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Efficacy and Persistence of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) Applied through a Center-Pivot Irrigation System Against Larval Corn Rootworms (Coleoptera: Chrysomelidae)

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ABSTRACT We evaluated control of natural and artificially infested populations of larval corn rootworms (*Diabrotica virgifera virgifera* LeConte and *D. barberi* Smith & Lawrence) in corn (*Zea mays* L.) treated with entomopathogenic nematodes, *Steinernema carpocapsae* (Weiser); All strain, applied after planting through a center-pivot irrigation system at 2.5 and 1.2 × 10^9 infective juvenile nematodes per hectare. We compared control achieved with nematodes with that achieved with planting time applications of terbufos (Counter 15G [granular]; 9.9 kg [AI]/ha) and chlorpyrifos (Lorsban 4E [emulsion]; 1.12 kg [AI]/ha) applied after planting through an irrigation system, and with an untreated (water only) control. In 1989 studies with natural rootworm populations, chlorpyrifos and both nematode rates provided significantly lower root damage ratings than terbufos and the untreated control. Chlorpyrifos was significantly more effective than the low nematode rate. In 1990 studies with a natural rootworm infestation, the high nematode rate, terbufos, and chlorpyrifos provided significantly lower root damage ratings than the untreated control. Effectiveness of the low rate of nematodes was not significantly different from that of the untreated control. In both years, all treatments were generally less effective under higher rootworm pressure in artificially infested plots. In 1989, the high rate of nematodes significantly reduced adult western corn rootworm emergence compared with terbufos, the low rate of nematodes, and the untreated control. In 1990, all treatments significantly reduced western corn rootworm adult emergence compared with the untreated control. In 1989, viable nematodes were commonly found 1–3 d after application and at low levels 28 d after application. However, in 1990, no viable nematodes were found >7 d after application.

KEY WORDS corn rootworms, entomopathogenic nematodes, biological control

ENTOMOPATHOGENIC NEMATODES in the family Steinernematidae have potential for biological control of a variety of insects (Kaya 1985). They have several useful characteristics (e.g., they have wide insect host range, are able to kill hosts relatively quickly, can be economically mass produced, are noninjurious to vertebrates and are currently exempted from EPA registration requirements [Gaugler & Kaya 1990, Kaya & Gaugler 1993]).

Previous research has documented the susceptibility of *Diabrotica* spp. larvae to *Steinernema* spp. Laboratory studies (Jackson 1985, Jackson & Brooks 1989) evaluated *S. carpocapsae* (Weiser) [see Poinar (1990) for current information on nomenclature and synonomies of *Steinernema* species] against larval western corn rootworms (*D. virgifera virgifera* LeConte). Third instars were more susceptible to *S. carpocapsae* (Mexican strain) than first or second instars. Several field studies have evaluated different strains of *S. carpocapsae* against corn rootworms, primarily western corn rootworms (Rohrbach 1969, Munson & Helms 1970, Poinar et al. 1983, Levine 1984, Oleson & Tollefson 1985, Peters 1986). Generally, nematodes did not perform as well as available soil insecticides in these studies. Poinar et al. (1983) reported positive results, but the study was unreplicated.

Recently, Thurston & Yule (1990) evaluated *S. carpocapsae* and *S. feltiae* (Filipjev) [=*S. bibionis* (Bovien)] against larvae of northern corn rootworms (*D. barberi* Smith & Lawrence) in laboratory and field studies. Laboratory studies indicated that *S. carpocapsae*, Mexican strain, was more virulent against first-instar *D. barberi*

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compared with *S. feltiae*. Field studies with the Mexican strain of *S. carpocapsae* and *S. feltiae* showed that nematodes applied in furrow at planting time at 10,000 and 100,000 infective juveniles per meter of row (= 130×10^6 and 1.3×10^9/ha) significantly reduced larval northern corn rootworm populations compared with an untreated control, but provided significantly less control than an in-furrow application of fonofos at planting.

Most published studies in which *S. carpocapsae* has been tested against corn rootworms in field studies have used applications of nematodes at the time of planting; with the exception of the recent work by Thurston & Yule (1990), these studies have obtained variable but mostly negative results. This may be a result of the relatively long period between planting dates and corn rootworm egg hatch, during which nematodes are potentially exposed to desiccation, cool temperatures, and ultraviolet radiation, all of which could decrease their survival (Kaya 1985, Gaugler 1988). In addition, many previous researchers used relatively low rates of nematodes ($<10^9$/ha).

In the study described here, we evaluated the commercially available All strain of *S. carpocapsae* applied after planting through a center-pivot irrigation system. Currently, some insecticides labeled for corn rootworm control in field corn can be applied by injection into center-pivot irrigation systems (=chemigation) (Wright et al. 1992). These applications are timed to occur shortly after the hatch of corn rootworm eggs, but before economic damage to the roots occurs. Application of nematodes in a manner analogous to the chemigation treatments has several benefits over treatments at the time of planting, including better timing of nematode application in relation to the occurrence of rootworm larvae and improved soil moisture conditions for nematode survival after irrigation at the time of application.

The objective of our study was to evaluate the persistence and efficacy of *S. carpocapsae* (All strain) against corn rootworms when applied through a center-pivot irrigation system in corn. We timed application to be similar to that used with chemigation of chlorpyrifos for rootworm larval control. Chemigated chlorpyrifos, a planting-time soil insecticide (terbufos), and an untreated (water only) control were included for comparison.

Materials and Methods

All field studies were conducted at the University of Nebraska's Northeast Research and Extension Center near Concord, NE (Dixon County). Although the natural population of corn rootworms consisted primarily of western corn rootworms, northern corn rootworms also were present. The soil type was a Crofton silty loam (fine-silty, mixed [calcareous], mesic Typic Ustorthents) with 6–11% slope, <1% organic matter, and soil pH of 7.0. The previous crop was corn.

Chlorpyrifos and the two nematode treatments were injected into the water stream with similar but separate injection systems and applied through a four-tower center-pivot irrigation system that covered 5.54 ha and used low-pressure, low-angle, impact nozzles. Nematodes were suspended in water at known concentrations and held in a canister with continuous agitation during the application period. The injection pumps were positive-displacement diaphragm pumps (PULSA-feeder Microflo 680, Interpace, Rochester, NY) with the injection port located midway into the irrigation mainstream. Irrigation volume during each application was ≈2.2 cm.

Corn ('Pioneer 3377') was planted in rows 76 cm wide on 11 May 1989 and 29 May 1990 at 47,400 seeds per hectare. Alachlor (Lasso 4 Emulsifiable Concentrate [EC]) at 2.24 kg (AI)/ha and cyanazine (Bladex 4 Liquid [L]) at 1.96 kg (AI)/ha were applied in a 36-cm band over the row in 56.8 liters of water at planting. Fertilization included anhydrous ammonia (134 kg/ha) applied before planting and a 10-34-0 starter fertilizer applied (102 kg/ha) at planting.

Dauer-stage larvae of *S. carpocapsae* (All strain) were used in these studies. Nematodes were reared by Biosys (Palo Alto, CA) with liquid culture techniques (Friedman 1990); they were used in field studies within 1 wk of shipment date. They were held at 5°C until use.

Parallel independent studies were done during both years, with treatments applied against the natural rootworm infestation and against natural infestations augmented with artificial infestation of western corn rootworm eggs (600 per 30.5 cm of row). Rootworm eggs were applied to corn by trickling a known concentration of eggs suspended in agar into a shallow trench next to the base of the corn plant and then covering the trench with soil. Treatments were arranged in a randomized complete-block design with two replications. Treatments were applied on different dates in the natural and infested plots, corresponding to the first field observation of second-instar rootworms based on repeated sampling of soil and corn roots. This treatment timing is standard for chemigated application of chlorpyrifos. Plots of each infestation type were covered with a plastic tarpaulin during treatment to prevent double treatment of the plots. Plot size of the natural infestations was four rows by 30.5 m long; the artificially infested plots were four rows (1989) or two rows (1990) by 6.1 m long.

In the natural infestations during both years, we evaluated the following treatments: *S. carpocapsae* at 1.2 and 2.5×10^9/ha applied through the irrigation system, chlorpyrifos (Lorsban 4 Emulsion) at 1.12 kg (AI)/ha applied through the irrigation system, terbufos (Counter 15 Granular) at 9.9 kg (AI)/ha applied in an 18-cm band at planting, and an untreated control. In the artificial infestation plots, the same treatments were applied except that terbufos was not included. Irrigation water (2.5 cm) was applied within 48 h after the irrigation treatments were applied.

In 1989, corn rootworm eggs were applied on 23 May. Treatments were applied through the irrigation system on 21 June in the naturally infested plots and on 27 June in the artificially infested plots. In 1990, corn rootworm eggs were applied on 18 June, and treatments were applied through the irrigation system on 26 June in both the naturally and artificially infested plots.

Nematode Distribution Along Length of Center Pivot. During application of the nematodes (at both rates) on 21 June 1989, 470-ml samples of water were collected from two nozzles per span of the center pivot to determine the uniformity of distribution throughout the irrigation system and nematode survival. A 15-ml subsample was taken from each sample and examined microscopically. Nematodes that moved were counted as alive.

Root Damage Ratings. On 20 July 1989 and 23 July 1990, roots from 10 randomly chosen plants from each treatment replicate were dug from the soil, thoroughly washed to remove the soil, and rated for rootworm feeding damage using the 1–6 Iowa root-rating scale (Hills & Peters 1971): (1) no damage or only a few minor feeding scars; (2) feeding scars evident, but no roots eaten off to within 4 cm of the stalk; (3) at least one root, but less than one node of roots, eaten off to within 4 cm of the stalk; (4) one node of roots eaten off to within 4 cm of the stalk; (5) two nodes of roots eaten off to within 4 cm of the stalk; and (6) three nodes of roots eaten off to within 4 cm of the stalk.

Nematode Persistence. Soil samples for nematode bioassays were collected from each plot of the natural infestation 1 d before and 1, 3, 7, 14, and 28 d after the 21 June 1989 treatment, and 2 h (from water-only treatment) and 3, 7, and 14 d after the 26 June 1990 treatment. One sample (10 cm diameter by 10 cm deep) each was taken from within the row and between the row in each plot, thoroughly mixed, and refrigerated. Samples were packed in dry ice and sent by overnight express to Biosys (Palo Alto, CA) for bioassay. A 200-g subsample from each soil sample was divided into five equal portions, which were placed in five petri dishes (100 by 15 mm). Ten *Galleria mellonella* (L.) larvae were added to each dish, and the petri dish top was sealed with paraffin film. Dishes were held at room temperature (≈22°C) for 5 d. Counts of the number of live and dead *G. mellonella* were made; dead larvae were dissected and checked for steinernematid nematodes.

Adult Rootworm Emergence. On 11 July 1989 and 13 July 1990, saran cages (1.8 by 1.8 by 1.8 m) were placed in selected plots of the natural rootworm infestation, sealed with soil at the base, and monitored three times a week until 1 September for corn rootworm beetle emergence. Beetles were identified to species. One cage per replicate was placed in plots treated with terbufos, with the high nematode rate, and in the untreated control. In 1989, however, cages were placed in plots treated with the high nematode rate twice by mistake and not at all in the low nematode rate.

Plant Lodging. Plant lodging (another indicator of severity of root damage by corn rootworms) evaluations were made on 25 September 1989 and 9 October 1990 by counting the number of plants leaning in excess of 30 degrees from vertical in a randomly selected group of 20 consecutive plants in each plot.

Environmental Conditions. Soil moisture samples (one core per plot: 2.5 cm diameter by 10 cm deep) were collected 1, 7, 14, 21, and 28 d after the 21 June 1989 application. Soil moisture was determined gravimetrically (Brady 1974, p. 176). Soil moisture data were not collected in 1990, but rainfall and irrigation amounts were recorded each year at the study site.

Data Analysis. Root rating data and beetle emergence data from both years were analyzed by analysis of variance (SAS Institute 1985); means were separated by a protected least significant difference test ($P = 0.05$).

Results

Nematode Distribution Along Length of Center Pivot. Examination of water collected from the center-pivot nozzles while nematodes were being injected into the system in 1989 indicated that the nematodes were viable and distributed down the length of the system approximately in proportion to the application rate (mean = 94 nematodes per 15 ml, SD = 13 at 2.5 billion/ha; mean = 41, SD = 14 at 1.2 billion/ha; $n = 6$ for both rates). However, this center pivot was only 126 m long, compared with the standard 366-m-long systems in use commercially.

Root Damage Ratings. In 1989 (Table 1) in the natural rootworm infestation, chlorpyrifos and the high nematode rate both provided similar levels of root protection, significantly better than that provided by terbufos or the untreated control. The low rate of nematodes also provided significantly better protection compared with the untreated plots and terbufos, but was significantly less effective than chlorpyrifos. The plots that were infested artificially with western corn rootworm eggs to augment the natural infestation had higher levels of rootworms, as shown by the higher root damage ratings in the absence of any treatment (5.2 compared with 3.9 in the natural

Table 1. Root damage ratings of corn plants after application of several treatments to plots naturally and artificially infested (600 per 30.5 cm of row) with corn rootworm (*Diabrotica* spp.) eggs, Concord, NE, 1989 and 1990

Yr	Treatment, rate	Root damage rating (1–6 scale)	
		Natural infestations	Artificial infestations
1989	Untreated	3.9 ± 0.4a	5.2 ± 0.1a
	Applied in soil at planting		
	Terbufos, 9.9 kg (AI)/ha	3.9 ± 0.5a	—
	Applied through irrigation system after rootworm egg hatch		
	S. carpocapsae (All strain)		
	2.5×10^9/ha	2.7 ± 0.1bc	4.2 ± 0.2b
	1.2×10^9/ha	2.9 ± 0.4b	3.6 ± 0.4bc
	Chlorpyrifos, 1.12 kg (AI)/ha	2.3 ± 0.1c	2.7 ± 0.2c
1990	Untreated	4.8 ± 0.6a	4.7 ± 0.5a
	Applied in soil at planting		
	Terbufos, 9.9 kg (AI)/ha	2.4 ± 0.4c	—
	Applied through irrigation system after rootworm egg hatch		
	S. carpocapsae (All strain)		
	2.5×10^9/ha	3.7 ± 0.8bc	4.7 ± 0.7a
	1.2×10^9/ha	4.0 ± 0.3ab	4.0 ± 0.4a
	Chlorpyrifos, 1.12 kg (AI)/ha	3.4 ± 0.3bc	4.4 ± 0.7a

Mean ± SD, $n = 2$. Means in a column for each year followed by the same letter are not significantly different ($P = 0.05$; protected least significant difference test [SAS Institute 1985]).

infestation). The nematodes were less effective against this higher rootworm population; neither nematode rate provided root ratings <3.0. All treatments were significantly more effective than the untreated control, and the nematodes at the low rate provided control equivalent to that provided by chlorpyrifos.

In the natural rootworm infestations in 1990, terbufos, chlorpyrifos, and the high rate of nematodes all provided significantly improved levels of root protection compared with the untreated plots (Table 1). Terbufos provided significantly improved root protection compared with the low nematode rate. In the artificially infested plots, we detected no significant differences among any of the treatments and the untreated control.

Rootworm populations in all studies were adequate to produce root damage ratings >3.0 in the untreated plots (Table 1). Although the relationship between root damage ratings and yield loss is quite variable, ratings >3.0 suggest the potential for economic loss (Mayo 1986).

Nematode Persistence. In 1989, viable nematodes were detected with the *G. mellonella* bioassay for as long as 28 d after application, although the level of nematode activity decreased sharply in samples taken >3 d after application (Table 2). In 1990, nematode persistence was shorter; no nematode activity was detectable >7 d after application. In both years, we detected greater levels of nematode activity at the higher application rate. Bacterial (*Serratia* spp.) and fungal (*Beauveria bassiana* [Bals.] Vuill.) pathogens commonly were observed both years in *G. mellonella* without nematode infection. These pathogens were the major cause of mortality on day 7 and 14 in 1990 (unpublished data).

Adult Rootworm Emergence. In 1989, one of two groups of plots treated with the high rate of nematodes showed significantly reduced emergence of western corn rootworm adults in the naturally infested plots, compared with untreated plots or those treated with terbufos or the high rate of nematodes (Table 3). In 1990 all treatments significantly reduced western corn rootworm emergence compared with the untreated control.

In both years, the northern corn rootworm population was 2.4–5.5% of the total adult rootworm emergence. Although some significant differences were detected between treatments in both years (Table 3), we cannot reject the possibility that these differences were the result of a low, variable native northern corn rootworm population, rather than of biological effects of the treatments.

Plant Lodging. In 1989, 6% of the plants in the untreated control plots of the natural infestation were lodged; no lodged plants occurred in the other treatments. In the artificially infested plots, 4% of plants in the untreated plots and 1% of the plants in the plots treated with the high rate of nematodes were lodged; no lodging occurred in the other treatments. No lodging was observed in 1990 in any treatments.

Environmental Conditions. In 1989, percentage soil moisture was as follows: in the top 10 cm 1 d after application, 33.6%; 7 d, 33.0%; 14 d, 12.0%; 21 d, 13.6%; 28 d, 19.3%; in the subsoil (10–15 cm) 1 d after application, 23.5%; 7 d, 23.0%; 14 d, 16.8%; 21 d, 13.6%; 28 d, 19.0%. In 1990, soil moisture data were not collected. Irrigation and rainfall data for both years during the study period are shown in Fig. 1.

Discussion

Our study documents the efficacy of nematodes applied through a center-pivot irrigation system for management of corn rootworm larvae.

Table 2. Persistence of *S. carpocapsae* (All strain) after application of 1.2 and 2.5 × 10⁹ nematodes per hectare through a center-pivot irrigation system to corn, as determined by a *Galleria mellonella* bioassay, Concord, NE, 1989 and 1990

Yr	Nematode rate per ha (× 10^9)	Days after application	% dead *G. mellonella* larvae: With *S. carpocapsae*	% dead *G. mellonella* larvae: Without
1989	—	−1	0	23.0 ± 27.4
	1.2	1	50.5 ± 18.3	15.6 ± 20.8
	2.5	1	53.9 ± 24.9	18.5 ± 27.0
	1.2	3	18.2 ± 13.8	32.7 ± 23.2
	2.5	3	47.0 ± 29.1	14.9 ± 7.5
	1.2	7	7.2 ± 7.0	6.7 ± 6.6
	2.5	7	11.3 ± 15.3	4.7 ± 2.6
	1.2	14	11.8 ± 29.8	15.2 ± 25.8
	2.5	14	1.4 ± 2.1	7.0 ± 9.1
	1.2	28	0.3 ± 0.6	19.5 ± 26.1
	2.5	28	0.8 ± 1.1	5.3 ± 3.4
1990	Water only	0[a]	0	42.6 ± 33.5
	1.2	0	5.6 ± 6.3	51.8 ± 21.7
	2.5	0	22.0 ± 11.7	42.2 ± 22.7
	1.2	3	2.8 ± 3.5	20.6 ± 15.9
	2.5	3	6.4 ± 5.4	24.2 ± 13.2
	1.2	7	0.6 ± 1.0	76.2 ± 15.1
	2.5	7	1.0 ± 1.7	77.4 ± 16.1
	1.2	14	0	61.0 ± 19.8
	2.5	14	0	76.0 ± 13.6

Values are means ± SD; $n = 10$, except for 1989, −1 d after application, where $n = 20$; 3 d after treatment, where $n = 9$; and 1990, 2 h after treatment, water-only treatment, where $n = 5$.
[a] 0, 2 h after application.

Early field studies (cited above) in which steinernematid nematodes were tested against corn rootworms generally provided poor levels of root protection, probably because nematodes were applied at planting time and at rates much lower than in our study.

Compared with application of nematodes at planting time, use of this application method allows nematodes to be applied with better timing in relation to the occurrence of susceptible rootworm stages and provides an improved environment for nematode survival because of the increased soil moisture after irrigation. This application method should be applicable to other crops and pests as a means to increase the efficacy of nematodes as biological control agents. Previous work (Reed et al. 1986) documented the

Table 3. Emergence of corn rootworm (*Diabrotica* spp.) adults as influenced by planting time applications of terbufos and application of *S. carpocapsae* (All strain) through center-pivot irrigation to plots naturally infested with corn rootworms, Concord, NE, 1989 and 1990

Treatment	No. of emerged corn rootworm adults per cage: 1989 WCR[a]	1989 NCR[b]	1990 WCR	1990 NCR
Untreated	65.8a	2.0b	49.8a	0.2b
Applied in soil at planting				
Terbufos, 9.9 kg(ai)/ha	62.7a	0.7b	13.7b	0.5ab
Applied through irrigation system after rootworm egg hatch				
S. carpocapsae				
1.2 × 10^9/ha	—	—	26.7b	1.2a
2.5 × 10^9/ha	54.3a	7.1a	24.7b	0.9a
2.5 × 10^9/ha	20.6b	2.0b	—	—

Means in a column followed by the same letter are not significantly different ($P = 0.05$; protected least significant difference test [SAS Institute 1985]).
[a] WCR, *D. virgifera virgifera*.
[b] NCR, *D. barberi*.

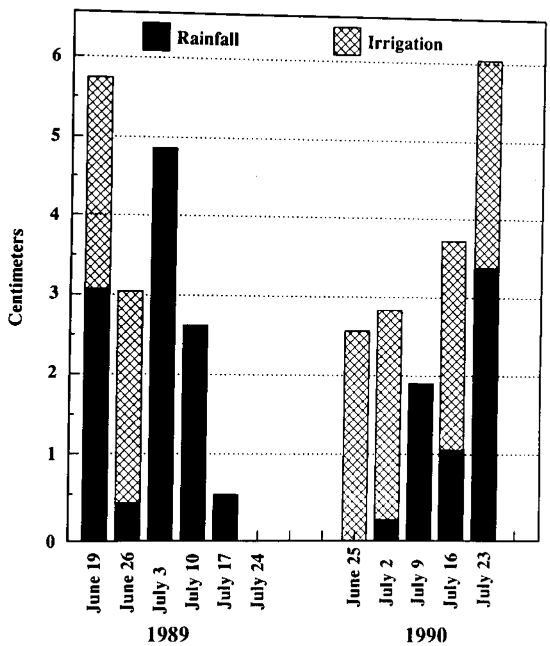

Fig. 1. Rainfall and irrigation amounts (cm) during study period, Northeast Research and Extension Center, Concord, NE, 1989 and 1990.

use of drip irrigation systems as a delivery method for nematodes used in soil insect control.

Published studies on nematode behavior may help explain some of the results of our study. *S. carpocapsae* usually moves <15 cm and often only 1–2 cm deep into the soil when applied to the surface, and movement is reduced in fine-textured soils (Moyle & Kaya 1981, Georgis & Poinar 1983, Schroeder & Beavers 1987). These studies have also documented some increase in downward movement when a host is present below ground. Limited downward movement by *S. carpocapsae* may explain in part why relatively high nematode rates (>10^9/ha) are needed for efficacy against rootworms.

Two previously published laboratory studies have examined the possible influence of plant roots on host-finding ability of *Steinernema* spp. In a study using young plants, Choo et al. (1989) found no reduction in the ability of *S. carpocapsae* to find *G. mellonella* larvae in the presence of corn, tomato, or marigold roots. However, Thurston & Yule (1990) concluded that *S. carpocapsae* and *S. feltiae* could not find *D. barberi* larvae feeding among and within corn roots, based on lack of reduction in larval densities when rates of nematodes expected to produce high levels of control were used. Additional studies are needed before conclusions can be made concerning whether corn roots interfere chemically or physically with the ability of nematodes to find rootworm larvae.

Based on results of a laboratory study, Thurston & Yule (1990) recommended that nematode applications be timed to coincide with the occurrence of first-instar northern corn rootworms so that larvae are controlled before they enter the roots and nematodes presumably are less able to find them. This contrasts with the strategy used in our study (i.e., application of nematodes to coincide with later rootworm instars). Our timing was chosen based on the results of Jackson (1985), whose laboratory bioassays indicated that western corn rootworm larvae increased in susceptibility to *S. carpocapsae* as they went through their three instars. In addition, the relatively short residual activity of entomopathogenic nematodes under the conditions of our study suggests that nematodes should be applied to coincide with the appearance of the most susceptible rootworm stages. Possible differences between *Diabrotica* spp. in instar-specific susceptibility to nematodes and the role of corn roots in nematode host finding must be clarified before the optimum nematode timing can be determined.

Use of adult rootworm emergence as an evaluation criterion for nematodes may not be a good indication of its value in corn rootworm management. Insecticides commonly are applied in a band centered on the seed furrow with the goal of protecting the central portion of the root system sufficiently to prevent lodging of the plant and to have enough roots for water and nutrient uptake, not to reduce adult numbers. For this reason, root damage ratings are commonly used to assess insecticide efficacy (Mayo 1986). Corn rootworm larvae may survive to adulthood even in insecticide-treated fields if they feed on roots growing outside the treated band. Data from Sutter & Lance (1991) show that reductions in adult emergence from use of soil insecticides at planting time may range from 16.5 to 81.1%, depending primarily on the insecticide and on soil moisture conditions during rootworm growth. Our results (Table 3) fall within this range. Similarly, some corn rootworms might survive a broadcast application of nematodes if they were feeding below the level of nematode activity, or if they were protected by feeding within or among corn roots.

Our study represents an extreme test for nematodes, because several environmental factors were not ideal. The soil type in these studies was fine textured, which does not favor nematode survival (Kung et al. 1990) or movement. Supplemental irrigation was applied only as needed for maintenance of the corn, and the soil surface dried out between irrigations. The lack of nematode persistence >7–14 d (Table 2) may be explained by the lower soil moisture during this period (generally <20%). In addition, efficacy against rootworms might be increased with different strains (Jackson 1985) or species of steinernematid nematodes.

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