Effect of Soil Texture on the Distribution and Infectivity of Neoaplectana carpocapsae (Nematoda: Steinernematidae)[1]

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Abstract: The vertical migration of *N. carpocapsae* infective juveniles applied to the soil surface or introduced 14 cm below the soil surface was studied in four different soil types (pure silica sand, coarse sandy loam, silty clay loam, and clay). The percentage of juveniles able to migrate and infect wax moth pupae placed in the soil decreased as the percentage of clay and silt increased. Most nematodes placed on the soil surface remained within 2 cm of the surface, but some penetrated to a depth of 10 cm in pure silica sand and coarse sandy loam to infect pupae. Some pupae at the same depth were also infected with nematodes in silty clay loam soil. In pure silica sand and coarse sandy loam, nematodes introduced 14 cm below the soil surface were able to infect wax moth pupae located between 4 and 24 cm. Movement was least in clay soil and limited in silty clay loam. Nematodes showed a tendency to disperse upwards from the point of application. In all cases the number of migrating nematodes was greatest when wax moth pupae were present. *Key words: Neoaplectana carpocapsae,* biological control, dispersal, attraction, nematode movement, entomogenous nematode.

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A wide variety of nematodes attack invertebrates. Among this assortment, members of the genus *Neoaplectana* appear especially promising as biological control agents of insects (8). *Neoaplectana carpocapsae* Weiser and its associated bacterium, *Xenorhabdus nematophilus* (Poinar and Thomas), have been tested against a number of soil pests in the field, but the results have not been consistent (9). Reed and Carne (10) obtained poor control of the pruinose scarab, *Sericesthis germinata,* Boisduvl, and the dark soil scarab, *Othnonius batesi* Olliff, because the DD-136 strain of *N. carpocapsae* migrated to the soil surface after being introduced into the soil. The results of Moyle and Kaya (7) were contrary to those obtained by Reed and Carne. When the DD-136 juveniles were placed at 2.5 cm or 5.0 cm below the surface, the majority remained at the point of application, but some showed a tendency to move 10 cm downward and to infect wax moth pupae *Galleria mellonella* (L.). Jaques et al. (5) reported that soil application of *N. carpocapsae* against the larvae of the pale apple leaf roller, *Pseudexentera mali* (Freeman), and cocoons of the winter moth, *Operophtera brumata* (L.), gave reasonable control. Harlan et al. (4) obtained 38% reduction in populations of white-fringed beetle larvae, *Graphognathus peregrinus* (Busch.), 11 months after applying *N. carpocapsae.* Lewis and Raun (6) reported that *N. carpocapsae* did not infect larvae of the European corn borer, *Ostrinia nubilalis* (Hübner), in soil-born corn debris. If these nematodes are to be used in the soil, then more information is needed regarding their mobility in this medium. The present study defines the migration of *N. carpocapsae* in different soil types under laboratory conditions.

MATERIALS AND METHODS

Nematode culturing: N. carpocapsae (Breton strain) was cultured in larvae of the greater wax moth *Galleria mellonella* L. (2). After extraction infective juveniles were stored in water for 3 weeks at 5 C.

Soil type: Vertical migration of *N. carpocapsae* juveniles was studied in four different steam-sterilized soils: (i) pure silica sand, (ii) coarse sandy loam (10% clay, 10% silt, and 80% sand), (iii) silty clay loam (20% clay, 15% silt, and 65% sand), and (iv) clay (34% clay, 24% silt, 42% sand). The soil was treated with 30 ml of distilled water per 100 cm^3 soil before the experiments.

Soil columns: Vertical columns, 10 cm in length consisting of 2-cm sections of plastic tubing (7-cm inner diameter), were joined together with adhesive tape and filled with moist soil. Ten wax moth pupae were enclosed in the last section of the

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tube. The tubes were capped with aluminum foil and maintained at 23–25 C. Surface applications were conducted by adding 30,000 infective juveniles in 0.2 ml of water in small drops to the surface of the soil at the top of the vertical column.

The ability of the nematode to move vertically upward and downward was also studied, using the method described in the previous experiment. In this test, plastic tubes 28 cm in length (7-cm inner diameter) consisted of 4-cm sections. Wax moth pupae were placed at the top and the bottom of the tube, and 30,000 infective stage juveniles were injected into the soil at a depth of 14 cm.

After 5 days the plastic tubes were carefully separated and the nematodes were recovered for counting by washing them through a 200-mesh sieve and trapping them on a 400-mesh sieve screen (7). Wax moth pupae were removed and dissected, and the number of nematodes they contained noted. Nematode distribution in the absence of the wax moth pupae was also compared. Five replications (columns of tubes) were used.

Nematode infectivity: Nematode infectivity was determined by the ability of the juveniles to reach and infect pupae of *Galleria mellonella* placed at various levels in the soil. Four wax moth pupae were placed in each section of the tube (28 cm in length), and approximately 30,000 third-stage infective nematodes were introduced 14 cm below the soil surface. After 6 days, the plastic tubes were separated into the 4-cm lengths and the pupae were washed, dissected, and examined for nematodes. Nematode development inside the pupae was recorded. Replication was threefold.

Data analysis: Data in all experiments were anlyzed by two-way analysis of variance and Duncan's multiple-range test. Percentages were transformed using arcin transformation before statistical analysis.

RESULTS

The percentage of juveniles able to migrate and infect wax moth pupae decreased as the proportion of clay and silt increased.

The results showed nematode distribution varied greatly with soil type (Table 1). The greatest dispersal and infectivity occured in pure silica sand and coarse sandy loam. Most juveniles remained within 2 cm of the surface, but some penetrated 10 cm in depth in pure silica sand and coarse sandy loam to infect pupae. A few pupae were also infected with nematodes in silty clay loam soil. In the above cases the presence of the wax moth pupae resulted in significant increase of nematode movement. No nematodes were recovered beyond 4 cm in clay soil either in the presence or absence of a host (Table 1).

In pure silica sand and coarse sandy loam, nematodes introduced 14 cm below the soil surface were able to infect wax

Table 1. Vertical distribution of *Neoaplectana carpocapsae* infective juveniles in four different soil types, 5 days after placement of 30,000 nematodes at the soil surface.

	x̄% of juveniles in four different soil types							
	Pure silica sand		Coarse sandy loam		Silty clay loam		Clay	
Depth (cm)	P*	A†	P	A	P	A	P	A
0–2	73.6a‡	78.1a	80.8a	87.5a	95.7a	94.3a	95.9a	94.3a
2–4	11.6b	14.4b	9.6b	10.1b	4.0c	5.3c	4.9c	5.5c
4–6	6.4bc	5.2c	5.4c	1.8d	0.2e	0.4e	0.2e	0.2e
6–8	6.3bc	2.1d	2.1d	0.5e	0.09e	0	0	0
8–10	2.1d	0.2e	2.1d	0.1e	0.01f	0	0	0
x̄ total no. of nematodes recovered	25,822	20,763	23,909	20,830	19,971	20,637	22,125	18,622

*P = pupae of wax moth present.

†A = pupae of wax moth absent.

‡Means followed by the same letter are not signficantly different at the 5% level using Duncan's multiple-range test.

moth pupae located 10 cm above (section 0–4 cm) and 10 cm below (section 24–28 cm) the point of application (Table 2). The number of nematodes moving downward was greatest when a host was present. At a depth of 24–28 cm, the number of nematodes recovered was significantly higher when insect pupae were present (Table 2). Movement was less in silty clay loam soil. In all cases the nematodes showed a tendency to disperse upward from the point of application.

Nematodes introduced 14 cm below the soil surface were able to infect wax moth pupae located 0–14 cm above and 14–28 cm below the point of application (Table 3). The migration area was less in silty clay loam soil and even more limited in clay soil. Most of infected pupae were found to contain nematode progeny which subsequently could disperse downward and upward to infect more pupae.

Table 3. Mortality of wax moth pupae (*Galleria mellonela*) in four different soil types, 6 days after placement of *Neoaplectana carpocapsae* at a depth of 14 cm.

Depth (cm)	x̄ no. of infected pupae* in four different soil types			
	Pure silica sand	Coarse sandy loam	Silty clay loam	Clay
0–4	1.0	0.7	0	0
4–8	1.7	1.3	0.7	0
8–12	4.0	3.7	3.0	0
12–16	4.0	4.0	4.0	3.7
16–20	3.3	3.7	3.7	0
20–24	2.7	2.0	0.3	0
24–28	1.3	1.0	0	0
Total % mortality	62.9	58.6	41.9	13.2

*Four pupae were placed in each soil section.

DISCUSSION

It is well known that both moisture and pore size influence the movement of soil nematodes (12). A nematode cannot move between soil particles when the pore diameters are less than the width of the nematode. This appears to be the case with the clay soil tested here. Although there was some movement, indicating that the Breton strain of *N. carpocapsae* can indeed migrate through clay soil, movement was much less than in sand and sandy loam. This fact should be kept in mind when making field applications of neoaplectanid nematodes for soil insects.

In all soil types, most nematodes remained near the point of application but many showed a tendency to move upward when placed 14 cm below the soil surface. These results are in agreement with those obtained by El-Sherif (cited in Poinar [9]) and Moyle and Kaya (7). Reed and Carne

Table 2. Vertical distribution of *Neoaplectana carpocapsae* infective juveniles in four different soil types, 5 days after placement of 30,000 nematodes at a depth of 14 cm.

Depth (cm)	x̄% of juveniles in four different soil types							
	Pure silica sand		Coarse sandy loam		Silty clay loam		Clay	
	P*	A†	P	A	P	A	P	A
0–4	3.4a‡	3.3a	3.4a	2.9a	0.02j	0	0	0
4–8	11.9b	5.1a	7.9a	4.6a	0.9e	0.2f	0	0
8–12	23.3c	16.9bc	21.3bc	19.3bc	8.6b	10.7b	0.01j	0.02j
12–16	33.7d	41.2d	44.6d	50.2d	87.7h	86.9h	99.9h	99.98h
16–20	19.1bc	28.7c	18.6bc	21.0bc	2.5a	2.2a	0	0
20–24	7.0ab	4.5a	3.2a	1.8e	0.01j	0	0	0
24–28	1.6e	0.3f	1.0e	0.2f	0	0	0	0
x̄ total no. of nematodes recovered	24,190	23,229	23,643	21,773	22,924	23,087	23,076	22,509

*P = pupae of wax moth present.
†A = pupae of wax moth absent.
‡Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple-range test.

(10) reported that juveniles of *N. carpocapsae* (DD-136 strain) moved to the surface after being introduced into the soil. The results of this study are contrary to those obtained by Reed and Carne (10). The reason for these differences is not known. It may be that placement of the nematodes 14 cm below the soil surface and the strain of *N. carpocapsae* result in differences in vertical dispersal of the nematode. The number of migrating nematodes was greater when a host was present, indicating an attractiveness of the host to the nematode. Schmidt and All (11) suggested that the attraction of *N. carpocapsae* to wax moth larvae was due to a chemical gradient around the larvae. Furthermore, Gaugler et al. (3) found that *N. carpocapsae* responds positively to carbon dioxide, and recently Byers and Poinar (1) reported that the infective stages of *N. carpocapsae* respond to heat conducted from wax moth larvae in the absence of carbon dioxide or chemical gradients.

Nematodes applied 14 cm below the soil surface were able to move upward and downward to infect wax moth pupae. After reproducing inside the hosts, the infective stages emerged. This is how nematode populations would increase in areas of high host density.

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