EVALUATION OF NATIVE ENTOMOPATHOGENIC NEMATODES FOR BIOLOGICAL CONTROL OF PLUM CURCULIO IN NEW YORK APPLE ORCHARDS

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Science

By Tessa Grace Lessord

January 2017

ABSTRACT

In New York State, apple growers face an increasing number of challenges throughout the season. In this region where water and sun are plentiful, insect pests are one of the most prominent concerns. Historically, growers have relied on a regular schedule of chemical insecticides applied with an airblast sprayer. However, recent legislation has brought on use restrictions and losses of commonly used insecticides. This along with the consumer's push for safer and more environmentally friendly produce is what inspires researchers and professionals to look for alternative pest management strategies.

Entomopathogenic nematodes are soil-dwelling roundworms with free-living and insect-dependent life stages. Since the early 20th century they have often been mass-reared and used as a biological control agent. Success has varied greatly among cropping systems, pest species, and nematode species. Plum curculio was chosen as a good candidate for control with entomopathogenic nematodes because plum curculio spends a large portion of its life cycle in close contact with the soil. Control with entomopathogenic nematodes is especially attractive because only the adult stage appears in the canopy where they are accessible by insecticides.

In this study, we set out to gain a better understanding of orchard characteristics conducive to the establishment and virulence of entomopathogenic nematodes in apple orchards. We collected data on soil texture, water holding capacity, water content, and carbon/nitrogen content. These data could then be utilized to help understand the results of our bioassays in both laboratory and field settings, in which we were able to compare

plum curculio emergence across different sites and nematode treatments.

Results from this study indicated, while *Steinernema carpocapsae* "NY-001" and *Steinernema feltiae* "NY-04" are virulent against plum curculio in laboratory bioassays, they may be less effective under natural orchard soil conditions. The plum curculio mortality in untreated and treated intact soil columns was not different. In sieved orchard soil, the *S. feltiae* "NY-04" treated columns had significantly higher mortality rates than untreated and *S. carpocapsae* "NY-001" cores. Sieved soil treated with both *S. feltiae* and *S. carpocapsae* concurrently was not different from *S. feltiae* treated soil. However, within the intact soil column category, there was no difference in plum curculio emergence in any of the treatments. This leads us to believe that overall soil structure affects the success of these nematode species in this particular system. Soil texture, water holding capacity, carbon content, and nitrogen content all proved to be different among sites.

BIOGRAPHICAL SKETCH

Tessa Lessord was born in Sodus, NY on September 25, 1991. While growing up on an apple farm in Upstate New York, she was always interested in all aspects of nature. After spending two summers during college as a crop scout for a Wayne County-based fruit tree consulting business, Tessa got a summer job as a field assistant working with Dr. Arthur Agnello at NYSAES Geneva. It was during that summer that Dr. Agnello offered Tessa an opportunity to do her Master's research under his supervision.

Tessa chose to concentrate her research on part of Dr. Agnello and Dr. Elson Shields' entomopathogenic nematode project because she is interested in the way that ecology fits into agriculture. Her interest in helping growers couples nicely with her extreme infatuation with ecology, nature, and dirt.

Tessa holds an A.A.S. in Agriculture Technology with a concentration in Plant Science from Alfred State College, a B.S. in Entomology from Cornell University, and this thesis marks the completion of her M.S. in Entomology.

This thesis is dedicated to the late Judith Hann.

ACKNOWLEDGEMENTS

I owe thanks to many people who assisted with the process of my research project and this thesis. First and foremost, I am grateful to my advisor, Dr. Art Agnello who has provided endless guidance with my academic and professional development. I thank Dr. Kyle Wickings and Dr. Huijie Gan for their advice regarding soil ecology and associated laboratory techniques. I also owe great thanks to Dr. Elson Shields and Antonio Testa for providing me with the nematodes that were necessary to execute my experiments, and for advice regarding my methods. Dr. David Soderlund also helped a great deal in the review and editing of my thesis. Lynn Johnson of the Cornell Statistical Consulting Unit was vital to the analysis of my data, and I am forever grateful.

I also received great help from our summer field assistants, Forrest English-Loeb, Emily Pennock, and Abagail Davis.

I am extremely thankful for my friends who supported me and guided me through this adventure – Zachary Thibault, Hanna Scott, Collis Malloy, Kristina Chyn, and my family, all of whom have encouraged my unorthodox interests for my entire life – SueAnne Garder, Sherrill Lessord, Kevin Lessord, and Maya Lessord.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE:

INTRODUCTION

Overview

While New York's main agricultural product is dairy, the state ranks second in the country in apple production. In recent history, there has been a growing interest in producing apples for the fresh market. However, in fresh fruit, a minor cosmetic defect can send the apple to processing rather than the fresh market, which greatly reduces its monetary value (Piñero et al. 2011, Agnello et al. 2014).

Plum curculio [*Conotrachelus nenuphar* (Herbst)] is a common weevil pest in fruit tree systems east of the Rocky Mountains. Adult plum curculio damage the fruit by feeding, which can create puncture scars on the apple skin as the fruit expands. Additionally, adult females cut a slit in the skin of the developing fruit and lay their eggs within it. In apple, if the fruit aborts, the eggs can continue their development within the dropped fruit. However, when the apple remains on the tree continuing to grow, the pressure from natural fruit expansion crushes the eggs (Racette et al. 1992, Vincent et al. 2008). A fan-shaped scar remains on the apple skin, cosmetically preventing it from sale in the fresh market. In heavily infested areas, there can be several scars per apple. Historically, this pest has been suppressed by several regular insecticide applications. Using insecticides has been found to be a rather impractical control method for this highly evasive pest, and, due to increasing pesticide restrictions, researchers have sought alternative control methods (Vincent et al. 2008, Agnello et al. 2014).

Entomopathogenic nematodes have had a place in the biological control realm for decades, and are known to be generalist insect predators (Dowds & Peters 2002, Shapiro-

Ilan et al. 2002a). Commercially available varieties of entomopathogenic nematodes are strains that are poorly adapted for establishing resident populations in New York State. This is due to many factors, including climate. Past research has explored the possibility of using native entomopathogenic nematodes, which are better adapted to persist under the native field conditions where they are found (Ishibashi & Kondo 1987, Shields et al. 2009, Atay & Kepenkci 2016, Foye et al. 2016). Two native New York nematode strains, *Steinernema carpocapsae* "NY-001", and *Steinernema feltiae* "NY-04", have been found to persist in New York soils for at least 8 years (Shields et al. 2009). Preliminary laboratory studies have shown that these nematode strains are virulent against plum curculio to varying degrees, dependent upon the insect's life stage (Alston et al. 2005, Agnello 2014).

The Apple Orchard System

The United States ranks second in the world for apple production, and New York ranks second in apple production within the United States, with 29.5 million bushels produced annually. There are five main apple production regions in New York: the Hudson Valley, Champlain Valley, the Niagara Frontier, Central New York, and Lake Country. These regions comprise approximately 700 individual growers and 18,200 hectares dedicated to apple production. New York's apple industry supports 10,000 direct jobs and 7,500 indirect jobs (handling, packing, etc.). In New York, approximately 13,250,000 bushels (53%) of apples are sent to the fresh market (NY Apple Association 2016; New York Environment Report 2016; Good Fruit Grower 2016). Due to the economic importance of high value fresh market apples in New York State, there is a low

tolerance for fruit irregularities.

Plum Curculio

Plum curculio (*Conotrachelus nenuphar*) is a native insect attacking pome and stone fruits in the eastern United States. This insect is part of the weevil family, Curculionidae, and possesses the characteristic modified mouthparts that form a "snout." Female plum curculio use their snouts as a cutting tool to aid them in oviposition, the main cause of fruit damage by this pest. Plum curculio is distributed from Québec, south to Florida, west to the Rockies, and in Utah (Quaintance & Jenne 1912, Racette et al. 1992, Vincent et al. 2008, Agnello et al. 2014).

At the beginning of the growing season when ambient temperature rises to approximately 15° C, adult plum curculios emerge from their overwintering sites in the soil. They usually remain at the soil surface for some time before moving to the trees to begin to feed on the newly formed buds and blossoms. As the fruits begin to develop, the insects begin their mating period. When the female plum curculio is ready to lay her eggs, she will cut a flap in the apple's epidermal layer using her snout and then oviposit in the crevice. After oviposition there are two possible outcomes. If the tree aborts the fruit, the eggs will hatch and the plum curculio larvae will begin to develop inside the apple fruitlet; on average, larval development occurs over a 15-18 day period. If the fruit remains on the tree, the pressure caused by fruit expansion will crush the plum curculio eggs, preventing any further insect development. While this thesis focuses on the management of plum curculio in apple, it is important to note that this pest behaves differently in softer fruit varieties, like peaches and cherries. In those softer fruits, the

eggs will not be crushed and larvae are capable of completing their development within fruit that remains on the tree (Racette et al. 2012, Shapiro-Ilan et al. 2002a, Shapiro-Ilan et al. 2013).

Once the plum curculio larva has reached its last larval instar, it exits the apple and burrows into the soil below for pupation. After an average of 12 days in the soil, the insect begins to pupate at a depth between 2 and 8 cm. The total time spent in the soil ranges from 3 to 5 weeks. When the adult emerges from the pupa, it tunnels to the surface where it moves to the trees to feed on the fruit. This feeding damage also causes scarring, and it may allow for pathogen entry. After this feeding period, most plum curculio adults return to the surrounding woodlands to overwinter, with a minor part of the population actually overwintering within the orchard (Racette et al. 2002, Vincent et al. 2008).

Plum curculio is one of the most serious pests of pome and stone fruit. Unsprayed orchards experience an average of 85% fruit damage. While the scarring left by plum curculio is the more obvious sign of the insect's damage, it has been estimated that about 60% of damage caused by plum curculio has already occurred by the time the trees have reached petal fall (Racette et al. 2002, Vincent et al. 2008). Normally insecticides are sprayed at petal fall, and then at 10-14-day intervals thereafter. This can be expensive, and regulatory actions no longer allow many of these insecticides to be used in New York (Agnello et al. 2003 Akotsen-Mensah et al. 2012).

Entomopathogenic Nematodes

Entomopathogenic nematodes are roundworms with both free-living and insect-dependent life stages. The relatively high virulence, ease of rearing, and broad host range

of Steinernematids and Heterorhabditids have made them a subject of study for biological control of pest insects (Gaugler 1988a, Gaugler 1988b, Dowds & Peters 2002, Shapiro-Ilan et al. 2002). Since the first attempt at culturing *Steinernema glaseri* in the early 20th century, successes with entomopathogenic nematodes as a biocontrol agent include control of the *Diaprepes* root weevil in citrus, black vine weevil in cranberry, fungus gnats in greenhouse plants, and billbugs in turf (Shapiro-Ilan et al. 2002, Kim & Alston 2008, Akotsen-Mensah et al. 2012, Lacey & Georgis 2016).

Entomopathogenic nematodes are not direct predators of the insect, but rather a vector of an insect pathogenic bacterium that resides in their gut. The exact placement of the bacterial reservoir, called a vesicle, varies among nematodes (Dowds & Peters 2002). In most cases, Steinernematids' vesicle is in the anterior part of the intestine, while Heterorhabditids house their bacteria in the midgut(Adams & Nguyen 2002, Forst & Clarke, 2002). This interaction is a mutualism because the bacteria, once regurgitated and replicated within an insect's body cavity, are also the nematodes' food source (Kaya & Gaugler 1993, Stock 2015).

The nematode species studied in this thesis are both in the genus *Steinernema*, in the family Steinernematidae. Each species of *Steinernema* is associated with its own unique species of Proteobacteria in the genus *Xenorhabdus*, which is a gram-negative, rod-shaped facultative anaerobe in the family Enterobacteriaceae. *Steinernema feltiae* and *Steinernema carpocapsae* are closely linked via cospeciation with their symbiotic bacteria, *X. bovenii* and *X. nematophila*, respectively (Boemare 2002, Stock 2015).

Entomopathogenic nematodes in the non-feeding infective juvenile stage will search for a possible insect host using a variety of cues, which have yet to be completely

defined (Dowds & Peters 2002). Studies have shown that entomopathogenic nematodes use CO_2 gradient as well as electromagnetic waves to locate their hosts, along with a myriad of other cues (Lewis et al. 1993, Ilan et al. 2013). Once entering the host through any natural opening or wound, the nematodes have to overcome the insect's immune system. The immune system can rapidly synthesize and send lysozymes or antibacterial peptides to fight nematode or bacterial infection. Often the insect's immune system will attempt to send haemocytes to the site of infection, so that the nematode/bacteria can be stopped via phagocytosis. If this attempt fails, the haemocytes will aggregate in a process called nodulation, in which they surround the infecting body and then melanize, preventing further attack. Many entomopathogenic nematodes are known to secrete proteins that suppress immune response. This allows them to reside in the insect long enough to cause an infection that the immune system is unable to overcome. *Xenorhabdus nematophila* bacteria, which are associated with *Steinernema carpocapsae*, are known to adhere to and kill haemocytes as soon as they are released (Forst & Clarke 2002). In the case of *Galleria mellonella* (L.) [Lepidoptera:Pyralidae], the insect has a lack of non-self recognition, preventing encapsulation and any further immune responses (Dowds & Peters 2002, Forst & Clarke 2002).

Once inside the insect haemocoel, the infective juvenile will regurgitate a culture of *Xenorhabdus* bacteria into the hemocoel of the insect. If the insect's immune system suppresses the infection at this point, some *Xenorhabdus* and *Photorhabdus* will multiply inside the nodules created by the insect's immune system. The nematode and bacteria can then overpower the haemocytes, break out of the nodule, and re-enter the haemolymph. As the bacteria grow and reproduce, the insect dies of septicemia, usually within 24–48

hours (Dowds & Peters 2002, Forst & Clarke 2002). The antimycotic and antibacterial properties of *Xenorhabdus* shield the insect from colonization by other decomposers. The bacteria consume the insect rather quickly and the increasing population of *Xenorhabdus* is the now third stage juvenile nematodes' food source.

Once ready, the nematodes will molt into fourth stage adults. Unlike some nematode species, *Steinernema* is not hermaphroditic therefore both genders need to be present for reproduction. Within the insect, the nematodes are now able to reproduce for several generations within the insect until the resources are depleted and the insect's cuticle bursts. The infective juveniles then exit the resource-depleted cadaver and enter the surrounding environment where they begin searching for a new host (Dowds & Peters 2002).

Virulence against a particular insect varies among nematode strains within a species, and each nematode species is best adapted to occupy a different environment and soil niche. Many species of entomopathogenic nematodes are able to exist because of differing habitat and host preference (Ferguson et al. 1995, Hominick 2002). For instance, *Steinernema carpocapsae* prefers to occupy the soil surface (0-4 cm in depth), whereas *Steinernema feltiae* prefers depths of 4-14 cm. In the wild, *S. carpocapsae* is more often found in woodlands, while *S. feltiae* can most often be found in fields and grasslands. Host finding behavior also varies among nematode species. *S. carpocapsae* is an ambusher, meaning that it stands upright, a process called nictating, and waits for a host to appear. Cruisers are nematodes that travel through the soil actively looking for a host (Hominick 2002). *S. feltiae* possesses an intermediate behavior between ambusher and cruiser (Hominick 2002, Shields et al 2009). In addition to these traits, the ability to

infect a specific host varies by nematode species and strain. Some entomopathogenic nematodes have natural associations with insects that prevent the immune system from attacking the nematode, but also do not allow the insect to be killed when infected by that particular nematode species (Forst & Clarke 2002).

There is a long list of biotic and abiotic factors affecting the survival and virulence of nematodes in the soil. In addition, cultural and pest management practices in agriculture can impact nematodes heavily (Gaugler & Kaya 1990, Gouge et al. 2000, Foye et al.2016). Nematodes have such high reproductive rates because in natural conditions, there are multiple forces keeping their populations in check. Even then, once a nematode finds a host, it might actually take more than one nematode to effectively kill an insect. Furthermore, there are many nematode predators in the environment including but not limited to Protozoans, mites, fungi, other nematodes, and insects (Hominick 2002, Kaya 2002). Among the various soil characteristics that can affect nematode movement and survival, two of great importance are soil moisture and soil texture/structure (Wallace 1968, Gouge et al. 2000, Glazer 2002, Hominick 2002, Rijal et al, 2014, Atay & Kepenkci 2016). Nematodes need moisture to be able to move through the soil, but too much moisture will drown them. Gouge et al. (2000) found that the ideal moisture content for nematode host infectivity about 30%. Soil texture and structure also plays an important part in nematode survivorship because of its influence on soil water. A soil too high in clay content may hold too high a level of water and not give the nematodes adequate oxygen levels. A sandy soil drains too well and becomes too dry for nematodes to move around (Wallace 1968, Gaugler 1988a, Gaugler 1988b, Glazer 2002, Hominick 2002).

Biological Control

An ideal sustainable pest management program takes into account all factors contributing to the success of a cropping system and uses the most applicable techniques to form a comprehensive, multi-tactic plan for managing pests. It is a conscious effort from start to finish, incorporating cultural, biological, mechanical, and chemical control methods to reach the intended goal. This type of management plan is different from traditional pest management methods, which have involved spraying pesticides on a calendar-based schedule (Hoyt 1969, Agnello et al. 2003).

The research described in this thesis is part of an ongoing effort to augment the current pest management protocols in New York orchards, specifically through the use of biological control. Biological control is a method of pest control that involves utilization of the pest's natural enemies: predators, parasites, and parasitoids. Classical biological control involves importing non-native enemies. Currently, this is done if the pest itself is non-native, and after extensive research on potential ecological impact (Glass et al. 1976). Augmentative biocontrol is the practice of rearing native natural enemies and releasing these organisms en masse, in an attempt to boost the already existing population. Since classical biological control is understandably more ecologically risky, modern researchers often first look for native biological control agents (Van Driesche & Bellows 1996, Ovruski 2000).

While it is the ultimate goal to create a successful method of pest control that will keep the pest insect's population below the level of economic injury, pest management specialists understand that there may not always be one individual fix-all technique. In many situations, there are several techniques to be implemented throughout the growing

season. This is also true with biological control; the biological control agent may be just a piece of the overall scheme (Glass 1976, Agnello et al. 2003).

Since insecticides applied using an airblast sprayer can only reach insects aboveground and mainly in the canopy, it is only logical that we attempt to find a belowground method of control for soil-dwelling insects such as plum curculio (Glass 1976). Entomopathogenic nematodes are a good prospective underground insect control method because they can be mixed with water and applied with a boom sprayer; thus, they are compatible for tank mixing with many agricultural chemicals (Shields et al. 2009). Plum curculio was chosen as a good candidate for control with entomopathogenic nematodes because this insect spends its last larval instar, pupal, and adult overwintering stage in the soil. All of these stages have been found to be susceptible to nematode attack (Shapiro-Ilan et al. 2002, Kim & Alston 2008). The use of native entomopathogenic nematodes to control plum curculio would be considered augmentative biological control, an attempt to strengthen a native biological control agent (Hoy & Herzog 1984, Van Driesche & Bellows 1996). Economic control of plum curculio using entomopathogenic nematodes has yet to be accomplished, and long-term efficacy of entomopathogenic nematodes in orchard soil conditions remains unknown. However, preliminary laboratory data suggest that control may be possible using native nematode strains adapted to the environmental conditions of New York State (Agnello et al. 2014).

Research Focus

The goal of this study was to determine the virulence and persistence of *Steinernema carpocapsae* "NY-001" and *Steinernema feltiae* "NY-04" within the apple orchard system. These strains are specifically directed as a pest management technique against plum curculio (*Conotrachelus nenuphar*). This project focused on the following research questions: (1) Can *Steinernema feltiae* "NY-04" and *Steinernema carpocapsae* "NY-001" establish and persist in New York orchard soils? (2) How effective are these strains at causing plum curculio mortality? (3) What soil characteristics might influence this interaction?

This knowledge will allow crop protection specialists to gain insight on whether to include *Steinernema carpocapsae* "NY-001" and *Steinernema feltiae* "NY-04" in their pest management regime. This information is not only useful within the fruit tree system, but also may influence researchers in other commodities who are combatting pests with similar life histories.

References

Adams, B., and K. Nguyen. 2002. pp. 1-33. In Entomopathog. Nematol., CAB Int., New York, NY.

Agnello, A. M., W. H. Reissig, J. Kovach, and J. P. Nyrop. **2003**. Integrated apple pest management in New York State using predatory mites and selective pesticides. Agric., Ecosyst. & Environ. 94: 183–195.

Agnello, A., H. Reissig, and K. Cox. **2012**. Development and validation of a "Real-Time" Apple IPM Website for New York. IOBC/WPRS Bulletin. 74: 57–60.

Agnello, A., P. Jentsch, E. Shields, A. Testa, M. Keller. 2014. Evaluation of Persistent Entomopathogenic Nematodes for Biological Control of Plum Curculio. New York Frui Quarterly. 22(1): 21-24.

Akotsen-Mensah, C., R. T. Boozer, and H. Y. Fadamiro. **2011**. Field evaluation of reduced insecticide spray programs for managing plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae), in Alabama peaches. Pest Manag. Sci. 67: 626–632.

Akotsen-Mensah, C., R. T. Boozer, and H. Y. Fadamiro. **2012**. Influence of orchard weed management practices on soil dwelling stages of plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae). Florida Entomol. 95: 882–889.

Alston, D. G., D. E. N. Rangel, L. A. Lacey, H. G. Golez, J. J. Kim, and D. W. Roberts. **2005**. Evaluation of novel fungal and nematode isolates for control of *Conotrachelus nenuphar (*Coleoptera: Curculionidae) larvae. Biol. Control. 35: 163–171.

Atay, T., and I. Kepenkci. **2016**. Biological Control Potential of Turkish Entomopathogenic Nematodes Against Holotrichapion pullum (Gyllenhal) (Coleoptera, Apionidae). Egypt. J. Biol. Pest Control. 26: 7–10.

Boemare, N. 2002. pp. 35-56. In Entomopathog. Nematol., CAB Int., New York, NY.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14–19.

De Bach, P. 1964. Biological control of insect pests and weeds. 844.

Carter, M. R. 1990. Relative Measures of Soil Bulk Density to Characterize Compaction in Tillage Studies on Fine Sandy Loams. Can. J. Soil Sci. 70: 425–433.

(Compost increases the water holding capacity of droughty soils) MSU Extension. **2016**. Compost increases the water holding capacity of droughty soils. (http://msue.anr.msu.edu/news/compost_increases_the_water_holding_capacity_of_droughty_soils).

Delate, K., A. McKern, R. Turnbull, J. Walker, R. Volz, A. White, V. Bus, D. Rogers, L. Cole, N. How, S. Guernsey, and J. Johnston. **2010**. Latest trends in insect and disease management in organic apple systems in the Midwestern USA and New Zealand., pp. 243–252. *In* Prange, R.K., Bishop, S.D. (eds.), Acta Horticulturae. Intern. Soc. Hort. Sci. (ISHS), Leuven, Belgium.

Downing, A. S. 1994. Effect of Irrigation and Spray Volume on Efficacy of Entomopathogenic Nematodes (Rhabditida: Heterorhabditidae) Against White Grubs (Coleoptera: Scarabaeidae). J. Econ. Entomol. 87: 643–646.

Dowds, B., and A. Peters. 2002. pp. 79-98. In Entomopathog. Nematol., CAB Int., New York, NY.

Duncan, L. W., D. G. Dunn, and C. W. McCoy. **1996**. Spatial Patterns of Entomopathogenic Nematodes in Microcosms: Implications for Laboratory Experiments. J. Nematol. 28: 252–258.

Ferguson, C. S., P. C. Schroeder and E. J. Shields. 1995. Vertical distribution, persistence and activity of entomopathogenic nematodes (Nematoda: Heterorhabditidae and Steinernematidae) in alfalfa snout beetle (Coleoptera: Curculionidae) infested fields. Environ. Entomol. 24: 149-158

Forst, S., and D. Clarke. 2002. pp. 57-77. In Entomopathog. Nematol., CAB Int., New York, NY.

Foye, S. D., C. M. Greenwood, and K. E. Risser. **2016**. Virulence of Entomopathogenic Nematodes Native to Western Oklahoma Against Diorhabda Carinulata (faldermann, 1837) (Coleoptera: Chrysomelidae). Coleopt. Bull. 70: 149–152.

Gaugler, R. 1988a. Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agric., Ecosys. & Environ., Proc of a Workshop on Interactions Between Soil-Inhabiting Invertebrates and Microorganisms in Relation to Plant Growth. 24: 351–360.

Gaugler, R. 1988b. Proceedings of a Workshop on Interactions Between Soil-Inhabiting Invertebrates and Microorganisms in Relation to Plant Growth Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agric., Ecosys. & Environ. 24: 351–360.

Gaugler, R. 2002. Entomopathog. Nematol. CABI.

Gaugler, R., and H. Kaya. **1990**. Gaugler, R. & Kaya, H.K. (Eds). 1990. "Entomopathogenic Nematodes in Biological Control." CRC Press. 365 pp. ResearchGate.

Glass, E. H. 1976. Pest Management: Principles and Philosophy, pp. 39–50. *In* Apple, J.L., Smith, R.F. (eds.), Integr. Pest Manag. Springer US.

Glazer, I. 2002. pp. 168-187. In Entomopathog. Nematol., CAB Int., New York, NY.

Good Fruit Grower. 2016. New York apple industry by numbers. (http://www.goodfruit.com/new-york-apple-industry-by-numbers/).

Gouge, D. H., K. A. Smith, L. L. Lee, and T. J. Henneberry. **2000**. Effect of Soil Depth and Moisture on the Vertical Distribution of Steinernema riobrave (Nematoda: Steinernematidae). J. Nematol. 32: 223–228.

Hoffmann, E. J., J. VanderJagt, and M. E. Whalon. **2007**. Pyriproxyfen activates reproduction in prediapause northern strain plum curculio (*Conotrachelus nenuphar* Herbst). Pest Manag. Sci. 63: 835–840.

Hominick, W. 2002. pp. 115-143. In Entomopathog. Nematol., CAB Int., New York, NY.

Hoy, M. 2012. Biology Control in Agriculture IPM System. Elsevier.

Hoyt, S. C. 1969. Integrated Chemical Control of Insects and Biological Control of Mites on Apple in Washington. J. Econ. Entomol. 62: 74–86.

Ilan, T., D. B. Kim-Shapiro, C. H. Bock, and D. I. Shapiro-Ilan. **2013**. Magnetic and electric fields induce directional responses in *Steinernema carpocapsae*. Int. J Parasitol. 43: 781–784.

Ishibashi, N., and E. Kondo. **1987**. Dynamics of the Entomogenous Nematode Steinernema feltiae Applied to Soil with and without Nematicide Treatment. J Nematol. 19: 404–412.

Johnson, D. T., B. Lewis, C. R. Rom, H. Friedrich, R. Bryant, and M. Pszczolkowski. **2010**. Organic fruit production needs and pest management practices in the southeastern United States., pp. 37–44. *In* Prange, R.K., Bishop, S.D. (eds.), Acta Horticulturae. International Society for Horticultural Science (ISHS), Leuven, Belgium.

Jones, F. G. W., D. W. Larbey, and D. M. Parrott. **1969**. The influence of soil structure and moisture on nematodes, especially *Xiphinema, Longidorus, Trichodorus and Heterodera* spp. Soil Biol. Biochem. 1: 153–165.

Jones, V. P., S. A. Steffan, L. A. Hull, J. F. Brunner, and D. J. Biddinger. **2010**. Effects of the Loss of Organophosphate Pesticides in the US: Opportunities and Needs to Improve IPM Programs. Outlooks Pest Manag. 21: 161–166.

Kaya, H. K., and R. Gaugler. **1993**. Entomopathogenic Nematodes. Annu. Rev. Entomol. 38: 181–206.

Kaya, H. 2002. pp. 189-203. In Entomopathog. Nematol., CAB Int., New York, NY.

Kim, H. G., and D. G. Alston. **2008**. Potential of two entomopathogenic nematodes for suppression of plum curculio (*Conotrachelus nenuphar*, Coleoptera: Curculionidae) life stages in northern climates. Environ. Entomol. 37: 1272–1279.

Koppenhöfer, A. M., and E. M. Fuzy. **2006**. Effect of soil type on infectivity and persistence of the entomopathogenic nematodes *Steinernema scarabaei, Steinernema glaseri, Heterorhabditis zealandica,* and Heterorhabditis bacteriophora. J. Invert. Pathol. 92: 11–22.

Kung, S.-P., R. Gaugler, and H. K. Kaya. **1990**. Soil type and entomopathogenic nematode persistence. J. Invert. Pathol. 55: 401–406.

Kung, S.-P., R. Gaugler, and H. K. Kaya. **1991**. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. J. Invert. Pathol. 57: 242–249.

Lacey, L. A., and R. Georgis. **2012**. Entomopathogenic Nematodes for Control of Insect Pests Above and Below Ground with Comments on Commercial Production. J Nematol. 44: 218–225.

Leskey, T. C., V. Hock, G. Chouinard, D. Cormier, K. Leahy, D. Cooley, A. Tuttle, A. Eaton, and A. Zhang. **2014**. Evaluating Electrophysiological and Behavioral Responses to Volatiles for Improvement of Odor-Baited Trap Tree Management of *Conotrachelus nenuphar* (Coleoptera: Curculionidae). Environ. Entomol. 43: 753–761.

Leskey, T. C., S. E. Wright, J. Saguez, and C. Vincent. **2013**. Impact of insecticide and fungicide residue contact on plum curculio, *Conotrachelus nenuphar* (Herbst), mobility and mortality: implications for pest management. Pest Manag. Sci. 69: 464–470.

Lewis, E. E., R. Gaugler, and R. Harrison. **1993**. Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. Can. J. Zool. 71: 765–769.

New York Environment Report. **2016**. New York Ranks High in Apple, Grape Production. (http://www.nyenvironmentreport.com/new-york-ranks-high-in-apple-grape-production/).

NY Apple Association. **2016**. NY Apple Industry Facts - NY Apple Association. (http://www.nyapplecountry.com/about/facts).

Ovruski, S., M. Aluja, J. Sivinski, and R. Wharton. **2000**. Hymenopteran Parasitoids on Fruit-infesting Tephritidae (Diptera) in Latin America and the Southern United States: Diversity, Distribution, Taxonomic Status and their use in Fruit Fly Biological Control. Integ. Pest Manag. Reviews. 5: 81–107.

Pereault, R. J., M. E. Whalon, and D. G. Alston. **2009**. Field Efficacy of Entomopathogenic Fungi and Nematodes Targeting Caged Last-Instar Plum Curculio (Coleoptera: Curculionidae) in Michigan Cherry and Apple Orchards. Environ. Entomol. 38: 1126–1134.

Piñero, J. C., A. M. Agnello, A. Tuttle, T. C. Leskey, H. Faubert, G. Koehler, L. Los, G. Morin, K. Leahy, D. R. Cooley, and R. J. Prokopy. **2011**. Effectiveness of Odor-Baited Trap Trees for Plum Curculio (Coleoptera: Curculionidae) Monitoring in Commercial Apple Orchards in the Northeast. J. Econ. Entomol. 104: 1613–1621.

Quaintance, A. L., and E. L. Jenne. **1912**. The Plum Curculio. U.S. Department of Agriculture, Bureau of Entomology.

R Core Team. 2014. R: A language and environment for statistical computing. R. Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/.)

Racette, G., G. Chouinard, C. Vincent, and S. B. Hill. **1992**. Ecology and management of plum curculio, *Conotrachelus nenuphar* [Coleoptera:Curculionidae], in apple orchards. Phytoprotection. 73: 85.

Rijal, J. P., C. C. Brewster, and J. C. Bergh. **2014**. Effects of Biotic and Abiotic Factors on Grape Root Borer (Lepidoptera: Sesiidae) Infestations in Commercial Vineyards in Virginia. Environ. Entomol. 43: 1198–1208.

Schroeder, W. J., and J. B. Beavers. **1987**. Movement of the Entomogenous Nematodes of the Families Heterorhabditidae and Steinernematidae in Soil. J Nematol. 19: 257–259.

Selby, R. D., S. H. Gage, and M. E. Whalon. **2014**. Precise and low-cost monitoring of plum curculio (Coleoptera: Curculionidae) pest activity in pyramid traps with cameras. Environ. Entomol. 43: 421–431.

Shapiro-Ilan, D. I., T. C. Leskey, and S. E. Wright. **2011**. Virulence of Entomopathogenic Nematodes to Plum Curculio, *Conotrachelus nenuphar*: Effects of Strain, Temperature, and Soil Type. J. Nematol. 43: 187–195.

Shapiro-Ilan, D. I., R. F. Mizell, and J. F. Campbell. **2002a**. Susceptibility of the Plum Curculio, *Conotrachelus nenuphar*, to Entomopathogenic Nematodes. J Nematol. 34: 246–249.

Shapiro-Ilan, D., D. Gouge, A. Koppenhöffer. 2002b. pp. 189-203. In Entomopathog. Nematol., CAB Int., New York, NY.

Shapiro-Ilan, D. I., S. E. Wright, A. F. Tuttle, D. R. Cooley, and T. C. Leskey. **2013**. Using entomopathogenic nematodes for biological control of plum curculio, *Conotrachelus nenuphar*: effects of irrigation and species in apple orchards. Biol Control. 67: 123–129.

Shetlar, D. J., P. E. Suleman, and R. Georgis. **1988**. Irrigation and Use of Entomogenous Nematodes, Neoaplectana spp. and Heterorhabditis heliothidis (Rhabditida: Steinernematidae and Heterorhabditidae), for Control of Japanese Beetle (Coleoptera: Scarabaeidae) Grubs in Turfgrass. J. Econ. Entomol. 81: 1318–1322.

Shields, E. J., A. Testa, G. Neumann, K. L. Flanders, and P. C. Schroeder. **2009**. Biological Control of Alfalfa Snout Beetle with a multi-species application of locally adapted persistent entomopathogenic nematodes: The first success. A. Entomol. 55: 250–257.

Stock, P. S. 2015. Ch 1 - Diversity, Biology and Evolutionary Relationships. In: R. Campos-Herrera (ed). Nematode pathogenesis of insects and other pests – ecology and applied technologies for sustainable plant and crop protection, Series: Sustainability in Plant and Crop Protection, Vol. 1 (R. Campos-Herrera ed). Pp. 3-28.

Van Driesche, R. V., and T. S. Bellows, Jr. **1996**. Biological Control. Springer Science & Business Media.

Vincent, C., G. Chouinard, and T. Leskey. **2008**. Plum Curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae), pp. 2947–2953. *In* Capinera, J.L. (ed.), Encyclopedia of Entomology. Springer Netherlands.

Wallace, H. R. 1968. The Dynamics of Nematode Movement. Annual Review of Phytopathology. 6: 91–114.

White, G. F. 1927. A Method for obtaining Infective Nematode Larvae from Cultures. American Association for the Advancement of Science. Science. 66: 302–303 pp.

Wise, J. C., K. Kim, E. J. Hoffmann, C. Vandervoort, A. Gökçe, and M. E. Whalon. **2007**. Novel life stage targets against plum curculio, *Conotrachelus nenuphar* (Herbst), in apple integrated pest management. Pest. Manag. Sci. 63: 737–742.

CHAPTER 2:

EVALUATION OF NATIVE ENTOMOPATHOGENIC NEMATODES FOR BIOLOGICAL CONTROL OF PLUM CURCULIO (*Conotrachelus nenuphar* [Herbst]) IN NEW YORK APPLE ORCHARDS

Introduction

While New York's main agricultural product is dairy, the state ranks second in the United States in apple production (Good Fruit Grower 2016; NY Apple Assoc. 2016). Over the past several years, there has been a growing interest in producing apples for the fresh market. However, in fresh fruit, a minor cosmetic defect can send the apple to processing rather than the fresh market, which greatly reduces its monetary value.

Plum curculio [*Conotrachelus nenuphar* (Herbst)] is a common weevil pest in tree fruit systems east of the Rocky Mountains. Adult plum curculio damage the fruit by feeding, which can create puncture scars on the apple skin as the fruit expands. Additionally, adult females cut a slit in the skin of the developing fruit and lay their eggs inside it. In apple, if the fruit aborts, the eggs can continue their development within the dropped fruit. However, when the apple remains on the tree continuing to grow, the pressure from natural fruit expansion crushes the eggs. A fan-shaped scar remains on the apple skin, cosmetically preventing it from sale in the fresh market. In heavily infested areas, there can be several scars per apple (Racette et al. 1992). Historically this pest has been suppressed by several regularly scheduled insecticide applications. Using insecticides has been found to be a rather impractical control method for this highly evasive pest, and, due to increasing pesticide restrictions, researchers have sought alternative control methods (Agnello et al. 2014).

Entomopathogenic nematodes have had a place in the biological control realm for many years, and are known to be generalist insect predators. Two native nematode strains, *Steinernema carpocapsae* "NY-001", and *Steinernema feltiae* "NY-04" have shown promise for control of plum curculio and other insects (Shields & Testa 2009, Agnello et al. 2014). Both species have been found to be persistent in New York soils for several years. Preliminary laboratory studies have shown that these nematode strains are virulent against plum curculio to varying degrees, dependent upon the insect's life stage (Agnello et al. 2014). In this study, we hope to characterize the orchard conditions conducive to nematode establishment and efficacy against plum curculio.

Materials and Methods

Site Selection

Four field sites were selected in Geneva, New York, based on history of apple production and incidence of plum curculio damage. Three blocks hereafter referred to as "Empire," "IdaRed," and "Loomis", are located at the New York State Agricultural Experiment Station (NYSAES) in Geneva; the fourth, the "Davies" farm is owned and operated by Red Jacket Orchards, Geneva, NY. The "Loomis" farm is managed according to USDA Certified Organic standards, and prior to the 2013 season, the "Davies" block was managed organically as well. The "IdaRed" and "Empire" blocks have a history of being managed conventionally. The Loomis farm is primarily Collamer silt loam, while Empire, IdaRed, and Davies are all composed of Lima loam and Honeoye loam soils (Table 1). All of the sites were bordered by unmanaged fields and woodlands on at least one side (Appendices I-IV).

Field Inoculation

On May 30, 2012, selected grassy aisles at the IdaRed and Empire blocks at the NYSAES Research North farm were inoculated with *S. carpocapsae* and *S. feltiae* using a boom sprayer mounted on an ATV. The ATV was equipped with a 25 gallon (94.6 L) tank and 0008 fertilizer stream nozzles. The application rate was equivalent to 450 million infective juveniles per acre, which has been shown to be an effective rate for achieving control of soil-dwelling insects (Shetlar et al. 1988, Shields et al. 2009, Agnello et al. 2014). On May 6, 2013, the NYSAES Loomis and Red Jacket Davies farm were inoculated following the same procedure.

Field Bioassays

Field bioassays to evaluate nematode establishment and infectivity were conducted during the 2014 and 2015 growing seasons. At each of the four sites, acrylic tubes [15.2 cm L X 10.2 cm diam] were driven into the soil to a depth of about 8 cm, allowing for a 2.5-cm portion of the tube to remain aboveground to create an arena for field bioassays. These arenas were created with 10 replicates per treatment. There were three treatments: sprayer inoculated, hand inoculated, and untreated. Spray inoculation was as described above, using an ATV-mounted boom sprayer. Hand inoculation was done to allow us to deliberately place the nematodes in the arenas and also to newly inoculate the given area, as well as, by comparison, to evaluate the effectiveness of the spray inoculation. Rows selected for hand-inoculated arenas were known to be free of entomopathogenic nematodes. The hand-inoculated arenas were treated in June of 2014 and then again in June of 2015. This was accomplished by pouring in 50–100 mL of a solution with a pre-determined concentration of nematodes comparable to that in the field

inoculation (5,000–7,000 nematodes/arena).

In each of the 30 field arenas at each of the sites, 10 last-instar plum curculio larvae were placed on the soil surface and allowed to tunnel into the soil. The plum curculio larvae were from a non-diapausing laboratory colony maintained at NYSAES since 2012. Next, a USDA Cotton Boll Weevil-style emergence trap top (Great Lakes IPM, Vestaburg, MI) was fitted to each arena. Each emergence trap contained a removable transparent sight-glass top so that emerging plum curculio adults could be collected and counted.

Laboratory Bioassays

Soil Columns

Intact soil columns were collected during both the 2014 and 2015 growing seasons, and used to conduct laboratory trials during the off-season. The soil columns were collected to maintain the integrity of the orchard soil profile as much as possible. The columns were taken from the orchard and housed within white opaque PVC tubes, 15.2 cm L X 10.2 cm diam. The tubes were beveled on one end using a belt sander to aid in inserting them into the soil.

At each of the four sites, we collected intact soil columns by driving beveled PVC tubes into the soil within the rows, until 2.5 cm of tubing remained aboveground. The tubes containing intact soil monoliths were then excavated from the soil using a shovel. This allowed us to maintain an accurate representation of the soil profile while minimizing disturbance. A piece of fiberglass screen was fitted across the bottom of each column and secured in place with a plastic cable tie to contain the sample. Once secured, all columns were then transferred to the lab for bioassays.

Sieved Soil

In addition to the intact columns, soil was collected from each of the four sites to create similar-sized mesocosms with sieved soil, in an attempt to remove soil "macrostructure" (e.g. cracks, rocks, large pieces of organic matter) as a factor. Soil was collected from several points in each orchard, to a depth of approximately 8–10 cm, using a shovel. The soil was then sieved at 6 mm, air-dried, and put into 1025-mL plastic deli cups (Fabri-Kal, Kalamazoo, MI) to be used for additional laboratory bioassays. Each of the cup bottoms was punctured with drainage holes using a dissecting probe to minimize water pooling.

Laboratory Soil Conditions

For each field site, newly collected soil columns were sorted into groups (by site) of 15 and sieved cups were sorted into groups of 10. All soil samples were placed in plastic storage bins [50.8 cm L X 36.83 cm W X 15.24 cm H]. A set of 15 intact cores and 10 sieved soil cups were inoculated with each of the nematode treatments described below. Soil cores and sieved cups were kept on steel racks in randomized order inside two walk-in misting chambers set at 23° C. In these chambers, there was a constant mist produced so that the cores would be kept close to water holding field capacity. Additional holes were drilled into the bottom of each storage bin to allow excess water to drain.

Groups of 15 intact columns and 10 sieved cups from each site were used to conduct bioassays to test three different research questions. (1) How long can *S. carpocapsae* "NY-001" and *S. feltiae* "NY-04" persist in orchard soil? (2) Are *S. carpocapsae* "NY-001" and *S. feltiae* "NY-04" effective in reducing plum curculio numbers? (3) Which species (or combination) of nematode is most effective?

The Shields laboratory at Cornell University provided *Galleria mellonella* L. (Lepidoptera: Pyralidae) cadavers inoculated with infective juveniles of either *Steinernema carpocapsae* "NY-001" or *Steinernema feltiae* "NY-04". Fourteen days post-inoculation, the *G. mellonella* larvae had deteriorated and infective juveniles were starting to emerge from the *G. mellonella* cadavers. Nematode infective juveniles were rinsed (through a 4-mm sieve) from the deteriorating *G. mellonella* larvae with deionized water to prepare a nematode solution. A dissecting microscope was used to estimate the approximate number of nematodes per mL of solution, and then the required volume of solution to approximate a rate of 450 million $acre^{-1}$ was immediately pipetted into each soil column or sieved cup. The rates for the lab nematode inoculation were intended to approximately match the rate at which they would be applied in the field. Each replicate received approximately 5,000–7,000 nematodes, which is equivalent 7–10 infective juveniles per cm^3.

Laboratory Plum Curculio Survivorship

To estimate nematode-induced plum curculio mortality, it was necessary to determine normal plum curculio development and survivorship in untreated soil. Fifteen intact soil columns and 10 sieved cups from each site were each infested with 10 last-instar plum curculio larvae, and all of these arenas were fitted with boll weevil emergence trap tops. Plum curculio mortality was determined by comparing the number of plum curculio adults emerged with the original 10 plum curculio larvae introduced to each core.

Virulence of S. carpocapsae "NY-001" and S. feltiae "NY-04"

To determine the efficacy of our strains of entomopathogenic nematodes as a

biological control agent, we inoculated a set of 15 soil columns and a set of 10 sieved soil cups from each of the four field sites with entomopathogenic nematodes. After inoculation, all soil columns and sieved cups were held in the misting chambers at constant conditions (23°C, 16 hrs light: 8 hrs dark) for at least one week to allow the nematodes to acclimate.

In the first set of experiments, two sets of 15 intact soil cores and 10 sieved soil samples were inoculated for each site with both *S. carpocapsae* "NY-001" and *S. feltiae* "NY-04" to mimic field conditions. In addition, there was a similar set of sieved cups and intact cores left untreated to determine plum curculio survivorship in untreated soil. The experimental treatments were formulated to test: 1) nematode persistence, 2) nematode virulence against plum curculio, and 3) plum curculio survivorship in untreated soil. All experimental treatments were replicated (15 for intact columns and 10 for sieved soil cups) across soil samples from all of the four field sites.

In the second set of experiments, individual trials were conducted for each of the two species of nematodes individually, as well as in a combined trial. Sets of 15 intact soil columns and sets of 10 sieved deli cup mesocosms were inoculated with *S. carpocapsae* "NY-001", *S. feltiae* "NY-04", and both species combined, mimicking the conditions in the field trials. One set for each site was left untreated. Experimental treatments (7) were administered to test: 1-3) persistence of *S. carpocapsae, S. feltiae*, and *S. carpocapsae/S. feltiae*; 4-6) virulence of *S. carpocapsae*, *S. feltiae,* and *S. carpocapsae/S. feltiae*; and 7) plum curculio survivorship in untreated soil. All experimental treatments were replicated across all of the four field sites.

After inoculation, nematode establishment in each soil column and sieved cup

was confirmed by exposing *G. mellonella* larvae to the laboratory soil samples, and then placing the dead larvae on white traps (White 1927). Nematode infective juvenile emergence from the insect cadavers verified successful nematode establishment.

White traps consist of a petri dish (10 cm diam) containing a plaster disk (~5 cm diam). The disk is soaked with deionized water and the petri dish is also filled with water. Within the white trap, the *Galleria mellonella* cadavers are placed upon the plaster disk and kept moist until infective juvenile entomopathogenic nematodes are ready to emerge and can be observed under a dissecting microscope, usually after 6–7 days (White 1928).

Each of the soil columns and sieved soil samples were then infested with 10 last-instar plum curculio larvae, and an emergence trap top was attached. The trap tops were equipped with a removable transparent cap to allow newly emerged plum curculio adults to be collected and counted. Plum curculio mortality was estimated by comparing the number of emerged plum curculio adults with the number of larvae originally infested.

Laboratory Persistence of Entomopathogenic Nematodes in Orchard Soil

The newly inoculated soil samples were held in the misting chambers at 25°C for at least one week to allow the nematodes to acclimate and establish. After this holding period, we were able to begin bioassays. Nematode persistence was measured by quantifying nematode-caused *G. mellonella* mortality over time.

For year 1 bioassays, fiberglass screen cages approximately 8 cm (average plum curculio burrowing depth) in length and 1 cm in width, containing two *G. mellonella* wax worm larvae were inserted into a hole of corresponding size in each of 15 intact columns and 10 sieved cups from each of the four field sites. Only a single cage was used per soil column to test for *Steinernema* persistence.

Year 2 bioassays used screen cages 4 cm and 14 cm in length and approximately 1 cm in width, constructed using fiberglass screen and sealed with staples. One of the narrow ends of these cages was left open to allow the insertion of two *Galleria* larvae. In these bioassays, two cages per soil sample were used to test the persistence of the two nematode species separately. One cage was inserted to approximately 3.8 cm to check for *S. carpocapsae* and the second cage was inserted to ~ 12 cm to check for *S. feltiae.* The species that caused death was inferred based on the depth of the larva. In year 2, all of the soil samples were bioassayed at 2 months, and then again at 8 months. In the interim months, a subsample of 6 cores were bioassayed, in the interest of time and resources.

The cages were inserted into holes of corresponding sizes in the soil columns/sieved mesocosms, made using a cork borer. Air-dried sieved soil from the corresponding site was used to backfill the remaining space around the screen cages. The *Galleria* remained in the soil for 6–7 days before being removed and assessed for mortality. Dead *Galleria* larvae were moved to white traps to confirm nematode-caused death. In both trials this process was repeated approximately every 4 weeks over a period of 8 months to determine the length of nematode persistence in the given soil conditions.

Abiotic Soil Characteristics

Water Holding Capacity

In the spring of 2014, soil samples at each of the four fields were collected using an Oakfield probe (Oakfield Apparatus, Fond du Lac, WI) to a depth of 15 cm. Five samples were taken in a one square meter area, and this was repeated at 10 different points in each field. The samples for each square meter were combined, sieved at 6 mm, and oven-dried. The dried samples were then weighed out into 10-g subsamples. A

wooden rack held funnels (~5 cm diam) lined with a (9 cm diam) circular filter paper (VWR International, Radnor, PA), which were weighed dry, then soaked with deionized water and weighed. The soil was added to these funnels, saturated with deionized water, and covered with cellophane to prevent evaporation. The soil in the funnels was allowed to fully drain overnight and then weighed. The final wet mass and dry mass were used to calculate the water holding capacity for each sample.

Carbon and Nitrogen

During the winter of 2015, air-dried soil from the 2014 bioassays was ground using a roller grinder (Norton Plastics and Synthetics, Akron, OH) and then stored in 20-mL plastic scintillation vials. Ten samples from each site were chosen for analysis of carbon and nitrogen analysis (Costech Instruments Elemental Combustion System CHNS-O, Costech Analytical Technologies, Valencia, CA). Subsamples of 15–20 mg were weighed and placed into 5x9 mm pressed tin capsules (Costech Analytical Technologies). The analysis results allow the carbon (C) content, nitrogen (N) content, and the C:N ratio to be calculated using the elemental mass output in mg g^{-1} soil.

Laboratory Bioassay Water Content

A subset of the cores and mesocosms was destructively sampled to obtain core water content. Approximately 30 g of soil was sieved and weighed into drying tins. The soil was allowed to air dry for several days and then weighed again. Gravimetric water content of the cores was calculated by dividing the dry mass by the original mass (g) and is reported here as % of total sample mass (Appendix V).

Soil Texture

Soil samples were collected using the same method as the soil water holding

capacity samples described above. Soil was processed and analyzed using the hydrometer method according to the KBS LTER protocol (Robertson et al. 1999). Soil texture could then be calculated according to the formula provided in the protocol, using the results provided from the particle size analysis. The particle size analysis provided results in terms of sand, silt, and clay content.

Statistical Analysis

The field bioassay results were analyzed using a generalized linear model with logit links, with site and nematode treatment (hand, spray, and control) as factors. Pairwise comparisons were made using Tukey's adjustments for multiple comparisons. The sieved and intact soil samples in the laboratory bioassays were analyzed separately using a generalized linear model, with site and nematode species as factors. Each of the abiotic soil conditions were analyzed using a linear model with site as a factor followed by Tukey's tests for multiple comparisons. The laboratory nematode persistence trials were analyzed using a generalized linear model using pairwise comparisons between treatments at each time point (R Core Team 2014).

Results

Soil Characteristics

There was no difference in sand or silt content among the Davies, Empire, and IdaRed sites. However, Loomis had higher silt content and lower sand content (p=5.3x10^{-8}) than the other three sites (Table 1). Clay content was different among all sites except in the cases of Empire and Loomis (p=0.09) and Davies and IdaRed (p=0.99). The Empire site had a marginally higher water holding capacity than IdaRed (p=0.013), but no other

sites varied from one another. The nitrogen content results showed a strong site effect (p=3.3x10^{-6}), with the highest nitrogen content at the Loomis site. Nitrogen content at Davies was lower than Empire, but neither were different from the IdaRed site. The carbon analysis results also showed a strong effect of site (p=1.8x10^{-8}). The carbon content was highest at Loomis, and lowest at Davies. The Empire and IdaRed sites were not different from one another. The carbon:nitrogen results were also different among sites, and was highest at Loomis and lowest at Davies. (p=0.001) (Table 1).

Field Plum Curculio Bioassays

There was a strong effect of both site (p=5.6x10^{-5}) and treatment (p=8x10^{-5}) in the 2014 field trials, as well as a site-by-treatment interaction (Figure 1; p=0.016). In 2014, plum curculio emergence in the spray-inoculated rows was not different from the untreated check rows at any site (p=0.861). Over all sites, the hand-inoculated treatment had the lowest plum curculio adult emergence (0.2 ± 0.02). The IdaRed site had higher plum curculio adult emergence than the other three sites, regardless of treatment.

In 2015, the field bioassays showed a strong effect of treatment (p=0.0005), site (p=1.84x10^{-9}), and site:treatment (Figure 2; p=0.0002). Plum curculio adult emergence in spray-inoculated rows was higher than both the hand-inoculated and untreated check rows (0.29 ± 0.023). Overall, there was no difference between the hand-inoculated treatment and the untreated check rows. Davies site had overall higher adult plum curculio emergence than the other three sites, regardless of treatment (0.36 ± 0.03). There were no differences in emergence among IdaRed, Empire, and Loomis (p=5.9x 10^{-5}).

Laboratory Plum Curculio Bioassays

In the 2014 laboratory studies there was no difference in plum curculio emergence between *S. carpocapsae*/*S. feltiae*-inoculated soil and the untreated soil (Figure 3, p=0.1747). Since there were no sieved cup trials during this year we were unable to compare emergence with regards to soil macrostructure.

Analysis showed a strong effect of nematode species (Figure 4, $p=3x10^{-12}$) and a strong site effect in 2015 (Figure 4, $p=2.8x10^{-6}$). Soil collected from the Loomis site provided lower plum curculio emergence (0.17 ± 0.02) than the other sites (Davies: 0.36 ± 0.03, Empire: 0.21 ± 0.02, IdaRed: 0.14 ± 0.02) in both sieved and intact soil (p<0.0001). There were no other differences among sites. Overall, regardless of soil macrostructure, in 2015, the plum curculio emergence from the *S. feltiae* "NY-04" inoculated soil samples (0.26 ± 0.01) was not different from the soil inoculated with both nematode species (0.27 ±0.01, p=0.7763). The plum curculio adult emergence for these trials (0.233 ± 0.014, 0.254 ± 0.016, respectively) was much lower than in the *S. carpocapsae* treated soil (0.423 ± 0.016). The *S. carpocapsae* treated soil's (0.41 ±0.02) adult plum curculio emergence was not different from the untreated soil (0.41 ± 0.02, p=0.977). The same was found when looking at the sieved soil mesocosms separately (Figure 4).

Entomopathogenic Nematode Persistence Bioassays

The nematode persistence bioassays (Figures 5, 6) showed that nematodes were indeed present in our soil post-inoculation, and that they can persist under controlled temperature and moisture for at least 8 months. Within the intact soil columns, there was a significant effect of nematode species (p=0.002, Figure 7a). Overall, the inoculation of

both nematode species led to higher *G. mellonella* mortality than *S. carpocapsae* alone (p=0.0087). There was also an effect of time, and site by time ($p<2.2 \times 10^{-16}$, 4.6×10^{-6}, respectively). Specifically, at 2 months, the Empire site had significantly higher *G. mellonella* mortality than at the Loomis site (p=0.0011).

In the sieved soil trials, there was a strong interaction effect of time and nematode species (p=0.0002 and $<2.2 \times 10^{-16}$, respectively, Figure 7b). At the 2 month time point, the soil inoculated with both nematode species showed significantly higher *G. mellonella* mortality than the soil inoculated with *S. carpocapsae* (p=0.0001) or *S. feltiae* alone (p=0.0163). At 3 months, again, the soil inoculated with both nematode species, or with *S. feltiae* alone, showed higher mortality rates than soils inoculated with *S. carpocapsae* alone (p=0.0606). At 5 months, the combined nematode species caused higher mortality than *S. carpocapsae* alone (p=0.0045).

Table 1. Abiotic soil characteristics among the four field sites

Characteristic	Davies	Empire	IdaRed	Loomis
Water Holding Capacity (%)	50.96 (±2.3) ab	57.95 (±2.3) b	47.48 (±2.3) a	55.55 (±2.3) ab
Carbon: Nitrogen	8.45 (±0.36) a	10.17 (±0.36) bc	8.9 (±0.4) ab	10.4 (±0.38) c
Nitrogen Content (%)	0.0013 (±9.2*10^-5) a	0.0017 (±9.2*10^-5) b	0.0017 (±1*10-4) ab	0.0023 (±1*10-4) c
Carbon Content (%)	0.0091 (±0.0012) a	0.017 (±0.0012) b	0.015 (±0.0014) b	0.024 (±0.0014) c
Sand Content (%)	50.25 (±3.37) b	46 (±3.37) b	53.63 (±2.39) b	25.63 (±2.39) a
Silt Content (%)	36.5 (±3.29) a	34.75 (±3.29) a	32.75 (±2.32) a	52.78 (±2.32) b
Clay Content (%)	13.25 (±1.16) a	19.25 (±1.16) b	13.625 (±0.82) a	22.75 (±0.82) b
Soil Type [a]	Honeyoye Loam/ Lima Loam	Lima Loam	Honeyoye Loam/ Lima Loam	Collamer Silt Loam

Across sites, different letters indicate statistically significant differences ($p<0.05$)

[a] Soil type was obtained using Web Soil Survey (Soil Survey Staff, Natural Resource Conservation Service 2016)

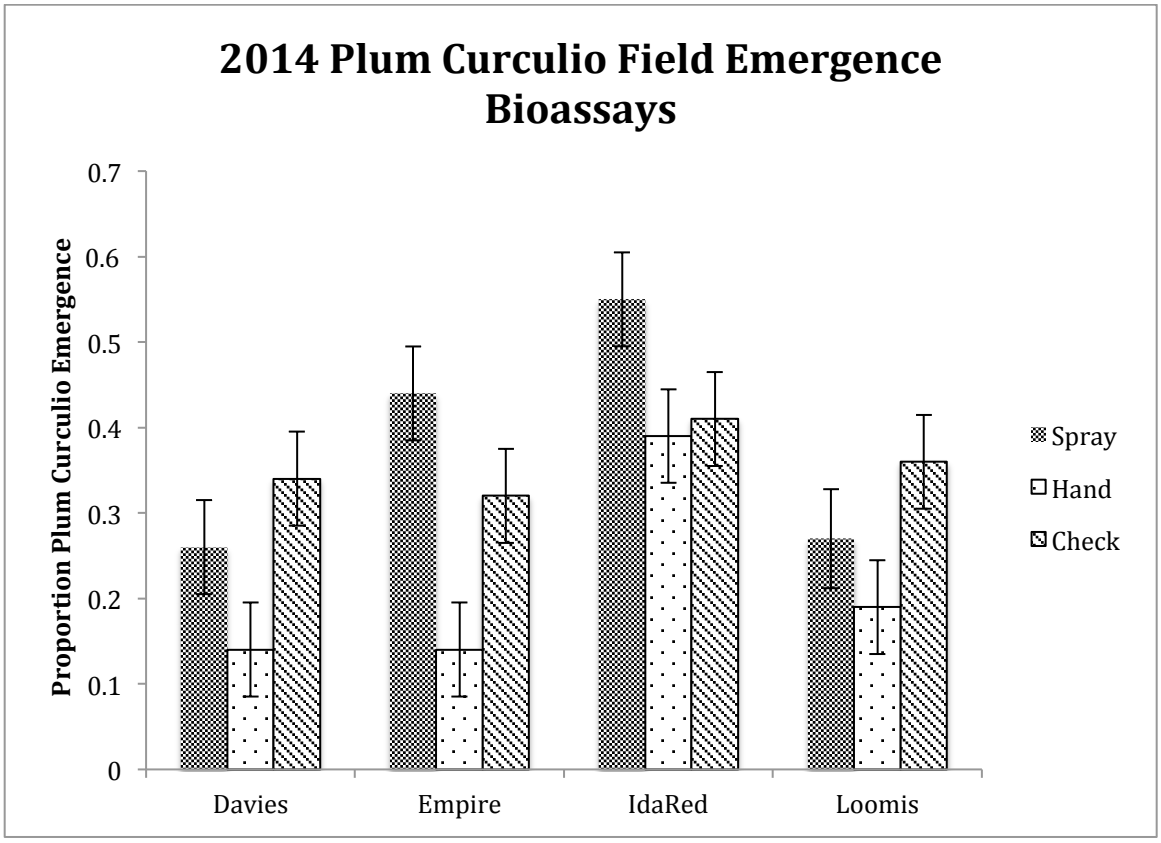


Figure 1. 2014 proportion of field plum curculio emergence in orchard rows untreated (Check), hand-inoculated (Hand), and sprayer-inoculated (Spray) with *Steinernema carpocapsae* "NY-001" strain and *Steinernema feltiae* "NY-04" strain, across four field sites

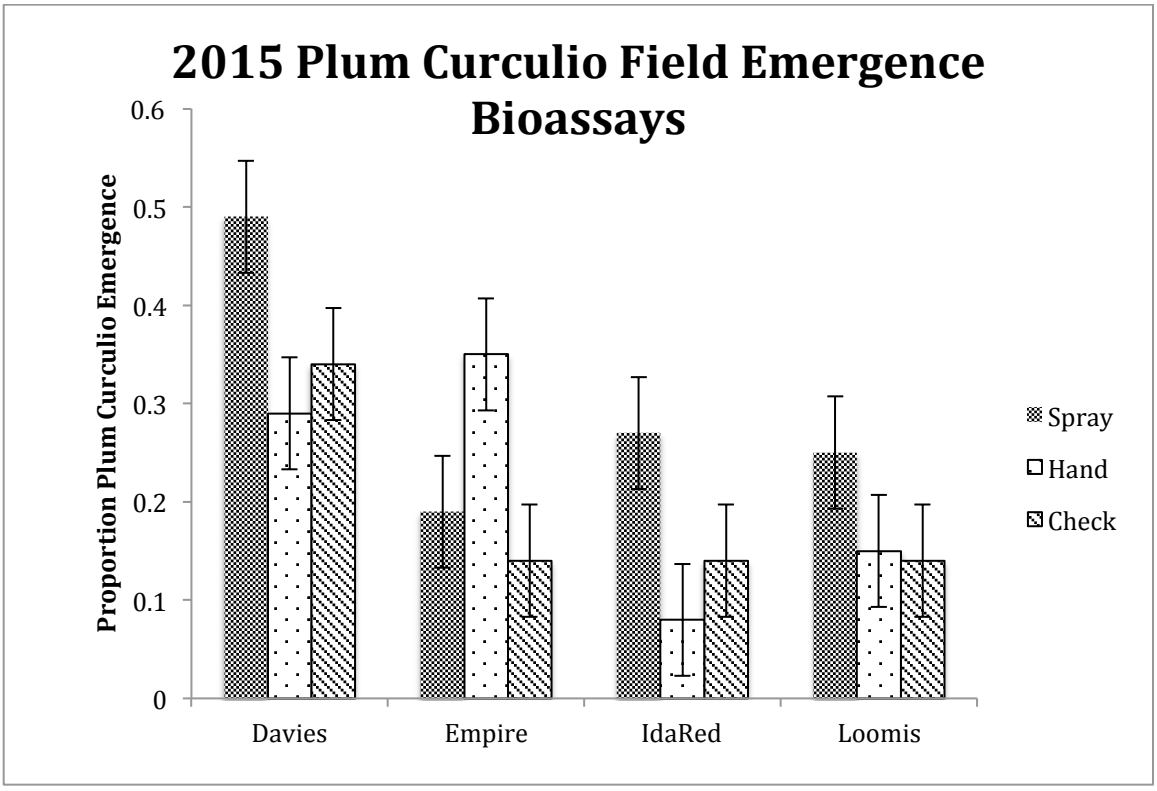


Figure 2. 2015 proportion of field plum curculio emergence in orchard rows untreated (Check), hand-inoculated (Hand), and spray-inoculated (Spray) with *Steinernema carpocapsae* NY 001 strain and *Steinernema feltiae* NY 04 strain, across four field sites

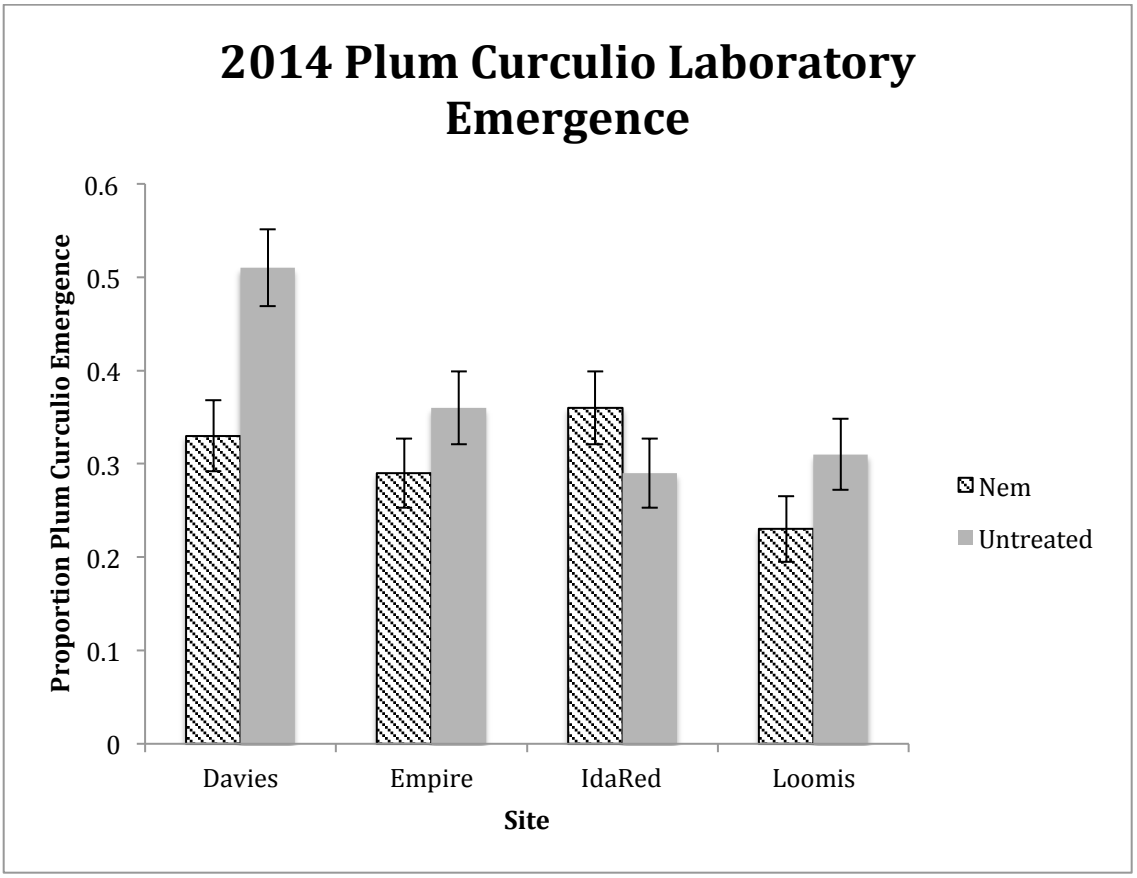


Figure 3. 2014 laboratory bioassays comparing plum curculio emergence in untreated intact soil columns with columns treated with *Steinernema carpocapsae* "NY-001" and *Steinernema feltiae* "NY-04" (Nem).

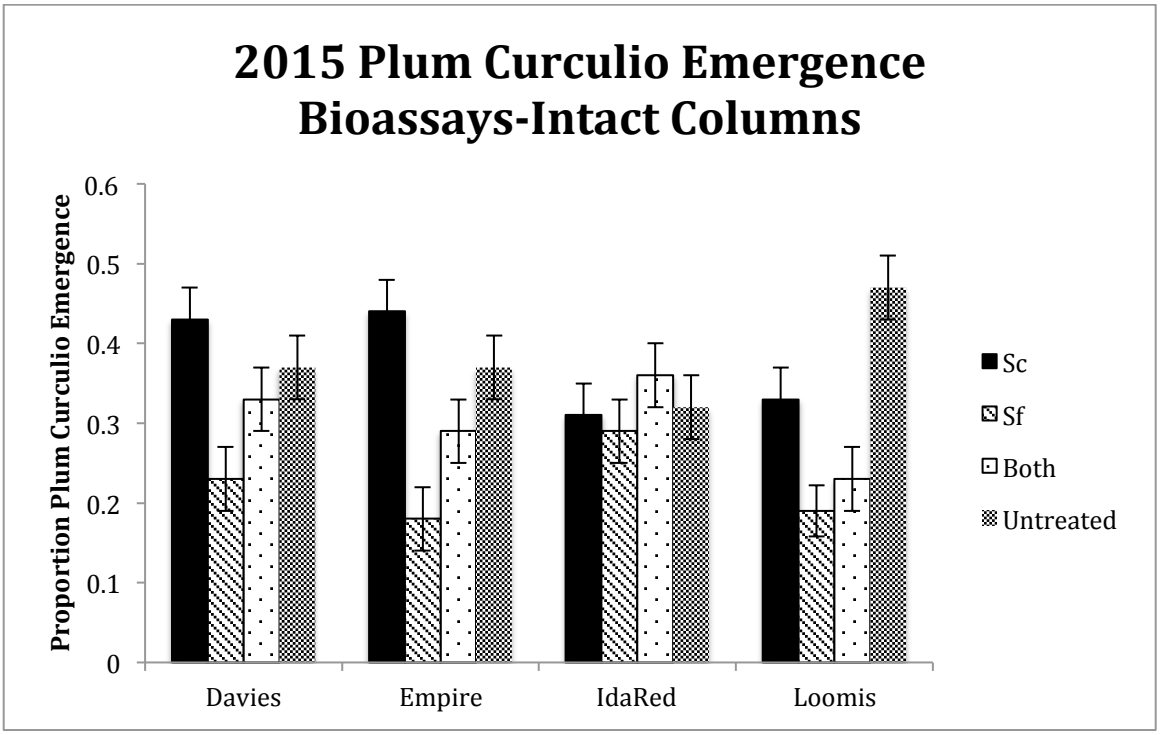


Figure 4a

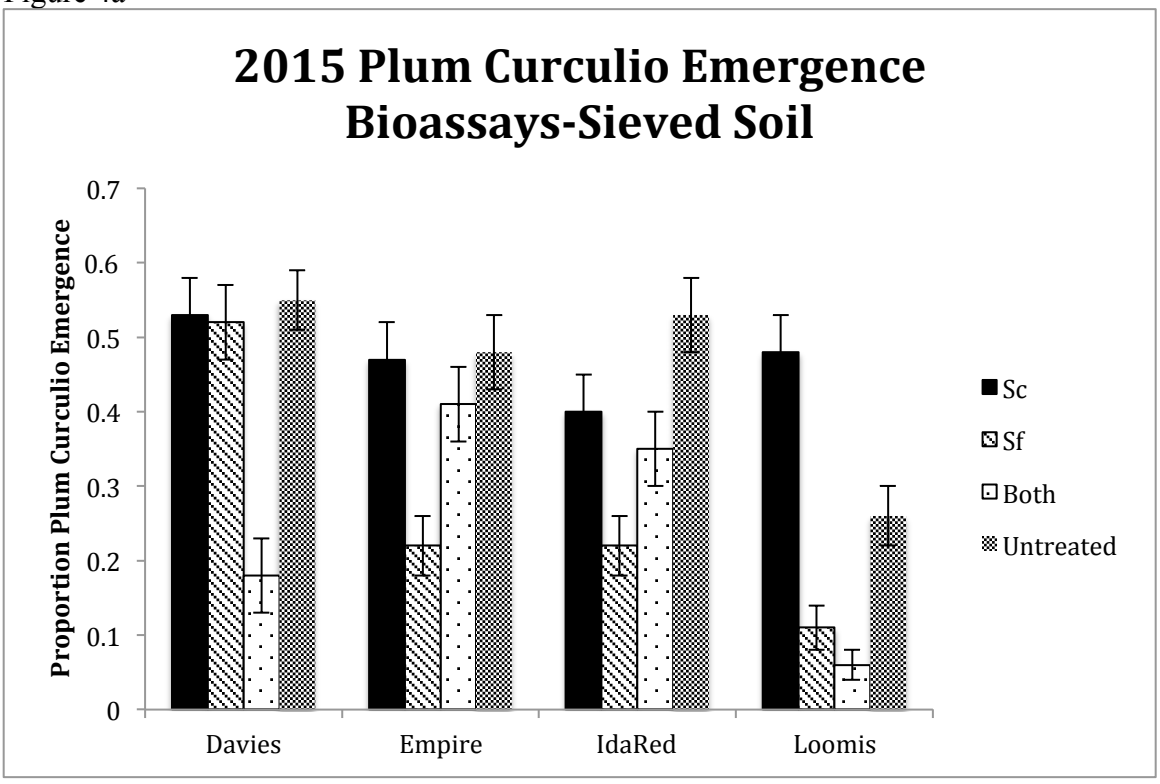


Figure 4b.

Figures 4a and 4b. Laboratory plum curculio emergence in untreated intact (4a) and sieved soil columns (4b) compared with columns treated with *Steinernema carpocapsae* "NY-001" strain and *Steinernema feltiae* "NY-04" alone (Sc or Sf), and in combination (Both)

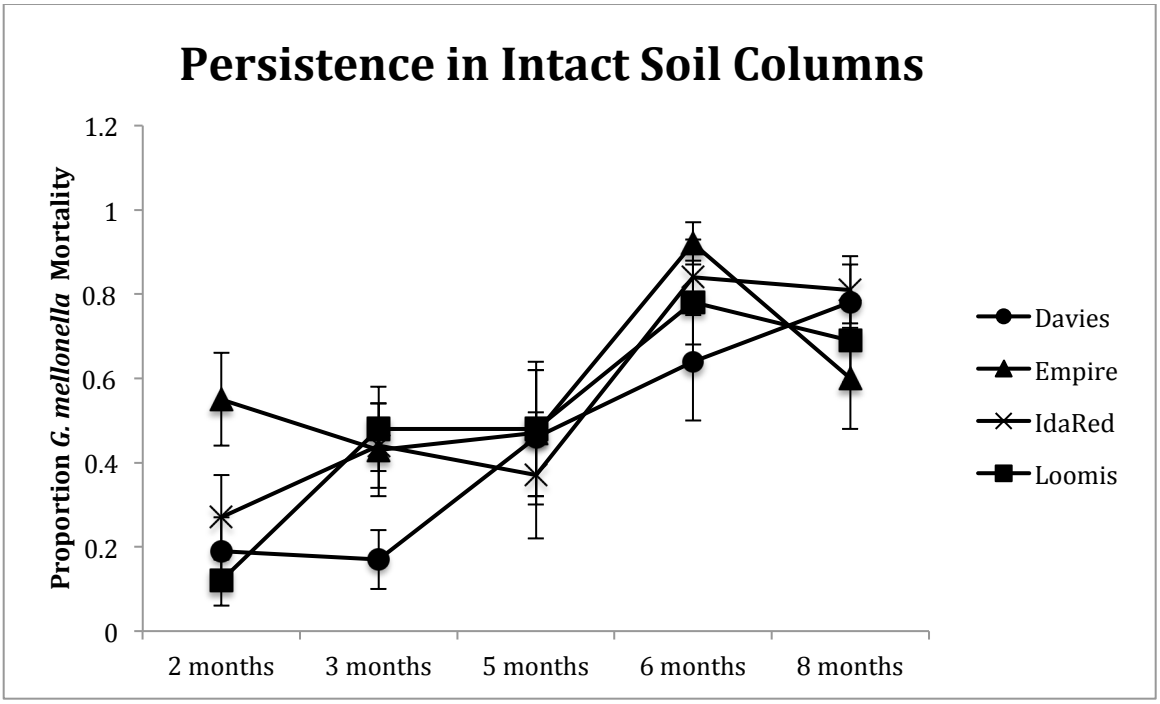


Figure 5a.

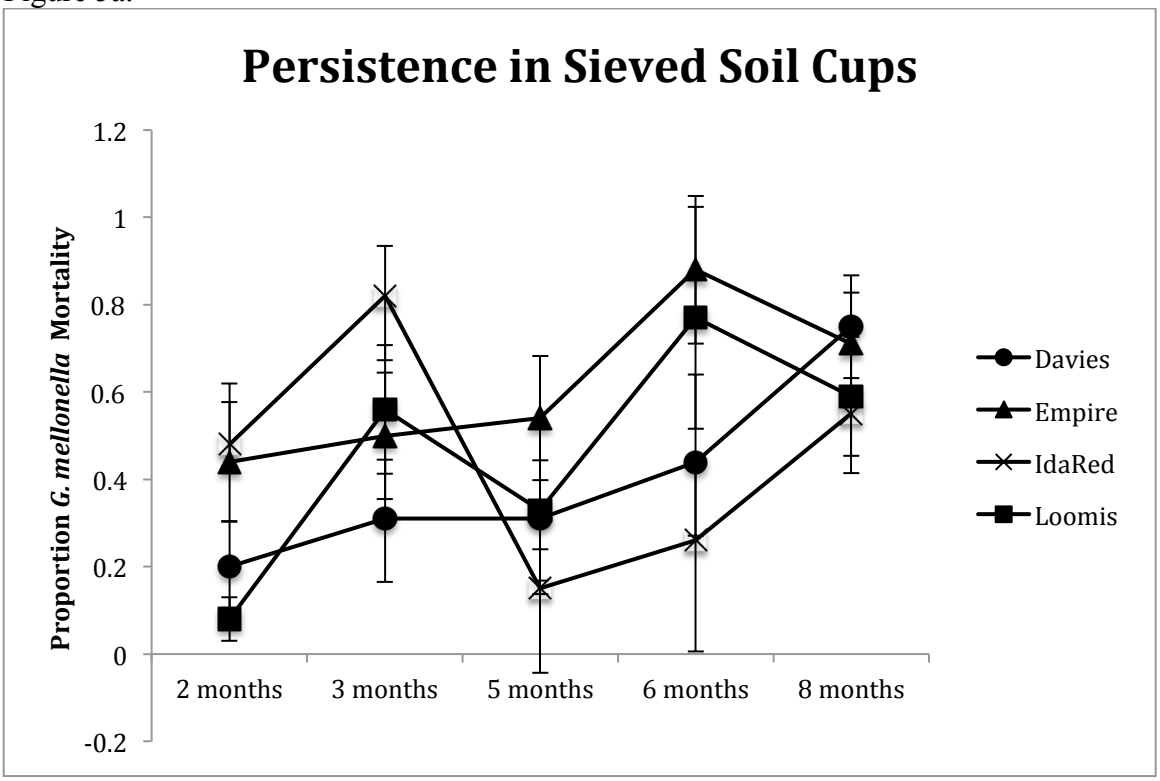
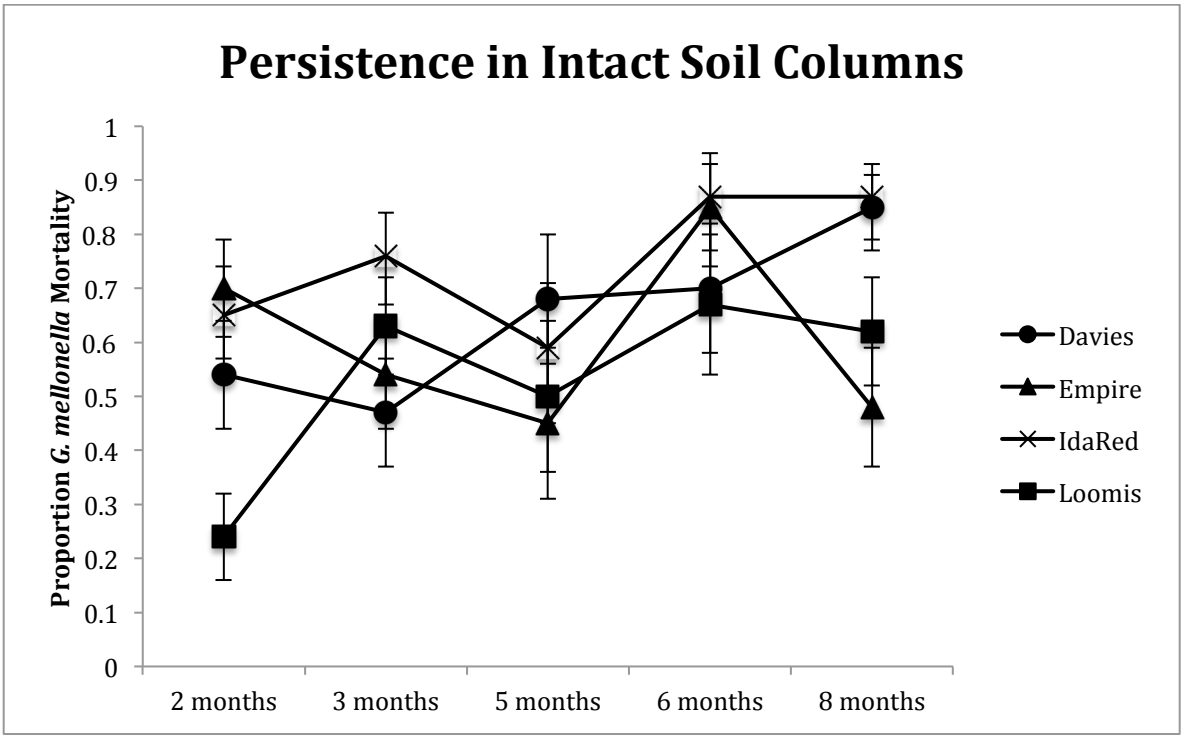


Figure 5b.

Figures 5a and 5b. Galleria mellonella mortality caused by Steinernema carpocapsae "NY-001" over time, in soils from all four field sites in the intact soil columns (a) and sieved soil (b).



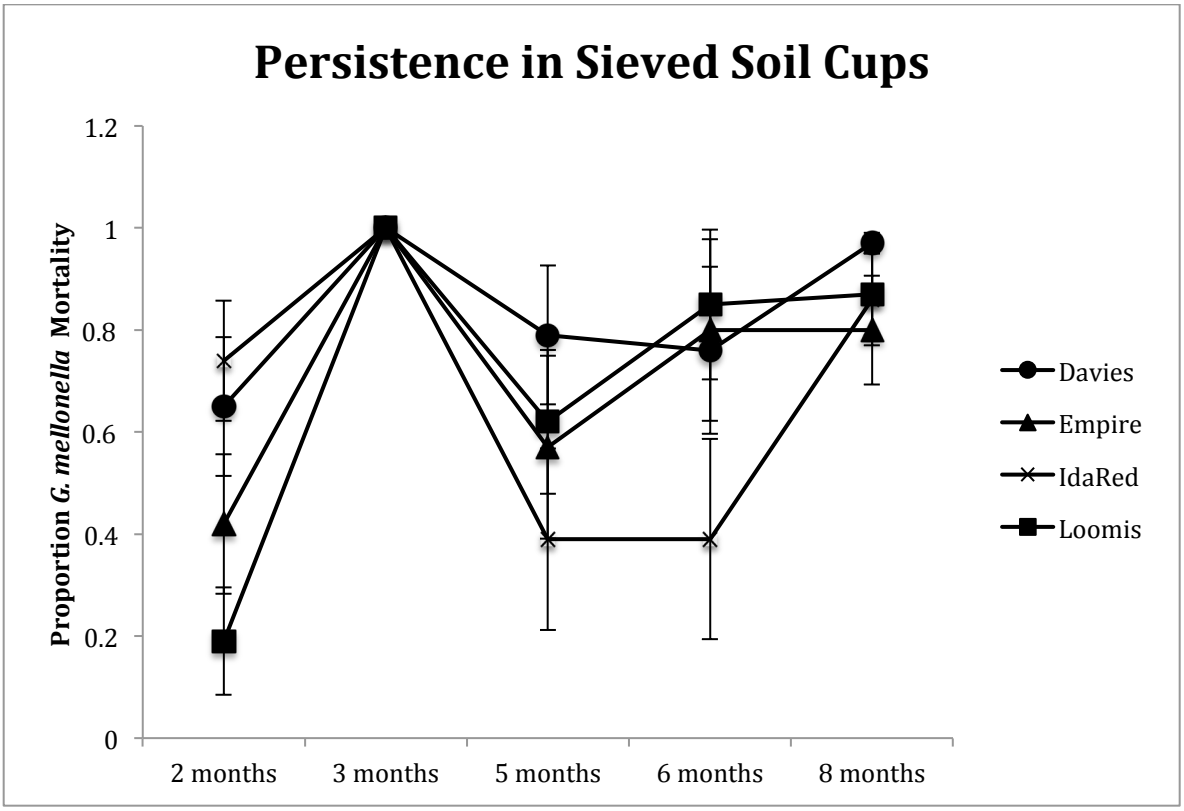


Figure 6a and 6b. *Galleria mellonella* mortality in intact (6a) and sieved soil (6b) caused by *Steinernema feltiae* "NY-04" over time

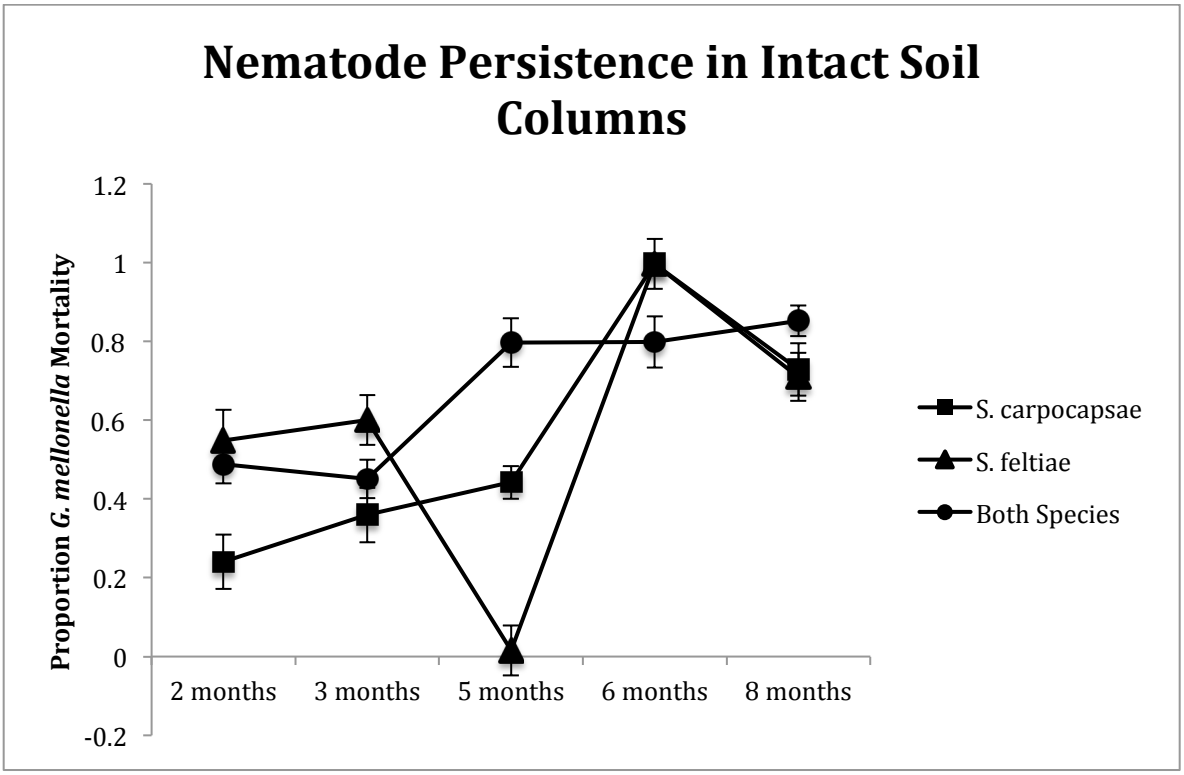


Figure 7a.

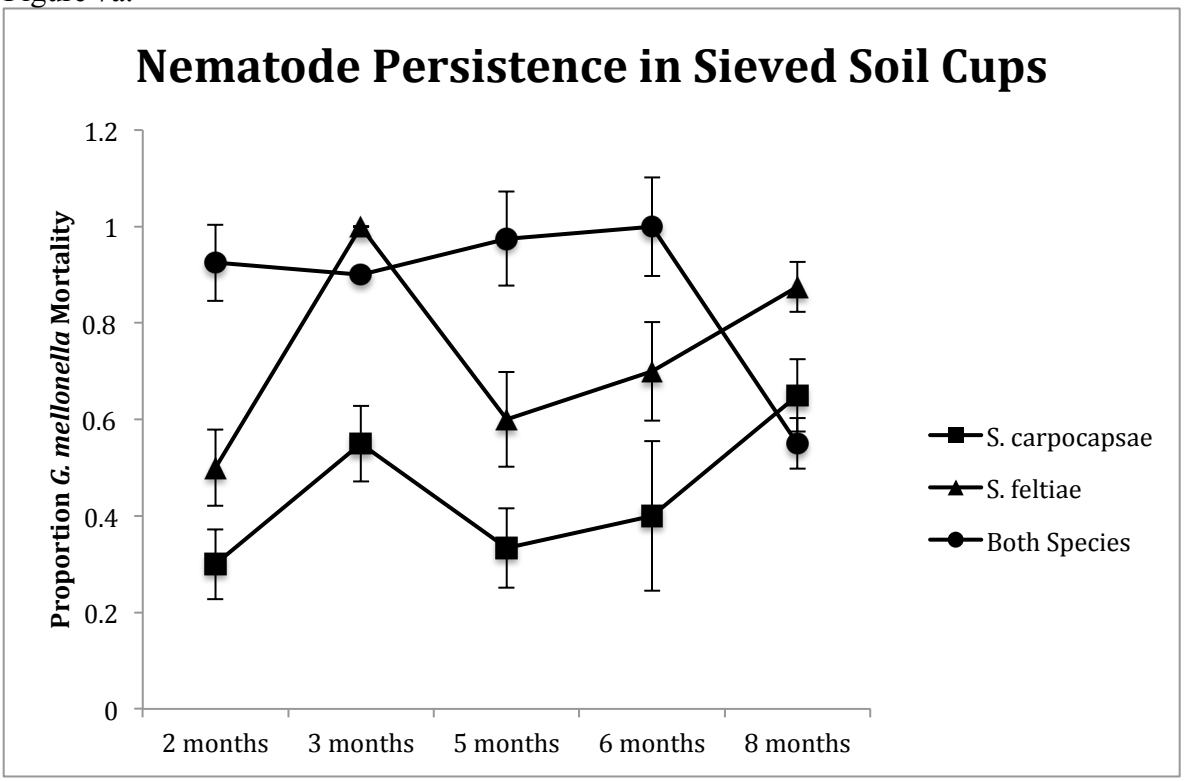


Figure 7b.

Figures 7a and 7b. *G. mellonella* mortality caused by entomopathogenic nematodes in intact (7a) sieved soil (7b) over time.

Discussion

The results of this study show that the nematode/insect/environment relationship is highly dynamic, and that there are many factors that limit nematode virulence against a given species. We demonstrate that both nematode species and soil structure can impact the virulence of entomopathogenic nematodes in the apple system.

The 2015 laboratory results show that there is a strong effect of nematode species on the control of plum curculio in the given soil conditions. It is well known that entomopathogenic nematodes vary in virulence among host species (Gaugler & Kaya 1990, Gaugler 1988a). Consequently, the nematode's potential as a biological control agent varies on an insect-to-insect basis. Since there was no difference in plum curculio emergence between the *S. feltiae* "NY-04" treated soil and the soil treated with both *S. carpocapsae* "NY-001" and *S. feltiae* "NY-04", we conclude that *S. feltiae* "NY-04" is most likely the nematode species responsible for causing the plum curculio mortality in the conditions of these trials. This is further supported by the fact that there was no difference in plum curculio mortality between the *S. carpocapsae* "NY-001" treated soil and the soil with no nematodes present. The higher efficacy of *S. feltiae* might be attributed to the depth at which plum curculio burrows. Plum curculio has a rather wide burrowing range, but tends to pupate at a depth of 2–8 cm. The maximum depth of *S. carpocapsae* is ~3.8 cm, while *S. feltiae* usually occupies soil depths between 5 and 14 cm (Shapiro-Ilan et al. 2002, Shapiro-Ilan et al. 2011).

The significant effect of site in the field bioassays and 2015 laboratory bioassay results suggests that site characteristics greatly influence nematode infectivity as well as the survivorship of the pest. This is consistent with the results of other similar previous

studies regarding entomopathogenic nematodes (Wallace 1968, Gaugler 1988a, Gaugler 1988b, Glazer 2002, Hominick 2002). What is novel about this study is that the soil macrostructure has been determined to likely be important in the agricultural efficacy of entomopathogenic nematodes as biological control agents.

Furthermore, the effect of nematode species was more extreme in sieved soil trials, suggesting that soil structure coupled with mineralogy can have strong effects on the successful use of entomopathogens for biocontrol belowground. In our experiments, the soil was sieved at 6 mm, which preserves the integrity of most soil aggregates, but reduces structural variability at larger scales (Gaugler 1988a, Koppenhöffer & Fuzy 2006). For instance, the intact columns preserve the overall soil structure present in the orchard, including rocks, large pieces of organic matter, cracks, and channels due to biotic activity and abiotic weathering. Throughout the study it was evident that the sites and individual samples varied greatly in rock and larger organic matter content.

The restriction of nematode species effects to trials conducted in sieved soils suggests that differences observed in nematode efficacy among sites may also be related to differences in soil texture and mineralogy. In 2015, the Loomis site had lower adult plum curculio emergence in the laboratory. Our soil data shows that Loomis is a very unique site overall. This site had significantly higher silt content and lower sand content than the Davies, Empire, and IdaRed sites. Loomis also had higher clay content than Davies and Empire. The Davies, Empire, and IdaRed sites are all described as loamy soil, and Loomis is a silt loam. The Loomis site also had higher carbon and nitrogen content than the other three sites, as well as a higher carbon:nitrogen ratio. Soil texture has been shown to influence the virulence of entomopathogenic nematodes, but the actual

mechanisms underlying the relationship between soil chemistry and entomopathogenic nematode infectivity is unclear (Gaugler 1988a, Koppenhöfer et al. 2006, Li et al. 2016**)** While our findings do not provide direct evidence of a relationship between soil physico-chemical traits and nematode infectivity, they do suggest that further investigation of this aspect of nematode ecology is warranted.

Kung et al. (1990) found that *S. carpocapsae* can persist in a somewhat wide range of soil moisture levels (2–16%) but 2% moisture provides *S. carpocapsae* with the highest combined survivability and infectivity. Even under the controlled water conditions in our laboratory trials, soil moisture ranged from 3–30%. Past studies have shown that optimum moisture for *S. carpocapsae's* insect infectivity has been found to be around 30%, so it seems that there is a disconnect between this factor and the optimum moisture level for nematode persistence (Gouge et al. 2000). Shetlar et al. 1988 found that entomopathogenic nematodes cause the highest rates of control of Japanese beetle grubs at moderate to high soil moisture levels. This means that while the nematodes will themselves be at their hardiest at low moistures, their ability to infect insects at an optimum rate requires a moisture of around 30% or greater. It is also important to note that there have not been any such studies done on the particular nematode strains used in this study.

The higher magnitude of differences in our results from sieved soil than from the intact soil may be attributed to effects of removing soil macrostructure upon sieving. The significant effect of site on nematode persistence in the intact soil columns (Figures 5, 6) and the lack of a site effect in the sieved columns shows that soil macrostructure is likely a significant contributing factor.

Shields et al. (2009) found success with *S. carpocapsae* "NY-001" and *Heterorhabditis bacteriophora* "Oswego" with alfalfa snout beetle in alfalfa plantings. Alfalfa differs from apples in many ways, one being that it is a cultivated crop, often implemented in a crop rotation plan. This means that the soil in the alfalfa system is much more disrupted, and likely more heterogeneous. Bulk density and water-related physical characteristics of the disrupted soil might explain why the nematodes had a higher infectivity in the sieved cup laboratory trials, as well as in preliminary petri dish trials (Agnello et al. 2014).

Conclusion

The results of this study indicate that there are many directions for further investigation that could be followed in the future. Specific to this study, I think it would be very valuable to collect reliable bulk density data at every site, because this characteristic is important to the movement of nematodes through the soil. It would also be useful to continue the field bioassays for additional years, to gain more knowledge on the reliability of the field inoculation progressing over time.

As discussed above, it has been noted in past studies that nematodes have host preferences. A possible survey of orchard alternative hosts within the soil may add some knowledge to this subject. Our nematodes may be more attracted to other insect species in the field setting.

To better understand our results, the consistent variables in this study could be manipulated. For instance, the soil moisture content within the laboratory bioassays could

be manipulated to exhibit an array of moistures. In addition, it would be of value to study how the nematodes tolerate different temperatures.

In the past, there has been success using entomopathogenic nematodes in systems that cultivate the soil much more often than is done in apple orchards. It would be beneficial to look at the interaction between entomopathogenic nematodes and other species of weevil that harm growing systems that are culturally more similar to apple orchards. Since all previous studies have been either in the field or in the lab using disrupted soil, more research needs to be done with intact soil under controlled conditions.

References

Agnello, A. M., W. H. Reissig, J. Kovach, and J. P. Nyrop. **2003**. Integrated apple pest management in New York State using predatory mites and selective pesticides. Agric., Ecosyst. & Environ. 94: 183–195.

Agnello, A., H. Reissig, and K. Cox. **2012**. Development and validation of a "Real-Time" Apple IPM Website for New York. IOBC/WPRS Bulletin. 74: 57–60.

Agnello, A., P. Jentsch, E. Shields, A. Testa, M. Keller. 2014. Evaluation of Persistent Entomopathogenic Nematodes for Biological Control of Plum Curculio. New York Frui Quarterly. 22(1): 21-24.

Akotsen-Mensah, C., R. T. Boozer, and H. Y. Fadamiro. **2011**. Field evaluation of reduced insecticide spray programs for managing plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae), in Alabama peaches. Pest Manag. Sci. 67: 626–632.

Akotsen-Mensah, C., R. T. Boozer, and H. Y. Fadamiro. **2012**. Influence of orchard weed management practices on soil dwelling stages of plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae). Florida Entomol. 95: 882–889.

Alston, D. G., D. E. N. Rangel, L. A. Lacey, H. G. Golez, J. J. Kim, and D. W. Roberts. **2005**. Evaluation of novel fungal and nematode isolates for control of *Conotrachelus nenuphar (*Coleoptera: Curculionidae) larvae. Biol. Control. 35: 163–171.

De Bach, P. 1964. Biological control of insect pests and weeds. 844.

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14–19.

Carter, M. R. 1990. Relative Measures of Soil Bulk Density to Characterize Compaction in Tillage Studies on Fine Sandy Loams. Can. J. Soil Sci. 70: 425–433.

(Compost increases the water holding capacity of droughty soils) MSU Extension. **2016**. Compost increases the water holding capacity of droughty soils. (http://msue.anr.msu.edu/news/compost_increases_the_water_holding_capacity_of_droughty_soils).

Delate, K., A. McKern, R. Turnbull, J. Walker, R. Volz, A. White, V. Bus, D. Rogers, L. Cole, N. How, S. Guernsey, and J. Johnston. **2010**. Latest trends in insect and disease management in organic apple systems in the Midwestern USA and New Zealand., pp. 243–252. *In* Prange, R.K., Bishop, S.D. (eds.), Acta Horticulturae. International Society for Horticultural Science (ISHS), Leuven, Belgium.

Downing, A. S. 1994. Effect of Irrigation and Spray Volume on Efficacy of Entomopathogenic Nematodes (Rhabditida: Heterorhabditidae) Against White Grubs (Coleoptera: Scarabaeidae). J. Econ. Entomol. 87: 643–646.

Duncan, L. W., D. G. Dunn, and C. W. McCoy. **1996**. Spatial Patterns of Entomopathogenic Nematodes in Microcosms: Implications for Laboratory Experiments. J. Nematol. 28: 252–258.

Gaugler, R. 1988a. Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agric., Ecosys. & Environ., Proc of a Workshop on Interactions Between Soil-Inhabiting Invertebrates and Microorganisms in Relation to Plant Growth. 24: 351–360.

Gaugler, R. 1988b. Proceedings of a Workshop on Interactions Between Soil-Inhabiting Invertebrates and Microorganisms in Relation to Plant Growth Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agric., Ecosys. & Environ. 24: 351–360.

Gaugler, R. 2002. Entomopathog. Nematol. CABI.

Gaugler, R., and H. Kaya. **1990**. Gaugler, R. & Kaya, H.K. (Eds). 1990. "Entomopathogenic Nematodes in Biological Control." CRC Press. 365 pp. ResearchGate.

Glass, E. H. 1976. Pest Management: Principles and Philosophy, pp. 39–50. *In* Apple, J.L., Smith, R.F. (eds.), Integr. Pest Manag. Springer US.

Gouge, D. H., K. A. Smith, L. L. Lee, and T. J. Henneberry. **2000**. Effect of Soil Depth and Moisture on the Vertical Distribution of Steinernema riobrave (Nematoda: Steinernematidae). J. Nematol. 32: 223–228.

Hoffmann, E. J., J. VanderJagt, and M. E. Whalon. **2007**. Pyriproxyfen activates reproduction in prediapause northern strain plum curculio (*Conotrachelus nenuphar* Herbst). Pest Manag. Sci. 63: 835–840.

Hoy, M. 2012. Biology Control in Agriculture IPM System. Elsevier.

Hoyt, S. C. 1969. Integrated Chemical Control of Insects and Biological Control of Mites on Apple in Washington. J. Econ. Entomol. 62: 74–86.

Ilan, T., D. B. Kim-Shapiro, C. H. Bock, and D. I. Shapiro-Ilan. **2013**. Magnetic and electric fields induce directional responses in *Steinernema carpocapsae*. Int. J Parasitol. 43: 781–784.

Ishibashi, N., and E. Kondo. **1987**. Dynamics of the Entomogenous Nematode Steinernema feltiae Applied to Soil with and without Nematicide Treatment. J Nematol. 19: 404–412.

Johnson, D. T., B. Lewis, C. R. Rom, H. Friedrich, R. Bryant, and M. Pszczolkowski. **2010**. Organic fruit production needs and pest management practices in the southeastern United States., pp. 37–44. *In* Prange, R.K., Bishop, S.D. (eds.), Acta Horticulturae. International Society for Horticultural Science (ISHS), Leuven, Belgium.

Jones, F. G. W., D. W. Larbey, and D. M. Parrott. **1969**. The influence of soil structure and moisture on nematodes, especially *Xiphinema, Longidorus, Trichodorus and Heterodera* spp. Soil Biol. Biochem. 1: 153–165.

Jones, V. P., S. A. Steffan, L. A. Hull, J. F. Brunner, and D. J. Biddinger. **2010**. Effects of the Loss of Organophosphate Pesticides in the US: Opportunities and Needs to Improve IPM Programs. Outlooks Pest Manag. 21: 161–166.

Kaya, H. K., and R. Gaugler. **1993**. Entomopathogenic Nematodes. Annu. Rev. Entomol. 38: 181–206.

Kim, H. G., and D. G. Alston. **2008**. Potential of two entomopathogenic nematodes for suppression of plum curculio (*Conotrachelus nenuphar*, Coleoptera: Curculionidae) life stages in northern climates. Environ. Entomol. 37: 1272–1279.

Koppenhöfer, A. M., and E. M. Fuzy. **2006**. Effect of soil type on infectivity and persistence of the entomopathogenic nematodes *Steinernema scarabaei, Steinernema glaseri, Heterorhabditis zealandica,* and Heterorhabditis bacteriophora. J. Invert. Pathol. 92: 11–22.

Kung, S.-P., R. Gaugler, and H. K. Kaya. **1990**. Soil type and entomopathogenic nematode persistence. J. Invert. Pathol. 55: 401–406.

Kung, S.-P., R. Gaugler, and H. K. Kaya. **1991**. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. J. Invert. Pathol. 57: 242–249.

Lacey, L. A., and R. Georgis. **2012**. Entomopathogenic Nematodes for Control of Insect Pests Above and Below Ground with Comments on Commercial Production. J Nematol. 44: 218–225.

Leskey, T. C., V. Hock, G. Chouinard, D. Cormier, K. Leahy, D. Cooley, A. Tuttle, A. Eaton, and A. Zhang. **2014**. Evaluating Electrophysiological and Behavioral Responses to Volatiles for Improvement of Odor-Baited Trap Tree Management of *Conotrachelus nenuphar* (Coleoptera: Curculionidae). Environ. Entomol. 43: 753–761.

Leskey, T. C., S. E. Wright, J. Saguez, and C. Vincent. **2013**. Impact of insecticide and fungicide residue contact on plum curculio, *Conotrachelus nenuphar* (Herbst), mobility and mortality: implications for pest management. Pest Manag. Sci. 69: 464–470.

Lewis, E. E., R. Gaugler, and R. Harrison. **1993**. Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. Can. J. Zool. 71: 765–769.

Li, N., F. Pan, X.-Z. Han, and B. Zhang. **2016**. Development of soil food web of microbes and nematodes under different agricultural practices during the early stage of pedogenesis of a Mollisol. Soil Biology and Biochemistry. 98: 208–216.

Robertson, G.P., D.C. Coleman, C.S. Bledsoe, P. Sollins, 1999. pp. 78-80. Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York, NY.

(New York Ranks High in Apple, Grape Production) New York Environment Report. **2016**. New York Ranks High in Apple, Grape Production. (http://www.nyenvironmentreport.com/new-york-ranks-high-in-apple-grape-production/).

(NY Apple Industry Facts - NY Apple Association). **2016**. NY Apple Industry Facts - NY Apple Association. (http://www.nyapplecountry.com/about/facts).

(NYFQ) 2016. New York apple industry by numbers. Good Fruit Grower. (http://www.goodfruit.com/new-york-apple-industry-by-numbers/).

Ovruski, S., M. Aluja, J. Sivinski, and R. Wharton. **2000**. Hymenopteran Parasitoids on Fruit-infesting Tephritidae (Diptera) in Latin America and the Southern United States: Diversity, Distribution, Taxonomic Status and their use in Fruit Fly Biological Control. Integ. Pest Manag. Reviews. 5: 81–107.

Pereault, R. J., M. E. Whalon, and D. G. Alston. **2009**. Field Efficacy of Entomopathogenic Fungi and Nematodes Targeting Caged Last-Instar Plum Curculio (Coleoptera: Curculionidae) in Michigan Cherry and Apple Orchards. Environ. Entomol. 38: 1126–1134.

Piñero, J. C., A. M. Agnello, A. Tuttle, T. C. Leskey, H. Faubert, G. Koehler, L. Los, G. Morin, K. Leahy, D. R. Cooley, and R. J. Prokopy. **2011**. Effectiveness of Odor-Baited Trap Trees for Plum Curculio (Coleoptera: Curculionidae) Monitoring in Commercial Apple Orchards in the Northeast. J. Econ. Entomol. 104: 1613–1621.

Quaintance, A. L., and E. L. Jenne. **1912**. The Plum Curculio. U.S. Department of Agriculture, Bureau of Entomology.

R Core Team. 2014. R: A language and environment for statistical computing. R. Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/.)

Racette, G., G. Chouinard, C. Vincent, and S. B. Hill. **1992a**. Ecology and management of plum curculio, *Conotrachelus nenuphar* [Coleoptera:Curculionidae], in apple orchards. Phytoprotection. 73: 85.

Rijal, J. P., C. C. Brewster, and J. C. Bergh. **2014**. Effects of Biotic and Abiotic Factors on Grape Root Borer (Lepidoptera: Sesiidae) Infestations in Commercial Vineyards in Virginia. Environ. Entomol. 43: 1198–1208.

Schroeder, W. J., and J. B. Beavers. **1987**. Movement of the Entomogenous Nematodes of the Families Heterorhabditidae and Steinernematidae in Soil. J Nematol. 19: 257–259.

Selby, R. D., S. H. Gage, and M. E. Whalon. **2014**. Precise and low-cost monitoring of plum curculio (Coleoptera: Curculionidae) pest activity in pyramid traps with cameras. Environ. Entomol. 43: 421–431.

Shapiro-Ilan, D. I., T. C. Leskey, and S. E. Wright. **2011**. Virulence of Entomopathogenic Nematodes to Plum Curculio, *Conotrachelus nenuphar*: Effects of Strain, Temperature, and Soil Type. J. Nematol. 43: 187–195.

Shapiro-Ilan, D. I., R. F. Mizell, and J. F. Campbell. **2002**. Susceptibility of the Plum Curculio, *Conotrachelus nenuphar*, to Entomopathogenic Nematodes. J Nematol. 34: 246–249.

Shapiro-Ilan, D. I., S. E. Wright, A. F. Tuttle, D. R. Cooley, and T. C. Leskey. **2013**. Using entomopathogenic nematodes for biological control of plum curculio, *Conotrachelus nenuphar*: effects of irrigation and species in apple orchards. Biol Control. 67: 123–129.

Shetlar, D. J., P. E. Suleman, and R. Georgis. **1988**. Irrigation and Use of Entomogenous Nematodes, Neoaplectana spp. and Heterorhabditis heliothidis (Rhabditida: Steinernematidae and Heterorhabditidae), for Control of Japanese Beetle (Coleoptera: Scarabaeidae) Grubs in Turfgrass. J. Econ. Entomol. 81: 1318–1322.

Shields, E. J., A. Testa, G. Neumann, K. L. Flanders, and P. C. Schroeder. **2009**. Biological Control of Alfalfa Snout Beetle with a multi-species application of locally adapted persistent entomopathogenic nematodes: The first success. A. Entomol. 55: 250–257.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/. Accessed [7/15/2015].

Van Driesche, R. V., and T. S. B. Jr. **1996**. Biological Control. Springer Science & Business Media.

Vincent, C., G. Chouinard, and T. Leskey. **2008**. Plum Curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae), pp. 2947–2953. *In* Capinera, J.L. (ed.), Encyclopedia of Entomology. Springer Netherlands.

Wallace, H. R. 1968. The Dynamics of Nematode Movement. Annual Review of Phytopathology. 6: 91–114.

White, G. F. 1927. A Method for obtaining Infective Nematode Larvae from Cultures. American Association for the Advancement of Science. Science. 66: 302–303 pp.

Wise, J. C., K. Kim, E. J. Hoffmann, C. Vandervoort, A. Gökçe, and M. E. Whalon. **2007**. Novel life stage targets against plum curculio, *Conotrachelus nenuphar* (Herbst), in apple integrated pest management. Pest. Manag. Sci. 63: 737–742.

APPENDIX I: Davies Farm, Red Jacket Orchards, Geneva, NY
"Geneva, New York." Map. Google Maps. 28 Feb 2016. Web. 28 Feb 2016
Rows and alleyways marked in red were inoculated with entomopathogenic nematodes May 6, 2013.

APPENDIX II: Ida Red Plot, Research North Farm, New York State Agricultural Experiment Station, Cornell University, Geneva, NY
"Geneva, New York." Map. Google Maps. 28 Feb 2016. Web. 28 Feb 2016
Rows and alleyways marked in red were inoculated with entomopathogenic nematodes May 30, 2012.

APPENDIX III: Empire Plot, Research North Farm, New York State Agricultural Experiment Station, Cornell University, Geneva, NY
"Geneva, New York." Map. Google Maps. 28 Feb 2016. Web. 28 Feb 2016
Rows and alleyways marked in red were inoculated with entomopathogenic nematodes May 30, 2012.

APPENDIX IV: Loomis Plot, Loomis Farm, New York State Agricultural Experiment Station, Cornell University, Geneva, NY

"Geneva, New York." Map. Google Maps. 28 Feb 2016. Web. 28 Feb 2016

Rows and alleyways marked in red were inoculated with entomopathogenic nematodes May 6, 2013

APPENDIX V: Soil moisture in 2014/2015 laboratory bioassays.

Characteristic	Davies	Empire	IdaRed	Loomis
Sieved 2014	19.7 (±1.5)	22.36 (±1.4)	18.33 (±0.91)	15.09 (±1.4)
Intact 2014	18.16 (±0.71)	16.77 (±0.75)	18.84 (±0.71)	20.03 (±0.76)
Sieved 2015	18.85 (±0.80)	21.07 (±0.77)	18.99 (±0.78)	23.9 (±0.75)
Intact 2015	20.78 (±0.67)	20.96 (±0.69)	20.9 (±0.66)	20.2 (±0.67)