The CERES Trust Organic Research Initiative Final Grant Report

Title: Development and delivery of "living pest management" for organic greenhouses and local food systems.

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Key Project Findings:

1. The addition of live waxworms to soil after entomopathogenic nematode applications does not appear a viable way to increase the residence time of nematodes. Growers should thus plan on reapply soil drenches of nematodes to maintain functional populations of these soil biological control agents.
2. Black Soldier flies are a potential rearing host for *H. bacteriophora* but not the Steinernematid species of entomopathogenic nematodes. Modification of black soldier fly 5th stage larvae improves host suitability. However, wax worms remain a superior rearing host for small scale production of entomopathogenic nematodes
3. Watering events can wash beneficial predatory mites off of plants in greenhouses, especially plants with simple leaf structures. The presence of trichomes may further help mites remain on plants after watering events. Growers should apply mites soon after watering events to provide mites with time to establish on their plants.
4. Maintenance of barley plants adjacent to vegetable crops may increase the rate of colonization by both pests (herbivores) and natural enemies (predators and parasitoids).
5. The predatory beetle *Dalotia coriaria* —a natural enemy of thrips and fungus gnats— is unlikely to be affected by the application of entomopathogenic nematodes. Nematodes appeared to have more of an impact on immature beetles than adults. Growers should be able to use both biological control approaches without fear of nematodes impacting the predator.
6. The predatory beetle *Dalotia coriaria* can be reared on potting mix or on compost. Weekly additions of chicken feed (7g/l/week) greatly improve population development. Rearing containers should be as uncovered as possible but need to be kept moist. Movable containers of *D. coriaria* could be used to inoculate multiple greenhouses/hoophouses by rotating them on a two week cycle.

PROJECT SUMMARY: The two goals of our project were to: provide organic greenhouse growers with improved biocontrol tactics and to adapt greenhouse open rearing tactics for use on small scale urban and rural organic farms. We approached our goals through four project objectives: development of on-site augmentative entomopathogenic nematode production, determination of the impact of irrigation and plant architecture on breeder pile systems, adaptation of EPNs, breeder pile, and banker plant systems for small scale farms, and the development of extension materials. The development of integrated insect biological control systems is expected to facilitate new and existing organic production and improve the profitability and sustainability of large organic greenhouses, smaller 3-4 season hoophouses, and outdoor organic vegetable production.

PROJECT OBJECTIVES: 1) The development and delivery of on site entomopathogenic nematode rearing and delivery systems; 2) Determine temporal and physical factors affecting the success of "breeder pile" thrips predator mite systems; 3) Adaptation and delivery of greenhouse biological control techniques to small scale organic vegetable production operations with a focus on urban agriculture; 4) Development of extension materials including: a flip book for greenhouse and hoophouse producers that identifies common insect pests and natural enemies and "how to" extension bulletins on the rearing of natural enemies.

PROJECT RESULTS BY OBJECTIVE AND YEAR:
OBJECTIVE 1: ***Development and delivery of on-site Entomopathogenic nematodes (EPN)***

First Year Progress: Our first year efforts for this objective focused on testing the efficacy of releasing EPN either in a traditional aqueous soil drench or via infected larval cadavers.

Steinernema feltiae **release experiment:** We conducted an experiment testing the efficacy of the entomopathogenic nematode (EPN), *S. feltiae* released in either aqueous solution or from infected insect cadavers as a fungus gnat biological control. Experimental units consisted of a standard 1020 flat planted with barley in a peat-based soil mix. Our treatments were: i) no entomopathogenic nematode application (control), ii) soil drench of 65000 infective juvenile EPN (*S. feltiae*) in a aqueous solution, iii) EPN applied by placing two infected wax worm cadavers onto soil surface, iv) soil drench of IJ's plus "booster" wax worms placed two weeks later, and v) cadaver plus "booster" wax worm placed two weeks later. The idea behind the "booster" wax worms is that by providing an additional host to nematodes applied through either aquaeous or cadaver release we may be able to maintain high numbers of infective juveniles within the treated flats —in effect this is an open rearing tactic for EPN. While others have researched cadaver releases the addition of "booster" wax worms is an entirely new approach. Treatments were replicated 5 times and arranged in a random block design. One yellow sticky card was replaced weekly to monitor adult fungus gnats over the whole experiment area. Potato discs were placed onto the soil surface for 72 hours to monitored fungus gnat larvae. The presence of EPN in the soil is being monitored weekly using a combination of Baermann funnel technique and sentinel wax worms.

Second Year Progress: Our major conclusions from our first year experiments were that: booster wax worms did not provide increased retention of nematodes, reductions in fungus gnat populations were caused by predation of fungus gnat larvae by predatory rove beetle, *Dalotia coriaria*. Based upon these results we redesigned our experiment and began exploring alternative rearing hosts for entomopathogenic nematodes.

2nd ***Steinernema feltiae*** **release experiment:** A second year experiment was set-up in caged greenhouse space to exclude small predatory beetles. This time the experimental unit was an aluminum baking pan (30.0 cm * 23.5 cm * 5.5 cm) filled with peat moss potting mix and planted with organic barley, inoculated with fungus gnats, and watered daily. A temperature data logger (HOBOware version 3.5.0, Onset Computer Corporation, Bourne, MA) was set to record air temperatures every ten minutes. The average daily temperature in the greenhouse was 24°C (range 15°C to 34°C). *Steinernema feltiae* were reared on wax worms in the lab. Wax worms were purchased from a local bait shop. The experiment was set-up as a randomized

complete block design with six blocks and six treatments: 1) aqueous solution of 17,625 *S. feltiae* juvenile nematodes in 250 ml of deionized water, 2) host wax worm cadaver with juvenile nematodes ready to emerge, 3) no nematode application, 4) aqueous solution plus two booster wax worms, 5) host cadaver plus two booster wax worm, and 6) no nematodes plus two booster wax worms. Booster wax worms are healthy wax worms placed on the soil surface to be infected by nematodes already present in the soil either natural occurring or from a previous application. Booster wax worms were caged in window screen cages (6 cm * 3.5 cm) and inserted into the soil surface. Nematode persistence was measured by collecting four soil cores into a 540 ml deli container (Fabri-Kal Corp, Kalamazoo, MI) with 10 bait wax worms and incubate for one week in a growth chamber at 20°C, 0:24 light:dark cycle. Bait wax worms were then dissected to count the number of adult nematodes. Fungus gnat larvae were monitored weekly by placing 2 potato discs (3.5 cm diameter) on the soil surface for 48 hours and counting the number of larvae present.

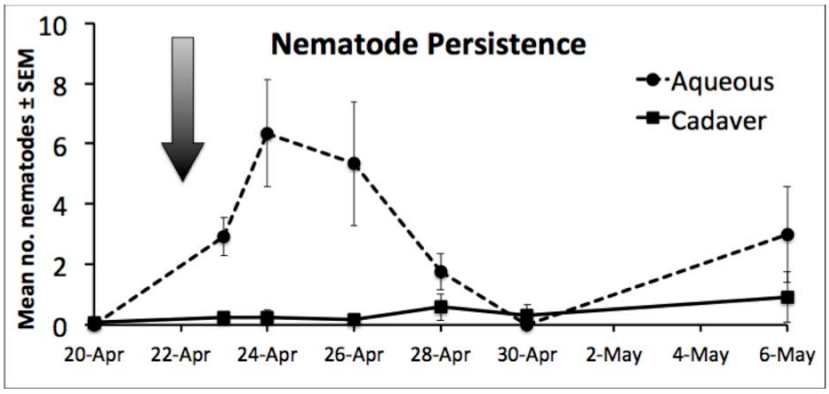


Figure 1. Number of adult nematodes recovered from bait Galleria. Control data not shown (no nematodes found). Treatments applied on 22 April 2013 indicated by arrow.

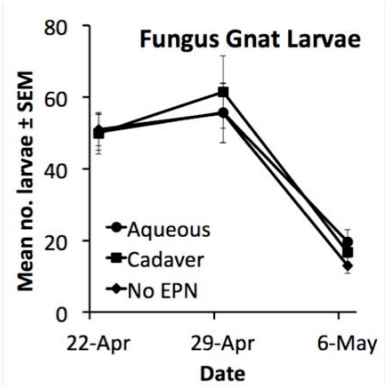


Figure 2. Number of fungus gnat larvae counted on two potato discs per pan.

After a week, none of the booster wax worms were infected by entomopathogenic nematodes, therefore booster wax worm treatments were combines with respective treatments that did not have booster wax worms for data analysis (Fig. 1). More adult nematodes were recovered from the aqueous solution treatment than the host cadaver treatment (Fig. 1). Since the host cadavers did not release as many juvenile nematodes as were applied in the aqueous solution, we suspect that the quality of nematodes in our lab colony was compromised from repeated rearing on wax worms. No nematodes were recovered from the control treatments, data not shown. The fungus gnat populations decreased in all treatments and cannot be attributed to the nematodes (Fig. 2). This experiment was deemed a failure.

3rd *Steinernema feltiae* release experiment "Booster Galleria": We set-up a third, smaller experiment in a growth chamber (20°C, 0:24 light:dark cycle) to address a simpler objective: determine if entomopathogenic nematodes populations can be maintained in the soil through the use of booster wax worms. Experimental units were aluminum baking pan (30.0 cm * 23.5 cm * 5.5 cm) filled with one liter of sterilized sand instead of potting mix. Sand was pre-moistened with 35 ml of boiled tap water. Nematodes were sourced from BeckerUnderwood and were

applied at rate of 1,000,000 nematodes/m^2 (70,500 nematodes in 35 ml of boiled tap water) to each baking pan. The experimental treatments were no booster wax worms placed on soil surface or 2 booster wax worms placed on the soil surface with 5 replicates of each treatment. To measure nematode persistence, the baking pans were sampled taking four sand cores and placing into a 10 cm petri dish (VWR, Radnor, PA) with 10 bait wax worms and incubate for two days in a growth chamber at 20°C, 0:24 light:dark cycle. Bait wax worms were the transferred to a clean Petri dish and incubate for additional four days in the growth chamber before dissection to count the number adult nematodes.

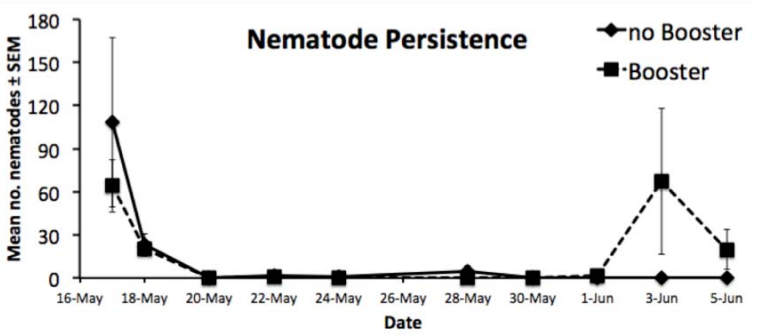


Figure 3. Number of adult nematodes recovered from bait Galleria.

There was no difference in the number of nematodes recovered from the bait wax worms due to addition of booster wax worm before the 3-Jun (Fig. 3). The increase in the number of nematodes and high variance seen on the 3-Jun is from nematodes emerging from only two of the booster wax worms. It may be possible to maintain a high nematode population in the soil ecosystem; however, the nematodes did not reliable emerge from the booster wax worms.

Black soldier fly as an EPN rearing host: Based on the negative results of our three attempts at managing EPN populations using "booster" Galleria larvae we decided to abandon this research approach and instead focus on the exploration of a new potential rearing host. One of the biggest challenges for onsite rearing of EPN is the need to either purchase or rear wax worm larvae as a host —largely because wax worms represent an additional financial or labor cost for already busy growers. In contrast, black soldier fly, *Hermetia illucens*, larvae offer multiple uses and benefits to diversified farming systems. One of the main benefits of Black Solider Fly (BSF) larvae is the rapid "arthro-composting" of organic matter including manures into finished compost or feedstock for vermicompost http://www.thebiopod.com/. Pre-pupal BSF are also an excellent feedstock for aquaculture, poultry, or exotic pets. Black soldier fly larvae have been used at CAFOs for manure management and have been increasingly popular on small organic farms. In CAFO operations larval feeding/composting has been shown to reduce the odor and the volume by 50%. House flies and other nuisance flies avoid laying eggs where BSF larvae are present because of their ravenous feeding behavior. Thus the use of BSF as an EPN rearing host would provide growers with three potential benefits: 1) rapid composting of organic matter, 2) feedstock for animal operations, 3) hosts for onsite EPN programs.

In preliminary trials, we infected BSF larvae with two EPN species *Steinernema carpocapsae* and *S. feltiae*. Compared to wax worms, BSF larvae had a lower infection rate and fewer juveniles were harvested, but a sufficient amount that we thought to continue (Fig 3). In a third trial, we used infective juveniles that were previously harvested from the BSF cadavers to show that the nematodes can be continually reared on the new host. Nematodes were harvested from only two of 100 BSF larvae in the experiment. Cadavers were dissected and dead nematodes were found inside. We postulated that the tough and leathery cuticle of the BSF

larvae was presenting a problem to the infective juveniles both entering the host and leaving the host. An experiment was conducted where larvae were punctured with an insect pin prior to infection and one week after infection, so that the BSF larvae more closely resembled a caterpillar with spiracles on almost every body segment. This trial yielded favorable results with BSF injured prior to infection having 91% infection compared with 50% infection for non-injured larvae and post infection injured larvae showing between 81% and 100% emergence compared to 46-66% emergence in un injured larva (Table 1).

Aqueous vs. cadaver based release of Steinernema feltiae conclusions: The use of cadavers for nematode release or booster Galleria did not prove to be an effective means of delivering *S. feltiae* under greenhouse conditions. Predation of Galleria larvae was preventable but nematodes did not emerge predictably nor was their retention over time improved by application of booster galleria (Fig. 1 &3). Fungus gnat (the target pest) populations were not impacted by any of our treatments (Fig. 2) but did seem to respond to invasion of experimental pots by *Dalotia coriaria*. Based on these results we suggest that growers use repeated soil drenches of entomopathogenic nematodes. These drenches should be timed around target pest phenology as nematodes do not persist in the environment for very long (Fig. 1).

Third Year Activities: In the final year of our project we focused objective one activities on the further development of black soldier fly into a potential rearing host. This was done in a series of experiments that evaluated establishment and production of four commonly used species of EPN on artificially damaged black soldier fly.

Evaluation of Black Soldier Fly as a Rearing Host for Entomopathogenic Nematodes: The first objective of our this process was to determine which instars of black soldier fly are susceptible to four commonly available species of entomopathogenic nematodes; *Heterorhabitis 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.

Experiment 1 – black soldier fly susceptibility by larval and pupa stages: 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

Table 1. The number of BSF larvae (n=12) that were successfully infected (row 1) and the number of BSF cadavers that had infective juveniles emerge out of the cadavers (row 2).

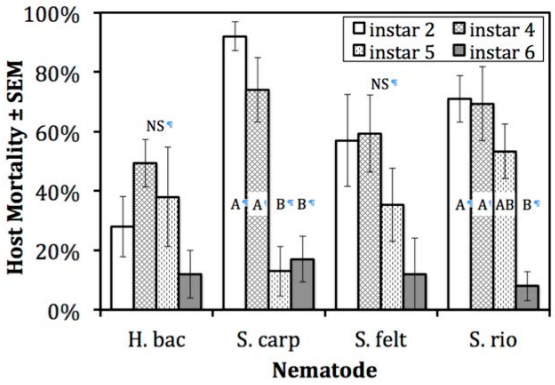
Treatments	Control	Post-infection injury	Pre-infection injury	Pre & post-infection injury
Number of Infections *(% infection (n=12))*	6 *(50%)*	7 *(58%)*	11 *(92%)*	11 *(92%)*
Number of cadavers producing juveniles *(% emergence (n=number infected))*	4 *(66%)*	7 *(100%)*	5 *(46%)*	9 *(81%)*

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. Insect mortality was assessed daily for eight days and on day eight all dead larvae were 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.

A similar, but separate experiment set-up was used to test if the pupal stage of black soldier fly was susceptible 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. Data were analyzed using ANOVA.

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

Figure 3. Black soldier fly % corrected mortality



Nematodes were only recovered from a few black soldier fly cadavers (Table 2). No *H. bacteriophora* nematodes were recovered from the second instars.

Table 2. Adult nematodes recovered from black soldier fly instar (n=25 per treatment combination). Data not shown for *G. mellonella*.

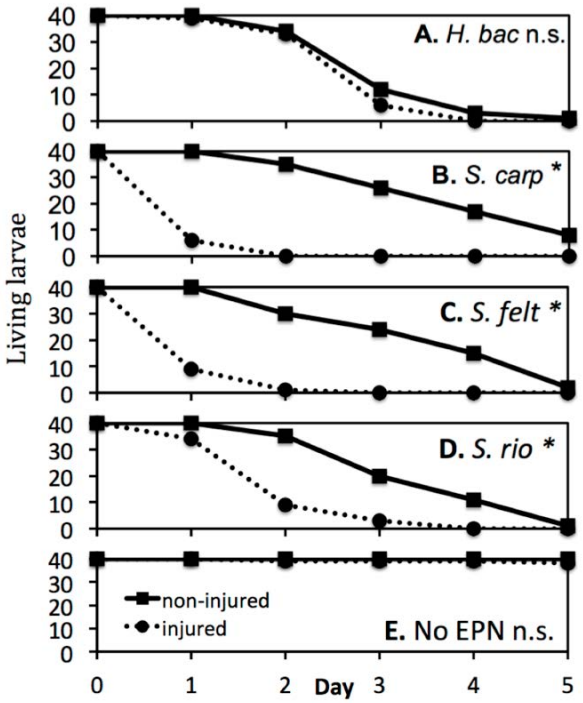
Nematode	Host instar	Percent Infected ± SEM	Nematodes ± SEM
H. bacteriophora	2	0.0 ± 0.0 ns	0.0 ± 0.0ns
	4	8.0 ± 4.9	2.5 ± 0.4
	5	4.0 ± 4.0	4.0 ± 0.0
	6	8.0 ± 8.0	2.0 ± 0.7
S. carpocapsae	2	32.0 ± 4.9a	6.0 ± 0.7ns
	4	12.0 ± 4.9a	1.7 ± 0.4
	5	0.0 ± 0.0b	0.0 ± 0.0
	6	8.0 ± 4.9a	2.0 ± 0.0
S. feltiae	2	12.0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

* Kruskal-Wallis test, different letters indicate significant differences, ns= no significant difference.

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 individual 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 2 – effect of larval injury on nematode infection: Fifth instars were selected for this experiment, which consisted of a 3x5 two-way factorial experiment to test whether injuring the black soldier fly larvae affects nematode infectivity. The levels of the first factor where 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.

Figure 5A-E. Black soldier fly survival curves. (*) denotes significant difference (P < 0.05).



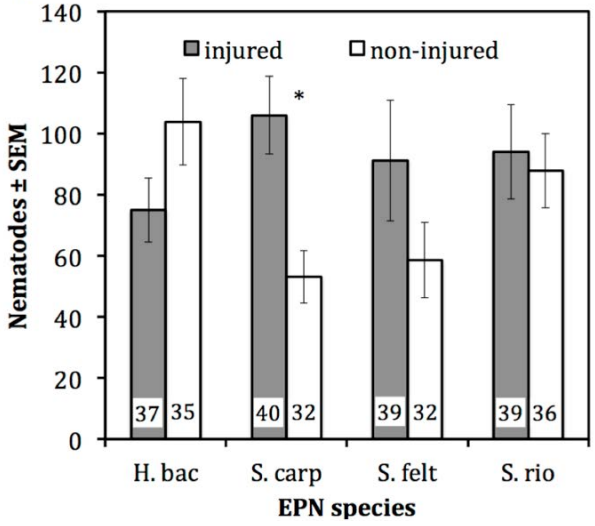
Petri dish infection arenas were prepared by inserting 60 mm diameter No. 1 Whatman filter paper circles into 60 mm diameter inverted Petri dishes. Infective juveniles (1000) were applied to the filter paper in 500 µl. Negative controls received just 500 µl of water. Under a dissecting microscope, a 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. Insect mortality was assessed as described above in experiment 1. 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 could occur. 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. Data were analyzed using a Kruskal Wallis means comparison.

Insect mortality was significantly affected by injury treatment and nematode species (χ^2 = 37.2, df = 1, $p < 0.001$, χ^2 = 443.8, df = 4, $p < 0.001$, respectively). The interaction term was also significant (χ^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). For *H. bacteriophora,* the injured larvae did not die any faster than the non-injured larvae (Fig. 2A) (χ^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. 4A). For *S. carpocapsae*, all of the injured larvae were dead by day 2 whereas only 5 of the non-injured larvae were dead (Fig. 4B) (χ^2 = 78.6, df = 1, $p < 0.001$). On day 5, 32 of the non-injured larvae were dead (Fig. 4B). A similar pattern was observed for *S. feltiae*. Only one injured larvae was still alive on day 2 whereas 30 non-injured larvae were still alive (Fig. 2C) (χ^2 = 65.4, df = 1, $p < 0.001$). All but two non-injured larvae died by day 5 (Fig. 4C). For *S. riobrave*, 31 injured larvae and 5 non-injured larvae were dead on day 2 and 40 injured larvae and 39 non-injured larvae were dead on day 5 (Fig. 4D) (χ^2 = 38.3, df = 1, $p < 0.001$). Only two injured larvae and none of the non-injured larvae died by the fifth day (Fig. 4E) (χ^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.

Injuring the larvae significantly increased the odds of the larvae being infected from 84% to 97% ($p < 0.001$). Of the 159 non-injured larvae treated with nematodes, 6.3% were alive after 5 d, 1.3% were alive and nematodes were found inside, 8.8% died but were not infected, and nematodes were successfully established in 84%. Compared to none that were living, 3.1% dead, and 97% infected from the 160 injured larvae treated with nematodes. Infection rate did not differ by nematode species (p = 0.76). The interaction term was not significant (p = 0.14). Blocking was not significant.

Figure 6. Nematodes recovered from dead BSF



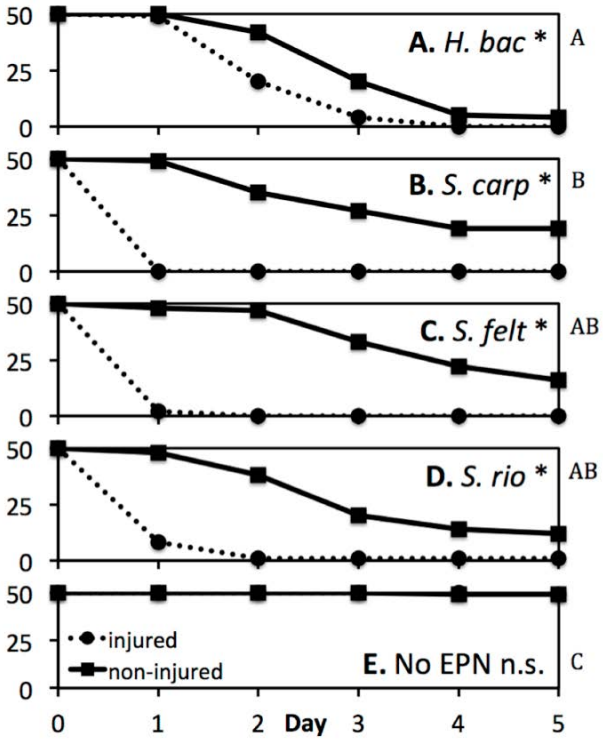
For the number of nematodes found in the infected larvae, the main effect of nematode species was not significant (F = 1.28, df = 3, p = 0.28). The main effect of injuring the larvae was significant (F = 5.35, df = 1, p = 0.02), as was the interaction term (F = 3.85, df = 3, p = 0.01). Injuring the larvae increased the number of recovered nematodes only *S. carpocapsae*, but not any of the other nematode species (p = 0.03) (Fig. 6). 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.

Experiment 3 – nematode production in black soldier fly: A 5x5 two-way factorial experiment was conducted to test the following hypotheses: (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.

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 and traps checked daily for juveniles. Juveniles were harvested on the first day they appeared in the White trap with subsequent harvesting occurred every other day. Cadavers were collected and frozen three days after juveniles stopped 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). Infective juveniles were counted under a dissecting microscope. Mortality data were analyzed using survival analysis and nematode presence and counts analyzed using ANOVA.

Figure 7A-E. Black soldier fly survival curves. (*) denotes significant difference (P < 0.05) within plots. Plots with different letters are significantly different (p< 0.05).



Injuring 5th 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 nematodes 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).

The survival rate of the non-injured larvae (42%) did not significantly differ from the non-injured larvae that were damaged as cadavers one week later (38%) (95% CI [34%, 51%],

[31%, 48%], respectively). Likewise, for the larvae that were injured immediately before nematode application, survival rate did not significantly differ between those damaged as cadavers (21%) or not (19%) (95% CI [15%, 29%], [13%, 28%], respectively). These two groups of larvae did differ in survival rate by the injury treatment. This response is the same as in the pre-infection injury experiment (i.e. experiment 2).

For the negative control, only 2% of the injured and 2% of the non-injured larvae died. This is significantly different than all of the nematode treatments (95% CI [95%, 100%])(Figure 6E). Only 4% (95% CI [1.5%, 10.4%]) of the larvae survived when treated with *H. bacteriophora*. Injuring the larvae did significantly affect the survival rate ($\chi^2 = 25$, df = 1, p-value < 0.001). *Steinernema carpocapsae* killed the least amount of black soldier fly larvae, 19% of them survived. This was significantly fewer than *H. bacteriophora* (95% CI [12.7%, 28.5%]). All of the injured were dead with in the first 24 h. Only one non-injured larvae died after 24 h. By day 5, 62% of the larvae died, which was significantly less than the injured larvae ($\chi^2 = 95.1$, df = 1, p-value < 0.001). Sixteen percent of the larvae treated with *S. feltiae* survived until day 5 (Fig. 7C). This is not significantly different from *H. bacteriophora* or *S. carpocapsae* (95% CI [10.2%, 25.1%]). Within first 48 h, 100% of the injured larvae died, but only three non-injured larvae died. By day 5, only 32% died, which is significantly less than the injured larvae ($\chi^2 = 96.4$, df = 1, p-value < 0.001) (Fig. 7C). For larvae treated with *S. riobrave*, the survival rate was 13%, which was not significantly different than any of the other nematode species (95% CI [7.8%, 21.6%]) (Fig. 7D). On day 2 only 24% of the non-injured larvae died, whereas 98% of the injured larvae died. Even by day 5, only 76% non-injured larvae died. The survival curve of the non-injured larvae is significantly different than the injured larvae ($\chi^2 = 67.9$, df = 1, p-value < 0.001) (Fig. 7). As expected, 100% of *G. mellonella* treated with nematodes died within 48 h while no *G. mellonella* died in the no nematode 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.

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) (Table 3). Injuring 5th 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 than the post-infection injury treatment (p = 0.99), nor was the pre-infection injury treatment different than 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 ($p \leq 0.04$) (Table 3). 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-infection, and post-infection injury treatments did not differ by

nematode species (Table 3). *Heterorhabditis bacteriophora* (91,084 ± 23,592) and 59,585 ± 23,679 *S. carpocapsae* from the pre+post-infection injury treatment were not significantly different for each other (p = 1)(Table 3). 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 ± 60,963). This was significantly different than *S. carpocapsae* (116,247 ± 41,167) (p < 0.001) and *S. feltiae* (137,666 ± 28,984) (p < 0.01), but not *S. riobrave* (296,713 ± 42,467) (p = 0.88) (Table 3).

From the non-injured black soldier fly, 7,835 ± 5119 *H. bacteriophora* were harvested. The pre-infection injury nor the post-infection injury treatments significantly altered the nematode harvest (1,948 ± 890 and 28,302 ± 16,848, respectively) (p ≥ 0.84) (Table 2). The pre+post-infection injury treatment significantly increased the amount of harvested nematodes (91,084 ± 23,592) over the single injury treatments and the non-injury treatment (p ≤ 0.023) (Table 3). *Galleria mellonella* produced 180 times more *H. bacteriophora* than any of the black soldier fly injury treatments (p < 0.001) (Table 3).

Table 3. Infective juveniles harvested from black soldier fly and *G. mellonella* (n=25 per treatment combination). Infective juveniles (1000) applied per host at 25°C. None of the negative controls were infected. BSF = black soldier fly, Gm = G. mellonella, no = non-injured, post = damaged as a cadavers, pre = larva injured before nematode application, and p+p = pre and post.

Nematode	Host injury trt	Cadavers	Nematodes ± SEM		
H. bacteriophora	BSF-no	23	7835	±	5119a
	BSF-post	23	28302	±	16484a
	BSF-pre	25	1948	±	890a
	BSF-p+p	25	91084	±	23592b
	Gm-no	25	941884	±	60963c
S. carpocapsae	BSF-no	19	184	±	113a
	BSF-post	20	3486	±	2083a
	BSF-pre	25	5160	±	2411a
	BSF-p+p	25	59585	±	23679b
	Gm-no	25	116247	±	41167b
S. feltiae	BSF-no	19	1840	±	1250a
	BSF-post	18	3814	±	3475a
	BSF-pre	25	1314	±	1169a
	BSF-p+p	25	68	±	23a
	Gm-no	25	137666	±	28984b
S. riobrave	BSF-no	16	658	±	178a
	BSF-post	22	10706	±	5897b
	BSF-pre	25	411	±	136a
	BSF-p+p	24	308	±	224a
	Gm-no	25	296713	±	42467c

* ANOVA, Tukey HSD a=0.05, different letters indicate significant differences, ns= no significant difference.

There were 116,247 ± 41,167 *S. carpocapsae* produced on *G. mellonella*. A similar amount (59,585 ± 23,679) of nematodes were 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 ≤ 0.098) (Table 3).

Galleria mellonella produced 100 times more *S. feltiae* than any of the black soldier fly injury treatments (p < 0.001). None of the injury treatments produced harvestable nematodes that were significantly different than not injuring (p ≥ 0.93) (Table 3).

The smallest amount of *S. riobrave* was harvested from the pre+post-infection injury treatment (308 ± 224). This was not significantly different than the pre-infection (411 ± 136) or no (658 ± 178) injury treatments (p ≥ 0.15), but it was significantly less than the post-infection injury treatment (10,706 ± 5, 897) (p = 0.022). The post-infection injury treatment was not significantly different than the pre-infection or the no injury treatments (p ≥ 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).

Evaluation of Black Soldier Fly as a Rearing Host for Entomopathogenic Nematodes
Conclusions: The primary goal of these experiments were 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 was that it has on-farm uses —including composting and livestock feed, whereas the traditional nematode rearing hosts do not. The major conclusions of the study were black soldier fly not especially susceptible to entomopathogenic nematodes (Fig. 1). While host modification did improve infection rates, it did not sufficiently improve host quality to use this insect as a rearing host. While this project did not yield promising results for the development of a new rearing host, it does raise some interesting questions regarding the biology of black soldier fly and the foraging strategies of entomopathogenic nematodes. The cuticle of the black soldier fly larvae may be an adaptation against infection by entomopathogenic nematodes. Damaging the cuticle increases the success of entomopathogenic nematodes. Entomopathogenic nematodes may be able to determine host suitability.

While this research indicates that black soldier fly larvae are not a suitable rearing host for entomopathogenic nematodes it does suggest that the relative susceptibility of the host to some steinernematids can be manipulated through injury (Fig. 3 and Table 2). Injuring the black soldier fly larvae before nematode application increased the mortality rate for *Steinernema spp.* but not *H. bacteriophora* (Figs. 4 and 6). Likewise, pre-infection injury also increased the infection rate across all nematode species. For *S. carpocapsae*, more infective juveniles entered the injured larvae than the non-injured larvae (Fig. 5). Surprisingly, the increase in mortality rate associated with pre-infection injured black soldier fly exposed to *Steinernema spp.* did not lead to an increase in the number of infective juveniles harvested (Table 3). However, injuring the cadavers after infection significantly increased the amount of nematodes harvested (Table 3). This suggests that the tough cuticle may prevent entry by infecting juveniles as well as egress by infective juveniles resulting from infection.

OBJECTIVE 2: ***Improving efficacy of open rearing predator mite systems for thrips management***.

First Year Activities: In year one we focused on how plant canopy structure —the macro environment— trichomes, — the microenvironment— and overhead irrigation affect foliar predatory mite populations.

Effect of micro and macro environmental complexity on N. cucumeris establishment on greenhouse plants: Two trials were conducted at Elzinga-Hoeksema Greenhouse using plants provided by the greenhouse. Plants were selected based upon leaf structure (simple vs compound) and trichomes (presence or absence). Basil and rosemary were used in the first trial. Basil has a simple, concave, smooth leaf with very sparse trichomes. Rosemary is a simple, need-like leaf that is densely covered in branched trichomes. A sample of leaves were collected and scanned to measure the area and perimeter (see figure). *Neoseiulus cucumeris* mites (n=975 ± 6) were applied by sprinkling a bran mixture onto plants. Mites were allowed to disperse for a couple of hours and then a single overhead irrigation event occurred. Twenty leaves from each experimental unit were collected and washed in ethanol to measure then number of mites before mite application, before, immediately, and 24 hours after the single watering event. Tomato, cilantro, oregano and basil were used in the second trial. Both tomato and cilantro have

compound leaves. Basil and oregano have simple leaves. Tomato and oregano leaves are covered in trichomes, whereas basil and cilantro lack them. Mites (n=820 ± 80) were applied is the same method and allowed to disperse overnight. Sampling time periods were the sample plus 6 hours after the single watering event.

Results are presented in figure 8. In trial one, watering did not have a negative effect on the mites in the rosemary canopy but did reduce mites in the basil canopy. The dense trichomes on the rosemary leaves or the more complex leaf architecture may have protected the mites as the water came through the plant canopy. In trial two, all mite populations dropped after the watering event and recovered within 6 to 24 hours, suggesting overhead irrigation negatively affects mites in the plant canopy. Mite populations on cilantro and basil, the smooth leaves, seem to face larger reductions (Fig. 8).

Effect of micro and macro environmental complexity on N. cucumeris establishment on greenhouse plants conclusions: Watering displaces mites at differential rates depending on plant species. Plants with complex leaves and trichomes (plant hairs) appear to retain predator mites better than plants with simple leaves. We suggest that growers should release mites immediately following watering to provide them with adequate time to establish on plants. Growers may also want to adjust their application rate depending on crop leaf structure: *i.e.* use higher release rates on plants that lack complex leaves and or trichomes.

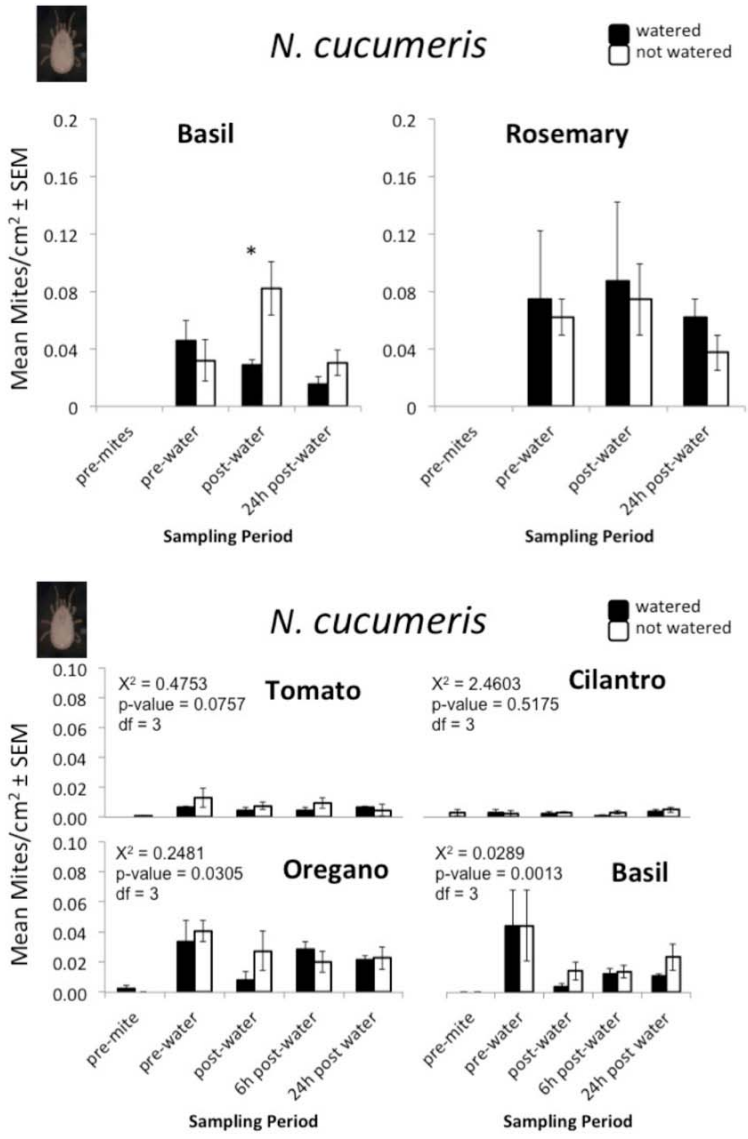


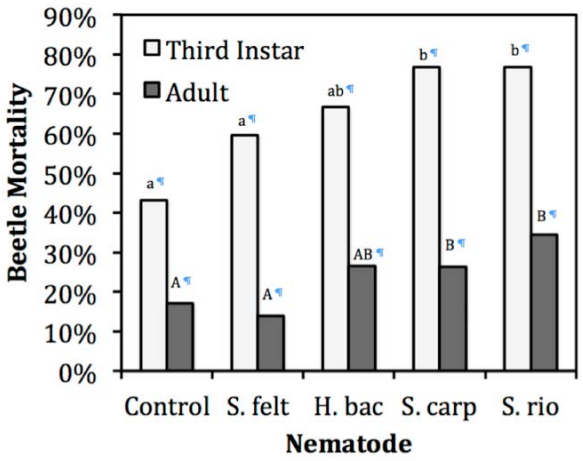
Figure 8. *Neosiulus cucumeris* after watering events on plants grown at Elzinga- Hoeksema greenhouse (updated figures).

Second and Third Year Activities: We encountered a major set back in this objective when our greenhouse collaborator —Elzinga Hoeksma Greenhouses declared bankruptcy in early 2013. The Elzinga-Hoeksma facilities were purchased by a new operation that chose not to maintain organic certification and prevented us from conducting further experiments at this site. We were unable to obtain adequate certified organic greenhouse space to run follow up experiments for

this objective, thus we shifted our year three efforts into assessing the compatibility of EPN with *Dalotia coriaria*. This experiment resulted in a paper recently published in the Journal Insects.

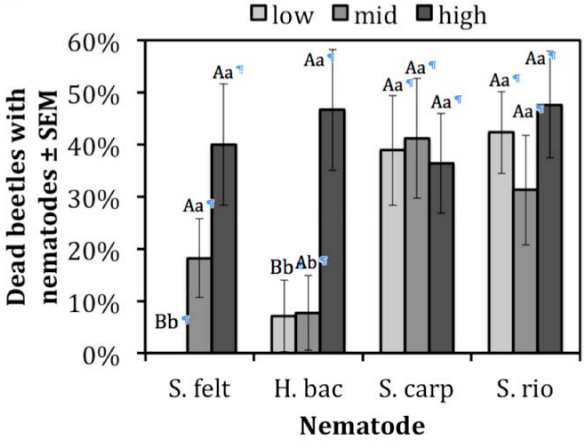
Susceptibility of Dalotia coriaria to entomopathogenic nematodes: Dalotia coriaria (Coleoptera: Staphylinidae) and entomopathogenic nematodes are two soil-dwelling biological control agents used to manage common greenhouse pests including: thrips, fungus gnats, and shore flies. Growers often utilize multiple natural enemies to achieve economic control but knowledge of interactions among natural enemies is lacking. The objective of this experiment was to determine the susceptibility of *D. coriaria* third instars and adults to four commonly utilized species of entomopathogenic nematodes: *H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave*.

Figure 9. % 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 differ at the 0.05 level



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 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. 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. 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. We evaluated an aqueous solution of infective juveniles at 1, 2, and 4 IJ/µl in order to apply 50 µl of nematode solution to all microcentrifuge tubes —this corresponds to the suggested application rate of 100IJ/cm^2. 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. Data were analyzed using Cox's proportional hazard function (Survival Analysis).

Figure 10. % dead *D. coriaria* with confirmed nematodes. Within each nematode, bars with different lowercase letters differ significantly. For rate across nematode species, bars with different uppercase letters differ significantly ($p < 0.05$).



Third instar *D. coriaria* were 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. 9). 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). *Steinernema feltiae* caused 14% mortality in the adults and 60% mortality in the third instars (Fig. 9). This was not significantly different than the control ($\chi^2 = 0.03$, df = 1, p = 0.873). The mortality of the adults treated with *H. bacteriophora* was 27% and third instars mortality was 69% (Fig. 9). This was not significantly different than the control or *S. feltiae* ($\chi^2 = 2.09$, df = 1, p = 0.148, and $\chi^2 = 2.084$, df = 1, p = 0.149, respectively). *Steinernema carpocapsae* caused significantly higher mortality – 26% for the adults and 77% of the third instars (Fig. 9) – than the control ($\chi^2 = 6.24$, 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). Adults (34%) and 77% of third instars (Fig. 9) died when incubated with *S. riobrave*. This is significantly higher than the control ($\chi^2 = 6.38$, 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 interactions terms were significant.

Not all of the dead beetles contained nematodes. An equal frequency of nematodes were found in third instars and adults (p = 0.121). The main effects of nematode species and rate were significant (p = 0.025 and 0.046, respectively). The two-way interaction term for nematodes species and rate was also significant (p = 0.028). Nematodes were recovered from 0%, 18%, and 40% of the beetles that died after being treated with *S. feltiae* at the low, intermediate, and high rates, respectively. The intermediate rate was not significantly different than the high rate (p = 0.22) (Fig. 10). For *H. bacteriophora*, nematodes were found in 7%, 8%, and 47% of the dead beetles treated with at the low, intermediate, and high rates, respectively (Fig. 10). The low and intermediate rates were not significantly different from each other; however, both were significantly different from the high rate (p = 0.034 and 0.049, respectively). For the *S. carpocapsae* treatment, 39%, 41%, and 36% of the dead beetles contained nematodes but were not significantly different (Fig. 10). For the *S. riobrave* treatment, nematodes were found in 42%, 31%, and 48% of the dead beetles but were not significantly different (Fig. 10).

Susceptibility of Dalotia coriaria to entomopathogenic nematodes conclusions:
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. The four nematode species tested in the present study were able to kill and infect third instar and adult *D. coriaria* to varying degrees. Adults were less susceptible than third instars, a pattern seen with other beetle hosts. *Dalotia coriaria* adults and third instars were less susceptible to *S. feltiae* than the other three species. Thus, *Steinernema feltiae* appears to be a good candidate to use with *D. coriaria* in biological control programs of greenhouse pests.

Objective 3. ***Adaption and delivery of greenhouse biocontrol tactics for small diversified organic farms***

Evaluation of barley as an outdoor banker plant: Our first year activities focused on developing good working relationships with local, urban diversified farms and market gardens and collecting preliminary data on the standing biological control potential at these sites. Barley plants were placed at the SOF and Lansing community gardens June thru August. At the SOF, ten 6" pots of barley were placed among 260 pepper plants in a hoop house, and 16 barley pots were placed among tomato plants in another hoop house. We monitored pest and natural enemy activity with weekly visual inspection of the barley and crop plants and replacement of yellow sticky cards. When barley plants were initially placed, they were inoculated with a small amount of cereal aphids. Natural predators quickly consumed these aphids (Fig 11). More cereal aphid did show up on the barley in later weeks, but populations were rarely greater than 100 aphids per barley pot due to constant pressure from natural enemies.

Figure 11. Barley plants adjacent to crop plans at Popping Fresh Farms (Lansing MI) (left) and a barley plant infested with cereal aphids (right).

Second Year Progress: All of the sticky cards from year one were processed. Sticky cards were either placed right next to a barley pot or placed 5-10 meters away from a barley pot. A similar amount of parasitoids were caught on the sticky cards near and far from barley plants (Fig. 12A). Numerically, there were a few more predators – spiders, minute pirate bugs, lacewings, rove beetles, ladybugs, and hover flies – present on the sticky cards closer to the barley plants early in the season (Fig. 12B). Numerically, there were fewer herbivores – thrips, whiteflies, and leafhoppers – caught on the sticky cards closer to the barley plants early in the season (Fig. 12C).

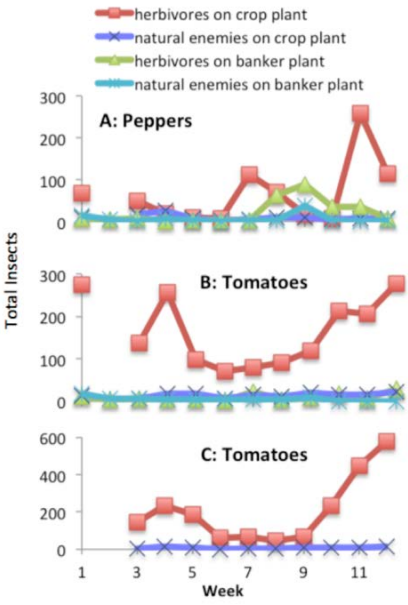


Figure 12. Total count of insects on plants by week at SOF. A) Banker plants among pepper plants. B) Banker plants among tomato plants. C) Tomatoes w/o banker plants

Evaluation of barley as an outdoor banker plant: Barley plants may increase the rate of colonization of both herbivores (pests) and predators/parasites (beneficials). However most of the herbivores observed were not primary pests. Barley and fast growing grain crops may provide additional natural enemy habitat when planted adjacent to vegetable beds.

***Atheta coriaria* rearing-release experiment:** In 2013 an experiment was conducted to determine how porous a rearing-release container for the soil-dwelling generalist predatory rove beetle, *Atheta coriaria*. Two containers – mesh bag and plastic ziploc container and lid with

Figure 13. Test containers for *Atheta coriaria* rearing-release experiment: A) plastic box, B) mesh pouch, and C) open pile.

holes – along with an open pile of substrate were compared. The experiment set-up a complete random block design with three blocks in each of two hoop houses (6 blocks total) at the MSU Student Organic Farm. The experimental unit was a white screen collapsible cage (14*14*24 inches) (BioQuip Products, Rancho Dominguez, CA). The test container was placed in the middle of the cage and on top of capillary matting and a shallow aluminum pan. Water (100 ml) was added 2-3 times per week to the capillary matting to keep substrate material moist. Yellow sticky cards were placed onto of the capillary matting around the test container to track the number of beetles leaving the container. An additional sticky card was suspended above the container to trap flying beetles. Adult beetles (160) (Syngenta Bioline LTD., Little Clacton, England) in one liter of moistened substrate (50:50 coir and vermiculite) were added to each test container. The first test container was 2.25L plastic rectangular Ziploc container with five 0.5 cm diameter holes in the bottom (Fig. 13A). Lids covered with aluminum foil to provide shade and two 2.5 cm diameter holes to allow beetles to leave were snapped onto the containers. The second container was an 18 by 24 inch piece of mosquito netting formed into pouch by gathering and twist-ting the corners together (Fig. 13B). The third treatment was an open pile of substrate with limited containment (Fig. 13C). Plastic rings were created by cutting the bottom out and top half off of the Ziploc containers. These rings were placed around the open pile and mesh pouch to prevent substrate material from spilling onto the surrounding sticky cards. Popsicle sticks (4) were attached at the corners of Ziploc lids covered with aluminum foil and two 2.5 cm diameter holes were places

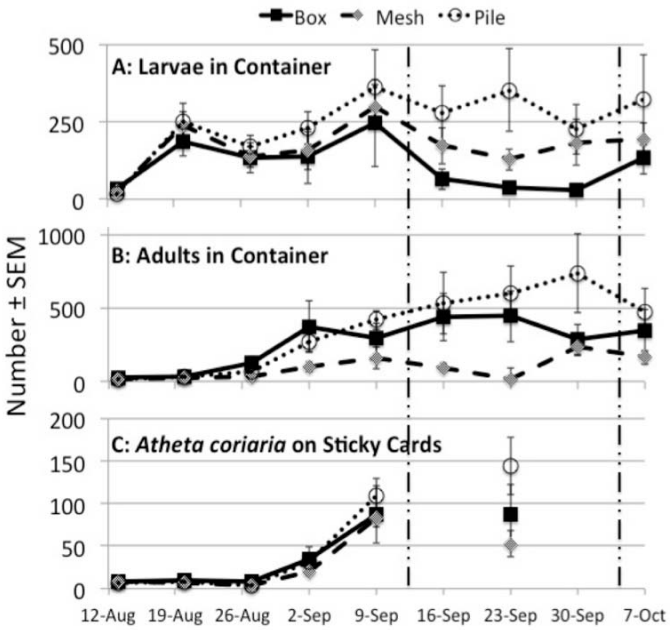


Figure 14. *Atheta coriaria* rearing-release container experiment. Vertical dashed lines represent windstorms. A) Number of larvae in container. B) Number of adults in container. C) Number of beetles on sticky cards. Not all cards have been processed yet.

over the open pile and mesh bags to provide the same amount of shade that the box treatment received. Chicken feed (15g) was added weekly to the substrate as a food source for the beetles.

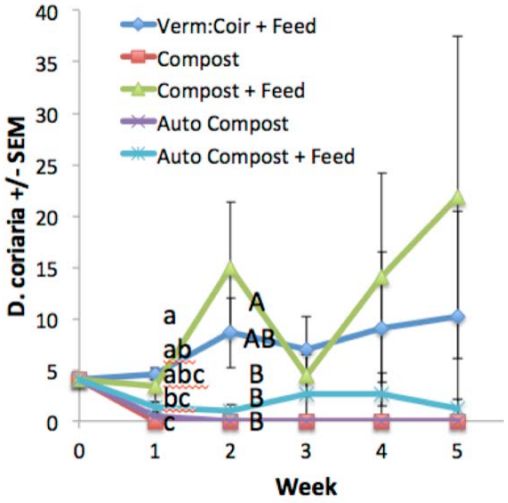
Beetles were sampled weekly by spreading a 15 ml scoop of substrate on to a cafeteria tray to aspirate and count the number of larvae and adult beetles. This step was repeated 10 times for a total sampled volume of 150 ml. Counted beetles were returned to their container. Yellow sticky card were placed once every two weeks; however, the beetles were counted every week. Hoop house temperature was recorded every 15 minutes with a HOBO data logger. On 12-Sept and 4-Oct, heavy windstorms blew several of the cages over (Fig. 13).

The type of container had an effect on the number of beetles in the container. Numerically, there were more adults and larvae in the open pile than in the mesh pouch or plastic box (Fig. 13). Based on our results open rearing containers with minimal coverage appear to be the most productive rearing apparatus for this natural enemy.

Third Year Activities: Our 2014 activities initially focused on a field scale experiment evaluating the impact of border winter annual grain plots on early season colonization of vegetables. Unfortunately the experiment failed due to an exceptionally cold winter that resulted in poor establishment of our border cover crops, thus we were unable to evaluate our treatments. Thus we shifted our summer 2014 activities to further refinement of on-site *Dalotia coriaria* rearing techniques.

Determining optimal rearing media for D. coriaria. In 2014 we ran an experiment evaluating the affect of media type and supplemental feeding on the production of *D. coriaria*. We tested five media conditions: the standard 50:50 vermiculite/coir used in typical rearing programs supplemented with 7 g/l of chicken feed per week; either active or autoclaved dairy compost + feed and active or autoclaved dairy compost without supplementary feed. The objective of this experiment was to evaluate the rearing quality of a potentially readily available medium (compost) vs. one that would require purchase from outside the farm (vermiculite/coir) as well as to evaluate whether soil flora/fauna in the compost would have a positive or negative impact on *D. coriaria* population development. We performed our experiment in a randomized complete block design with the three treatments replicated five times. Initial colonies were established by releasing 10 D. coriaria into a 200 ml deli container with 150 ml of the appropriate media. Experimental arenas were evaluated weekly for a period of five weeks by sieving the media and counting the total number of adult and larval *D. coriaria*. Beetles were returned to their arena following evaluation. Data were analyzed using ANOVA at each time step.

Figure 15. Mean *D. coriaria* beetles produced by different rearing media. Points with different letters of the same case differ significantly (P<0.05)



Results from our experiment show that inclusion of a supplementary diet source is critical to rearing *D. coriaria* but media appears to be less important. Only two of the five weeks had significant differences among treatments. In week

one the vermiculite:coir media with additional feed had significantly more beetles present than any of the media lacking supplementary feed as well as the autoclaved compost with supplementary feed (F= 78.46, d.f. = 4,20, P = 0.002) (Fig. 15). In week two both the "live" compost with supplementary feed had significantly more beeltes than any of the treatments except the vermiculite:coir mix with feed (F= 894.6, d.f. = 4,20, P = 0.012) (Fig. 15). In weeks 3-5 there was extreme variability among replicates but the vermiculite:coir media with additional media and "live" compost with additional media had much higher numerical values compared to the other treatments.

On site rearing of D. coriaria conclusions: Our research indicates that growers wishing to rear *D. coriaria* would be best advised to use a container with minimal coverings. Evaluation of rearing media suggests that "live" but not autoclaved compost is capable of providing an adequate environment but the regular addition of small amounts of supplementary diet is critical to beetle population development. Interestingly the addition of diet to autoclaved compost did not appear to improve its function as a rearing media. Thus, our recommended rearing procedure would be to establish colonies of the beetle in 2 l or larger containers with open or largely open lids using either potting mix or compost as a media. Containers should have 7 g of chicken or poultry feed added per l of media per week. Containers should also be watered occasionally to maintain adequate moisture for beetle development.

Objective 4: Development of extension materials

We produced a number of extension outputs throughout the course of the project with a large quantity of materials produced in 2014 including 4 publications (1 in prep), 4 presentations at scholarly meetings and 12 presentations in extension venues. Project results were presented to Lansing Urban Farmers at field days at the Lansing Roots urban farm in 2014 and 2014 at the 2011-2014 Great Lakes Fruit and Vegetable EXPO, at the MSU organic reporting session, at the 2011-2013 MOSES meetings. A nematode rearing demonstration was provided at the 2014 Great Lakes Horticultural Expo. Two extension bulletins have are in draft form and are being converted into a single home-printable booklet: one highlighting home production of EPN and the other rearing of Atheta Coriaria. An instructional YouTube video highlighting EPN culture was posted at https://www.youtube.com/watch?v=kSDQbJRh0Ss. A project Webinar was held in Fall of 2014. A final output of this project was the training of a M.S. level entomologist, Joseph Tourtois, who defended his degree in December 2014 and was awarded the "Rhodes Outstanding M.S. Student Award" by the MSU department of Entomology in April 2015. Joe is now employed as a research technician with Vesteron International where he is assisting in the discovery of new biological insecticides.

List of Project Outputs (Presentations, media and publications):

Publications (4 to date *1 in prep*):

1. Tourtois, J., and M.J. Grieshop. 2015. Susceptibility of *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae) to Entomopathogenic Nematodes (Rhabditida: Heterorhabditidae and Steinernematidae). Insects 6(1): 224–235.
2. Tourtois, J., J. Ali and M.J. Grieshop. In Prep. Exploring the use of black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) as an in vivo entomopathogenic nematode host.

3. Tourtois, J., and M.J. Grieshop. 2015. On farm rearing of *Dalotia coriaria* a soil predator. An extension handout.
4. Tourtois, J., and M.J. Grieshop. 2015. Rearing Entomopathogenic Nematodes with Household Items. An extension handout.

Scholarly Presentations (4 to date):

5. Tourtois, J. (Presenter & Author), Grieshop, M.J. (Author Only). 2014, November. *Evaluating entomopathogenic nematode production on injured black soldier fly, Hermetia illucens (L.) (Diptera: Stratiomyidae)*. Paper presented at Entomological Society of America Annual Meeting, Portland, Oregon. (November 17, 2014).
6. Tourtois, J. (Presenter & Author), Grieshop, M.J. (Author Only), 2013 Annual Meeting, Entomological Society of America, Austin TX, "Rearing entomopathogenic nematodes on Black Soldier Fly, Hermetia illucens (L.) (Diptera: Stratiomyidae)." (November 11, 2013).
7. Tourtois, J. (Presenter & Author), Grieshop, M.J. (Author Only), 2013 Annual Meeting, North Central Branch Entomological Society of America, Rapid City SD, "The persistence of Steinernema feltiae (Rhabditidia:Steinernematidae) by adding host cadavers and sentinel hosts to greenhouse soil.." (June 17, 2013).
8. Riddle, J. S. (Presenter & Author), Grieshop, M.J. (Author Only), 2012 Annual Meeting, Entomological Society of America, Reno NV, "The effects of overhead irrigation and leaf structure on foliar microarthropods including Neoseiulus cucumeris." (November 12, 2012)

Extension Presentations (12 to date):

9. Tourtois, J. (Presenter & Author), Grieshop, M.J. (Author Only). 2014. *On farm rearing of entomopathogenic nematodes*. Great Lakes Fruit and Vegetable Expo, Grand Rapids, MI. (December 11, 2014).
10. Tourtois, J. (Presenter & Author), **Grieshop, M.J.** (Presenter & Author). 2014. *Harnessing "Micro-Livestock" for on-Farm Biological Control and Nutrient Cycling*. presented at: Webinar, East Lansing, MI. (November 25, 2014). https://connect.msu.edu/p9lm4rvqlig/.
11. Tourtois, J. (Presenter & Author), Grieshop, M.J. (Author Only). 2014. *Organic pest management and biological control for small scale farms*. CERES Trust Field Day, Lansing, MI. (August 04, 2014).
12. Tourtois, J. (Presenter & Author), Grieshop, M.J. (Author Only). 2014. *Rear your own predatory beetles: rearing-release systems for Atheta coriaria*. Poster session presented at Midwest Organic and Sustainable Education Service (MOSES) 25th Annual Organic Farming Conference, La Crosse, WI. (March 01, 2014).
13. Dindia J., J. Tourtois and M.J. Grieshop. 2014. How to Rear Nematodes for Biocontrol. Online Video: https://www.youtube.com/watch?v=kSDQbJRh0Ss
14. Tourtois, J. (Presenter & Author), Grieshop, M.J. (Author Only), 2013 Great Lakes Fruit and Vegetable Expo, Grand Rapids, MI, "Rear your own predatory beetles: rearing-release systems for Atheta coriaria." (December 10, 2013).
15. Matlock, J. (Presenter & Author), Tourtois, J. (Author Only), Grieshop, M.J. (Author Only). 2014. *Processing Pre-Consumer Food Waste and Chicken Manure Bedding with Black Soldier Fly Larvae*. Poster session presented at Midwest Organic and Sustainable

Education Service (MOSES) 25th Annual Organic Farming Conference, La Crosse, WI. (March 01, 2014).
16. Riddle, J. (Presenter & Author), Grieshop, M.J. (Author Only), MOSES 24th Annual Organic Farming Conference, La Crosse WI, "Boom Irrigation Affects Predatory Mites in Plant Canopies. Midwest Organic & Sustainable Education Service." (February 22, 2013).
17. Riddle, J. (Presenter & Author), Grieshop, M.J. (Author Only), MOSES 24th Annual Organic Farming Conference, La Crosse WI, "Boom Irrigation Affects Predatory Mites in Plant Canopies. Midwest Organic & Sustainable Education Service." (February 22, 2013).
18. Pochubay, E. (Presenter & Author), Riddle, J. (Author Only), Grieshop, M.J. (Author Only), 2012 Great Lakes Fruit and Vegetable Expo, Grand Rapids, MI, "Scenarios of Greenhouse Biocontrol: Pests, Predators, Challenges, and Outcomes." (December 4, 2012).
19. Pochubay, E. (Presenter & Author), Riddle, J. (Author Only), Grieshop, M.J. (Author Only), 2012 Great Lakes Fruit and Vegetable Expo, Grand Rapids, MI, "Scenarios of Greenhouse Biocontrol: Pests, Predators, Challenges, and Outcomes." (December 4, 2012).
20. Riddle, J. (Presenter & Author), Pochubay, E. (Author Only), Himmelein, J. (Author Only), Grieshop, M.J. (Author Only), MOSES 23rd Annual Organic Farming Conference, La Crosse WI, "Protecting the Good Guys from the Good Guys: Optimizing Multiple Predator Biological Control Systems." (February 22, 2012).

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