Journal of Nematology 25(2):189–192. 1993.


# Interactions between Nematodes and Earthworms: Enhanced Dispersal of *Steinernema carpocapsae*[1]

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

*Abstract:* Dispersal of the nematode *Steinernema carpocapsae* (All strain), applied on the top or the bottom of soil columns, was tested in the presence or absence of two earthworm species, *Lumbricus terrestris* or *Aporrectodea trapezoides*. Nematode dispersal was estimated after a 2-week period with a bioassay against the greater wax moth, *Galleria mellonella*. Vertical dispersal of nematodes was increased in the presence of earthworms. When nematodes were placed on the surface of soil columns, significantly more nematodes dispersed to the lower half of the columns when either earthworm species was present than when earthworms were not present. When nematodes were placed on the bottom of soil columns, significantly more nematodes dispersed to the upper half of the columns when *L. terrestris* was present than when *A. trapezoides* was present or in the absence of earthworms. Because nematodes were found on the exterior and in the interior of earthworms, nematode dispersal may be enhanced by direct contact with the earthworms.

*Key words:* dispersal, earthworm, nematode, nematode dispersal, *Steinernema carpocapsae*, Steinernematidae.

The nematode *Steinernema carpocapsae* (Weiser) has good potential as a biological control agent of insects, especially in the soil environment (5). More than 90 percent of all insect pests spend part of their life cycle in soil (9). *Steinernema carpocapsae* has a wide host range of more than 250 insects (15). One of the characteristics of nematodes that make them attractive for use in biological control is their ability to seek out their hosts. However, a limited capacity for nematodes to disperse in their habitat has occasionally been cited as a hindrance to the success of certain biological control applications (6,12,16). Generally, *S. carpocapsae* will disperse very little from the site of application in soil (7,13). Movement that does occur is affected by soil moisture (18), soil texture, and presence or absence of hosts (7).

Earthworms improve soil by modification of soil structure, by aeration and drainage, and by breaking down and distributing organic matter (3). The ability of earthworms to change soil structure and move large amounts of soil (10) indicates that nematode movement may increase in the presence of earthworms. Furthermore, entomopathogenic nematodes have been found to have no deleterious effects on earthworms (2,14). The present study was conducted to determine the effects of earthworms on vertical dispersal of entomopathogenic nematodes.

## Materials and Methods

Vertical dispersal of nematodes in the presence or absence of earthworms was tested in soil columns over a 2-week period. *Steinernema carpocapsae* (All strain) was obtained from Biosys (Palo Alto, CA) and was reared in the last instar of the greater wax moth, *Galleria mellonella* (L.). Earthworms, *Lumbricus terrestris* (L.) and *Aporrectodea trapezoides* (Duges), were obtained from the National Soil Tilth Laboratory, Ames, Iowa. Both species were from colonies that were reared on partly decomposed horse manure (1). Soil columns were constructed by joining six 4-cm-long sections of 5-cm-d polyvinyl chloride (PVC) pipe and filling them with soil (34% sand, 28% silt, 38% clay) to a bulk density of approximately 1.2 g/cm$^3$. Soil

Received for publication 5 November 1992.
[1] Journal Paper No. J-15106 of the Iowa Agricultural and Home Economics Experiment Station, Ames; Project No. 3130.
[2] Department of Entomology, Iowa State University, Ames, IA 50011.
[3] USDA ARS, National Soil Tilth Laboratory, 2150 Pammel Dr., Ames, IA 50011.
[4] USDA ARS, Corn Insects Research Unit, Genetics Laboratory, c/o Insectary Bldg., Iowa State University, Ames, IA 50011.
We thank M. Abbas for assistance in the laboratory and J. J. Jackson, T. B. Parkin, and G. L. Tylka for constructive criticism of an earlier draft of this manuscript. This research was supported in part by the Leopold Center for Sustainable Agriculture, Ames, Iowa, Grant No. 92-18.

moisture was standardized to field capacity in each column. The soil columns received one of three treatments: the addition of either two *L. terrestris* or *A. trapezoides,* or no earthworms. All treatments were applied to soil columns testing both upward and downward dispersal with three replications (soil columns) for each treatment–direction combination (18 columns total). Earthworms were allowed to tunnel for 1 week before nematodes were added to each column. Soil columns were incubated at 21 C and approximately 92% relative humidity.

To test downward movement, $1.0 \times 10^4$ nematodes were applied in 0.1 ml water to the surface of each soil column. *Steinernema carpocapsae* tends to move upward (7,13,17); therefore, to encourage downward migration of nematodes, two wax moth larvae (hereafter referred to as "bait larvae") were enclosed in cloth mesh and placed on the bottom end of each column. After 14 days the soil columns were dismantled. The bait larvae in each column were checked for mortality, and the soil from each 4 cm-long section of PVC pipe was removed and placed in a plastic petri dish (150 × 25 mm) with 20 fresh *G. mellonella* larvae (hereafter referred to as "assay larvae"). The petri dishes were placed at 27 C and approximately 60% relative humidity for 3 days, after which mortality of assay larvae was recorded. Differences in mortality of assay larvae were used to indicate differences in numbers of nematodes.

To test upward movement, $1.0 \times 10^4$ nematodes in 0.1 ml water were applied to soil on the bottom end of the column and allowed to migrate for 2 weeks, after which the columns were dismantled and soil from each section was exposed to assay larvae as described previously. Because *S. carpocapsae* tend to disperse upward, bait larvae were not required in the columns testing upward dispersal of nematodes.

The experiments testing upward and downward dispersal of nematodes were completely randomized designs with repeated measures. Statistical differences in nematode dispersal among treatments were determined by doing an analysis of variance on the numbers of dead larvae infected from soil in the bottom three sections of columns testing downward dispersal, and in the top three sections of columns testing upward dispersal. The numbers of dead larvae, caused by nematodes that dispersed to the most distant three sections of the columns, were then compared among the treatments using contrasts.

## Results

*Steinernema carpocapsae* dispersed significantly farther downward when either *L. terrestris* or *A. trapezoides* were present than when no earthworms were present. The numbers of assay larvae killed when exposed to soil from column sections from the lower half of the columns were significantly greater when earthworms were present than when they were not (Table 1), indicating that the nematodes dispersed farther downward with earthworms present. Bait larvae were always infected with nematodes after 2 weeks when earthworms were present but never infected when earthworms were absent.

Nematodes moved further upward when earthworms were present than when no earthworms were present. The number of assay larvae killed by nematodes when exposed to soil from column sections in the upper half of the columns was significantly greater when *L. terrestris* was present than when either *A. trapezoides* was present or when no earthworms were present (Table 1). Nematodes were detected in the opposite-most section of the columns when earthworms were present but not when earthworms were not present.

## Discussion

More of the nematodes associated with *L. terrestris* were dispersed upward compared with those associated with *A. trapezoides.* The differences in nematode distributions in the soil columns may be caused by differences in earthworm behavior.

TABLE 1. Number of dead *Galleria mellonella* (assay) larvae in soil columns containing soil with no earthworms, *Lumbricus terrestris*, or *Aporrectodea trapezoides*.

| | Upward dispersal | | | Downward dispersal | | |
|---|---|---|---|---|---|---|
| Depth (cm) | Lt† | At | 0 | Lt | At | 0 |
| 0–4 | 3 | 1.7 | 0 | 10.3 | 15 | 15.7 |
| 4–8 | 11 | 5.3 | 1.3 | 17.7 | 19.7 | 19.7 |
| 8–12 | 14 | 11 | 9 | 18.3 | 19.3 | 20 |
| 12–16 | 17.3 | 16.7 | 13 | 18.3 | 19.3 | 11.3 |
| 16–20 | 19.6 | 13.3 | 13 | 13.7 | 17 | 5.3 |
| 20–24 | 19.3 | 20 | 19.3 | 0.3 | 2.7 | 0.3 |
| m‡ = | 9.3 b | 6 a | 3.4 a | 10.7 b | 13 b | 5.6 a |
| b§ = | | | | 6.0 | 6.0 | 0 |

Numbers represent means of three replications. *Steinernema carpocapsae* were introduced 24 cm below the soil surface in the upward dispersal experiment and on the soil surface in the downward dispersal experiment. Twenty *G. mellonella* larvae were exposed to soil removed from column sections 2 weeks after nematodes were introduced. *G. mellonella* mortality indicates relative nematode densities.

† Lt = *Lumbricus terrestris;* At = *Aporrectodea trapezoides;* 0 = no earthworms.

‡ m = average mortality of assay larvae in the bottom three sections in downward dispersal and top three sections in upward dispersal experiment. Means followed by the same letter are not significantly different.

§ b = total mortality of (bait larvae) *G. mellonella* placed at 20–24 cm in each column.

Channels produced by *L. terrestris* are vertically oriented, whereas *A. trapezoides* generally produces horizontally oriented burrows (11). It may be expected that *A. trapezoides* would be most suitable in enhancing nematode dispersal horizontally. If earthworms were incorporated into biological control applications to enhance nematode dispersal, the species of earthworm used would need to be considered.

Increased dispersal of *S. carpocapsae* may have resulted from direct or indirect effects of earthworms. Dispersal may have been increased directly by transportation of nematodes on the surface of the earthworms, or in the digestive tract of the earthworms. Some evidence of this phenomenon was observed in this study. Several earthworms (five *A. trapezoides* and three *L. terrestris*) were examined after exposure to *S. carpocapsae* in soil columns. Live nematodes were found in dissected earthworms, in earthworm casts, and in the debris washed from the surface of earthworms. Mortality was observed in *G. mellonella* larvae exposed to nematodes isolated from dissected earthworms, but nematodes were not observed to reproduce and emerge. Nematodes found in the casts and debris of earthworms were able to infect and reproduce in larvae of the *G. mellonella.* Although positive identification of *S. carpocapsae* was not made, the nematodes did cause mortality in the *G. mellonella* larvae characteristic of nematodes in the family Steinernematidae (e.g., typical coloration). Therefore, these observations suggest that earthworms may serve as phoretic hosts of *S. carpocapsae.* Phoresy of entomopathogenic nematodes has been observed on nematophagous mites (4). *Steinernema carpocapsae* (Breton strain) avoided predation by bridging onto the dorsum of mites and were able to leave the mites to infect and reproduce in prepupae of *G. mellonella.* (4). Phoresy may have adaptive value for entomopathogenic nematodes by allowing them to disperse further than they could on their own energy (8).

Another hypothesis is the *S. carpocapsae* dispersal may have been increased indirectly. Nematode dispersal is affected by soil moisture, structure, and texture (18). For example, the dispersal of *S. carpocapsae* is greater in soils with coarser textures than in soils with high clay contents (7). Soil texture, moisture, and structure are altered by earthworm burrowing (11). Therefore, nematode dispersal may be different in earthworm burrows than in soil void of earthworms. Further research will be required to test this hypothesis and to determine what benefits an enhanced capacity for nematodes to disperse in the presence of earthworms may offer to biological control applications.

## Literature Cited


1. Berry, E. C., and D. L. Karlen. 1993. Comparison of alternate farming systems: II. Earthworm population and density and species diversity. Journal of Alternative Agriculture 8:23–28.

2. 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.

3. 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.

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

5. Gaugler, R. 1981. Biological control potential of Neoaplectanid nematodes. Journal of Nematology 13:241–249.

6. Georgis, R., B. A. Mullens, and J. A. Meyer. 1987. Survival and movement of insect parasitic nematodes in poultry manure and their infectivity against *Musca domestica.* Journal of Nematology 19: 292–295.

7. 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.

8. 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.

9. Klein, M. G. 1990. Efficacy against soil-inhabiting insect pests. Pp. 195–214 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

10. Kretzschmur, A. 1983. Soil transport as a homeostatic mechanism for stabilizing the earthworm environment. Pp. 59–66 *in* J. E. Satchell, ed. Earthworm ecology: From Darwin to vermiculture. London: Chapman and Hall.

11. Lee, K. E. 1985. Earthworms: Their ecology and relationship with soils and land use. Sydney, Australia: Academic Press.

12. Lewis, L. C., and E. S. Raun. 1978. Laboratory and field evaluation of DD-136 strain of *Neoaplectana carpocapsae* for control of the European corn borer, *Ostrinia nubilalis.* Iowa State Journal of Research 52: 391–396.

13. Moyle, P. L., and H. K. Kaya. 1981. Dispersal and infectivity of the entomogenous nematode, *Neoaplectana carpocapsae* Weiser (Rhabditida: Steinernematidae), in sand. Journal of Nematology 13:295–300.

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

15. Poinar, G. O., Jr. 1979. Nematodes for biological control of insects. Boca Raton, Florida: CRC Press.

16. Reed, E. M., and P. B. Carne. 1967. The suitability of a nematode (DD-136) for the control of some pasture insects. Journal of Invertebrate Pathology 9:196–204.

17. 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.

18. Wallace, H. R. 1958. Movement of eelworms. 2. A comparative study of the movement in soil of *Heterodera schachtii* Schmidt and of *Ditylenchus dipsaci* (Kühn) Filipjev. Annals of Applied Biology 46:86–94.