

Effect of Soil Texture on the Distribution and Infectivity of *Neoaplectana glaseri* (Nematoda:Steinernematidae)

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Abstract: The vertical migration of infective juveniles of *Neoaplectana glaseri* applied to the soil surface or introduced 16 cm below the soil surface was studied in pure silica sand, coarse sandy loam, silty clay loam, and clay. The number of juveniles that migrated and infected wax moth pupae placed in the soil decreased as the proportion of clay and silt increased. The majority of nematodes moved downwards 2-6 cm from the surface, but some penetrated to a depth of 14 cm in pure silica sand and coarse sandy loam. In pure silica sand and coarse sandy loam, nematodes introduced 16 cm below the soil surface were able to infect wax moth pupae located at depths of 0-4 cm and 28-32 cm. Nematodes showed a greater tendency to disperse downwards from the point of application. Movement of the nematode was least in clay soil and limited in silty clay loam soil. The number of migrating nematodes was greatest when wax moth pupae were present. **Key words:** *Neoaplectana glaseri*, biological control, dispersal, attraction, nematode movement, entomogenous nematode.

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The entomogenous nematode *Neoaplectana glaseri* Steiner 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). In summarizing field experiments with *N. glaseri*, Glaser et al. (7) stated that of 73 different experimental plots treated at various seasons since 1931, parasitized Japanese beetle grubs *Popillia japonica* (Newm) were recovered from 72. Parasitism varied from 0.3 to 81% depending on soil moisture, nematode dosage, soil temperature, and host density. Recently, soil application of *N. glaseri* against the larvae of black vine weevil, *Otiorynchus sulcatus* Fab., gave high control under greenhouse conditions (6). Further, field application of a mixture of *N. glaseri* and *N. carpocapsae* Weiser (1:1) resulted in 62% reduction in population of black vine weevil larvae, *Nemoctes incomptus* Fab., (4). If *N. glaseri* is to be used as a biological control agent, more information is needed regarding its ability to move in soil. The present study defines the migration of *N. glaseri* in different soil types under laboratory conditions.

MATERIALS AND METHODS

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

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

Soil columns: Vertical columns, 14 cm in length and consisting of 2-cm sections of plastic tubing (7-cm inner diam.), were joined together with adhesive tape and filled with moist soil. Each section held approximately 180-220 g of moistened soil. Ten wax moth pupae were enclosed in the bottom section of each tube. The tubes were capped with aluminium 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 soil columns were not compacted more than would occur from the natural weight of the soil.

The ability of the nematode to move

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vertically upward and downward was studied, using the method described in the previous experiment, except plastic tubes, 32 cm in length (7-cm inner diam.) and consisting of 4-cm sections, were used. Ten wax moth pupae were placed at the top (section 0-4 cm) and the bottom (section 28-32 cm) of the tube, and 30,000 infective-stage juveniles were injected into the soil at a depth of 16 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 (8). Wax moth pupae were removed and dissected, and the number of nematodes was determined. 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 *G. mellonella* placed at various levels in the soil. Four wax moth pupae were placed in each section of the tube (32 cm in length), and approximately 30,000 third-stage infective nematodes were introduced 16 cm below the soil surface. After 6 days, the plastic tubes were separated into sections and the pupae washed, dissected, and examined for the presence of developing nematodes.

Data analysis: Data collected were ana-

lyzed by two-way analysis of variance and Duncan's multiple-range test. Percentages were transformed using arcsin transformation before statistical analysis.

RESULTS

The percentage of juvenile nematodes that migrated and infected wax moth pupae decreased as the proportion of clay and silt increased (Tables 1, 2).

The greatest dispersal and infectivity occurred in pure silica sand and coarse sandy loam. When the nematodes were applied to the soil surface, most juveniles remained within 2-6 cm of the surface but some were able to penetrate in the pure silica sand and coarse sandy loam and infected all pupae located at the bottom (section 12-14 cm). The presence of the wax moth pupae resulted in a significant increase of nematode movement. When the hosts were present in pure silica sand, 3.3% of the nematodes were found at 12-14 cm depth, compared to 0.3% in the absence of hosts. No nematodes were recovered below 6 cm in clay soil, and hosts did not affect nematode distribution (Table 1).

In pure silica sand and coarse sandy loam, nematodes introduced 16 cm below the soil surface were able to infect wax moth pupae located 16 cm above (section 0-4 cm) and 16 cm below (section 28-32 cm) the

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

Depth (cm)	Mean % of juveniles in four different soil types*							
	Pure silica sand		Coarse sandy loam		Silt clay loam		Clay	
	P†	A†	P	A	P	A	P	A
0-2	13.5ac	16.3a	19.2a	16.7a	36.4f	38.6f	56.8h	61.6h
2-4	25.1b	23.8b	24.4ab	24.2b	41.7f	47.3g	43.1g	38.2f
4-6	33.0b	29.5b	27.7b	30.5b	16.5a	10.5ac	0.1e	0.2e
6-8	12.4ac	16.6a	11.3ca	16.4a	5.2d	3.6d	0	0
8-10	8.1ca	10.1c	11.6ca	9.2c	0.2e	0	0	0
10-12	4.6d	3.4d	2.4d	2.7d	0	0	0	0
12-14	3.3d	0.3e	3.4d	0.3e	0	0	0	0
Mean total no. of nematodes recovered	28,710	26,813	24,791	28,012	27,426	24,762	27,331	25,811

*Means followed by the same letters are not significantly different at the 5% level, Duncan's multiple-range test.

†P = pupae of wax moth present at 12-14 cm depth. A = pupae of wax moth absent.

Table 2. Vertical distribution of *Neoplectana glaseri* infective juveniles in four different soil types, 5 days after placement of 30,000 nematodes at a depth of 16 cm.

Depth (cm)	Mean % of juveniles in four different soil types*							
	Pure silica sand		Coarse sandy loam		Silt clay loam		Clay	
	P†	A†	P	A	P	A	P	A
0-4	1.4a	0.6ah	1.1a	0.3h	0	0	0	0
4-8	2.9ae	3.1e	3.8e	2.8e	0.2h	0	0	0
8-12	13.3b	15.6b	9.3g	11.6bg	6.7ge	5.8	0	0
12-16	20.6c	26.3c	22.7cb	30.1d	84.3j	81.6j	100.00k	100.00k
16-20	31.1d	33.7d	34.6d	28.9d	6.4	12.3	0	0
20-24	18.6b	14.5b	17.5b	19.8b	0.3	0.3	0	0
24-28	8.6g	4.3e	7.2g	5.3ge	0.1	0	0	0
28-32	4.1e	1.9f	3.8e	1.2f	0	0	0	0
Mean total no. of nematodes recovered	26,821	27,081	27,315	24,689	27,516	23,907	25,733	27,991

*Means followed by the same letters are not significantly different at the 5% level, Duncan's multiple-range test.

†P = pupae of wax moth present at 0-4 cm and 28-32 cm depth. A = pupae of wax moth absent.

point of application (Table 2). Again the number of migrating nematodes was significantly greater when hosts were present in these soil types. The percentage of nematodes recovered at the 0-4-cm and 28-31-cm depths was higher in the presence of wax moth pupae than when pupae were not available. Movement was limited in the silty clay loam soil and did not occur in the clay soil. In all cases the nematodes showed a significantly greater tendency to disperse downward.

In pure silica and coarse sandy loam, *N. glaseri* introduced 16 cm below the soil surface were able to infect wax moth pupae located between 0 and 16 cm above and be-

tween 16 and 32 cm below the point of application (Table 3). The number of infected pupae was less in silty clay loam soil and even more limited in clay soil. Most infected pupae contained nematode progeny.

DISCUSSION

Soil texture is important for vertical distribution and infectivity of juveniles of *N. glaseri*. Differences in migration patterns in soils of different structure probably occur because the nematodes have an optimum particle size for movement (11). The structure of clay and silt appears to limit move-

Table 3. Mortality of wax moth pupae in four different soil types, 6 days after placement of *Neoplectana glaseri* at a depth of 16 cm.

Depth (cm)	Mean % of infected pupae* in four different soil types			
	Pure silica sand	Coarse sandy loam	Silt clay loam	Clay
0-4	0.3	0.3	0	0
4-8	1.7	1.3	0.3	0
8-12	4.0	3.7	3.0	0
12-16	4.0	4.0	4.0	3.7
16-20	4.0	4.0	3.7	1.0
20-24	3.7	3.0	0.3	0
24-28	3.7	3.0	0.3	0
28-32	3.0	3.0	0	0
Total % mortality	76.1	72.8	36.3	15.0

*Four wax moth pupae were placed in each section (five replications).

ment of *N. glaseri*; similar behavior was reported for *N. carpocapsae* (5).

It appears that the behavior of *N. glaseri* in the soil is different from *N. carpocapsae*. When infective-stage nematodes of *N. carpocapsae* were applied to the soil surface, most nematodes remained near the point of application but showed a tendency to move upwards when placed 14 or 15 cm below the soil surface (5,8).

The number of migrating nematodes was greater when hosts were present. This indicates an attractiveness of the host to the nematode. Schmidt and All (10) suggested that the attraction of *N. carpocapsae* to wax moth larvae was due to a chemical gradient around the larvae. Further, Gaugler et al. (3) found that *N. carpocapsae* responds positively to carbon dioxide, and 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.

Greater movement in some soils may be possible because nematodes multiplied inside the host and infective stages emerged. These infective stages in turn have the potential to disperse further and build up population in an area when host density is high. The results of this study throw light on nematode movement which is important in the placement of *N. glaseri* in field applications.

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