Dispersal and Infectivity of the Entomogenous Nematode, Neoaplectana carpocapsae Weiser (Rhabditida: Steinernematidae), in Sand[1]

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Abstract: Laboratory tests determined the lateral and vertical dispersal patterns of *Neoaplectana carpocapsae* in sand. In the vertical tests, placement of infective juveniles 15 cm below the sand's surface resulted in the majority (77%) being recovered above the point of placement after 48 h. Placement of the nematodes on the sand's surface resulted in the majority (90.4%) remaining within 1 cm of the sand's surface. Placement of nematodes at depths of 2.5 cm and 5.0 cm below the sand's surface also resulted in little nematode dispersal. However, vertical bioassay tests showed that juvenile nematodes placed on the sand's surface dispersed 12 cm down to infect 67% of the *Galleria mellonella* pupae placed at the depth. Conversely, when nematodes were placed 11 cm below the insect pupae no infection was observed, but 53% infection occurred when nematodes were 7 cm below the site of the insect pupae. In lateral dispersal, 87% of the nematodes remained within 2 cm of the placement site, although 0.5% were recovered at 12–14 cm away from the point of placement. Lateral bioassay tests indicated that the nematodes were capable of infecting 90, 35, and 5% of the *G. mellonella* pupae at 7 cm, 10 cm, and 14 cm from the point of placement, respectively. *Key words:* DD-136 nematode, biological control, entomophilic nematode, dispersal, nematode movement.

The entomogenous nematode, *Neoaplectana carpocapsae* Weiser, and its associated bacterium, *Xenorhabdus nematophilus* (Poinar and Thomas), are effective against a number of insect pests in the laboratory (2,7,11) but their use in the field has resulted in varying degrees of control (1,12). In above ground applications, the infective juvenile nematodes die within a short time if not protected from desiccation (10,19,20). Simons and Poinar (17) suggested that this nematode may be more effective as a biological control agent against soil-inhabiting insects because adequate moisture is present in soil. Although *N. carpocapsae* has been used against soil-inhabiting insects, the results have not been consistent. Reed and Carne (13) reported that the nematode was effective against the pruinose scarab, *Sericesthis geminata* Boisduval, and the dark soil scarab, *Othnonius batesi* Olliff, under laboratory conditions, but no mortality could be attributed to *N. carpocapsae* in field trials. Jaques et al. (5) applied infective nematodes to the soil surface beneath apple trees and found that the nematode reduced survival of the larvae of the pale apple leafroller, *Pseudexentera mali* (Freeman), and of the cocoons of the winter moth, *Operophtera brumata* (L.), to 33 and 12% of the controls, respectively. Lewis and Raun (8) reported that *N. carpocapsae* could be found in nematode-treated soil but did not infect larvae of the European corn borer, *Ostrinia nubilalis* (Hübner), in soil-borne corn debris.

A closer examination of the behavior of this nematode in soil is required. Preliminary studies reported by El-Sherif and by van Bracht (cited in 12) indicate that infective juveniles of *N. carpocapsae* and *Heterorhabditis bacteriophora* Poinar behave differently when placed in the soil or on the surface of the soil. This paper reports laboratory tests on the lateral and vertical dispersal of *N. carpocapsae* in sand. This information may provide a basis for future work in the field to maximize the use of this nematode as a biological control agent.

MATERIALS AND METHODS

Nematode rearing: Stock suspensions of infective juveniles of *N. carpocapsae* were obtained by infecting larvae of the wax moth, *Galleria mellonella* (L.), according to the method described by Dutky et al. (3). Juvenile nematodes which emerged from the larvae were processed through a Baermann funnel apparatus into 250-ml Erlenmeyer flasks. Stock suspensions were maintained at 10 C in 0.1% formalin at a concentration of ca 1,000 nematodes per ml. Nematodes were concentrated by centrifuga-

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tion (ca 500 rpm) as needed. New nematode stocks were obtained every 3–4 wk.

Sand type: Sand used in this study was 0.2–0.5 mm particle size which was washed 10 times—five hot water (56 C) washings and five distilled water washings—to remove debris and any free salts, oven dried, and remoistened with 30 ml of distilled water per 100 cc of sand.

Vertical dispersal: Vertical dispersal of *N. carpocapsae* was determined by using a 30- × 5-cm plexiglass column as described by Johnson and Lear (6). The column could be broken down into individual 5- × 5-cm sections (inside diameter = 4.4 cm), and each section held ca 100 g of moistened sand (76.3 cm^3). For the first test, ca 15,000 *N. carpocapsae* juveniles were injected into the sand at a depth of 15 cm. The top of the column was capped with aluminum foil to retard the sand from drying. After 48 h, the column was dismantled into six sections and a 50- × 16-mm core sample of sand was taken with a No. 7 cork borer from the center of each section. Center samples were taken to eliminate the possibility of recovering nematodes which may have moved along the plexiglass/sand interface. Nematodes were recovered for counting by washing them through a 200 mesh sieve (74 μm pores) and trapping them on a 400 mesh (25 μm) sieve screen. The percentage of nematodes recovered per section, based on the total number of nematodes recovered from the samples, was recorded. There were three trials with three replicates per trial.

Tests in which the juvenile nematodes were placed on the surface of the sand and at depths of 2.5 cm and 5 cm were also conducted. Columns used in these tests were composed of two 5-cm sections, with the upper 5-cm section composed of five 1-cm rings. Tests were conducted for 48 h as described above, using ca 15,000 nematodes per column. There were three trials per depth with one column per trial.

Lateral dispersal: Lateral dispersal of *N. carpocapsae* was studied by using a modified technique of Marban-Mendoza and Viglierchio (9). A 28-cm diameter glass plate was covered to a depth of 0.5 cm with oven-dried sand and moistened with 15 ml distilled water with the aid of a separatory funnel. The tip of the stopcock stem was placed at the edge of the sand disc, which was maintained at a slight angle to allow the water to wet the sand by capillary action. Approximately 10,000 nematodes were placed in the center of the plate, and the plate placed in a humidity chamber at 25 ± 2 C. The plate was removed after 48 h and placed on a slide-ringing apparatus and seven concentric 2-cm-wide bands of sand collected (9). Nematodes were recovered and counted as described above. There were three trials with three replicates in each trial.

Data analysis: Data collected from vertical and lateral dispersal tests were analyzed by two-way analysis of variance and Duncan's multiple-range test. Percentages were transformed using arcsin transformation before statistical analysis.

Bioassay: Bioassay techniques were also utilized to determine vertical and lateral dispersal. Vertical dispersal bioassays were conducted as follows. In the first experiment, five 3–5-d-old *G. mellonella* pupae per 30-cm column were placed at depths of 0, 1, 4, 8, and 12 cm. Approximately 15,000 juvenile nematodes per column were placed on the sand's surface to determine the ability of the nematode to disperse and infect pupae. The columns were capped with aluminum foil and maintained at 26 ± 2 C. After 7 d, each column was dismantled and the pupae were recovered, dissected, and examined for nematodes. The stages of nematode development were recorded. There were two trials with one to three replicates per trial. The second experiment was similar to the first except that five pupae per column were placed at depths of 0, 4, 8, and 12 cm, and ca 15,000 nematodes were introduced 15 cm below the surface of the sand to determine whether the nematodes could disperse upward and infect the pupae. There were three replicates. Pupae placed at a depth of 12 cm in columns without nematodes served as controls in both experiments.

Lateral bioassay dispersal experiments were conducted using the 28-cm glass plate. In these experiments four sets of five *G. mellonella* pupae each were placed at distances of 5, 10, and 14 cm along the radii and approximately 10,000 nematodes were introduced at the center of the plate. The

plate was placed in a humidity chamber. After 7 d pupae were removed and examined for nematode presence and development. There were two replicates.

RESULTS

When infective juveniles of *N. carpocapsae* were placed 15 cm below the sand's surface in a column (Table 1), there were highly significant differences in the distribution of the nematodes among the six sections ($F = 319.1$, $P < 0.01$). The majority (77%) of the juveniles were found above the point of placement. A comparison of sections equidistant from the point of placement showed significantly more nematodes in the upper sections than in the lower sections. For example, 5.5% of the nematodes were found at the 0–5 cm depth in comparison to 0.2% at 25–30 cm. Thus, the nematodes showed a tendency to disperse upwards from the point of placement.

Placement of the nematodes on the sand's surface (Table 2) showed that there were highly significant differences in the distribution of the nematodes ($F = 521.1$ $P < 0.01$). The majority (90.4%) remained within 1 cm of the surface while the remainder were recovered 1–10 cm below the surface. When the nematodes were placed 2.5 cm below the surface, 55% were recovered from the upper 2 cm of the sand's surface, while ca 40% were found below the point of placement down to 10 cm. Upon comparing the two sections at 0–5 cm and 5–10 cm, the majority (94%) of the nematodes were in the 0–5 cm section, which indicated that very little dispersal had occurred. When the nematodes were placed 5 cm below the sand's surface, ca 43% were recovered near the placement point and ca 56% were found below the point of placement. Less than 1% were found above the point of placement. In comparing the 0–5 cm section with the 5–10 cm section, more were in the lower section (56%) than in the upper section (44%).

Sufficient nematodes dispersed downward to infect *G. mellonella* pupae when juveniles were placed on the surface of the sand. Thus, 100% (n = 25) infection occurred at 0 cm, 80% (n = 10) at 1 cm, 80% (n = 10) at 2 cm, 72% (n = 25) at 4 cm, 67% (n = 15) at 8 cm, and 67% (n = 15) at 12 cm. Furthermore, at 12 cm 54% of the infected pupae contained nematode progeny which demonstrated that both male and female juveniles dispersed downward. Conversely, when nematodes were placed 15 cm below the sand's surface, none (n = 15) of the pupae located 11 cm above the point of placement (pupae at 4 cm) was infected. However, 47% (n = 15) of the pupae located 7 cm above the point of placement (at 8 cm) and 73% (n = 15) located 3 cm above the point of placement (at 12 cm) were infected. Only 10% of all infected pupae from the upward dispersal trials had progeny.

In the lateral dispersal studies (Table 3), highly significant differences were observed in the number of nematodes recovered from various distances from the point of placement ($F = 304.4$, $P < 0.01$). Although the majority (87%) of the nematodes were recovered within 2 cm of the site of placement, 0.5% of the nematodes dis-

Table 1. Vertical distribution of *Neoaplectana carpocapsae* juveniles 48 h after placement at depth of 15 cm in sand.

Depth (cm)†	x̄%(x̄ total no.) nematodes recovered*			
	Trial 1	Trial 2	Trial 3	Overall x̄%‡
0-5	5.0 (186)	5.6 (204)	5.9 (217)	5.5 c
5-10	10.3 (407)	13.3 (471)	15.0 (555)	12.9 d
10-15	63.0 (2,424)	57.6 (2,098)	55.3 (2,148)	58.6 f
15-20	20.0 (770)	21.6 (785)	21.5 (791)	21.0 e
20-25	1.4 (52)	1.8 (67)	2.2 (79)	1.8 b
25-30	0.3 (11)	0.1 (3)	0.1 (3)	0.2 a

*x̄ of three replicates in each trial.

†Dashed line indicates point of placement of 15,000 nematodes.

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

Table 2. Vertical distribution of *Neoaplectana carpocapsae* juveniles 48 h after placement at different depths in sand.

Depth (cm)	% (total no.) nematodes recovered						x̄%*
	Trial 1		Trial 2		Trial 3		
	Placement of nematodes on surface						
0-1	86.6	(4,861)	92.8	(7,185)	91.7	(6,018)	90.4 d
1-2	10.2	(573)	5.4	(414)	6.0	(394)	7.2 c
2-3	1.9	(106)	0.5	(42)	1.1	(73)	1.2 ab
3-4	1.0	(53)	0.8	(61)	0.7	(45)	0.8 ab
4-5	0.2	(13)	0.4	(30)	0.4	(29)	0.3 ab
5-10	0.1	(8)	0.1	(11)	0.1	(6)	0.1 a
	Placement of nematodes at 2.5 cm below surface						
0-1	0.5	(43)	3.2	(140)	2.6	(175)	2.1 a
1-2	2.2	(180)	2.8	(124)	4.2	(284)	3.1 a
2-3	47.4	(3,926)	54.3	(2,380)	63.4	(4,246)	55.0 d
3-4	30.0	(2,484)	20.4	(896)	13.3	(891)	21.2 c
4-5	17.3	(1,435)	10.3	(450)	10.2	(683)	12.6 bc
5-10	2.6	(212)	9.0	(396)	6.3	(417)	6.0 ab
	Placement of nematodes at 5.0 cm below surface						
0-1	0.2	(3)	0.0	(0)	0.3	(6)	0.2 a
1-2	0.1	(1)	0.0	(0)	0.4	(8)	0.2 a
2-3	0.1	(1)	0.0	(0)	0.2	(3)	0.1 a
3-4	0.4	(8)	0.1	(3)	0.5	(11)	0.3 a
4-5	53.6	(896)	26.6	(1,769)	48.4	(966)	42.8 b
5-10	45.6	(762)	73.3	(4,872)	50.2	(1,002)	56.4 c

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

persed 12–14 cm away from the placement site.

Bioassay tests indicated that sufficient numbers of nematodes dispersed laterally to infect 90% (n = 40) of the pupae at 7 cm, 35% (n = 40) at 10 cm, and 5% (n = 40) at 14 cm from the point of placement. The nematodes dispersed in different directions because the *G. mellonella* pupae were placed at four different points on the plate and some of the pupae from all points at 7 and 10 cm away were infected. Furthermore, these nematodes were capable of infecting some pupae after dispersing laterally up to 14 cm.

Table 3. Lateral distribution of *Neoaplectana carpocapsae* juveniles after 48 h on a sand-covered 28-cm plate.

Distance from center of plate cm†	x̄% (x̄ total no.) nematodes recovered*						x̄%‡
	Trial 1		Trial 2		Trial 3		
0-2	95.0	(2,279)	76.7	(3,003)	89.3	(6,931)	87.2 d
2-4	2.9	(376)	14.0	(535)	6.9	(530)	7.8 c
4-6	1.1	(123)	3.5	(145)	1.9	(148)	2.2 ab
6-8	0.5	(61)	2.4	(93)	0.9	(68)	1.2 ab
8-10	0.2	(30)	1.3	(44)	0.4	(27)	0.6 ab
10-12	0.1	(19)	0.9	(29)	0.4	(26)	0.5 a
12-14	0.2	(21)	1.2	(32)	0.5	(12)	0.5 a

*x̄ of three replicates.

†10,000 nematodes placed in center of plate.

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

DISCUSSION

In studies on the dispersal of *N. carpocapsae* in a soil medium, El-Sherif (cited in 12) demonstrated that the DD–136 strain had a tendency to disperse upward when placed 15 cm below the surface of a sandy loam soil and the Agriotos strain dispersed upwards and downwards in about equal numbers. However, both strains of the nematode stayed near the point of placement. Reed and Carne (13) reported that most DD–136 juveniles disperse toward the soil's surface. On the soil surface, *N. carpocapsae* is reported to be able to leap from one soil particle to another (14). Using another nematode species, *Heterorhabditis bacteriophora,* van Bracht (cited in 12) reported that when infective juveniles were placed 15 cm below the soil surface, the majority dispersed upwards, but some dispersed downwards.

In our studies with the DD–136 strain, the majority of the nematodes remained near the point of placement, but those which dispersed showed a tendency to disperse upwards when placed 15 cm below the surface of the sand. These data are in agreement with those obtained by El-Sherif (cited in 12). When the DD–136 juveniles were placed at 2.5 cm or 5.0 cm below the surface, the majority again remained at the point of placement, but the migrating nematodes showed a tendency to disperse downwards. These data are contrary to those obtained by Reed and Carne (13). The reason for these differences is not known. It may be that placement of the nematodes at different levels below the surface and the strain of *N. carpocapsae* result in differences in vertical dispersal of the nematode. Different species of nematodes also show differences in vertical dispersal. Therefore, tests with various strains of *N. carpocapsae* placed at different depths should be conducted before generalizations, if any, are made on vertical dispersal of the infective nematodes.

When placed on the sand's surface, *N. carpocapsae* juveniles displayed little downward movement. Reed and Carne (13) and Reed and Wallace (14) concluded that *N. carpocapsae* is better adapted for dispersal on the soil surface and does not penetrate deeply into the soil.

Lateral dispersal of *N. carpocapsae* juveniles in sand apparently has not been assessed before. The results indicate that the nematode is able to disperse laterally to 14 cm, although the majority remained within 6 cm of the placement site.

In the vertical bioassay tests, sufficient juvenile nematodes dispersed in either direction to infect the host pupae, but infectivity was higher when the nematodes were placed on the surface of the sand. Similarly, the lateral bioassay trials showed that the nematode was capable of dispersing 14 cm and infecting the host, although the highest percentage of infection occurred within 7 cm. This occurred even though the vertical and lateral dispersal studies showed that nematodes tended to remain at the placement site.

The ability of *N. carpocapsae* to orientate to host insects was demonstrated by Schmidt and All (15) and they suggested that chemical attractants might be involved. Furthermore, *N. carpocapsae* orientate to insect by-products including uric acid (16) and CO^2 (4). Plant-parasitic nematodes also respond to chemical gradients that form in the rhizosphere of host roots (18). This ability to orient to chemical attractants may explain that while DD–136 juveniles tend to remain at the point of placement, they can infect hosts located 14 cm away.

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