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Soil Applications of Steinernematid and Heterorhabditid Nematodes for Control of Colorado Potato Beetles, *Leptinotarsa decemlineata* (Say)[1]

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Abstract: Three strains of *Steinernema feltiae* Filipjev (All, Mexican, and Breton strains) and one of *Heterorhabditis heliothidis* (Khan, Brooks, and Hirschmann) were evaluated for their potential to control Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), larvae and pupae in the soil. In laboratory studies, *H. heliothidis* and *S. feltiae* (Mexican strain) produced the highest mortality (6 days posttreatment) of CPB when applied to the surface of a soil column containing mature CPB larvae 5 cm below. Mortality ranged from 80 to 90% at rates of 79–158 nematodes/cm². Similar results were seen in a field microplot study with all four nematodes; *S. feltiae* (Mexican strain) and *H. heliothidis* were most effective. Adult CPB emergence was reduced 86.5–100% after application of 31–93 *H. heliothidis*/cm² and 88.4–100% with 93–155 *S. feltiae* (Mexican strain)/cm². The All strain of *S. feltiae* was moderately effective (ca. 80% reduction at 93–155 nematodes/cm²), while the Breton strain was ineffective (< 40% reduction at 155 nematodes/cm²). In small plots of potatoes enclosed in field cages, application of *H. heliothidis* and *S. feltiae* (Mexican strain) at rates of 93–155 nematodes/cm² before larval CPB burial in the soil resulted in 66–77% reduction in adult CPB emergence. Soil applications of these nematodes show potential for biological control of CPB.

Key words: Leptinotarsa decemlineata, Steinernema feltiae, Heterorhabditis heliothidis, potato, entomogenous nematode, biological control.

Chemical control of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), has led to high levels of resistance in CPB to most available insecticides (5) and to contamination of groundwater with aldicarb, carbofuran, and oxamyl (11). Entomogenous nematodes offer an attractive alternative to chemical insecticides for CPB control (18–20). These nematodes are safe to nontarget vertebrate organisms (6,14,15), their cost of production is rapidly decreasing (1), and they can be applied with commercially available sprayers (12).

Previous studies evaluating entomogenous nematodes for CPB control in North America have used either the DD-136 (13,20) and Mexican (18) strains of *Steinernema feltiae* Filipjev, or *S. glaseri* Steiner (18). Because entomogenous nematodes vary in their pathogenicity to insects (2), it is important to determine if other species or strains of entomogenous nematodes are pathogenic to CPB. Accordingly, we evaluated the All and Breton strains of *S. feltiae* and *Heterorhabditis heliothidis* (Khan, Brooks, and Hirschmann) for CPB control. The Mexican strain of *S. feltiae* was used for comparison with previous studies.

MATERIALS AND METHODS

H. heliothidis and *S. feltiae* (Mexican, All, and Breton strains) were produced by Biosis (Palo Alto, CA) using a modification of the method described by Dutky et al. (4) and stored on moist sponge pads at 6 C. Nematodes were washed out of sponge pads and suspensions were made just before their use. Subsamples of a concentrated nematode suspension were examined microscopically to determine the number of live infective juveniles per milliliter. The desired nematode rates were obtained by diluting the concentrated suspensions.

Laboratory screening test

All four nematodes were studied in the laboratory to select the best treatments for the field cage study. Field soil (sandy loam) from the Long Island Horticultural Research Lab (LIHRL) was screened (0.32-

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cm-pore sieve) to remove coarse gravel and lumps and put in plastic tubes (5 cm tall, 5 cm d). Ten milliliters of water was added to each tube to moisten the soil. Fourth-stage CPB larvae were collected from untreated potato plants at LIHRL and fed fresh potato leaves daily until larvae completed feeding and entered the prepupal stage (the inactive, nonfeeding stage prior to pupation). Then, one CPB larva was placed at the bottom of each tube by inverting the soil column on top of the larva and the tube bottom covered with aluminum foil. The appropriate nematode species or strain being studied was then applied to the tubes at rates of 0, 1.2, 2.5, 4.9, 9.9, 19.7, 39.5, 79.0, and 158.0 infective juveniles/cm^2. Nematodes were applied in ascending concentration to the top of the soil tube in 1 ml water. Each treatment was applied to 10 tubes. Tube tops were covered with plastic wrap to retard evaporation, and tubes were misted with water every 2–3 days to maintain soil moisture. Air temperature was recorded with a thermograph continuously during the study; soil moisture was measured gravimetrically at the beginning and end of the study. After 6 days the number of living and dead CPB was recorded.

Field studies

Microplot study: Circular microplots (2,374 cm^2) were constructed at LIHRL with sections of fiberglass sheeting (180 × 30 cm) sunk 15 cm into recently tilled soil. Twenty-five fourth-stage CPB larvae handled as in the laboratory test were placed in each microplot and covered with 5 cm of sieved field soil on 19 July. Plots were irrigated 3 days before introduction of larvae, and the soil in each microplot was moistened with 1 liter of water immediately after burial of larvae.

Nematode suspensions were made as in the laboratory test and applied in 2 liters of water evenly with a watering can to microplots on 21 July. All four nematodes were evaluated in the microplot study at the following rates: 31, 93, and 155 infective juveniles/cm^2. Control treatments of water were also applied. Each treatment was replicated five times in a randomized complete block design.

A cone-shaped aluminum screen emergence cage was placed over the top of each microplot and sealed at the base with soil to trap emerging CPB adults. After emergence began (2 August), traps were checked every 1–2 days until emergence was completed (12 August).

Because the means and variances of the number of CPB emerged per treatment were positively correlated, data were transformed by $\log_{10}(x + 1)$ before analysis of variance; mean separation was by Fisher's protected least significant difference (LSD) test ($P = 0.05$) (17). Data shown are back-transformed values.

Soil temperatures at depths of 5 and 15 cm were monitored continuously with a recording thermograph at the experimental site. Soil samples for moisture measurements were taken at weekly intervals at these depths from additional microplots treated similarly to those used in the experiments except that no CPB or nematodes were added.

Field cage studies: Small plots (two rows, 122 cm long) of potatoes (*Solanum tuberosum* L. cv. Katahdin) were planted at LIHRL and maintained using standard cultural practices. CPB were controlled in the plots with rotenone and piperonyl butoxide, sprayed as needed, before the start of the study. A fabric cage (180 × 180 × 180 cm) was placed over each plot and sealed with soil around the base just before nematode applications. Irrigation was applied the day before nematode applications.

H. heliothidis and *S. feltiae* (Mexican strain) were chosen for further evaluation on the basis of the laboratory screening test (Table 1). They were applied to the soil at 93 and 155 infective juveniles/cm^2 on 8 August. Nematodes were applied in 5 liters of water evenly with a watering can to the plot area of each cage. At the time of the application, potato plants were full grown and the vines were sprawled over the plot area, so nematodes were applied to the foliage

as well as to bare ground. Control treatments of water were also applied.

One hundred fourth-stage CPB larvae collected from untreated potato plants were introduced into each cage on 9 August. By 12 August most of these larvae had completed feeding, dropped off the plants, and burrowed into the soil to pupate. Later, emerging adults were removed and counted at 1–2-day intervals until emergence was complete.

Treatments were replicated four times in a randomized complete block design. Data were analyzed and presented as in the microplot study.

Soil moisture at depths of 5 and 15 cm was sampled weekly during the study from additional uninfested plots covered with tents and treated similarly to experimental plots. Soil temperature data at these depths were obtained from the thermograph established at the site of the microplot study which was adjacent to the field cage studies.

Results

Laboratory screening test

The results indicate that *H. heliothidis* and *S. feltiae* (Mexican strain) provided the best control of CPB larvae (80–90% mortality at 79–158 nematodes/cm²). Mortality in larvae treated with *S. feltiae* (All strain) reached 50%, whereas mortality with *S. feltiae* (Breton strain) did not exceed 30% at the highest rate applied (158 nematodes/cm²) (Table 1). The variable mortality of the control treatments may have been due to the extensive handling of CPB larvae required to set up this bioassay.

Soil moisture was 6.5 ± 0.5% (mean ± standard error) at the beginning of the study and 7.3 ± 0.7% at the end. Daily maximum and minimum air temperatures averaged 27.2 C (26–28) and 23.9 (23–26), respectively, during the study.

Field studies

Microplot study: The results of the microplot study (Table 2) were consistent with the results of the laboratory study. The two best treatments were *S. feltiae* (Mexican strain) and *H. heliothidis*. Intermediate levels of control were seen with *S. feltiae* (All strain), and low levels of control were seen with *S. feltiae* (Breton strain). The lower degree of control seen at the highest rate of *H. heliothidis* is puzzling when compared with the control achieved at the two lower rates of this nematode.

Table 1. Percentage of mortality (6 days post-treatment) in a laboratory screening test of nematode efficacy against Colorado potato beetle larvae.

Application rate (nematodes/cm²)	*H. heliothidis*	*S. feltiae* strain		
		Mexican	All	Breton
0	20	30	0	0
1.2	40	10	30	20
2.5	20	0	10	20
4.9	20	0	20	30
9.9	30	30	20	20
19.7	10	40	10	10
39.5	40	50	0	0
79.0	80	40	20	10
158.0	70	90	50	30

Nematodes were applied to the surface of plastic tubes (5 cm d, 5 cm tall) filled with soil; one mature fourth-instar CPB larva was placed at the bottom of each tube. Ten tubes were treated with each nematode at each rate.

Soil moisture at the 5-cm depth was 16.3 ± 0.5% (mean ± standard error) on 25 July and 13.9 ± 0.4% on 10 August; at the 15-cm depth, soil moisture was 17.4 ± 0.4% on 25 July and 18.6 ± 0.1% on 10 August. Daily maximum and minimum temperatures at the 5-cm depth averaged 28.3 C (24–32) and 21.1 (18–23), and at the 15-cm depth averaged 22.2 (20–27) and 17.8 (15–20).

Field cage study: Nematode application to the soil before larval burial and pupation resulted in a significant reduction in the number of emerging CPB adults with all nematode treatments (Table 3). The lower degree of control at the high rate of *S. feltiae* (Mexican strain) was unexpected. Except for very good control in one of the replicates at the lower rate, levels of control at both rates were about equal.

Weekly measurements of soil moisture for the period of the field cage study averaged 11.7% (9.5–14.2%) at the 5-cm

TABLE 2. Efficacy of nematodes applied in a microplot against Colorado potato beetle (CPB) larvae buried in soil, Riverhead, NY, 1985.

Nematode strain	Application rate (nematodes/cm²)	Number CPB adults emerged†	Percentage of reduction
Control	0	8.7	
H. heliothidis	31	1.2	86.5
	93	0	100.0
	155	3.2	62.9
S. feltiae	31	2.2	74.6
(Mexican strain)	93	1.0	88.4
	155	0	100.0
S. feltiae	31	3.2	62.9
(All strain)	93	1.7	80.4
	155	1.8	79.3
S. feltiae	31	9.0	−4.3
(Breton strain)	93	9.1	−5.0
	155	5.4	37.9
LSD ($P = 0.05$)		1.1	

Nematodes applied to soil surface after 25 last-stage potato beetle larvae per plot were buried 5 cm deep in soil; five replications.
† Data shown are backtransformed values.

TABLE 3. Efficacy of nematodes against Colorado potato beetles (CPB) in a field cage study, Riverhead, NY, 1985.

Nematode strain	Application rate (nematodes/cm²)	Number CPB adults emerged†	Percentage of reduction
Control	0	45.6	
H. heliothidis	93	27.3	40.2
	155	15.2	66.8
S. feltiae	93	9.7	78.7
(Mexican strain)	155	16.1	64.8
LSD ($P = 0.05$)		1.9	

Nematodes applied to soil surface 1 day before addition of 100 last-stage potato beetle larvae per cage; four replications.
† Data shown are backtransformed values.

depth and 12.2% (11.1–13.9%) at 15 cm. During this period daily measurements of maximum and minimum soil temperatures at the 5-cm depth averaged 27.2 C (22–34) and 21.1 (17–26), and at the 15-cm depth averaged 22.2 (16–29) and 17.8 (13–22).

DISCUSSION

The results of the laboratory and microplot studies were consistent in the identification of the two best nematode treatments. There were differences, however, in efficacy at equal rates of nematodes in the different studies.

H. heliothidis and *S. feltiae* were less effective in the field cage study than in the microplot study at equivalent nematode rates. This may have been due to several differences between the studies. Larvae were allowed to dig into the soil and bury to various depths in the field cage study, whereas they were all placed 5 cm deep in the microplot study. Thus larvae in the microplot study were distributed more uniformly, and this probably increased the uniformity of their exposure to nematodes. CPB larvae have been reported to bury from 1.3 to 15 cm deep in sandy loam soils (8). Thus some larvae in the field cage study could have buried to a depth that resulted in their being exposed to a lower nematode density than larvae in the microplot study. Another difference was that the field cage study soil was compacted by foot traffic in and around the plots while cages were set up and nematodes applied. This probably caused a less uniform distribution of nematodes than in the microplot study, either through uneven passage of the nematodes into the soil, or through soil compaction effects on soil pore size, which might have hindered nematode movement (7), resulting in pockets of low nematode density.

In both field studies (Tables 2, 3), it was noted that an increase in nematode application rates produced no significant increase in mortality. This has also been reported in other field studies (9,16).

In previous studies, Toba et al. (18) evaluated applications of *S. feltiae* (Mexican strain) to the soil against CPB larvae using laboratory and field methodologies somewhat different from ours. Even so, our laboratory and field results are similar to theirs; our 6-day laboratory bioassay resulted in 50% mortality at 39.5 nematodes/cm² (Table 1), compared with their 6-day LC_{50} of 47.5 nematodes/cm², and our field cage study (Table 3) resulted in 65% mortality compared with 59% in their

field cage study, both at 155 nematodes/cm². Our field cage study, however, had a higher control percentage at a lower rate (78.7% control at 93 nematodes/cm²).

These studies have demonstrated that soil applications of both *H. heliothidis* and *S. feltiae* (Mexican strain) are potentially useful in CPB management. The methodology of the field cage study simulates the use of nematodes under conditions of commercial potato production. We suggest that soil applications of nematodes could be made most effectively just before the time that first generation CPB larvae drop off potato plants to burrow in the soil and pupate. Soil applications would provide a favorable environment for survival of nematodes until CPB larvae enter the soil, as noted by Kaya (10). In the northeast region of the United States this application often could be combined with the last cultivation of potatoes to avoid a separate field operation. This also might help to ensure a uniform distribution of nematodes throughout the upper soil layer where most CPB pupate.

The rates of CPB control seen in our field cage study (60–80%) should be useful as part of a pest management program. This rate of control on the first larval CPB generation would have an important longer term impact on CPB population dynamics during the rest of the season, similar to the effect of soil applications of the fungal pathogen, *Beauveria bassiana* (3). Although preliminary studies by Toba et al. (18) could not demonstrate residual activity of two species of nematodes against CPB 6 weeks after application to field plots, any residual activity, perhaps over shorter periods, would be an additional advantage over many chemical insecticides. Because of these potential long-term effects, biological control agents such as nematodes should not be evaluated using the same short-term criteria as chemical insecticides.

Literature Cited

1. Bedding, R. A. 1981. Low cost in vitro mass production of *Neoaplectana* and *Heterorhabditis* species (Nematoda) for field control of insect pests. Nematologica 27:109–114.

2. Bedding, R. A., A. S. Molyneux, and R. J. Akhurst. 1982. *Heterorhabditis* spp., *Neoaplectana* spp. and *Steinernema kraussei*: Interspecific and intraspecific differences in infectivity for insects. Experimental Parasitology 55:249–257.

3. Campbell, R. K., T. E. Anderson, M. Semel, and D. W. Roberts. 1985. Management of the Colorado potato beetle using the entomogenous fungus, *Beauveria bassiana*. American Potato Journal 62:29–38.

4. Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1964. A technique for mass propagation of the DD-136 nematode. Journal of Insect Pathology 6: 417–422.

5. Forgash, A. J. 1985. Insecticide resistance in the Colorado potato beetle. Pp. 33–52 *in* D. N. Ferro and R. H. Voss, eds. Proceedings of the Symposium on the Colorado potato beetle, 17th International Congress of Entomology, Massachusetts Agricultural Experiment Station Research Bulletin No. 704, University of Massachusetts at Amherst.

6. Gaugler, R., and G. M. Boush. 1979. Nonsusceptibility of rats to the entomogenous nematode, *Neoaplectana carpocapsae*. Environmental Entomology 8:658–660.

7. Georgis, R., and G. O. Poinar, Jr. 1983. Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). Journal of Nematology 15:308–311.

8. Gibson, A., R. P. Gorham, H. F. Hudson, and J. A. Flock. 1925. The Colorado potato beetle in Canada. Dominion of Canada, Department of Agriculture Bulletin No. 52—New Series, Ottawa.

9. Harlan, D. P., S. R. Dutky, G. R. Padgett, J. A. Mitchell, Z. A. Shaw, and F. J. Bartlett. 1971. Parasitism of *Neoaplectana dutkyi* in white-fringed beetle larvae. Journal of Nematology 3:280–283.

10. Kaya, H. K. 1985. Entomogenous nematodes for insect control in IPM systems. Pp. 283–302 *in* M. A. Hoy and D. C. Herzog, eds. Biological control in agricultural IPM systems. New York: Academic Press.

11. Leach, S. S., W. E. Fry, R. T. Jones, R. Loria, R. H. Storch, R. D. Sweet, J. P. Tette, G. B. White, and R. J. Wright. 1986. Integrated systems for managing potatoes in the Northeast. Maine Agricultural Experiment Station Technical Bulletin No. 116, University of Maine at Orono.

12. Lindegren, J. E., J. E. Dibble, C. E. Curtis, T. T. Yamashita, and E. Romero. 1981. Compatibility of NOW parasite with commercial sprayers. California Agriculture 35:16–17.

13. MacVean, C. M., J. W. Brewer, and J. L. Capinera. 1982. Field tests of antidesiccants to extend the infection period of an entomogenous nematode, *Neoaplectana carpocapsae*, against the Colorado potato beetle. Journal of Economic Entomology 75:97–101.

14. Obendorf, D. L., B. Peel, R. J. Akhurst, and L. A. Miller. 1983. Non-susceptibility of mammals to the entomopathogenic bacterium, *Xenorhabditis nematophilus*. Environmental Entomology 12:368–370.

15. Poinar, G. O., Jr., G. M. Thomas, S. B. Presser, and J. L. Hardy. 1982. Inoculation of entomogenous nematodes, *Neoaplectana* and *Heterorhabditis* and their

associated bacteria, *Xenorhabditis* spp., into chicks and mice. Environmental Entomology 11:137–138.

16. Sosa, O., Jr., and J. B. Beavers. 1985. Entomogenous nematodes as biological control organisms for *Ligyrus subtropicus* (Coleoptera: Scarabaeidae) in sugarcane. Environmental Entomology 14:80–82.

17. Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics, 2nd ed. New York: McGraw-Hill Book Co.

18. Toba, H. H., J. E. Lindegren, J. E. Turner, and P. V. Vail. 1983. Susceptibility of the Colorado potato beetle and the sugarbeet wireworm to *Steinernema feltiae* and *S. glaseri*. Journal of Nematology 15: 597–601.

19. Welch, H. E. 1958. Test of a nematode and its associated bacterium for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Annual Report of the Entomological Society of Ontario 88: 53–54.

20. Welch, H. E., and L. H. Briand. 1961. Tests of the nematode DD 136 and an associated bacterium for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Canadian Entomologist 93:759–763.