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Comparative vertical migration of twenty one isolates of the insect parasitic nematode *Heterorhabditis* spp. in sand at 20 °C

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Summary – Twenty-one heterorhabditid isolates, most likely belonging to six taxonomic groups and originating from ten countries, were tested for downward migration in 9 cm vertical sand columns at 20 °C with or without a larva of the greater wax moth, *Galleria mellonella,* at the bottom. The fractions of nematodes in the top layer (1.5 cm), middle section (6 cm) and bottom layer (1.5 cm) of the columns were determined at intervals over 8 h and occasionally at 48 h. All isolates except *Heterorhabditis zealandica* NZH3 migrated better than isolates of *H. bacteriophora.* Differences in migration were congruent with putative taxonomic groups within the genus. In most cases, the presence of a *G. mellonella* larva increased the downward movement. Nematodes of the North West European isolates were the most mobile. However, migration also varied between batches of the same isolate. In the presence of *G. mellonella,* 95 to 99 % of the nematodes of a number of Dutch isolates left the top layer of the sand column and were recovered from the bottom layer 6 h later.

Résumé – ***Migration verticale comparée de vingt et un isolats du nématode entomoparasite* Heterorhabditis, *dans le sable, à 20 °C*** – Vingt et un isolats d'Hétérorhabditides, appartenant principalement à six groupes taxonomiques et provenant de dix pays différents, ont été testés pour leur migration vers le bas, à 20 °C, dans des colonnes remplies de sable et pourvues ou non d'une larve de *Galleria mellonella* déposée au fond. Les nématodes présents dans la couche supérieure (1,5 cm), la partie médiane (6 cm) et la partie inférieure (1,5 cm) des colonnes sont dénombrés à des intervalles de 8 h et, occasionnellement, de 48 h. Tous les isolats, à l'exception d'*Heterorhabditis zealandica* NZH3, migrent plus aisément que les isolats de *H. bacteriophora.* Les différences observées dans la migration sont en accord avec les groupes supposés taxinomiques définis à l'intérieur du genre. Dans la plupart des cas, la présence d'une larve de *G. mellonella* augmente la migration vers le bas. Les nématodes appartenant aux isolats du Nord-Ouest de l'Europe sont les plus mobiles, encore que la migration puisse varier entre les différents lots d'un même isolat. En présence de *G. mellonella,* 95 à 99 % des nématodes de la plupart des isolats néerlandais quittent la couche superficielle de la colonne de sable et sont récupérés au fond 6 h plus tard.

Key-words : *Heterorhabditis,* insect parasitic nematodes, migration, activity, migration rate, *Galleria mellonella.*

Insect parasitic nematodes of the genus *Heterorhabditis* Poinar are used commercially in the Netherlands and other North West European countries for control of the black vine weevil, *Otiorhynchus sulcatus* F., in indoor ornamentals. The larvae of this weevil cause serious damage to the root system and base of the plants. The insect parasitic nematodes, which naturally inhabit the soil, favour a dark and moist environment and are eminently suited for control of this type of soil-dwelling pest.

The normal practice in horticulture is to apply nematodes to the soil surface as a curative biological insecticide. Quick movement of the nematodes into the soil is necessary to escape solar radiation and desiccation (Gaugler, 1988). Since the nematodes search actively for their hosts, their use for controlling soil-borne insects has an advantage over chemical pesticides whose contact with the insect depends on diffusion, water percolation or insect movement. However, application of insect parasitic nematodes must result in high levels of parasitism even when the host insects are scarce in order to be economically attractive. This makes exacting demands on the ability of the nematodes to search for and reach the host. A high host searching ability is generally regarded as the prime requisite of an effective natural enemy (Doutt & DeBach, 1964). Westerman and Stapel (1992) found that migration of the Dutch heterorhabditid HFr86 was related to the degree of efficacy against the black vine weevil. No data are available yet to allow extrapolation of this relationship to other heterorhabditid species and isolates.

In the present study, downward movement by twenty-one *Heterorhabditis* isolates originating from ten countries, including a large number of isolates from NW Europe (the Netherlands, U.K., Germany and Ireland), were measured in sand columns at 20 °C in the presence and absence of a larva of the greater wax moth, *Galleria mellonella* (L.).

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Materials and methods

Nematode cultures

The isolates of *Heterorhabditis* tested (Table 1) were grouped according to their currently proposed taxonomic status (Smits *et al.*, 1991; Dix *et al.*, 1992; Joyce *et al.*, 1994). All nematodes were propagated on *G. mellonella* at 20 °C and harvested in modified White traps (Poinar, 1975). Nematode suspensions were cleaned by decanting and adding fresh tap water, after which the nematodes were allowed to migrate overnight through a cotton-wool filter into a water film below. The infective juveniles were stored in 500 ml water in 1 l bottles in a dark cold room at 4-5 °C under constant aeration with sterile-filtered air. Eight isolates (B1, NC1, V16, HDa, HP88, HI82, NZH3 and M198), however, were stored at 9-12 °C, because 4-5 °C proved lethal to them (Westerman, unpubl.). Three days before the beginning of the experiments the nematodes were transferred to 20 °C to let them adjust to the test temperature.

Migration

Nematode migration was assessed in 9 cm high PVC cylinders (diam. 4.5 cm), made of six, 1.5 cm rings, connected with adhesive tape. The bottom of the cylinder consisted of a Petri dish (5.5 cm diam.) fixed to the bottom ring with synthetic modelling clay. The cylinders were filled with fine sterile sand (particle size; 72 % 180-300 μm, 21 % 300-425 μm), moistened with demineralized water (8 % w/w), and kept at 20 °C. Immediately after preparation, cylinders with and without a last instar larva of *G. mellonella* at the bottom, were inoculated on top with approximately 2000 living nematodes in 0.5 ml water and covered with a Petri dish lid. After 2-8 h or 48 h at 20 °C the rings were separated and the sand of each ring was rinsed in 50 ml water. The number of nematodes in ring 1, rings 2-5 and ring 6 were determined by counting the nematodes in four samples of 3 ml of the rinse water. Fifty to 60 % of the applied nematodes were recovered, except after a period of 48 h when only 20 to 40 % were recovered. The percentage of nematodes in each layer was calculated, based on the number of recovered nematodes per cylinder. Nematodes found in a ring were considered to have moved halfway through the ring. Accordingly, migration was defined here as the average distance migrated :

$$migration = \frac{0.75R_1 + 4.5R_{2-5} + 8.25R_6}{R_1 + R_{2-5} + R_6}\ cm,$$

where R_1, R_{2-5} and R_6 are the numbers of nematodes recovered at 4 h in ring 1, rings 2-5, and ring 6, respectively. Migration and the average migration rate at 4 h *[migration/4 (cm/h)]* were used to compare migration and rates of migration among isolates and different batches of isolates tested.

Table 1. *Origin of the nematodes isolates.*

Isolates	Code	Location	Sources *
H. zealandica	NZH3	Auckland, New Zealand	Akhurst
H. bacteriophora	B1	Brecon, South Australia	Kaya/Smits
NC1	NC1	Clayton, NC, USA	Burnell/
V16	V16	Geelong, Victoria, Australia	Brooks
HDa	HDa	Darmstadt, Germany	Ehlers/Bathon
HP88	HP88	Logan, Utah, USA	Glazer
HI82	HI82	Italy	Deseö
H. megidis	HO1	Ohio, USA	Akhurst/ Griffin
Heterorhabditis sp. (Irish group)			
IRL-H-K122	K122	Wexford, Ireland	Griffin
IRL-H-M145	M145	Wexford, Ireland	Griffin
IRL-H-M198	M198	Cork, Ireland	Griffin
Heterorhabditis sp. (NW European group)			
NL-H-W79	HW79	Wageningen, The Netherlands	Vlug
NL-H-L81	HL81	Limburg, The Netherlands	Galle
NL-H-F85	HF85	Flevoland, The Netherlands	
NL-H-Fr86	HFr86	Friesland, The Netherlands	
NL-H-Nb87	HNb87	N-Brabant, The Netherlands	
NL-H-B87.1	HB1'87	Bergeyk, The Netherlands	Smits
NL-H-E87.3	HE87	Eindhoven, The Netherlands	Smits
GB-H-UK211	HUK211	United Kingdom	Rodgers
D-H-HSH	HSH	Schleswig Holstein, Germany	Ehlers
USSR-H-Kem	HKem	Kemerovo, Siberia, Russia	

* R. Akhurst, CSIRO, Canberra, Australia; H. K. Kaya, Univ. California, Davis, USA; P. H. Smits and H. Vlug, Research Institute for Plant Protection Wageningen, The Netherlands; A. M. Burnell and C. T. Griffin, St. Patrick's College, Maynooth, Ireland; W. M. Brooks, NC State Univ., Raleigh, NC, USA; R.-U. Ehlers, C. A. Univ., Kiel, Germany; H. Bathon, Inst. biologische Schädlingsbekämpfung, Darmstadt, Germany; I. Glazer, The Volcani Center, Bet Dagan, Israel; K. V. Deseö, Univ. Bologna, Italy; F. Galle, De Groene Vlieg Company, Nieuwe Tonge, The Netherlands; P. B. Rodgers, Agricultural Genetics Company Ltd, Cambridge, UK.

Five isolates (*H. zealandica* NZH3, *H. bacteriophora* B1, NC1, V16, and HDa) were tested at both a 4 h and 48 h incubation period and were inoculated on the same date with the same batch of nematodes. The batch of HDa was also used in the time series (see below). Five more isolates (HI82, HW79, HSH, HKem and M198) were tested at a 4 h incubation period only; M198 was tested twice using different batches. All other isolates, including HDa and batches of NZH3, and B1, were tested in a time series of 2, 4, 6 and 8 h in two to four replicates, with the exception of HL81 and HFr86 (which were tested hourly from 2 to 7 h), and HF85 and

B1 (which were not tested at a 2 h period). Of these isolates HF85 and B1, HL81 and HFr86, HI82 and HW79, and HDa and HE87 were tested in pairs on the same date. Additional data on migration at 4 h of HF85, HUK211, and K122 were obtained from experiments conducted for other purposes (partly obtained from K. Jung).

Migration data from the time series were analysed separately for each batch on effects of incubation time and of the absence and presence of the host using linear regression analysis. Standard errors of the mean (sem) were calculated about migration at 4 h to permit comparison among isolates. χ^2 test was used to check for uniform distributions of nematodes in cylinders without *G. mellonella.*

After completion of the experiments the *G. mellonella* larvae from the cylinders were usually kept at 20 °C for another 3-4 days and checked for parasitism. Parasitized larvae were recognized by the characteristic colour change induced by the symbiotic bacteria. Occasionally dead larvae were opened after 6-7 days under a dissection microscope to count the number of hermaphrodite nematodes in the cadaver (done for NZH3, HDa, HP88, K122, M145, HO1, HUK211 and HE87 in the time series, and NZH3, B1, NC1, V16 and HDa at both 4 h and 48 h). The numbers of nematodes recovered from the insects were usually small (≤ 35/insect at 8 h) compared to the total numbers recovered (1000-1200) and were not included in calculations of the distance migrated, except for NZH3, B1, NC1, V16 and HDa at the 48 h incubation period and for HE87 in the time series.

Quality

The experiments were performed in the course of several years (1990-1994), in which the influence of culturing, time and storage conditions on motility became gradually clear (Westerman, 1992; Westerman & Stapel, 1992). Therefore, from 1991 onwards, the visual appearance of the nematode batches was evaluated using two samples of approximately 300 nematodes as described by Westerman and Stapel (1992) and, except where noted, only batches with a good visual appearance were used in experiments. Fourteen batches and six of the batches that were used in the additional migration experiments were subjected to this test (Tables 2, 3). Criteria used were mortality less than 5 %, less than 5 % exsheathed nematodes and less than 10 % of the nematodes without ample food reserves. Occasionally these criteria were not met, particularly in the case where the isolates were stored at 9 °C, viz. NZH3 (72 % with ample food reserves and 32 % exsheathed in the second batch), B1 (17 % mortality, 76 % with ample food reserves, 16 % exsheathed in the second batch), NC1 (12 % exsheathed), V16 (81 % with ample food reserves, 19 % exsheathed), M198 (13 % mortality, 86 % with ample food reserves in the first batch; 27 % mortality, 71 % with ample food reserves in the second batch), and both batches of HUK211 in the additional experiments (17 % mortality in the first batch and 27 % with ample food reserves and 12 % exsheathed in the second batch). Percentage mortality was the only characteristic assessed for the first batch of *H. zealandica* NZH3 (53 %). The time of storage from the onset of emergence from the host to the start of the experiments ranged from 10 to 62 days (average 28 days).

Results

H. zealandica NZH3

In the time series 15 % of the recovered NZH3 had migrated from the first layer in the absence of an insect at 8 h; 23 % in the presence of *G. mellonella* (Fig. 1A). Downward movement was very slow (0.22-0.25 cm/h at 4 h) and the first nematodes reached the bottom layer not earlier than 8 h (≤ 1 % of the recovered nematodes). Both test insects at 8 h contained two hermaphrodites. Migration was not significantly increased by the presence of *G. mellonella* (e.g. Table 2). The other batch of NZH3 yielded similar results at both 4 h (Table 2) and 48 h (1.0 ± 0.0 cm ($x \pm$ sem) without insect and 1.1 ± 0.1 cm in the presence of *G. mellonella*). None of the test insects was parasitized.

H. bacteriophora B1, NC1, V16 and HDa

In the time series only 5 % of the recovered B1 and 17 % of HDa had dispersed from the first layer in the absence of an insect at 8 h; in the presence of *G. mellonella* these percentages were 14 % and 23 %, respectively (Fig. 1B, C). Downward movement was slow (0.20-0.25 cm/h at 4 h) and the first nematodes reached the bottom layer at 4 h (B1) and 2 h (HDa). Migration of HDa seemed to increase at 6 h but this was not followed up at 8 h. At 8 h 1 % and 7 % of the recovered B1 and HDa, respectively, were found in the bottom ring. Test insects exposed to HDa at 6 and 8 h contained negligible numbers of hermaphrodites (≤ 7/insect). Migration was not significantly increased by the presence of *G. mellonella* for either isolate ($P \geq 0.2$).

The nematodes of the other batch of B1, tested at 4 h, migrated better than those used in the time series; 2 % and 10 % of the recovered nematodes were found in the bottom layer at 4 h in cylinders without and with *G. mellonella,* respectively, resulting in higher distances migrated (Table 2). The mean distance migrated by NC1, V16 and HDa at 4 h did not differ from that of either batch of B1 at 4 h (Table 2). Two and 5 % of the recovered nematodes were found in the bottom layer for NC1 and V16, respectively. Test insects either survived exposure to the nematodes in sand columns (HDa and V16) or they contained ≤ 1 hermaphrodite per insect (B1 and NC1).

Data of the four isolates tested at 48 h were less reliable than those at 4 h since percentage recovery from

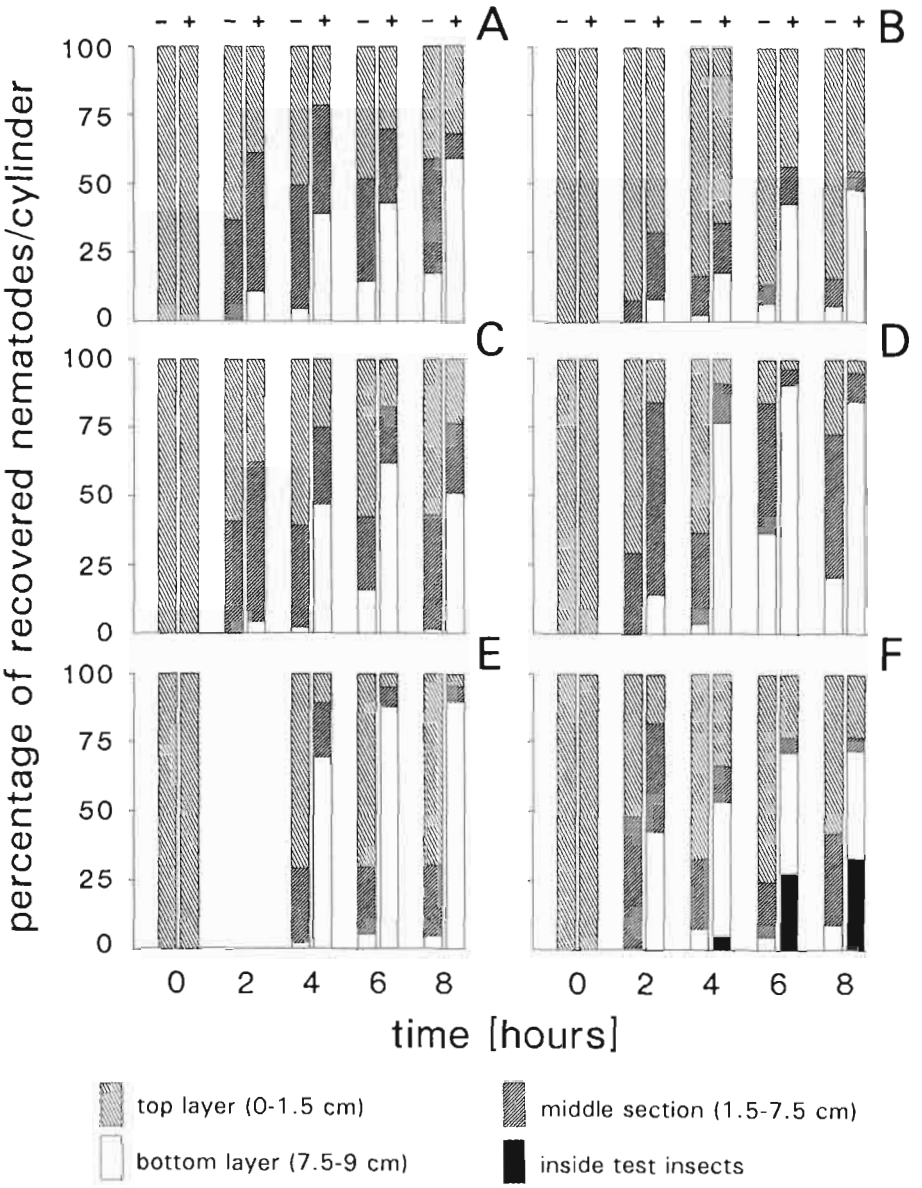

Fig. 1. *Percentage of recovered nematodes in the course of time in three sections of 9 cm high sand columns, in the absence (–) and presence (+) of a last instar of* Galleria mellonella *at the bottom of the cylinders, after application of approximately 2000 living heterorhabditid nematodes on top of the cylinders. A :* Heterorhabditis zealandica *NZH3; B :* H. bacteriophora *B1; C :* H. bacteriophora *HDa; D : HP88; E : K122; F : M145.*

the sand was low (20-40 %). In the absence of *G. mellonella* 10, 21, 2 and 16 % of the nematodes were found in the bottom layer for B1, NC1, V16 and HDa, respectively (mean distances migrated (x ± sem); 2.0 ± 0.4 cm, 2.7 ± 0.2 cm, 1.2 ± 0.2 cm, and 2.5 ± 0.6 cm, respectively). Relatively high numbers of hermaphrodites were recovered from the insect cadavers (x ± sem); 45 ± 6, 281 ± 49, 144 ± 25, and 184 ± 58 for B1, NC1, V16 and HDa, respectively, thus boosting the values for migration in these cylinders (2.8 ± 0.2 cm, 4.0 ± 0.4 cm, 3.4 ± 0.3 cm and 2.9 ± 0.5 cm, respectively).

HP88 AND HI82

In the time series 53 % and 68 % of HP88 had left the top layer of sand at 8 h in the absence and presence of *G. mellonella,* respectively (Fig. 1D). At the same time 4 % and 33 %, respectively, were found in the bottom layer. The nematodes moved slowly (0.3-0.5 cm/h at 4 h). Ten hermaphrodites were recovered from the one insect killed at 6 h and one and two hermaphrodites from the

Table 2. *Migration (x; average distance covered [cm]), with standard error of the mean (sem), at 20 °C in 4 h of a number of (batches of) isolates of* Heterorhabditis *in a number of 9 cm sand columns (n) without (–) and with (+) a* G. mellonella *larva at the bottom.* (For each isolate migration followed by different letters are significantly different from each other, $P \leq 0.05$).

	n	x ± sem –	x ± sem +
H. zealandica NZH3	2	0.9 ± 0.1 *a*	1.0 ± 0.0 a^t
	3	1.1 ± 0.2 *a*	0.9 ± 0.1 $a^{q,d}$
H. bacteriophora			
B1	4	1.0 ± 0.1 *a*	0.8 ± 0.0 a^t
	3	1.6 ± 0.2 *a*	2.5 ± 0.1 $a^{q,d}$
NC1	3	1.2 ± 0.1 *a*	1.9 ± 0.1 $a^{q,d}$
V16	3	1.0 ± 0.0 *a*	1.7 ± 0.2 $a^{q,d}$
HDa	3	1.2 ± 0.1 *a*	2.1 ± 0.1 $a^{q,t}$
	3	1.4 ± 0.2 *a*	2.1 ± 0.2 $a^{q,d}$
HP88	2	1.1 ± 0.2 *a*	2.1 ± 0.8 a^t
HI82	3	2.0 ± 0.3 *a*	3.8 ± 0.7 b^q
H. megidis HO1	4	2.8 ± 0.2 *a*	5.2 ± 0.5 b^t
Heterorhabditis sp. (Irish group)			
K122	2	2.5 ± 0.1 *a*	2.6 ± 0.4 a^t
M145	2	1.3 ± 0.0 *a*	1.4 ± 0.2 a^t
M198	3	1.7 ± 0.2 *a*	4.2 ± 0.3 b^q
	3	0.9 ± 0.0 *a*	1.3 ± 0.1 a^q
Heterorhabditis sp. (NW European group)			
HW79	3	1.9 ± 0.1 *a*	7.5 ± 0.3 b^q
HL81	4	3.0 ± 0.2 *a*	7.7 ± 0.1 b^t
HF85	4	1.9 ± 0.3 *a*	6.7 ± 1.0 b^t
HFr86	3	2.7 ± 0.1 *a*	6.7 ± 0.7 b^t
HNb87	3	2.3 ± 0.2 *a*	7.0 ± 0.4 $b^{q,t}$
HB1'87	3	2.3 ± 0.2 *a*	5.3 ± 0.6 $b^{q,t}$
HE87	3	2.3 ± 0.1 *a*	5.2 ± 0.5 $b^{q,t}$
HUK211	2	1.5 ± 0.2 *a*	2.8 ± 0.3 a^t
HSH	3	3.1 ± 0.3 *a*	7.0 ± 0.1 b^q
HKem	3	3.3 ± 0.1 *a*	6.3 ± 0.6 b^q

[q] Batch subjected to evaluation of quality (see text), [t] data from time series, 4 h, [d] batch also tested at 48 h (see text).

two insects killed at 8 h. The mean distance covered by HP88 significantly increased in the presence of *G. mellonella* ($P \leq 0.02$) and in time ($P \leq 0.05$). Fewer nematodes of HI82 had left the top layer at 4 h (22-46 %), but they moved at a higher rate (0.5-0.9 cm/h at 4 h), resulting in higher migration compared to HP88 (Table 2).

The Irish isolates M145, K122 and M198

In the time series only 34-35 % of K122 and 20-21 % of M145 had left the top layer at 8 h (Fig. 1E, F), and nematodes moved slowly (0.6 cm/h and 0.3 cm/h at 4 h for K122 and M145, respectively). The percentage of recovered nematodes that remained in the top layer stabilized at 60 % for K122 and 80 % for M145. At 8 h, 30 % of K122 reached the bottom layer, compared with 8-11 % for M145. Test insects exposed to K122 were killed from 4 h onwards and contained 1.5, 10 and 11 hermaphrodites per insect at 4, 6 and 8 h, respectively. Those exposed to M145 contained 7 ± 3 (x ± sem) hermaphrodites per insect at 6 and 8 h. In the additional experiments, more nematodes of K122 had left the top layer at 4 h (52-86 %), resulting in higher distances covered (Table 3). Migration of K122 was enhanced by the presence of *G. mellonella,* but only in the additional experiments (Table 3). One batch of M198 dispersed better than the other two Irish isolates, but the other batch did not (Table 2).

H. megidis HO1

At 8 h 59 and 68 % of *H. megidis* HO1 had left the top layer in the absence and presence of *G. mellonella,* respectively (Fig. 2A). In the presence of *G. mellonella* 59 % of the recovered nematodes were found in the bottom layer. These nematodes moved relatively fast (1.3 cm/h). From 4 h onwards all test insects were killed and contained equal numbers of hermaphrodites per insect (35 ± 7 (x ± sem)). In the cylinders without *G. mellonella,* a more or less stable situation was reached at 4 h with 45 % in the top layer, 40 % in the middle part and 15 % in the bottom layer. HO1 responded significantly to the presence of *G. mellonella* and to time ($P \leq 0.002$).

The NW European isolates

Migration of the six Dutch isolates and the British HUK211 in the time series resembled that of *H. megidis* HO1, but the fractions of nematodes leaving the first layer of sand at 8 h were much greater, except for HUK211 (Figs. 2, 3). Sixteen and 55 % of HUK211 had left the top layer at 8 h in the absence and presence of *G. mellonella,* respectively (Fig. 2B). In the presence of *G. mellonella* virtually all nematodes (95-99 %) of HL81, HF85, HFr86, and HNb87 left the first layer within 4 h and arrived in the bottom layer at 6 h. These nematodes moved fast (1.7-1.9 cm/h at 4 h). Since nematodes were already found in the bottom layer at 2 h, some individuals " raced " through the cylinder at an average rate of 4.5 cm/h. Test insects exposed to HE87 in the time series contained 3 ± 3, 58 ± 19, 256 ± 21 and 337 ± 120 (x ± sem) hermaphrodites per insect at 2, 4, 6, and 8 h, respectively. Unfortunately only test insects exposed to HE87 were dissected. The fraction of recovered nematodes leaving the top layer in cylinders without *G. mellonella* varied from 31 % for HF85 to 77 % for HL81 and HFr86. Additional results on other batches of HF85 at 4 h demonstrated a considerable variation in migration (Table 3). Migration of the German HSH, the Russian HKem and the Dutch HW79 at 4 h was comparable to that of the other NW European isolates

Table 3. *Migration (x; average distance covered [cm]), with standard error of the mean (sem), at 20 °C in 4 h of a number of batches of three isolates of* Heterorhabditis *in a number of 9 cm sand columns (n) without (–) and with (+) a* G. mellonella *larva at the bottom.* (For each isolate migration data followed by different letters are significantly different from each other, $P \leq 0.05$).

	n	x ± sem –	x ± sem +
K122	3	3.4 ± 0.2 *a*	5.1 ± 0.2 *b**q
	3	2.5 ± 0.2 *a*	5.7 ± 0.1 *b**q
	3	3.1 ± 0.1 *a*	5.9 ± 0.2 *b**q
HF85	3	3.7 ± 0.1 *a*	8.0 ± 0.1 *b**q
	2	2.4 ± 0.2 *a*	6.4 ± 0.5 *b*
	2	3.3 ± 1.3 *a*	7.4 ± 0.1 *b*
	2	2.6 ± 0.5 *a*	7.5 ± 0.4 *b*
	2	3.7 ± 1.0 *a*	7.1 ± 0.1 *b*
	4	3.1 ± 0.2 *a*	6.6 ± 0.4 *b*
HUK211	3	2.1 ± 0.1 *a*	3.4 ± 0.2 *b*q
	3	2.8 ± 0.2 *a*	3.6 ± 0.1 *b*q

* Data obtained from K. Jung, Friesland College of Agriculture, Leeuwarden, The Netherlands.
q Subjected to evaluation of quality (see text).

(Table 2). In the time series all Dutch isolates and HUK211 responded significantly to the presence of the host insect ($P \leq 0.001$). In fact, all except HF85, responded more than proportionally to *G. mellonella* in the course of time(host x time interaction, $P \leq 0.05$).

Variation in migration between experiments was assessed for isolates that were tested frequently. Variance of mean migration between experiments was generally larger than within experiments; e.g. 2.12 *versus* 0.90 for HF85 (n = 5), 3.44 *versus* 0.12 for K122 (n = 4), and 1.34 *versus* 0.09 for HUK211 (n = 3).

The isolates could be roughly ranked in order of descending migration at 4 h; NW European group (except HUK211) ≥ *H. megidis* > Irish group ≥ HP88, HI82, *H. bacteriophora* > *H. zealandia.*

Uniform dispersal would result in a 1 : 4 : 1 ratio in the number of nematodes in the three layers of a cylinder. Although this distribution was never found (χ^2-test, $P \leq 0.05$), the distributions of HL81 (1 : 2.3 : 1.2), HFr86 (1.1 : 2.3 : 1) and HNb87 (1.3 : 2.5 : 1) in the absence of *G. mellonella* came very near. However, relatively large proportions of the nematodes were consistently recovered from both ends of the cylinder for these most rapidly migrating isolates.

Discussion

In NW Europe *O. sulcatus* is an increasingly serious pest of nursery stock, ornamentals and soft fruit such as strawberries. Hanula (1993) demonstrated that a biological control agent must penetrate to depths of 15 cm to be 95 % effective against larvae of *O. sulcatus* on field grown yews. Although the actual vertical distribution of larvae may vary with plant species, the above data illustrate the need for actively migrating nematodes if insect parasitic nematodes were to be applied for effective black vine weevil control.

H. bacteriophora (syn. *H. heliothidis,* see Poinar, 1990) and HP88, which are so far the only heterorhabditids included in studies on migration, generally outperformed steinernematids (e.g. Georgis & Poinar, 1983 *a, b, c;* Alatorre-Rosas & Kaya, 1990). However, the present study showed that most heterorhabditids tested in this study, except *H. zealandica* NZH3, outperformed *H. bacteriophora* in the ability to migrate through a sand column at 20 °C, both in the rate of migration and in the proportion of actively migrating nematodes. In fact, the four *H. bacteriophora* isolates used here, including the type strains B1 and NC1, migrated better than previously reported for *H. bacteriophora* or *H. heliothidis* in sandy loam (20-25 °C, five days; Georgis & Poinar, 1983 *c*). Soil type, isolate or quality of the batch used may all partially explain the observed differences.

Up to 99 % of some of the heterorhabditids migrated when placed on a sand surface, contradicting Kaya's (1990) generalization that only a small proportion of steinernematid or heterorhabditid populations disperse when placed on the soil surface. Inactivity of a certain proportion of a population of nematodes is well documented among insect parasitic nematodes (Ishibashi & Kondo, 1990), and is assumed to be a survival strategy. When inactive, they can be stimulated to become active by various mechanical and chemical stimuli, such as CO_2 and other attractants from insects (Ishibashi & Kondo, 1990). In the present study activation by substances released by *G. mellonella* may have contributed to the increase in the percentage actively migrating nematodes that most isolates exhibited when *G. mellonella* was present (chemo-orthokinesis).

Most heterorhabditid isolates tested here migrated at a higher rate than *H. bacteriophora* or *H. zealandica :* an 8 h period was generally sufficient to follow a large proportion of the population down to the bottom layer of the 9 cm sand column. The nematodes of most NW European isolates migrated at an incredible rate. The rapid downward migration could not have resulted from passive movement caused by water drainage or gravity, because low water content (8 %) in the sand did not allow water to drain. In fact, upward migration to a similar degree as downward migration has been obtained in identical cylinders when placed upside down (Westerman & Godthelp, 1991). Size of the nematodes in relation to particle size and the available pore space may also affect migration (Wallace, 1958). Indeed, isolates with long infective juveniles (NW European group, Irish group, *H. megidis*) generally migrated more rapidly than isolates with short infectives *(H. bacteriophora, H.*

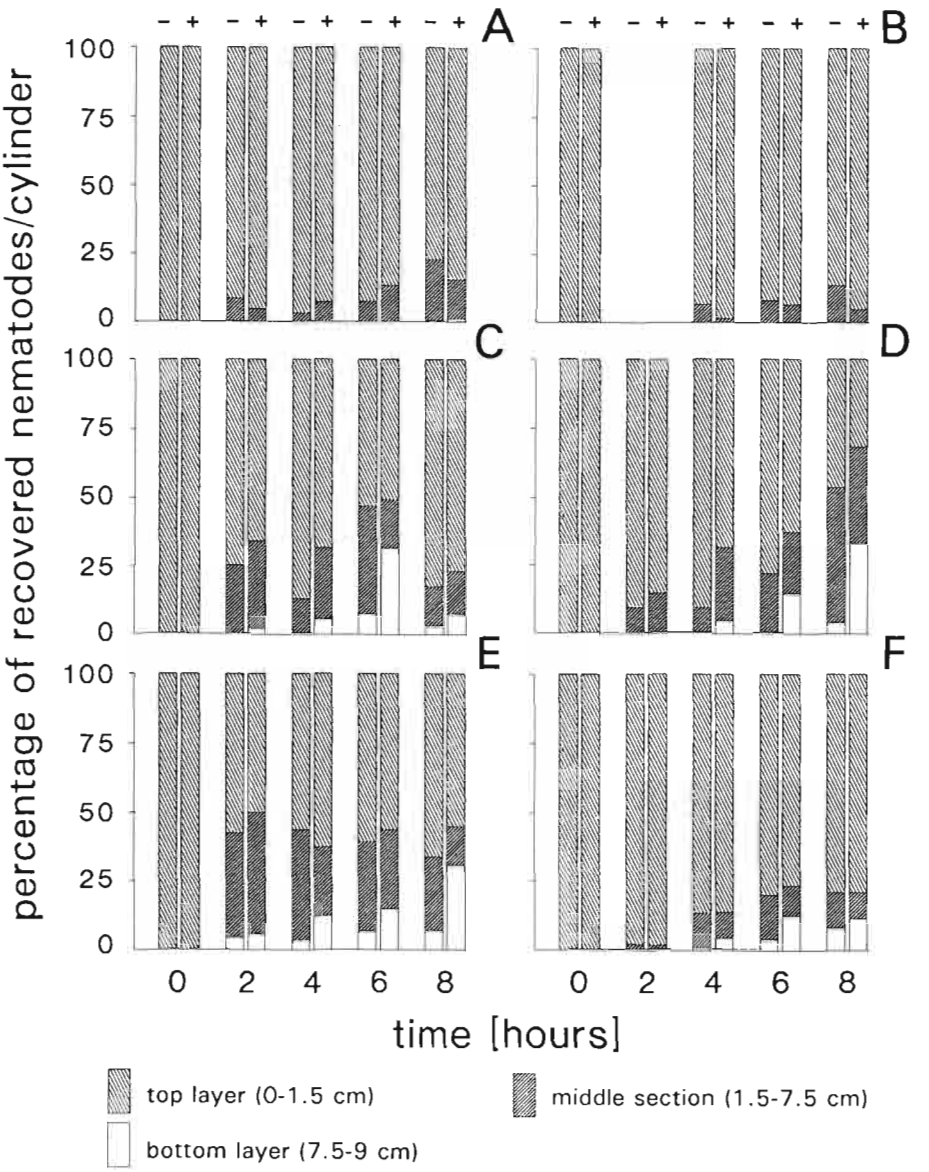

Fig. 2. *Percentage of recovered nematodes in the course of time in three sections of 9 cm high sand columns or inside* Galleria mellonella, *in the absence* (–) *and presence* (+) *of a last instar of* G. mellonella *at the bottom of the cylinders, after application of approximately 2000 living heterorhabditid nematodes on top of the cylinders. A :* Heterorhabditis megidis *HO1; B : HUK211; C : HB1'87; D : HNb87; E : HF85; F : HE87.*

zealandica). However, of the related isolates HP88 and HI82, which are both approximately the same size, the former had the highest percentage nematodes leaving the top layer, but the nematodes of the latter isolate migrated more rapidly.

The rate of migration was doubled in the presence of *G. mellonella* for most isolates, which may have resulted from mechanisms such as chemotaxis and chemo-klinokinesis. Lei *et al.* (1992) observed that the individuals of *H. zealandica* T327 moved randomly on agar dishes, but their movements became directed in the presence of puparia or larvae of the cabbage maggot, *Delia radicum.* Similarly, Gaugler *et al.* (1980) observed that on agar most individuals of *S. carpocapsae* oriented to a CO_2 gradient. It is likely that the nematodes in this study oriented to a concentration gradient of volatile chemicals since the duration of the experiment was short for the formation of a gradient of water-soluble host cues, ex-

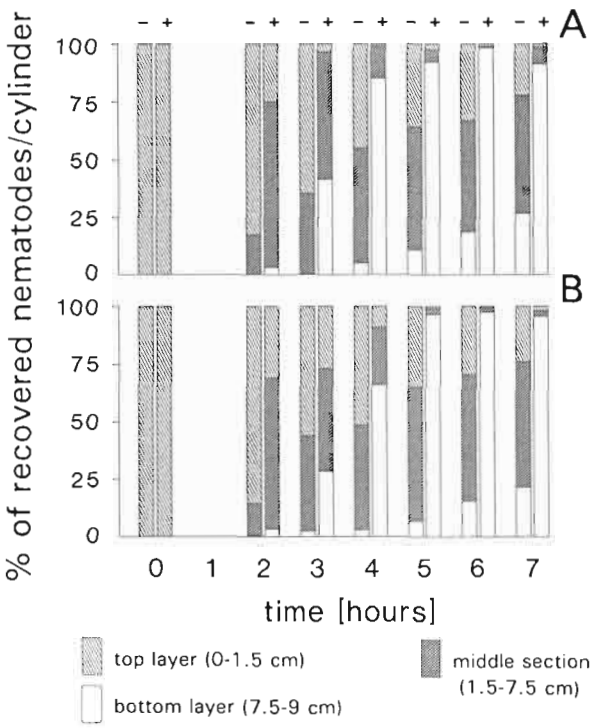

Fig. 3. *Percentage of recovered nematodes in the course of time in three sections of 9 cm high sand columns, in the absence (–) and presence (+) of a last instar of* Galleria mellonella *at the bottom of the cylinders, after application of approximately 2000 living heterorhabditid nematodes on top of the cylinders. A : HL81; B : HFr86.*

cept in case of the 48 h experiments. The presence of *G. mellonella* corroborated the differences in migration between isolates without essentially altering the order. Lewis *et al.* (1992, 1993), who investigated the behaviour of steinernematids on an agar surface with respect to host searching strategies, predicted and found that species that moved in search of hosts, such as *S. glaseri*, would be more responsive to volatile host cues than species that adopted a " sit-and-wait " strategy, such as *S. carpocapsae*. *H. bacteriophora* and *H. zealandica*, although active searchers in comparison to most steinernematids, would be classified as " sit-and-wait " strategists in comparison to, for instance, heterorhabditid isolates of the NW European group, which were more active and responded more strongly to volatile cues of *G. mellonella* than *H. bacteriophora* or *H. zealandica*. Lewis *et al.* (1992) further suggested that these actively searching isolates may be better adapted for finding and parasitizing subterranean, sedentary insect larvae such as *O. sulcatus*.

Mean migration at 4 h, which was chosen to compare migration among all batches tested, was not determined at a stable situation but, for most isolates, during the course of rapid movement. In this way laborious dissecting of insect cadavers could be avoided. However, migration defined in this manner is bound to be variable as it is sensitive to factors that influence the rate of migration or the proportion of actively migrating nematodes, such as subtle changes in the particle size composition, pH, moisture content, solute concentrations, condition, size and attractiveness of the test insects, or condition of the nematodes. Nevertheless, migration of HF85 (NW European group) proved unaffected by, for instance, particle size (range 105-425 μm) or pH (range 3-10) of the sand (Westerman, unpubl.). Probably variation in migration between replicates of one isolate can mainly be attributed to differences between batches of the nematodes, due to production and storage conditions (Kaya, 1990), as was demonstrated for heterorhabditids by Westerman and Stapel (1992). Moreover, migration will only give an indication of the ability of a batch to migrate at that time. For example, Westerman and Stapel (1992) demonstrated that migration decreased with storage time of the batch used and Lei *et al.* (1992) observed that newly collected nematodes did not orient to host cues, whereas one-month-old nematodes did.

Differences in mean migration were more or less congruent with putative taxonomic groups within the genus (Tables 2, 3). For example, isolates of the NW European group, with the exception of HUK211, exhibited almost identical courses of migration in time, distinct from those of the other groups. The behaviour of the related *H. megidis* and Irish isolates (Smits *et al.*, 1991; Dix *et al.*, 1992, Joyce *et al.*, in press) resembled most closely that of the NW European group. The migration curves of the isolates of *H. bacteriophora* seem to differ from those of the taxonomically different, but closely related, isolate HP88 and *H. zealandica* (Dix *et al.*, 1992; Joyce *et al.*, 1994).

Since conditions may have been nearly optimal for migration, the results of this study only give an indication of the migration potential of the isolates examined. Migration and host searching in natural soils will depend on many other factors, such as the distribution and attractiveness of the target insect, soil texture, moisture and particle size composition, the presence and density of root systems and soil temperature. For example, larvae of *O. sulcatus* were not attractive to some NW European heterorhabditids (Westerman & Godthelp, 1991). Furthermore migration of NW European heterorhabditids is seriously impaired in humous soils (Westerman, unpubl.). Shape and size of the root system of the plant will not only influence the distribution of target insects but may also influence migration and host finding of nematodes, as was demonstrated by Choo *et al.* (1989) and Choo and Kaya (1991). Although dense roots reduced infectivity of *H. bacteriophora* NC1 in sandy soils, sparse roots increased nematode infectivity in humic soils. Twenty °C, as chosen in this study, might be close to optimal for application to indoor ornamen-

tals. Outdoors, however, soil temperatures would vary over a wide range and would be appreciably cooler during much of the year in NW Europe. Since temperature affects efficacy to a large extent, these experiments should be continued at lower temperatures for comparison with the available efficacy data (e.g. Simons & Van der Schaaf, 1986; Westerman & Van Zeeland, 1989).

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