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Heterorhabditis sp. (Nematoda: Heterorhabditidae): A Nematode Parasite Isolated from the Banded Cucumber Beetle *Diabrotica balteata*

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Abstract: A nematode identified as *Heterorhabditis* sp. was discovered in June 1982 in larval cadavers of the banded cucumber beetle, *Diabrotica balteata,* in soil on wooded land. Effective beetle control (over 95%) was obtained when larvae were exposed to potted soil containing infective stage nematode juveniles or infected larval cadavers. The nematode was propagated in vivo on larvae of *D. balteata, Diaphania nitidalis* (the pickleworm), and *Galleria mellonella* (the greater wax moth). This *Heterorhabditis* sp. has promising potential as a biocontrol agent for the banded cucumber beetle.

Key words: banded cucumber beetle, biocontrol, ecology, efficacy, entomology, pickleworm, propagation, wax moth.

Many insects attack corn and sweet potatoes in the United States. Larvae of *Diabrotica* spp. are among the most destructive soil insect pests attacking these crops. Currently, there are no practical chemical means of controlling *Diabrotica* in regions with severe infestations of this insect.

In June 1982 the wooded land on the U.S. Vegetable Laboratory farm at Charleston, South Carolina, was surveyed for the mermithid nematode, *Filipjevimermis leipsandra.* We discovered that larvae of the banded cucumber beetle, *Diabrotica balteata,* that had been buried in the soil at one sampling site a week earlier were all dead. The cadavers were a reddish color and when dissected were found to contain numerous nematodes. Specimen nematodes submitted to Dr. George Poinar, University of California, were determined to be an unidentified species in the genus *Heterorhabditis.*

The genus *Heterorhabditis* was erected by Poinar (4) in 1976 and presently comprises four species: *H. bacteriophora* Poinar, 1976; *H. heliothidis* Kahn, Brook, and Hirschmann, 1976; *H. hambletoni* Pereira, 1937; and *H. hoptha* Turco, 1970. Infective juveniles of *Heterorhabditis* spp. possess a symbiotic bacterium, *Xenorhabdus* sp., in the anterior of their intestine which is lethal to the host insect (4).

Recovery of a new *Heterorhabditis* sp. from dead larvae of *D. balteata* in soil suggests that this nematode might be a useful biological control agent for this and possibly other *Diabrotica* species. Therefore, we initiated laboratory studies on the culture of *Heterorhabditis* and its efficacy against *D. balteata.* The results are reported here.

Materials and Methods

Cultures: Using techniques similar to those reported earlier (3), the *Heterorhabditis* sp. was cultured in vivo in larvae of the banded cucumber beetle, the pickleworm (*Diaphania nitidalis*), and the greater wax moth (*Galleria mellonella*). We obtained late instar larvae of the banded cucumber beetle and pickleworm from our insect rearing facility and wax moth larvae from Grubco, Hamilton, Ohio.

Infective juveniles penetrated insect larvae placed on damp filter paper in petri dishes killing them within 48 hours. Within 2 weeks, large numbers of juveniles emerged from the cadavers. Juveniles collected in a water trap (6) were harvested 1–3 times a week. The suspension was poured into a flask and after the nematodes had settled, the supernatant was discarded and additional water was added to wash the nematodes. The number of infective juveniles in a 0.1-ml aliquot were counted under a stereoscopic microscope, and the volume of the suspension was adjusted to deliver the desired number of nematodes in a given amount of water. As suggested by Dr. Poinar (pers. comm.), the nematode suspension was stored at 15 C until needed to minimize detrimental effects on the associated bacterium. Twenty infected cadavers of each of the three insect species

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TABLE 1. Yield of *Heterorhabditis* sp. juveniles from larvae of three insect species.

Insect	n	Avg. no. juveniles/larva ± SD	Range
Diabrotica balteata	20	13,905 ± 2,570	10,000–20,000
Diaphania nitidalis	20	16,962 ± 7,258	8,500–30,000
Galleria mellonella	20	106,550 ± 22,809	90,000–120,000

were isolated and the juveniles exiting from each cadaver were collected in a water trap and counted to determine yields of juveniles from larvae of each insect species.

Infectivity: Five laboratory tests were conducted in 8-cm-d clay pots containing various media to assess the efficacy of this *Heterorhabditis* sp. to control larvae of the banded cucumber beetle. Tests 1–3 were conducted in a room maintained at 22.5 ± 2.5 C.

Test 1 was conducted with various numbers (0, 5, 10, 20) of infected *D. balteata* cadavers buried in damp (ca. 20% moisture) commercial blasting sand on 19 August 1982. Four days later each pot was inoculated with third-instar *D. balteata* larvae and sprouted wheat added for food. The beetle larvae were caged for 7 days after which time the pots were emptied and the soil combed for living larvae. Survivors were removed and counted. Each treatment was replicated five times.

On 31 August, 100 third-instar larvae were again added to each pot with more sprouted wheat added. These larvae were caged for 5 days after which the pots were emptied and the surviving larvae counted.

Tests 2 and 3 were conducted in field soil (ca. 17% moisture) that had been passed through hardware cloth (8 mesh) to remove coarse debris and placed in pots, 200 cm^3/pot. The pots were inoculated with 200–5,000 juveniles on 14 June 1983 (Test 2) and with 800–20,000 juveniles on 25 July 1983 (Test 3). Each level of nematodes (treatment) was replicated five times. One day following each inoculation, 50 third-instar *Diabrotica* larvae and sprouted wheat were buried in each pot. The larvae were caged, and the surviving larvae were counted 6 days later.

Tests 4 and 5 were conducted in soil, prepared as in Tests 2 and 3, in pots inoculated with 10,000 juveniles on 31 January 1984 (Test 4) and 25,000 juveniles on 7 February 1984 (Test 5) and held at 15, 22, and 30 C. Nematode levels (treatments) at each temperature were replicated five times. One day following nematode inoculation of the soil, 50 third-instar *Diabrotica* larvae and sprouted wheat were buried in each pot. Five days later, the surviving larvae were counted.

RESULTS

Culture: Heterorhabditis sp. is apparently host nonspecific, as it increased in banded cucumber beetle, pickleworm, and wax moth larvae. Wax moth larvae yielded about eight-fold more nematodes than did banded cucumber and pickleworm larvae. More than 100,000 infective juveniles were obtained from one wax moth larva (Table 1).

Infectivity: Test 1. Inoculum levels of 5–

TABLE 2. Efficacy of *Diabrotica balteata* cadavers containing nematodes of *Heterorhabditis* sp. to live *D. balteata* larvae in potted soil.

No. parasitized *D. balteata* cadavers per pot	Bioassay 1		Bioassay 2	
	Avg. no. surviving larvae	Reduction (%)	Avg. no. surviving larvae	Reduction (%)
5	4.4 a*	95.6	0.8 a*	99.2
10	5.4 a	94.6	0.0 a	100.0
20	0.4 a	99.6	1.4 a	98.6
Untreated (0)	100.0 b	0.0	100.0 b	0.0

* Mean separation within columns by Duncan's multiple-range test ($P = 0.05$). Five replicates of 50 larvae.

TABLE 3. Reduction of *Diabrotica balteata* larvae exposed to pottted soil inoculated with *Heterorhabditis* sp.

No. juveniles per pot	Avg. no. surviving larvae*	Reduction (%)
Test 2		
200	6.3 c	87.3
1,000	4.0 b	91.9
2,000	2.8 b	94.4
5,000	0.5 a	99.0
Untreated	49.5 d	
Test 3		
800	10.5 c	77.8
4,000	5.0 b	89.7
8,000	0.5 a	99.0
20,000	0.3 a	99.5
Untreated	48.3 d	

* Mean separation in columns by Duncan's multiple-range test ($P = 0.05$). Five replicates of 50 larvae.

TABLE 4. Reduction of *Diabrotica balteata* larvae exposed to potted soil inoculated with *Heterorhabditis* sp. at various temperatures.

No. juveniles per pot	Temp. (C)	Avg. no. surviving larvae*	Reduction (%)
Test 4			
10,000	15	49.2 c	1.2
0	15	49.8 c	
10,000	22	14.0 b	71.9
0	22	49.8 c	
10,000	30	6.2 a	87.6
0	30	49.8 c	
Test 5			
25,000	15	48.2 c	2.8
0	15	49.6 c	
25,000	22	7.4 b	85.1
0	22	49.6 c	
25,000	30	0.6 a	98.8
0	30	49.2 c	

* Mean separation in columns by Duncan's multiple-range test ($P = 0.05$). Five replicates of 50 larvae.

20 nematode-infected beetle cadavers/pot reduced the population of *Diabrotica* larvae by over 95% (Bioassay No. 1) (Table 2). Extremely effective reductions (99–100%) of *Diabrotica* were also achieved about 2 weeks later after additional beetle larvae were added to the pots (Bioassay No. 2). In Test 2, 5,000 juveniles/pot reduced larvae 99% and were significantly more effective than lower numbers of juveniles (Table 3). In Test 3, 8,000 or 20,000 juveniles/pot achieved larval reductions of over 99%, whereas 800 juveniles provided a 78% reduction.

Temperature greatly influenced mortality of *Diabrotica* larvae infected with *Heterorhabditis* (Table 4). In Test 4, larval mortality was greatest in pots incubated at 30 C. No significant reduction of *Diabrotica* larvae was obtained in pots incubated at 15 C. Test 5 corroborated the results of Test 4.

DISCUSSION

Our tests indicate that this *Heterorhabditis* sp. is a highly effective parasite of *Diabrotica* larvae. Death of the beetle larvae occurs within 2 days of parasitism. The minimum number of juveniles needed per pot to obtain a 99% reduction of *Diabrotica* larvae was about 5,000. As the nematode reproduces abundantly in larval cadavers, its residual capability for infecting beetle larvae in the soil could be highly effective. This was evidently the case in our first test, as the infective juveniles emerging from larvae killed in the first soil bioassay probably accounted for the high degree of mortality of beetle larvae in a second bioassay conducted 2 weeks later.

As this nematode appears to be host nonspecific, it may prove effective against many soil-inhabiting insects, including other species of *Diabrotica.* The infectivity of this nematode will apparently depend on soil temperature at application. Low soil temperatures (about 13 C) and insect location below the ground surface can reduce infections of the large pine weevil, *Hylobius abietis,* by the nematode *Neoaplectana carpocapsae* (5). Mobility of *N. carpocapsae* was also low at 15 C (1).

In mass rearing the mermithid nematode *F. leipsandra,* we use first-instar larvae of *D. balteata* which apparently are very susceptible to penetration (2); since *Heterorhabditis* sp. kills the later instars rapidly, the possibility of utilizing both nematode species together in a biocontrol research program may be worth pursuing.

Cultivation of *Heterorhabditis* sp. in vitro is relatively easy. Once mass cultivation techniques are perfected, large amounts of nematodes for inoculating soil will be available. Biocontrol of the banded cucumber beetle and the related corn rootworm by this nematode should be explored further.

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