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Heterorhabditid Behavior in the Presence of the Cabbage Maggot, *Delia radicum*, and its Host Plants

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Abstract: The behavior of *Heterorhabditis zealandica* Poinar strain T327 was investigated in the presence of the cabbage maggot, *Delia radicum* L., and plants that are susceptible to *D. radicum* infestation. Newly formed puparia and freeze-killed third instar larvae were attractive to infective nematodes. Newly harvested infective nematodes did not respond to the puparia, whereas 1-month-old and 2-month-old nematodes reached the insect targets within 15 minutes. There were no significant differences in the ability of similar-sized, third instar larval *D. radicum* and *Galleria mellonella* L., the greater wax moth, to attract nematodes. There was a tendency for a greater number of insects to attract more nematodes. The roots of ball cabbage and radish were equally attractive to nematodes, but rutabaga roots neither attracted nor repelled the nematodes. Germinated seeds of radish attracted nematodes, and there was a tendency for more numerous germinated seeds to attract more nematodes.

Key words: biological control, chemoattraction, *Heterorhabditis zealandica*, host-parasite interaction, nematode.

The cabbage maggot, *Delia radicum* L., is a common pest in vegetable-growing areas (2) throughout many temperate regions. Larval *D. radicum* cause damage by feeding and tunneling, mostly in the main roots but sometimes in the aerial parts of many cruciferous species, cultivated and wild (6), and by increasing the prevalence of bacterial soft rot. Control of this pest has been through the use of organochlorine, organophosphorus, and carbamate insecticides (3).

Heterorhabditid nematodes have been studied as biological control agents for many insect pests (9). Third-stage, infective juvenile (J3) nematodes are attracted to an insect host and penetrate its cuticle (1). Once in the hemocoel they release the symbiotic bacterium *Xenorhabdus luminescens* Thomas & Poinar, which multiplies rapidly, causes septicemia, and kills the host within 24–48 hours. Little is known of the behavior of heterorhabditid nematodes except for the report of Bedding and Molyneux (1). Carbon dioxide (7), insect fecal and excretory products (12), heat (5), and pH gradients (11) are attractive to the J3 of another rhabditid, *Steinernema carpocapsae* Weiser. Larvae of the greater wax moth, *Galleria mellonella* L., are attractive to the J3 of *S. carpocapsae* DD136 strain (10).

Eggs, laid by adult *D. radicum* close to the plant stems, hatch within 2–14 days, and the newly hatched larvae penetrate the plant roots within hours (6). The very small size of the first instar larvae and their short duration in the soil makes them an elusive target for entomopathogenic nematodes (4). Hence, our experiments were aimed at determining the behavior of the nematode in relation to the final (third instar) larval and the puparial stages of the insect.

Understanding the behavior of heterorhabditid nematodes in the presence of the cabbage maggot and plants that are susceptible to infestation by this pest may help to increase the efficacy of these nematodes against this insect and make them economically viable as control agents. This investigation reports on qualitative and quantitative experiments done to determine the behavior of heterorhabditids in the presence of different developmental stages of the target insect and of the crop plant.

Materials and Methods

Cabbage maggots were obtained from J. Whistlecraft, Agriculture Canada Re-

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search Center, London, Ontario, and reared in the laboratory. Adult flies were maintained in a cage (40 × 25 × 25 cm) at 20–25 C and 50–70% RH, with a photoperiod of 16 hours per day. Flies were fed powdered food composed of sugar, skim milk powder, soya flour, yeast, and yeast hydrolysate. An oviposition site was made in a cage from a petri dish lid containing a thin layer of autoclaved sand (ca. 1.0-mm particle size) and a piece of fresh rutabaga (5 × 5 × 0.5 cm). Dry sand containing 200–400 of the newly laid eggs was poured around a caged, potted rutabaga in moist sand, and after 10–12 days third-instar larvae were obtained by cutting the open rutabaga. Newly formed puparia were obtained by collecting mature larvae as they emerged from the rutabaga, washing them with water, keeping them overnight at room temperature in a dish with moist filter paper, and collecting the pupae the following day. Puparia and larvae were surface sterilized at room temperature with 80% ethanol for 30 seconds, rinsed five times with sterilized water, and dried on filter paper.

Heterorhabditis zealandica Poinar strain T327, isolated in Tasmania, Australia, was obtained from Dr. R. A. Bedding in 1987 and reared in larvae of *G. mellonella.* The J3 were collected (15) and stored in moist, oxygenated sponge in sealed packages at 12 C. Cultures were passaged through the laboratory host every 6 months to ensure that vigorous nematodes were used for this study. Before each experiment, the nematodes were removed from the storage incubator and allowed 2–4 hours to adapt to the prevailing room temperature. Infective juveniles were extracted from storage by rinsing the sponge with distilled water. They were washed and the number of nematodes in a suspension was estimated volumetrically. Individual nematodes were handled with a fine, mounted hair. All experiments were done at 22–24 C in petri dishes (100 × 15 mm) containing 1.5% Bacto Agar (Difco Laboratories, Detroit), and they were then sealed with Parafilm "M" (American Can, Greenwich, CT). For the qualitative experiments, the very thin layer of agar was obtained by pouring off surplus agar before it set.

Rutabaga (*Brassicae napus* var. *napobrassica*), radish (*Raphanus sativus*), and ball cabbage (*B. oleracea* var. *capitata*) were used for this study. Radish and ball cabbage were grown outdoors from seed, but fresh rutabagas were bought locally.

Experiment 1: Experiments were done to observe the behavior of *Heterorhabditis* juveniles in the presence of puparia and larvae of *D. radicum.* The underside of each of 12 petri dishes was marked at the center and also at the apexes of an equilateral triangle. The apexes were 2 cm from the center. A thin layer of agar was poured into each dish. After the agar had cooled, two surface-sterilized puparia or two killed larvae of *D. radicum* were placed at the center of each of six dishes and three J3 were placed one at each of the marked apexes of each of the 12 dishes; the 6 dishes without *D. radicum* functioned as the control. The surface-sterilized, third-instar larvae were killed by freezing for 8 minutes, after which they were thawed for 30 minutes. At 21 C, photographs were taken (13) at intervals of 5, 15, and 30 minutes after the nematodes were introduced. Subsequently, seven more photographs of each dish were taken at 30-minute intervals, for a total of 10 photographs of each dish. Later, 10 more replicates with puparia and nine more with the larvae were set up using the same methods and under similar conditions.

The number of nematodes that were present at the targets of puparia or larvae was determined by following the nematode tracks in the agar as recorded on the photographic plates.

Experiment 2: An experiment was done to test if freshly emerged nematodes from in vivo culture oriented to newly formed puparia of *D. radicum.* Each experiment was replicated five times. Newly formed, washed, surface-dried puparia were transferred to 7-mm-d discs of Whatman #1 filter paper on a thin layer of agar in a petri dish (Fig. 1a). Dishes were sealed with

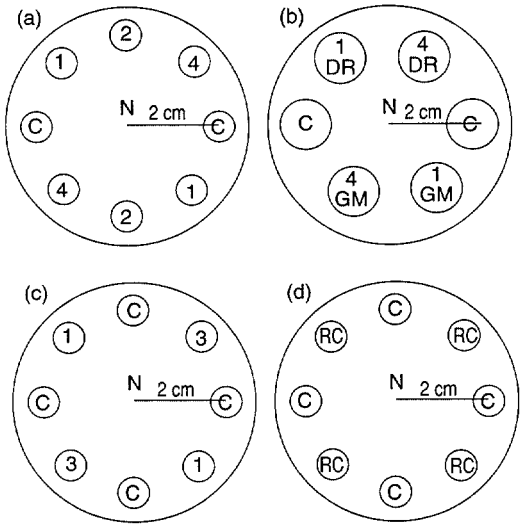

FIG. 1. Arrangement of filter paper discs in petri dishes of agar in relation to the point of introduction (N) of infective juveniles of *Heterorhabditis zealandica* strain T327 (a) with pupal or larval *Delia radicum* at points 1, 2, and 4 or blank controls (C), (b) with larval *D. radicum* (DR), *Galleria mellonella* (GM), or blank controls, (c) with germinated seeds of radish at points 1 and 3 or blank controls and (d) with cylinders of radish and rutabaga roots, or of radish and ball cabbage roots (RC), or blank controls.

parafilm and incubated at room temperature for 1 hour before approximately 250 newly harvested J3 were placed at the center of each of five dishes. The dishes were resealed and incubated at 24–24.5 C under fluorescent room light. Twenty-four hours later, the puparia and paper discs were removed and washed separately with water, and the nematodes in the washes were counted under a dissecting microscope. Nematodes present in and on the agar beneath the paper discs were counted immediately after the puparia and paper discs were removed.

Another experiment with juveniles that had been stored for 1 month was done under similar conditions and divided into two subsets (each with ten replicates), one ending 4 hours and the other 24 hours after nematode introduction.

Experiment 3: Experiments were done to test if the larvae of *D. radicum* and *G. mellonella* influenced T327 differently. *Delia radicum* larvae were collected, washed, killed by freezing, and then thawed; in another experiment, larvae of *D. radicum* and *G. mellonella* of about equal weight were washed (not sterilized) with distilled water, killed by freezing, and then thawed. For each experiment, these larvae were arranged on filter paper discs on agar in each of 10 petri dishes (Fig. 1A,B). The twenty controls contained no larvae. Infective juveniles were introduced after 2 hours, and the sealed dishes were maintained under fluorescent room light for 24 hours. Nematodes that reached the targets were counted. The total number of nematodes at the targets included those that had entered the larvae, which were dissected 4 days after being removed from the target sites.

Experiment 4: To study the influence of the host plants of *D. radicum* on the juveniles of *Heterorhabditis,* radish seeds were sterilized with 80% ethanol for 1 minute, rinsed five times with sterilized water, and kept overnight on wet filter paper in a petri dish. They germinated quickly, and the radicles grew to about 3 mm in length within 24 hours at room temperatures. These germinated seeds, complete with testa, were transferred to filter paper discs that had been arranged on agar in petri dishes (Fig. 1C). One or three seeds, with the filter paper discs and the agar underneath, were the treated targets; the paper discs alone and the agar underneath were the control targets. The sealed dishes were incubated in the dark for 2 hours and then 100–200 infective juvenile T327, which had been stored in an incubator at 12 C for 2 months, were placed at the center of each of the dishes. The dishes were resealed and incubated for 3 hours under fluorescent room light, after which nematodes were counted on the seeds, on the filter paper discs, and in the agar beneath the paper discs.

Experiment 5: To compare the attractiveness of *D. radicum* host plant roots for juvenile *Heterorhabditis,* two tests were done on separate days using roots of either radish and rutabaga (test #1) or roots of radish and ball cabbage (test #2). Roots of radish, rutabaga, and ball cabbage were

cut transversely with a blade and punched with a core punch into cylinders of 5 mm d and 2 mm height, not including root skin. The cylinders were arranged on filter paper discs on agar in petri dishes (Fig. 1D), sealed, and incubated under fluorescent room light for 1.5 hours. From 300 to 600 nematodes were placed at the center of each dish, and the dishes were resealed and incubated under light for 2.5 hours; at this time the nematodes reaching the treated and control targets were recorded.

Comparisons between treatments and controls were tested for significance using Student's *t*-test, in terms of the mean percentage number of nematodes that had reached the insect or plant target.

Results

Experiment 1: Soon after the nematodes were placed on the agar, responding nematodes moved toward the insect targets. Some reached the puparia or the larvae within 15 minutes, and this number increased with time (Fig. 2). Within 4 hours, 33% and 64% of the introduced nematodes reached the puparia and the larvae, respectively. Nematodes in the six control dishes moved randomly.

Experiment 2: One day after approximately 250 newly harvested J3 were introduced, only three nematodes had reached three targets of two puparia and one nematode had reached one target of four puparia. No nematodes were present at the remaining targets. However, J3 that had been stored for 1 month after being collected from the same culture reached the targets of four, two, and one puparia after 4 hours, and significantly more reached the larger number of puparia after 24 hours ($P < 0.05$ between two puparia and one, and between four and two; $P < 0.01$ between four and one; Fig. 3A).

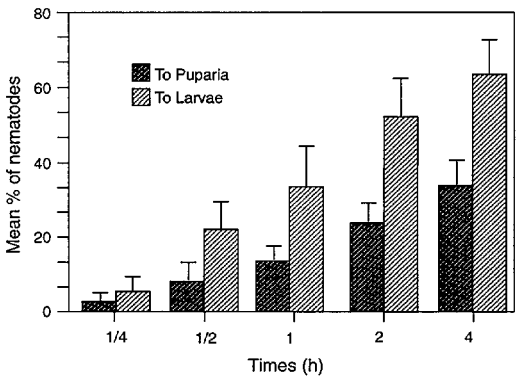

FIG. 2. Mean percentages of introduced, infective juvenile *Heterorhabditis zealandica* strain T327 that reached newly formed puparia or freeze-killed, third-instar larvae of *Delia radicum* over time. Top bars show standard errors (SE; n = 13 for puparia; n = 12 for larvae).

Experiment 3: Delia radicum larvae were attractive to the nematodes and multiple larvae attracted more nematodes than did a single larva (Fig. 3b). More nematodes were attracted to four larvae than to lower numbers ($P < 0.01$ between four larvae and one; $P < 0.05$ between four larvae and two) or to the controls ($P < 0.05$).

In this experiment on larval *D. radicum* and *G. mellonella,* the nematodes began to move randomly immediately after they were placed on the agar. Later, some of them oriented to the insects, but few oriented to the controls. More nematodes oriented to the targets as the numbers of target insects were increased (Fig. 3c). Although some nematodes reached the controls soon after they were introduced, none stayed there after 24 hours, and differences occurred between the treatments with insects and the controls ($P < 0.05$). There were no significant differences between the percentages of nematodes that were attracted to *D. radicum* and to *G. mellonella* (Fig. 3c).

Experiment 4: Within 3 hours, on average, 0.5%, 1.7%, and 3.2% of the introduced nematodes reached zero-seed, one-seed, and three-seed targets, respectively (Fig. 4a). Although most of the nematodes moved randomly on the agar or had moved to the walls or lids of the dishes, the germinated radish seeds appeared to be slightly attractive to them, because there were more nematodes at the one-seed ($P < 0.05$) and three-seed ($P < 0.001$) targets than at the controls (Fig. 4a). More nematodes accumulated at the three-seed than at the one-seed targets ($P < 0.05$).

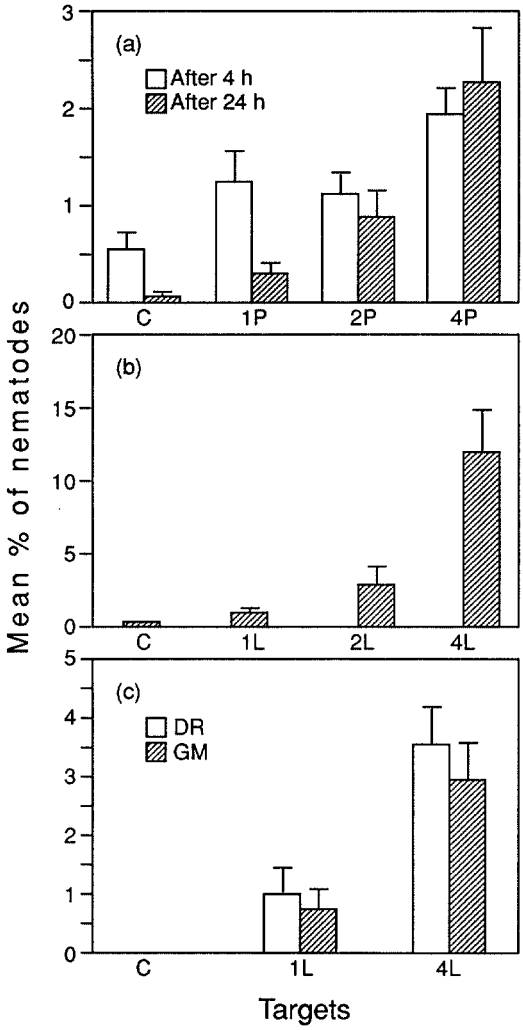

FIG. 3. Mean percentages (±SE) of introduced, infective, juvenile *Heterorhabditis zealandica* strain T327 that reached the targets of (a) one (1P), two (2P), or four (4P) newly formed puparia of *Delia radicum* after 4 and 24 hours, (b) one (1L), two (2L), or four (4L) frozen, dead, 3rd-instar larval *D. radicum* within 24 hours, and (c) one (1L) or four (4L) larval *D. radicum* (DR) and *Galleria mellonella* (GM) within 24 hours. In each case, the controls (C) contained zero organisms.

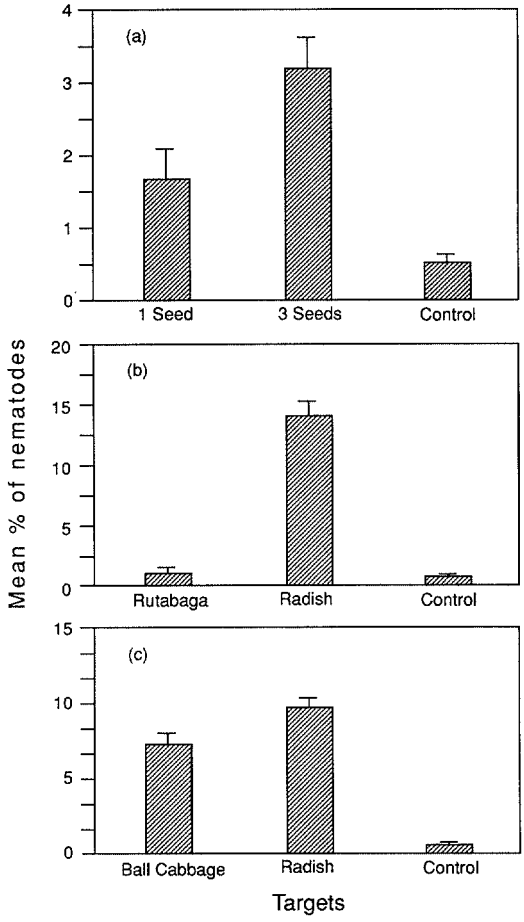

FIG. 4. Mean percentages (±SE) of infective juvenile *Heterorhabditis zealandica* strain T327 that were attracted to different targets. (a) Targets of germinated seeds of radish (10 replicates of each treatment) and blank controls (20 replicates), within 3 hours. (b) Target cylinders of radish and rutabaga roots (10 replicates of each) and blank controls (20 replicates) for 2.5 hours. (c) Target cylinders of radish and ball cabbage roots (10 replicates of each) and blank controls (20 replicates) for 2.5 hours.

Experiment 5: Within 2.5 hours, on average, 13.8%, 0.8%, and 0.5% of the nematodes introduced in test #1 had reached the roots of each of radish, rutabaga, and the control targets, respectively (Fig. 4b); 9.4%, 7.2%, and 0.3% of the nematodes in test #2 had reached the roots of each of radish, ball cabbage, and the control targets, respectively (Fig. 4c). In both tests, more nematodes reached radish than the controls, and in the first test more nematodes reached radish than rutabaga. However, there were no significant differences between the attractiveness of rutabaga and the control targets in the first test and between radish and ball cabbage targets in the second test.

DISCUSSION

The tracks of the infective juvenile T327 on the agar strongly suggested that the

nematodes were attracted toward the living puparia and dead larvae of *D. radicum* rather than encountering them randomly. Responding nematodes reacted to the attractants soon after they were introduced onto the agar and moved quickly to the targets, which they often reached within 15 minutes.

Because more nematodes reached the targets over time, there seemed to be a difference in speed of response to the attractants among individuals. Within 4 hours, many more nematodes reached targets with dead larvae than with living puparia. Dead larval integument is probably more permeable to attractive chemicals than that of puparia. Recently (4), laboratory experiments and field trials showed very limited success of *H. heliothidis* against first-instar larvae and pupariae. In our experience, the very small first-instar larvae are not readily attacked by *H. heliothidis*.

In our study, orientation of nematodes to *D. radicum* and *G. mellonella* could have been caused by the combined effect of various factors, some of which may predominate. Microbes on the surface of insect targets in the qualitative experiments had probably been eliminated by sterilization with ethanol, and there was no visible contamination. In the quantitative experiments, 26 hours after the insects were introduced, there was slight visible bacterial contamination on the agar around some of the filter paper discs with unsterilized insects, but not around sterilized ones. Unsterilized and sterilized insects were equally and strongly attractive to the nematodes. Heat, produced from the respiration of living pupae or from the decomposition of dead larvae, may attract the nematodes. Perhaps more likely, chemical gradients responsible for the attraction developed around the insects, such as carbon dioxide from the respiration of living pupae and (or) chemicals released from decomposing larvae. There were no significant differences in attraction between dead larvae of *D. radicum* and *G. mellonella*.

Newly collected juveniles did not orient to pupal *D. radicum,* whereas others from the same cultures stored at 12 C for 1 month did. This suggests that these nematodes may need some conditioning before they are able to recognize physical or chemical attractants from their hosts. This requirement could be a factor allowing for more effective dispersal for synchronizing the development of this nematode to that of its natural host and ensuring that only a few J3 are attracted to each host. It is suggested that newly harvested infective juvenile T327 be stored for a period before they are used to control insect pests.

In nature, plant roots liberate various substances, such as carbon dioxide and amino acids, which form chemical gradients around the root system. These substances in turn modify the quantitative and qualitative microbial composition of the rhizosphere (14). Similarly, heat given off by respiring roots and microorganisms of the rhizosphere establish temperature gradients around the root system. The chemical and temperature gradients that are attractive to the entomopathogenic nematodes may increase their opportunities to obtain access to larval *D. radicum*. The combined attractiveness of *D. radicum* and some of its host plants to these nematodes may aid the nematodes considerably in locating their insect hosts. This has been suggested (8) for corn roots in regard to their influence on the pathogenicity of *H. zealandica* on the insect pest. Although the third instar larvae and puparia may be more attractive to *H. zealandica* than are the first instar larvae, the respective biologies of the insect and nematode are probably not sufficiently complementary for the nematode to be an effective biological control agent.

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