

ON ENTOMOPATHOGENIC NEMATODES (RHABDITIDA:
STEINERNEMATIDAE AND HETERORHABDITIDAE): A POTENTIAL
REARING HOST, BLACK SOLDIER FLY *HERMETIA ILLUCENS* (L.)
(DIPTERA: STRATIOMYIDAE) AND COMPATIBILITY WITH A PREDATORY
BEETLE, *DALOTIA CORIARIA* (KRAATZ) (COLEOPTERA: STAPHYLINIDAE)

By

Joseph S. Tourtois

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Entomology – Master of Science

2014

ABSTRACT

ON ENTOMOPATHOGENIC NEMATODES (RHABDITIDA: STEINERNEMATIDAE AND HETERORHABDITIDAE): A POTENTIAL REARING HOST, BLACK SOLDIER FLY *HERMETIA ILLUCENS* (L.) (DIPTERA: STRATIOMYIDAE) AND COMPATIBILITY WITH A PREDATORY BEETLE, *DALOTIA CORIARIA* (KRAATZ) (COLEOPTERA: STAPHYLINIDAE)

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Entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) are soil-dwelling insect parasitic round worms used in augmentative biological control to manage western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and fungus gnats *Bradysis spp.* (Diptera: Sciaridae) in greenhouses. Reducing production costs is one way of increasing their adoption by growers. Black soldier fly larvae *Hermetia illucens* (L.) (Diptera: Stratiomyidae) are evaluated as a potential nematode rearing host. They are not highly susceptible to entomopathogenic nematodes; however, damaging the cuticle before and after infection increases mortality rate, infection rate, nematode entry, and nematode emergence. Even with modification, black soldier fly larvae produce only 10% of the nematodes produced on the standard rearing host *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). The soil-dwelling predatory rove beetle *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae) is also used to manage populations of western flower thrips and fungus gnats. Its compatibility with entomopathogenic nematodes is evaluated in a laboratory bioassay. *Dalotia coriaria* appears to be most compatible with *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae).

ACKNOWLEDGEMENTS

I thank my major professor Dr. Matthew Grieshop for all of his encouragement and advice over the past four years I spent in the Organic Pest Management Laboratory. I thank my committee members Dr. Zsafia Szendrei and Dr. John Biernbaum for their guidance and approval. I am grateful for Dr. Anne Nielsen, Nate Walton, Pete Nelson, and Dr. David Shapiro-Ilan. They provided support in rearing and working with entomopathogenic nematodes. I appreciate Morgan Burnette, Yvonne Millar, Paul Owen-Smith, Mirijam Garske, and other numerous undergraduate students who spent countless hours counting nematodes, dissecting black soldier fly, and assessing insect mortality. Without their hard work, this project would not have been finished in a timely manner. I also thank Brad Baughman and Emily Pochubay for being excellent role models. Lastly, I need to thank my wife Krys Tourtois for all of her support and understanding.

TABLE OF CONTENTS

LIST OF TABLES VI

LIST OF FIGURES VII

CHAPTER 1. LITERATURE REVIEW 1

Biological Control..... 1

Augmentative Biological Control..... 2

Limitations to application of augmentative biological control 4

Economics..... 4

Intraguild Predation..... 5

Compatibility with other management strategies 6

Entomopathogenic Nematodes and *D. coriaria* in biological control..... 6

Entomopathogenic Nematodes 7

Biology..... 7

Life Cycle 11

Applications 12

Rearing..... 13

In vivo rearing..... 13

In vitro rearing..... 14

Alternative Rearing Host..... 14

Black Soldier Fly 15

Biology..... 15

History..... 16

Potential Benefits 16

Potential Limitations..... 17

***Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae)..... 18**

History..... 18

Biology..... 18

Life Cycle 19

Feeding habits..... 19

Rearing 20

Compatibility with nematodes..... 21

Thesis Objectives..... 22

**CHAPTER 2. EXPLORING THE USE OF BLACK SOLDIER FLY *HERMETIA ILLUCENS*
(L.) (DIPTERA: STRATIOMYIDAE) AS AN *IN VIVO* ENTOMOPATHOGENIC NEMATODE
REARING HOST..... 23**

Introduction..... 23

Methods and Materials	26
Black Soldier Fly colony.....	26
Entomopathogenic Nematode colonies.....	27
Experiment 1a – black soldier fly susceptibility by larval stage.....	28
Experiment 1b – black soldier fly susceptibility at pupal stage.....	29
Statistical Analysis.....	30
Experiment 2 –effect of larval injury on nematode infection.....	30
Statistical Analysis.....	32
Experiment 3 – nematode production in black soldier fly.....	32
Statistical Analysis.....	34
Results	36
Experiment 1a – black soldier fly susceptibility by larval stage.....	36
Experiment 1b – black soldier fly susceptibility at pupal stage.....	38
Experiment 2 – effect of larval injury on nematode infection.....	40
Experiment 3 – nematode production.....	45
Discussion	50
CHAPTER 3. SUSCEPTIBILITY OF <i>DALOTIA CORIARIA</i> (KRAATZ) (COLEOPTERA: STAPHYLINIDAE) TO ENTOMOPATHOGENIC NEMATODES.	55
Introduction	55
Methods and Materials	59
Insect and Nematode Culture:.....	60
Experimental Methods:.....	61
Statistical Analysis.....	62
Results	64
Beetle Mortality.....	64
Presence of nematodes in cadavers.....	67
Discussion	68
CHAPTER 4. SYNTHESIS AND CONCLUSIONS	73
Black Soldier Fly as a Rearing Host	73
<i>Dalotia coriaria</i> Susceptibility to Entomopathogenic Nematodes	76
Conclusions	77
APPENDIX.....	78
BIBLIOGRAPHY.....	81

LIST OF TABLES

Table 2-1. Mean \pm SEM adult nematodes recovered from four black soldier fly instars (n=25 per treatment combination) in susceptibility bioassay. Infective juveniles (100) applied per host at 20°C. None of the negative controls were infected. Data not shown for <i>G. mellonella</i> . Amounts with different letters indicate statistical difference within nematode treatment (Kruskal-Wallis, $p < 0.05$). ns = no statistical difference.	37
Table 3-1. Model selection based on AIC values using the step function in R 3.1.1. ...	63

LIST OF FIGURES

Figure 1-1. Life cycle of entomopathogenic nematodes. See text for details.....10

Figure 2-1. Abbott’s corrected mortality for black soldier fly larvae. Nematodes applied at 100 infective juveniles per host at 20°C. Mortality for the second instar is from day 7 since there was high mortality on day 8 in the control. *G. mellonella* mortality was >95% for all nematode species and 24 ± 10% for the control on day 5 (data not shown). Bars with different letters indicate statistical difference within nematode treatment (ANOVA, Tukey HSD, p < 0.05). NS = no statistical difference. 35

Figure 2-2. Survival curves for injured (dotted line) and non-injured (solid line) fifth instar black soldier fly for each nematode species: A) *H. bacteriophora*, B) *S. carpocapsae*, C) *S. feltiae*, D) *S. riobrave*, and E) control (no nematodes). Infective juveniles (1000) applied per host at 25°C. All *G. mellonella* treated with nematodes were dead on day 2 and only 5 *G. mellonella* died in the control treatment (data not shown). Asterisk (*) denotes significant difference (p < 0.05). n.s. = not statistically different.39

Figure 2-3. Percent fifth instar black soldier fly infected with entomopathogenic nematodes (n=40). Infective juveniles (1000) applied per host at 25°C. Asterisk (*) denotes significant difference (p < 0.05).41

Figure 2-4. Mean number of entomopathogenic nematodes (first generation adults and juveniles) recovered from fifth instar black soldier fly cadavers. Data not shown for *G. mellonella*. Infective juveniles (1000) applied per host at 25°C. Numbers within the bars indicate number of infected larvae (max=40). Asterisk (*) denotes significant difference (P < 0.05).42

Figure 2-5. Survival curves for injured (dotted line) and non-injured (solid line) fifth instar black soldier fly before nematode application for each nematode species: A) *H. bacteriophora*, B) *S. carpocapsae*, C) *S. feltiae*, D) *S. riobrave*, and E) control (no nematodes). Infective juveniles (1000) applied per host at 25°C. All *G. mellonella* treated with nematodes were dead on day 2 and none died in the control treatment (data not shown). Asterisk (*) denotes significant difference (p < 0.05) for injury treatments, n.s. = not statistically different. Plots with different letters are significantly different (p < 0.05).44

Figure 2-6. Mean number of infective juveniles harvested from fifth instar black soldier fly and *G. mellonella*. no = non-injured, post = damaged as a cadavers, pre = larva injured before nematode application, and p+p = pre and post. Bars labeled

with different letters are significantly different, ANOVA, Tukey HSD, $\alpha = 0.05$, n.s. = not significantly different.....47

Figure 3-1. Percent mortality of *D. coriaria* on Day 4. H. bac = *H. bacteriophora*, S. carp = *S. carpocapsae*, S. felt = *S. feltiae*, S. rio = *S. riobrave*. Bars with different letters are significantly different ($p < 0.05$).65

Figure 3-2. Percent dead *D. coriaria* with confirmed nematodes. H. bac = *H. bacteriophora*, S. carp = *S. carpocapsae*, S. felt = *S. feltiae*, S. rio = *S. riobrave*. Within each nematode group, bars with different lowercase letters are significantly different ($p < 0.05$). For each rate across nematode species, bars with different uppercase letters are significantly different ($p < 0.05$).66

Chapter 1. Literature Review

Biological Control

Biological control is the practice of using living organisms to suppress the population or impact of a pest organism (Eilenberg et al. 2001). A pest is any organism that causes direct harm to humans or economic losses to human endeavors (Hajek 2004). The living organisms used to suppress the pest organism are collectively referred to as biological control agents or natural enemies (Hajek 2004). The goal of biological control in Integrated Pest Management (IPM) is to minimize the use of pesticides and harmful effects on the environment and human health (Eilenberg et al. 2001; Hajek 2004; Heinz et al. 2004). Biological control is a proactive ecological approach employing knowledge of food-web relationships to manage pest populations. When successful, biological control can provide stable and continuous pest management (Hajek 2004; Vincent et al. 2007).

There are three main types of biological control: classical, conservation, and augmentative (Hajek 2004; Lazarovits et al. 2007). Classical biological control is most commonly employed against invasive plant and animal pest species and is when exotic natural enemies are introduced to manage the invasive pest (Caltagirone 1981; Eilenberg et al. 2001). Some authors refer to classical biological control as inoculation biological control since a natural enemy is being established in a new location (van Lenteren 2012). Conservation biological control is the management or manipulation of the environment to support and maintain higher populations of natural enemies or increase their effectiveness (Barbosa 1998; Eilenberg et al. 2001). Augmentative biological control is when natural enemies are

mass reared and then released into a cropping system, often multiple times during the growing season (Collier and Van Steenwyk 2004; van Lenteren 2012).

Farmers may adopt biological control for many reasons including: consumer demand for pesticide free foods (Magnusson and Cranfield 2005), a desire to provide a safer worker environment (Bailey et al. 2009), environmental stewardship (Bailey et al. 2009; Kogan 1998), or a smaller pesticide tool kit due to increased government regulation (Heinz et al. 2004). Another reason growers adopt biological control is that pest populations have developed resistance to the available pesticides (Jensen 2000; Shipp et al. 2007; Zhao et al. 1995). In cases where pesticide resistance has not yet developed, the efficacy, predictability, and cost of natural enemies are the main issues affecting implementation of biological control (Heinz et al. 2004).

Augmentative Biological Control

Augmentative biological control is divided into two subcategories: — inoculation and inundation— that describe the manner in which natural enemies are released (Hajek 2004). Inoculation biological control is the release of a natural enemy into a system with the expectation that it will reproduce to provide control of a target population (Eilenberg et al. 2001). An example of inoculation is the release of predatory rove beetles in a greenhouse with the expectation that they will establish and future releases are not required (Bennison et al. 2009; Bennison et al. 2008). Inundation biological control is where a large quantity of natural enemies are released to target a pest population with little expectation of reproduction (Eilenberg et al. 2001). An example of inundation biological control is the mass

release of 2.5 billion entomopathogenic nematodes per hectare to target soil-dwelling pests (Barbercheck 2004). Entomopathogenic nematodes naturally occur in the soil, but it is assumed that there are rarely enough to provide economic control (Stuart et al. 2006). In either case, the goal of releasing natural enemies is to augment the natural population to obtain sufficient biological control (Hajek 2004).

One of the earliest examples of augmentative biological control is the mass production and release of the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) to control the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) in Canadian greenhouses (Shipp et al. 2007). After the invention of DDT and other chemical pesticides, rearing of *E. formosa* was suspended. Then in the 1970s with a rise in insecticide resistance, interest in biological control was reinvigorated (Shipp et al. 2007). Since then the number of natural enemies that are mass produced for use in augmentative biological control has increased, most noticeably in the 1980s and 1990s (van Lenteren 2012). What was once a cottage industry producing only a handful of natural enemies now produces 230 species at a commercial professional industry level (van Lenteren 2012).

Greenhouse growers who use biological control typically use augmentative biological control with an emphasis on inundation of pest populations. Greenhouses provide ideal conditions for rapid insect population growth due to high temperatures and relative humidity (Van Lenteren and Woets 1988). Greenhouses are also isolated, semi-enclosed spaces with vents that open and close throughout the day to regulate temperature (Lindquist and Short 2004). These are ideal

conditions to use augmentative biological control – natural enemies are lacking and conditions are optimal for prey build up.

Limitations to application of augmentative biological control

Economics

Augmentative biological control is dependent upon the availability of mass-reared natural enemies (van Lenteren 2012; Warner and Getz 2008). The mass reared natural enemy industry is still quite small with an estimated \$25-30 million wholesale income in North America in 2005-2007 compared to the \$3.1 billion pesticide industry in 2001 (Warner and Getz 2008). Worldwide, there are 230 natural enemy species that are produced and sold on a commercial scale worth \$394 million (€300 million) (Cock et al. 2010; van Lenteren 2012). Hymenopterans account for over half of the species (120 species), followed by Acari (30 species), Coleoptera (28 species), Heteroptera (19 species), Nematoda (10 species), Neuroptera (8 species), Diptera (8 species), Thysanoptera (6 species), and 1 species of Mollusca and Chilopoda each (van Lenteren 2012). Many new species were brought onto the market during the 1990s following a decade of research in mass-rearing insects and augmentative biological control (van Lenteren 2012; Warner and Getz 2008). Since that time only a relatively small number of new natural enemy species have been brought to the market (e.g. *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae)) (van Lenteren 2012).

The companies that produce natural enemies generally consist of between 1 to 10 employees with only 5 companies employing more than 10 people (van Lenteren 2012). These companies are also interdependent, organized in

associations by continent. One result of this is that natural enemies are often bought and repackaged by producers and distributor before the end-consumer receives them (Warner and Getz 2008). This relative scarcity of biological control providers and repackaging of biological control products can lead to high prices.

Van Lenteren (2012) makes the argument that biological control is more economically viable than chemical pest management. Even though there are fewer insects than synthetic chemical compounds to test for pest management, the success ratio for finding an effective natural enemy is higher, 1:10 vs. 1: 140,000 – especially given the governmental regulator restrictions and testing costs for developing new chemical pesticides. Even though the price of pesticides are becoming more expensive, it is still cheaper for farmers to use pesticides than purchase commercial natural enemies from an insectary. As an example, entomopathogenic nematodes cost from \$200-300/application/hectare whereas a single pesticide application may cost as little as \$40/hectare.

Intraguild Predation

A potential limitation to augmentative biological control is intraguild predation, which occurs when two or more natural enemies feed upon or negatively interact each other in a non-competitive fashion (Polis et al. 1989). Intraguild predation can be either bidirectional or unidirectional. Bidirectional predation occurs when two predators consume each other. For an example, the predatory mite *Hypoaspis aculeifer* (Canestrini) (Mesostigmata: Laelapidae) preys on the first instars of *D. coriaria* and *D. coriaria* predate on the eggs and early developmental stages of the predatory mite (Jandricic et al. 2006). Unidirectional intraguild

predation occurs when a predator eats another, but the second predator does not prey on the first. Two examples of unidirectional intraguild predation are when the two soil predators, *Stratiolaelaps miles* (Berlese) (Mesostigmata: Laelapidae) and *D. coriaria* feed on the foliar predatory mite *Neoseiulus cucumeris* Oudemans (Phytoseiidae) when placed on the soil surface (Pochubay and Grieshop 2012).

Compatibility with other management strategies

Biological control is often not compatible with pesticides (Hajek 2004). The active ingredients in pesticides often kill not only the pests but the predators and parasites as well (Cloyd and Bethke 2011; Hassan and Veire 2004). Imidacloprid and benicarb are toxic to multiple stages of *D. coriaria* and insect growth regulators are toxic to third instars (Jandricic et al. 2006; Cloyd et al. 2009). Many pesticides have been tested for compatibility to entomopathogenic nematodes – some are toxic others are not (Rovesti et al. 1988; Rovesti and Deseö 1990; Rovesti and Deseo 1991). One way to integrate pesticides and biological control is to separate the organism temporally and spatially from pesticide applications (Hassan and Veire 2004).

Entomopathogenic Nematodes and *D. coriaria* in biological control

Both entomopathogenic nematodes and *D. coriaria* are promising augmentative biological control agents that have seen some use in greenhouse and small scale agriculture. Both natural enemies can be used to manage western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and fungus gnats *Bradysis spp.* (Diptera: Sciaridae) populations. A rearing-release system has been developed for *D. coriaria* for use in greenhouses and nurseries. One possible

way to expand natural enemy use is to provide framers with the knowledge to rear their own biological control organisms and how these organisms interact with each other.

Since the typical application rate of entomopathogenic nematodes is 2.5 billion infective juveniles (IJ) per hectare, their use in augmentative biological control is dependent on mass production (Barbercheck 2004; Shapiro-Ilan et al. 2002b). Entomopathogenic nematodes can be reared *in vivo* on *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) or *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Shapiro-Ilan et al. 2002a; Shapiro-Ilan and Gaugler 2002). These insects have limited uses other than as rearing hosts. Framers would benefit from an alternative rearing host that has multiple on-farm uses.

Dalotia coriaria and entomopathogenic nematodes are two soil-dwelling organisms used in augmentative biological control programs to manage greenhouse pests. Current information about the interactions between these two is lacking. Possible negative interactions could lead to less successful biological control.

The simple life cycle and wide host range make entomopathogenic nematodes easily adaptable rearing *in vivo*. *Dalotia coriaria* is a fierce predator. Both natural enemies are conducive to augmentative biological control.

Entomopathogenic Nematodes

Biology

Entomopathogenic nematodes have been used in augmentative biological control as a biopesticide since the 1930s (Glaser et al. 1935). In order for entomopathogenic nematode to be successful in managing a pest population, they

are applied at rates of 2.5 billion entomopathogenic nematodes per hectare (Barbercheck 2004). This requires rearing them en mass. *In vitro* rearing systems have been developed but require large capital investments and a strong supporting market (Shapiro-Ilan et al. 2014). *In vivo* rearing of entomopathogenic nematodes with an insect host is feasible at the cottage industry scale. The greater wax moth *Galleria mellonella* is the most commonly used rearing host while the yellow mealworm *T. molitor* has also been used in some systems (Shapiro-Ilan et al. 2002a; Shapiro-Ilan and Gaugler 2002).

Entomopathogenic nematodes are soil-dwelling round worms that infect and rapidly kill insect hosts with the aid of bacterial partners (Dillman et al. 2012). As of 2012, there are 15 described species of Heterorhabditis, 63 described species of Steinernema, and three species in the family Rhabditidae (Zhang et al. 2012). There is disagreement in the literature about whether the last three species belong to the genus *Heterorhabditoides* or *Osccheius* (Ye et al. 2010; Liu et al. 2012; Zhang et al. 2012). Each nematode species has a symbiotic bacteria species, i.e. *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) and *Photorhabdus luminescens* (Thomas and Poinar) (Enterobacteriales: Enterobacteriaceae), *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) and *Xenorhabdus nematophila* (Thomas and Poinar). The bacteria aid in breakdown of the host into a nutrient soup, and the nematodes vector the bacteria from one host to the next.

G. Steiner described the first entomopathogenic nematode *Steinernema* (= *Aplectana*) *kraussei* (Rhabditida: Steinernematidae) in 1923 (Poinar and Grewal 2012). In 1929, *S.* (= *Neoplectana*) *glaseri* (Steiner) was the second

entomopathogenic nematode to be described (Steiner 1929). Throughout the 1930s, it was the first nematode to be tested in augmentative biological control programs against the Japanese beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae) (Glaser 1932; Glaser et al. 1935; Glaser 1940). Researchers at the time had not yet established the symbiotic relationship between the nematode and bacteria and the colonies were lost (Poinar and Grewal 2012). Renewed interest in entomopathogenic nematodes was sparked when *S. carpocapsae* was isolated from codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in two separate locations in 1955 (Dutky and Hough 1955). For many years the nematode isolate in Eastern United States was referred to as the DD-136 strain, and the nematode from Europe was referred to as *Neoplacenta carpocapsae*. In 1967, Poinar established that these two populations were conspecifics (Poinar 1967). Poinar started a new family of entomopathogenic nematodes when he discovered and described *H. bacteriophora* in 1975 (Poinar 1975a). Today, there are 78 identified species of entomopathogenic nematodes in three families: Steinernematidae, Heterorhabditidae, and Rhabditidae (Zhang et al. 2012). Only nine species are mass-produced on a commercial scale, – four *Heterorhabditis* and five *Steinernema* (van Lenteren 2012).

Nematode: Life Cycle

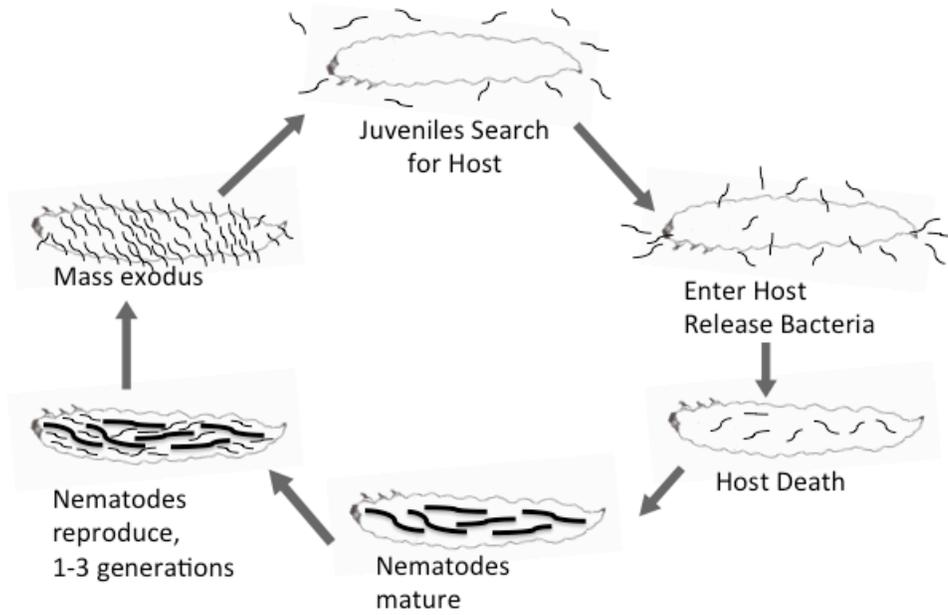


Figure 1-1. Life cycle of entomopathogenic nematodes. See text for details

Life Cycle

The life cycle of entomopathogenic nematodes consists of: eggs, four juvenile stages, and an adult stage. Immature stages of nematodes are referred to as juveniles so as not to be confused with the immature stage of insects known as larvae. Most of the nematode life cycle occurs within a host. The third stage infective juvenile (or dauer juvenile) is the only stage that is found outside of a host and does not feed. This stage vectors the symbiotic bacteria and infects new hosts. To protect itself in the environment, the infective juvenile continues to wear the cuticle of the second stage as an extra sheath (Kaya and Gaugler 1993). Infective juveniles enter insect hosts through natural openings – mouth, anus, and spiracles (Kaya and Gaugler 1993).

Some Heterorhabditis have a dorsal tooth and can penetrate through insect integument (Bedding and Molyneux 1982). On the way into the host the infective juvenile sheds the sheath. Once inside the insect haemocoel, the symbiotic bacteria are released from the infective juvenile. *Photorhabdus spp.* bacteria leave their *Heterorhabditis spp.* vector by the mouth (Adams et al. 2006; Ciche and Ensign 2003); whereas *Xenorhabdus spp.* bacteria leave their *Steinernema spp.* vector by the anal opening (Martens et al. 2003; Poinar 1966). Together the nematode-bacteria complex overcome the host's immune system and kill it within 24-48 h (Kaya and Gaugler 1993) and proceed to release enzymes that breakdown the host into a nutrient soup. Bacteria multiply as the host is digested and nematodes complete their development feeding on the bacteria and digested host. Within 2-4 d, the founding nematodes reach the adult stage.

Steinernematids are male and female; whereas the founding nematodes of Heterorhabditids are hermaphroditic (Poinar 1975b). Progeny in the following generations within the same host can be hermaphroditic or amphimictic (Strauch et al. 1994). The nematodes proceed through one to three generations dependent upon the size of the host (Kaya and Gaugler 1993). When host resources are depleted and the nematodes reach a certain density, juveniles develop into infective juveniles, store symbiotic bacteria in their intestinal cavity and emerge from the cadaver by the thousands in search of a new host (Kaya and Gaugler 1993).

Applications

Entomopathogenic nematodes are used as a bio-insecticide in a limited number of agriculture systems, including turfgrass, greenhouse and nursery, mushrooms, and a handful of field crops and orchards (Grewal et al. 2005). Turfgrass was the earliest system to adopt the use of nematodes to control Japanese beetles (Glaser et al. 1935). White grubs (Coleoptera: Scarabaeidae) feed on the roots of many grasses and are considered the most damaging insect pests of turfgrass (Jackson 1992). A great deal of research has gone into the control of white grubs with the use of nematodes.

Another benefit of using entomopathogenic nematodes is that they can be applied using traditional spray equipment (Grewal et al. 2005). Beneficial nematodes are often applied at high rates – 2.5 billion IJ/hectare in an aqueous solution (Barbercheck 2004). Because of these two characteristics of application (i.e. high numbers of infective juveniles and applied by traditional equipment), they are often thought of as a biopesticide even though they are not regulated by the EPA.

Rearing

Entomopathogenic nematodes can be reared with a host insect (i.e. *in vivo*) or without a host (i.e. *in vitro*). Either way the nematodes need to be reared with their symbiotic bacteria to produce infective juveniles that are effective as biological control organisms over multiple generations (Han and Ehlers 2000).

In vivo rearing

The most common *in vivo* rearing host for entomopathogenic nematodes is the mature larvae of the highly susceptible greater wax moth *G. mellonella*. Another rearing host is the yellow mealworm *T. molitor* (Shapiro-Ilan et al. 2002a; Shapiro-Ilan and Gaugler 2002). *In vivo* rearing capitalizes on the biology of the nematode-bacteria complex and is performed in a system of trays of shelves (Shapiro-Ilan et al. 2014).

The production of infective juveniles from one host is about 50,000 to 200,000 IJ per *G. mellonella* larva depending on nematode species (Dutky et al. 1964; Selvan et al. 1993). Rearing entomopathogenic nematodes *in vivo* is typically done at small scales (i.e. laboratory colonies and cottage industry) (Shapiro-Ilan et al. 2014). *Galleria mellonella* and infective juveniles in aqueous solution are placed onto a piece of Whatman filter paper inside an inverted Petri dish. The infection rates range from 20-100 infective juveniles per *G. mellonella* larva (Selvan et al. 1993). For other hosts —such as the yellow mealworm— the infective rate is much higher, 400-800 IJ/host (Shapiro-Ilan et al. 2008). *G. mellonella* typically die within 1-2 d of infection and change color. *G. mellonella* infected with *Steinernema spp.* typically become a gray, brown, or beige color and hosts infected with

Heterorhabditis spp. typically turn a brick red to orange color. About 7-10 d after host death, cadavers are transferred to a White trap (White 1927). The Petri dish holding the cadavers is placed in a larger Petri dish and water is added surrounding the smaller dish. As the infective juveniles emerge from the host cadaver, they travel over the filter paper, up-and-over the Petri dish edges, and into the surrounding water. This allows the nematodes to be easily harvested and stored in an aqueous solution. The optimal temperature for rearing nematodes *in vivo* is 25° C and 75-100% relative humidity (Grewal et al. 1994b).

***In vitro* rearing**

Rearing nematodes *in vitro* first requires the establishment of symbiotic bacteria and nematodes in separate pure cultures, then inoculating a medium with the bacteria and nematodes, which requires high startup capital to purchase fermentation tanks (Shapiro-Ilan et al. 2012). *In vitro* rearing of nematodes comes in two forms – solid and liquid. There are four major steps to *in vitro* solid rearing: preparing the solid media, inoculating with symbiotic bacteria, inoculation with axenic nematodes, and finally harvesting. The solid medium is a crumbled or shredded polyether polyurethane sponge that is coated with a homogenate growth medium of various animal parts, grain products, or yeast extracts (Bedding 1981; Gaugler and Han 2002; Shapiro-Ilan et al. 2012).

Alternative Rearing Host

Cost is one of the main reasons why more growers do not adopt entomopathogenic nematodes in augmentative biological control programs (Grewal

et al. 2005; Shapiro-Ilan et al. 2014). Reducing the cost of the rearing host and related production practices is one way to potentially increase grower adoption. *Galleria mellonella* costs about \$0.04 - \$0.07 per larva at bait-and-tackle shops and pet stores. Criteria to selecting an alternative host include: negligible to no cost to rear, highly susceptible, and large body size. Black soldier fly larvae *Hermetia illucens* (L.) (Diptera: Stratiomyidae) has the potential to be an ideal alternative rearing host. Black soldier flies are reared on farm wastes, allowing for nutrient cycling (Newton et al. 2005; Sheppard et al. 1994). Mature larvae are about two-thirds the mass of *G. mellonella*. Thus, the susceptibility to entomopathogenic nematodes is a question that needs to be addressed.

Black Soldier Fly

Biology

Hermetia illucens is a hypermetamorphotic insect; the last instar is morphologically unlike the earlier instars. The life cycle begins with adult female flies laying eggs in dry cracks and crevices above food sources (Booth and Sheppard 1984). Larvae hatch from the eggs, fall to the food source, and feed gregariously (Bradley 1930). Larvae develop through six instars within three weeks (May 1961; Tomberlin et al. 2009). The sixth instar differs from the previous instars and is known as a prepupa (Sheppard et al. 1994). The mouthparts are reduced and immobilized into a hook; the ocelli are more prominent; and the cuticle is darker covered in more pubescence and lacking the ventral spicules (May 1961). The prepupa is a non-feeding, wandering stage that searches for dry substrates for

pupation (Newton et al. 2005; Sheppard et al. 1994). Adults live for about 10-14 d and do not feed (Tomberlin et al. 2002).

History

Since the beginning of the 20th century, authors have reported the appearance of black soldier flies in beehives (Copello 1926), latrines and privies (Johannsen 1922; Bradley 1930), animal manures (Furman et al. 1959), carrion, and various other decaying organic matter (May 1961). Sheppard et al. (1983) explored the use of black soldier fly larvae to manage house fly populations and the large volumes of manure produced on poultry farms. Newton et al. (1977) determined that black soldier fly meal is a good dietary supplement for swine. Forensic entomologists have also been interested in using black soldier fly to determine postmortem interval (Lord et al. 1994; Pujol-Luz et al. 2008). Most recently, the medical field has taken an interest in them to looking for new antimicrobial substances (Choi et al. 2012; Park et al. 2014).

Potential Benefits

Black soldier fly are a powerful nutrient management tool, allowing nutrients to be cycled multiple times. Young larvae feed on organic farm and food wastes. Mature larvae can be feed to livestock such as swine, poultry, and fish (Newton et al. 2005; Sheppard et al. 1994). Compost resulting from the rearing process can be used as a soil amendment (Newton et al. 2005; Sheppard et al. 1994). A small number of larvae could be diverted from livestock feed to rear entomopathogenic nematodes.

Presently, on farm rearing of entomopathogenic nematodes relies on purchased or reared *G. mellonella*, requiring additional investments of money and/or on-farm labor. Diet requirements for *G. mellonella* include honey, wheat bran and dogfood —items that are rarely produced on farm. In contrast, black soldier flies are reared on farm wastes. Rearing entomopathogenic nematodes on black soldier fly larvae would thus add a tertiary on-farm use for black soldier fly, making flies even more attractive for small farm and greenhouse operations. A free, ready supply of entomopathogenic nematode rearing hosts could also increase the availability of this relatively underutilized form of augmentative biological control for small-scale farmers.

Potential Limitations

Some potential limitations of using black soldier fly as a rearing host are morphological and immunological. Black soldier fly larvae are amphipneustic; two anterior spiracles are located on the first thoracic segment and two posterior spiracles are located inside pouch on the last abdominal segment. This is a reduced amount of spiracles compared to the highly susceptible *G. mellonella*, which is peripneustic with nine spiracles on each side. The lower number of spiracles means fewer entry points for entomopathogenic nematodes. The larvae of Stratiomyidae also incorporate calcium carbonate into their cuticle (Johannsen 1922). This gives the cuticle a shagreened pattern of hexagons and makes the cuticle thick and tough yet flexible (Newton et al. 1977). For nematodes such as *H. bacteriophora* which likes to enter its host not by natural openings but puncturing a hole through the integument, the shagreened cuticle of the black soldier fly may inhibit entry

(Bedding and Molyneux 1982). Due to their life history with decaying organic matter, black soldier flies have a well-developed immune system and strong responses to non-self organisms (Choi et al. 2012; Park et al. 2014). The morphology and immune system of the black soldier fly larvae may limit its use as an entomopathogenic rearing host.

***Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae)**

History

In the early 1980s, *D. coriaria* was discovered preying upon *Stelidota geminate* (Say) (Nitidulidae) in a laboratory colony (Miller and Williams 1983). However, it was not until the 21st century that its potential as a biological control organism in greenhouses was explored (Carney et al. 2002). It is one of the few new organisms biological control companies have added to the market since the 1990s (van Lenteren 2012). Today, *Dalotia coriaria* is sold for the management of western flower thrips, fungus gnats, and shore flies *Scatella stagnalis* (Fallen) (Diptera: Ephydriidae). The knowledge of interactions between *D. coriaria* and entomopathogenic nematodes is lacking.

Biology

Dalotia coriaria is a small (3-4 mm), highly mobile, soil-dwelling polyphagous predator. Larvae are a pale yellow to cream color; and the adults are a glossy, dark color (Miller and Williams 1983). Body posture is typically S-shaped with their heads pointed down and their abdomens upturned. Both the larvae and adults are

fiercely polyphagous – feeding on several greenhouse pests including western flower thrips, fungus gnats, and shore flies (Carney et al. 2002; Jandricic et al. 2006).

Life Cycle

Dalotia coriaria is a holometabolous insect with four life stages: egg, larva, pupae, and adult. Adult females lay eggs in the soil and larvae hatch 2 to 3 d later (Echegaray and Cloyd 2013; Miller and Williams 1983). Larvae develop through three instars in about 7 d (range 4.5 to 11 d) (Echegaray and Cloyd 2013; Miller and Williams 1983). The third instar spins a cocoon of silk strands and soil particles before pupation (Miller and Williams 1983). Adults emerge 5 to 8 d later (Echegaray and Cloyd 2013; Miller and Williams 1983). The development time from egg to adult is about 2.5 to 3 w (Carney et al. 2002; Echegaray and Cloyd 2013; Miller and Williams 1983). Adults beetles live for 4 to 12 w (Echegaray and Cloyd 2013). Females can lay up to 14 eggs a day with a mean fecundity of 90 eggs during their life (Echegaray and Cloyd 2013).

Feeding habits

Dalotia coriaria has been reported feeding on a variety of organisms. Miller and Williams (1983) recorded *D. coriaria* feeding on the eggs of *Musca domestica* L. (Diptera: Muscidae) and several Nitidulid species. Messelink and Van Wensveen (2002) reported *D. coriaria* feeding on the eggs and first larval stages of *Duponchelia fovealis* Zeller (Lepidoptera: Pyralidae), a widespread pest in Dutch greenhouses as well as collembolans but not woodlice or millipedes. Adult *D. coriaria* have been shown to consume as many as 95 second instar thrips, 78 thrips pupae, 154 fungus

gnat eggs, and 150 first instar fungus gnats within 24 h (Carney et al. 2002). Third instar beetles can consume an equally impressive 100 eggs and 100 first instar fungus gnats in a 24 h period (Carney et al. 2002). In a laboratory bioassay, one adult rove beetle can consume 68 – 78% of the second and third instar fungus gnats presented to them in Petri dishes within 24 h (Echegaray Wilson 2012).

Rearing

Dalotia coriaria has been reared on a variety of artificial diets under various conditions. Carney et al (2002) first reared *D. coriaria* on trout food in coconut fiber (coir). They tried multiple artificial diets including raw and cooked ground beef, cat and dog food, and trout food. *Dalotia coriaria* performed equally well on all food substrates, but the authors decided that a commercialized trout food was the easiest to use. Others have reared *D. coriaria* on turkey feed (Bennison et al. 2009; Bennison et al. 2008) and oatmeal (Birken and Cloyd 2007). *Dalotia coriaria* has been reared at multiple temperatures ranging from 20-25°C, relative humidity ranging from 30-90% and different photoperiods ranging from: no light, 12:12 h, 16:8 h and seasonal cycles.

Bennison et al. (2008) developed a rearing-release system for *D. coriaria*. Rearing containers were 3 L plastic boxes half filled with moistened coir and vermiculite (50:50 mixture) and snap on lids with two ventilation holes. Initial populations were started with 60 adults and fed with 2.5 g of turkey feed every 3-4 d. Turkey feed was mixed into the coir and vermiculite to prevent fungal growth. Beetle populations increased 20-fold in 23 d. Beetles reared in these containers

could be deployed in cropping systems and were reported to disperse up to 30 m within a week (Bennison et al. 2009).

Authors have reported several problems when rearing *D. coriaria*. The most noted is that the immature stages are cannibalistic, especially the third instars, which feed on the younger instars when rearing containers are over crowded (Carney et al. 2002; Echegaray and Cloyd 2013; Miller and Williams 1983). Authors have also noted the presence of mites that appeared detrimental to *D. coriaria* colonies (Carney et al. 2002; Echegaray Wilson 2012). Carney et al. (2002) described a phoretic deutonymph resembling *Rhizoglyphus spp.* (Astigmata: Acaridae) that restricted the mobility, feeding, and mating of the beetles. Echegaray (2012) observed mites (unknown species) feeding on multiple stages of the beetle (eggs, larvae, and adults). However, not all mites present in *D. coriaria* colonies are a cause for concern. Birken and Cloyd (2007) observed the presence of an astigmatid mite *Sancassania aff. sphaerogaster* (Acaria: Acaridae) in their rove beetle colonies and thought it likely that *D. coriaria* was feeding on the mites since *D. coriaria* is a generalist predator.

Compatibility with nematodes

Dalotia coriaria and entomopathogenic nematodes are both soil-dwelling organisms used as biological control agents to manage the same pests, i.e. thrips, fungus gnats, and shore flies. Both could be applied at the same time in augmentative biological control programs and come into contact with each other. A single study has tested the compatibility of the predatory beetle with only one species of nematode, *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae)

(Jandricic et al. 2006). Adult *D. coriaria* was not susceptible to *S. feltiae* but third instar mortality was dose dependent. Mortality at the highest dose rate of 50 IJ/cm² was 27%, which was significantly greater than the two lower rates and control (Jandricic et al. 2006).

Thesis Objectives

The development of adequate rearing techniques has been a consistent challenge for the development of augmentative biological control programs. This is especially true for growers that wish to rear biological control organisms in cases where specific natural enemies cannot be sourced reliably from commercial insectaries. Intraguild predation and other negative interactions are another area where research is needed.

The goals of my thesis project were to: 1. Determine if black soldier fly could be used as mass rearing host for four common entomopathogenic nematodes and 2. Test the compatibility of entomopathogenic nematodes with another soil-dwelling biological control organism *D. coriaria*.

Chapter 2. Exploring the use of black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae) as an *in vivo* entomopathogenic nematode rearing host

Introduction

Entomopathogenic nematodes have been used in biological control of insect pests for many years in multiple agricultural systems including: turfgrass, greenhouses, nurseries, mushrooms, and orchards (Grewal et al. 2005). However, nematodes have not seen widespread adoption in any of these systems. One of the factors limiting the use of nematodes is high production costs (Grewal et al. 2005; Shapiro-Ilan et al. 2014).

Entomopathogenic nematodes are reared either in large scale *in vitro* cultures or small scale *in vivo* operations. Rearing nematodes *in vitro* first requires the establishment of the symbiotic bacteria and nematodes in separate pure cultures, then inoculating a medium with the bacteria and nematodes, which requires high startup capital to purchase fermentation tanks (Shapiro-Ilan et al. 2014). *In vivo* production requires the use of insect hosts, since entomopathogenic nematodes are obligate parasites of insects. The free-living stage known as infective juveniles (or dauer larvae) enter insect hosts through natural openings – mouth, anus, and spiracles. Inside the insect heomcel, the juvenile nematodes release their symbiotic bacteria and kill the host within 24-48 h. Nematodes complete their development within the host, proceeding through one to three generations. When host resources are depleted, thousands of infective juveniles emerge from the

cadaver in search of a new host (Kaya and Gaugler 1993). *In vivo* production of nematodes is often done on a small scale suitable for cottage industry or niche markets (Shapiro-Ilan et al. 2014).

The most commonly used hosts for rearing nematodes *in vivo* are mature larvae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) and the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Shapiro-Ilan et al. 2002a; Shapiro-Ilan and Gaugler 2002). Farmers can purchase wax worms at local bait-and-tackle shops or both at pet stores. However they represent an additional cost to the farmer without providing any services beyond a rearing host. Black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae), larvae have a history of on-farm use and may serve as an alternative entomopathogenic nematode host.

Black soldier fly larvae are detritivores and have several agriculture uses including: manure management (Sheppard et al. 1994), house fly suppression (Sheppard 1983; Bradley and Sheppard 1984), composting (Newby 1997), and livestock feed (Newton et al. 1977; Newton et al. 2005). The black soldier fly life cycle begins with adult female flies laying eggs in dry cracks and crevices above food sources (Booth and Sheppard 1984). Larvae hatch from the eggs and feed on various manures – chicken, swine, dairy (Sheppard et al. 1994; Newton et al. 2005; Myers et al. 2008), carrion (Tomberlin et al. 2005), coffee grounds (Lardé 1990), and rotting vegetation (Newby 1997). Larvae develop through six instars within three weeks (Tomberlin et al. 2009). The last instar is a non-feeding wandering stage known as a prepupa (Sheppard et al. 1994). They are easily collected from feeding containers

as they seek out dry substrates for pupation (Newton et al. 2005; Sheppard et al. 1994). Adults live for about 10-14 d and do not feed (Tomberlin et al. 2002).

Black soldier fly are a potentially powerful nutrient management tool allowing nutrients to be cycled multiple times —feed larvae organic farm wastes then feed reared larvae to livestock (Newton et al. 2005; Sheppard et al. 1994). A small number of larvae could be diverted from livestock feed to rear entomopathogenic nematodes. Nematodes could then be used to control various insect pests (e.g. fungus gnats, thrips, weevils, white grubs, etc.) (Grewal et al. 2005). The goal of this research was to determine the feasibility of using black soldier fly as an entomopathogenic nematode rearing host.

In preliminary experiments infective juveniles emerged from only a few black soldier fly cadavers (Tourtois and Grieshop, unpublished data); however, upon dissecting the cadavers thousands of dead juveniles were found inside the cadaver. The tough, calcium carbonate-studded larval cuticle may be presenting a two-fold problem to the infective juveniles entering and leaving the host (Johannsen 1922; Newton et al. 1977). Black soldier fly larvae have fewer spiracles than *G. mellonella*, thus less natural openings for nematodes to enter and exit. Based on preliminary results, injuring the black soldier fly larvae greatly increased the infectivity of *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) (Tourtois and Grieshop, unpublished data).

The first objective was to determine which instars of black soldier fly are susceptible to four commonly available species of entomopathogenic nematodes; *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhbditidae), *Steinernema*

carpocapsae (Weiser) (Rhabditida: Steinernematidae), *S. feltiae* (Filipjev), and *S. riobrave* Cabanillas, Poinar & Raulston. The second objective was to determine if injuring the black soldier fly changed the infectivity of entomopathogenic nematodes. The third objective was to determine if damaging the cadavers affects the number of infective juvenile nematodes that emerge.

Methods and Materials

Black Soldier Fly colony

Black soldier fly larvae were originally obtained from a commercial source (Compost Mania, North Carolina). Larvae were reared in batches of 300 individuals in 946 ml plastic deli containers (WNA, Chattanooga, TN). The deli containers were covered with a brown paper towel held in place with a rubber band. Larvae were fed 10 g of Gainesville house fly diet (5:3:2 ratio of wheat bran, alfalfa meal and cornmeal) (Hogsette 1992) and 17 ml of water, 5 days a week (Tomberlin et al. 2002). In 2013, larvae were reared in a growth chamber at $25.5 \pm 3.8^\circ\text{C}$, $79 \pm 12.1\%$ RH, and in the dark (0:24, light: dark). Rearing containers were placed in aluminum baking pans (49 by 30 by 7.5 cm, GFS, Grand Rapids, MI) to catch escapees. In 2014, larvae were reared in an insectary room at $25.6 \pm 1.1^\circ\text{C}$ and $44 \pm 15.9\%$ RH. An additional aluminum-baking pan was placed over rearing containers to block light. Feeding stopped when >50% of the larvae had molted to prepupa. Rearing containers remained in the growth chamber or insectary for an additional week or until larvae started to pupate.

Prepupae along with digested food were dumped into one of two aluminum baking pans (30 by 23.5 by 6 cm, GFS, Grand Rapids, MI) in a screened cage (60 by

60 by 60 cm, BioQuip, Rancho Dominguez, CA) in a greenhouse ($24.9 \pm 3.8^{\circ}\text{C}$, $35.8 \pm 15.8\%$ RH, and natural light cycle). Prepupae pupated in the baking pans and emerged as adults. No food was provided to the adults. Water was misted onto the top of the screen cage with a mist nozzle at least once a day, several times a week. Deli containers with 1 cm diameter cardboard rolls suspended above wet Gainesville fly diet (30 g diet mixed with 75ml of water) was included in the cage for adult females to lay eggs (Sheppard et al. 2002).

Entomopathogenic Nematode colonies

Heterorhabditis bacteriophora Oswego strain was obtained from Anne Nielsen, Rutgers Agriculture Research and Extension Center, Bridgeton, NJ. *Steinernema carpocapsae* and *S. feltiae* were obtained from BeckerUnderwood (Ames, IA). *Steinernema riobrave* 355 strain was obtained from David Shapior-Illan, USDA-ARS, Byron, GA. The four nematode species were reared on the host *G. mellonella* in inverted Petri dishes (Kaya and Stock 1997). *Galleria mellonella* was purchased from a local bait shop and a pet store. Approximately 100 infective juveniles in 500 μl of water were applied to five *G. mellonella* on filter paper in one Petri dish. One week later, infected cadavers were transferred to a White trap (White 1927). Harvested infective juveniles were stored in tissue culture flasks in laboratory drawers at ambient room temperature. Each nematode species was stored in a separate cabinet drawer under 24 h dark conditions. All infective juveniles used in the experiments described below were less than 14 days old.

Experiment 1a – black soldier fly susceptibility by larval stage

A 5x5 two-way factorial experiment was set-up to test the susceptibility of black soldier fly instars to multiple species of entomopathogenic nematodes. The levels of the first factor were multiple instars (2, 4, 5, 6) of black soldier fly plus the positive control –late instar *G. mellonella*. The levels of the second factor were four species of nematodes (*H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave*) plus a negative control –water without nematodes. There was a total of 25 treatment combinations.

Fourth through sixth instars were refrigerated at $9.9 \pm 2.4^{\circ}\text{C}$ and $84.9 \pm 12.0\%$ RH for 11 d until second instars arrived. Larvae were separated from feed by dumping the contents of a rearing container out onto a cafeteria tray and picking larvae out by hand. They were washed by placing them into #5 soil sieve, submerging in tub of tap water, and swirling around to wash away pieces of wheat bran. Larvae were transferred on to brown paper towel to dry. Larvae were distributed to inverted 60 mm diameter Petri dish containing one piece of No. 1 Whatman filter paper, one larva per dish. Using a micropipette, 100 infective juveniles in 500 μl of deionized water were applied to the filter paper. A total of 25 replicates were arranged on five cafeteria trays (block) with five replicates of each treatment combination on every tray. Each block was a shelf in the growth chamber ($20.4 \pm 0.6^{\circ}\text{C}$, $70.4 \pm 10.4\%$ RH, no light, model I-35L, Percival, Perry, IA). A HOBO® data logger (model U23-001, Onset Computer Corporation, Bourne, MA) was used to verify the environmental conditions in the growth chamber.

Insect mortality was assessed daily for 8 d. It was difficult to assess black soldier fly larvae mortality because they did not immediately respond to handling even when they were alive. If a larva was actively crawling about the Petri dish arena, then it was recorded as alive. If the larva was not active, the bottom of the inverted Petri dish was used to roll the larva over onto its dorsal side and gently pressed down. If the larva responded by rolling back over onto its ventral side, then it was recorded as alive. If the larva did not respond to manipulation and felt soft when squeezed, it was recorded as dead. If we were not certain that the larva was dead, it was recorded as alive.

On the eighth day, any insect that was not actively moving about its Petri dish arena was selected and frozen at -20°C for later dissection. At least one insect from each treatment and block combination was selected. To estimate the number of founding nematodes, cadavers were dissected in deionized water under a dissecting microscope.

Experiment 1b – black soldier fly susceptibility at pupal stage

Black soldier fly pupae were not available at the same time as the larvae; therefore, a separate experiment was set-up to test the susceptibility of the pupal stage to entomopathogenic nematodes. A simpler 2x5 factorial experiment was set-up. The levels of the first factor were black soldier fly pupae and the positive control – *G. mellonella*. The levels of the second factor were four species of nematodes (*H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave*) plus a negative control – water without nematodes. There was a total of 10 treatment combinations.

Statistical Analysis

All statistical analyses were performed in the R statistical language (version 3.1.1, R Core Team 2014). *Galleria mellonella* was excluded from all statistical analyses since they were only included in the experiment design to verify that the nematodes were indeed infective. Insect mortality was corrected using Abbott's formula (Abbott 1925). Any values calculated as less than zero, were entered as zero. Due to high mortality in the control treatment on day 8, mortality for the second instars was compared using mortality from day 7. Mortality was calculated as a proportion for each block. Data was transformed by taking the arcsine of the square root to fit ANOVA assumptions of normality and variance and analyzed using a two-way ANOVA model. Tukey HSD was used to separate means, p-value < 0.05. Percent infected hosts and the number of nematodes per host were analyzed using Kruskal-Wallis test since data did not meet assumptions of normality.

Experiment 2 –effect of larval injury on nematode infection

Based on the results from the previous experiment, fifth instars were selected for all following experiments. A 3x5 two-way factorial experiment was set-up to test whether injuring the black soldier fly larvae affects nematode infectivity. The levels of the first factor were injured black soldier fly larvae, non-injured black soldier fly larvae, and the positive control *G. mellonella*. The levels of the second factor were four species of entomopathogenic nematodes (*H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave*) plus a negative water control. There were a total of 15 treatment combinations. Five replicates of each treatment were represented in each of eight blocks for a total of 40 replicates for each treatment.

The experiment took place in a growth chamber ($24.7 \pm 0.8^\circ\text{C}$, $96.0 \pm 4.8\%$ RH, no light, model I-41VL, Percival, Perry, IA) and blocked by space (two blocks per shelf). HOBO® data loggers (model U23-001, Onset Computer Corporation, Bourne, MA) were used to verify the environmental conditions in the growth chamber.

Petri dish infection arenas were prepared by inserting 60 mm diameter No. 1 Whatman filter paper circles into 60 mm diameter inverted Petri dishes. Infective juveniles (1000) were applied to the filter paper in 500 μl . Negative controls received just 500 μl of water. Meanwhile, black soldier fly larvae were separated from feeding material and washed in a tub of lukewarm tap water. Under a dissecting microscope, a size 0 insect pin was used to puncture two holes in the cuticle on the ventral lateral edges of nine segments – the mesothorax, metathorax, and first seven abdominal segments. A total of 18 holes were punctured into each larva. After the puncturing operation, larvae were immediately transferred onto the prepared Petri dishes. Insect pins were sterilized by boiling in water for 1 min and storing in 80% ethanol. Non-injured larvae and *G. mellonella* were placed into appropriate Petri dishes at the same time. One block of replicates was completed before proceeding onto the next block.

Insect mortality was assessed as described above in experiment 1. Any larvae that were alive and previously recorded as dead were reevaluated daily. On the fifth day of mortality assessment, all dead individuals plus at least one replicate from each control block was frozen (-20°C) to arrest nematode development until dissection.

Cadavers were washed in water to remove exterior nematodes and dissected in Ringers solution (Kaya and Stock 1997) under a dissecting microscope. Nematodes were counted. Negative controls were dissected to verify a lack of nematode contamination.

Statistical Analysis

Survival analysis of the black soldier fly was completed using Cox proportional hazard function in R statistical language (version 3.1.1, R Core Team 2014). Factors included in the model were injury treatment, nematode species, and the two-way interaction term. Blocking was not significant. A logistic regression was used to test the effect of injury treatment and nematode species on the number of infected larvae. The number of nematodes recovered from cadavers was $\log(1+x)$ transform to meet normality assumptions and compared among treatments in a two-way ANOVA. Tukey HSD was used for mean separation with $\alpha = 0.05$. *Galleria mellonella* were excluded from all analyses.

Experiment 3 – nematode production in black soldier fly

A 5x5 two-way factorial experiment was conducted to test the following objectives: (1) determine if damaging the black soldier fly cadavers one week post-infection affects infective juvenile emergence and (2) compare the number of infective juveniles that emerge from black soldier fly versus *G. mellonella*. The five levels of the first factor were non-injured larvae, larvae injured prior to nematode application, cadavers injured after infection, larvae injured both before and after infection, and non-injured *G. mellonella*. Henceforth, injury that occurred

immediately before nematode application will be referred to as pre-infection injury; damaged done to the cadavers one week after the start date will be referred to as post-infection injury; the combination of both treatments will be referred to as pre+post-infection injury. The five levels of the second factor were the four nematode species (*H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave*) and water as a negative control. There was a total of 25 treatments.

Larvae (25 per treatment) were weighed. Fifth instar black soldier fly were injured as described in experiment 2. Larvae were placed individually on a piece of filter paper in an inverted 60 mm diameter petri dish with 1000 infective juveniles and held in a growth chamber ($24.8 \pm 0.2^{\circ}\text{C}$, $92.0 \pm 13.5\%$ RH, no light, model I-41VL, Percival, Perry, IA). HOBO® data loggers (models U23-001 and UA-002-08, Onset Computer Corporation, Bourne, MA) were used to verify the environmental conditions in the growth chamber.

Insect mortality was assessed daily for 5 d. On day seven, black soldier fly cadavers were damaged in the post-infection injury treatments with an insect pin. They were damaged in the same manner as the larvae were as described in experiment 2. At this time, all cadavers were transferred to a White trap to collect emerging juveniles (White 1927). White traps were checked daily for juveniles. On the first day that juveniles appeared in the White trap, they were harvested into 30 ml tissue culture flasks (Corning Inc., Tewksbury, MA). Subsequent harvesting occurred every other day until no more juveniles were present in the White trap or the end date of the experiment. Cadavers were collected and frozen three days after juveniles were no longer emerging from the cadaver or at the end of the experiment,

which was 28 d from the start date (i.e. cadavers were observed for three weeks for infective juveniles).

Tissue culture flasks were filled to the 30 ml fill mark and laid down on an orbital shaker (model S500, VWR, Radnor, PA) at speed 2 for at least 10 sec to uniformly suspend nematodes in water. Six subsamples (50 – 3000 μ l) were transferred to 60 mm Petri dishes with a micropipette. Infective juveniles were counted under a dissecting microscope.

Statistical Analysis

Survival analysis of the black soldier fly was tested using Cox proportional hazard function in the survival package version 2.37-7 of R 3.1.1 (R Core Team 2014; Therneau 2000). The factors included in the model were injury treatment, nematode species, and the two-way interaction term. A two-way ANOVA to compare the number of harvested juveniles from black soldier fly and *G. mellonella*.

Nematode harvest data was standardized to facilitate direct comparison among individuals of different mass by dividing the harvested juveniles by the mass of the host. Data were transformed ($\log(1+x)$) to meet model requirements of normality and homoscedasticity and fitted to a two-way factorial ANOVA consisting of injury treatment, nematodes species, and the two-way interaction term. Tukey's HSD was used to separate treatment means with a critical value = 0.05.

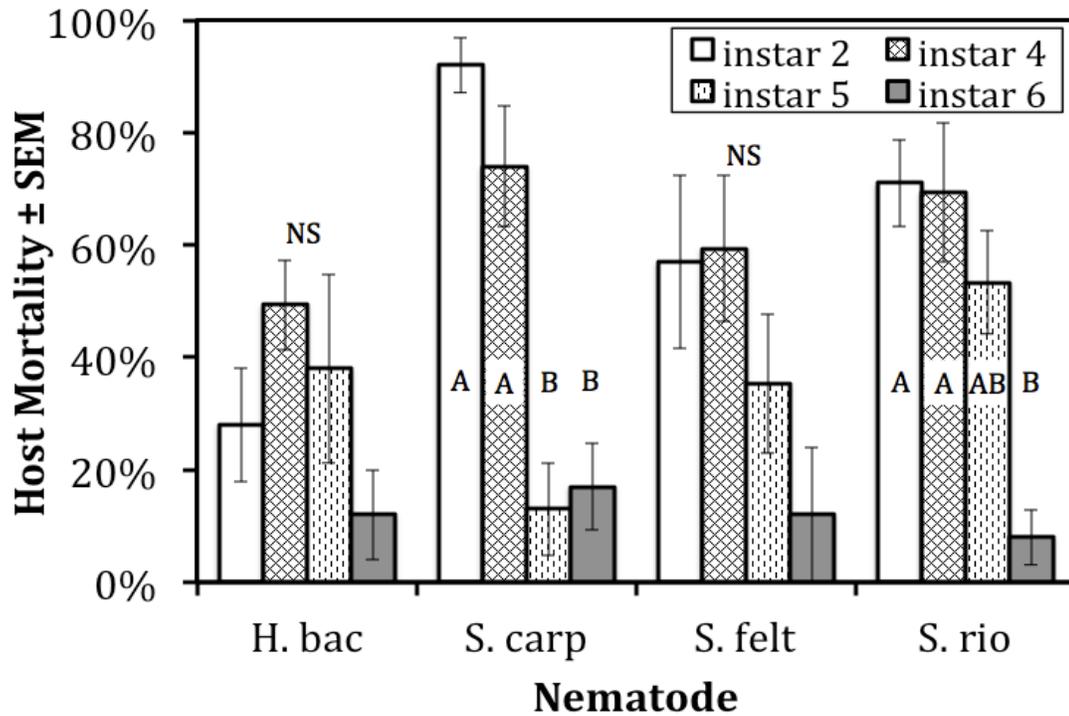


Figure 2-1. Abbott's corrected mortality for black soldier fly larvae. Nematodes applied at 100 infective juveniles per host at 20°C. Mortality for the second instar is from day 7 since there was high mortality on day 8 in the control. *G. mellonella* mortality was >95% for all nematode species and 24 ± 10% for the control on day 5 (data not shown). Bars with different letters indicate statistical difference within nematode treatment (ANOVA, Tukey HSD, $p < 0.05$). NS = no statistical difference.

Results

Experiment 1a – black soldier fly susceptibility by larval stage

Black soldier fly mortality was stage-dependent ($F = 20.994$, $df = 3$, $p\text{-value} < 0.001$) and there was an interaction between nematode species and black soldier fly instar ($F = 2.217$, $df = 9$, $p\text{-value} = 0.032$). Mortality was not significantly different among instars when infected with *H. bacteriophora* or *S. feltiae* (Fig. 2-1). Second instars ($92\% \pm 5\%$ SEM) and $74\% \pm 11\%$ SEM of fourth instars were killed by *S. carpocapsae*, which was significantly more than fifth and sixth instars, $13\% \pm 8\%$ SEM and $17\% \pm 8\%$ SEM, respectively (Fig. 2-1). Of the larvae treated with *S. riobrave*, $71\% \pm 8\%$ SEM of the second instars and $69\% \pm 12\%$ SEM of the fourth instars died, which was significantly more than sixth instars ($8\% \pm 5\%$ SEM), but not significantly different than fifth instars ($53\% \pm 9\%$) (Fig. 2-1). Second instars were most susceptible to *S. carpocapsae* ($92\% \pm 5\%$ SEM), intermediate susceptible to *S. feltiae* and *S. riobrave* ($57\% \pm 15\%$ SEM and $71\% \pm 8\%$ SEM, respectively), and less susceptible to *H. bacteriophora* ($28\% \pm 10\%$ SEM). Fourth through sixth instar susceptibility did not vary by nematode species.

Table 2-1. Mean \pm SEM adult nematodes recovered from four black soldier fly instars (n=25 per treatment combination) in susceptibility bioassay. Infective juveniles (100) applied per host at 20°C. None of the negative controls were infected. Data not shown for *G. mellonella*. Amounts with different letters indicate statistical difference within nematode treatment (Kruskal-Wallis, $p < 0.05$). ns = no statistical difference.

Nematode	Host instar	Percent Infected \pm SEM	Nematodes \pm SEM
<i>H. bacteriophora</i>	2	0.0 \pm 0.0 ns	0.0 \pm 0.0ns
	4	8.0 \pm 4.9	2.5 \pm 0.4
	5	4.0 \pm 4.0	4.0 \pm 0.0
	6	8.0 \pm 8.0	2.0 \pm 0.7
<i>S. carpocapsae</i>	2	32.0 \pm 4.9a	6.0 \pm 0.7ns
	4	12.0 \pm 4.9a	1.7 \pm 0.4
	5	0.0 \pm 0.0b	0.0 \pm 0.0
	6	8.0 \pm 4.9a	2.0 \pm 0.0
<i>S. feltiae</i>	2	12.0 \pm 4.9ns	1.0 \pm 0.0ns
	4	4.0 \pm 4.0	2.0 \pm 0.0
	5	0.0 \pm 0.0	0.0 \pm 0.0
	6	12.0 \pm 4.9	1.7 \pm 0.2
<i>S. riobrave</i>	2	12.0 \pm 8.0ns	5.0 \pm 2.0ns
	4	4.0 \pm 4.0	1.0 \pm 0.0
	5	8.0 \pm 4.9	1.0 \pm 0.0
	6	0.0 \pm 0.0	0.0 \pm 0.0

Nematodes were only recovered from a few black soldier fly cadavers (Table 2-1). No *H. bacteriophora* nematodes were recovered from the second instars. Nematodes were found in the fourth through sixth instars, but the mean was four or less nematodes from one or two cadavers. For *S. carpocapsae*, an average of six nematodes were recovered from eight second instars, which was significantly more than the two nematodes recovered from three fourth-instars and two sixth-instars. No *S. carpocapsae* nematodes were recovered from fifth instars. For *S. feltiae*, only one nematode was recovered from three second instars, two nematodes from one fourth instar, and a mean of 1.7 nematodes from three sixth instars. No nematodes were found in the fifth instars. A mean of five *S. riobrave* were recovered from three second instars, which was not significantly different from the one nematode in one fourth instar and one nematode each in two fifth instars. No nematodes were recovered from sixth instars. *Heterorhabditis bacteriophora* infected 56% of *G. mellonella*, *S. carpocapsae* infected 96%, *S. feltiae* infected 48%, and *S. riobrave* infected 72% of the *G. mellonella*. No nematodes were recovered from the untreated controls.

Experiment 1b – black soldier fly susceptibility at pupal stage

Nematodes were not recovered from any pupae. Pupae (18%) completed metamorphosis and emerged as adults.

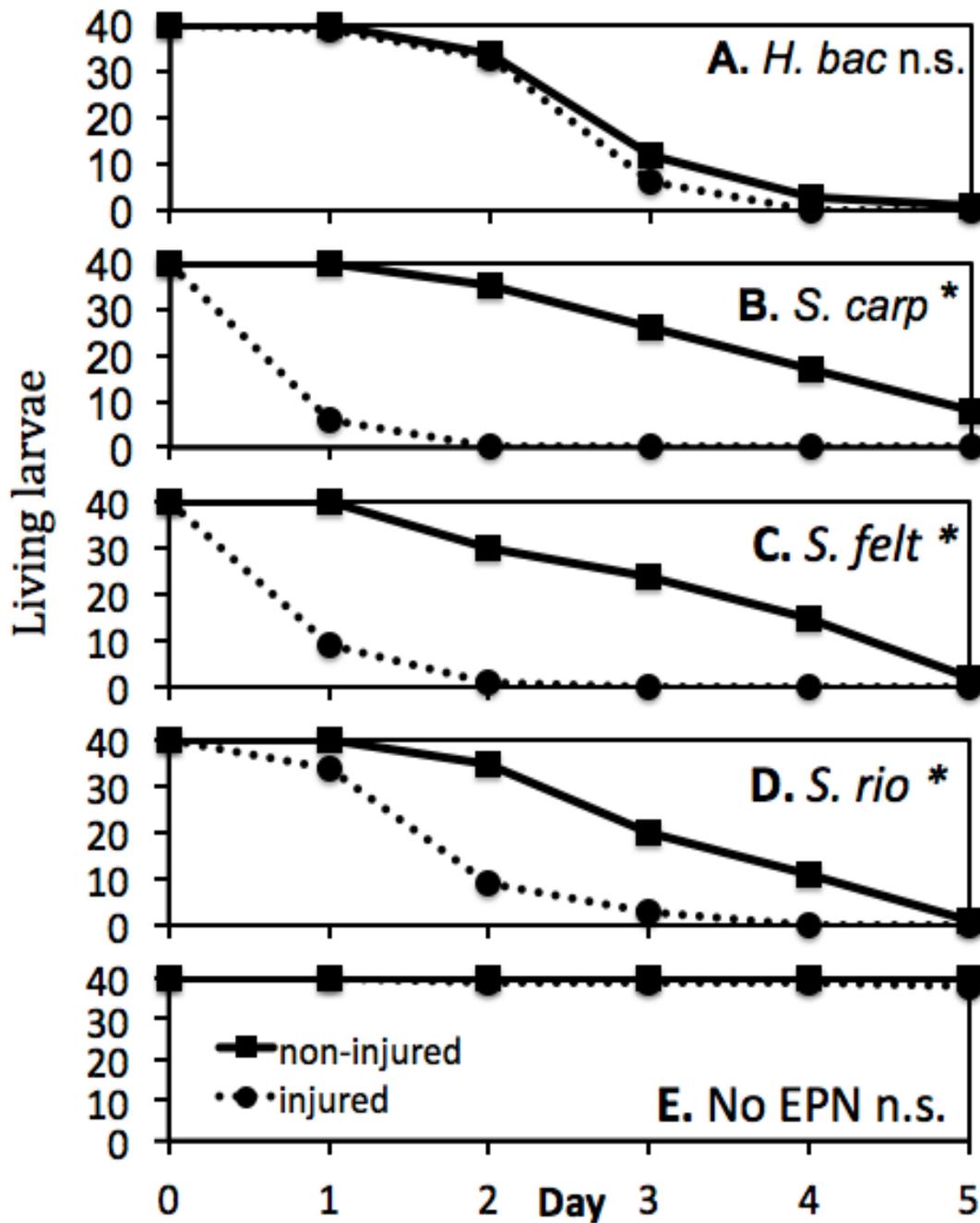


Figure 2-2. Survival curves for injured (dotted line) and non-injured (solid line) fifth instar black soldier fly for each nematode species: A) *H. bacteriophora*, B) *S. carpocapsae*, C) *S. feltiae*, D) *S. riobrave*, and E) control (no nematodes). Infective juveniles (1000) applied per host at 25°C. All *G. mellonella* treated with nematodes were dead on day 2 and only 5 *G. mellonella* died in the control treatment (data not shown). Asterisk (*) denotes significant difference ($p < 0.05$). n.s. = not statistically different.

Experiment 2 – effect of larval injury on nematode infection

Insect mortality was significantly affected by injury treatment and nematode species ($\chi^2 = 37.2$, $df = 1$, $p < 0.001$, $\chi^2 = 443.8$, $df = 4$, $p < 0.001$, respectively).

Injuring the larvae did not consistently increase infection by nematode species ($\chi^2 = 113.2$, $df = 4$, $p < 0.001$). Injuring the black soldier fly larvae increased the mortality rate when *Steinernema spp.* was applied but not *H. bacteriophora* (Fig. 2-2). For *H. bacteriophora*, the injured larvae did not die any faster than the non-injured larvae (Fig. 2-2A) ($\chi^2 = 3.5$, $df = 1$, $p = 0.06$). On day 5, all of the injured larvae and all but one non-injured larvae were dead (Fig. 2-2A). For *S. carpocapsae*, all of the injured larvae were dead by day 2 whereas only five of the non-injured larvae were dead (Fig. 2-2B) ($\chi^2 = 78.6$, $df = 1$, $p < 0.001$). On day 5, 32 of the non-injured larvae were dead (Fig. 2-2B). A similar pattern was observed for *S. feltiae*. Only one injured larva was still alive on day 2 whereas 30 non-injured larvae were still alive (Fig. 2-2C) ($\chi^2 = 65.4$, $df = 1$, $p < 0.001$). All but two non-injured larvae died by day 5 (Fig. 2C). For *S. riobrave*, 31 injured larvae and five non-injured larvae were dead on day 2 and 40 injured larvae and 39 non-injured larvae were dead on day 5 (Fig. 2-2D) ($\chi^2 = 38.3$, $df = 1$, $p < 0.001$). Only two injured larvae and none of the non-injured larvae died by the fifth day in the untreated control (Fig. 2-2E) ($\chi^2 = 2$, $df = 1$, $p = 0.155$). All of the *G. mellonella* treated with nematodes were dead on day 2, and only five *G. mellonella* died in the untreated control.

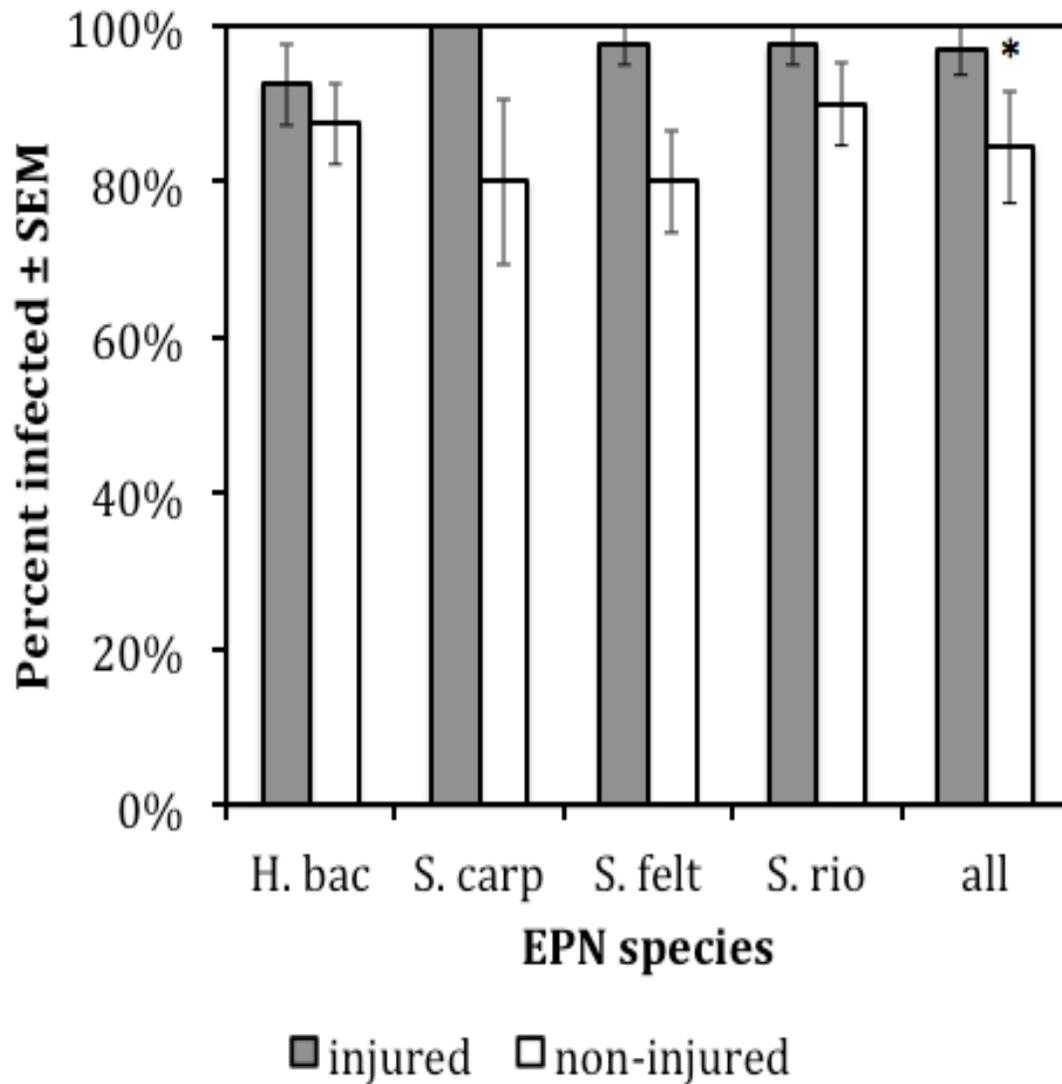


Figure 2-3. Percent fifth instar black soldier fly infected with entomopathogenic nematodes (n=40). Infective juveniles (1000) applied per host at 25°C. Asterisk (*) denotes significant difference ($p < 0.05$).

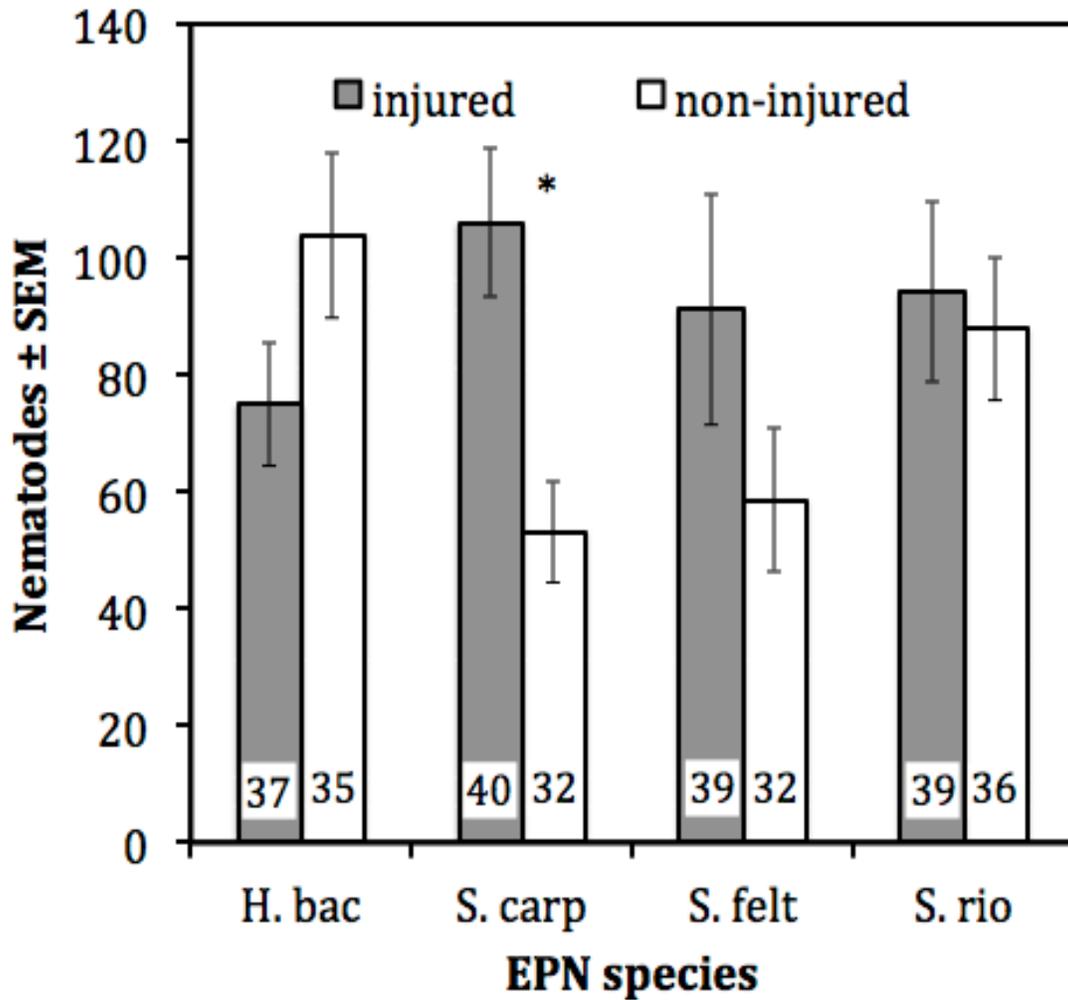


Figure 2-4. Mean number of entomopathogenic nematodes (first generation adults and juveniles) recovered from fifth instar black soldier fly cadavers. Data not shown for *G. mellonella*. Infective juveniles (1000) applied per host at 25°C. Numbers within the bars indicate number of infected larvae (max=40). Asterisk (*) denotes significant difference ($P < 0.05$).

Injuring the larvae significantly increased larval infection by nematodes from 84% to 97% ($p < 0.001$) (Fig. 2-3). Five days post infection 6.3%, 1.3%, 8.8%, and 84% of non-injured larvae treated with nematodes were: alive, alive with nematodes, dead without nematodes, and dead with nematodes, respectively. Five days post infection 3.1% and 96.9% of injured larvae treated with nematodes were: dead without nematodes or dead with nematodes, respectively. Infection rate did not differ by nematode species ($p = 0.76$). The interaction term was not significant ($p = 0.14$).

Neither the main effects of injury nor nematode species were significant for the number of nematodes found in the infected larvae ($F = 1.12$, $df = 1$, $p = 0.29$ and $F = 1.95$, $df = 3$, $p = 0.12$, respectively), however, a significant interaction was detected ($F = 5.47$, $df = 3$, $p = 0.001$). Injuring the larvae increased the number of recovered nematodes only for *S. carpocapsae*, but not for any of the other nematode species ($p = 0.047$) (Fig. 2-4). Blocking was significant and retained in the model. Not all of the *G. mellonella* were dissected, but at least one from each block was dissected to verify nematode infection. All of the *G. mellonella* that were treated with nematodes were infected. None of the dissected *G. mellonella* from the negative control ($n=13$) were infected.

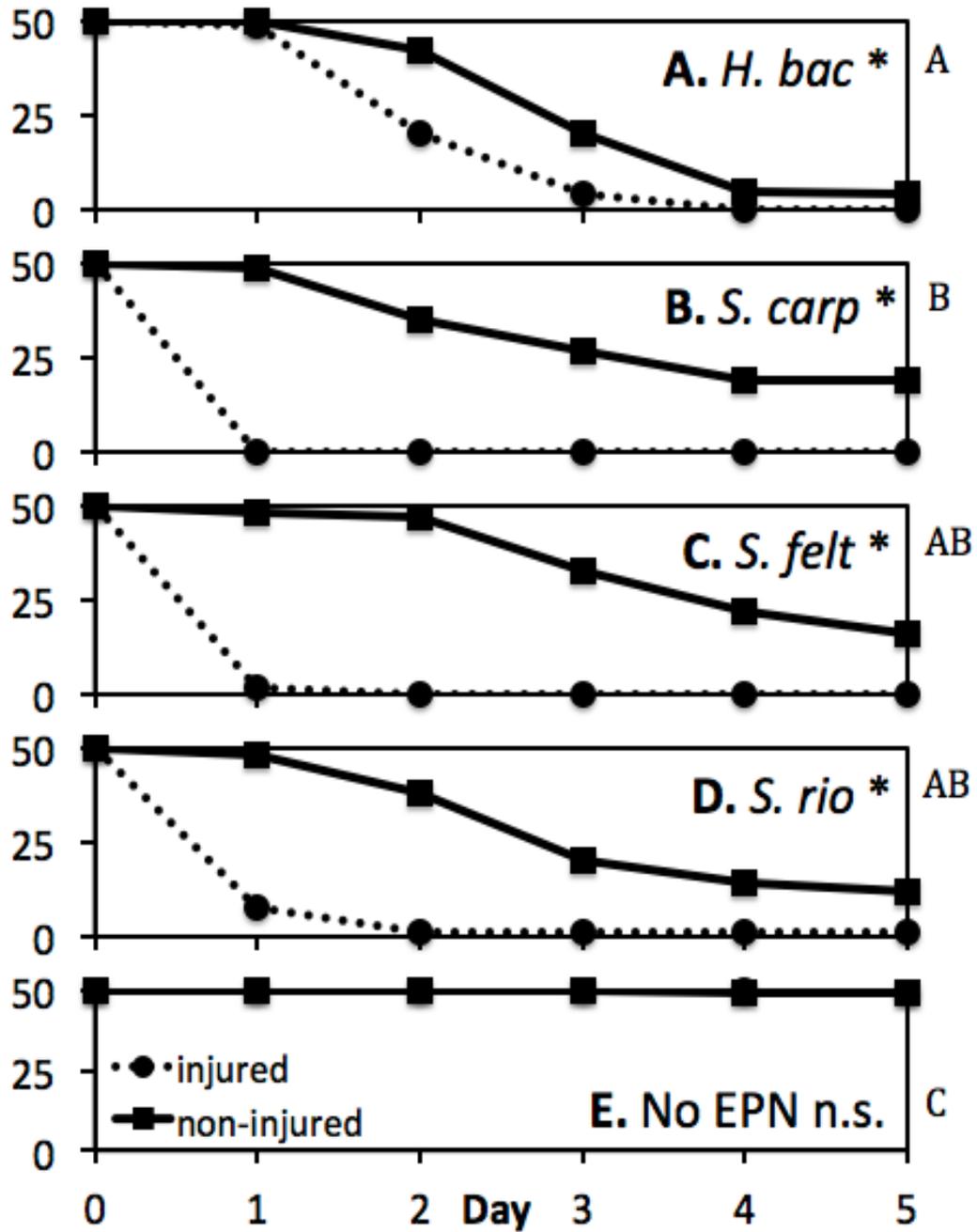


Figure 2-5. Survival curves for injured (dotted line) and non-injured (solid line) fifth instar black soldier fly before nematode application for each nematode species: A) *H. bacteriophora*, B) *S. carpocapsae*, C) *S. feltiae*, D) *S. riobrave*, and E) control (no nematodes). Infective juveniles (1000) applied per host at 25°C. All *G. mellonella* treated with nematodes were dead on day 2 and none died in the control treatment (data not shown). Asterisk (*) denotes significant difference ($p < 0.05$) for injury treatments, n.s. = not statistically different. Plots with different letters are significantly different ($p < 0.05$).

Experiment 3 – nematode production

Injuring the fifth instar black soldier fly in this experiment showed the same mortality response as in the previous experiment. Injuring the black soldier fly was significant ($\chi^2 = 85.4$, $df = 3$, p -value < 0.001). The nematode species also had a significant effect on mortality ($\chi^2 = 493.2$, $df = 4$, p -value < 0.001). The two-way interaction term was also found to be significant ($\chi^2 = 124.5$, $df = 12$, p -value < 0.001).

As expected, the survival rate of the non-injured larvae (42%, 95% CI [34%, 51%]) did not significantly differ from the larvae injured post-infection (38%, 95% CI [31%, 48%]). Likewise, the larvae injured pre-infection (19%), 95% CI [13%, 28%]) did not survive longer than the larvae injured pre+post-infection (21%, 95% CI [15%, 29%]). The larvae injured pre-infection and pre+post-infection died significantly sooner than the larvae not injured before infection (i.e. post-infection and non-injured larvae). This response was consistent with the pre-infection injury experiment (Experiment 2).

For the negative control, only 2% of the injured and 2% of the non-injured larvae died (Fig. 2-5E). This is significantly different from all of the nematode treatments (95% CI [95%, 100%]). Only 4% (95% CI [1.5%, 10.4%]) of the larvae survived when treated with *H. bacteriophora* (Fig. 2-5A) and injury significantly decreased their survival ($\chi^2 = 25$, $df = 1$, p -value < 0.001). In contrast, 19% (95% CI [12.7%, 28.5%]) of larvae survived when treated with *S. carpocapsae*, significantly more than those treat with *H. bacteriophora* (Fig. 2-5B). Furthermore, all of the injured larvae treated with *S. carpocapsae* died within the first 24 h, while only one

non-injured larva died within 24 h. By day 5, 62% of the non-injured larvae treated with *S. carpocapsae* died, significantly fewer than the injured larvae ($\chi^2 = 95.1$, $df = 1$, p -value < 0.001). Sixteen percent (95% CI [10.2%, 25.1%]) of the larvae treated with *S. feltiae* survived until day 5 (Fig. 2-5C). This is not significantly different from *H. bacteriophora* or *S. carpocapsae*. Within the first 48 h, 100% of the injured larvae treated with *S. feltiae* died, but only three non-injured larvae died. By day 5, only 32% of the non-injured larvae treated with *S. feltiae* died, significantly fewer than the injured larvae ($\chi^2 = 96.4$, $df = 1$, p -value < 0.001) (Fig. 2-5C). For larvae treated with *S. riobrave*, the survival rate was 13% (95% CI [7.8%, 21.6%]), which was not significantly different from any of the other nematode species (Fig. 2-5D). On day 2 only 24% of the non-injured larvae treated with *S. riobrave* died, whereas 98% of the injured larvae died. Even by day 5, only 76% non-injured larvae treated with *S. riobrave* died. The survival curve of the non-injured larvae treated with *S. riobrave* was significantly different from the injured larvae ($\chi^2 = 67.9$, $df = 1$, p -value < 0.001) (Fig. 2-5). As expected, 100% of *G. mellonella* treated with nematodes died within 48 h while no *G. mellonella* died in the negative control.

Galleria mellonella were 0.14 grams heavier than black soldier fly ($F = 637.2$, $df = 4$, $p < 0.001$). The mass of the black soldier fly was not different among injury treatments. The mean mass of black soldier fly larvae was 0.171 g with a range of 0.121 – 0.248 g. The mean mass of *G. mellonella* was 0.311 g with a range of 0.262-0.401 g.

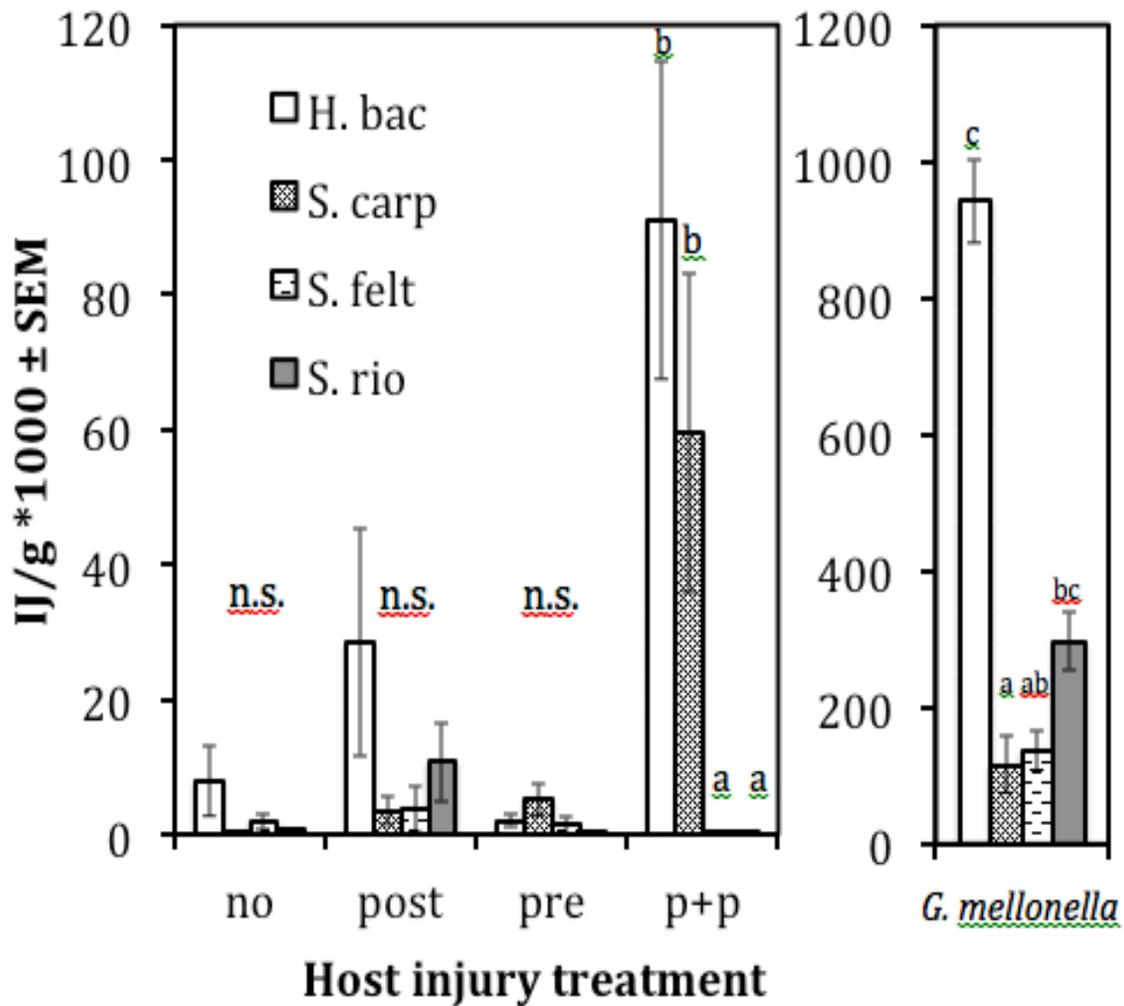


Figure 2-6. Mean number of infective juveniles harvested from fifth instar black soldier fly and *G. mellonella*. no = non-injured, post = damaged as a cadavers, pre = larva injured before nematode application, and p+p = pre and post. Bars labeled with different letters are significantly different, ANOVA, Tukey HSD, $\alpha = 0.05$, n.s. = not significantly different.

The main effect of host injury treatment significantly affected the amount of nematodes that were harvested ($F = 75.8$, $df = 4$, $p < 0.001$). At least 10 times more nematodes were produced per gram of *G. mellonella* than black soldier fly ($p < 0.001$) (Fig. 2-6). Injuring fifth instar black soldier fly post-infection but not pre-infection greatly increase nematode harvest. The post-infection injury increased the nematode harvest by a factor of 3.2 compared to no injury ($p = 0.048$) and by a factor of 2.7 compared to pre-infection injury ($p = 0.058$). The pre+post-infection injury increase nematode harvest by a factor of 4.1 compared to no injury ($p = 0.011$) and by a factor of 3.5 compared to the pre-infection injury ($p = 0.011$). The pre+post-infection injury treatment was not significantly different from the post-infection injury treatment ($p = 0.99$), nor was the pre-infection injury treatment different from no injury ($p = 0.99$).

The main effect of nematode species was significant ($F = 6.5$, $df = 3$, $p < 0.001$). More *H. bacteriophora* juveniles were harvested than all three *Steinernema* spp. for three of the four injury treatments ($p \leq 0.04$) (Fig. 2-6). None of the *Steinernema* spp. were significantly different from each other ($p \geq 0.47$).

The two-way interaction term between injury treatment and nematode species was significant ($F = 9.2$, $df = 12$, $p < 0.001$). The amounts of nematodes are reported in infective juveniles per gram of host. The harvested nematodes (< 30,000 per gram of fifth instar black soldier fly) from the no-, pre-, and post-infection injury treatments did not differ by nematode species (Fig. 2-6). *Heterorhabditis bacteriophora* ($91,084 \pm 23,592$) and $59,585 \pm 23,679$ *S. carpocapsae* from the pre+post-infection injury treatment were not significantly different for each other

($p = 1$)(Fig. 2-6). They were significantly greater than *S. feltiae* (68 ± 23) and *S. riobrave* (308 ± 224) ($p < 0.001$). The nematode species that was harvested the most from *G. mellonella* was *H. bacteriophora* ($941,884 \pm 60,963$). This was significantly different from *S. carpocapsae* ($116,247 \pm 41,167$) ($p < 0.001$) and *S. feltiae* ($137,666 \pm 28,984$) ($p < 0.01$), but not *S. riobrave* ($296,713 \pm 42,467$) ($p = 0.88$) (Fig. 2-6).

From the non-injured black soldier fly, $7,835 \pm 5119$ *H. bacteriophora* were harvested. Neither the pre-infection injury nor the post-infection injury treatments significantly altered the nematode harvest ($1,948 \pm 890$ and $28,302 \pm 16,848$, respectively) ($p \geq 0.84$) (Fig. 2-6). The pre+post-infection injury treatment significantly increased the amount of harvested nematodes ($91,084 \pm 23,592$) over the single injury treatments and the non-injury treatment ($p \leq 0.023$) (Fig. 2-6). *Galleria mellonella* produced 180 times more *H. bacteriophora* than any of the black soldier fly injury treatments ($p < 0.001$) (Fig. 2-6).

There were $116,247 \pm 41,167$ *S. carpocapsae* produced on *G. mellonella*. A similar amount ($59,585 \pm 23,679$) of nematodes was harvested from the pre+post-infection injury treatment to the black soldier fly ($p = 0.99$). The pre-infection, post-infection, and no injury treatments are not significantly different from each other ($p > 0.85$), but they all were significantly less than the pre+post-infection injury treatment ($p \leq 0.098$) (Fig. 2-6).

Galleria mellonella produced 100 times more *S. feltiae* per g of host than any of the black soldier fly injury treatments ($p < 0.001$). None of the injury treatments increased *S. feltiae* harvest. ($p \geq 0.93$) (Fig. 2-6).

The smallest number of *S. riobrave* were harvested from the pre+post-infection injury treatment (308 ± 224). This was not significantly different from the pre-infection (411 ± 136) or no injury (658 ± 178) treatments ($p \geq 0.15$), but it was significantly less than the post-infection injury treatment ($10,706 \pm 5,897$) ($p = 0.022$). The post-infection injury treatment was not significantly greater than the pre-infection or the no injury treatments ($p \geq 0.73$). There was 500 times more *S. riobrave* harvested from *G. mellonella* than any of the black soldier fly injury treatments ($p < 0.001$).

Discussion

The primary goal of this research project was to assess the feasibility of using black soldier fly larvae as a rearing host for entomopathogenic nematodes and whether physical modification of larvae could improve host quality. The appeal of using black soldier fly as a rearing host is that it has on-farm uses—including composting and livestock feed (Lardé 1990; Newby 1997; Newton et al. 1977; Newton et al. 2005; St-Hilaire et al. 2007), whereas the traditional nematode rearing hosts do not. The major conclusion of the study was black soldier fly were not susceptible to entomopathogenic nematodes (Fig. 2-1). Host modification improved infection rates, but it did not sufficiently improve host quality to use this insect as a rearing host. While this project did not develop a new nematode rearing host, it did raise some interesting questions regarding the biology of black soldier fly and the foraging strategies of entomopathogenic nematodes.

Black soldier fly's tough cuticle may prevent entry by invading infective juveniles as well as egress by infective juveniles resulting from previous infection.

Injuring black soldier fly larvae before nematode application increased their mortality (Figs. 2-2 and 2-5) and infection rates (Fig. 2-3). Surprisingly, these increases did not lead to increases in the number of infective juveniles harvested from black soldier fly larvae exposed to *Steinernema spp.* (Fig. 2-6). Post-infection injury of black soldier fly larvae increased the amount of *H. bacteriophora* and *S. carpocapsae* harvested (Fig. 2-6). Thus, compromising the black soldier fly larval cuticle varies the effect of entomopathogenic nematode reproduction by species.

Few published studies have reported the response of entomopathogenic nematodes to injured hosts. In a study with pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), the pupae were not infected by *S. carpocapsae* unless the cuticle was punctured (Lindgren et al. 1993; Henneberry et al. 1995). In the case of the present study, injuring the black soldier fly larvae was not necessary for nematode entry; however, it greatly increased nematode entry for *S. carpocapsae*.

One possible explanation for why host injury was not beneficial to *H. bacteriophora* (Figs. 2-2 and 2-5) is the presence of a dorsal tooth on the infective juveniles (Bedding and Molyneux 1982). The dorsal tooth aids them in entering a host's hemocoel by creating an entrance hole through the insect integument (Bedding and Molyneux 1982). However, Bedding and Molyneux (1982) also photographed that multiple nematodes entered the same hole. This is a bit paradoxical as it suggests that *H. bacteriophora* should be pre-adapted to utilizing wounds as entry points.

Mortality of black soldier fly was stage-dependent with the earlier instars more susceptible than later instars and pupae entirely resistant to infection. Other researchers have reported this trend in a variety of insects. For example, 100 day-old *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) larvae were less susceptible than 50-day-old or younger larvae to infection by *H. bacteriophora*, *H. indica* Poinar et al., and *S. riobrave* (Shapiro et al. 1999). First instar fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) were more susceptible than later instars and pupae to *S. carpocapsae* Mexican strain (Fuxa et al. 1988). Larger *S. littoralis* Boisduval larvae were less susceptible than smaller larvae to *S. carpocapsae* All and Mexican strain, *S. glaseri* (Steiner), and *H. bacteriophora* HP88 strain (Glazer 1992). Third instar *Liriomyza trifolii* (Diptera: Agromyzidae) was less susceptible to *S. carpocapsae* than second instars (LeBeck et al. 1993).

One hypothesis for this phenomenon is that later instars have a more developed immune system (Kaya 1990). Physical evidence of an immune response was observed in some of the black soldier fly larvae with the inside of the larvae taking on a gray appearance—an indication of activation of melatonin to encapsulate the nematode-bacteria complex (Dunphy and Thurston 1990). A formal conclusion on immune response would require a more detailed study.

Dissections of infected black soldier fly larva showed that 10% or less of the nematodes applied actually entered the larvae (Fig. 2-4). Other authors report higher rates of nematode recovery (40 to 45%) from *G. mellonella* (Fan and Hominick 1991; Grewal et al. 1994a), but recovery rates are rarely reported higher

than 45%. Hominick and Reid (1990) suggested a “phased-infectivity” hypothesis to explain this phenomenon.

The “Phased Infectivity” hypothesis posits that some infective juveniles have an innate non-infectious period and delay their infectiousness by entering a dormant period (Campbell et al. 1999; Hominick and Reid 1990). An alternative hypothesis for phased infectivity is that nematodes make infection decisions based on measures of expected host quality. Campbell et al. (1999) provide evidence against the phase-infective hypothesis for *S. carpocapsae*, *S. feltiae*, and *S. glaseri* and instead conclude that perceived phase-infectivity is a function of the number of hosts presented to a given population of infective juveniles. In contrast, the same experiment provides support for the phase infectivity hypothesis for *H. bacteriophora*. In the present study more *S. carpocapsae* and *S. feltiae* juveniles infected the more susceptible host. *Steinernema carpocapsae* (20%, 11%, and 5%) and 16%, 9%, and 6% of *S. feltiae* infected *G. mellonella*, injured and non-injured black soldier fly, respectively (Fig. 2-4). Likewise, 29%, 7.5%, and 10% of the *H. bacteriophora* responded to *G. mellonella*, injured and non-injured black soldier fly, respectively (Fig. 2-4). Since fewer juveniles responded to the black soldier fly than *G. mellonella*, this suggests that *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora* may be waiting for a more suitable host, i.e. infectiousness is host dependent. In contrast, similar amounts of *S. riobrave* (11%, 9%, and 9%) infected *G. mellonella*, injured and non-injured black soldier fly, respectively. Thus, the results from our trial further support Campbell et al.’s conclusions for *S. carpocapsae* and *S. feltiae* but not *H. bacteriophora* and suggest that *S. riobrave* may demonstrate phase infectivity.

Intraspecific competition may explain the relatively low production of the Steinernematids on *G. mellonella* (Fig. 2-6). Selvan et al. (1993) demonstrated that nematode production increases as the number of founding nematode increases up to about 100 nematodes per host, after which production falls more severely for *S. carpocapsae* than *H. bacteriophora*. *In vivo* production of *S. carpocapsae* and *H. bacteriophora* on *G. mellonella* is approximately 200,000 infective juveniles per host (Dutky et al. 1964; Flanders et al. 1996; Selvan et al. 1993). In the present study, 942,000 *H. bacteriophora* and only 116,000 *S. carpocapsae* infective juveniles per gram of host were produced in *G. mellonella* (Fig. 2-6). These results may indicate that *S. carpocapsae* and the two other *Steinernema spp.* are more sensitive to intraspecific competition than *H. bacteriophora*.

In conclusion, through the life history in the soil, black soldier fly larvae may have developed morphological –thick cuticle and fewer spiracles—and immunological adaptations to prevent infection from entomopathogenic nematodes. Higher mortality of the injured black soldier fly larvae was correlated with *Steinernema spp.*, but not *H. bacteriophora*. The success of rearing *S. carpocapsae* and *H. bacteriophora* on black soldier fly larvae can be increased by pre and post infection injury. *Steinernema carpocapsae*, *S. feltiae*, and *H. bacteriophora* may be better at searching for susceptible hosts than *S. riobrave*.

Chapter 3. Susceptibility of *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae) to entomopathogenic nematodes.

Introduction

Dalotia coriaria (Kraatz) (Coleoptera: Staphylinidae) and entomopathogenic nematodes are two soil-dwelling biological control agents used to manage common greenhouse pests including: thrips, fungus gnats, and shore flies. The use of biological control in greenhouses has become more desirable due to the development of pesticide resistance in pest populations (Shipp et al. 2007). Growers often use multiple natural enemies to achieve economic control but knowledge of interactions among natural enemies is lacking.

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is a major pest of vegetable production and floriculture in greenhouses and nurseries (Jensen 2000; Reitz 2009). Due to their cryptic behaviors (eggs laid in plant tissue, pupation occurs in the soil, and feeding on developing tissues) and their resistance to many insecticides, biological control has become increasingly important to successful western flower thrips management programs (Jensen 2000; Reitz 2009; Xu et al. 2006; Manners et al. 2013). Both entomopathogenic nematodes and *D. coriaria* are employed to target the soil-dwelling stage of western flower thrips (i.e. prepupae and pupae).

Fungus gnats *Bradysia spp.* (Diptera: Sciaridae) are also major pests in greenhouses. Fungus gnat larvae feed on many plant roots. Nematodes and *D.*

coriaria are recommended soil-dwelling biological control agents to target this pest. Five species of Heterorhabditis and four species of Steinernema have been tested for controlling fungus gnats (Gouge and Hague 1995; Harris et al. 1995; Jagdale et al. 2007). *Steinernema feltiae* is often the leading performer in controlling fungus gnats. Both larval and adult *D. coriaria* feed on fungus gnat eggs and larvae (Carney et al. 2002).

Dalotia coriaria is a small (3-4 mm), highly mobile, soil-dwelling polyphagous predator. Larvae are a pale yellow to cream color; and the adults are a glossy, dark color (Miller and Williams 1983). The body posture of the adults is typically S-shaped with their heads pointed down and their abdomens upturned. Both the larvae and adults are polyphagous – feeding on multiple life stages of mites and other insects (Miller and Williams 1983; Carney et al. 2002; Jandricic et al. 2006).

Adult *D. coriaria* are voracious predators and have been shown to consume as many as 95 second instar thrips, 78 thrips pupae, 154 fungus gnat eggs, or 150 first instar fungus gnats within 24 h (Carney et al. 2002). Third instar beetles can consume an equally impressive 100 eggs and 100 first instar fungus gnats in a 24 h period (Carney et al. 2002). In a laboratory bioassay, one adult rove beetle can consume 68 – 78% of the second and third instar fungus gnats presented to them in Petri dishes within 24 h (Echegaray Wilson 2012).

In a screened greenhouse trial with *Impatiens* (L.) (Ericales: Balsaminaceae), *D. coriaria* reduced western flower thrips populations by 53 – 82% (Bennison et al. 2008). On caged *Gerbera jamesonii* (Bolus ex Hook) (Asterales: Asteraceae) and *Chrysanthemums spp.* (Asterales: Asteraceae), *D. coriaria* did not reduce western

flower thrips populations (Manners et al. 2013). In a field experiment with five or 10 adult rove beetles per caged parsley *Petroselinum crispum* (Mill.) Nyman ex A. W. Hill (Apiales: Apiaceae) pot, there were 75% and 85% fewer fungus gnat adults on yellow sticky cards compared to the control over a 22 d period (Bennison et al. 2008). In a follow-up experiment, two adult rove beetles reduced the number of fungus gnats on yellow sticky cards by 48% (Bennison et al. 2009).

Entomopathogenic nematodes in the families Heterorhabditidae and Steinernematidae are soil-dwelling round worms that are obligate parasites of insects. Infective juveniles (IJ) (or dauer larvae) enter insect hosts through natural openings – mouth, anus, and spiracles. Inside the insect haemocoel, the juvenile nematodes release their symbiotic bacteria and kill the host within 24-48 h. Nematodes complete their development within the host, proceeding through one to three generations. When host resources are depleted, thousands of infective juveniles emerge from the cadaver in search of a new host (Kaya and Gaugler 1993). *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae), *S. feltiae* (Filipjev), and *S. riobrave* Cabanillas et al. are four commonly available entomopathogenic nematodes.

Heterorhabditis bacteriophora, *S. feltiae*, and *S. carpocapsae* can infect > 50% of second instar thrips and prepupae and 11% - 54.5% of the pupae (Ebssa et al. 2001). *Heterorhabditis* (12 strains) and 16 strains of *Steinernema* have been screened for infectivity in western flower thrips in several laboratory studies (Chyzik et al. 1996; Ebssa et al. 2001; Premachandra et al. 2004; Ebssa et al. 2004).

Thrips mortality ranged from 0% - 75%. Chyzik et al. (1996) found that *H. bacteriophora* HP88 was the most effective at reducing western flower thrips populations by 39%. Ebssa et al. (2001) reported > 50% thrips prepupae mortality for several nematode strains. They chose *H. bacteriophora* HK3, *S. feltiae* Sylt, and *S. carpocapsae* DD136 as the strain that caused the highest mortality for each species. Premachandra et al. (2003) chose *H. bacteriophora* HK3 and *S. feltiae* Nemaplus® as the most effective nematodes. Ebssa et al. (2004) identified *H. indica* Poinar, Karunakar & David and *S. bicornutum* Tallosi, Peters & Ehlers as the nematode species from each genus for causing the highest western flower thrips mortality. Thus, multiple nematode species and strains in both genera can be used for thrips management.

Entomopathogenic nematode host searching strategy is generally considered to be on a continuum from cruiser to ambusher (Kaya and Gaugler 1993; Lewis et al. 2006). Cruisers such as *H. bacteriophora* move through the soil in search of a host (Baweja and Sehgal 1997). Ambushers like *S. carpocapsae*, perform a behavior known as nictitating, where they elevate 95% of their body and wave back and forth, waiting for a host to pass by (Campbell and Gaugler 1993). Nematodes such as *S. feltiae* and *S. riobrave* are intermediate in their search strategy and display both behaviors (Grewal et al. 1994a). Ambushers are often applied to target mobile hosts and cruisers are often applied to target stationary hosts. Thus it is possible that, *D. coriaria*, which is highly mobile, may more likely be infected by an ambusher, *S. carpocapsae*, than the cruiser, *H. bacteriophora*.

Dalotia coriaria and entomopathogenic nematodes are both soil-dwelling organisms used as biological control agents to manage the same pests, i.e. western flower thrips, fungus gnats, and shore flies; therefore, both could be applied at the same time and come into contact with each other. To date a single study has tested the compatibility of the predatory beetle with only one species of nematode, *S. feltiae* (Jandricic et al. 2006). Biological control companies recommend the use of *S. feltiae* to control both thrips and fungus gnats; however, other nematode species have also been shown to be effective against western flower thrips. A handful of laboratory studies screened multiple nematode species against western flower thrips and found that nematode species such as *H. bacteriophora*, *H. indica*, and *S. bicornutum* killed similar numbers or more thrips prepupae than *S. feltiae* (Chyzik et al. 1996; Ebssa et al. 2004; Ebssa et al. 2001; Premachandra et al. 2004). Jandricic et al. (2006) showed that *S. feltiae* is capable of infecting third instar *D. coriaria* in a laboratory bioassay, but only 16% of the mortality confirmed nematode infection at the highest dose – 50 infective juveniles (IJ)/cm². In a microcosm bioassay, 100 IJ/cm² caused 25% mortality of third instars (Jandricic et al. 2006). The objective of this study was to determine the susceptibility of *D. coriaria* third instars and adults to four commonly used species of entomopathogenic nematodes: *H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave*.

Methods and Materials

A 4x3x2 factorial experiment was conducted to test the pathogenicity of four nematode species – *H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave* – at multiple doses – one-half, one, and two times the recommended rate of application

(BeckerUnderwood 2013)– for two life stages of *D. coriaria* —third instar and adult. There were 24 treatments plus two controls – adult and third instar beetles without nematodes.

Insect and Nematode Culture:

Laboratory colonies of *D. coriaria* were established with beetles purchased from BioBest (Leamington, Ontario) and Syngenta (Little Clacton, England). Beetles used for this study were either laboratory reared or purchased from IPM Laboratories Inc. (Locke, New York). Laboratory colonies were reared in two different plastic containers. One plastic container type was a 9.4 L capacity from Rubbermaid, (High Point, NC). Two ventilation holes (dia 7.62 cm) were drilled into the lid and covered with bridal veil. The other plastic container was a 2.25 L rectangle box from Ziploc (Racine, WI). Two ventilation holes (dia 2.54 cm) were drilled into the lid and covered with bridal veil. Both containers contained grounded coconut husk (coir) (Canna Continental, Los Angeles, CA) and vermiculite (Good Earth Horticulture, Inc. Lancaster, NY) (50:50 ratio) as a substrate. The larger container held 6 L of substrate, and the smaller container held 1 L of substrate. Containers were kept on a laboratory bench under ambient conditions (22.0 ± 0.9 °C, 47.9 ± 18.4 %RH) near windows and subjected to the natural light cycle. Certified organic chicken feed (HiLo Acres, Portland MI) was added to each container on a weekly schedule – 15 ml to the smaller and 30 ml to the larger containers. Chicken feed was mixed into the media and water was added as needed to maintain moisture (Bennison et al. 2009; Bennison et al. 2008).

Heterorhabditis bacteriophora Oswego strain was obtained from a laboratory culture (Anne Nielsen, Rutgers Agriculture Research and Extension Center, Bridgeton, NJ). *Steinernema carpocapsae* and *S. feltiae* were obtained from BeckerUnderwood (Ames, IA). *Steinernema riobrave* 355 strain was obtained from David Shapiro-Illan, USDA-ARS, Byron, GA. The four nematode species were reared on late instar *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) in laboratory colonies. Five *G. mellonella* were placed on filter paper in an inverted Petri dish and infected with 500 infective juveniles (IJ) in aqueous solution (Kaya and Stock 1997). Infective juveniles were harvested using a White trap (White 1927) and stored in 600 ml tissue culture flasks with vented cap (Corning Inc., Tewksbury, MA) in the dark, under ambient laboratory conditions.

Experimental Methods:

The test arena consisted of a 1.7 ml microcentrifuge tube (Denville Scientific Inc., South Plainfield, NJ) with a hole (approx. 0.045 mm) in the lid to allow air exchange (Ramos-Rodríguez et al. 2006). A piece of No. 1 Whatman filter paper (dia 55 mm) was cut into eight equal radial slices. A slice was inserted into each tube to provide a substrate for nematodes and to help regulate relative humidity. One grain of organic rolled oats was also added to each tube as supplemental food for the beetles. Infective juveniles were applied in aqueous solution (50 µl) to the filter paper and one beetle was added per tube (Ramos-Rodríguez et al. 2006).

The recommended rate of nematode application is 100 IJ/cm² for western flower thrips management (BeckerUnderwood 2013) and the cap of the microcentrifuge tubes used in the experiment were approximately 1 cm². Thus, one-

half, one, and two times the recommended rate was calculated at 50, 100, 200 IJ/cm². Infective juveniles were used within 14 d of harvest from laboratory colonies.

Experimental timing was determined by beetle availability. From June to August 2014, two to four replicates were set-up and run at a time, until 20 replicates were completed. Nematode viability was assessed for each run by infecting *G. mellonella*. For each nematode species, eight *G. mellonella* were placed in an inverted 9 cm Petri dish with 160 infective juveniles. Arenas and Petri dishes were placed in a growth chamber set at 24.4 ± 0.3 °C, $92.7 \pm 15.2\%$ RH, with 24 h darkness. Beetle mortality was assessed daily for 4 d and on the fourth day, all tubes were placed in the freezer (-20°C). Dead beetles were later dissected to check for the presence of nematodes.

Statistical Analysis

The last four replicates of beetles exposed to *H. bacteriophora* were excluded from the analysis because the *G. mellonella* were poorly infected (i.e. < 88% infection). Three replicates each of adult and larval beetles in the control were excluded from the analysis since holes were not punctured into the lid of the microcentrifuge tubes. Lastly, one entire block was excluded from analysis since nematodes were found in the dead beetles in the control treatments.

Survival analysis was tested with Cox's proportional hazard function using PROC PHREG in SAS 9.3 (SAS Institute Inc. 2008; Therneau 2000). Beetle mortality was modeled by insect stage, nematode species, dose rate, and interaction terms. Terms with a p-value > 0.15 were dropped from the model. A logistic regression was

performed using the R statistical language (version 3.1.1, R Core Team 2014) to compare the number of infected beetles per treatment. Beetle infection was modeled by insect stage, nematode species, dose rate, and interaction terms. The step function was used to select a reduced model based on the lowest AIC value (Table 3-1). Multiple comparisons of the slopes were conducted using the contrast package (Kuhn 2013).

Table 3-1. Model selection based on AIC values using the step function in R 3.1.1.

Model	AIC
EPN + Rate + stage + EPN:Rate + EPN:stage + Rate:stage + EPN:Rate:stage + Block	263.33
EPN + Rate + stage + EPN:Rate + EPN:stage + Rate:stage + EPN:Rate:stage	255.15
EPN + Rate + stage + EPN:Rate + EPN:stage + Rate:stage	252.82
EPN + Rate + stage + EPN:Rate + EPN:stage	250.80
EPN + Rate + stage + EPN:Rate	249.30

EPN = entomopathogenic nematode, Rate = dose of infective juveniles, stage = developmental stage of *D. coriaria*.

Results

Beetle Mortality

Third instar *D. coriaria* are approximately three times more susceptible to the nematodes than the adults ($\chi^2 = 77.54$, $df = 1$, $p < 0.001$). In the control, there was 17% adult beetle mortality and 43% mortality for the third instars (Fig. 3-1). The main effect of nematode species was significant ($\chi^2 = 13.54$, $df = 4$, $p = 0.009$). The dosage rate of the nematodes was not significant ($\chi^2 = 5.16$, $df = 2$, $p = 0.076$). Mortality for *D. coriaria* adults and third instars treated with *S. feltiae* and *H. bacteriophora* was not significantly different than the control ($\chi^2 = 0.03$, $df = 1$, $p = 0.873$ and $\chi^2 = 2.084$, $df = 1$, $p = 0.149$, respectively) (Fig. 3-1). Mortality for *D. coriaria* adults (26%) and third instars (77%) treated with *S. carpocapsae* was significantly higher than the control (Fig. 3-1) ($\chi^2 = 6.24$, $df = 1$, $p = 0.013$) and *S. feltiae* ($\chi^2 = 7.06$, $df = 1$, $p = 0.008$), but not *H. bacteriophora* ($\chi^2 = 1.22$, $df = 1$, $p = 0.269$). Mortality for *D. coriaria* adults (34%) and third instars (77%) treated with *S. riobrave* was significantly higher than the control (Fig. 3-1) ($\chi^2 = 6.38$, $df = 1$, $p = 0.012$) and *S. feltiae* ($\chi^2 = 7.09$, $df = 1$, $p = 0.008$), but not significantly different than *H. bacteriophora* ($\chi^2 = 1.18$, $df = 1$, $p = 0.278$) or *S. carpocapsae* ($\chi^2 = 0.002$, $df = 1$, $p = 0.965$). None of the interaction terms were significant.

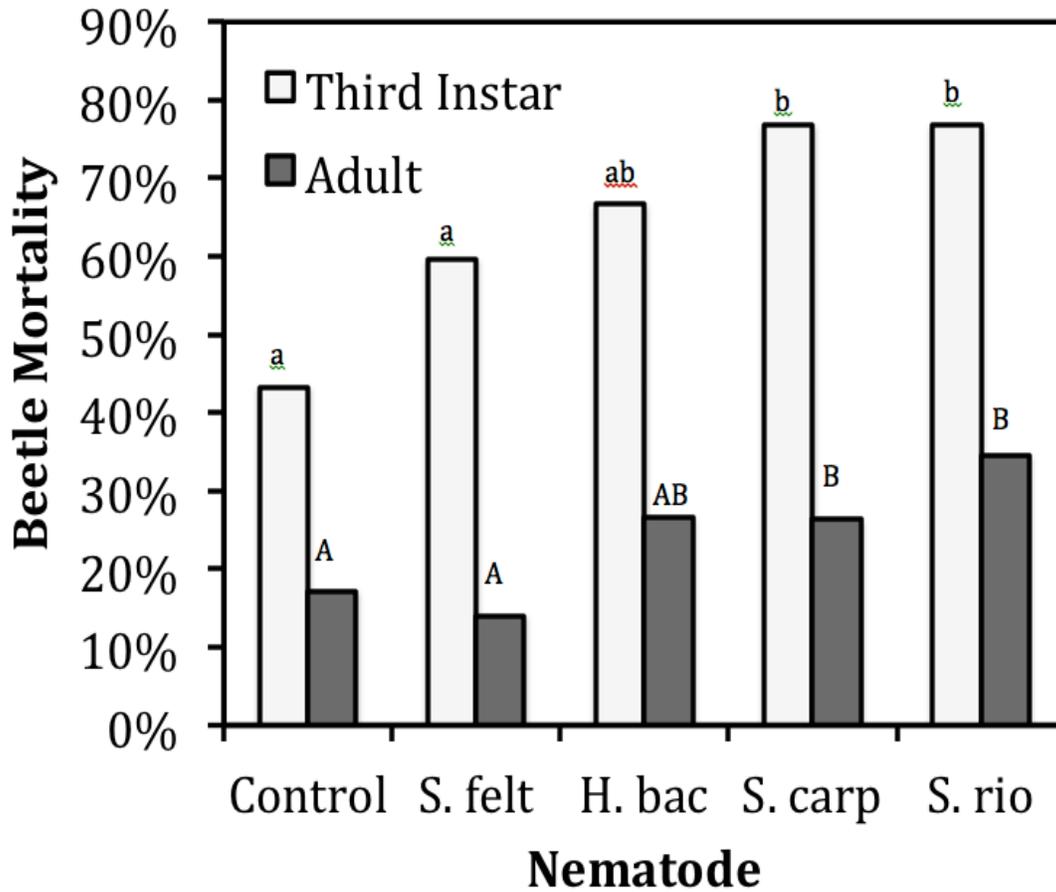


Figure 3-1. Percent mortality of *D. coriaria* on Day 4. *H. bac* = *H. bacteriophora*, *S. carp* = *S. carpocapsae*, *S. felt* = *S. feltiae*, *S. rio* = *S. riobrave*. Bars with different letters are significantly different ($p < 0.05$).

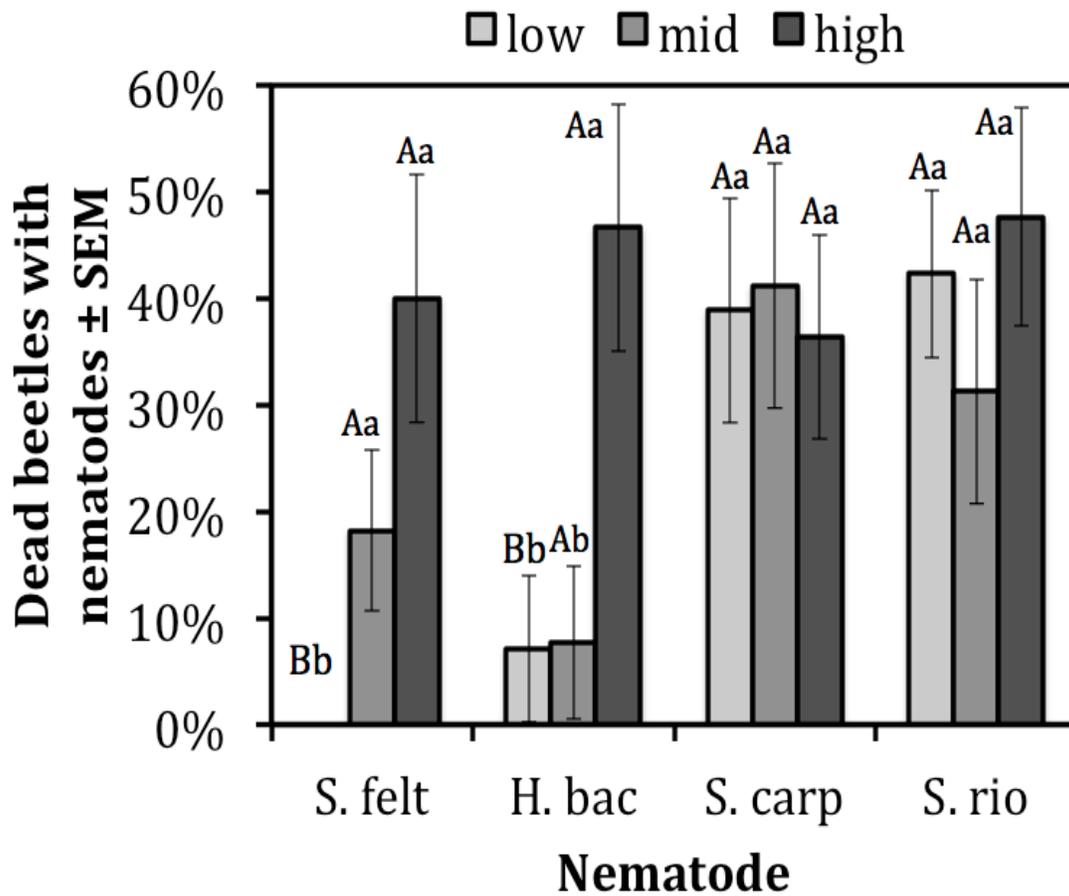


Figure 3-2. Percent dead *D. coriaria* with confirmed nematodes. H. bac = *H. bacteriophora*, S. carp = *S. carpocapsae*, S. felt = *S. feltiae*, S. rio = *S. riobrave*. Within each nematode group, bars with different lowercase letters are significantly different ($p < 0.05$). For each rate across nematode species, bars with different uppercase letters are significantly different ($p < 0.05$).

Presence of nematodes in cadavers

Not all of the dead beetles contained nematodes. Even though third instar mortality was higher in the presence of entomopathogenic nematodes than adult mortality, a similar number of nematodes were recovered from both third instars and adults ($\chi^2 = 237$, $df = 197$, $p = 0.121$). The main effects of nematode species and dose were significant for the number of nematodes recovered from adults and third instars ($\chi^2 = 246$, $df = 200$, $p = 0.025$ and $\chi^2 = 240$, $df = 198$, $p = 0.046$, respectively). The two-way interaction term for nematodes species and rate was also significant ($\chi^2 = 223$, $df = 191$, $p = 0.028$). There was an increasing dosage effect for *S. feltiae* and *H. bacteriophora* but not *S. carpocapsae* and *S. riobrave*. Nematodes were recovered from 0%, 18%, and 40% of the adult and larval beetles that died after being treated with *S. feltiae* at the low, intermediate, and high rates, respectively, with significantly more nematodes recovered at the high rate relative to the low rate ($t = 2.13$, $df = 191$, $p = 0.034$) (Fig. 3-2). For *H. bacteriophora*, nematodes were found in 7%, 8%, and 47% of the dead adult and larval beetles treated at the low, intermediate, and high rates, respectively, with significantly more nematodes recovered from the high rate relative to the low and intermediate rates ($t = 2.13$, $df = 191$, $p = 0.034$ and $t = 1.98$, $df = 191$, $p = 0.049$, respectively) (Fig. 3-2). For the *S. carpocapsae* treatment, nematodes were found in 39%, 41%, and 36% of the dead adult and larval beetles treated at the low, intermediate, and high rates, respectively, without any significant differences between rates (Fig. 3-2). For the *S. riobrave* treatment, nematodes were found in 42%, 31%, and 48% of the dead adult

and larval beetles treated at the low, intermediate, and high rates, respectively, without any significant differences between rates (Fig. 3-2).

Discussion

Entomopathogenic nematodes and *D. coriaria* are soil-dwelling biological control organisms that could come into contact with each other, especially when used as augmentative biological control tactics. A previous study tested the laboratory susceptibility of *D. coriaria* to only one nematode, *S. feltiae*, and concluded that third instar mortality is dose dependent but not adult beetle mortality (Jandricic et al. 2006). The four nematode species tested in the present study were able to infect third instar and adult *D. coriaria* with varying success, but only *S. carpocapsae* and *S. riobrave* significantly increased mortality (Fig. 3-1). Adult beetles were less susceptible than third instars, a pattern seen in other beetle hosts. *Dalotia coriaria* adults and third instars were less susceptible to *S. feltiae* than the other three species. Thus, *S. feltiae* appears to be a good candidate to use with *D. coriaria* in biological control programs of greenhouse pests.

In their laboratory studies, Jandricic et al. (2006) showed that adult *D. coriaria* was not susceptible to *S. feltiae* but third instar mortality was dose dependent. Mortality at the highest dose rate of 50 IJ/cm² was 27%, which was significantly greater than the two lower rates and control (Jandricic et al. 2006). In contrast the present study did not show a dosage effect for the mortality. This inconsistency may be due to the higher doses tested. In Jandricic et al. (2006), third instars were treated with 12 IJ/cm², 25 IJ/cm², and 50 IJ/cm². Whereas, in this

study, the doses were 50 IJ/cm², 100 IJ/cm², and 200 IJ/cm² and resulted in higher mortality, 74%, 47%, and 58% for each dose respectively.

Third instars were two to four times more likely to die than the adults (Fig. 3-1). For both phytophagous and predatory beetles, adults are typically less susceptible to nematode infection, but not always (Doucet et al. 1999). Adult carrot weevil *Listronotus oregonensis* (LeConte) (Coleoptera: Curculionidae) is less susceptible to *H. heliothidis* (Khan, Brooks, and Hirschmann), *S. bibionis* (Steiner), and *S. carpocapsae* DD-136 than third instars (Belair and Boivin 1985). Adult lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) is less susceptible to *S. carpocapsae* DD-136 than late instars, but not *H. heliothidis* or *S. glaseri* (Steiner)(Geden et al. 1985). Larvae of the confused flour beetle *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) are generally more susceptible to *S. feltiae* than the adults (Athanassiou et al. 2008). Multiple adult predatory beetles including *Philonthus sp.* (Coleoptera: Staphylinidae) were less susceptible to *H. bacteriophora* and *S. carpocapsae* than last instars (Georgis et al. 1991). It is not known why adults are generally less susceptible than larvae, but it could be due to cuticle thickness, morphological differences in body openings, or behavior (Georgis et al. 1991).

Insect mortality due to entomopathogenic nematodes is correlated with behavior and morphology of both the host and nematode (Campbell and Gaugler 1993; Lewis et al. 1996). Infective juveniles that cruise are better adapted to search for sedentary hosts; whereas, ambushers are better adapted to search for mobile hosts at the soil surface. Spiracles smaller than infective juvenile body width

(Henneberry et al. 1995) or fitted with sieve plates restrict nematodes entry (Koppenhöfer et al. 2007). In the present study, neither search strategy nor size correlates with beetle mortality. Aspects of beetle movement behavior could explain their susceptibility to nematodes.

If infective juvenile foraging strategy was a significant factor in causing *D. coriaria* mortality then there would be higher susceptibility to an ambusher than a cruiser because *D. coriaria* is highly mobile in both the larval and adult stages. Of the nematodes assayed, *H. bacteriophora* is a cruiser and is more effective at finding sedentary hosts (Campbell and Gaugler 1993); *S. carpocapsae* is an ambusher and is more effective at finding a mobile hosts (Campbell and Gaugler 1993). *Steinernema feltiae* and *S. riobrave* both exhibit an intermediate behavior in the search continuum (Grewal et al. 1994a; Millar and Barbercheck 2001). The observed mortality pattern is not consistent with nematode foraging behavior. Mortality caused by the ambusher *S. carpocapsae* was not significantly greater than the mortality from the cruiser *H. bacteriophora* (Figs. 3-1). Mortality from *S. feltiae* and *S. riobrave*, the intermediates, was lower than cruiser and higher than the ambusher, respectively, not in between them. Thus, the results do not support the hypothesis that nematode foraging behavior explains *D. coriaria* susceptibility. This pattern holds for both the mortality data and frequency of nematode establishment.

If *D. coriaria* mortality could be explained by the size of the infective juvenile, then *D. coriaria* would likely be more susceptible to the narrowest nematodes. The observed mortality does not correlate to infective juvenile size. The infective juvenile with the greatest width is *S. riobrave* at 28 microns (Cabanillas et al. 1994).

With a mean body width of 26 microns, *S. feltiae* is the second largest infective juvenile (Poinar 1990). Followed by *S. carpocapsae* at 25 microns and *H. bacteriophora* at 23 microns (Poinar 1990). *Dalotia coriaria* showed higher susceptibility to *S. riobrave* and *S. carpocapsae* even though *H. bacteriophora* is the narrowest nematode (Figs. 3-1). Thus, the size of natural openings did not prevent or allow certain nematode species entry into the host. The size of the beetle spiracles, anal, and oral openings is unknown.

Dalotia coriaria may be a poor host due to its relative size to nematodes. Nematodes need a host that is large enough to provide sufficient resources for reproduction. *Dalotia coriaria* is only 3-4 mm long and provides much less resources than *G. mellonella*, 12-20 mm in length. From the perspective of the beetle, the nematodes are likely large enough – 14% to 28% the size of the beetle – to be perceived. *Dalotia coriaria* may have developed behaviors to avoid or groom nematodes before they can enter.

In conclusion, *D. coriaria* appears to be most likely compatible with applications of *S. feltiae* and *H. bacteriophora*. These nematodes did not cause significantly higher mortality than the control, and established in < 20% of the beetles at or below the recommended rate. The biological control organisms *S. feltiae* and *D. coriaria* could be applied at the same time to manage greenhouse pests such as fungus gnats and western flower thrips. However, this laboratory study set-up a worse case scenario for the potential host; a homogeneous habitat with limited potential refuges from foraging nematodes. The two-dimensional piece of vertical filter paper provided a simple environment for the infective juveniles to search.

Since, cruising nematodes can find hosts more effectively in a three-dimensional space than a two-dimensional space (Grewal et al. 1994a), these results should be confirmed in experiments that provide or approximate field conditions.

Chapter 4. Synthesis and Conclusions

My thesis research project had two major objectives: 1. Determine whether black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae) could be used in an entomopathogenic nematode rearing system and 2. Determine if entomopathogenic nematodes are compatible with *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae), when used as an augmentative natural enemy for management of western flower *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and fungus gnats *Bradysis spp.* (Diptera: Sciaridae). In addition to satisfying my objectives, this research has raised some interesting and important questions for entomopathogenic nematode ecology and behavior.

Black Soldier Fly as a Rearing Host

My research demonstrated that black soldier fly is not an ideal host for rearing entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) due to low susceptibility to infection and low production (Tourtois, 2014a). While modification of black soldier fly by puncturing the integument did increase mortality, nematode entry, and nematode production, it did not increase nematode production to a level comparable to that observed in the greater wax moth larva *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). Although black soldier fly larvae were not found to be a suitable alternative host for rearing entomopathogenic nematodes, some interesting new questions arose from this study about host entry and preference. The thick cuticle, few spiracles, and robust immune system are likely characteristics of black soldier fly larvae that limit the

success of entomopathogenic nematode entry and establishment. The presence of a dorsal tooth on the infective juveniles of *H. bacteriophora* may determine how the nematode interacts with wounded hosts. Entomopathogenic nematodes given the choice may prefer to infect more suitable hosts than black soldier fly.

Three characteristics of black soldier fly larvae – thick cuticle, few spiracles, and robust immune system – may make them a less suitable host. Like all stratiomyids, black soldier fly larvae incorporate calcium carbonate into their cuticle making the cuticle thick and tough (Johannsen 1922). Similar to most dipteran larvae, black soldier fly larvae have a reduced number of spiracles. They are amphipneustic with two anterior spiracles on the prothorax and two posterior spiracles inside a pocket (Barros-Cordeiro et al. 2014). With fewer spiracles, they have fewer entry sites than *G. mellonella*, which is a caterpillar with 18 spiracles. Soil-borne pathogens are commonly associated with soils and decaying matter (Baker 1968). Black soldier flies have a life history with multiple forms of decaying organic materials and may have developed a robust immune system to deal with these conditions (Choi et al. 2012; Park et al. 2014). Damaging the cuticle is one way to overcome the physical barrier and lack of spiracles to allow nematode entry.

Based on my research, injuring the black soldier fly benefited the two genera of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*, in different ways. Infective juvenile morphology could determine how they enter wounded hosts. The lack of increased mortality for the injured black soldier fly suggests that *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) did not enter through the punctured wounds or at the very least did not directly benefit from the

punctures. Infective juveniles of *H. bacteriophora* and other *Heterorhabditis spp.* have a dorsal tooth and can penetrate directly through the cuticle into the hemocoel (Bedding and Molyneux 1982; Nguyen et al. 2006; Nguyen et al. 2004; Poinar Jr et al. 1987). *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae), *S. feltiae* (Filipjev) and *S. riobrave* Cabanillas, Poinar & Raulston caused higher mortality of the injured black soldier fly larvae. More *S. carpocapsae* were recovered from the injured larvae than the non-injured larvae suggesting that they entered through the wounds. In a study performed by Henneberry et al. (1995), *S. carpocapsae* entered pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) pupae through artificial wounds. Most *Steinernema spp.* are not equipped with a dorsal tooth. The presence of a dorsal tooth may determine how an infective juvenile enters a wounded host. *Steinernema spp.* may be opportunists that take advantage of wounds.

Given the fact that black soldier fly appear well adapted to avoid infection by entomopathogenic nematodes an interesting question is: “would entomopathogenic nematodes even try infecting black soldier fly in a natural setting?” This question would be best addressed in a choice trial comparing infective juvenile nematode response to either a highly susceptible host (e.g. *G. mellonella*) or black soldier fly larvae. Entomopathogenic nematodes respond to plant root and host volatiles (Hiltpold et al. 2011; Lewis et al. 1995) but whether it is possible for them to determine the potential susceptibility of a host from a distance is not well understood. Most current work on nematode foraging has focused on host cues (Grewal et al. 1994a; Lewis et al. 1996; Lewis et al. 1992) but how this affects host

preference is another aspect of entomopathogenic nematode ecology that has not been well researched.

***Dalotia coriaria* Susceptibility to Entomopathogenic Nematodes**

Dalotia coriaria vary in their susceptibility to entomopathogenic nematodes by species. They were least susceptible to *S. feltiae* and *H. bacteriophora*. Neither infective juvenile search strategy nor size correlated with beetle susceptibility. The nematodes accounted for < 35% of the beetle mortality. Entomopathogenic nematodes may have an evolutionary history to avoid relatively smaller hosts since they provide fewer resources for reproduction. Nematodes were recovered from 45% or less of the dead beetles. When trying to enter the beetle through the mouth, the mandibles may crush and damage the nematode (Gaugler and Molloy 1981). The nematodes may survive long enough to deliver the bacteria to the hemocoel to kill the host, but perish soon afterwards. These negative intraguild interactions could be detrimental to augmentative biological control programs incorporating these two organisms. Confirming the laboratory results under field conditions is needed to provide a definitive description of the intraguild interactions between *D. coriaria* and entomopathogenic nematodes.

Entomopathogenic nematode host preference as it relates to other guild members would be valuable information when managing multiple pests (e.g. western flower thrips and fungus gnats) in greenhouses. *Steinernema feltiae*, the ideal host to manage fungus gnats (Gouge and Hague 1995; Harris et al. 1995; Jagdale et al. 2007) may prefer to infect fungus gnats over western flower thrips and a second nematodes species such *H. bacteriophora* may need to be included in the

augmentative biological control program. Likewise, when employing a predator such as *D. coriaria* to manage fungus gnats host preference could be pertinent. *Steinernema feltiae* may prefer to infect the predator to the target host, fungus gnats.

Conclusions

Black soldier fly are not recommended for entomopathogenic nematode rearing compared to the highly susceptible host *G. mellonella*. Wounding the black soldier fly larvae was not enough to overcome reduced nematode production relative to *G. mellonella*. The thick cuticle and few spiracles may be physical adaptations of the black soldier fly to limit pathogen entry. *Steinernema carpocapsae* but not *H. bacteriophora* may exploit wounded insects. *Dalotia coriaria* is likely more compatible with *S. feltiae* and *H. bacteriophora*, than *S. carpocapsae*, or *S. riobrave*. Host suitability is likely predicated by host preference.

APPENDIX

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2014-8

Author and Title of thesis: Joseph S. Tourtois
On entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae):
a potential rearing host, black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae)
and compatibility with a predatory beetle, *Dalotia coriaria* (Kraatz) (Coleoptera:
Staphylinidae)

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:				
Family	Genus-Species	Life Stage	Quantity	Preservation
Staphylinidae	<i>Dalotia coriaria</i>	adult	6	pointed
Staphylinidae	<i>Dalotia coriaria</i>	adult	7	alcohol
Staphylinidae	<i>Dalotia coriaria</i>	larva	7	alcohol
Pyralidae	<i>Galleria mellonella</i>	larva	5	alcohol
Stratiomyidae	<i>Hermetia illucens</i>	adult	8	pinned
Stratiomyidae	<i>Hermetia illucens</i>	6 th instar	6	alcohol
Stratiomyidae	<i>Hermetia illucens</i>	5 th instar	6	alcohol
Stratiomyidae	<i>Hermetia illucens</i>	2 nd instar	10	alcohol

Family	Genus-Species	Life Stage	Quantity	Preservation
Heterorhabditidae	<i>Heterorhabditis bacteriophora</i>	infective juvenile	5	picture
Heterorhabditidae	<i>Heterorhabditis bacteriophora</i>	adult	5	picture
Steinernematidae	<i>Steinernema carpocapsae</i>	infective juvenile	5	picture
Steinernematidae	<i>Steinernema carpocapsae</i>	adult female	5	picture
Steinernematidae	<i>Steinernema carpocapsae</i>	adult male	5	picture
Steinernematidae	<i>Steinernema feltiae</i>	infective juvenile	5	picture
Steinernematidae	<i>Steinernema feltiae</i>	adult female	5	picture
Steinernematidae	<i>Steinernema feltiae</i>	adult male	5	picture
Steinernematidae	<i>Steinernema riobrave</i>	infective juvenile	5	picture
Steinernematidae	<i>Steinernema riobrave</i>	adult female	5	picture
Steinernematidae	<i>Steinernema riobrave</i>	adult male	5	picture

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