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Target Host Finding by *Steinernema feltiae* and *Heterorhabditis bacteriophora* in the Presence of a Non-Target Insect Host[1]

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Abstract: The ability of *Steinernema feltiae* or *Heterorhabditis bacteriophora* infective juveniles (IJ), when applied to the soil surface, to infect a *Galleria mellonella* larva at the base of a soil-filled cup (276 cm^3) was evaluated in the presence and absence of 100 larvae of a non-target insect, the aphid midge *Aphidoletes aphidimyza,* near the soil surface. In all four trials with either *S. feltiae* or *H. bacteriophora, A. aphidimyza* presence did not affect the number of IJ finding and infecting a *G. mellonella* larva. *Steinernema feltiae* and *H. bacteriophora* IJ movement (as measured by the percentage of IJ aggregating on either side of an experimental arena) in the presence of one or many *A. aphidimyza* larvae was evaluated in agar- and soil-filled petri dishes, respectively. Infective juvenile movement in the presence of *A. aphidimyza* did not differ from random, indicating that IJ were not attracted to *A. aphidimyza.* It is suggested, therefore, that *A. aphidimyza* does not reduce IJ efficacy when these two forms of biological control agent are present together in a field situation even though it is known that *A. aphidimyza* is susceptible to IJ of these species.

Key words: Aphidoletes aphidimyza, entomopathogenic nematodes, *Galleria mellonella, Heterorhabditis bacteriophora,* host finding, non-target, *Steinernema feltiae.*

As biological control becomes more prevalent in pest management, it will become increasingly important to anticipate interactions between biological control agents (Rosenheim et al., 1995). Infective juveniles (IJ) of entomopathogenic nematodes (EPN) are applied as inundative biological control agents of soil insect pests on a variety of crops (Shapiro-Ilan et al., 2002). The efficacy of such IJ applications may be reduced by interactions with other soil organisms that result in IJ mortality (Timper and Kaya, 1992), inhibition of movement, and (or) prevention of IJ from finding target hosts (Kaya and Koppenhöfer, 1996). Aggregation of IJ on agar has been observed as a response to various cues, including carbon dioxide (Gaugler et al., 1980), feces (Schmidt and All, 1979), and temperature gradients (Byers and Poinar, 1981), that are associated with insect hosts and other soil organisms. Individual IJ aggregating near false stimuli from non-target arthropods would not be immediately available to infect and kill the target host. In this case, non-target arthropods would represent a "sink" for the IJ population because of the diminished number of IJ, reducing the host infection rate by the IJ population and, potentially, the death rate of the host population. Therefore, the presence of non-target arthropods in the soil could reduce EPN efficacy as a biological control agent.

The aphid midge, *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), is a biological control agent of aphids in greenhouses. *Aphidoletes aphidimyza* is distributed throughout much of the northern hemisphere (Harris, 1973; Yukawa et al., 1998), and wild populations are often present in fields where aphids are present (Stewart and Walde, 1997). Larvae prey upon aphids on the foliage of plants and, once satiated, the larvae drop to the soil surface, burrow to a depth of up to 2 cm, and form cocoons in which they continue their development. Entomopathogenic nematodes and *A. aphidimyza* can occur together in the soil under the same crops (e.g., strawberry, ornamentals) in both greenhouse and field situations (Georgis, 2002; Harris, 1973; Raworth, 1984). *Heterorhabditis bacteriophora* and, to a lesser extent, *Steinernema feltiae* have been shown to infect and kill *A. aphidimyza* in laboratory bioassays and in greenhouse experiments, demonstrating the potential for an IJ application to reduce *A. aphidimyza* efficacy (Powell and Webster, 2004). Infective juveniles are typically applied to the soil surface and actively foraging IJ migrating downward in search of a host might encounter *A. aphidimyza* prior to a target pest around plant roots at a lower depth. The objectives of this study were (i) to evaluate the potential impact on IJ efficacy of *A. aphidimyza* as a non-target by determining the effect of the presence of *A. aphidimyza* on the ability of two EPN species, *S. feltiae* 'B27' and *H. bacteriophora* 'D-H-Dal', to find and infect a target host, and (ii) to evaluate the effect of non-target derived stimuli on this interaction by determining if *S. feltiae* and *H. bacteriophora* IJ aggregated near *A. aphidimyza.*

Materials and Methods

Source and rearing of nematodes and insects: Entomopathogenic nematodes used in this study had been cultured in the laboratory on *Galleria mellonella* larvae for many generations, following standard procedures (Kaya and Stock, 1997). Infective juveniles used in experiments were collected from White traps (White, 1927) and stored in sterile, distilled water at 25 °C for up to 1 week following emergence from the host.

Galleria mellonella larvae were obtained from the De-

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partment of Biological Sciences Insectary, Simon Fraser University, where they were reared at 28 °C to 30 °C under a 16 hours light:8 hours dark photoperiod on a diet of 1.2 liters Mixed Cereal (J. H. Heinz, Leamington, ON), 119 ml glycerine, 100 ml refined sucrose, 5 drops of multi-vitamin supplement (Polyvisol, Mead Johnson & Company, Evansville, IN), and 98 ml water.

Aphidoletes aphidimyza was reared as a laboratory colony on pea aphid (*Acyrthosiphon pisum*)-infested broad beans (*Vicia faba* 'Windsor'; from West Coast Seeds, Vancouver, BC, and T&T Seeds, Headingly, MB). The initial population of adult *A. aphidimyza* was obtained from Applied Bionomics (Sidney, BC), and the colony was supplemented with additional adult *A. aphidimyza* from Koppert Canada Ltd. (Scarborough, ON). Rearing methods were based on Morse (1981) and Gilkeson (1986), as modified by Powell and Webster (2004). Only those *A. aphidimyza* larvae that had dropped to the colony water tray ≤3 days previously were used in experiments.

Experimental methods: The ability of *S. feltiae* and *H. bacteriophora* IJ to find a "target" *G. mellonella* larva in soil, in the presence of non-target *A. aphidimyza,* was tested in 276-cm^3 styrofoam cups. Cups were filled to within 1 cm of the top with sterile soil (53% sand, 38% silt, 9% clay; high organic matter content; 37% to 41% moisture content (w/w)). A cork borer was used to make a 1-cm-diam. hole in the center of the bottom of the cup. None or 100, third-instar *A. aphidimyza* were added to the soil surface, and one *G. mellonella* larva (200 to 300 mg) within a perforated 1.5-ml microcentrifuge tube was inserted through the hole in the bottom of the cup. The exposed surface of the cup was covered with two layers of plastic film secured with an elastic band. After 24 hours, a 1-ml suspension of IJ in water (containing <0.1 µl Triton X-100) was added to the soil surface and the cup was covered again with the plastic film. In each case, approximately 500 IJ in aqueous suspension were added to each cup, except for the second *S. feltiae* trial in which an unknown number of IJ were added. Cups were incubated at 22 °C for 3 days. Each day, the target insect larva was removed from each cup and replaced by a new target larva. The collected larvae were placed on moist filter paper in separate 60-mm-diam. petri dishes and transferred to 25 °C. After 3 days, dead target larvae were cut open and incubated at 37 °C in ~10 ml pepsin solution (8 g pepsin; 23 g NaCl; 20 ml, 1.0 N HCl; 940 ml distilled water) (Mauleon et al., 1993) on a shaker (200 rpm) for 2 hours before the number of nematodes per target was counted. Due to their small size and cryptic cocoon, it was not possible to reliably recover *A. aphidimyza* larvae from the soil; therefore, the number of nematodes infecting *A. aphidimyza* was not determined. Each cup was monitored for emergence of *A. aphidimyza* adults, which varied from 17% to 79% (mean: 46%); therefore, at least some *A. aphidimyza* remained viable in each cup. Four trials were conducted for each EPN species. An equal number of replicates were used per treatment, ranging from 4 to 15/trial. To ensure that any lack of effect was not due to the experimental design, an additional trial was conducted as a control for each EPN species. The experimental design was similar to that described above except that, instead of 100 *A. aphidimyza* larvae, one *G. mellonella* larva within a perforated, 1.5-ml microcentrifuge tube was placed in the top 2 cm of soil. The number of nematodes within the target larva at the bottom of the cup was recorded as above.

An experiment was conducted to evaluate IJ movement in the presence of *A. aphidimyza. Heterorhabditis bacteriophora* IJ are very active on agar, often migrating up the side of the dish and becoming trapped in water droplets; thus, the number of IJ remaining on the agar surface is insufficient to obtain precise estimates of IJ aggregation. Consequently, IJ of this species were evaluated in petri dishes (150-mm-diam.) filled with moist sterile soil to a depth of 2 cm. A perforated, 1.5-ml microcentrifuge tube containing only a cotton plug was embedded into the soil 4 cm to the left of the center of the petri dish, while another containing 1 or 30 *A. aphidimyza* larvae, none (negative control), or one 200 to 300-mg *G. mellonella* larva (positive control), and a cotton plug to secure insects was embedded directly opposite (designated the "insect" side of the arena). These experimental arenas were incubated at 22 °C for 24 hours. Approximately 250 IJ were added in a small drop of water (containing <0.1 µl Triton X-100) to the center of each arena. After incubating the arenas at 22 °C for an additional 24 hours, the microcentrifuge tubes were removed and a 150-mm long barrier, consisting of two overlapping glass slides held together with rubber cement, was placed in the center of the arena perpendicular to an imagined straight line connecting the microcentrifuge tubes, dividing the soil into two halves. *Aphidoletes aphidimyza* and *G. mellonella* were maintained at 25 °C for 3 days following removal from soil, and dead *A. aphidimyza* were cut open and examined for IJ infection. The number of nematodes within the *G. mellonella* larva was recorded as described above. After removing the initial insects from the arena, four *G. mellonella* larvae in perforated microcentrifuge tubes (baits) were added, two to each side. The number of nematodes within the baits was recorded 3 days later using the method described above. Percentage of IJ migrating toward the insect(s) was calculated by dividing the number of IJ counted in baits (not including the initial *G. mellonella* larva in the case of the positive control treatment) from the insect side of the arena by the total number of nematodes counted in all baits from both sides of the arena and multiplying by 100%. The experiment was repeated once; each treatment was replicated 10 times/trial.

Movement of *S. feltiae* IJ was evaluated on 100-mm-

diam. petri dishes filled with ~35 ml of 2% agar (w/v). A 1-cm-diam. hole was placed in the center of each petri dish lid using a hot cork borer and two 5-mm-diam. holes were placed 18 mm from the edge on either side of the center using a hot dissecting needle. Petri dishes were sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL). The center hole was covered with adhesive tape and one 2.5-ml pipet tip was inserted through each of the other two holes so that its tip was imbedded ~2 mm below the agar surface. Pipet tips containing 1 or 75, third-instar *A. aphidimyza* larvae, one *G. mellonella* larva (positive control), or no insects (negative control) secured in place by a cotton plug were placed on one side of the arena, chosen at random, while the other side contained only a cotton plug. Twenty third-instar *A. aphidimyza* were weighed individually on a CAHN 21 Automatic Electrobalance prior to the *S. feltiae* experiment. Mean mass (±SEM) of *A. aphidimyza* larvae was 0.79 ± 0.02 mg. All *G. mellonella* larvae used in the *S. feltiae* experiment weighed approximately 75 to 100 mg (similar in total weight to 75 *A. aphidimyza* larvae). Petri dishes were incubated at 22 °C for 24 hours and then the tape was removed, 150 to 300 IJ were added through the center hole to the agar surface in 20 µl of water (containing <0.1 µl Triton X-100), and the center hole was covered again with adhesive tape. The plates were incubated at 22 °C for an additional 24 hours, and then the number of IJ found within a 15-mm radius circle surrounding each pipet tip was recorded. Percentage of IJ aggregating near the pipet tip containing one or more insects was calculated by dividing the number of IJ observed in the circle on the insect side of the arena by the total number of IJ observed in both circles and multiplying by 100%. In negative control dishes, the "insect" side of the arena was chosen randomly from the two sides. The experiment was repeated once; each treatment was replicated six and seven times in the first and second trial, respectively.

Data were analyzed using JMP 4.0.3 (SAS Institute, Duxbury Press, Cary, NC). Data reported as percentages were transformed with arcsin (√y), and counts were transformed with log (y+1) before analysis. Backtransformed means and confidence intervals are reported in the results section. Comparisons between two means were performed using Student's *t*-test. For the aggregation experiments, Student's *t*-test was used to determine if IJ displayed non-random movement, indicated by the percentage of IJ found near the insect(s) differing from 50%. The level of significance was set at $P = 0.05$.

Results and Discussion

More IJ were recovered from the target insect larvae in the bottom of the cups when no alternate host was present at the soil surface than in those cups in which a *G. mellonella* larva was at the surface (*S. feltiae:* 103 IJ (95% CI: 49–216) vs. 20 IJ (10–41); $t = 3.53$, df = 10, $P = 0.006$; *H. bacteriophora:* 16 IJ (13–21) vs. 9 IJ (7–11); $t = 3.67$, df = 17, $P = 0.002$). This amounted to an 81% and 44% reduction in the number of *S. feltiae* and *H. bacteriophora* IJ, respectively, infecting the target larva in the presence of an alternate *G. mellonella* larva. However, *A. aphidimyza* did not have the same effect on IJ host finding behavior. In all four trials for both *H. bacteriophora* (Fig. 1A) and *S. feltiae* (Fig. 1B), there was no difference in the number of IJ infecting target insect larvae whether *A. aphidimyza* were present or absent (*H. bacteriophora:* trial 1: $t = 0.41$, df = 6, $P = 0.70$; trial 2: $t = 0.22$, df = 10, $P = 0.81$; trial 3: $t = 0.72$, df = 22, $P = 0.48$; trial 4: $t = 0.63$, df = 26, $P = 0.53$; *S. feltiae:* trial 1: $t = 0.53$, df = 10, $P = 0.61$; trial 2: $t = 1.77$, df = 14, $P = 0.10$; trial 3: $t = 1.06$, df = 11, $P = 0.31$; trial 4: $t = 1.51$, df = 28, $P = 0.14$). Therefore, EPN efficacy in finding and infecting the target insect deeper within the soil should

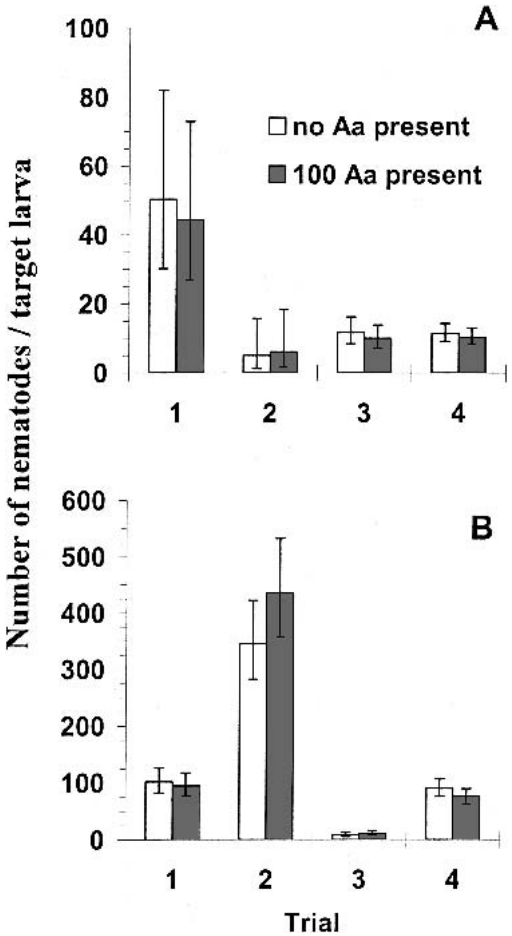

Fig. 1. Mean number of *Heterorhabditis bacteriophora* (A) and *Steinernema feltiae* (B) recovered from target *Galleria mellonella* larvae at the base of a soil-filled, styrofoam cup (276 cm^3) containing 100 or no *Aphidoletes aphidmyza* (Aa) larvae near the soil surface. All bars represent sample sizes $n = 4$, 6, 12, and 14 (A) and $n = 6$, 8, 6–7, and 15 (B) for trials 1 to 4, respectively. No differences were observed between the two treatments in all four trials for both EPN species ($P > 0.05$, Student's *t*-test). Backtransformed means and 95% confidence intervals are presented.

not be reduced following an inundative soil application when *A. aphidimyza* are present at the soil surface. Published estimates of *A. aphidimyza* abundance under greenhouse (van Schelt and Mulder, 2000) and field (Raworth, 1984) conditions indicate that the larval/pupal density in soil of a natural or augmented population is much smaller than the density that was used in this study. *Heterorhabditis bacteriophora* infection of *A. aphidimyza* did not differ among host stages of uncocooned third-instar larvae, uncocooned pupae, or either stage within a cocoon (Powell and Webster, 2004); therefore, the experiment was not attempted with *A. aphidimyza* at different developmental stages.

It is hypothesized that the lack of effect in the presence of *A. aphidimyza* was due to a relative lack of attraction by IJ to the *A. aphidimyza* in soil, even though they are known to be susceptible to EPN infection (Powell and Webster, 2004). The results of the aggregation experiment support this hypothesis (Fig. 2A,B). Within each of the EPN species-insect treatment combinations in the aggregation experiment, there was no difference among the different trials in the mean percentage of IJ found on the insect side of the arena. Therefore, within each treatment combination, the data from the different trials were pooled for analysis. Infective juveniles of both species displayed nonrandom movement in the presence of a *G. mellonella* larva (*H. bacteriophora:* $t = 2.74$, df = 19, $P = 0.01$; *S. feltiae:* $t = 2.42$, df = 12, $P = 0.03$). However, IJ of both species displayed random movement in the presence of *A. aphidimyza,* and increasing the number of *A. aphidimyza* in the arena did not alter this behavior (*S. feltiae:* 1 larva: $t = 0.40$, df = 12, $P = 0.70$; 75 larvae: $t = 0.12$, df = 12, $P = 0.91$; *H. bacteriophora:* 1 larva: $t = 0.47$, df = 19, $P = 0.65$; 30 larvae: $t = 0.03$, df = 19, $P = 0.98$). Infective juveniles in the negative control moved also at random (*S. feltiae:* $t = 0.48$, df = 12, $P = 0.64$; *H. bacteriophora:* $t = 0.46$, df = 19, $P = 0.65$). Of the total number of *S. feltiae* IJ added to the agar arenas, about 13% were found within 15 mm of the two pipet tips, whereas in the petri dishes containing soil, about 10% of *H. bacteriophora* IJ were recovered using *G. mellonella* larvae as bait. Few *A. aphidimyza* (<1%) were infected in the *H. bacteriophora* experiment; therefore, it is not likely that the observed lack of effect was due to IJ infecting *A. aphidimyza* not being counted. Since IJ aggregation in the *H. bacteriophora* experiment was measured indirectly, using *G. mellonella* larvae as bait insects, it is possible that the difference between the positive control and the other treatments is due to an effect on IJ infectivity following exposure to different types of insect hosts (Lewis et al., 1996). However, this is unlikely because the average number of IJ recovered per *G. mellonella* larvae examined (including the initial *G. mellonella* larva in the case of the positive control treatment) did not differ among the treatments (one-way ANOVA: $F = 1.22$, df = 3, 76, $P = 0.31$).

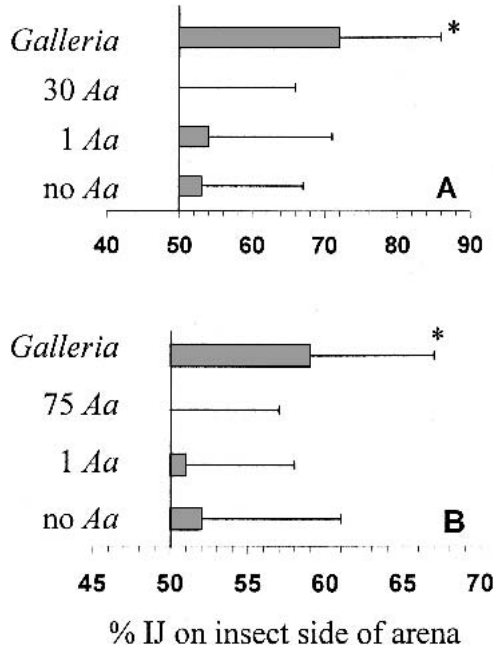

FIG. 2. Mean percentage of *Heterorhabditis bacteriophora* (A) and *Steinernema feltiae* (B) infective juveniles migrating toward the "insect" side of an attraction arena in the presence of no, one, or multiple *Aphidoletes aphidmyza* (Aa) larvae or one *Galleria mellonella* larva. Bars to the right of 50% indicate net movement toward insects. All bars based on sample size $n = 20$ (A) and $n = 13$ (B). Within each treatment, arcsine-transformed data were analyzed using Student's *t*-test to evaluate if the pattern of IJ movement was significantly different from random movement (i.e., 50%). *: $P < 0.05$. Backtransformed means and 95% confidence intervals are presented.

Galleria mellonella was used as a target host in these experiments even though, in the field, it is normally not a target in control programs using EPN. The IJ used in these experiments had been reared on *G. mellonella* for many generations, possibly resulting in selection for enhanced ability to find *G. mellonella* under artificial conditions (Gaugler and Campbell, 1991). The use of a less attractive (to IJ) target host species might have resulted in reduced IJ movement toward the target host at the bottom of the cup. Nevertheless, the lack of IJ aggregation near *A. aphidimyza* larvae suggests that no *A. aphidimyza* effect would be observed regardless of the target used.

Infective juveniles of *H. bacteriophora* have demonstrated potential to reduce *A. aphidimyza* efficacy as a biological control agent of aphids in greenhouse experiments; in particular, *A. aphidimyza* emergence was reduced in pots containing *Triticum aestivum* treated with a high density of IJ (250 IJ/cm^2) relative to untreated controls (Powell and Webster, 2004). In the present study, *A. aphidimyza* did not reduce target host finding by *S. feltiae* and *H. bacteriophora;* therefore, there is no evidence to suggest that *A. aphidimyza* reduces EPN efficacy when these two biological control agents are present together in a field situation. If these two agents are used together in an integrated pest management program, any antagonism that occurs will likely

be asymmetric, with a small number of IJ infecting *A. aphidimyza* by chance such that aphid control is impaired but EPN inoculum potential is not. However, other non-target arthropod species might have a negative effect because a reduction in target host finding by IJ was observed in the presence of an alternate *G. mellonella* larva. The abundance of other non-target arthropods, such as collembolans, mites, and other microarthropods, could be high at the time of an IJ application. If IJ were to orient toward non-target derived stimuli, the relative attractiveness to IJ of the target and nontarget(s) would take on growing importance with regard to EPN efficacy, as would differential responses to these stimuli among EPN species. Consequently, identifying those arthropods that IJ contact while searching for a host and determining the effect that their presence has on IJ target host finding and EPN efficacy merits further research.

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