Vertical Migration of Heterorhabditis bacteriophora and H. heliothidis (Nematoda: Heterorhabditidae) in Sandy Loam Soil

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The entomogenous nematodes *Heterorhabditis bacteriophora* Poinar and *H. heliothidis* (Khan, Brooks, and Hirschmann) and their associated bacterium *Xenorhabdus luminescens* (Poinar and Thomas) have been tested against a number of insect pests with some encouraging results (11). Simons (14) obtained 90% control of the black vine weevil, *Otiorhynchus sulcatus* Fab., after applying *Heterorhabditis* sp. to the soil surface in greenhouses. Similar results were reported by Bedding and Miller (1) and Georgis et al. (7) using *H. heliothidis*. Laboratory tests using *H. heliothidis* against Mediterranean fruit fly adults, *Ceratitis capitat* (Wied.), gave 85% control (12). Recently, confined field tests showed that the application of *H. bacteriophora* to brussels sprout seedlings have protected 88% of the plants from damage caused by the cabbage root maggot, *Hylemya brassicae* (Weid.) (8). If these nematodes are to be used as biological control agents, then more information is needed regarding their ability to move in soil. The present study defines the migration of *H. bacteriophora* and *H. heliothidis* in sandy loam soil under laboratory conditions.

Heterorhabditis bacteriophora and *H. heliothidis* were cultured separately in larvae of the greater wax moth *Galleria mellonella* L. (13). After extraction, infective juveniles were stored in water for 3 weeks at 8–10 C.

Vertical migration of *H. bacteriophora* and *H. heliothidis* juveniles was studied in sandy loam soil (10% clay, 10% silt, 80% sand). The soil had been steam sterilized and treated with 30 ml of distilled water per 100 cc soil.

Vertical columns 10 cm in length 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 ca. 180 g of moistened sand. Ten wax moth pupae were enclosed in the bottom section of each tube. The tubes were capped with aluminum foil and maintained at 20–23 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. Nematode migration in the absence of the wax moth pupae was also conducted. The experiment was replicated three times.

The ability of the nematode to move vertically upward was also studied, using the method described in the previous experiment with plastic tubes 28 cm in length (7-cm inner diam.) consisting of 4-cm sections. Ten wax moth pupae were placed at the top (section 0–4 cm) and the bottom (section 24–28 cm) 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 screen and trapping them on a 500-mesh sieve screen (10).

Placement of the nematodes on the soil surface showed that there were highly significant differences in the migrational pattern of the heterorhabditid species (Table 1). More than 70% of the infective juveniles of *H. heliothidis* were recovered within 0–2 cm of the surface, while only approximately 50% of *H. bacteriophora* was found at this depth. The remainder of both nematode species were recovered between 2 and 10 cm. Infective juveniles of *H. bacteriophora* recovered at 8–10 cm from the surface were significantly more numerous than *H. heliothidis*. Both heterorhabditid nematodes were able to infect all wax moth pupae located at the 8–10-cm depth from the point of application, and most pupae contained nematode progeny. The presence of wax moth pupae significantly increased the number of migrating

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nematodes. For example, when the host was present, 4.3% of *H. bacteriophora* infective juveniles were recovered at a depth of 8–10 cm compared to 0.7% in the absence of the host (Table 1).

When infective juveniles of both heterorhabditid nematodes were placed 14 cm below the soil surface (Table 2), there were highly significant differences in the migration of nematodes among the six sections. At the 0–12-cm depth, 46.9% and 36.6% of *H. bacteriophora* and *H. heliothidis*, respectively, were recovered in comparison to 22.3% and 26.5%, respectively, at the 16–28-cm depth. Thus the nematodes showed a tendency to disperse upwards from the point of application. The percentage of *H. bacteriophora* recovered above the point of placement (0–12 cm) was significantly higher than *H. heliothidis*. Again both nematode species were able to infect all wax moth pupae placed at the top (section 0–4 cm) and the bottom (section 24–28 cm) of the tube, and most pupae were found to contain nematode progeny.

Table 1. Vertical migration of *H. bacteriophora* and *H. heliothidis* infective juveniles in sandy loam soil, 5 days after placement of 30,000 nematodes at the soil surface (average of three replications).

	Mean % of juveniles recovered*			
	H. bacteriophora		*H. heliothidis*	
Depth (cm)	P†	A‡	P†	A‡
0–2	48.8a	53.5a	71.4f	70.9f
2–4	30.1b	25.3b	13.5cg	17.5c
4–6	13.1c	17.6c	11.0cg	9.8cg
6–8	3.7d	2.9d	2.2d	1.6h
8–10	4.3d	0.7e	1.9h	0.2e
Mean total no. of nematodes recovered	28.907	28.218	29.169	28.713

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

†P = Wax moth pupae present at 8–10-cm depth.

‡A = Wax moth pupae absent.

Table 2. Vertical distribution of *H. bacteriophora* and *H. heliothidis* infective juveniles in sandy loam soil, 5 days after placement of 30,000 nematodes at a depth of 14 cm (average of three replications).

	Mean % of juveniles recovered*	
Depth (cm)†	*H. bacteriophora*	*H. heliothidis*
0–4	4.3a	2.9f
4–8	15.4b	13.1b
8–12	27.2c	20.6g
12–16	30.8c	36.9h
16–20	12.9b	15.8b
20–24	8.2d	9.3d
24–28	1.2e	1.4e
Mean total no. of nematodes recovered	28.692	28.306

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

†Ten wax moth pupae were placed at 0–4 cm and 24–28 cm deep.

The results of this study have shown the migration patterns of *H. bacteriophora* and *H. heliothidis* infective juveniles in sandy loam soil. When the infective juveniles of *H. heliothidis* were placed on the soil surface, most of the nematodes remained at the 0–2-cm depth and the nematodes showed a tendency to move downwards to infect wax moth pupae located at the 8–10-cm depth. The behavior of this heterorhabditid nematode appears to be similar to *Neoaplectana carpocapsae* Weiser (5,6,10). The infective juveniles of *H. bacteriophora* showed a greater tendency to move downwards, in contrast to *N. carpocapsae*.

When both heterorhabditid species were introduced 14 cm below the soil surface, migrating juveniles of *H. bacteriophora* showed a greater tendency to move upwards than did *H. heliothidis*.

Some preliminary experiments were conducted by Van Bracht (cited in 11) to study the movement of *H. bacteriophora*. He observed that most of the juveniles migrated to the soil surface, or remained there, instead of remaining where they had been placed below the surface, as in the present study. Details of the experiment were not reported.

The movement of both heterorhabditid species was greater when wax moth pupae were present. This indicates an attractiveness of the host to the nematode. The ability of *N. carpocapsae* to orientate to chemical stimuli and heat provided by the host was reported by several workers (2,4,13). This response to the host was also noted in

similar experiments with infective juveniles of *N. carpocapsae* and *N. glaseri* (6,9).

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