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Short note

Comparative vertical migration of insect parasitic nematodes *Heterorhabditis* spp. and *Steinernema* spp. in sand at 9°C

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Insect parasitic nematodes of the genus *Heterorhabditis* Poinar can provide effective control of the black vine weevil, *Otiorhynchus sulcatus* F; up to 100 % mortality of the larvae can be obtained in strawberries and ornamentals in the greenhouse. However, the nematodes are not very effective when soil temperatures are below 12-13°C (*e.g.*, Rutherford *et al.*, 1987), which limits the extension of the application of *Heterorhabditis* in regions of lower soil temperatures. Nematodes of the genus *Steinernema* Travassos tend to be more effective at low temperatures than heterorhabditids (*e.g.*, Molyneux, 1986); however, heterorhabditids are generally more pathogenic to *O. sulcatus* and other soil dwelling coleopterans (*e.g.*, Rutherford *et al.*, 1987; Hanula, 1993). Nematode mobility is required for black vine weevil control, as larvae of *O. sulcatus* can be found at great depths, down to 15 cm in yew, for instance (Hanula, 1993). Species and isolates of *Heterorhabditis* differ in their migration at 20°C (Westerman, 1995). Therefore, in the present study, the mobility of sixteen isolates of *Heterorhabditis* and three isolates of *Steinernema* was studied in sand columns at 9°C.

Mean migration was assessed in 9 cm vertical sand columns at 9°C with or without a larva of *Galleria mellonella* at the bottom as described in an earlier paper on migration at 20°C (Westerman, 1995). Origin, culture, and storage method of the nematode species and isolates were as described by Westerman (1995). *S. feltiae* OBSIII (Nagele, Netherlands) was isolated by Dr F. Galle, *S. feltiae* Mr (Austria) was kindly provided by Dr R.-U. Ehlers and *S. carpocapsae* UK (UK) was provided by Dr N.G.M. Hague. The nematodes were left at the cold storage temperature (4-5 or 9°C) until inoculation. Immediately after preparation, each sand column was inoculated on top with approximately 2000 nematodes in 0.5 ml water. Isolates were either tested in time series of 16, 24, 32, 40, 48, and 56 h or only after a 48 h incubation period. The number of nematodes in the top layer (0-1.5 cm), the middle section (1.5-7.5) and the bottom layer (7.5-9.0) was estimated, and used to calculate the average distance migrated [cm] (Westerman, 1995). 40 to 60%, and for HP88 70%, of the applied nematodes were recovered. The number of nematodes recovered from dead insects was so small (≤ 25 on average) that they were omitted from calculations.

Migration over time is presented in Figs 1 and 2 for eleven heterorhabditid and one steinernematid isolates. Mean migration data, analysed in a similar way as the 20°C data (Westerman, 1995), significantly increased with time ($P \leq 0.05$) for all isolates tested in time series, except for HF85, HNb87 and HSH, and most isolates significantly responded to the presence of *G. mellonella;* HB1'87, HUK211 and *H. megidis* HOI responded more than proportionally to *G. mellonella* over time (host × time interaction, $P = 0.005$, 0.03, and 0.001, respectively). Five isolates did not respond significantly to the presence of *G. mellonella*; HNb87, HSH, HKem, M145, and *S. feltiae* OBSIII. 56 h were often insufficient to follow the proportion mobile of nematodes in the population down to the bottom layer. This was mainly due to rate differences, because generally only a small proportion of the nematodes was inactive and remained in the top layer. Mean migration at 9°C was higher in cylinders with than in cylinders without *G. mellonella* for most isolates, and the presence of *G. mellonella* corroborated differences in migration between isolates (Table 1).

In contrast to migration at 20°C, differences at 9°C do not seem to be related to putative taxonomic groups within *Heterorhabditis*. Isolates identified here as being relatively mobile at 9°C belonged to different species and groups, *e.g.*, the Irish K122, the NW European UK211 or *H. megidis* HO1. In addition, there were differences between isolates from the same locality (*e.g.*, compare Dutch or Irish isolates), which indicates that the ability to move in cold sand columns is not directly related to the geografic origin. However, not all species and groups were equally represented in this study.

The three steinemematids tested in this study, *S. carpocapsae* UK, *S. feltiae* Mr, and *S. feltiae* OBSIII, were relatively immobile at 9°C, especially when compared to the Irish and NW European heterorhabditids

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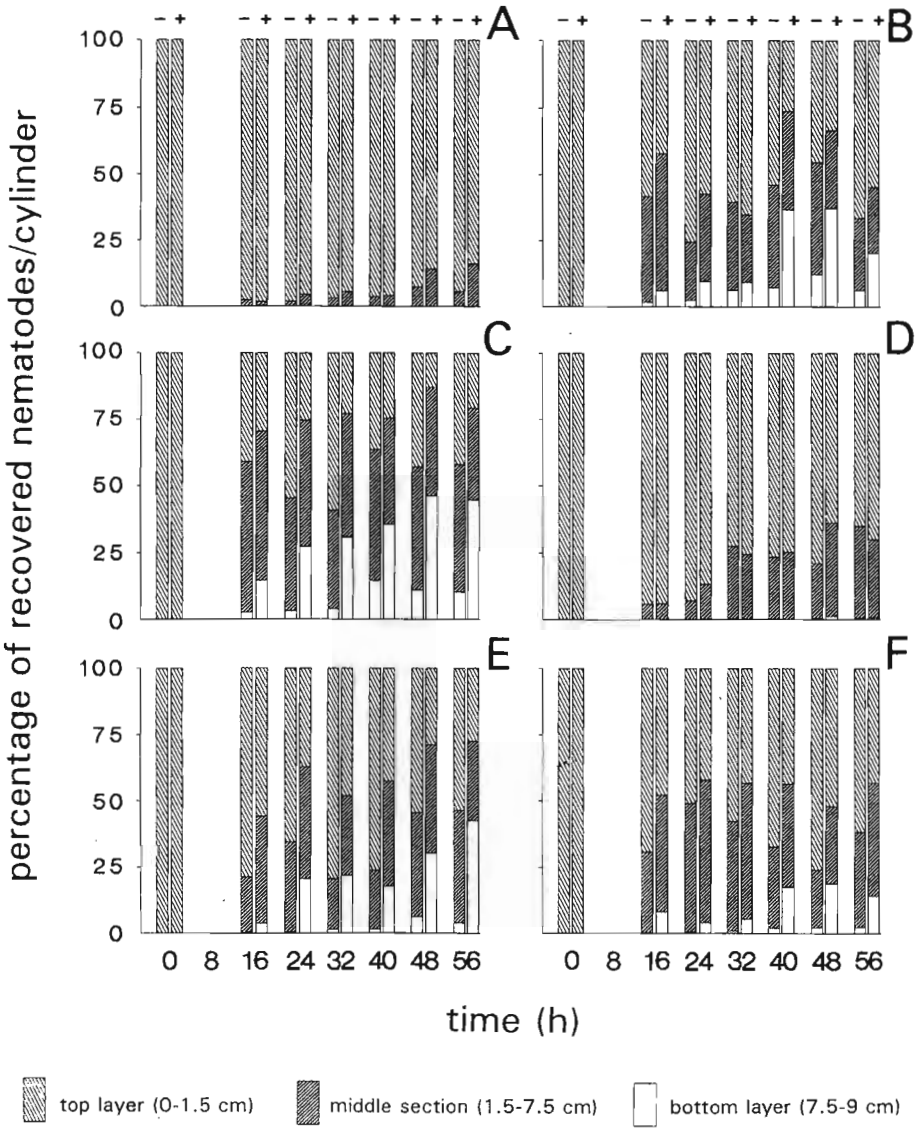

Fig. 1. *Percentage of recovered nematodes over time in three sections of 9 cm high sand columns, in the absence (–) or presence (+) of a last instar of* Galleria mellonella *at the bottom of the cylinders, after application of approximately 2000 living heterorhabditid nematodes on top of the cylinders. A: HP88; B:* Heterorhabditis megidis *HO1; C: K122; D: M145; E: HL81; F: HF85.*

at 9°C, or when compared to *S. feltiae* Mr at 20°C (3.2 ± 0.6 and 5.7 ± 0.1, in cylinders without and with *G. mellonella* at the bottom, respectively; unpubl.). However, steinernematids constitute a large group of species and isolates. Other isolates, *e.g.*, the cold active isolate LIC (Morris *et al.*, 1990), may be more mobile at 9°C.

The migration assay provides an indication of the migration potential in sand at 9°C of the isolates (batches) examined. It is not possible to identify iso-

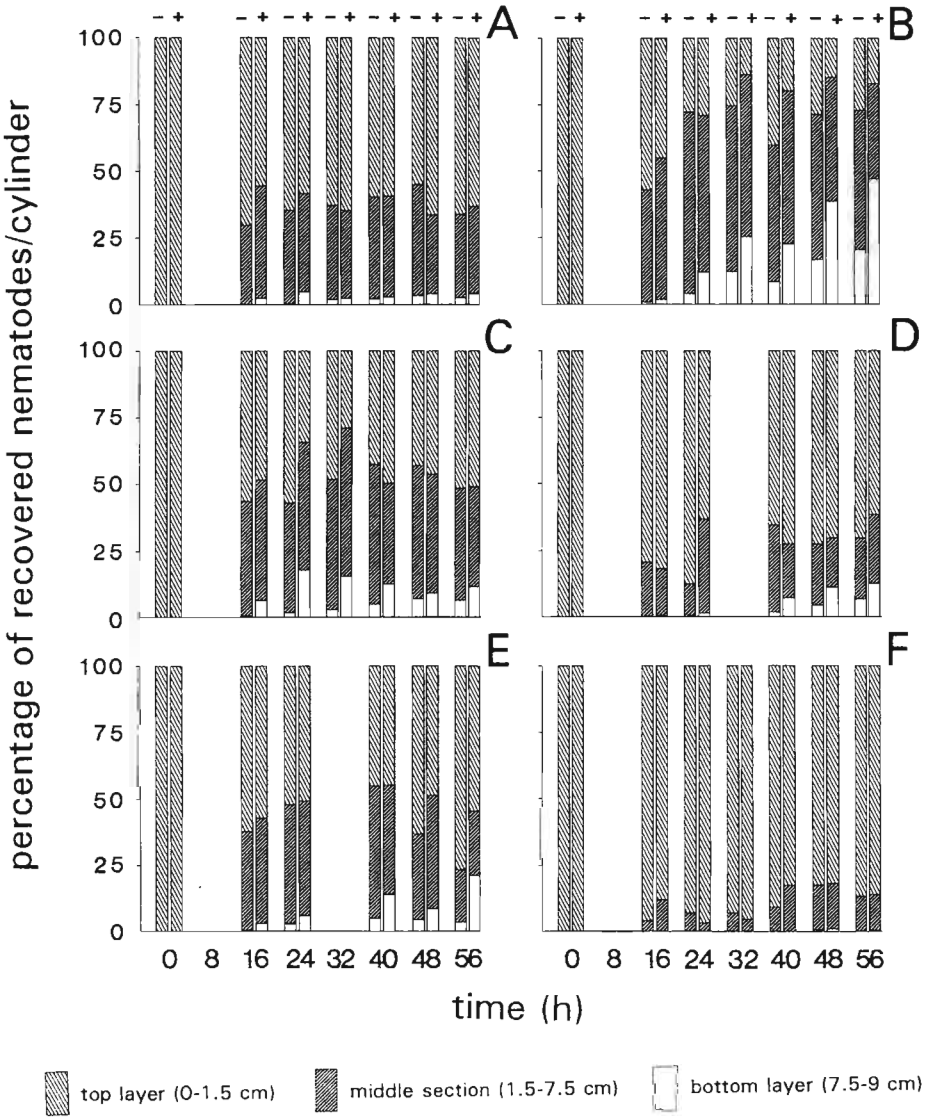

Fig. 2. *Percentage of recovered nematodes over time in three sections of 9 cm high sand columns, in absence (–) and presence (+) of a last instar of* Galleria mellonella *at the bottom of the cylinders, after application of approximately 2000 living heterorhabditid or steinernematid nematodes on top of the cylinders. A: HNb87; B: HUK211; C: HB1'87; D: HSH; E: HKem; F:* Steinernema feltiae *OBSIII.*

lates that are mobile or relatively immobile at 9°C solely on the basis of the present data, because the batches used may have differed in quality. However, those isolates qualified as relatively mobile at 9°C are worth further testing. Whether migration in sand columns reflects the ability of the nematodes to move in natural soils, and whether this is relevant to efficacy in the field remains to be tested.

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Table 1. *Migration, (mean distance covered [cm] + SE) at 9°C in 48 h of a number of* Heterorhabditis *and* Steinernema *species and isolates in a number (n) of 9 cm sand columns in the presence or absence of a* Galleria mellonella *larva. (Migration data per isolate followed by the same letter are not significantly different from each other; Tukey's test, P ≥ 0.05).*

	n	*G. mellonella* larva	
		–	+
H. zealandica NZH3	3	1.1 ± 0.0 *a*	1.0 ± 0.1 a^{q}
H. bacteriophora B1	3	0.9 ± 0.0 *a*	0.9 ± 0.1 a^{q}
HP88	3	1.0 ± 0.1 *a*	1.3 ± 0.1 $a^{q,t}$
HI82	3	0.8 ± 0.0 *a*	0.9 ± 0.0 a^{q}
H. megidis HO1	4	3.3 ± 0.3 *a*	4.6 ± 0.4 a^{t}
Heterorhabditis sp.			
(Irish group)			
K122	4	3.3 ± 0.3 *a*	5.8 ± 0.3 b^{t}
M145	4	1.6 ± 0.1 *a*	2.2 ± 0.3 a^{t}
M198	3	1.2 ± 0.1 *a*	1.4 ± 0.1 a^{q}
Heterorhabditis sp			
(NW Europ. group)			
HW79	3	1.4 ± 0.1 *a*	2.7 ± 0.2 b^{q}
HL81	3	2.7 ± 0.0 *a*	4.6 ± 0.2 $b^{q,t}$
HF85	4	1.7 ± 0.2 *a*	3.3 ± 0.3 b^{t}
HNb87	4	2.6 ± 0.1 *a*	2.2 ± 0.2 a^{t}
HB1'87	4	3.1 ± 0.1 *a*	3.1 ± 0.2 a^{t}
HUK211	4	4.1 ± 0.2 *a*	5.4 ± 0.2 b^{t}
HSH	3	2.3 ± 0.2 *a*	3.0 ± 0.4 $a^{q,t}$
HKem	3	2.0 ± 0.4 *a*	2.3 ± 0.2 $a^{q,t}$
Steinernema spp.			
S. feltiae OBSIII	3	1.4 ± 0.1 *a*	1.5 ± 0.0 $a^{q,t}$
S. feltiae Mr	3	1.7 ± 0.4 *a*	1.6 ± 0.2 a^{q}
S. carpocapsae UK	3	1.1 ± 0.3 *a*	0.9 ± 0.1 a^{q}

^{q} Batch subjected to evaluation of quality (see text)

^{t} Data obtained from time series, 48 h

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