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Susceptibility of the Carrot Weevil (Coleoptera: Curculionidae) to *Steinernema feltiae*, *S. bibionis*, and *Heterorhabditis heliothidis*[1]

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Abstract: Larvae, pupae, and adults of the carrot weevil (*Listronotus oregonensis*) were infected and killed by the three entomophagous nematodes (*Steinernema feltiae, S. bibionis,* and *Heterorhabditis heliothidis*) under controlled conditions. Third-stage larvae were more susceptible than pupae or adults. *S. feltiae* and *S. bibionis* were the most aggressive nematode species, causing larval mortality after 24–48 hours in both continuous and 2-hour contact with nematode suspension. The nematodes multiplied sufficiently in all insects at all stages of development; however, production of infective-stage larvae per host cadaver was variable.

Key words: entomogenous nematodes, *Listronotus oregonensis,* control.

The carrot weevil, *Listronotus oregonensis* Le Conte (Coleoptera: Curculionidae), is an important pest of carrot, parsley, and celery in eastern Canada and northeastern United States (7,8,10). Chemical insecticides, applied as granules at seeding time or as foliar sprays, are the only method available to control this insect. If integrated pest management (IPM) programs are to be applied in these crops, alternative control methods are needed. Biological control could potentially replace some chemical treatments, but no information is available on pathogens of *L. oregonensis.*

Many entomophagous nematode members of the family Steinernematidae and Heterorhabditidae have wide host ranges. Some are promising biological control agents in IPM programs (6). This laboratory study was undertaken to determine the susceptibility of larvae, pupae, and adults of *L. oregonensis* to infection by *Heterorhabditis heliothidis* (Khan, Brooks, and Hirschmann), *Steinernema feltiae* Filipjev, and *S. bibionis* Steiner under continuous and short exposure periods in the laboratory.

Materials and Methods

The three nematode species *H. heliothidis, S. feltiae* DD-136 strain, and *S. bibionis* T335 were obtained from Dr. J. W. Webster, Simon Fraser University, Vancouver, Canada, and reared on larvae of the greater wax moth, *Galleria mellonella* (L.), by the method of Dutky et al. (2).

Carrot weevils were reared on carrots by the method of Martel et al. (4). Adults were originally collected in carrot fields in southwestern Quebec; the weevil was in its sixth to eighth generation in laboratory culture. Third-instar larvae, pupae less than 48 hours old, and adults 2–3 months old were used in the tests.

For infection experiments, 20 insects (larvae, pupae, or adults) were placed in each 10-cm-d sterile petri dish lined with two #2 Whatman filter papers. Three milliliters of water containing approximately

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TABLE 1. Mortality of carrot weevil exposed to *Steinernema feltiae, S. bibionis,* and *Heterorhabditis heliothidis* in the laboratory.

	Mean % cumulative mortality after indicated day											
	Continuous contact (Exp. 1)						2-hour contact (Exp. 2)					
	1	2	4	6	8	10	1	2	4	6	8	10
Larva												
S. feltiae	90 a	99 a	100 a				49 a	67 a	72 a	75 a	78 ab	
S. bibionis	97 a	100 a	100 a				57 a	82 a	83 a	87 a	94 a	
H. heliothidis	10 b	71 b	100 a				9 b	21 b	34 b	51 b	63 b	
Nontreated	0 c	0 c	0 b				0 b	1 c	1 c	7 c	23 c	
Pupa												
S. feltiae	20 a	46 a	74 a	76 a			43 a	55 a	88 a			
S. bibionis	8 b	46 a	80 a	82 a			40 a	55 a	92 a			
H. heliothidis	6 b	11 b	40 b	79 a			11 b	32 b	90 a			
Nontreated	9 b	11 b	36 b	39 b			11 b	26 b	35 b			
Adult												
S. feltiae	22 ab	37 ab	69 ab	74 a	84 a	85 a	14 a	17 b	29 a	33 a	34 a	37 a
S. bibionis	35 a	53 a	79 a	83 a	86 a	86 a	16 a	28 ab	32 a	35 a	39 a	40 a
H. heliothidis	15 bc	31 b	59 b	69 a	81 a	83 a	18 a	31 a	34 a	38 a	40 a	40 a
Nontreated	1 c	2 c	12 c	13 b	23 b	26 b	1 b	1 c	4 b	5 b	7 b	11 b

Means followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

8,000 infective-stage nematodes were added to each plate. Control plates each contained 20 insects and 3 ml distilled water. Treatments were replicated five times, and experiments were carried out at 27 ± 1 C in the dark. Immobile insects were placed in petri dishes designed to collect the infective-stage nematodes in 0.1% aqueous formaldehyde (6). Nematodes were collected every 4 days and counted; new solution was then added to the dishes. In Experiment 1, susceptibility of *L. oregonensis* was evaluated under constant contact of the three species of nematodes in suspension. In Experiment 2, weevils were exposed to the nematodes for 2 hours after which the insects were placed in petri dishes on filter paper saturated with distilled water.

RESULTS

Under continuous contact, larvae of *L. oregonensis* were highly susceptible to infection by *S. feltiae, S. bibionis,* and *H. heliothidis,* showing 100% cumulative mortality after 4 days (Table 1). High larval mortality occurred within 24 hours exposure to *S. feltiae* and *S. bibionis,* but not until 48 hours after exposure to *H. heliothidis.* In the 2-hour contact experiment, larvae were more susceptible to *S. feltiae* and *S. bibionis* infections than to *H. heliothidis* even after 8 days, with 78%, 94%, and 63% mortality, respectively.

Pupae of *L. oregonensis* were less susceptible than larvae to nematode infection. *S. feltiae* was the most aggressive, causing 20% mortality after 24 hours. No significant differences in mortality were observed between the three nematode species after 6 days continuous contact. Pupal mortality in the control was high (36%) after 4 days.

Adults of *L. oregonensis* were also susceptible to infection by all three nematodes. Under continuous exposure to nematodes, adults also were more susceptible to *S. feltiae* and *S. bibionis* than to *H. heliothidis* at 4 days but not after 6 days or longer. In the 2-hour nematode contact, adults were equally susceptible to all three nematode species, although mortality was only about half that under continuous contact with nematodes.

All three nematodes multiplied within larvae, pupae, and adults of *L. oregonensis* (Table 2). Infective-stage juveniles began emerging after 7–9 days from larvae and after 12–14 days from pupae and adults. The infectivity of juveniles of *S. feltiae, S. bibionis,* and *H. heliothidis* from dead carrot

TABLE 2. Number of infective-stage nematodes emerging from infected carrot weevil (continuous contact) after a 1-month recovery period.

Insect stage	Nematodes/insect		
	S. feltiae	*S. bibionis*	*H. heliothidis*
Larva	2,870 a	6,538 a	2,410 a
Pupa	350 b	552 b	692 b
Adult	136 b	350 b	345 b

Means of five replicates per treatment. Means followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

weevils was verified by plating them with larvae of *G. mellonella* and *L. oregonensis*, which were killed within 96 hours.

DISCUSSION

Our study showed that larvae, pupae, and adults of *L. oregonensis* become infected by three different entomophagous nematodes under experimental laboratory conditions. Insect death occurred 24–48 hours after exposure to the infective *S. feltiae* and *S. bibionis* but not until 72–96 hours after exposure to *H. heliothidis*, confirming an earlier report (6). Actively feeding larvae were shown to be more susceptible than pupae and adults, possibly because larvae were eating the filter papers and thus assuring entry of the nematodes through the alimentary tract. In adults, the main routes of nematode entry are thought to be the spiracles and anus and, perhaps, orally as well. The lower mortality of pupae, compared with larvae and adults, could be related to the difficulty encountered by nematodes in infecting pupae. High mortality in the untreated pupae probably occurred because they were exhausted from continuous abdominal wiggling in efforts to form a pupal cell.

The carrot weevil is a soil-inhabiting insect with a 1-year life cycle which may be controlled biologically by application of a parasite such as a nematode. Spring migration of adult weevils from ditches surrounding carrot fields for feeding and reproduction raises the possibility of infecting them with nematodes by spraying or baiting before egg laying. Because the adults are not killed promptly, dispersion of the nematodes is increased and so is the area of insect control in the field. Soil applications of entomophagous nematodes have been shown to effectively reduce field populations of other root weevil species (1,3,9).

Our results demonstrated that the three nematode species studied reproduced well in carrot weevil larvae. Based on the average body weight of third-stage larvae (16 mg), the reproductive capacity on *L. oregonensis* was ca. 300 nematodes/mg compared to 1,100/mg reported for large *G. mellonella* larvae (2). The total emergence averaged over stages was markedly less from pupae and adults than larvae in the constant contact experiment for all three nematode species. However, differences were not significant between the total infective larvae emergence of stages recovered from the 2-hour contact experiment. From our results and those of others (5,6), nematode production varies among different host insects and among different developmental stages of a given insect.

Field studies will be required to determine the practicality of using these entomophagous nematodes to control the carrot weevil.

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