Susceptibility of Prepupae of the Colorado Potato Beetle (Coleoptera: Chrysomelidae) to Entomopathogenic Nematodes (Rhabditida: Steinernematidae, Heterorhabditidae)[1]

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ABSTRACT This research investigated the ability of five species or strains of parasitic nematodes to control the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), when beetle prepupae were released on potting soil containing the nematodes. The nematodes tested were: *Steinernema carpocapsae* Weiser (All strain); *S. carpocapsae* (Mexican strain); *S. feltiae* (=bibionis) (Filipjev) (strain #27); *S. feltiae* (strain #980) and *Heterorhabditis bacteriophora* Poinar. Six dosage levels ranging from 10.3 to 329.2 nematodes/cm², in addition to the water-only controls, were used in these laboratory tests. Each dosage-strain combination was replicated four times with 20 prepupae exposed to each individual treatment. The five strains demonstrated 100% kill at 164.6 nematodes/cm². Except for the Mexican strain of *S. carpocapsae,* every strain produced over 98% mortality at 82.3 nematodes/cm². Dosage response regression equations were calculated for each strain. Although field applications probably would require higher rates to obtain the same mortality, the use of parasitic nematodes has potential for suppressing Colorado potato beetle populations. The cost or dosage rate of nematodes may have to be reduced before the control costs would be acceptable to a grower.

KEY WORDS Biological control, *Heterorhabditis bacteriophora,* pest, *Steinernema carpocapsae, Steinernema feltiae, Xenorhabdus,* Colorado Potato Beetle, *Leptinotarsa decemlineata.*

The genetic lability of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), has enabled the beetle to overcome various efforts to subdue it. The CPB has developed resistance to all major types of insecticides (Forgash 1985). It has been able to adapt to allelochemicals in wild plant species that normally are resistant to CPB attack (Groden and Casagrande 1986). Under laboratory conditions, the CPB has developed a tolerance to microbially produced toxins (Rie et al. 1990). Thus, diverse methods may need to be incorporated into a CPB IPM program to delay or prevent the occurrence of resistance. Entomopathogenic nematodes appear to have potential as a component of such an IPM program.

Efforts to control the CPB with nematodes have concentrated on the steinernematids and the heterorhabditids, for which mass rearing procedures in liquid fermentation have been developed (Friedman 1990). Death of the attacked insect results from the penetration and feeding by the nematode and from the septicemia produced by *Xenorhabdus* spp. bacteria released from the gut of the nematode into the insect's hemolymph.

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In previous laboratory tests designed to kill the prepupae, Welch and Briand (1961) were able to produce 60% mortality using *Steinernema carpocapsae* (DD-136 strain) at the rate of 115 nematodes/cc of soil; Toba et al. (1983) obtained 100% mortality with *S. carpocapsae* (Mexican strain) at the rate of 157 nematodes/cm^2 of soil; Wright et al. (1987) obtained 70% mortality with *Heterorhabditis heliothidis*, 90% mortality with *S. carpocapsae* (Mexican strain), 50% mortality with *S. carpocapsae* (All strain), and 30% mortality with *S. carpocapsae* (Breton strain) at the rate of 158 nematodes/cm^2 for each strain.

Although nematodes have great potential as pest control agents, they have been rarely used because of their cost and their inability to survive desiccation in an exposed environment (Welch and Briand 1961). The most suitable stages of the CPB for the application of nematodes appear to be the prepupal and pupal stages. The prepupa drops from the plant to the soil, which is usually shaded by the foliage, and burrows into the soil to pupate. Application of nematodes in water to the soil at this time would be expected to result in frequent contacts by the nematode with the CPB. The research described herein compared the efficacy of five strains of parasitic nematodes applied under laboratory conditions to potting soil that was subsequently infested with CPB prepupae. Two new strains of *S. feltiae* (=bibionis) were used for the first time against the CPB. The other three strains that showed promise earlier (Toba et al. 1983, Wright et al. 1987) were evaluated again in this larger test. Identification of efficacious nematodes would contribute to the development of an IPM program for the CPB.

Materials and Methods

Late fourth instar CPB were field collected and held with potato foliage until they exhibited burrowing behavior, at which time they were used in the experiments. Twenty prepupae each were added to plastic trays (22.5 by 13.5 by 7.5 cm deep) that contained one liter of potting soil mix. By volume, the mix contained 30% peat, 26% perlite, and 44% loam soil. Water was added to the mix to raise the moisture level to ca. 30%. Immediately before infesting with prepupae, the nematodes in 20 ml of water were poured onto the surface of the mix. The nematodes had been held at 5°C. The application rate of nematodes ranged from 3,125 to 100,000 per tray. The dosage data are reported in units of nematodes/cm^2 of surface area. One or more trays treated with water only were included as controls for each day's test. The 112 applications were made over a 9-day period as a sufficient number of CPB prepupae became available and included 28 controls. Each strain and dosage combination was replicated four times except for *S. feltiae* #980 and *H. bacteriophora* that were not tested at 329.2/cm^2 because both strains at the 164.6/cm^2 dosage resulted in 100% mortality. The infested trays were held in a controlled temperature cabinet at 24°C until all of the surviving beetles had emerged, a maximum of 17 days for a tray. Beetles emerging from treated mixes were dissected to determine if nematode infection had occurred.

The five nematode species/strains used in this experiment were: *Steinernema carpocapsae* Weiser (All strain); *S. carpocapsae* (Mexican strain); *S. feltiae* (=bibionis) (Filipjev) (strain #27); *S. feltiae* (strain #980); and *Heterorhabditis bacteriophora* Poinar. The taxonomy follows that of Poinar (1990).

An asymptotic growth curve relating mortality to dose was fitted for each nematode strain by nonlinear least squares, using the SAS NLIN procedure (SAS

Institute 1988). The percent mortality was adjusted for mortality in the untreated using the formula of Abbott (1925). The nonlinear model was $AM_{ij} = 1 - \exp(-R_j(\text{dose}_j - Z_j)) + \varepsilon_{ij}$ where AM_{ij} is adjusted mortality, R_j is the exponential for the jth strain, Z_j is the dose-axis intercept mortality for the jth strain, and ε_{ij} is the random error term. Estimates of the LD50 and LD95 for each strain were calculated from the nonlinear least squares estimates of R_j and Z_j. The parameters $[R_j]$, [LD50], and [LD95] were compared using asymptotic t-tests.

Results

There was a clear difference between nematode strains in their ability to kill the CPB, with the Mexican strain being the least capable. However, at dosages of 164.6 nematodes/cm^2, all five nematode strains killed all of the CPB (Table 1). The variations obtained could be ascribed to differences in the ability of the nematodes to move through the soil and find a host, ability to enter the host, the virulence of the bacteria released by the nematodes, or differences in nematode survival. Death appeared to occur in the prepupal or pupal stage. CPB killed as prepupae were darkened, with body cavities swollen with nematodes and bacteria. Nematodes were observed entering the spiracles. The trays were examined for emerged beetles five days a week. The number of dead adults found in the emergence trays by dose level across the five nematode strains (a total of 20 trays containing 400 prepupae per dose) was: 55 at 10.3 nemas/cm^2, 33 at 20.6 nemas/cm^2, 25 at 41.2 nemas/cm^2, and 3 at 82.3 nemas/cm^2. The two highest doses had no dead adults because all of the CPB had been killed in the prepupal or pupal stage. Nematodes were found in 24 of the 116 dead adults and one of the living adults (Table 1).

Table 1. Percent mortality, adjusted with Abbott's formula, of CPB exposed as prepupae to five strains of nematodes at six different concentrations using four replicates of CPB prepupae per each combination.

	Nematodes/cm^2					
Strain	10.3	20.6	41.2	82.3	164.6	329.2
S. carpocapsae All	52.3(4)	79.4(3)	95.2(1)	98.7	100.0	100.0
S. feltiae #27	15.9(1)	47.7(4E)	81.0(4E)	100.0	100.0	100.0
S. feltiae #980	49.2(2E)	58.7(2E)	90.5	100.0	100.0	—
H. bacteriophora	49.3(1)	77.8	88.8	98.5	100.0	—
S. carpocapsae Mex.	0	0	19.0	58.7(2)	100.0	100.0
Untreated n = 560; mean mortality = 21.25%						

Within parentheses are the number of emerged adults with nematode infection. An 'E' following a number indicates nematodes were encapsulated by the CPB defensive system although the beetles were dead.

Some nematodes of the *S. feltiae* strains were found to be encapsulated in the dead adult CPB. Webster (1973) reported encapsulation of *S. carpocapsae*, DD-136 strain, by CPB. We found no encapsulation of *S. carpocapsae* or *H. bacteriophora*, which is similar to the findings of Dunphy and Thurston (1990) and Seryczynska and Kamionek (1974). The small percentage of encapsulation observed suggests that this factor had little affect on total mortality. Jackson and Brooks (1989) found this to be true for *S. feltiae* attacking the western corn rootworm also. Because of the uncertainty of the cause of death of these 116 adults, in the analysis they were all treated as not killed by the nematodes. Therefore, the dose given to obtain specific mortality may be somewhat high, but some of the living may have been infected, which would partially compensate for the high dosages.

The R^2 of the overall model was 0.88, which indicates a good overall fit of the predicted mortality to the observed mortality. Table 2 contains the parameter estimates from the nonlinear regression for each nematode strain.

Discussion

Analyses of the rates and intercepts (Table 2) indicate that four of the nematode strains produced similar CPB mortality with the fifth nematode, the Mexican strain of *S. carpocapsae,* being less efficacious. The lethal doses to obtain 95% mortality are provided as a guideline for further research. The All strain, which produced the highest mortality, is commercially available at a cost of ca. $US 0.10 per million infective stage nematodes (Ramon Georgis, Biosys Inc., Palo Alto, California, personal communication). If 95% control was sought and this could be achieved at a dose rate of 42 nematodes/cm^2, it would require 4,200 million nematodes to treat a hectare at at cost of $420. If one application could achieve 95% control, this would be a practical method of treatment. Farmers have been reported spending up to $684 per hectare for insecticides to control mainly the CPB and have made a profit (Ferro et al. 1983). However, a long emergence period of the overwintering beetles can result in a diversity of stages being present at a time. To infect a majority of the prepupae as they enter the soil may require two to four applications, and may not be economical. Application when the majority of the first generation prepupae were entering the soil would have the greatest effect on the population. The efficacy of the nematodes would be dependent on the synchrony of CPB development, timing of the application, the weather (such as temperature, wind speed, cloud cover, solar radiation, relative humidity, rainfall, etc.), and soil factors (texture, pore size, moisture content, aeration, temperature, and chemistry [Kaya 1990, Georgis and Poinar 1983]).

These experiments were conducted under conditions favorable for nematode survival. Field application of nematodes would be expected to require higher dose rates because of the lethal effects of solar radiation (Gaugler and Bousch 1978), pathogenic soil fungi, and because of the desiccating effect of air movement that would reduce the active life of the nematodes. However, MacVean et al. (1982) have shown that the use of an antidesiccant in a morning application extended the life of nematodes on foliage from 20 minutes with water alone to two hours with water plus antidesiccant. If the nematode application was made in the evening, survival of the nematodes with antidesiccant was extended to 10 or 11 hours. Current research no doubt will reveal methods or materials that will extend even further the infective life of nematodes. New application techniques, such as applying the nematodes in irrigation water, have the potential to improve CPB control (Reed et al. 1986). Nematode strains naturally more tolerant to desiccation may yet be discovered or developed by a selection process (Gaugler 1988). The potential for parasitic nematodes to become an important component of an IPM program for the CPB, and other insect pests, is very high if the cost of the nematodes or the dosage required is reduced to an economically profitable level and the infective life of the nematode is substantially extended.

Table 2. Asymptotic growth curve of nematode density fitted to corrected Colorado potato beetle mortality. Percent mortality $= 1 - \exp(R_j(\text{dose}_j - Z_j))$; Z = intercept on dose axis; R = exponential rate parameter.

			Nematode/cm^2		
Strain	Intercept (± S.E.)	Rate (± S.E.)	LD50 (± S.E.)	LD95 (± S.E.)	R^2
S. carpocapsae All	- 3.859 (3.109) bc	0.065 (0.012) a	6.78 (1.44) c	42.10 (5.28) c	0.89
S. feltiae #27	6,829 (1.553) b	0.048 (0.007) a	21.17 (0.05) b	68.82 (8.43) b	0.91
S. feltiae #980	- 3.996 (4.528) c	0.043 (0.010) a	11.99 (1.85) c	65.11 (11.42) bc	0.83
H. bacteriophora	- 1.768 (5.283) bc	0.061 (0.020) a	9.61 (2.22) c	47.40 (11.54) bc	0.68
S. carpocapsae Mex.	17.451 (3.245) a	0.013 (0.002) b	70.80 (0.002) a	248.04 (33.03) a	0.88

Data in a column followed by the same letter are not significantly different at the P = 0.05 level according to asymptotic t-tests.

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References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Dunphy, G. B. and G. S. Thurston. 1990. Insect Immunity. Pages 301-323 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press: Boca Raton, FL.

Ferro, D. N., B. J. Morzuch, and D. Margolies. 1983. Crop loss assessment of the Colorado potato beetle (Coleoptera: Chrysomelidae) on potatoes in western Massachusetts. J. Econ. Entomol. 76: 349-356.

Forgash, A. J. 1985. Insecticide resistance in the Colorado potato beetle. Pages 33-52 *in* D. N. Ferro and R. H. Voss, eds. Proceedings of the Symposium on the Colorado Potato Beetle, XVIIth International Congress of Entomology, Univ. of Mass. Amherst, Res. Bull. No. 704.

Friedman, M. J. 1990. Commercial production and development. Pages 153-172 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL.

Gaugler, R. 1988. Entomogenous nematodes and their prospects for genetic improvement. Pages 457-484 *in* Biotechnology in Invertebrate Pathology and Cell Culture. Academic Press, San Diego, CA.

Gaugler, R. and G. M. Boush. 1978. Effects of ultraviolet radiation and sunlight on the entomogenous nematode, *Neoplectana carpocapsae.* J. Inver. Path. 32: 291-296.

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

Groden, E. and R. A. Casagrande. 1986. Population dynamics of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) on *Solanum berthaultii.* J. Econ. Entomol. 79: 91-97.

Jackson, J. S. and M. A. Brooks. 1989. Susceptibility and immune response of western corn rootworm larvae (Coleoptera: Chrysomelidae) to the entomogenous nematode, *Steinernema feltiae* (Rhabditida: Steinernematidae). J. Econ. Entomol. 82: 1073-1077.

Kaya, H. K. 1990. Soil Ecology. Pages 93-115 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL.

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. J. Econ. Entomol. 75: 97-101.

Poinar, G. O., Jr. 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. Pages 23-61 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL.

Reed, D. K., G. L. Reed, and C. S. Creighton. 1986. Introduction of entomogenous nematodes into trickle irrigation systems to control striped cucumber beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 79: 1330-1333.

Rie, J. van, W. H. McGaughey, D. E. Johnson, B. D. Barnett, and H. van Mellaert. 1990. Mechanism of insect resistance to the microbial insecticide *Bacillus thuringiensis.* Science 247: 72-74.

SAS Institute. 1988. SAS/STAT User's Guide, Release 6.03 Edition. Cary, NC: SAS Institute Inc., 1028 pp.

Seryczynska, H. and M. Kamionek. 1974. Defensive reactions of *Leptinotarsa decemlineata* Say in relation to *Neoaplectana carpocapsae* Weiser (Nematoda: Steinernematidae) and *Pristionchus uniformis* Fedorko et Stanuszek (Nematoda: Diplogasteridae). Bull. Acad. Pol. Sci. 22: 95.

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*. J. Nematol. 15: 597-601.

Webster, J. M. 1973. Manipulation of environment to facilitate use of nematodes in biocontrol of insects. Exper. Parasitol. 33: 197-206.

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

Wright, R. J., F. Agudelo-Silva and R. Georgis. 1987. Soil applications of Steinernematid and Heterorhabditid nematodes for control of Colorado potato beetles, *Leptinotarsa decemlineata* (Say), J. Nematol. 19: 201-206.