Susceptibility of the Colorado Potato Beetle and the Sugarbeet Wireworm to Steinernema feltiae and S. glaseri[1,2]

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Abstract: In laboratory tests, larvae of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), and the sugarbeet wireworm (SBW), *Limonius californicus* (Mannerheim), were exposed to the nematodes *Steinernema feltiae* Filipjev (Mexican strain) (= *Neoaplectana carpocapsae*) and *S. glaseri* Steiner in soil. *S. feltiae* caused significantly higher mortality in SBW larvae than did *S. glaseri*, but both nematode species were equally effective against CPB larvae. The minimum concentration of *S. feltiae* for 100% mortality of CPB larvae after 13 days was 157 nematodes/cm² of soil, and the LC_{50} based on 6-day mortality was 47.5 nematodes/cm²; in contrast, 100% mortality of SBW larvae was not achieved with even the highest concentration tested, 393 nematodes/cm². CPB adults emerging from nematode-contaminated soil were not infected. In field cage tests, *S. feltiae* applied to the soil surface at the rates of 155 and 310 nematodes/cm² soil caused 59% and 71% mortality, respectively, of late-fourth-instar spring-generation CPB, and 28% and 29% mortality, respectively, of SBW. No infection was obtained when larvae of summer generation CPB and SBW were placed in the same cages approximately 6 weeks after nematodes were applied to the soil. Inundative soil applications of *S. feltiae*, though cost prohibitive at present, were effective in reducing caged CPB and SBW field populations. *Key words: Leptinotarsa decemlineata, Limonious californicus,* control, soil application, entomogenous nematodes. Journal of Nematology 15(4):597-601. 1983.

The sugarbeet wireworm (SBW), *Limonius californicus* (Mannerheim), and the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), are important pests of potatoes. Larvae of SBW cause feeding damage to underground parts of the plants, particularly tubers, whereas the adults and larvae of CPB are generally foliage feeders, the older fourth instars entering the soil primarily to pupate.

Entomogenous nematodes show promise as biological control agents, and one of them, *Steinernema feltiae* Filipjev (= *Neoaplectana carpocapsae*), has a wide host range (10). Welch (14) first reported on the susceptibility of CPB to *S. feltiae*. Subsequently, some success was obtained in controlling CPB larvae by spraying aqueous suspensions of nematodes on potato foliage (13,15), but the feasibility of foliar applications in the field is questionable because of low nematode survival due to desiccation (15) and to sunlight sensitivity (4). However, MacVean et al. (8) reported increased infection rates with the addition of antidesiccants. Application of nematodes to moist soil affords them a more suitable environment for survival and infectivity, as demonstrated with CPB larvae (13,15). Likewise, Danilov (3) reported on the infectivity of soil-applied *S. feltiae* in tests with the wireworms *Agriotes lineatus* (L.) and *Selatosomus aeneus* (L.), and Kovacs et al. (5) reported a significant reduction in corn seedling damage by an Elateridae spp. with an application of *S. feltiae* (Breton strain). However, work has not been reported on the effect of *S. feltiae* on SBW and the effect of *S. glaseri* Steiner on either CPB or SBW.

We therefore conducted laboratory tests to compare the susceptibilities of CPB and SBW larvae to *S. feltiae* and *S. glaseri*. We also conducted field cage tests to determine the effectiveness of *S. feltiae* applied to the soil in controlling these two pests.

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[1] *Steinernema* = *Neoaplectana*, and *S. feltiae* = *N. carpocapsae*.

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MATERIALS AND METHODS

A Mexican strain of *S. feltiae* originally collected from diseased codling moth larvae, *Laspeyresia pomonella* (L.) (11), was used in the test. Infective stage nematodes were propagated and stored as described by Lindegren et al. (7). Insect larvae used in the tests were field collected: SBW were of medium size (7–10th instars) and CPB were in the late fourth instar. The tests were conducted at Yakima, Washington, in Ritzville (Calciorthidic Haploxerolls) silt loam soil. Data were analyzed by analysis of variance and Duncan's multiple-range test. Percentages were transformed to arcsine $\sqrt{x}$ before analysis.

Laboratory tests: The first test was conducted to determine the susceptibilities of SBW and CPB larvae to either *S. feltiae* or *S. glaseri* in soil. For testing CPB larvae, 10-ml aliquots of an aqueous suspension of 25, 250, 2,500, or 25,000 nematodes were added to 9 × 6 cm paper cups containing 100 g of moistened soil (16 ml water/100 g dry soil). The control cups received 10 ml of water in place of the nematode suspension. Thus, the cups yielded concentrations of 0, 0.4, 3.9, 39.3, and 393.1 nematodes/cm² of soil. Five prepuparial larvae were placed in each of the cups, and the cups covered with plastic lids. SBW larvae (5/cup) were tested as described for CPB larvae, except that 50 g of soil and a 5-ml aliquot of nematode suspension were used per cup. Each cup was considered a replicate, and each treatment was replicated three times. Cups, held in polyethylene bags to prevent desiccation, were checked for insect mortality 1, 2, and 3 weeks later. At the end of 3 weeks, all insects were dissected and examined for presence of nematodes.

The second test was conducted with only *S. feltiae* to determine the effectiveness of concentrations higher than those previously tested against SBW larvae and to determine the minimum concentration needed to obtain 100% mortality of CPB larvae. This test was similar to the first test, except that the concentrations were 0, 31, 79, 157, 314, and 629 nematodes/cm² of soil, and mortality was checked 6 and 13 days after treatment. The LC_{50} was calculated using probit analysis.

Field cage tests: Screen cages measuring 61 × 61 × 30 cm high with open bottoms were placed ca. 5 cm deep in ground that had been previously rototilled and irrigated. In the center of each cage, a 32-mesh screen envelope measuring 15 ×15 × 2 cm and containing soil and five SBW larvae was buried horizontally 5 cm deep. Nematodes were applied to the soil in the cages with a sprinkling can containing the desired amount of nematode suspension in 2 liters of water. The soil was covered with potato foliage to provide food for larvae that may not have been ready to pupate, and CPB larvae were introduced into the cages. Immediately and periodically thereafter, the cages were sprinkler-irrigated as needed to keep the soil moist. Emerging CPB adults were counted and removed from the cages. At the end of 3 weeks, the SBW larvae were retrieved, examined for mortality, and dissected for presence of nematodes.

In 1980, two tests were conducted with concentrations of 0, 1.5, 15.5, and 155 nematodes/cm² of soil. In the first test, which was started on 3 July, 55 CPB larvae were used per cage and each treatment was replicated six times. In the second test, which was started on 6 August, 20 larvae were used per cage and each treatment was replicated four times.

In 1981, a test was conducted with concentrations of 0, 3.1, 31, and 310 nematodes/cm² of soil. Starting on 17 July, 50 spring-generation CPB larvae were used per cage and each treatment was replicated 12 times. On 20 August, the same cages were reinfested with summer-generation CPB and SBW larvae.

Bioassay: A technique described by Bedding and Akhurst (2) for detecting entomogenous nematodes in soil was modified as follows: A 1.9-cm-d soil core, 15 cm deep, was obtained from within each cage and the cores were placed separately in Petri dishes. Ten alfalfa looper larvae, *Autographa californica* (Speyer), were placed in each of the dishes, the dishes were then covered and examined for nematodes 3 days later. The cores were obtained 4 weeks after application of the nematodes in 1980, and both immediately after and 4 weeks after nematode application in 1981.

RESULTS

Laboratory tests: In the first test, *S. glaseri* was less effective than *S. feltiae* against SBW larvae (Table 1). However, even the highest concentration of *S. feltiae* (393 nematodes/cm²) caused only 58% mortality in 3 weeks. At that concentration, both nematode species caused 100% mortality of CPB larvae in 3 weeks. All dead specimens except those in the controls were infected with nematodes. None of the surviving SBW larvae and emerging CPB adults were infected. In the second test, the minimum concentration of *S. feltiae* that caused 100% mortality of CPB larvae after 13 days was 157 nematodes/cm² (Table 2), and the LC_{50} based on mortality after 6 days was 47.5 nematodes/cm². Only dead larvae from treated soil were infected with nematodes. The SBW larvae were infected with an extraneous nematode, probably belonging to the family Diplogasteridae; therefore, the results are not reported.

Field cage tests: In 1980, the mean mortality of CPB larvae was 59% for the highest nematode concentration tested (155 nematodes/cm² of soil), based on the number of adults recovered from the cages; doubling that concentration in 1981 increased mortality to 71% (Table 3). In 1981, reinfestation of the cages with summer-generation CPB larvae resulted in no differences in mortality between treatments since there were no differences in the number of adults recovered. Similar results were obtained with SBW larvae (Table 4), and reinfestation in 1981 resulted in the recovery of only one infected larva from a cage treated with 31 nematodes/cm².

Bioassay: In 1980, tests of soil from cages 4 weeks after treatment with nematodes showed that none of the alfalfa looper larvae were infected. In 1981, soil bioassayed immediately after application of nematodes in the cages resulted in combined totals of 100%, 74.6%, 16.6%, and 0% dead and infected alfalfa looper larvae for soil treated with 310, 31, 3, and 0 nematodes/cm² soil. However, test of soil from the same cages

Table 2. Mortality of Colorado potato beetle larvae (late fourth instars) exposed to various concentrations of *Steinernema feltiae* in cups of soil.

Nematode concentration (No./cm² of soil surface)	% Cumulative mortality* after indicated days†	
	6	13
31	40.0 c	86.7 a
79	60.0 bc	83.3 a
157	86.7 ab	100.0 a
314	100.0 a	100.0 a
629	100.0 a	100.0 a

*Corrected for control mortality with Abbott's formula. Means followed by the same letter within a column are not significantly different at the 5% level, Duncan's multiple-range tests.

†Means of three replicates/treatment; five larvae/replicate. All dead larvae, except those in the control, were infected with nematodes.

Table 1. Mortality of sugarbeet wireworm (SBW) and Colorado potato beetle (CPB) larvae exposed to various concentrations of *Steinernema feltiae* and *S. glaseri* in cups of soil.

Nematode concentration (No./cm² of soil surface)	% Cumulative mortality* after indicated week†					
	7–10th instar SBW			late 4th instar CPB		
	1	2	3	1	2	3
S. feltiae						
0.4	6.7 ab	13.3 b	13.3 bc	0.0 c	0.0 b	0.0 b
3.9	6.7 ab	28.3 ab	28.3 abc	6.7 c	6.7 b	0.0 b
39.3	20.0 a	33.3 ab	46.7 ab	0.0 c	13.3 b	6.7 b
393.1	26.7 a	58.3 a	58.3 a	86.7 a	100.0 a	100.0 a
S. glaseri						
0.4	6.7 ab	6.7 b	6.7 c	0.0 c	0.0 b	0.0 b
3.9	6.7 ab	6.7 b	6.7 c	0.0 c	6.7 b	8.3 b
39.3	6.7 ab	6.7 b	6.7 c	0.0 c	0.0 b	0.0 b
393.1	0 b	6.7 b	6.7 c	66.7 b	93.3 a	100.0 a

*Corrected for control mortality with Abbott's formula. Means followed by the same letter within a column are not significantly different at the 5% level, Duncan's multiple-range test.

†Means of three replicates/treatment; five larvae/replicate. All dead larvae, except those in the control, were infected with nematodes.

Table 3. Mortality of Colorado potato beetle larvae (late fourth instars) exposed to various concentrations of *Steinernema feltiae* applied to the soil in field cages.

Year	Nematode concentration (No./cm² of soil surface)	x̄ % Mortality*	Corrected mortality†
1980‡	0.0	28.7 c	
	1.5	25.2 c	2.8 c
	15.5	42.2 b	21.5 b
	155.0	71.7 a	58.6 a
1981§	0.0	45.8 c	
	3.1	43.5 c	12.3 c
	31.0	54.7 b	24.2 b
	310.0	81.8 a	71.0 a

*Means followed by the same letter for each year are not significantly different at the 5% level, Duncan's multiple-range test.

†Corrected for control mortality with Abbott's formula.

‡One test had six replicates (55 larvae/replicate) and another had four replicates (20 larvae/replicate).

§Twelve replicates (50 larvae/replicate).

4 weeks after treatment showed only one alfalfa looper larva infected, which was from a cage treated with 31 nematodes/cm².

DISCUSSION

Welch (14) reported that the DD-136 strain of *S. feltiae* infected and killed CPB adults in the laboratory. Veremchuk and Danilov (13) demonstrated that fourth-instar and adult CPB that were fed potato leaves infected with the agriotos strain of *S. feltiae* were susceptible to the nematodes. However, when we placed fourth-instar CPB in cups containing soil treated with a Mexican strain of *S. feltiae*, we found nematodes only in dead larvae and not in adults. Thus, it appears that infected CPB larvae did not mature to adults, and the surviving adults apparently escaped infection in treated soil.

Table 4. Mortality of sugarbeet wireworm larvae exposed to various concentrations of *Steinernema feltiae* applied to the soil in field cages.

Year	Nematode concentration (No./cm² of soil surface)	x̄ % Mortality*
1980†	0.0	0.0 b
	1.5	5.0 ab
	15.5	10.0 ab
	155.0	28.0 a
1981‡	0.0	0.0 b
	3.1	2.1 b
	31.0	10.1 b
	310.0	29.2 a

*Means followed by the same letter for each year are not significantly different at the 5% level, Duncan's multiple-range test.

†One test had six replicates and another had four replicates (five larvae/replicate).

‡Twelve replicates (five larvae/replicate).

In the second laboratory test with CPB larvae, each cup contained soil 2.2 cm deep, or 140 cm³ of soil. Thus, applying 157 nematodes/cm² was equivalent to applying 71 nematodes/cm³; this application rate caused 100% mortality, whereas Welch and Briand's (15) application of 115 nematodes/cm³ soil caused 60% infection. In field cage tests, we obtained 59% mortality of CPB with 1.55×10^6 nematodes/m² soil (= 155 nematodes/cm² soil) in 1980 and 71% mortality with 3.1×10^6 nematodes/m² soil (= 310 nematodes/cm² soil). Veremchuk and Danilov (13) obtained 66.8% mortality with 1×10^6 nematodes applied to the soil around a potato plant. Assuming a plant covers ca. 0.26 m² of soil (86-cm row spacing and 30-cm plant spacing), the rate that they used was 3.86×10^6 nematodes/m². Different strains of *S. feltiae*, different biotypes of CPB, and different soil types may account for the differences in mortality cited above.

In the 1981 tests, the expected persistence of the original nematodes applied to the soil in cages and the reproduction of nematodes in spring-generation CPB larvae were not evident in the summer exposures. The original nematodes could have been depleted by infecting larvae, by migration, or by death. Furthermore, in a separate laboratory study, nematode reproduction occurred in fall-generation larvae, but it was inhibited in spring-generation larvae (Lindegren, unpublished data). Therefore, the lack of the original nematodes along with the inhibition of reproduction in spring-generation larvae would account for the low mortality of the summer-generation larvae placed in the cages and of alfalfa looper larvae in the bioassays 4 weeks after treatment.

Only 28% control of SBW larvae was obtained when *S. feltiae* was applied to the

soil at a rate of 1.55×10^6 nematodes/m^2 in 1980. However, Danilov (3) obtained ca. 75% control of *Agriotes lineatus* and *Selatosomus aeneus* when the agriotos strain of *S. feltiae* was applied at a rate of 1.5×10^6 nematodes/m^2. These differences may have been due to a difference in nematode strains or in susceptibility of wireworm species or larval stages. Furthermore, what effect confining SBW larvae in screen envelopes had on their mortality is not known.

The movement of nematodes in the soil after they are applied to the soil surface undoubtedly influences the rate at which they infect the insect host. Reed and Carne (12) reported that infective stages of the DD-136 strain in the field tended to move toward the soil surface and thus failed to infect insect larvae in the soil. However, Moyle and Kaya (9) demonstrated that *S.* feltiae juveniles placed at different depths in sand remained more or less at those depths after 48 hours. When they placed nematodes on the sand surface, 90% were recovered at the 0–1-cm depth after 48 hours; yet, bioassays showed that 67% of *Galleria mellonella* (L.) pupae placed at the 8-cm depth were infected after 5 days. In field cage tests, we demonstrated that *S. feltiae* applied to the soil surface moved to a depth of 5 cm because SBW larvae placed at that depth were infected. The variable results obtained by different investigators pointed to the need for more research on factors affecting dispersal and survival of nematodes in soil under field conditions.

Inundative soil applications of the Mexican strain of *S. feltiae,* though cost prohibitive at present, were effective in reducing caged field CPB and SBW larval populations. Considering the availability of commercial sources of *S. feltiae* (6) and recently reported cost effective rearing methods (1), soil applications of entomogenous nematodes may be feasible as part of an integrated pest management program for control of CPB and SBW larvae in potatoes. Future investigations will be directed toward the evaluation of other entomogenous nematode species for their soil persistence and comparative mortality response and reproductive potential in CPB and SBW hosts.

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