Comparative Vertical Migration of Three Different Strains of *Heterorhabditis bacteriophora* and a Single Strain of *Heterorhabditis megidis* in Sand at 25°C

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SUMMARY

Four heterorhabditid isolates (three different strains of Heterorhabditis bacteriophora and Heterorhabditis megidis) were tested for downward migration in 9.5 cm vertical sand columns at 25°C with and without a larva of the wax moth, Galleria mellonella, at the bottom. The number of infective juveniles (IJs) in the upper section decreased since the IJs gradually migrated down toward the Galleria larvae into the lower section of the shell vials. Only the isolate OH 25 behaved differently, because the number of IJs decreased this isolate in the lower section. This isolate was the quickest, since after 12 hrs, most IJs could be found in the lower section of the vials from this isolate. The number of IJs was so small in the lower section that 12 hrs after injection, only the isolates MHG 3 and OH 25 could kill the Galleria larvae.

Besides the fact that mortality occurred among the Galleria larvae with the above isolates, neither IJs nor adults could be detected in either living or dead Galleria larvae 12 hrs after IJ injection. It is noteworthy that the behavior of the isolate Megidis was different from the other isolates: the number of IJs was so negligible in the lower section of the vials that even after 36 hrs none of the Galleria died and, consequently, neither adults nor IJs could be detected after dissecting the Galleria larvae. Each isolate could reach the lower section of the vials, yet only the isolate Megidis could not infect and kill the host. After dissecting the larvae, most IJs and adults could be found in the isolate MHG 3 (95 IJs and adults altogether) 36 hrs after injection.

INTRODUCTION

Laboratory studies show that nematodes respond positively to many chemical stimuli produced by insects (Gaugler et al., 1980). Many factors influence nematode movement in soil. Probably the most important factors are soil texture (Georgis and Poinar, 1983), temperature (Steiner, 1996), behavior of the nematode species (Grewal et al., 1994), genetic differences of species and strains (Glazer et al., 1991), presence or absence of a host (Molyneux, 1983) and moisture (Molyneux and Bedding, 1984). Although there are many stimuli that play an important role in the host-finding tactic of the nematodes, it seems that neither of the stimuli is predominant, but rather more stimuli influence and integrate together this process (Gaugler et al., 1980).

The host-finding tactic of entomopathogenic nematodes can be "cruiser" (actively searching foragers), "ambusher" (sit-and-wait foragers) or "intermediate" (Kaya and Gaugler, 1993). Direct response to host chemical cues (Lewis et al., 1992), active dispersal and an inability to nictate are the characteristics of cruisers, such as *Heterorhabditis bacteriophora* (Poinar) and *Heterorhabditis megidis* (Poinar), whereas less dispersing activity and active nictating are characteristic features among ambushers (Campbell and Gaugler, 1993).

This paper reports on laboratory tests of the vertical dispersal of four heterorhabditid isolates. The objective of this experiment was to examine the comparative movement of three different strains of *H. bacteriophora* and *H. megidis* in sand. The information gained from this experiment may enable us to use the above strains and species more effectively in the field as a biological control agent.

MATERIALS AND METHODS

Stock cultures of infective juveniles (IJs) of *H. bacteriophora* (strains: MHG 3, KMD 19, OH 25) and *H. megidis* were maintained by infecting fourth instar larvae of the greater wax moth (*Galleria mellonella*). The IJs were collected daily from cadavers placed on White traps (White, 1924). The emerged IJs were stored in Ehrlenmeyer flasks and maintained at 9°C in tap water, at a concentration of ca. 2000 nematodes/ml. Nematode suspensions were cleaned by decanting and adding fresh tap water. All IJs were used between five and ten days after their emergence from host cadavers.

Sand was used in this experiment. The sand was sterilized in an autoclave at 90°C for 99 min. After cooling, the moisture content was measured and adjusted to seven%.

Shell vials (diameter 2.5 cm, height 9.5 cm) were filled with sand and used as a container. A plastic sieve was used to make an upper (upper seven cm) and a lower section (the bottom 2.5 cm) in the vials. This assay was conducted twice (five replicates each) and the data were merged, making a total of ten replicates at each time period (12, 24 and 36 hrs). A total of ten controls were also conducted at each time period. A total of 120 vials per trial were used with each nematode isolates. The vials were separated into three groups. Each group contained ten replicates and ten control vials. The first group of vials was placed in a 25°C incubator for 12 h, the second group for 24 h and the third group for 36 h.

Immediately after preparation, vials with and without a last instar larva of *G. mellonella* at the bottom, approximately 500 IJs in 0.5 ml water were injected into the upper layer and covered with a plastic lid to retard the sand from drying. The IJs were tested for downward migration: nematode response to the host insect was monitored by recording the number of IJs found in the upper (seven cm) and in the lower section (2.5 cm) of the vials.

After 12, 24 and 36 h, the upper and the lower sand column in the vials were separated and put onto Baerman funnels for 72 h. After 72 h, the samples were collected separately into screw-capped glass tubes (O.D. x L = 1.5 cm x 15 cm) and stored in a refrigerator at 9°C till counting. The recovered insect larvae were washed and incubated for 72 hours in Petri dishes (five cm diameter) taped with parafilm for reduced dehydration at 25°C.

After 72 hours, all *G. mellonella* larvae were dissected and both IJs and adults were counted separately. Petri dishes (nine cm diameter) were used as containers for nematode solutions during nematode counting. The experiment was repeated two times giving a total of 960 samples. All data were subject to analysis of variance (ANOVA) at $P<0.05$ using Statistica statistical package. The significant differences among the different strains were calculated. The experiment was carried out in Wooster, OH (USA) at the OARDC Lab. of the Ohio State University.

RESULTS

The majority of IJs remained in the upper section of the shell vials where they were injected previously. The number of IJs recovered in the lower section in most of the isolates increased as time went by.

MHG 3

At 12 hrs, an average of 271 IJs were recovered out of 500 (the extraction efficiency was 54%) whereas at 24 hrs, an average of 235 IJs (47%) and, at 36 hrs, an average of 224 IJs (45%) were recovered out of 500. The number of IJs recovered in the lower section increased as time went by. At 12 hrs, an average of 94.1% of the IJs were recovered in the upper section and only 5.9% in the lower section, whereas at 24 hrs 91.5% were recovered in the upper and 8.5% in the lower and, at 36 hrs, 91.9% in the upper and only 8.5% in the lower section on the average (*Table 1*).

Table 1

Infective juveniles of *Heterorhabditis bacteriophora* (MHG 3) detected in the lower and upper sections of the vials and in the Galleria larvae 12, 24 and 36 after injection

Serial number of replicates	12 hrs						24 hrs						36 hrs					
	Number of IJs in				Total nematode	%	Number of IJs in				Total nematode	%	Number of IJs in				Total nematode	%
	upper section	lower section	Galleria				upper section	lower section	Galleria				upper section	lower section	Galleria			
			Adult	IJs					Adult	IJs					Adult	IJs		
1.	313	17	0	0	330	66	196	8	1	6	204	41	203	23	29	88	226	45
2.	204	7	0	0	211	42	121	22	5	18	143	29	181	15	53	76	196	39
3.	206	19	0	0	225	45	302	17	10	15	319	64	178	21	36	81	199	40
4.	267	11	0	0	278	56	226	14	2	9	240	48	166	19	44	93	185	37
5.	248	16	0	0	264	53	143	24	8	16	167	33	191	16	49	84	207	41
6.	192	26	0	0	218	44	297	26	2	9	323	65	268	14	21	23	282	56
7.	295	16	0	0	311	62	236	31	4	14	267	53	192	27	16	21	219	44
8.	296	8	0	0	304	61	276	12	5	7	288	58	272	23	32	46	295	59
9.	226	19	0	0	245	49	184	24	3	19	208	42	196	12	38	51	208	42
10.	301	23	0	0	324	65	169	19	7	21	188	38	209	16	26	49	225	45
Mean	255	16	0	0	271	54	215	20	5	13	235	47	206	19	34	61	224	45
Control																		
1.	226	76			302	60	232	23			255	51	224	21			245	49
2.	189	59			248	50	194	10			204	41	285	17			302	60
3.	177	43			220	44	238	13			251	50	164	15			179	36
4.	151	64			215	43	212	17			229	46	234	19			253	51
5.	202	71			273	55	246	19			265	53	259	18			277	55
6.	212	34			246	49	298	21			319	64	254	26			280	56
7.	191	34			225	45	206	16			222	44	214	31			245	49
8.	196	28			224	45	241	18			259	52	242	22			264	53
9.	202	46			248	50	162	9			171	34	266	16			282	56
10.	187	37			224	45	271	11			282	56	234	24			258	52
Mean	193	49			243	49	230	16			246	49	238	21			259	52

KMD 19

At 12 hrs, an average of 230 IJs were recovered out of 500 (the extraction efficiency was 46%), whereas at 24 hrs, an average of 282 IJs (56%) and, at 36 hrs, an average of 296 IJs (59%) were recovered out of 500. The number of IJs recovered in the lower section increased as time went by. At 12 hrs, an average of 97.8% of the IJs were recovered in the upper section and only 2.6% in the lower section whereas at 24 hrs, 90.8% were recovered in the upper and 9.2% in the lower and, at 36 hrs, 91.2% in the upper and only 8.8% in the lower section on the average (*Table 2*).

Table 2

Infective juveniles of *Heterorhabditis bacteriophora* (KMD 19) detected in the lower and upper sections of the vials and in the Galleria larvae 12, 24 and 36 after injection

Serial number of replicates	12 hrs						24 hrs						36 hrs					
	Number of IJs in				Total nema tode	%	Number of IJs in				Total nema tode	%	Number of IJs in				Total nema tode	%
	upper section	lower section	Galleria				upper section	lower section	Galleria				upper section	lower section	Galleria			
			Adult	IJs					Adult	IJs					Adult	IJs		
1.	201	1	0	0	202	40	301	12	2	7	313	63	285	22	8	9	307	61
2.	237	8	0	0	245	49	204	37	3	11	241	48	224	29	6	3	253	51
3.	192	6	0	0	198	40	251	14	1	5	265	53	297	34	2	16	331	66
4.	212	4	0	0	216	43	289	27	1	9	316	63	249	27	12	0	276	55
5.	226	7	0	0	233	47	267	31	2	8	298	60	324	31	4	31	355	71
6.	211	7	0	0	218	44	264	29	1	6	293	59	267	21	11	12	288	58
7.	224	5	0	0	229	46	295	19	4	8	314	63	241	13	17	23	254	51
8.	281	6	0	0	287	57	167	34	2	9	201	40	362	23	9	37	385	77
9.	267	4	0	0	271	54	246	18	1	3	264	53	219	34	14	26	253	51
10.	194	9	0	0	203	41	271	41	2	11	312	62	231	27	6	31	258	52
Mean	225	6	0	0	230	46	256	26	2	8	282	56	270	26	9	19	296	59
Control																		
1.	179	70			249	50	306	49			355	71	336	30			366	73
2.	270	48			318	64	331	20			351	70	279	38			317	63
3.	238	52			290	58	262	21			283	57	261	47			308	62
4.	214	63			277	55	294	37			331	66	306	36			342	68
5.	231	57			288	58	318	28			346	69	289	41			330	66
6.	246	38			284	57	271	25			296	59	317	62			379	76
7.	268	46			314	63	319	58			377	75	297	49			346	69
8.	205	67			272	54	247	24			271	54	218	38			256	51
9.	198	49			247	49	269	29			298	60	342	24			366	73
10.	301	34			335	67	302	38			340	68	338	43			381	76
Mean	235	52			287	57	292	33			325	65	298	41			339	68

OH 25

At 12 hrs, an average of 299 IJs were recovered out of 500 (the extraction efficiency was 60%), whereas at 24 hrs, an average of 318 IJs (64%) and, at 36 hrs, an average of 243 IJs (49%) were recovered out of 500. At 12 hrs, an average of 89.97% of the IJs were recovered in the upper section and only 10.03% in the lower section, whereas at 24 hrs, 91.5% were recovered in the upper and 8.5% in the lower and, at 36 hrs, 91.4% in the upper and only 8.6% in the lower section on the average (*Table 3*).

MEGIDIS

At 12 hrs, an average of 282 IJs were recovered out of 500 (the extraction efficiency was 56%), whereas at 24 hrs, an average of 283 IJs (57%) and, at 36 hrs, an average of 256 IJs (51%) were recovered out of 500. The number of IJs recovered in the lower section increased as time went by. At 12 hrs, an average of 98.6% of the IJs were recovered in the upper section, and only 1.4% in the lower section, whereas at 24 hrs, 97.2% were recovered in the upper and 2.8% in the lower and, at 36 hrs, 91.8% in the upper and only 8.2% in the lower section on the average (*Table 4*).

Table 3

Infective juveniles of *Heterorhabditis bacteriophora* (OH 25) detected in the lower and upper sections of the vials and in the Galleria larvae 12, 24 and 36 after injection

Serial number of replicates	12 hrs						24 hrs						36 hrs					
	Number of IJs in				Total nema tode	%	Number of IJs in				Total nema tode	%	Number of IJs in				Total nema tode	%
	upper section	lower section	Galleria				upper section	lower section	Galleria				upper section	lower section	Galleria			
			Adult	IJs					Adult	IJs					Adult	IJs		
1.	263	22	0	0	285	57	304	33	6	0	337	67	213	11	2	0	224	45
2.	287	17	0	0	304	61	318	14	1	0	332	66	273	14	5	3	287	57
3.	263	54	0	0	317	63	301	24	3	1	325	65	278	24	3	1	302	60
4.	248	31	0	0	279	56	273	38	2	0	311	62	189	16	6	4	205	41
5.	301	28	0	0	329	66	288	22	4	1	310	62	176	22	8	2	198	40
6.	267	29	0	0	296	59	287	31	2	1	318	64	241	26	5	5	267	53
7.	254	18	0	0	272	54	266	26	4	0	292	58	198	23	6	1	221	44
8.	238	22	0	0	260	52	311	19	6	1	330	66	212	19	3	2	231	46
9.	269	51	0	0	320	64	291	27	4	1	318	64	226	29	8	3	255	51
10.	304	24	0	0	328	66	274	32	4	0	306	61	211	24	7	4	235	47
Mean	269	30	0	0	299	60	291	27	4	1	318	64	222	21	5	3	243	49
Control																		
1.	254	28			282	56	267	42			309	62	279	17			296	59
2.	242	18			260	52	310	51			361	72	178	25			203	41
3.	275	39			314	63	301	47			348	70	133	33			166	33
4.	231	31			262	52	237	36			273	55	204	42			246	49
5.	261	29			290	58	212	32			244	49	238	36			274	55
6.	229	28			257	51	204	45			249	50	199	21			220	44
7.	242	12			254	51	261	51			312	62	208	34			242	48
8.	263	36			299	60	224	66			290	58	234	61			295	59
9.	241	26			267	53	249	38			287	57	221	44			265	53
10.	255	21			276	55	286	59			345	69	243	36			279	56
Mean	249	27			276	55	255	47			302	60	214	35			249	50

Table 4

Infective juveniles of *Heterorhabditis megidis* detected in the lower and upper sections of the vials and in the Galleria larvae 12, 24 and 36 after injection

Serial number of replicates	12 hrs						24 hrs						36 hrs					
	Number of IJs in				Total nema tode	%	Number of IJs in				Total nema tode	%	Number of IJs in				Total nema tode	%
	upper section	lower section	Galleria				upper section	lower section	Galleria				upper section	lower section	Galleria			
			Adult	IJs					Adult	IJs					Adult	IJs		
1.	261	5	0	0	266	53	253	6	0	0	259	52	217	13	0	0	230	46
2.	299	1	0	0	300	60	291	2	0	0	293	59	228	21	0	0	249	50
3.	257	3	0	0	260	52	287	7	0	0	294	59	236	14	0	0	250	50
4.	289	4	0	0	293	59	301	5	0	0	306	61	267	19	0	0	286	57
5.	316	2	0	0	318	64	238	8	0	0	246	49	194	23	0	0	217	43
6.	242	6	0	0	248	50	259	9	0	0	268	54	232	21	0	0	253	51
7.	268	2	0	0	270	54	267	11	0	0	278	56	217	16	0	0	233	47
8.	284	3	0	0	287	57	274	14	0	0	288	58	268	24	0	0	292	58
9.	277	4	0	0	281	56	288	6	0	0	294	59	253	27	0	0	280	56
10.	291	8	0	0	299	60	296	8	0	0	304	61	236	31	0	0	267	53
Mean	278	4	0	0	282	56	275	8	0	0	283	57	235	21	0	0	256	51
Control																		
1.	228	2			230	46	230	15			245	49	205	22			227	45
2.	249	1			250	50	249	11			260	52	248	17			265	53
3.	231	5			236	47	231	14			245	49	231	15			246	49
4.	258	4			262	52	213	13			226	45	207	19			226	45
5.	261	6			267	53	279	11			290	58	269	24			293	59
6.	212	2			214	43	224	21			245	49	231	27			258	52
7.	226	3			229	46	257	11			268	54	219	24			243	49
8.	238	4			242	48	224	16			240	48	227	19			246	49
9.	243	1			244	49	243	12			255	51	216	18			234	47
10.	252	2			254	51	261	19			280	56	198	26			224	45
Mean	240	3			243	49	241	14			255	51	225	21			246	49

Figure 1: **The percentage of infective juveniles of *Heterorhabditis bacteriophora* strains MHG3, KMD 19, OH 25, and a single strain of *Heterorhabditis megidis* recovered in the lower section of the vials at 12, 24 and 36 hrs after injection**

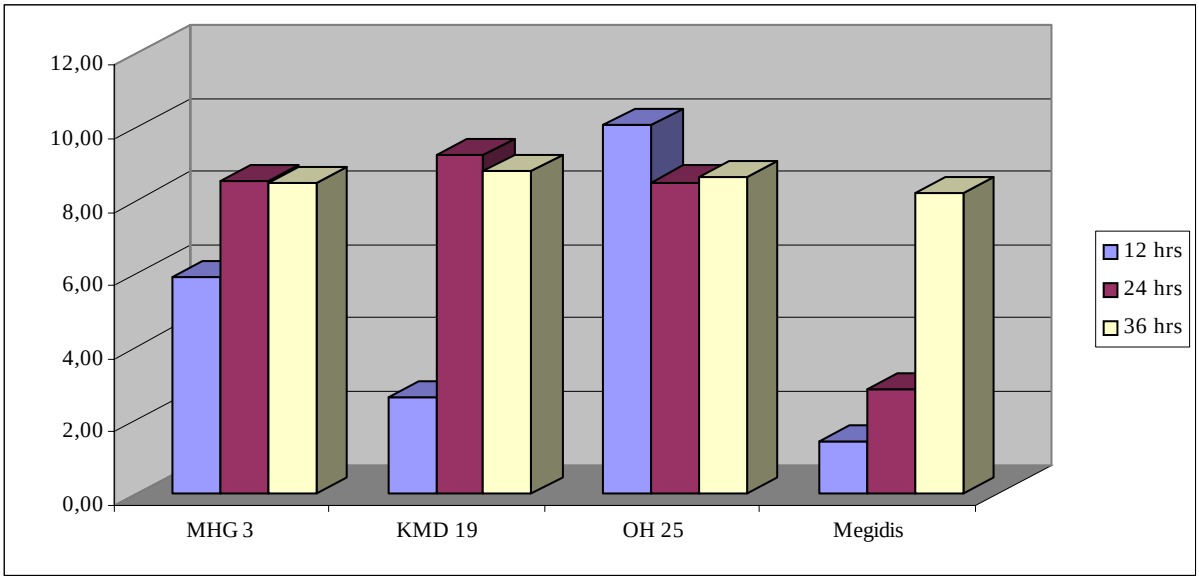
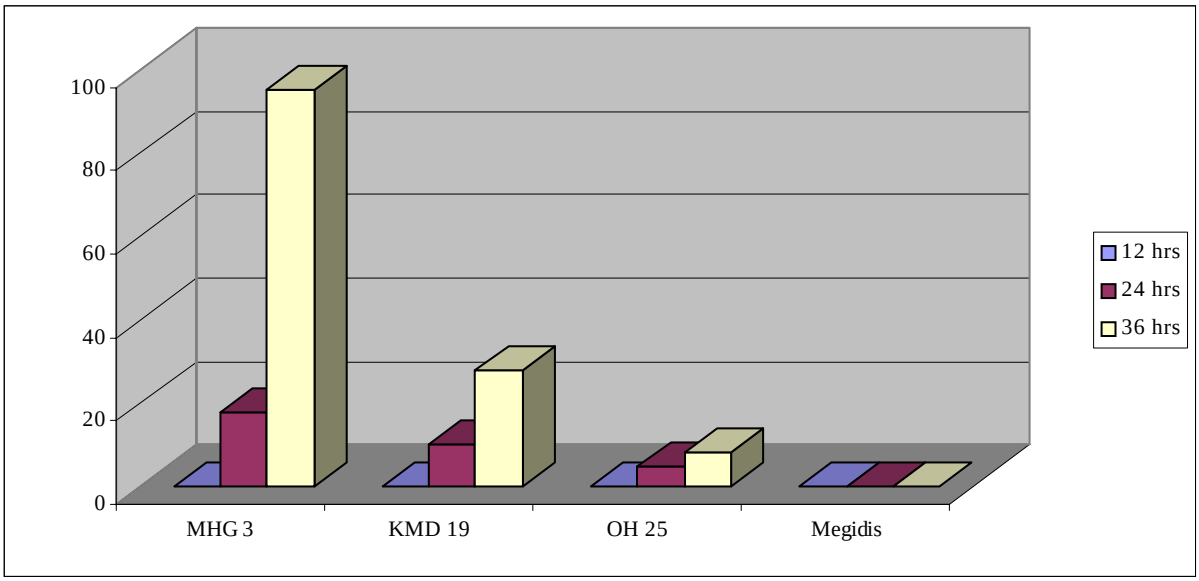


Figure 2: **Adult and infective juveniles of *Heterorhabditis bacteriophora* strains MHG3, KMD 19, OH 25, and a single strain of *Heterorhabditis megidis* found in Galleria larvae dissected 72 hrs after an experiment that was conducted for 12, 24 and 36 hrs**



DISCUSSION

The number of the IJs in the upper section decreased in case of every isolate as time went by, except KMD 19. The most of IJs in the upper section could be found in the isolate Megidis, since 12 hrs after injection, an average of 278 IJs were recovered out of 500 (extraction efficiency: 55.6%). The least number of IJs could be found in the upper section of the vials 12 hrs after injection in the case of the isolate OH 25 (only an average of 269 IJs were recovered out of 500). As time went by, the IJs started their downward migration toward the *Galleria* larvae, therefore, their number decreased in the upper section of the shell vials and increased in the lower section of the vials (*Figure 1*). Only the isolate OH 25 behaved differently, because the number of IJs decreased in the case of these isolates in the lower section. This isolate was the quickest since, after 12 hrs, most IJs could be found in the lower section of the vials (an average of 30). The least IJs could be found in the case of Megidis (an average of 4). The number of IJs was so small in the lower section that 12 hrs after injection, only the isolates MHG 3 and OH 25 could kill the *Galleria* larvae.

Besides the fact that mortality occurred among the *Galleria* larvae with the above isolates, neither IJs nor adults could be detected in neither living nor dead *Galleria* larvae 12 hrs after IJs injection. The number of IJs increased in every isolate in the lower section of the shell vials as time went by. The least IJs could be found in the lower section after 36 hrs in case of the isolate MHG 3: only an average of 19 IJs could be detected.

It is noteworthy that the behavior of the isolate Megidis was different from the other isolates: the number of the IJs was so negligible in the lower section of the vials that even after 36 hrs, none of the *Galleria* died and consequently neither adults nor IJs could be detected after dissecting the *Galleria* larvae.

According to the results of the present study we can conclude that the quickest nematode was the isolate OH 25, whereas the slowest was the isolate Megidis. The most IJs could be detected in the lower section of the vials in the case of OH 25, and the least in the case of Megidis and KMD 19. Each isolate could reach the lower section of the vials, yet only the isolate Megidis could not infect and kill the host. After dissecting the larvae most IJs could be found in case of the isolate MHG 3 (95 IJs and adults) 36 hrs after injection (*Figure 2*).

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