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# Natural Occurrence of Entomogenous Nematodes in Tennessee Nursery Soils[1]

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*Abstract:* To isolate potential insect biocontrol agents, entomogenous nematodes were surveyed in Tennessee plant nurseries in 1991. Soil samples from 113 nursery sites were baited with greater wax moth (*Galleria mellonella*) larvae, house cricket (*Acheta domesticus*) adults, lesser mealworm (*Alphitobius diaperinus*) adults, and house fly (*Musca domestica*) larvae. *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* were each recovered from 17 soil samples. *Heterorhabditis bacteriophora* was more common in habitats with crape myrtle (*Lagerstroemia indica*) and Chinese juniper (*Juniperus chinensis*) than other nursery plants, and *S. carpocapsae* was more frequently recovered from habitats with juniper and Southern magnolia (*Magnolia grandiflora*). Bulk density, electrical conductivity, organic matter, pH, temperature, and moisture content of the entomogenous-nematode positive soil samples were compared. Other nematode genera recovered with insect baits included *Rhabditis* sp., *Pelodera* sp., *Cryptaphelenchoides* sp., and *Mesodiplogaster* sp., which was recovered from a greater percentage of soil samples than the other five genera.

*Key words: Acheta, Alphitobius,* biological control, *Cryptaphelenchoides,* distribution, entomogenous nematode, *Heterorhabditis, Mesodiplogaster, Musca,* nursery plant, *Pelodera, Rhabditis, Steinernema,* survey.

Heterorhabditid and steinernematid nematodes are potentially useful biological control agents for numerous pests (11,17, 23). Following the introduction of the insect-bait trap method (6), numerous surveys and seasonal distributional studies of entomogenous nematodes were conducted worldwide, including Australia (1), Czechoslovakia (19), Great Britain (15), Hungary (20), Ireland (8,13), Sweden (10), Puerto Rico (26), Florida (5), North Carolina (2), and Hawaii (14). These nematodes were recovered from a variety of habitats (e.g., pastures, forests, row crop fields, orchards, beaches) with different soil characteristics and textures (2,14). The purposes of this study were to investigate the occurrence of entomogenous nematodes in various nursery plant habitats and soil types in Tennessee and to obtain locally adapted isolates for possible development of biological control programs in plant nurseries.

## Materials and Methods

*Sample collection:* A total of 113 soil samples was collected from selected plant nurseries in three counties (Warren, Franklin, and Cumberland) of Tennessee in August 1991 for about 2 weeks. The collection sites were predominantly covered with the following 10 woody ornamental plants: American arborvitae (*Thuja occidentalis*), Chinese juniper (*Juniperus chinensis*), prairie crabapple (*Malus ioensis*), weeping cherry (*Prunus subhirtella* var. *pendula*), boxwood (*Buxus sempervirens*), Japanese maple (*Acer palmatum*), Southern magnolia (*Magnolia grandiflora*), sycamore (*Platanus occidentalis*), crape myrtle (*Lagerstroemia indica*), and shrub althea (*Hibiscus syriacus*). In addition, soil samples were collected from areas covered with grasses, predominantly crabgrass (*Digitaria sanguinalis*) and quackgrass (*Elytrigia repens*). Ten soil samples were gathered from near each plant species, except for crape myrtle (13 samples). Each sample was collected to a 15-cm depth with a 8-cm-d soil corer. Each sample consisted of six random subsamples taken 30–90 cm from the base of

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the woody plants over an area of 100 $m^2$. The six subsamples were mixed thoroughly in a plastic bag. About 5 liters of each mixed sample was placed in another bag and taken to the laboratory in an air-conditioned vehicle. Soil temperature at sampling was measured at a depth of 5 cm.

*Nematode isolation and identification:* Soil samples were tested for entomogenous nematodes within 12 hours after sampling by baiting with four species of insects: sixth-instar larvae of the greater wax moth (*Galleria mellonella*), adults of the house cricket (*Acheta domesticus*), adults of the lesser mealworm (*Alphitobius diaperinus*), and third-instar larvae of house fly (*Musca domestica*). The *G. mellonella* larvae and *A. domesticus* adults were obtained from fish bait shops (Northern Bait Co., Chetek, WI, and The Bait Shop, Hendersonville, TN, respectively); the *A. diaperinus* adults were collected from a poultry house (Shelbyville, TN); and the *M. domestica* larvae were from our laboratory cultures, established from adult flies collected from another poultry house (Shelbyville, TN). Five individuals of each insect species were placed on the soil surface of each sample in a petri dish (14 × 2.5 cm), following a modification of published techniques (6,14). Each petri dish contained about 200 ml soil, and a total of 20 dishes (five dishes per insect species) were used per soil sample. The petri dishes were inverted and held at 24–26 C. Insect mortality was recorded 1 week later. The assay was repeated using fresh insects in the same soil samples for another week. Dead insects from each sample were rinsed in sterile deionized water and incubated in modified White (31) traps for 1–3 weeks to collect emerging infective juvenile nematodes. Cadavers without emerging juveniles were dissected to detect nematodes remaining in the insect. The juveniles that migrated into the water were exposed to five fresh *G. mellonella* larvae on a filter paper in a petri dish to verify pathogenicity (28). The pathogenic isolates were used to establish laboratory cultures. Entomogenous nematodes were morphologically identified by R. E. Harrison using pertinent literature (22–24).

*Nematode cross-breeding:* To complement and confirm identification based on morphology, TN1 (our Tennessee isolate recovered from a habitat containing *T. occidentalis*, with *G. mellonella* bait) was crossed with *S. carpocapsae* strain "Mexican" in vitro. Nematodes were co-cultured with their symbiotic bacteria on nutrient agar supplemented with 0.1% sunflower seed oil. For mating experiments, individual preadult females were transferred to NGM plates (9) containing 25 μg/ml cholesterol and seeded with bacteria isolated from *S. carpocapsae* strain "Mexican" or TN1. At this time or on the following day, three males of the desired strain were added to some of the plates; the remaining plates were controls. Each treatment combination was represented by three to eight plates. After 2 days, the nematodes were transferred to bacteria-seeded nutrient agar–oil plates. Plates were observed periodically until $F_2$ progeny appeared.

A similar procedure was used for cross-breeding our *H. bacteriophora* isolates TN4 and TN23 (Tennessee isolates recovered from habitats with *L. indica* and *J. chinensis*, respectively, with *G. mellonella* bait), except the plates were seeded with bacteria isolated from TN4 and TN23, the nematodes were left on NGM plates for the duration of the test, and the treatments were not replicated.

*Soil and statistical analyses:* All soil samples were analyzed for bulk density, electrical conductivity, pH, organic matter content, moisture content, and soil texture with modifications of published techniques (7,16). Differences in the associations between nursery plant species or soil texture and incidence of nematodes were analyzed statistically with Fisher's exact test (27).

## Results

*Nematode crossbreeding and identification:* *Heterorhabditis bacteriophora* Poinar was the only recovered heterorhabditid, as determined by morphology. It was present in

15% (17 of 113) of the nursery soil samples (Table 1). *Steinernema carpocapsae* Weiser, the only recovered steinernematid, also occurred in 15% of the samples. The morphological identification of these two species was confirmed through crossbreeding experiments.

Reciprocal crosses of *S. carpocapsae* strain "Mexican" and Tennessee isolate TN1 in vitro resulted in fertile progeny. Seven of eight TN1 females crossed with "Mexican" males were fertile, compared with eight of eight TN1 females crossed with TN1 males and zero of six unmated TN1 females. Five of five "Mexican" females crossed with TN1 males were fertile, compared with three of three "Mexican" females crossed with "Mexican" males and zero of three unmated "Mexican" females.

A preliminary test with our *H. bacteriophora* isolates TN4 and TN23 suggests that males are required at least in some cases, and that the two isolates are interfertile or capable of interbreeding. Neither control female produced progeny, whereas all four mated females produced fertile progeny.

As insect baits, *G. mellonella* larvae recovered *H. bacteriophora* from 15 of the 17 positive soil samples in the two bioassays (Table 1). The larvae also retrieved *S. carpocapsae* from 16 of the 17 positive samples. *Acheta domesticus* adults yielded *H. bacteriophora* and *S. carpocapsae* from only three samples each. One soil sample was positive for *S. carpocapsae* in this insect species only. *Alphitobius diaperinus* adults and *M. domestica* larvae, like *A. domesticus*, were poorly effective baits for *H. bacteriophora* and *S. carpocapsae*. Of the 31 soil samples positive for these two species, all but two were positive in the first assay. The other two samples were positive for only *S. carpocapsae* in the second assay and only with *G. mellonella* as bait.

TABLE 1. Number of 113 soil samples positive for entomogenous nematodes with four insect species as baits.

| Bait | Number of positive samples† | | | | | |
|---|---|---|---|---|---|---|
| | Hb | Sc | M | R | P | C |
| *Galleria mellonella* | 15 | 16 | 25 | 1 | 3 | 0 |
| *Acheta domesticus* | 3 | 3 | 66 | 3 | 5 | 1 |
| *Alphitobius diaperinus* | 3 | 1 | 1 | 0 | 0 | 0 |
| *Musca domestica* | 0 | 0 | 5 | 1 | 1 | 0 |
| All insects combined | 17 | 17 | 72 | 5 | 7 | 1 |

† Hb = *Heterorhabditis bacteriophora*, Sc = *Steinernema carpocapsae*, M = *Mesodiplogaster* sp.; R = *Rhabditis* sp.; P = *Pelodera* sp.; C = *Cryptaphelenchoides* sp.

Other nematodes recovered from the insect baits included *Mesodiplogaster* sp., *Rhabditis* sp., *Pelodera* sp., and *Cryptaphelenchoides* sp. (Table 1). *Mesodiplogaster* sp. was encountered most often. It was recovered from a majority of soil samples baited with *A. domesticus* adults. *Rhabditis* and *Pelodera* were found in a few soil samples baited with *G. mellonella* larvae, *A. domesticus* adults, or *M. domestica* larvae. *Cryptaphelenchoides* sp. was recovered from only one soil sample. All nematodes from each sample were passaged successfully a second time through a *G. mellonella* host.

*Heterorhabditis bacteriophora* and *S. carpocapsae* were found in soil samples from most of the Tennessee plant nursery sites surveyed (Table 2). Habitats with *J. chinensis* and *M. grandiflora* had the highest number of soil samples containing *S. carpocapsae*, whereas *L. indica* and *J. chinensis* yielded more soil samples positive for *H. bacteriophora*. Of 11 plant habitats surveyed, only *B. sempervirens* and *H. syriacus* soil samples yielded no steinernematid nematodes. Heterorhabditids were not recovered in nursery habitats with *T. occidentalis*, *A. palmatum*, *M. grandiflora*, and grasses. The relationship between nursery plant species and frequency of nematode recovery was significant ($P < 0.05$), either when the *H. bacteriophora* and *S. carpocapsae* were tested separately or together with Fisher's exact test.

*Mesodiplogaster* sp., the most common nematode in the survey, was recovered most frequently in soil samples from habitats with *B. sempervirens*. *Platanus occidentalis* yielded the least number of soil samples positive for *Mesodiplogaster*. *Rhabditis* sp. was found in soil samples with *A. palmatum*, *J. chinensis*, *M. grandiflora*, and grasses, whereas *P. subhirtella* var. *pendula*, *M. gran-*

TABLE 2. Number of soil samples containing entomogenous nematodes from different nursery plant habitats.

| Plant species | Samples tested | Positive samples† | | | | | |
|---|---|---|---|---|---|---|---|
| | | Hb | Sc | M | R | P | C |
| *Thuja occidentalis* | 10 | 0 | 1 | 5 | 0 | 0 | 0 |
| *Buxus sempervirens* | 10 | 1 | 0 | 10 | 0 | 0 | 0 |
| *Malus ioensis* | 10 | 1 | 1 | 8 | 0 | 0 | 0 |
| *Lagerstroemia indica* | 13 | 6 | 1 | 6 | 0 | 0 | 0 |
| *Acer palmatum* | 10 | 0 | 1 | 8 | 1 | 0 | 0 |
| *Juniperus chinensis* | 10 | 4 | 5 | 7 | 2 | 0 | 0 |
| *Magnolia grandiflora* | 10 | 0 | 4 | 8 | 1 | 1 | 1 |
| *Platanus occidentalis* | 10 | 1 | 2 | 3 | 0 | 0 | 0 |
| *Prunus subhirtella* var. *pendula* | 10 | 1 | 1 | 5 | 0 | 1 | 0 |
| *Hibiscus syriacus* | 10 | 3 | 0 | 7 | 0 | 0 | 0 |
| Grasses (predominantly *Digitaria saguinalis* and *Elytrigia repens*) | 10 | 0 | 1 | 5 | 1 | 5 | 0 |

† Hb = *Heterorhabditis bacteriophora*, Sc = *Steinernema carpocapsae*, M = *Mesodiplogaster* sp., R = *Rhabditis* sp., P = *Pelodera* sp.; C = *Cryptaphelenchoides* sp.

*diflora*, and grasses yielded soil samples positive for *Pelodera* sp. *Cryptaphelenchoides* sp. was recovered in one sample from a habitat with *M. grandiflora*.

*Soil analyses:* In *H. bacteriophora*-positive soil samples, bulk density averaged 1.4 g/cm$^3$; electrical conductivity ranged from 0.006–0.013 S/m; organic matter, 2.1–2.6%; pH, 5.6–6.2; moisture content, 10.8–15.7%; and soil temperature at the time of sampling, 21.5–26.9 C (Table 3). In *S. carpocapsae*-positive soil samples, bulk density averaged 1.4 g/cm$^3$; electrical conductivity ranged from 0.004–0.006 S/m; organic matter, 1.8–2.5%; pH 5.6–6.1; moisture content, 8.4–15.9%; and soil temperature, 23.3–26.4 C. In *Mesodiplogaster*-positive soil samples, bulk density averaged 1.4 g/cm$^3$; electrical conductivity ranged from 0.006–0.012 S/m; organic matter, 2.1–2.4%; pH, 5.4–6.1; moisture content, 11.1–13.9%; and soil temperature, 24–27.9 C. In entomogenous nematode-negative soil samples, bulk density averaged 1.4 g/cm$^3$; electrical conductivity ranged from 0.004–0.018 S/m; organic matter, 1.9–2.5%; pH, 5.8–6.1; moisture content, 8.6–11.1%; and soil temperature, 25–26.1 C.

*Heterorhabditis bacteriophora* was recovered most frequently from sandy loam soils (59%), followed by silt loam (18%) (Table 3). In contrast, *S. carpocapsae* occurred most frequently in silt loam (35%), followed by clay loam (29%) and sandy loam (29%). *Mesodiplogaster* sp., like *H. bacteriophora*, was recovered most often from sandy loam (47%), followed by silt loam (28%). The relationship between soil texture and incidence of *H. bacteriophora* and *S. carpocapsae* recovery was not significant either for individual species or together. On the other hand, there was significant relationship between soil textures and *Mesodiplogaster* recovery ($P < 0.05$; Fisher's exact test).

Entomogenous fungi, such as *Metarrhizium, Beauveria,* and *Hirsutella,* were recovered from *G. mellonella* larval baits in 8, 4, and 2 soil samples, respectively. *Heterorhabditis bacteriophora* and *S. carpocapsae* were not present in any *G. mellonella* fungal-infected larvae.

## DISCUSSION

Entomogenous nematodes have been detected with various frequencies in most terrestrial habitats. In our survey of plant nurseries, heterorhabditids and steinernematids occurred in identical percentages (15%) of the total number of samples. The incidence of these nematodes in our survey (27%) was higher than in surveys from habitats with different vegetation and soil conditions in Australia (11%) (1), Ireland

TABLE 3. Mean (±SE) of different parameters of plant nursery soils containing *Heterorhabditis bacteriophora, Steinernema carpocapsae,* and *Mesodiplogaster* sp.

| Soil parameter | Clay | Clay loam | Silt loam | Sandy loam |
|---|---|---|---|---|
| | | *H. bacteriophora* | | |
| Bulk density (g/cm$^3$) | 1.4 ± 0.05 | 1.4 ± 0.03 | 1.4 ± 0.05 | 1.4 ± 0.04 |
| Electrical conductivity (S/m) | 0.006 ± 0.001 | 0.013 ± 0.011 | 0.006 ± 0.0 | 0.007 ± 0.003 |
| pH | 5.6 ± 0.9 | 6.1 ± 0.3 | 5.7 ± 0.6 | 6.2 ± 0.6 |
| Organic matter (%) | 2.3 ± 0.3 | 2.1 ± 0.6 | 2.4 ± 0.1 | 2.6 ± 0.9 |
| Moisture content (%) | 15.7 ± 4.1 | 10.8 ± 0.1 | 13.6 ± 2.0 | 14.5 ± 4.8 |
| Temperature (C) | 24.4 ± 2.4 | 26.9 ± 2.8 | 24.8 ± 2.2 | 21.5 ± 3.7 |
| % of positive samples | 11.8 | 11.8 | 17.6 | 58.8 |
| Number of positive samples | 2 | 2 | 3 | 10 |
| Plant cover | b,e† | g,i,j | c,d | e,g,d |
| | | *S. carpocapsae* | | |
| Bulk density (g/cm$^3$) | 1.4 | 1.4 ± 0.05 | 1.4 ± 0.1 | 1.4 ± 0.1 |
| Electrical conductivity (S/m) | 0.005 | 0.004 ± 0.005 | 0.005 ± 0.002 | 0.006 ± 0.0 |
| pH | 5.8 | 6.1 ± 0.5 | 6.0 ± 0.5 | 5.6 ± 0.2 |
| Organic matter (%) | 1.8 | 2.4 ± 0.6 | 2.5 ± 0.6 | 2.3 ± 0.4 |
| Moisture content (%) | 8.4 | 12.6 ± 2.5 | 12.5 ± 2.1 | 15.9 ± 2.7 |
| Temperature (C) | 26.4 | 26.3 ± 2.6 | 26.2 ± 2.2 | 23.3 ± 1.2 |
| % of positive samples | 5.9 | 29.4 | 35.3 | 29.4 |
| Number of positive samples | 1 | 5 | 6 | 5 |
| Plant cover | f,g,h,j | g,h,i | a,d,k | c,h,i |
| | | *Mesodiplogaster* sp. | | |
| Bulk density (g/cm$^3$) | 1.4 ± 0.04 | 1.4 ± 0.04 | 1.4 ± 0.1 | 1.4 ± 0.06 |
| Electrical conductivity (S/m) | 0.006 ± 0.001 | 0.012 ± 0.010 | 0.010 ± 0.0 | 0.008 ± 0.003 |
| pH | 5.6 ± 0.9 | 5.6 ± 0.6 | 6.1 ± 0.3 | 5.4 ± 0.6 |
| Organic matter (%) | 2.2 ± 0.3 | 2.1 ± 0.5 | 2.4 ± 0.6 | 2.1 ± 0.6 |
| Moisture content (%) | 13.9 ± 4.1 | 12.2 ± 2.4 | 11.1 ± 2.4 | 13.5 ± 3.2 |
| Temperature (C) | 24.0 ± 1.8 | 27.9 ± 3.6 | 27.1 ± 1.9 | 27.2 ± 5.0 |
| % of positive samples | 4.1 | 20.8 | 27.8 | 47.2 |
| Number of positive samples | 3 | 15 | 20 | 34 |
| Plant cover | e,j | b,c,d,h,j | a,c,d,e,f,g,i,k | a,b,c,d,e,f,g,h,i |
| | | *Negative samples* | | |
| Bulk density (g/cm$^3$) | 1.4 ± 0.01 | 1.3 | 1.4 ± 0.1 | 1.4 ± 0.01 |
| Electrical conductivity (S/m) | 0.005 ± 0.0 | 0.004 | 0.018 ± 0.002 | 0.005 ± 0.002 |
| pH | 6.0 ± 0.3 | 6.1 | 5.8 ± 0.3 | 6.0 ± 0.6 |
| Organic matter (%) | 2.5 ± 0.1 | 2.3 | 2.1 ± 0.4 | 1.9 ± 1.1 |
| Moisture content (%) | 8.6 ± 0.7 | 11.1 | 9.0 ± 2.2 | 11.1 ± 4.0 |
| Temperature (C) | 25.7 ± 2.1 | 25.8 | 26.1 ± 1.3 | 25.0 ± 1.9 |
| % of negative samples | 15.8 | 5.3 | 52.6 | 26.3 |
| Number of negative samples | 3 | 1 | 10 | 5 |
| Plant cover | j | c | c,e,f,i | a,d,i,k |

† a = *Thuja occidentalis,* b = *Buxus sempervirens,* c = *Malus ioensis,* d = *Lagerstroemia indica,* e = *Hibiscus syriacus,* f = *Acer palmatum,* g = *Juniperus chinensis,* h = *Magnolia grandiflora,* i = *Platanus occidentalis,* j = *Prunus subhirtella* var. *pendula,* k = *Digitaria sanguinalis, Elytrigia repens.*

(11%) (13), or North Carolina (20%) (2). In North Carolina, 32% of the soil samples from soybean fields were positive for heterorhabditids and steinernematids, 17% from orchards or vineyards, and 5% from forests (2). In Hawaii, the associated vegetation of 73% of *Heterorhabditis* and *Steinernema*-positive sites was common ironwood (*Casuarina equisetifolia*) or mesquite (*Prosopis pallida*) (14). In our survey, more soil samples positive for *H. bacteriophora, S. carpocapsae,* and *Mesodiplogaster* were recovered from habitats with *L. indica, J. chinensis,* and *B. sempervirens,* respectively. These differences in nematode distribution possibly reflect differences in the presence of suitable hosts, vegetation, environmental factors (e.g., soil type) (2), or cultural practices.

*Galleria mellonella* recovered *H. bacteriophora* and *S. carpocapsae* from all but three positive soil samples. Two samples were

positive for nematodes in the second bioassay only, with *G. mellonella* as bait. These results again justify the popularity of *G. mellonella* as the bait insect of choice for recovering *H. bacteriophora* and *S. carpocapsae* from soil. *Acheta domesticus,* however, was a more effective bait for *Mesodiplogaster.* A second bioassay may be performed for exhaustive recovery of entomogenous nematodes from soil. In other reports (13,14), second bioassays revealed an additional 17–21% positive soil samples, with *G. mellonella* as baits.

*Heterorhabditis bacteriophora* and *S. carpocapsae* occurred in equal proportions in plant nursery soils in Tennessee. In North Carolina, steinernematids formed 26% of the heterorhabditid and steinernematid fauna from all habitats surveyed except for orchards or vineyards, where steinernematids constituted about 73% of the site fauna (2). Steinernematid nematodes formed 85% of the fauna in Australia (1) and 98% in Ireland (13) but only 8% in the Hawaiian Islands (14). Steinernematids may be more adapted to temperate areas, whereas heterorhabditids may prevail in tropical areas (14). Similarly, these two nematode groups may vary in their seasonal patterns of activity. Further study is needed to determine whether populations of these nematodes vary seasonally in Tennessee.

In Tennessee nurseries, *H. bacteriophora* occurred more in sandy loam soils than in other soil textures, and *S. carpocapsae* was recovered most frequently from silt loam soils. Neither of these associations was statistically significant, however. In Ireland, steinernematids were retrieved more frequently from sandy soils than from soils with high clay content, but differences between soil types were not significant (8). In our survey, soil characteristics, including bulk density, electrical conductivity, organic matter, pH, temperature, and moisture content were within previously published tolerance limits for the survival and pathogenicity of these nematodes (4,18).

Under suitable conditions, *Heterorhabditis* infective juveniles develop into hermaphrodites capable of producing both male and female progeny (24), but there is a question about the necessity of males for further reproduction. Zervos and Webster (32) propagated *H. zealandica* (=*H. heliothidis*) T327 through multiple "female-only" generations, and Glazer et al. (12) reported similar results with *H. bacteriophora* HP88. In our experiment with Tennessee isolates TN4 and TN23, females did not reproduce in the absence of males. Our females probably developed from eggs that were laid on the substrate; these females were not maintained in starved or unduly crowded conditions, factors that promote the formation of the infective stage. In our cultures, nematodes hatching from retained eggs seemed to be incapable of escaping from the parental cuticle unaided and were thus excluded from the experiment. Because progeny developing within the parental cuticle are crowded and have a limited food supply, many, or all, would be expected to arrest development as infective juveniles. This phenomenon may account for the difference between our results and other published reports.

Other nematode genera present in the nursery soils, particularly *Mesodiplogaster, Rhabditis,* and *Pelodera,* are possibly capable of infecting various insects. In our survey, *Mesodiplogaster* was the most common genus attracted to *A. domesticus* and *G. mellonella* baits. *Mesodiplogaster* sp. has been observed infecting larvae of *Korscheltellus gracilis* (30). *Rhabditis* includes free-living species whose dauer stages enter healthy insects (e.g., palm weevils, cerambycid beetles, rhinocerus beetles) and kill them (23). *Cryptaphelenchoides* sp., a known insect associate (21), was very rare in soil samples. In our study, nematodes of these genera that had been recovered from soil with insect baits were passaged a second time through insects (i.e., *G. mellonella*). We have propagated selected isolates of these three genera for multiple generations in monoxenic culture with the bacterium *Escherichia coli* OP50 on NGM plates as described for *Caenorhabditis elegans* (9). The nature of the

relationship between these nematodes and insects warrants further investigation.

Fungi recovered from the nursery soils, particularly *Beauveria, Metarrhizium,* and *Hirsutella,* infect many insect species (25). Barbercheck and Kaya (3) reported the negative interactions between *B. bassiana* and entomopathogenic nematodes. Some species of *Hirsutella* (e.g., *H. rhossiliensis*) parasitize nematodes in soil and may reduce the effectiveness of entomogenous nematodes (29).

The occurrence of heterorhabditid and steinernematid nematodes in soils from all nursery crops surveyed indicates a possible significant role of these nematodes in regulating the populations of soil insect pests. Further studies are needed to determine whether these nematodes, either alone or in combination with other soil fauna or flora, significantly suppress insect pest populations and how best to use them in formulating pest management strategies for nursery crops.

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