soil treatments. This difference was expected as plant disease damage increased or worsened as the observational time increased (Figure 2).

Numerous insects were found on tobacco plants throughout the field. For example, an aphid (Hemiptera: Aphidae) invasion resulted in thousands of aphids on some plants at one time, which contributes to large increases in insect density late in the season (Figure 3). The infestation of aphids also resulted in large ranges in insect density on the selected plants as can be seen in Figure 4. No significant differences ($p=0.191$) between soil treatments and insect densities over the observational time were observed, thus the null hypothesis is accepted. However, as with plant disease damage, a significant difference ($p<0.001$) was detected between the observational time and insect density. This difference over time was also expected because the number of insects in the field increased throughout the season, especially when the tobacco started to bloom in mid-August around observation 7 of this study (Figure 3). A rapid increase in insect populations occurred towards the last couple of observations, this increase was due to an aphid infestation. In observation 10, one plant had approximately 4,000 insects, which included mostly aphids.

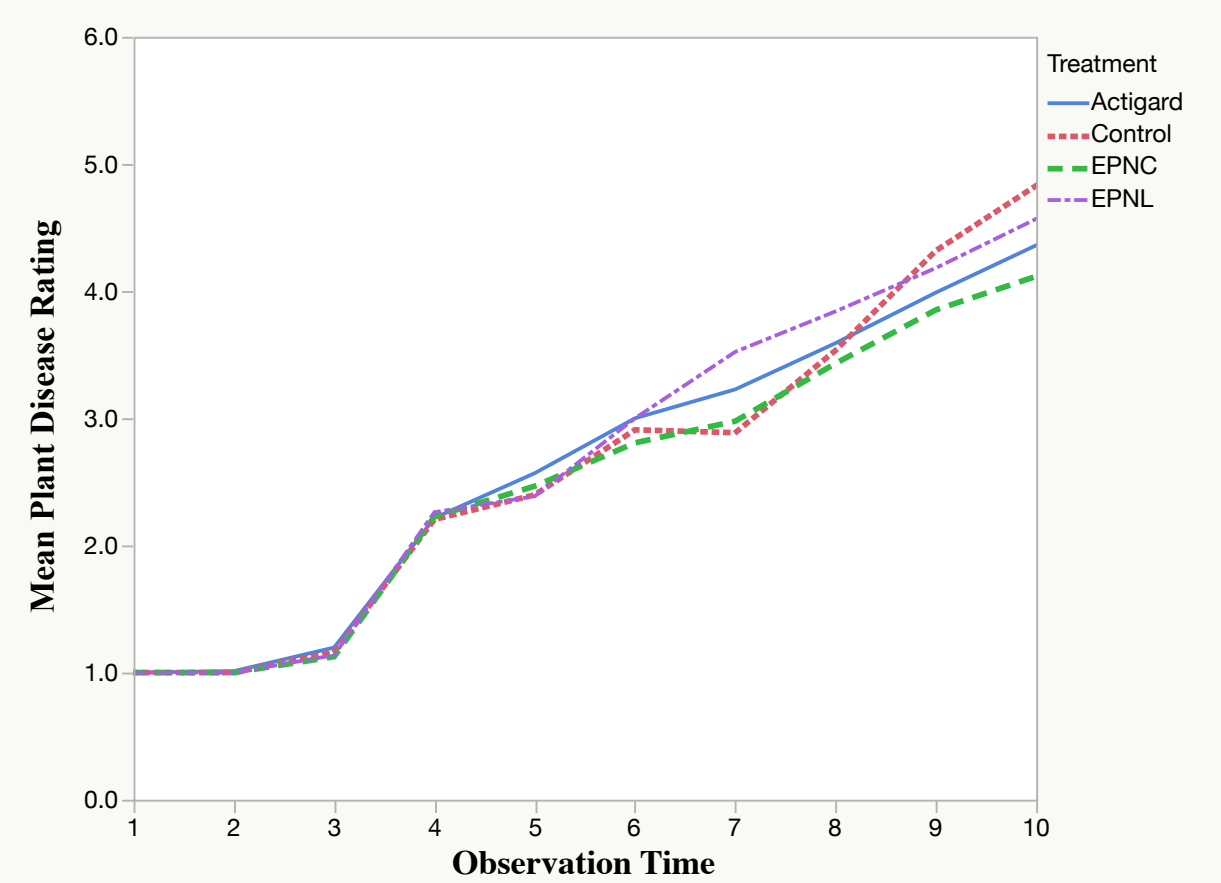


Figure 2. Mean disease rating (Horsfall-Barratt, scale 1-12) on tobacco in the four soil treatments over the observational time, Greeneville, TN, 2015. Observation time represents biweekly observations from July 17-August 20, 2015.

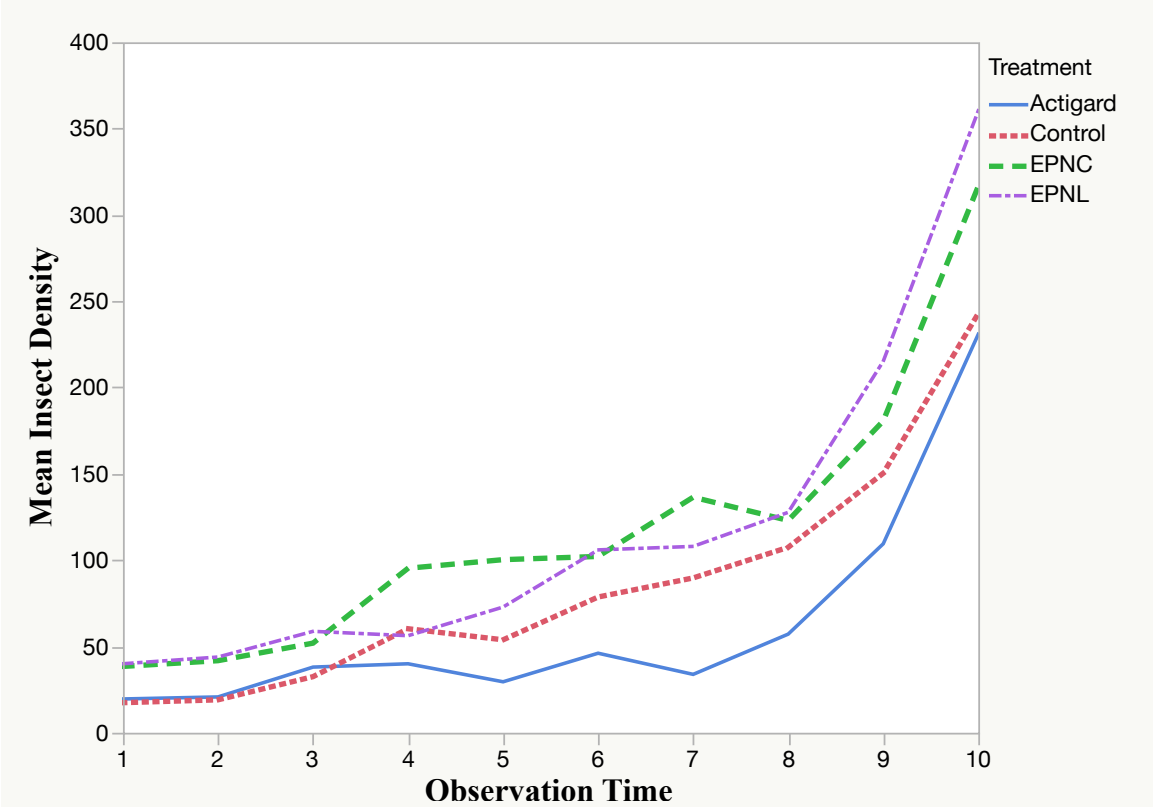


Figure 3. Mean insect density per tobacco plant over the observational time between the four soil treatments, Greeneville, TN, 2015. Observation time represents biweekly observations from July 17-August 20, 2015.

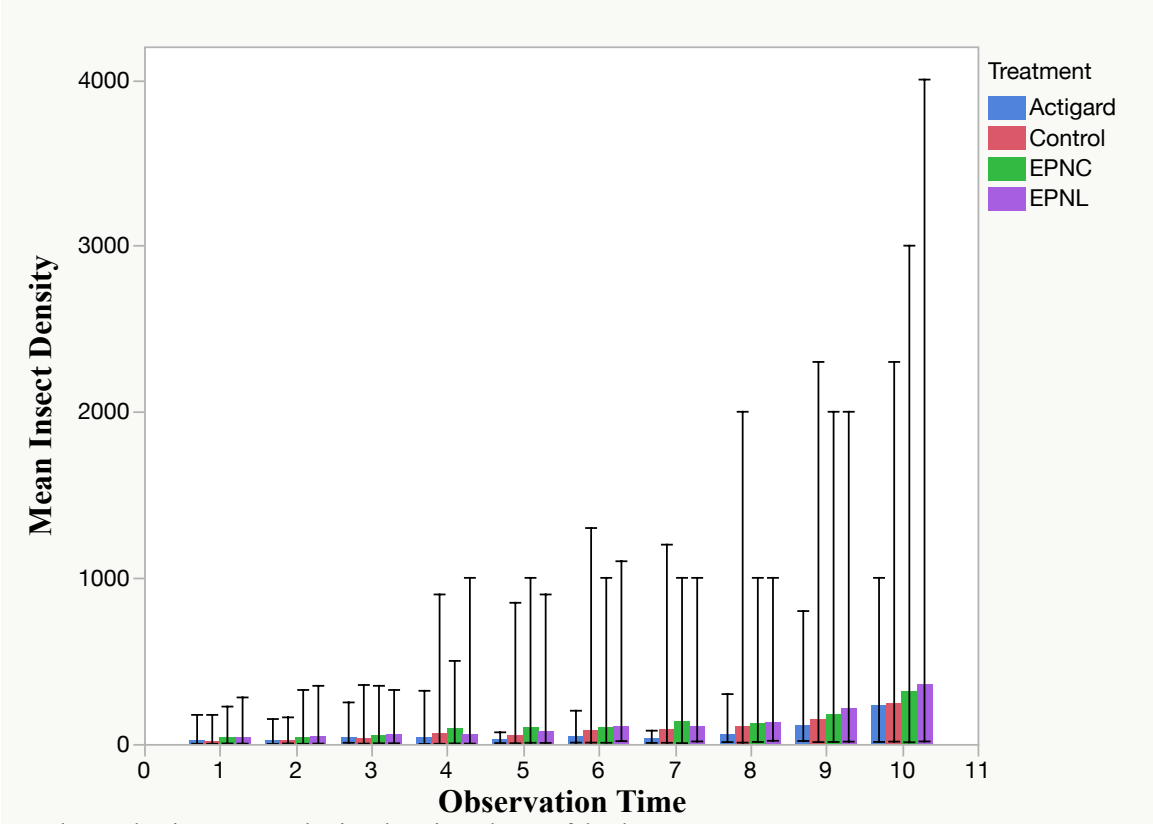


Figure 4. Mean insect density per tobacco plant throughout the observational period, Greeneville, TN, 2015. Observation time represents biweekly observations from July 17-August 20, 2015. Error bars show minimum and maximum insect density on each date.

iv. Summary

In this study, the effects of four soil treatments on inducing resistance in tobacco in a field setting with natural-occurring factors were evaluated. The factors that were evaluated included insect density, insect percent leaf damage, and plant disease percent leaf damage. The soil treatments were EPN aqueous solution, EPN-infected *G. mellonella* cadavers, Actigard 50WG, and a control of non-infected *G. mellonella* cadavers. However, in this particular experiment none of the soil treatments had any effect on controlling the factors evaluated in the field. No differences in soil treatments on percent leaf damage by plant diseases or insect density were detected. Differences in leaf damage by insects among soil treatments could not be analyzed because of lack of variation in the resulting data. An aphid infestation and increasing blue mold created opportunity for the soil treatments to control pests and diseases and their related damage, however that was not observed in this study. Additional research is needed to confirm if the soil treatments can induce resistance in a field setting for this variety and/or other varieties of tobacco.

Chapter III

Laboratory and Greenhouse Assessment of the Effectiveness of Entomopathogenic Nematode-Induced Systemic Resistance on Soybean and Tobacco

i. Introduction

Damage from foliage and fruit feeding by insect pests can cause huge economic losses as well as losses to the productivity and quality of tobacco and soybean plants. Typically, one type of control used against these pests that farmers implement is treating with pesticides. However, large-scale application of pesticides can and has resulted in increased concerns about the hazards to human health, non-target organisms and the environment (Abrol and Shankar 2012). Entomopathogenic nematodes (EPNs) are nematodes that are naturally found in soil. These nematodes are increasingly used worldwide as a biological control for soil-dwelling pests. The future use of these nematodes as IPM tools for further control of pests and pathogens is promising.

Previous studies have shown that applications of EPNs to the soil have resulted in non-target reduction in plant parasitic nematodes on turfgrass as well as other plant species. Studies also showed other non-target effects in above-ground pests and pathogen with a reduction of foliar nematodes on hostas and decelerating development of an insect pest, as well as a bacterium, on tomato plants (Lewis and Grewal 2005; Somasekhar *et al.* 2002; Perry *et al* 1998; Jagdale *et al*. 2002; Molina *et al.* 2007). An additional study on hostas considered mechanisms of EPNs in causing this plant-pest relationship and found

that EPNs induce resistance in hosta plants (Jagdale and Grewal 2008). This study investigates if this phenomenon can be seen in other plants in a laboratory setting. Plants tested were tobacco (*Nicotania tobacum*) and soybean (*Glycine max*) which were given applications of EPNs to soil; larvae of a model pest, corn earworm (*Helicoverpa zea*), were then placed on the leaves of treated plants. The development of the larvae was examined to test for an induced resistance effect by the soil treatments.

ii. Materials and Methods

Tobacco and soybean plants were grown from seeds in flat trays and transplanted individually into pots containing commercial potting mix [MiracleGro Organic Choice]. The pelletized seed cultivars TN-90 for burley tobacco and Ellis for soybean were used. MiracleGro water-soluble all-purpose plant food was applied to the potted plants every 7 days at a concentration of 2.46 ml per 4.54 L of water following the product label. Tobacco plants were grown to the sixth leaf stage at minimum before applying soil treatments. Soybean plants were grown to the R1 stage at minimum before applying soil treatments. Plants were grown in both greenhouse and growth chamber settings. Both were set at 23°C and typically had a photoperiod of 16 hrs light and 8 hrs dark.

Three soil treatments were examined: (1) freeze-killed cadavers of greater wax moth larvae (*Galleria mellonella)* as control; (2) EPN-infected cadavers; and (3) Actigard 50 WG. Last-instar greater wax moth larvae were used as cadavers. For the control soil treatment, wax moth larvae were freeze-killed and then buried 3-4 cm into soil at roots of selected plants. EPN-infected cadavers were obtained by infecting live last-instars of *G. mellonella* with EPN infective juveniles. To infect the larvae, infective juvenile EPNs

Steinernema carpocapsae carrying the symbiotic bacteria *Xenorhabdus nematophila* were placed in a petri dish (100 mm x 15 mm) with filter paper as aqueous solution. In each dish, approximately 10 larvae would be placed and exposed to 3 ml of a 500-nematode/ml solution. Larvae of *G. mellonella* were then left for 48-72 hrs, which is the average amount of time for the infective juvenile EPNs to infect and kill the host larvae (Kaya and Gaugler 1993). Once a larva is infected and EPNs begin to reproduce it will produce, on average, 200,000 infective juvenile nematodes (Grewal *et al*. 1994). Three infected cadavers were then buried around 4 cm in the soil at the base of the plant close to the roots. The active ingredient Acibenzolar-S-methyl in Actigard stimulates systematic acquired resistance in plants (Syngenta US 2014). Acibenzolar-S-methyl is an analog of salicylic acid, a plant hormone that is involved in signaling and mediating plant defense against pathogens and systematic acquired resistance (Myresiotis *et al.* 2014). A mixture with 10 mg of Actigard 50WG and 25 mL of water was applied to the soil of each selected plant at the base of the above-ground plant. In each experiment, one plant species (soybean or tobacco) was used and five plants with each soil treatment (5 plants x 3 soil treatments=15 plants total).

The model chewing pest used to evaluate and measure the effects of induced resistance in both tobacco and soybean was *H. zea*, also known as corn earworm, tomato fruitworm, and soybean podworm. Three sub-treatments represented the amount of days after applying soil treatments to the plants before placing *H. zea* larvae onto the plants. The three sub-treatments were 5, 10 and 15 days after treatment (DAT). Each sub-treatment was repeated twice with each plant species. On each plant per soil treatment, five larvae were placed in clip cages (Figure 5) with 25 larvae in total per five plants per

one soil treatment. Clip cages were made using 30 ml cups with an approximately 2 cm diameter hole cut into them and a lid made to fasten cages on to the leaves. The *H. zea* that were used in this study were obtained as first-instar larvae from Benzon Research in Carlisle, PA. The second-instar larvae were placed into the clip cages and placed onto plant leaves. Every 48 hours, the insect in the clip cage was removed from the plant and weighed and then returned to the plant leaf. To follow and evaluate the effect of soil treatments on insect development, weight (mg) was taken for 30 days or until the insect reached pupation or died. Survival time as well as time to pupation were also recorded. Data were recorded and analyses were made.

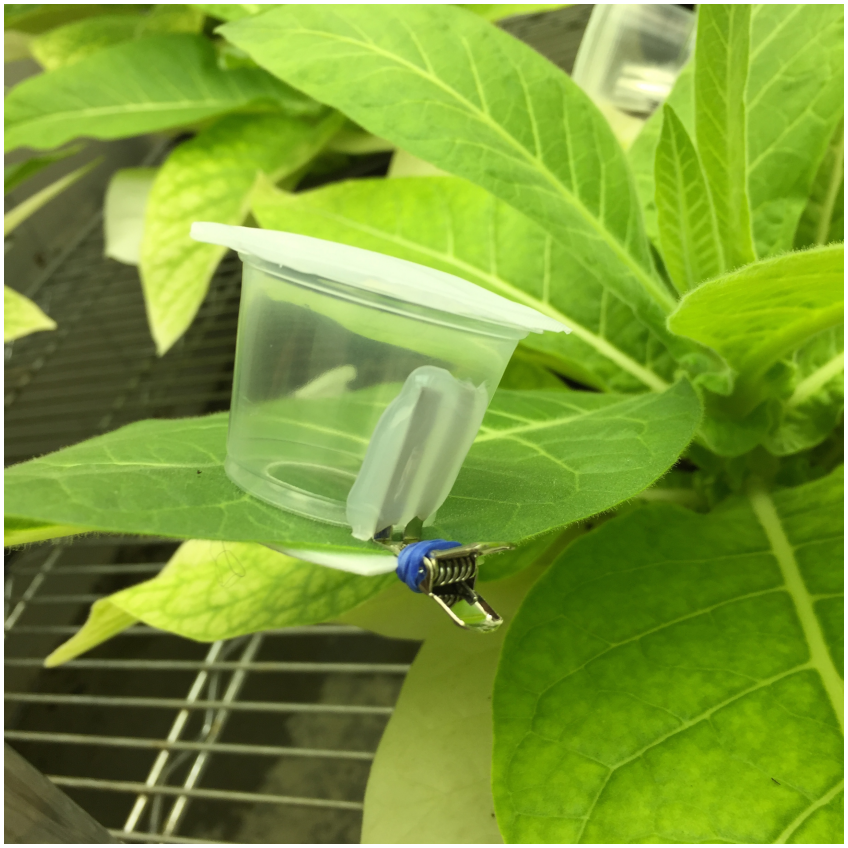


Figure 5. A clip cage on a tobacco plant.

A randomized complete block design factorial with replication and repeated measures on the two plant types was used with three main soil treatments applied to five pots of each plant type and five larvae applied to each potted plant. Larval weight data were collected and analyzed to determine effects of the soil treatments and sub-treatments on larval growth (their weight over the observational time period). A mixed model analysis with a rank transformation and least square means were compared using Tukey HSD tests in JMP Pro 12 (SAS Institute Inc., Cary, NC). To test the effect of induced resistance on soybean and tobacco three main null hypotheses were proposed: there are no differences in larval weight over the observational time period between the soil treatments; there are no differences in larval mortality over the observational time period between the soil treatments; and there are no differences in pupal weight over the observational time period between soil treatments. Another proposed null hypothesis examining differences of persistence in the induced resistance effect between soil treatments was that there are no differences in larval weight between sub-treatments (DATs) over the observational time period and soil treatments.

iii. Results and Discussion

Tobacco

Larval weights of corn earworm on tobacco were not significantly different ($p= 0.2395$) among soil treatments over the observational period. A mean separation of the least square means comparing just the soil treatments showed significant difference in larval weights between the EPN treatment (mean weight 11.25 mg) and Actigard (mean weight 9.91 mg); however, no difference in larval weights between EPN treatment and

the control (mean weight 12.69 mg) or between Actigard and the control were observed (Table 4). Overall, larval development was not significantly different among soil treatments compared to the control over the observational time.

The larval weights among soil treatments and between sub-treatments (DAT) did differ significantly ($p= 0.0036$) from one another over the observational time period. However, at each sub-treatment level (5,10, and 15 DAT), there were no significant differences ($p=0.122$, 0.599, and 0.061, respectively) in larval weights between soil treatments over the observational time period. Larval weights at 5 and 15 DAT comparing the soil treatments to one another were not statistically different (Table 5). However, at 10 DAT, larval weights were significantly different between the EPN treatment (mean weight 19.14 mg) and Actigard (mean weight 18.88 mg), but no differences between the EPN treatment and control (mean weight 21.68 mg) or Actigard and the control were documented (Table 5).

A high frequency of larval death at earlier observations was seen, and no statistical differences (p-value>0.05) were documented among soil treatments or sub-treatments (DAT) over the observational time period in larval death or mortality. Only 12 larvae successfully pupated in the experimental time between both plant types. Because of this low number, statistical comparisons were not available to evaluate the effects on soil treatments on pupal weight. Larval weights were significantly different ($p<0.001$) among observations (Figure 6). The development and growth of larvae throughout the observational time period explain the differences seen between observations. In other words, as larvae grow and get larger they weigh more, which is evident as the time period progresses. Individual sub-treatments (5, 10, and 15 DAT) also showed significant

Table 4. Comparison of mean larval weights of *Helicoverpa zea* among soil treatments (Actigard, Control, and Entompathogenic Nematodes [EPNs]) on tobacco in the greenhouse, 2015.

Soil Treatment	Mean Larval Weight (mg) (±SE)
Actigard	9.91 (2.83) B[1]
Control	12.69 (1.89) AB
EPN	11.25 (1.52) A

[1]Least Square Means Separation, Tukey HSD, α =0.05; means followed by the same letter are not significantly different; differences were determined using transformed data. Untransformed means are presented in table.
SE = standard error.

Table 5. Comparison of mean larval weights of *Helicoverpa zea* among soil treatments (Actigard, Control, and Entompathogenic Nematodes [EPNs]) at 5, 10, and 15 days after treatment (DAT) on tobacco in the greenhouse, 2015.

Mean Larval Weight (mg) (±SE)			
Soil Treatment	5 DAT	10 DAT	15 DAT
Actigard	0.053 (0.278) A	18.88 (1.86) B	14.76 (2.11) A
Control	0.191 (0.256) A	21.68 (1.58) AB	21.15 (1.97) A
EPN	0.693 (0.248) A	19.14 (1.55) A	15.99 (2.06) A

[1]Least Square Means Separation, Tukey HSD, α =0.05; means within a column followed by the same letter are not significantly different; differences were determined using transformed data. Untransformed means are presented in table. SE = standard error.

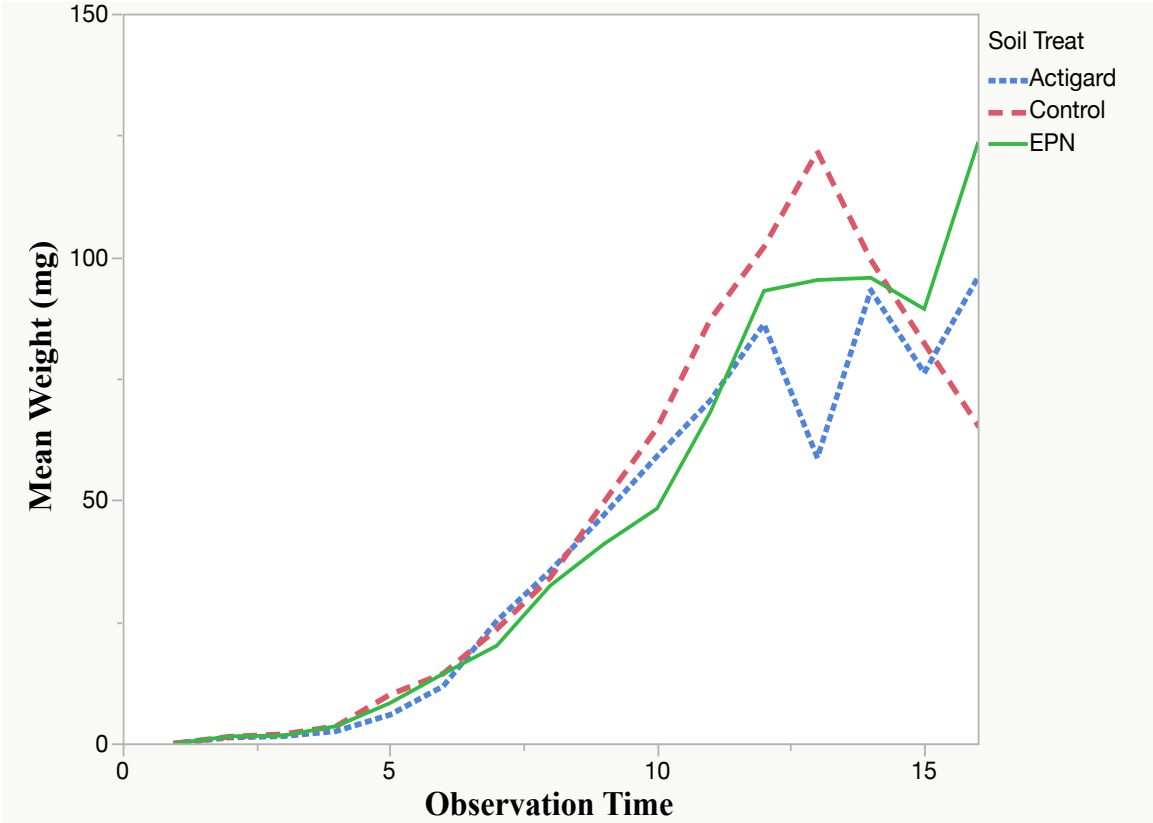


Figure 6. Mean larval weight of *Helicoverpa zea* on tobacco in the greenhouse over the observation time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.

differences (all $p<0.001$) in larval weight between observations. Again, the growth and development of the larvae between observations can explain the differences over the observational time (Figure 7, 8, 9,and 10).

Soybean

Larval weights of corn earworm were significantly different ($p = 0.0003$) between soil treatments over the observational time. Larval weights were significantly different between Actigard (mean weight 19.35 mg) and EPN treatment (mean weight 18.15 mg) and between Actigard and control (mean weight 27.52 mg). However, no significant difference between EPNs and the control were observed (Table 6).

Larval weights were significantly different ($p = 0.005$) over the observational time period among soil treatments and sub-treatments (DAT) (Table 7). Larval weights among soil treatments at all sub-treatment levels (5, 10, and 15 DAT), when observed individually, showed significant differences ($p<0.05$ at all three levels) over the observational time period. Larval weights at 5 DAT were significantly different between Actigard (mean weight 4.92 mg) and control (mean weight 3.85 mg) as well as Actigard and the EPN treatment (mean weight 3.33 mg). However, no differences were observed between the control and EPN soil treatments. Larval weights at 10 and 15 DAT were not significantly different among soil treatments (Table 7). As with tobacco, a higher frequency of larval death was observed at the earlier observations but larval death was not significantly different (p-value<0.05) among soil treatments over the observational time period.

Larval weights were significantly different ($p<0.001$) among the observations (Figure 11). The growth and development of larvae during the observational time period

Table 6. Comparison of mean larval weights of *Helicoverpa zea* among soil treatments (Actigard, Control, and Entompathogenic Nematodes [EPNs]) on soybean in the greenhouse, 2015.

Soil Treatment	Mean Larval Weight (mg) (±SE)
Actigard	19.35 (1.28) A[1]
Control	27.52 (1.31) B
EPN	18.15 (1.23) B

[1]Least Square Means Separation, Tukey HSD, α =0.05; means followed by the same letter are not significantly different; ; differences were determined using transformed data. Untransformed means are presented in table.
SE = standard error.

Table 7. Comparison of mean larval weights of *Helicoverpa zea* among soil treatments (Actigard, Control, and Entompathogenic Nematodes [EPNs]) at 5, 10, and 15 days after treatment (DAT) on soybean in the greenhouse, 2015.

	Mean Larval Weight (mg) (±SE)		
Soil Treatment	5 DAT	10 DAT	15 DAT
Actigard	4.92 (0.31) A	32.02 (2.99) A	23.13 (1.53) A
Control	3.85 (0.30) B	61.53 (3.21) A	20.08 (1.68) A
EPN	3.33 (0.29) B	35.66 (2.94) A	17.14 (1.51) A

[1]Least Square Means Separation, Tukey HSD, α =0.05; means within a column followed by the same letter are not significantly different; ; differences were determined using transformed data. Untransformed means are presented in table. SE = standard error.

explain the differences seen among observations. Individual sub-treatments (5, 10, and 15 DAT) also showed significant differences (all $p<0.001$) in larval weight between observations. Again, the larval growth and development between observations can explain the differences over the observational time (Figure 12, 13, 14, and 15).

iv. Summary

In this experiment, no evidence was observed of an induced resistance effect by any of the soil treatments on larvae of *H. zea*. Larval weights did not change over time among the soil treatments so there was no observation of change in the development of larvae. Soil treatments also had no effect on larval death, as soil treatments did not differ. Larvae were particularly slow in developing in the greenhouse setting with only around 1% of larvae reaching pupation. Previous studies suggest that total larval development ranges from 12-32 days depending on food (Butler 1976). These larvae, especially late instars, tend to eat more and faster as they develop. Because they were only moved to a new location on the leaf every 48 hrs, the development time of larvae on all plants was most likely delayed. Overall, soil treatments did not differ from the control treatment in development of larvae or larval death. Data of soil treatment effects on pupae could not be determined because of lack of pupation among the larvae in both plant species. More research is needed to confirm if soil treatments can induce resistance in a greenhouse setting on tobacco and soybean before being included in pest management programs.

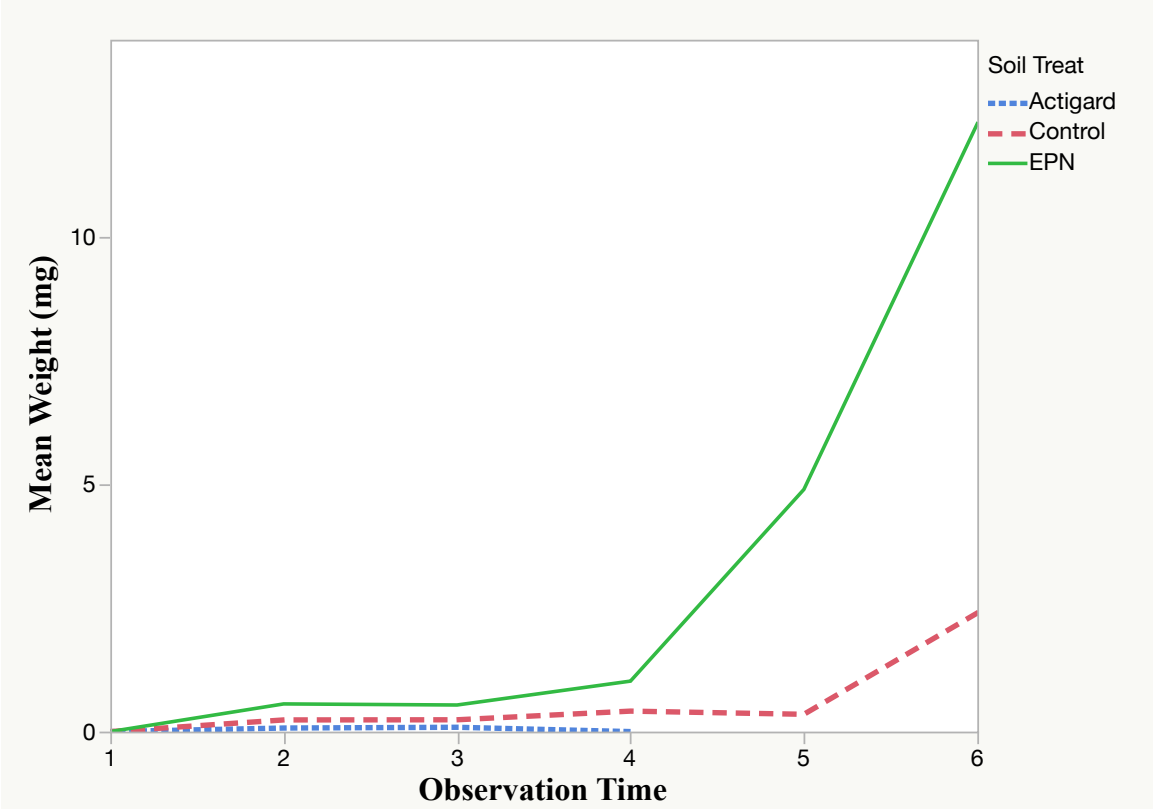


Figure 7. Mean larval weight of *Helicoverpa zea* on tobacco 5 DAT in greenhouse over the observational time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.

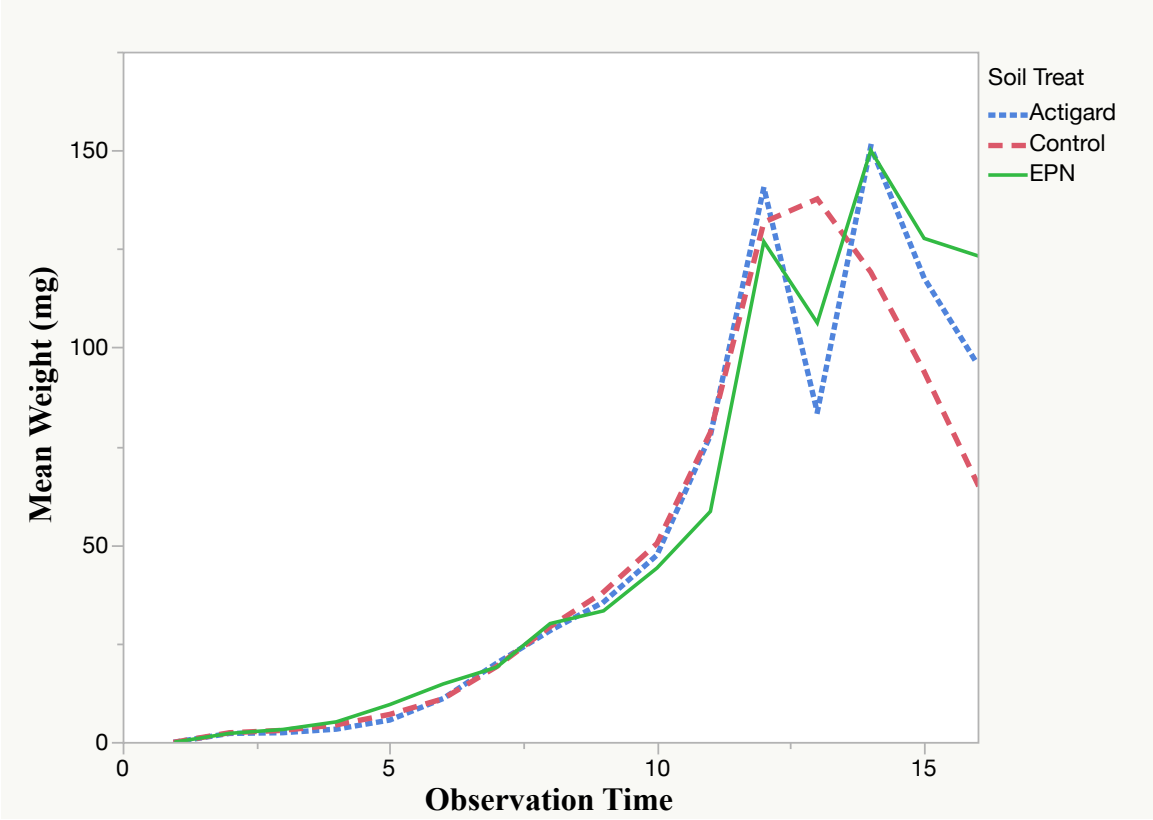


Figure 8. Mean larval weight of *Helicoverpa zea* on tobacco 10 DAT in the greenhouse over the observational time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.

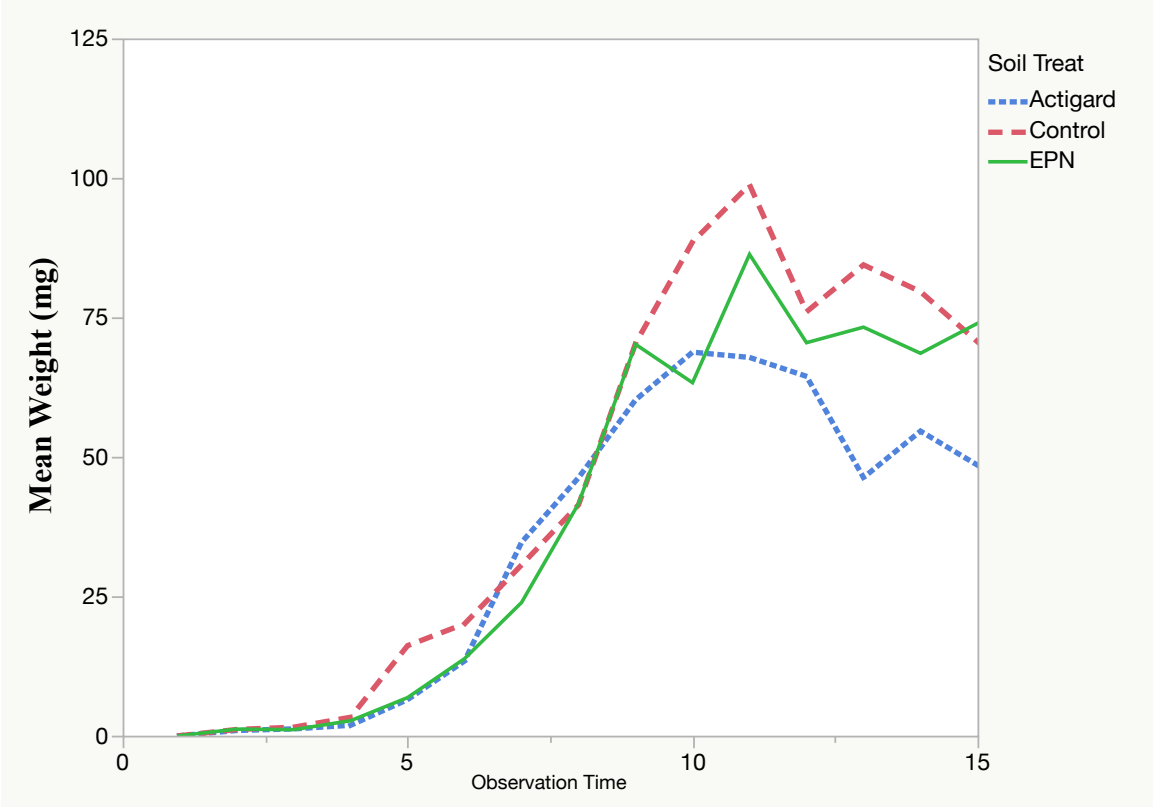


Figure 9. Mean larval weight of *Helicoverpa zea* on tobacco 15 DAT in the greenhouse over the observational time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.

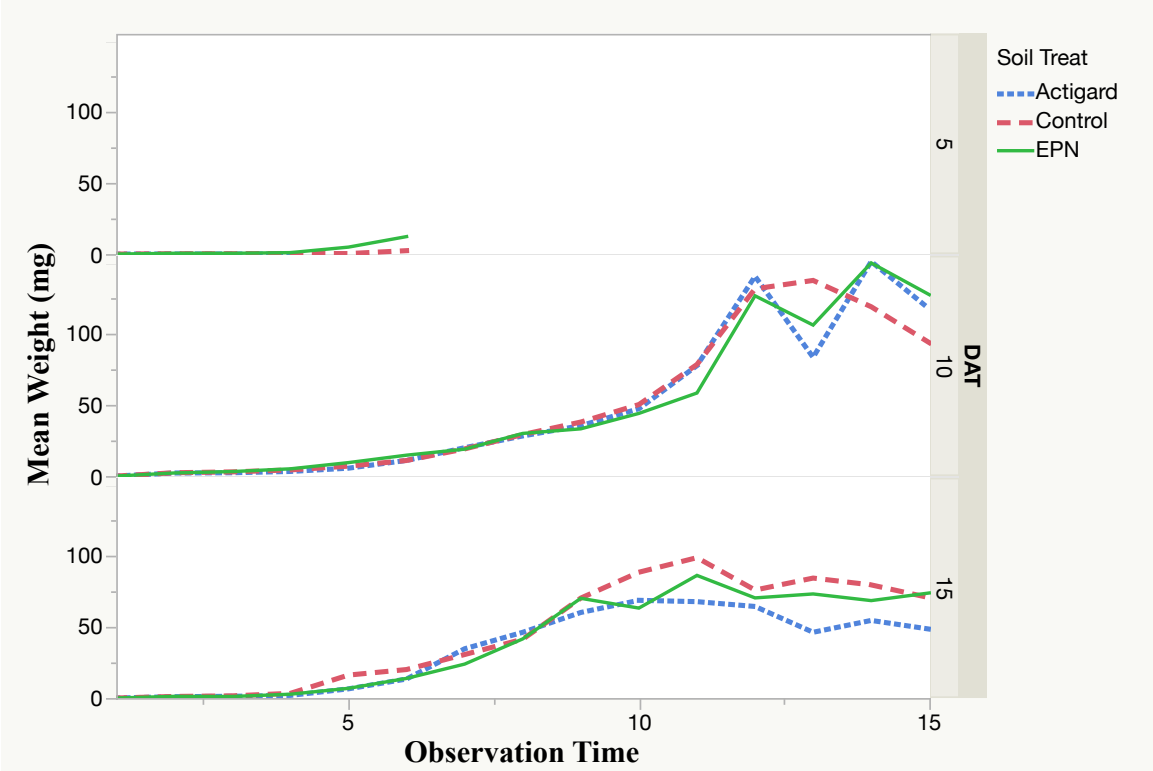


Figure 10. Mean larval weight of *Helicoverpa zea* on tobacco in the greenhouse over the observation time between soil treatments and separated by sub-treatments (DAT). Observation time represents larval weight observed every 48 hrs over a 30 day period.

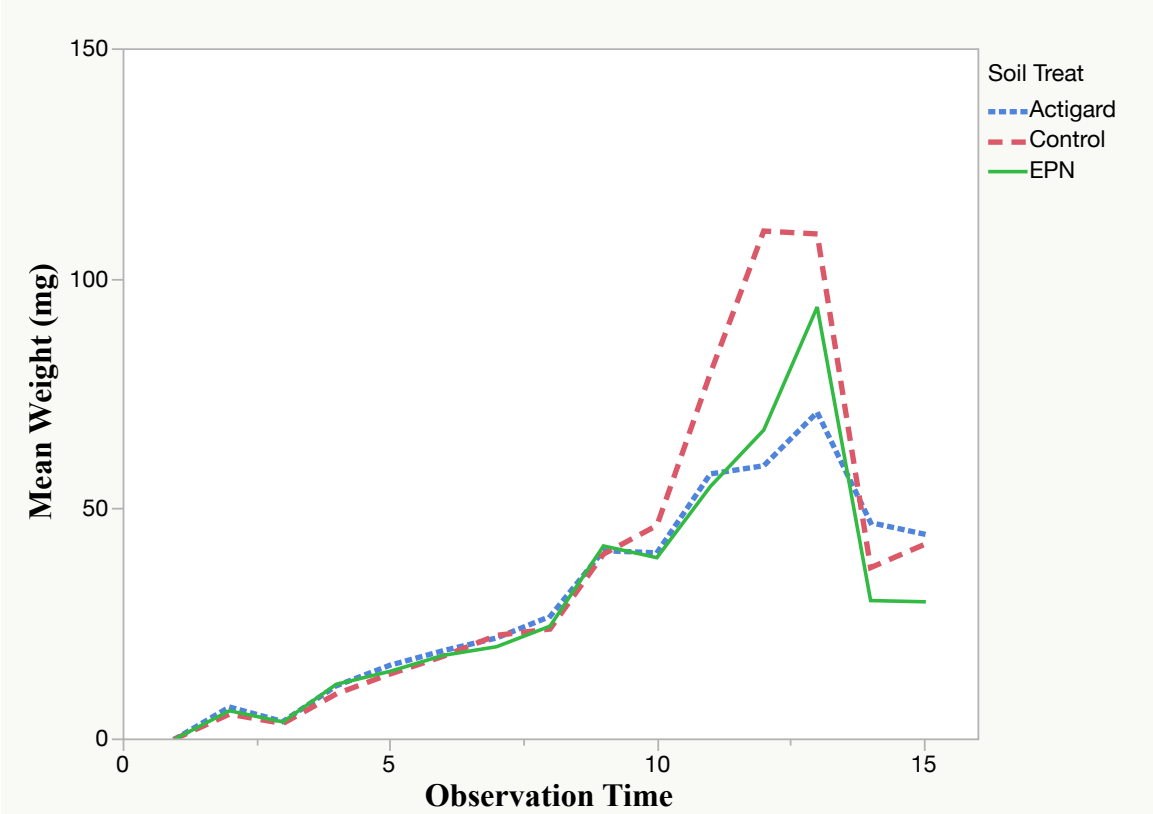


Figure 11. Mean larval weight of *Helicoverpa zea* on soybean in the greenhouse over the observation time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.

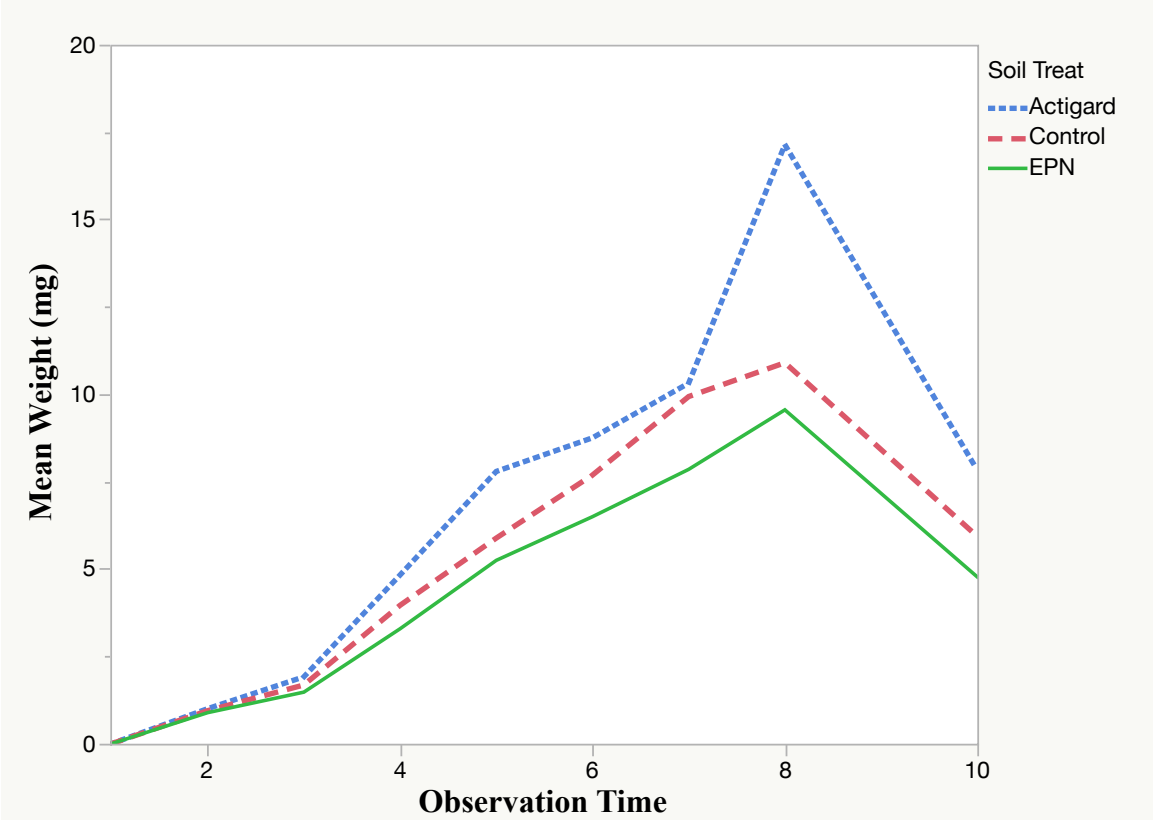


Figure 12. Mean larval weight of *Helicoverpa zea* on soybean 5 DAT in the greenhouse over the observation time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.

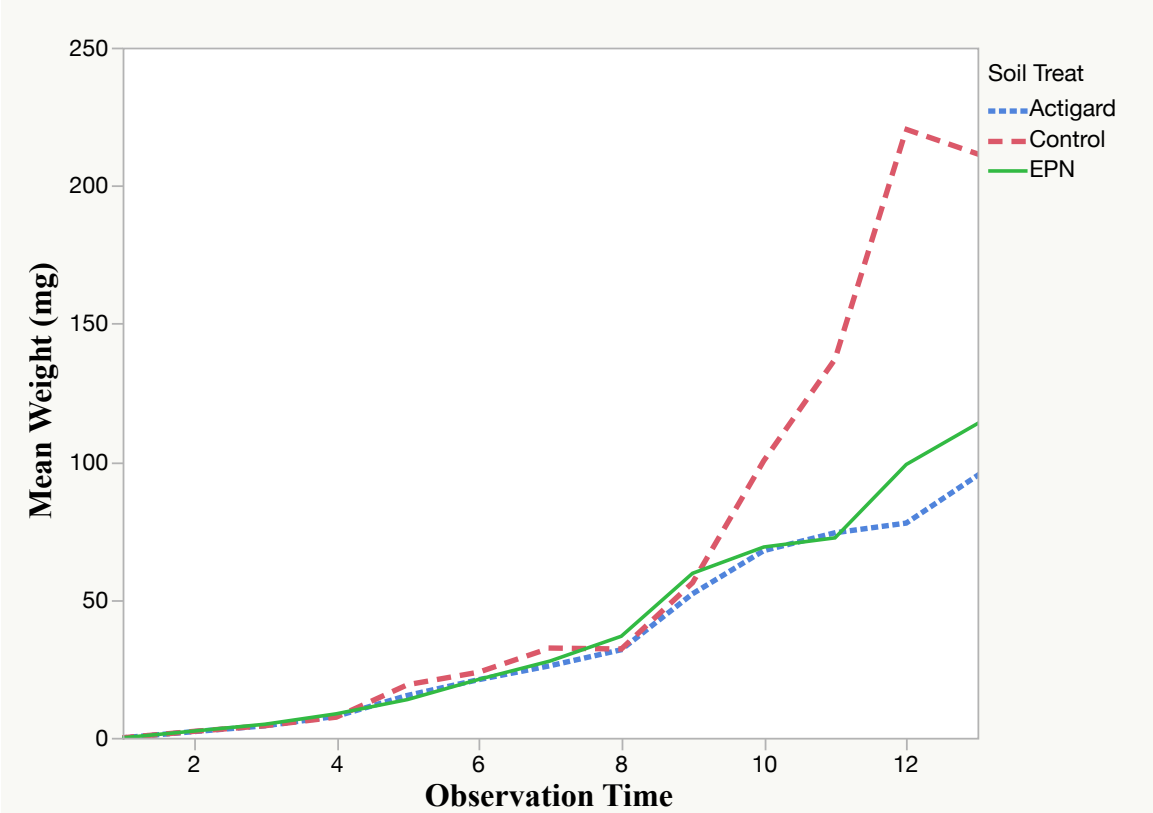


Figure 13. Mean larval weight of *Helicoverpa zea* on soybean 10 DAT in the greenhouse over the observational time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.

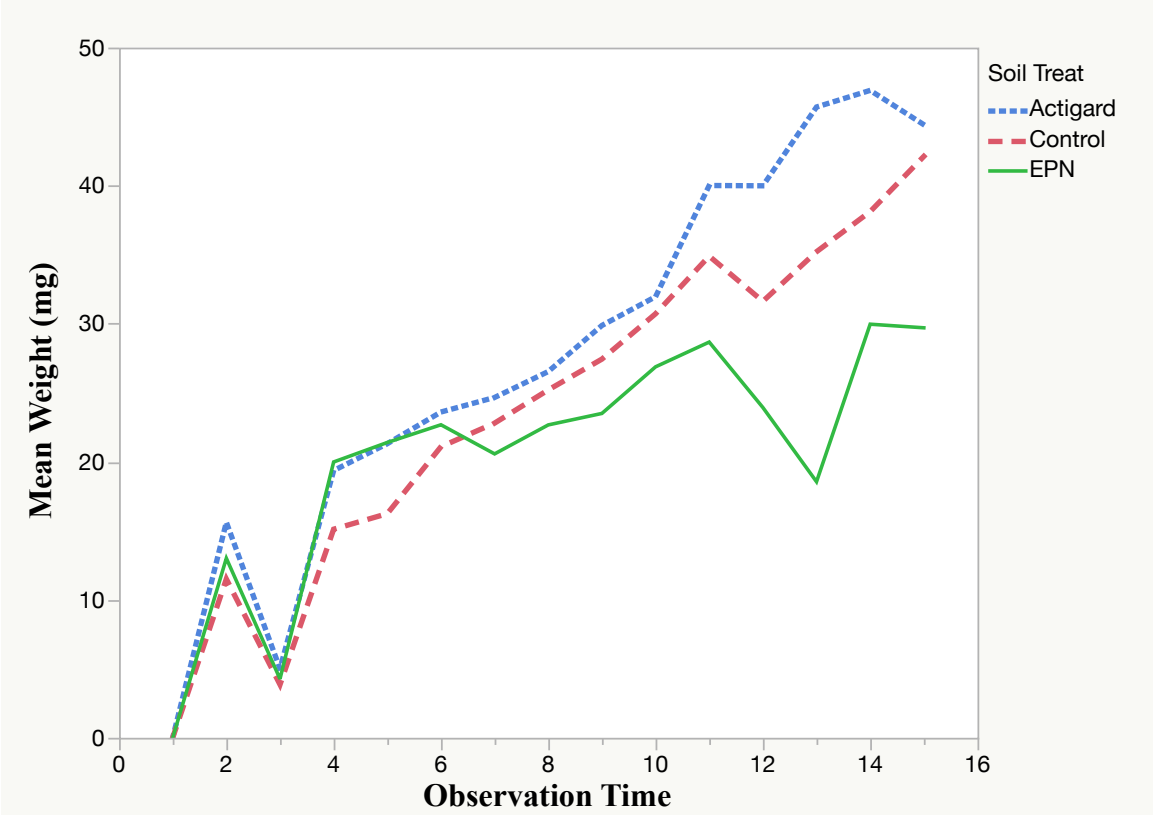


Figure 14. Mean larval weight of *Helicoverpa zea* on soybean 15 DAT in the greenhouse over the observation time between soil treatments. Observation time represents larval weight observed every 48 hrs over a 30 day period.

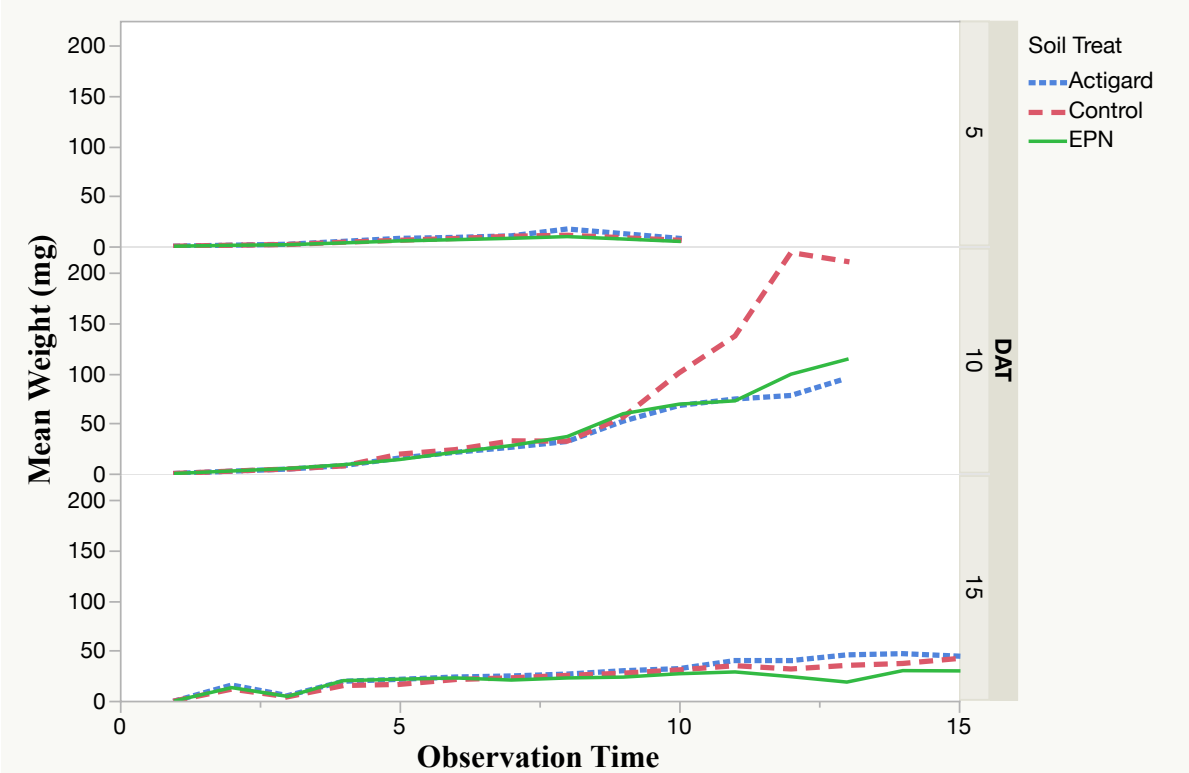


Figure 15. Mean larval weight of *Helicoverpa zea* on soybean in the greenhouse over the observation time between soil treatments among sub-treatments (DAT). Observation time represents larval weight observed every 48 hrs over a 30 day period.

Chapter IV

Conclusions

To test the effects of entomopathogenic nematode (EPN)-induced resistance, a field study on tobacco and a greenhouse study on tobacco and soybeans were completed using EPN soil applications. EPNs have been shown to induce resistance in other plants, including tomato and hosta, as well as having other non-target effects when applied to the soil, such as reducing plant parasitic nematode populations and foliar nematode populations. However, this study did not see evidence of plant defense or induced resistance.

The objective in the field study was to assess EPN-induced resistance in a field setting on tobacco against naturally-occurring pests and diseases. Examination of insect damage and disease damage on selected leaves, as well as number of insects on each plant, yielded no significant differences among soil treatments over the observational period. Induced resistance effects take time to occur in a plant and when the soil treatments were applied to the tobacco plants, pests and diseases, particularly blue mold, were already present. The initial presence of the diseases and pests could be one reason no reduction, and therefore no resistance in the plant by the soil treatments, of pests or diseases were observed in this study. Another possible factor in the lack of statistical significance in pest damage or number of insects on the plants is because of the insecticide that was applied to the plants prior to the study. If this study were to be repeated, using a field with no previous or ongoing insecticide use might produce different results, as well as provide clearer observations of the relationship between soil

treatments and pests. Additionally, if the study were to be repeated the leaves selected to be assessed for pest damage as well as disease damage should be much younger than the eighth or ninth leaf stage as in this study. Younger leaves were more likely to be damaged by pests in the field. While collecting data, it was often observed that the majority of the top of the plant, the youngest part, would be heavily damaged by pests while the leaves selected in this study, the older leaves, were not damaged.

In the greenhouse study, the objective was to determine if EPNs could induce resistance against a chewing pest, the corn earworm, *H. zea*, in tobacco and soybean in a greenhouse setting. Larvae were exposed to plants in clip cages 5, 10, or 15 days after applying soil treatments (DAT). Larval weight (mg) was monitored every 48 hrs for 30 days or until death or pupation to assess development. No differences in larval weights were detected among soil treatments to tobacco over the observational time. However, significant differences in larval weights among soil treatments to soybean were detected over the observational time. Upon closer evaluation, however, EPNs did not differ from the control. In both tobacco and soybean, significant differences in larval weights were detected between sub-treatments (DATs), but with a closer evaluation of each sub-treatment group of each plant species there were no differences in EPNs and the control.

Both plant species grown in the greenhouse were not as ideally healthy as if grown in a field setting. The health of the plants is a concern and may lead to misguided results. All larvae were particularly slower in development. This slow development could be a result of the food available to the larvae. Later-instar larvae tend to consume more and at a faster rate. In this study, larvae were only relocated on the leaves every 48 hrs to provide more food. This time frame of each relocation could have been a problem for

later-instar larvae and their dietary needs for development. If repeated, clip cages should be moved more frequently to provide for the dietary needs of later-instar larvae or use larger cages. An alternative would be to conduct the study using petri dishes providing leaves from treated plants to the larvae in the dishes. This method would also reduce incidences of escape from the clip cages onto the plants. This study did have a high initial level of larval mortality in both plant species that was not significantly different among soil treatments. Pupation of *H. zea* on either plant species was rare; sufficient data on the effects of the soil treatments on pupation could not be obtained.

Actigard 50WG was used as a soil treatment in each study and is a commercially available product whose mode of action induces resistance in plants. The active ingredient in Actigard 50WG is an analog of salicylic acid that plays an important role in plant defense mechanisms often associated with induced resistance against pathogens (Myresiotis *et al.* 2014). Also, Actigard is typically suggested as a foliar spray (Syngenta US 2014). This study applied Actigard as a soil treatment to be more comparable to the soil treatments of EPNs; however, using Actigard as a foliar spray is proposed for use in future studies since that method is recommended on the label.

Currently, little is known regarding the mechanisms of EPN-induced resistance in plants. Plants protect themselves against attack from pests and pathogens with several resistance strategies. Jasmonic acid and salicylic acid signaling pathways are two responses that protect plants against pests and pathogens (Heil and Bostock 2002; Walters 2011). A salicylic response is associated with pathogen attack and a jasmonic response is associated with insect herbivores (Pieterse *et al.* 2014). The active ingredient in Actigard is a salicylic acid analog and the reason the product is recommended is to

reduce loss caused by pathogens. The salicylic acid response is possibly another reason we did not see evidence of a resistance effect against pests in Actigard soil treatments as opposed to if the product was a jasmonic acid analog. Additional mechanistic studies examining both salicylic and jasmonic acid are needed to further understand EPN-induced resistance.

Although previous studies showed evidence of EPN-induced resistance, this study did not show such evidence in tobacco or soybean plants. EPNs are a novel approach and have many benefits and positive attributes as a possible biological control agent. However, additional research is needed to better understand the phenomenon of EPN-induced resistance before developing and applying them for pest and diesase management on plants.

Literature Cited

Abrol, D.P., and Shankar, U. (2012). History, overview and principles of ecologically-based pest management. In: D.P Arbol and U. Shankar, eds. *Integrated Pest Management Principles and Practice*. Oxfordshire, UK: CABI publishing. CAB International.

An, R., Orellana, D., Phelan, L., Cañas, L., and Grewal, P. (2016). Entomopathogenic nematodes induce systemic resistance in tomato against *Spodoptera exigua*, *Bemisia tabaci* and *Pseudomonas syringae*. *Biological Control* 93: 24-29.

Butler, G.D. (1976). Bollworm: Development in relation to temperature and larval food. *Environmental Entomology* 5: 520-522.

Carson, R. (1962). *Silent Spring*. Boston: Houghton-Mifflin.

Ehlers, R-U, and Shapiro-Ilan D.I. (2005). Mass production. In: P. Grewal, R-U Ehlers, and D.I. Shapiro-Ilan, eds. *Nematodes as Biological Control Agents*. Wallingford, UK: CABI Publishing. CAB International. 65-79.

(FAO) Food and Agriculture Organization. (2011). How to feed the world in 2050. (http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf).

Georghiou, G.P. (1990). Overview of insecticide resistance. In: M.B. Green, H.M. Lebaron, and W.K. Moberg, W.K., eds. *Managing Resistance to Agrochemicals.* American Chemical Society, Washington, DC. 18-41.

Glaser, R.W., and Farrell, C.C. (1935). Field experiments with Japanese beetle and its nematode parasite. *Journal of the New York Entomological Society* 43: 345-371.

Glaser, R.W., and Fox, H. (1930). A nematode parasite of the Japanese beetle. *Science* 70: 16-17.

Grewal, P.S. (2012). Entomopathogenic nematodes as tools in integrated pest management. In: D.P Arbol, and U. Shankar, eds. *Integrated Pest Management Principles and Practice*. Oxfordshire, UK: CABI Publishing. CAB International.

Grewal, P.S., Ehlers, R-U, and Shapiro-Ilan, D. I., eds. (2005). *Nematodes as Biocontrol Agents*. Wallingford, Oxfordshire, UK: CABI Publishing, CAB International. 505.

Grewal, P.S., Selvan, S., and Gaugler, R. (1994). Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction. *Journal of Thermal Biology* 19: 245-253.

Heil, M., and Bostock, R.M. (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defenses. *Annals of Botany* 89: 503-512.

Horsfall, J.G., and Barratt, R.W. (1945). An improved grading system for measuring plant diseases. *Phytopathology* 35: 655.

Jagdale, G.B., Kamoun, S., and Grewal, P.S. (2009). Entomopathogenic nematodes induce components of systemic resistance in plants: Biochemical and molecular evidence. *Biological Control* 51: 102-109.

Jagdale, G.B., and Grewal, P.S. (2008). Influence of the entomopathogenic nematode *Steinernema carpocapsae* in host cadavers or extracts from cadavers on the foliar nematode *Aphelenchoides fragariae* on hosta. *Biological Control* 44: 13–23.

Jagdale, G.B, Somassekahr, N., Grewal, P.S., and Klein, M.G. (2002). Suppression of plant parasitic nematodes by application of live and dead entomopathogenic nematodes on boxwood (*Buxus* spp). *Biological Control* 24: 42-49.

Kaya, H., and Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Entomology* 38: 181-206.

Lewis, E.E., and Grewal, P. S. (2005). Effects of entomopathogenic nematodes on plant-parasitic nematodes. In: P. S. Grewal, R. U. Ehlers, and D. Shapiro-Ilan, eds. *Nematodes as Biocontrol* Agents. Wallingford, UK: CABI Publishing. CAB International. 349-361.

Liebhold, A.M., and Tobin, P.C. (2008). Population ecology of insect invasions and their management. *Annual Review of Entomology* 53: 387-408.

Miles, C., Blethen, C., Gaugler, R., Shapiro-Ilan, D., and Murray, T. (2012). Using entomopathogenic nematodes for crop insect pest control. *A Pacific Northwest Extension Publication* 544.

Molina, J.P., Dolinski, C., Souza, RM., and Lewis, E.E. (2007). Effect of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterobdtidae) on *Meloidogyne mayaguenis* Rammah and Hirschmann (Tylenchida: Meloidoginidae) infection in tomato plants. *Journal of Nematology* 39: 338-342.

Murphy, P. (2005). *What a book can do: the publication and reception of Silent Spring.* Boston, MA: University of Massachusetts Press.

Myresiotis, C., Vryzas, Z., and Papadopoulou-Mourkidou, E. (2014). Enhanced root uptake of acibenzolar-S-methyl (ASM) by tomato plants inoculated with selected *Bacillus* plant-growth promoting rhizobacteria (PGPR). *Applied Soil Ecology* 77: 26-33.

Norris, R.F., Caswell-Chen, E.P., and Kogan, M. (2003). *Concepts in Integrated Pest Management*. Upper Saddle River, NJ: Prentice Hall.

Panda, N., and Khush, G.S. (1995). *Host Plant Resistance*. Wallingford, UK: CABI Publishing. CAB International.

Perry, R.N., Hominick, W.M., Beane, J., and Briscose, B. (1998). Effects of the entomopathogenic nematodes, *Stienernema feltiae* and *S. carpocapsae*, on the potato cyst nematode, *Globodera rostochiensis*, in pot trials. *Biocontrol Science and Technology* 8: 175-180.

Pieterse, C.M., Zamloudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C., and Bakker, P.A. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology* 52: 347-375.

Rizvi, P.Q., Ahmad, S.K., Choudhury, R.A., and Ali, A. (2012). Biopesticides in ecologically-based integrated pest management. In: D.P. Arbol, and U. Shankar, eds. *Integrated Pest Management Principles and Practice*. Oxfordshire, UK: CABI Publishing. CAB International.

Smart, G.C. (1995). Entomopathogenic nematodes for the biological control of insects. *Supplement to the Journal of Nematology* 27(4S): 529-534.

Somasekhar, N., Grewal, P.S, De Nardo, E.A.B, and Stinner, B.R. (2002). Non-target effects of entomopathogenic nematodes on the soil nematode community. *Journal of Applied Ecology* 39: 735-744.

Sygenta US. (2014). Label sheet Actigard. http://www.syngentacropprotection.com/Labels/p-644/Actigard_50WG

United States. Population Reference Bureau. Agency of Internal Development. (2015). 2015 world population data sheet. http://www.prb.org/pdf15/2015-world-population-data-sheet_eng.pdf

Walters, D. (2011). *Plant Defense: Warding off Attack by Pathogens, Herbivores, and Parasitic Plants*. Ames, IA: Blackwell Pub. Wiley-Blackwell.

Vita

Julia Ferguson was born in Canton, OH as the only child of Mary and Frank Ferguson. She attended Baylor School in Chattanooga, TN for middle and high school. After graduation, she continued on to the University of Tennessee at Knoxville (UTK) where she began her degree and continued interest in biology and science. She obtained a Bachelor of Science degree in Ecology and Evolutionary Biology from the UTK in May 2014. While obtaining her Bachelor's degree, Julia worked in the Department of Entomology and Plant Pathology as an undergraduate student worker. She accepted a Graduate Research Assistantship with the Department of Entomology and Plant Pathology in the summer of 2014. Julia graduated in May 2016 with a Master of Science degree in Entomology and Plant Pathology with a concentration in Entomology as well as a minor in Statistics.