Journal of Nematology 27(1):21–28. 1995.

Effects of Earthworms on the Dispersal of *Steinernema* spp.[1]

D. I. Shapiro,[2] G. L. Tylka,[3] E. C. Berry,[4] and L. C. Lewis[5]

Abstract: Previous studies indicated that dispersal of *S. carpocapsae* may be enhanced in soil with earthworms. The objective of this research was to determine and compare the effects of earthworms on dispersal of other *Steinernema* spp. Vertical dispersal of *Steinernema carpocapsae, S. feltiae,* and *S. glaseri* was tested in soil columns in the presence and absence of earthworms (*Lumbricus terrestris*). Dispersal was evaluated by a bioassay and by direct extraction of nematodes from soil. Upward dispersal of *S. carpocapsae* and *S. feltiae* increased in the presence of earthworms, whereas upward dispersal of *S. glaseri* was not affected by earthworms. No significant differences were detected in downward dispersal of *S. carpocapsae* and *S. feltiae* in soil with earthworms compared to soil without earthworms. Downward dispersal of *S. glaseri,* however, was greater in soil without earthworms relative to soil with earthworms. In soil void of earthworms, dispersal of *S. glaseri* was greatest followed by dispersal of *S. carpocapsae.* The presence of earthworm burrows in soil did not influence nematode dispersal. Nematodes were recovered from the surface, interior, and casts of earthworms. Therefore, nematodes may have a phoretic association with earthworms.

Key words: earthworm, entomophyllic nematode, nematode, nematode dispersal, *Steinernema* spp.

Entomopathogenic nematodes in the genus *Steinernema* have many attributes as biological control agents, including wide host ranges, safety to nontarget organisms, and vectoring a bacterium, *Xenorhabdus* sp., which rapidly kills insect hosts (15). Despite their great potential as biological control agents, results of nematode applications have been inconsistent (11). Biotic and abiotic factors that influence the efficacy of nematode applications must be investigated (8).

Increased dispersal may increase the efficacy of nematodes against insect pests (9). Adverse environmental conditions can decrease nematode mobility, thereby decreasing their effectiveness (8). Factors that may affect nematode dispersal include host presence, temperature, soil texture, and soil moisture (14).

Dispersal characteristics vary among *Steinernema* spp. *Steinernema carpocapsae* (Weiser) moves little from the site of application, whereas *S. glaseri* (Steiner) is a relatively active disperser (18). *Steinernema carpocapsae* applied to the surface of vertical soil columns remained within the upper 15 cm after 30 days, but *S. glaseri* was recovered 90 cm below the application point (18). Because of these differences in behavior, *S. carpocapsae* is classified as an ambusher and *S. glaseri* is classified as a cruise forager (15). The foraging behavior of *S. feltiae* (Filipjev) is considered intermediate between *S. carpocapsae* and *S. glaseri* (1). Differences in dispersal behavior may be important in selecting nematode species for use against pests occupying different niches in soil habitats (16).

Interactions with soil invertebrates may increase nematode dispersal (14). For example, a phoretic relationship was observed between nematophagous mites and *S. carpocapsae* (6). Shapiro et al. (19) reported increased dispersal of *S. carpocapsae* in the presence of earthworms. Earthworms are not adversely affected by entomopathogenic nematodes (4,17). Therefore, it may be possible to exploit the association between *S. carpocapsae* and earthworms in the soil to increase the effi-

Received for publication 8 August 1994.

[1] Journal Paper No. 15954 of the Iowa Agricultural and Home Economics Experiment Station, Ames; Project No. 3130.

[2] Department of Entomology, Iowa State University, Ames, IA 50011.

[3] Department of Plant Pathology, Iowa State University, Ames, IA 50011.

[4] USDA ARS, National Soil Tilth Laboratory, Ames, IA 50011.

[5] USDA ARS, Corn Insects Research Unit, Ames, IA 50011.

We thank M. Abbas, R. D. Gunnarson, D. Soh, and S. Souhrada for assistance in the laboratory and Drs. I. Glazer, E. E. Lewis, and J. J. Obrycki for reviewing an earlier draft of this manuscript. We also thank Dr. G. O. Poinar for nematode identification. This research was supported in part by the Leopold Center of Sustainable Agriculture, Ames, IA, Grant No. 92–18.

cacy of the nematode as a biological control agent. The influence of earthworms on the dispersal of other *Steinernema* species and the mechanism(s) of how earthworms affect nematode dispersal have not been examined. Because of differences in dispersal behavior, the effects of earthworms on dispersal may vary among nematode species. Shapiro et al. (19) suggested two hypotheses relating to the earthworm–nematode relationship: the nematodes are dispersed by direct contact with earthworms and (or) nematodes disperse at different rates in soil with earthworm burrows than in soil without burrows. The objectives of this research were to determine and compare the effects of earthworms on dispersal of *S. carpocapsae, S. glaseri,* and *S. feltiae* and to investigate the mechanisms involved in how earthworms influence nematode dispersal.

Materials and Methods

The effects of earthworms on dispersal of three nematode species were determined in vertical soil columns. *Steinernema carpocapsae,* All strain, *S. glaseri,* NC strain, and *S. feltiae,* strain 27, were originally obtained from biosys (Palo Alto, CA) and were reared in last instars of the greater wax moth, *Galleria mellonella* (L.). Experimental units were polyvinyl chloride (PVC) pipe consisting of six 4-cm-long sections and 5-cm-d pipe joined with duct tape and filled with soil (34% sand, 28% silt, and 38% clay, pH 7.0). Soil moisture in columns was adjusted to approximately field capacity. The column sections were numbered vertically; the top section was one and the bottom section was six.

The experiments were organized as randomized block designs with depth as a repeated measure. Experiments testing upward and downward movement of nematodes were run simultaneously. Six treatments (three nematode species, with and without earthworms) were each applied to eight soil columns for upward movement and eight columns for downward movement (96 columns total). Two earthworms, *Lumbricus terrestris* (L.), obtained from the USDA ARS National Soil Tilth Laboratory (Ames, IA), were added to the selected columns and allowed to burrow for 1 week before nematode application. Subsequently, 10,000 infective juveniles of each species were applied in 0.3–0.4 ml of distilled water to the bottom surface of the columns (section 6) to test upward movement, and to the top surface of the columns (section 1) to evaluate downward movement. Soil columns were then incubated for 14 days at approximately 21 C and 54% relative humidity, after which they were dismantled to evaluate nematode dispersal.

Nematode dispersal was evaluated using two methods: a bioassay against *G. mellonella* larvae, and direct extraction and counting of nematodes. Four replicate soil columns per treatment were used for each method of evaluation. For the bioassay, soil from each column section was removed and placed in plastic petri dishes (150 × 25 mm) with 20 last instar *G. mellonella* (19). The petri dishes were incubated at approximately 27 C and 80% relative humidity for 72 hours, at which time nematode-induced larval mortality was recorded. Mortality was considered to be nematode induced if cadavers exhibited flaccidity and coloration characteristic of *Steinernema* infections. In the second method, nematodes were extracted from the soil in each column section using sucrose centrifugation (13), and *Steinernema* spp. were quantified by observation with an inverted compound microscope at a magnification of ×40.

Analysis of nematode movement was accomplished by determining the number of nematodes reaching the half of the column opposite where the nematodes were placed (sections 1–3 for upward movement and 4–6 for downward movement). Bioassay data were analyzed through ANOVA of mean numbers of dead *G. mellonella* larvae obtained from soil in the three sections opposite to where the nematodes were placed. Because differences in virulence against *G. mellonella* among the three

nematode species were not determined, interspecific comparisons of dispersal could not be made using the bioassay data. For the direct extraction method, ANOVA was performed on mean proportions of nematodes recovered. Proportions were calculated based on the number of nematodes recovered from the half of the column opposite to where the nematodes were placed relative to the total number of nematodes recovered in that column. Using these proportions, extraction efficiency for each species was not a factor; hence comparisons of dispersal among *Steinernema* spp. could be made. If significant differences were found with the ANOVA, then multiple range tests (LSD) were used to distinguish treatment effects.

An experiment was conducted to investigate whether nematodes were moved through soil via a direct contact relationship with earthworms. Approximately 1.5×10^6 *S. carpocapsae* were placed in 3 liters of soil with 10 *L. terrestris* and incubated at 27 C for 7 days. Subsequently, the earthworms were removed and washed vigorously using distilled water. The wash from each earthworm was poured onto filter paper in 100 × 15 mm petri dishes. The earthworms were rinsed further in running tap water, placed in separate petri dishes with filter paper, and allowed to cast for approximately 1 hour. Five earthworms were then placed at −10 C for 7 minutes to immobilize them and were subsequently cut into 1-cm-long sections; nematodes were extracted from the dissected material through a Baermann funnel (2). The extracted nematode suspensions were poured onto filter paper in five petri dishes. Three last instar *G. mellonella* were placed in each petri dish (from the wash, casts, and dissections), and the petri dishes were incubated at 27 C and 80% relative humidity. If larval mortality occurred, the cadavers were placed on White traps (20) to verify nematode infection. To test whether a natural population of *Steinernema* spp. may have been associated with earthworms, five additional *L. terrestris* (that were not incubated in soil infested with *S. carpocapsae*) were dissected and exposed to *G. mellonella* as previously described.

An additional experiment was conducted to determine whether nematode dispersal is greater in soil with earthworm burrows than in soil without burrows. Upward dispersal of *S. carpocapsae* was determined in vertical soil columns in an experiment with a design similar to previous experiments. Soil columns and experimental conditions were as previously described, but treatments were columns with earthworms and burrows, columns with burrows only (earthworms removed), and columns with no earthworms or burrows. There were five replications per treatment (15 columns total). Two earthworms (*L. terrestris*) were added to ten randomly selected columns and allowed to burrow for 1 week, after which five columns were dismantled. Earthworms were manually removed from the dismantled columns, which were then reconstructed with the earthworm burrows realigned. Realignment of column sections was accomplished by aligning straight vertical lines, which were drawn on the columns prior to separation of the column sections. Ten thousand nematodes were then applied in 0.4 ml distilled water to each column, and columns were incubated for 2 weeks as described herein. Following incubation, nematode dispersal was estimated and analyzed using the bioassay method described herein. This experiment was repeated with no changes in procedure except that all columns were dismantled and then realigned, and nematode dispersal was evaluated using the direct extraction method.

RESULTS

The *G. mellonella* bioassays indicated increased upward dispersal of *S. carpocapsae* and *S. feltiae* in columns with earthworms relative to columns without earthworms (Table 1). No effects of earthworm presence were detected using the bioassay method to determine upward dispersal of

TABLE 1. Number of dead *Galleria mellonella* larvae from soil columns testing upward dispersal of three *Steinernema* spp. in the presence and absence of the earthworm, *Lumbricus terrestris*.

	S. carpocapsae		S. feltiae		S. glaseri	
Section depth (cm)	L+†	L−	L+	L−	L+	L−
1. 0–4	13.5	0.8	7.3	2.8	12.5	17.3
2. 4–8	13.8	6.3	7.0	1.3	13.3	19.0
3. 8–12	16.0	12.3	9.3	1.0	16.0	18.3
4. 12–16	13.8	14.0	10.8	2.5	19.5	19.8
5. 16–20	15.5	17.0	19.0	14.0	19.5	19.3
6. 20–24	19.4	20.0	19.8	19.3	19.0	20.0
m‡ =	14.6 a	6.4 b	7.8 b	1.7 c	13.9 a	18.2 a

Numbers represent means of four replications. Nematodes were introduced 24 cm below the soil surface. Twenty *G. mellonella* larvae were exposed to soil removed from column sections 2 weeks after nematodes were introduced. *Galleria mellonella* mortality indicates relative nematode densities.

† L+ = presence and L− = absence of the earthworm, *L. terrestris*.

‡ m = average mortality of *G. mellonella* larvae in the top three sections (1–3). Means from within-species comparisons followed by different letters are significantly different at $P \leq 0.05$.

S. glaseri (Table 1). Results from the direct extraction method evaluating upward nematode dispersal followed the same trends as bioassay data, but differences between earthworm presence and absence were not significant (Table 2). Interspecific comparisons made from direct extraction data indicated that in the absence of earthworms, upward dispersal of *S. glaseri* was greater than dispersal of *S. carpocapsae* and *S. feltiae* (Table 2). In the presence of earthworms, dispersal of *S. carpocapsae* was not different than that of *S. glaseri*.

Bioassay results indicated no significant effect of earthworms on downward dispersal for the three nematode species (Table 3). Results of direct extraction indicated greater downward dispersal of *S. glaseri* in the absence of earthworms than in the presence of earthworms (Table 4). Interspecific comparisons indicated downward dispersal abilities in the absence of earthworms to be greatest in *S. glaseri* followed by *S. carpocapsae*. In the presence of earthworms, dispersal of *S. glaseri* and *S. carpocapsae* were comparable and were significantly different from dispersal of *S. feltiae* (Table 4).

Nematodes were isolated from the surface, casts, and interior of earthworms. Emerged nematodes were observed in 1 of 10, 6 of 10, and 5 of 5 White traps that contained *G. mellonella* exposed to nematodes from the wash of earthworm surfaces, earthworm casts, and dissected earthworms, respectively. The emerged

TABLE 2. Number of nematodes recovered from soil columns testing upward dispersal of three *Steinernema* spp. in the presence and absence of the earthworm, *Lumbricus terrestris*.

	S. carpocapsae		S. feltiae		S. glaseri	
Section depth (cm)	L+†	L−	L+	L−	L+	L−
1. 0–4	28.0	0	3.5	0.5	74.0	222.0
2. 4–8	22.8	0.5	6.0	0	213.5	525.0
3. 8–12	27.0	9.0	10.5	5.0	427.5	614.5
4. 12–16	9.0	4.5	28.5	26.0	465.0	530.5
5. 16–20	34.5	52.5	123.0	48.0	904.5	550.5
6. 20–24	515.0	77.5	807.5	478.0	304.0	803.0
p‡ =	18.0 ab	7.1 b	2.7 b	0.7 b	29.5 a	37.5 a

Numbers represent means of four replications. Nematodes were introduced 24 cm below the soil surface.

† L+ = presence and L− = absence of the earthworm, *L. terrestris*.

‡ p = mean percentage of nematodes recovered from the top three sections (1–3) relative to the total number of nematodes recovered. Means followed by the same letter are not significantly different at $P \leq 0.05$.

TABLE 3. Number of dead *Galleria mellonella* larvae from soil columns testing downward dispersal of three *Steinernema* spp. in the presence and absence of the earthworm, *Lumbricus terrestris*.

	S. carpocapsae		S. feltiae		S. glaseri	
Section depth (cm)	L+†	L−	L+	L−	L+	L−
1. 0–4	20.0	20.0	20.0	19.8	10.8	16.0
2. 4–8	19.8	19.8	19.8	20.0	20.0	19.8
3. 8–12	19.3	20.0	19.0	18.8	19.8	20.0
4. 12–16	17.8	19.8	12.0	12.8	19.3	19.8
5. 16–20	18.3	19.3	10.8	1.5	16.3	19.8
6. 20–24	19.5	16.3	4.5	0.8	16.3	19.5
m‡ =	18.5 a	18.4 a	9.1 b	5.0 b	17.3 a	19.7 a

Numbers represent means of four replications. Nematodes were introduced on the soil surface. Twenty *G. mellonella* larvae were exposed to soil removed from column sections 2 weeks after nematodes were introduced. *Galleria mellonella* mortality indicates relative nematode densities.

† L+ = presence and L− = absence of the earthworm, *L. terrestris*.

‡ m = average mortality of *G. mellonella* larvae in the bottom three sections (4–6). Means from within-species comparisons followed by different letters are significantly different at $P \leq 0.05$.

nematodes were identified as *Steinernema* sp. No *Steinernema* nematodes were recovered from the five earthworms that were not exposed to soil infected with nematodes.

Dispersal of *S. carpocapsae* was greater in columns with earthworms than in columns with earthworm burrows (earthworms removed) and columns without earthworms (Table 5). Dispersal of *S. carpocapsae* was not different in columns with earthworm burrows compared to columns void of earthworms (Table 5).

DISCUSSION

Upward, but not downward, dispersal of *S. carpocapsae* and *S. feltiae* was enhanced by the presence of the earthworm *L. terrestris*. In previous research, downward dispersal of *S. carpocapsae* was increased in the presence of *L. terrestris* (19). The reasons for discrepancy between previous results and results reported herein are unknown. Upward dispersal of *S. glaseri* was not affected by earthworm presence, but downward dispersal was increased when earthworms were absent. Reasons for different effects of earthworms on upward and downward dispersal of these nematode species are unclear. Perhaps consistent differences would have been found in upward and downward nematode dispersal experiments if more replications had been used. Indeed, the trends observed in all experiments were consistent, regardless of statistical significance. Alternatively, if a phoretic relationship exists between the *L.*

TABLE 4. Number of nematodes recovered from soil columns testing downward dispersal of three *Steinernema* spp. in the presence and absence of the earthworm, *Lumbricus terrestris*.

	S. carpocapsae		S. feltiae		S. glaseri	
Section depth (cm)	L+†	L−	L+	L−	L+	L−
1. 0–4	83.5	249.0	886.5	1,115.0	47.0	207.0
2. 4–8	58.0	75.0	659.5	737.0	1,171.5	416.0
3. 8–12	36.5	46.5	109.5	198.0	1,082.0	970.5
4. 12–16	15.0	49.0	76.5	36.5	590.0	621.0
5. 16–20	33.0	43.0	30.5	3.0	170.0	325.0
6. 20–24	30.0	10.5	28.5	6.5	93.5	167.5
p‡ =	31.5 ab	22.0 b	7.3 c	2.2 c	26.5 b	42.3 a

Numbers represent means of four replications. Nematodes were introduced to the soil surface.

† L+ = presence and L− = absence of the earthworm, *L. terrestris*.

‡ p = mean percentage of nematodes recovered from the bottom three sections (4–6) relative to the total number of nematodes recovered. Means followed by the same letter are not significantly different at $P \leq 0.05$.

TABLE 5. *Galleria mellonella* larval mortality from bioassays, and numbers of *S. carpocapsae* recovered by direct extraction, from soil with earthworms, earthworm burrows, and no earthworms.

	G. mellonella mortality			S. carpocapsae recovered		
Section depth (cm)	L†	L0	0	L	L0	0
1. 0–4	3.4	0	0	66.8	0.4	0
2. 4–8	5.2	0.2	0	18.4	2.8	0.8
3. 8–12	6.6	0	0	28.8	3.6	0
4. 12–16	4.6	3.4	2.2	6.0	3.6	19.2
5. 16–20	9.4	8.6	6.2	21.2	48.0	45.6
6. 20–24	11.6	16.6	14.4	952.4	812.4	300.4
m‡ =	5.1 a	0.7 b	0 b			
p§ =				15.0 a	3.1 b	0.3 b

Numbers represent means of five replications. *Steinernema carpocapsae* were introduced 24 cm below the soil surface. For bioassay, 20 *G. mellonella* larvae were exposed to soil removed from column sections 2 weeks after nematodes were introduced. *Galleria mellonella* moratlity indicates relative nematode densities.

† L = presence of two *L. terrestris*, L0 = burrows present with earthworms removed, 0 = no earthworms or burrows.

‡ m = average mortality of *G. mellonella* larvae in the top three sections (1–3).

§ p = mean percentage of nematodes recovered from the top three sections (1–3) relative to the total number of nematodes recovered. Means followed by the same letter are not significantly differentat $P \leq 0.05$.

terrestris and *S. carpocapsae* and *S. feltiae*, then perhaps the nematodes tend to associate more with earthworms moving upward than with earthworms moving downward. *Steinernema carpocapsae* has a natural tendency to disperse upwards (10,18). Increased upward, but not downward, dispersal has been reported previously when *S. carpocapsae* was in the presence of *Heterorhabditis bacteriophora* Poinar, a nematode species with greater dispersal ability than *S. carpocapsae* (1).

This research found the natural dispersal ability of *S. glaseri* to be greater than that of *S. carpocapsae* and *S. feltiae*, which is consistent with previous studies confirming the dispersal behavior of *S. glaseri* as a cruiser. Because of the high natural dispersal ability of *S. glaseri*, it is not unexpected that earthworms would not enhance dispersal of this species. Perhaps the increased downward dispersal of *S. glaseri* observed in soil without earthworms relative to soil with earthworms may have been caused by earthworm burrows or earthworms obstructing the paths of these nematodes.

This study found the dispersal of *S. carpocapsae* to be greater than that of *S. feltiae*, which is contrary to the study of Alatorre-Rosas and Kaya (1), in which the authors found dispersal ability of *S. feltiae* to be intermediate between *S. glaseri* and *S. carpocapsae*. The disparity may be due to differences in soil texture or nematode strain. Nematode dispersal may be affected by soil texture; for example, *S. carpocapsae* dispersal increases with increased soil particle size (10). The study of Alatorre-Rosas and Kaya (1) was conducted in sand, whereas this research was conducted in soil (34% sand, 28% silt, and 38% clay). In both studies, *S. carpocapsae*, All strain, was used, but the strain of *S. feltiae* was not given in the report of Alatorre-Rosas and Kaya (1).

This study has provided evidence that earthworms may serve as phoretic hosts of *Steinernema* spp. Nematode dispersal was not different in soil with earthworm burrows compared to soil without burrows, and results indicated that the relationship between nematodes and earthworms is one of direct contact. *Steinernema carpocapsae* may be dispersed on the surface of earthworms, or may be passed through the earthworm digestive system and remain viable. Evidence strongly suggests that the nematodes that were recovered from earthworms and reared in *G. mellonella* were the *S. carpocapsae* that were placed in the infested soil, because the nematodes recovered were identified as belonging to the genus *Steinernema*, and because no *Steinernema* were found in earthworms that

were not incubated in soil infested with *S. carpocapsae*. Because a high nematode concentration was used to bait the soil, additional research will be required to determine to what extent *S. carpocapsae* is associated with earthworms in soil with lower nematode densities. Future studies may also determine if other entomopathogenic species are capable of being dispersed by earthworms through direct contact.

Two methods were used to evaluate nematode dispersal in this study. In general, the bioassay method was more sensitive in finding differences and less labor intensive than the direct extraction method. However, data from direct extraction allowed interspecific comparisons. Recent studies have found that LT_{50} (time required to cause 50% host mortality) and efficiency of invasion (number of nematodes entering the host per unit time) to be very sensitive methods of evaluating nematode activity (7,12). Perhaps more differences among treatments would have been detected in this study if LT_{50}s or invasion efficiencies had been determined.

Enhanced dispersal of *S. carpocapsae* and *S. feltiae* in the presence of earthworms may improve the efficacy of these nematodes as biological control agents. The dispersal ability of *S. carpocapsae* has been reported to be limited (1,10,18), but our research has established that dispersal of *S. carpocapsae* is comparable to that of *S. glaseri* in the presence of earthworms. Because host finding is necessary for successful biological control with nematodes and increased dispersal will improve host finding abilities (9), it is important to determine if increased earthworm population densities will increase the efficacy of biological control of insects with nematodes. Additionally, earthworms improve soil quality through aeration and by breaking down and distributing organic matter (5). Earthworm population densities can be increased through cultural practices (3).

Literature Cited

1. Alatorre-Rosas, R., and H. K. Kaya. 1990. Interspecific competition between entomopathogenic nematodes in genera *Heterorhabditis* and *Steinernema* for an insect host in sand. Journal of Invertebrate Pathology 55:179–188.

2. Ayoub, S. M. 1980. Plant nematology: An agricultural training manual. Sacramento, CA: Nemaid Publications.

3. Berry, E. C., and D. L. Karlen. 1993. Comparison of alternative farming systems. II. Earthworm population density and species diversity. American Journal of Alternative Agriculture 8:21–26.

4. Capinera, J. L., S. L. Blue, and G. S. Wheeler. 1982. Survival of earthworms exposed to *Neoaplectana carpocapsae* nematodes. Journal of Invertebrate Pathology 39:419–421.

5. Edwards, C. A. 1983. Earthworm ecology in cultivated soils. Pp. 123–138 *in* J. E. Satchell, ed. Earthworm ecology from Darwin to vermiculture. London: Chapman and Hall.

6. Epsky, N. D., D. E. Walter, and J. L. Capinera. 1988. Potential role of nematophagous microarthropods as biotic mortality factors of entomogenous nematodes (Rhabiditida: Steinernematidae, Heterorhabditidae). Journal of Economic Entomology 81: 821–825.

7. Epsky, N. D., and J. L. Capinera. 1993. Quantification of invasion of two strains of *Steinernema carpocapsae* (Weiser) into three lepidopteran larvae. Journal of Nematology 25:173–180.

8. Gaugler, R. 1988. Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agriculture, Ecosystems and Environment 24:351–360.

9. Gaugler, R., T. McGuire, and J. Campbell. 1989. Genetic variability among strains of the entomopathogenic nematode *Steinernema feltiae*. Journal of Nematology 21:247–253.

10. Georgis, R., and G. O. Poinar, Jr. 1983. Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). Journal of Nematology 15:308–311.

11. Georgis, R., and. R. Gaugler. 1991. Predictability in biological control using entomopathogenic nematodes. Journal of Economic Entomology 84: 713–720.

12. Glazer, I. 1992. Invasion rate as a measure of infectivity of Steinernematid and Heterorhabditid nematodes to insects. Journal of Inverterbrate Pathology 59:90–94.

13. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

14. Kaya, H. K. 1990. Soil ecology. Pp 93–115 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

15. Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. Annual Review of Entomology 38:181–206.

16. Kaya, H. K., T. M. Burlando, and G. Thurston. 1993. Two entomopathogenic nematode species with different search strategies for insect suppression. Environmental Entomology 22:859–864.

17. Nuutinen, V., J. Tyni-Juslin, I. Vänninen, A. Vainio. 1991. The effects of four entomopathogenic fungi and an entomoparasitic nematode on the hatching of earthworm (*Aporrectodea caliginosa*) cocoons in the laboratory. Journal of Invertebrate Pathology 58: 147–149.

18. Schroeder, W. J., and J. B. Beavers. 1987. Movement of the entomogenous nematodes of the families Heterorhabditidae and Steinernematidae in soil. Journal of Nematology 19:257–259.

19. Shapiro, D. I., E. C. Berry, and L. C. Lewis. 1993. Interactions between nematodes and earthworms: Enhanced dispersal of *Steinernema carpocapsae*. Journal of Nematology 25:189–192.

20. White, G. F. 1927. A method for obtaining infective nematode larvae from cultures. Science 66: 302–303.