Biological Control of the Western Corn Rootworm (Coleoptera: Chrysomelidae) Using the Entomopathogenic Nematode, *Steinernema carpocapsae*

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ABSTRACT A series of field trials evaluated the efficacy of the entomopathogenic nematode, *Steinernema carpocapsae* Weiser All strain for control of western corn rootworm, *Diabrotica virgifera virgifera* LeConte, larvae. Separate trials examined the effects of nematode application rate and timing on corn rootworm control in 1991. In the rate trial, *S. carpocapsae* (0, 10^4, 10^5, 10^6, and 10^7 nematodes per 30.5 row-cm) was applied as the insect population entered the second instar. Nematode treatment significantly reduced corn root injury and adult emergence. High application rates (10^6 and 10^7 nematodes per 30.5 row-cm) significantly outperformed low rates (10^4 and 10^5 nematodes per 30.5 row-cm). Nematode treatment (500,000 *S. carpocapsae* per 30.5 row-cm) reduced root injury and adult emergence slightly in the timing trial; later applications (second, third instar) were numerically, but not significantly, more effective than early (egg, first instar) ones. In 1992, a factorial trial combined four application dates (egg; first, second, third instar) and five rates (0, 10^4, 10^5, 10^6, 10^7 *S. carpocapsae* per 30.5 row-cm). Later applications (second, and particularly, third instar) were significantly more effective than early (egg, first instar) ones. Root injury and adult emergence in control and low rate (10^4 and 10^5 nematodes per 30.5 row-cm) plots significantly exceeded that in high rate (10^6 and 10^7 nematodes per 30.5 row-cm) plots. Commercially acceptable root protection (root injury ratings below 3.0) was provided by 10^6 nematodes per 30.5 cm of row applied to third instars, and by 10^7 nematodes per 30.5 cm of row applied to second and third instars.

KEY WORDS *Diabrotica virgifera virgifera*, *Steinernema carpocapsae*, biological control, western corn rootworm, field trial, entomopathogenic nematode

THE WESTERN CORN rootworm, *Diabrotica virgifera virgifera* LeConte, is an oligophagous, univoltine chrysomelid beetle, that damages corn (*Zea mays* L.) roots as a larva. Loss of root tissue may disrupt water balance and nutrient uptake, reduce silage and grain yield, and accentuate the risk of lodging (Branson et al. 1980, Mayo 1986, Spike and Tollefson 1988, 1989, Davis 1994). Corn rootworm management is currently limited to two basic strategies: annual rotation with a nonhost crop to break the insect life cycle; and soil insecticide application to limit corn root injury. Widespread adoption of crop rotation in Minnesota has decreased the acreage treated with soil insecticides by nearly 67% since the mid-1970s (Ostlie 1987). However, livestock feed demands, farm program limitations, and soil conservation restrictions can force farmers to plant continuous corn. Furthermore, the threat of corn rootworm attack often induces an "insurance" approach to insecticide use. In 1991, 328,000 ha were treated in Minnesota, making corn rootworm the primary insecticide target in the state (Subramanyam 1993).

Existing corn rootworm management strategies may not be sustainable. Northern corn rootworm, *Diabrotica barberi* Smith & Lawrence, populations in Minnesota, Iowa, and South Dakota have developed significant resistance to crop rotation through extended diapause (Krysan et al. 1986). Corn following soybeans has sustained severe root injury in an expanding area centered in east-central Illinois and northwestern Indiana, where strict adherence to a corn-soybean rotation has apparently favored western corn rootworm females that feed on and oviposit in soybeans (Sammons et al. 1997, Spencer et al. 1998). Reliance on soil insecticides also presents increasing difficulties, including groundwater contamination, phytotoxic interactions with herbicides, and toxicity to applicators and nontarget organisms (Ostlie 1987). Recent legislative action has further complicated corn rootworm management. Most of the insecticides labeled for use against corn rootworm are organophosphates, which will fall under the food residue restrictions imposed by the Food Quality Protection Act and will incur regulatory scrutiny by the U.S. Environmental Protection Agency. In the face of diminishing insecticide alternatives for corn rootworm control, an effective biological control agent would diversify and increase corn rootworm management options. The need for flexible "conventional" management strategies is highlighted by the advent of transgenic, corn rootworm-resistant corn. If resistance management plans parallel those for *Bt* corn and European corn borer, *Ostrinia nubilalis* (Hübner), growers will be required to plant refuge acres to maintain susceptible populations of corn rootworms (USEPA 1999). It is unlikely that they will chose to incur economic losses in these refuge acres.

Laboratory bioassays consistently demonstrate the susceptibility of western corn rootworm larvae to entomopathogenic nematodes, in particular, to the Steinernematidae (Jackson and Brooks 1989). However, the results of field trials evaluating *Steinernema carpocapsae* Weiser as a biological control agent for corn rootworm have been variable (Rohrbach 1969, Munson and Helms 1970, Levine 1984, Oleson and Tollefson 1985, Peters 1986, Thurston and Yule 1990, Wright et al. 1993, Jackson and Hesler 1995, Ellsbury et al. 1996, Jackson 1996). Numerous abiotic and biotic factors may negatively affect the field performance of *S. carpocapsae* against soil insects, including soil moisture and texture, temperature, solar radiation, pesticides, host defenses, natural enemies, host-seeking capability, and nematode persistence (Kaya 1990, Levine and Oloumi-Sadeghi 1992). Experimental design may also have contributed to inconsistent corn rootworm control in previous field trials. Nematode application rates used in some studies have been low compared with laboratory-determined LC_{50} values. In addition, application timing has often made agronomic, rather than phenological, sense, despite marked stadial differences in western corn rootworm susceptibility to *S. carpocapsae* (Jackson and Brooks 1995).

In light of the apparent contradictions between laboratory and field results, we undertook two studies in 1991: a rate trial to establish effective nematode application rates in the field; and a preliminary timing trial to investigate the effect of nematode application timing on larval western corn rootworm control. The results of these trials led us to conduct a more intensive rate and timing interaction trial in 1992 to investigate the individual and interactive effects of nematode application rate and timing on western corn rootworm control, and to establish optimal application parameters.

Materials and Methods

1991 Rate Trial. A completely random design with six replicates was used in this study. Treatments included five application rates (0 [agar control], 10^4, 10^5, 10^6, and 10^7 nematodes per 30.5 cm of row). Thirty experimental and three monitoring plots, each consisting of 30 plants ('Northrup King 3624', relative maturity 95 d, planted 10 June 1991 at 64,500 plants per hectare) in three rows of 10 plants (76 cm row spacing), were established in a Waukegan silt loam soil at the University of Minnesota's Rosemount Research and Outreach Center. The field was fall-tilled with a moldboard plow, and was field cultivated twice in the spring. Fertilizer was applied according to soil test results (N:P:K at 109:29:87 kg/ha), and the field was treated with the herbicides metolachlor at 2.24 kg [(AI)]/ha (Dual, Novartis Crop Protection, Greensboro, NC) and bromoxynil ocatanoate at 0.28 kg [(AI)]/ha (Buctril, Rhône-Poulenc, Research Triangle Park, NC) herbicides. Because northern corn rootworm eggs previously collected at Rosemount have displayed significant extended diapause capacity (Krysan et al. 1986), a field that had not been planted in corn during the last six years was used.

The experimental and monitoring plots were infested with 600 western corn rootworm eggs per 30.5 cm of row on 28 June. The plots were separated by 3.1 m within rows and by four rows. Eggs suspended in a 0.15% agar solution were applied with a 35-ml syringe (without needle) into freshly dug trenches on both sides of the row, ≈10 cm deep and 8 cm from the row, according to Chaddha (1990). The egg infestation rate was selected to maximize damage to the corn, while minimizing competitive stress to the corn rootworm larvae (Branson and Sutter 1985). Most plants were at the three- to four-leaf stage when infested. Eggs of western corn rootworm used in 1991 and 1992 were obtained from French Agricultural Research, Lamberton, MN.

Treatments of *S. carpocapsae* All strain were prepared 24 h before use by serial dilution of a stock suspension of 435,000 infective juveniles per milliliter (Biovector, Biosys, Columbia, MD) in 0.15% agar. Nematodes were applied in the early evening of 17 July. Approximately 12 ml of nematode suspension or agar was applied to the soil around the base of each plant with a 35-ml syringe (without needle). Because there had been no rain during the four previous days, the plots were then watered until the top 10 cm of soil appeared evenly moist, to ensure adequate moisture for nematode movement into the soil. Five plants were dug from the monitoring plots to determine western corn rootworm population age structure at nematode application. Sixteen late first and 14 second instars were found, as determined from measurements of head capsule width (Branson et al. 1975). Degree-day accumulations from western corn rootworm egg infestation to each nematode application in 1991 and 1992 are presented in Table 1. Degree-days were calculated by the sine wave method from air temperatures, using the computer program DEGDAY (Higley et al. 1986). Minimum and maximum developmental threshold temperatures of 11 and 18°C, respectively, were used (Davis et al. 1996). Temperature and precipitation data for the period starting one week before the first nematode application and ending the week after the last one in 1991 and 1992 are presented in Table 2.

Nematode treatment efficacy in the 1991 and 1992 studies was determined using corn root injury ratings and cumulative western corn rootworm adult emergence. Three emergence cages (one per row, covering two plants apiece) were installed in each plot on 24 July (Chaddha et al. 1993). The cages were monitored weekly until 23 September. On 17 September, 10 randomly selected root systems per plot were dug, washed, and rated using the Iowa 1–6 scale (Hills and Peters 1971). The sensitivity of the scale to subtle differences in corn rootworm control was increased

Table 1. Degree-days accumulated between western corn rootworm egg infestation and nematode application, 1991–1992

Year	Julian date	Days since western corn rootworm eggs infested	Targeted insect stage/instar	Degree-days, °C
1991	193	14	Egg	100
	196	17	1	119
	198	19	2 (early, rate trial)	133
	200	21	2 (middle)	147
	203	24	3 (early)	167
1992	170	9	Egg	105
	181	20	1	110
	190	29	2 (early)	160
	197	36	3 (early)	201

by subdivision into half-point increments (Chaddha 1990).

Data were analyzed by analysis of variance (ANOVA) using PC-SAS version 6.0 (PROC ANOVA, SAS Institute 1990). Adult emergence data were subjected to fifth-root transformation to stabilize variance before ANOVA. Transformations used for 1991 and 1992 data were selected according to Hinz and Eagles (1976). Orthogonal contrasts were used to isolate treatment differences. Treatment means resulting from this analysis were back-transformed. A "field LC_{50}" was calculated from the linear regression of percent mortality versus $\log_{10}$ (nematode application rate). Percent mortality for treated plots (untransformed data) was determined using the mean adult emergence from the control plots.

1991 Preliminary Timing Trial. A completely random design with three replicates was used in this study. Treatments included four nematode application dates at a single rate (5×10^5 nematodes per 30.5 cm of row) and agar control. Experimental plots, consisting of 20 plants in a single row, were laid out next to the rate trial and infested as previously described.

Nematode application dates were chosen to coincide approximately with western corn rootworm egg stage (12 July), first instar (15 July), second instar (19 July), and third instar (22 July), based on previous field observations of western corn rootworm development in Minnesota (L. French, personal communication). Application timing was confirmed with five-plant samples from the monitoring plots. Approximately 15 ml of an agar suspension of *S. carpocapsae* was applied, as above. The same suspension was used for all treatments; it was stored at 5°C between dates. Regular visual examination of stored nematodes revealed inconsequential mortality or loss of mobility over this period. Soil moisture was considered adequate on 12 July (1.30 cm of rain during the 48 h preceding application) and 22 July (3.51 cm of rain during the 48 h preceding application). On these dates, the top 10 cm of soil appeared evenly moist. However, postapplication watering was needed on 15 July (no rain during the previous 3 d) and 19 July (0.94 cm of rain early that morning, following six dry days).

Two emergence cages were installed in each plot on 24 July, and were monitored weekly until 23 September. Ten randomly selected root systems from each plot were dug, washed, and rated on 17 September. The data were analyzed as described above.

1992 Rate and Timing Interaction Trial. This two-way factorial experiment combined four nematode application dates, determined by western corn rootworm development (before hatch, and as the population entered first, second, and third instar) and five application rates (0 [agar control], 10^4, 10^5, 10^6, and 10^7 nematodes per 30.5 cm of row). Four replicates of these treatments were arranged in a randomized com-

Table 2. Temperature and precipitation during nematode application period, 1991–1992

Year	Julian date	Temp, °C				Total rainfall, cm	
		Daily mean		30-yr mean		Actual	30-yr mean
		High	Low	High	Low		
1991	186–192	26.6	15.8	29.6	15.7	1.24	2.21
	193–199[a]	29.9	18.3	29.5	16.0	0.84	2.00
	200–206[b]	28.4	17.5	29.3	15.9	5.26	2.99
	207–213	24.0	13.8	28.5	15.1	3.56	2.06
1992	163–169	28.3	15.6	26.3	12.8	9.65	3.30
	170–176[c]	21.3	10.2	26.9	13.5	0.20	2.62
	177–183[d]	23.8	11.8	28.6	15.0	1.70	2.66
	184–190[e]	24.5	13.3	29.2	15.6	9.68	1.65
	191–197[f]	23.7	14.0	29.2	15.7	3.96	2.35
	198–204	23.9	12.2	29.8	16.4	1.96	2.29

[a] 1991 timing trial egg stage (day 193) and first-instar (day 196) applications, rate trial (day 198) application.

[b] 1991 timing trial second- (day 200) and third-instar (day 203) applications.

[c] 1992 egg stage (day 170) application.

[d] 1992 first-instar (day 181) application.

[e] 1992 second-instar (day 190) application.

[f] 1992 third-instar (day 197) application.

plete block design. Experimental plots, consisting of 30 plants (Northrup King 3624, planted 19 May 1992 at 64,500 plants per hetare) in three rows of 10 plants (76 cm row spacing), were laid out as described for the 1991 trials in a field that had not been planted in corn during the previous 7 yr. The field was fall-tilled with a moldboard plow, and was disced and field cultivated in the spring. Fertilizer was applied according to soil test results (N:P:K at 114:30:90 kg/ha), and the field was treated with metolachlor at 2.24 kg [(AI)]/ha and bromoxynil ocatanoate at 0.28 kg [(AI)]/ha.

Eighty experimental plots and two monitoring plots were infested with 800 western corn rootworm eggs per 30.5 cm of row on nine June. The egg infestation rate was increased from that used in 1991 to ensure root injury ratings >3.0 in the controls. Most plants were at the one- to two-leaf stage when infested. The corn was infested at an earlier stage to increase the chances that western corn rootworm larvae would most severely damage roots of the fifth and sixth nodes, as opposed to the sixth and seventh nodes in 1991.

Nematodes were applied in the early evening of 18 June (egg), 29 June (first instar), 8 July (second instar), and 15 July (third instar). Monitoring of western corn rootworm development commenced on 22 June; four-plant samples were taken at 3-d intervals until 10 July and at 2-d intervals thereafter until the last nematode application. The egg stage application was made when the corn was still small enough to cultivate, but before western corn rootworm eggs were expected to hatch, based on previous field observations of western corn rootworm development in Minnesota (L. French, personal communication). Later applications were made on the day that each larval stage, or evidence thereof (e.g., feeding scars from first instars), was discovered. The second-instar application occurred when roughly equal numbers of first and second instars were present (12 late first instars and 13 early to mid-second instars, four root systems). The timing of the second-instar nematode application is comparable to that of the 1991 rate trial application. At third-instar application, most of the sampled larvae were large second instars (14 late first instars, 108 second, and 14 early thirds, three root systems).

Nematode treatments were prepared as previously described. Fresh suspensions were made for each application date. Approximately 14 ml of nematode suspension or agar was applied to the soil around the base of each plant. Soil moisture was considered adequate for nematode movement into the soil on 18 June (9.19 cm of rain in the 3 d preceding application), 29 June (1.55 cm of rain in the 48 h preceding application), and 15 July (0.56 cm of rain earlier that day). In each case, the top 10 cm of soil appeared evenly moist. In contrast, the plots were watered before nematode application on 8 July (no rain during the previous 6 d).

Three emergence cages were placed in each plot on 29 July. The cages were monitored weekly from 10 August to 14 October. On 3 September, 10 randomly selected root systems from each plot were dug, washed, and rated using the modified Iowa 1–6 scale. Data were analyzed as previously described. Adult emergence data were subjected to fifth-root transformation to stabilize variances before ANOVA. A "field LC_{50}" was calculated for the second- and third-instar applications.

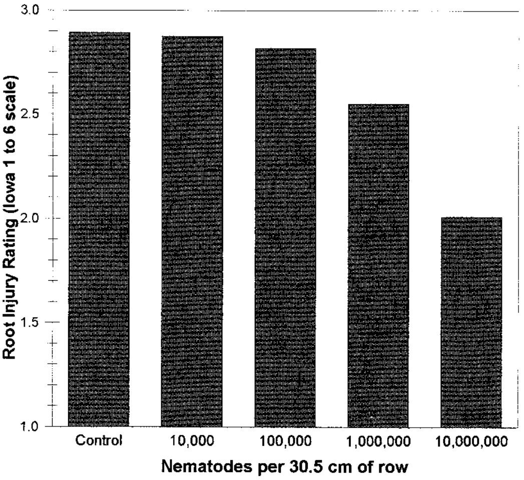

Fig. 1. Effect of *S. carpocapsae* application rate on corn root injury, 1991.

Results

Corn Root Injury. The extent of corn root injury inflicted by western corn rootworm larvae was significantly affected by the rate of *S. carpocapsae* application in 1991 ($F = 59.38$; df = 4, 25; $P < 0.0001$; Fig. 1). Root injury ratings in the control plots (2.89) were significantly higher ($F = 45.51$; df = 1, 25; $P < 0.0001$) than those in the treated plots, taken as a whole (2.56). However, root injury ratings in the low rate treatments (10^4, 10^5 nematodes per 30.5 cm of row) could not be statistically distinguished from the controls ($F = 0.89$; df = 1, 25; $P = 0.35$). High rate applications (10^6, 10^7 nematodes per 30.5 cm of row) were significantly more effective than low rate applications ($F = 168.61$; df = 1, 25; $P < 0.0001$). Within the high rate treatments, 10^7 nematodes per 30.5 cm of row provided root protection superior to that of 10^6 nematodes per 30.5 cm of row ($F = 77.03$; df = 1, 25; $P < 0.0001$).

In the timing trial, all four nematode applications reduced root injury slightly compared with the controls (2.89). Mean root injury ratings for the egg, first-instar, second-instar, and third-instar applications were 2.55, 2.57, 2.63, and 2.65, respectively. However, no application timing effect could be discerned on the basis of root injury ratings ($F = 0.31$; df = 3, 8; $P = 0.82$).

Western corn rootworm pressure was much higher in 1992 (Fig. 2). The mean control root injury rating was 4.10, signifying the loss of an entire whorl of roots. The experiment-wide mean root injury rating (3.76) indicated that many treatments provided little, if any, root injury protection. Nevertheless, *S. carpocapsae* did significantly affect corn root injury. Nematode

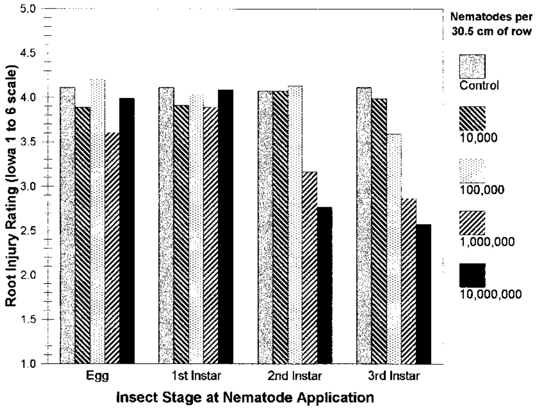

Fig. 2. Effects of *S. carpocapsae* application rate and timing of application relative to western corn rootworm development on corn root injury, 1992.

application rate (F = 15.18; df = 4, 57; P = 0.0001), timing of application relative to western corn rootworm development (F = 10.97; df = 3, 57; P = 0.0001), and rate-by-timing interaction (F = 3.95; df = 12, 57; P = 0.0002) were all highly significant factors.

The pattern of nematode effect evident in Fig. 2 is best addressed by first examining application timing. Egg and first-instar applications performed equivalently across rates (F = 0.18; df = 1, 57; P = 0.67), and both applications were significantly less effective than second- and third-instar treatments (F = 29.37; df = 1, 57; $P < 0.0001$). Third-instar applications provided better overall root injury protection than second-instar treatments (F = 3.35; df = 1, 57; P = 0.07).

The effect of nematode application rate was equally distinct. Corn plants in the agar control and low rate (10^4 and 10^5 nematodes per 30.5 cm of row) treatments suffered severe root injury compared with those in high rate (10^6 and 10^7 nematodes per 30.5 cm of row) plots (F = 59.41; df = 1, 57; $P < 0.0001$). The low rate treatments were equally ineffective on all application dates (F = 0.03; df = 1, 57; P = 0.87), and could not be distinguished from the controls (F = 1.24; df = 1, 57; P = 0.27). Notably, 10^6 nematodes per 30.5 cm of row provided root protection equivalent to the highest application rate (F = 0.04; df = 1, 57; P = 0.85). This was not the case in 1991.

Orthogonal contrasts on root injury ratings revealed two significant interactions between nematode application rate and timing relative to western corn rootworm development. The overall rate-by-timing interaction was highly significant (F = 34.82; df = 1, 57; $P <$ 0.0001), reflecting the inability of early and low-rate nematode treatments to reduce corn rootworm feeding injury. In addition, application timing strongly influenced nematode performance at effective rates. The root injury protection provided by 10^6 nematodes per 30.5 cm of row differed significantly from that of 10^7 nematodes per 30.5 cm of row when early (egg and first-instar) and late (second- and third-instar) applications were compared (F = 5.89; df = 1, 57; P = 0.02).

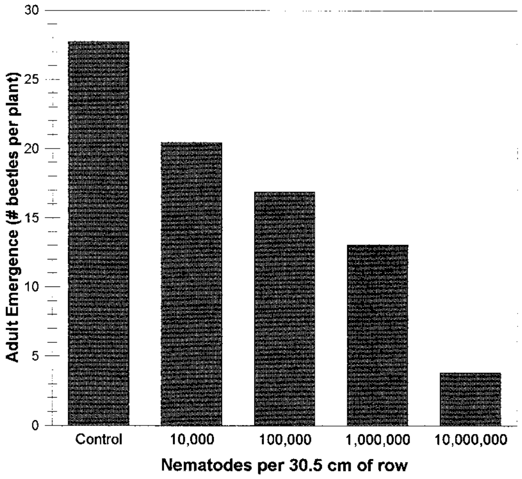

Fig. 3. Effect of *S. carpocapsae* application rate on western corn rootworm adult emergence, 1991.

Applying 10^6 nematodes per 30.5 cm of row had little effect on corn root injury until late second and early third instars comprised the majority of the western corn rootworm population. At 10^7 nematodes per 30.5 cm of row, however, root injury ratings declined sharply when nematodes were applied to late first and early second instars.

Adult Emergence. The effect of *S. carpocapsae* application rate on western corn rootworm adult emergence was highly significant in 1991 (F = 21.20; df = 4, 25; P = 0.001). Survival to adulthood decreased significantly when nematodes were applied at any rate (F = 38.20; df = 1, 25; $P < 0.0001$; Fig. 3). Adult emergence in the lowest rate plots (10^4 nematodes per 30.5 cm of row) was reduced 26.3% from emergence in the control plots. At 10^5 nematodes per 30.5 cm of row, adult emergence decreased 39.1% compared with the controls. This decrease represented an insignificant change from the lowest rate (F = 1.24; df = 1, 25; P = 0.28). High rate applications (10^6, 10^7 nematodes per 30.5 cm of row) performed significantly better than the low rates (F = 48.75; df = 1, 25; $P < 0.0001$), with 53.0 and 86.2% mortality occurring at 10^6 and 10^7 nematodes per 30.5 cm of row, respectively. These treatments also differed significantly (F = 34.68; df = 1, 25; $P < 0.0001$). A "field LC_{50}" of 2.85×10^5 nematodes per 30.5 cm of row was estimated from these results.

Western corn rootworm emergence in the 1991 timing trial declined numerically, but not significantly (F = 1.38; df = 3, 8; P = 0.32), with increasing larval age at the time of nematode application. Mean adult emergence in the egg, first-instar, second-instar, and third-instar treatments was 8.7, 6.8, 6.8, and 3.5 beetles per plant, respectively. These results suggested an increase in susceptibility to *S. carpocapsae* infection with larval age, which is consistent with laboratory results (Jackson and Brooks 1995).

Steinernema carpocapsae also significantly affected western corn rootworm adult emergence in 1992 (Fig.

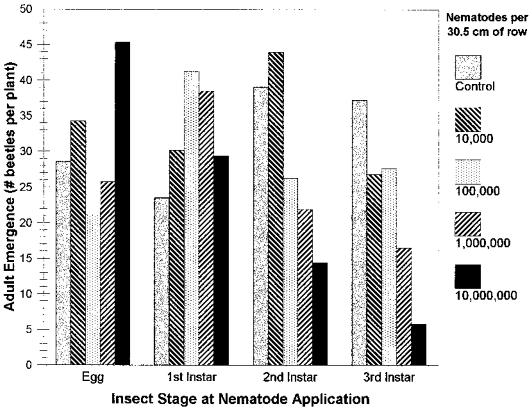

Fig. 4. Effects of *S. carpocapsae* application rate and timing of application relative to western corn rootworm development on western corn rootworm adult emergence, 1992.

4). The timing of nematode application ($F = 5.06$; df = 3, 57; $P = 0.0035$), rate ($F = 4.43$; df = 4, 57; $P = 0.0034$), and rate-by-timing interaction ($F = 4.59$; df = 12, 57; $P = 0.0001$) were all highly significant factors. The general pattern of nematode effect observed in root injury ratings was evident in adult emergence data, as well. Egg and first-instar applications reduced adult emergence less than second- and third-instar applications ($F = 9.43$; df = 1, 57; $P = 0.003$), and the early applications could not be distinguished from one another ($F = 0.23$; df = 1, 57; $P = 0.63$). However, the difference in performance between second- and third-instar applications was more apparent when adult emergence data, rather than root injury ratings, were examined. Significantly fewer beetles emerged from third-instar applications than from second-instar applications ($F = 5.53$; df = 1, 57; $P = 0.02$).

The effect of nematode application rate on western corn rootworm adult emergence observed in 1992 was similar to that seen in 1991. Significantly more beetles emerged from the control and low rate (10^4 and 10^5 nematodes per 30.5 cm of row) plots than from plots treated with 10^6 or more *S. carpocapsae* per 30.5 cm of row ($F = 13.83$ df = 1, 57; $P = 0.0005$). Over all application dates, the low rates did not reduce adult emergence compared with the controls ($F = 0.04$; df = 1, 57; $P = 0.84$), and could not be statistically differentiated from each other ($F = 1.32$; df = 1, 57; $P = 0.25$). The performance of 10^6 and 10^7 nematodes per 30.5 cm of row was statistically equivalent ($F = 2.54$; df = 1, 57; $P = 0.12$). This was initially surprising, beause adult emergence from plots treated with 10^7 nematodes per 30.5 cm of row at third instar was so low (six beetles per plant) compared with that observed in all other treatments. However, the success of third-instar applications was offset by the high emergence (45 beetles per plant) from egg stage applications of 10^7 nematodes per 30.5 cm of row.

Orthogonal contrasts of adult emergence data revealed two significant interactions between nematode application rate and timing relative to western corn rootworm development. The overall interaction of rate and timing was highly significant ($F = 29.22$; df = 1, 57; $P < 0.0001$), primarily reflecting the failure of the low rates and the effectiveness of high rate (10^6 and 10^7 nematodes per 30.5 cm of row), late date (second- and third-instar) treatments. In addition, the effects of 10^6 and 10^7 nematodes per 30.5 cm of row on adult emergence differed significantly when early (egg and first-instar) and late (second- and third-instar) treatments were compared ($F = 7.16$; df = 1, 57; $P = 0.009$). This difference may be partly attributed to the large number of western corn rootworm beetles that emerged from plots treated with 10^7 nematodes per 30.5 cm of row at egg stage.

Table 3. Percent reduction in western corn rootworm adult emergence, 1992

Application rate	Nematode application timing			
	Egg	First instar	Second instar	Third instar
10^4	−20.0	−28.6	−12.6	27.9
10^5	26.1	−75.8	32.7	25.8
10^6	10.0	−63.8	44.0	55.9
10^7	−58.4	−25.3	63.0	84.4

The complex mortality response observed in 1992 complicated estimation of the "field LC_{50}" values. No LC_{50} was calculated for egg or first-instar applications because a negative mortality response was observed in six of eight nematode treatments made on these dates (Table 3). For second-instar application, the LC_{50} was 3.08×10^6 nematodes per 30.5 cm of row. The third-instar application LC_{50} was 4.49×10^5 nematodes per 30.5 cm of row, reflecting an 85% increase in the susceptibility of the western corn rootworm population to *S. carpocapsae* infection with seven additional days of development.

Discussion

These trials demonstrated that *S. carpocapsae* All strain can reliably control western corn rootworm larvae in dryland corn, and that nematode performance is greatly enhanced by proper application timing. The significant application timing effect we observed in 1992 leads us to conclude that coordination of nematode application with corn rootworm development is the principal factor governing the success or failure of biological control efforts using *S. carpocapsae.* Age-specific host susceptibility is believed to be the primary contributor to this timing effect.

Western corn rootworm susceptibility to infection by steinernematid nematodes varies markedly with age (Jackson and Brooks 1995). Eggs are impervious to *S. carpocapsae* Mexican strain attack. In laboratory bioassays, the LC_{50} for first instars was 2,904 nematodes per insect, and the LC_{50} for pupae was 2,408 nematodes per insect. Susceptibility increased dramatically for second and third instars, with LC_{50}s of 172 and 35 nematodes per insect, respectively. On this basis, Jackson and Brooks (1995) asserted that nematode applications should be made when second and third instars constitute the majority of the population.

Data obtained in numerous studies, including our 1992 experiment, support this conclusion.

Field trials using entomopathogenic nematodes for larval corn rootworm control can be divided into two groups: those in which agricultural concerns determined nematode application timing; and those in which corn rootworm phenology determined nematode application timing. The first group is composed primarily of at-planting treatments. Some of the application methods used in these trials, such as tank-mixing with fertilizer, represent serious efforts to make nematode use compatible with standard corn-growing practices. Although limited successes have been reported (Peters 1986, Thurston and Yule 1990), at planting and other egg stage applications of *S. carpocapsae* have generally failed to significantly reduce corn root injury and yield loss or larval and emerging adult corn rootworm populations (Rohrbach 1969, Munson and Helms 1970, Levine 1984, Oleson and Tollefson 1985, Peters 1986, Wright et al. 1993 [1990 artificial infestation], Jackson 1996). Our 1992 egg stage treatments were also ineffective, even at much higher application rates than those used in the other trials.

Eclosion presents another early season opportunity for corn rootworm control. Under conventional management practices, postemergence liquid insecticide (e.g., carbofuran) may be applied at peak hatch. At this point in the growing season, it is still possible to drive through the rows without damaging corn, an important consideration in unirrigated fields. Rohrbach (1969) made surface, subsurface, and spray nematode applications at first hatch and 1 wk thereafter (spray only). All failed to reduce root injury and larval western corn rootworm populations. We also found hatching time (first instar) applications to be ineffective in 1992, although high rate treatments (10^6 and 10^7 *S. carpocapsae* per 30.5 cm of row) did reduce adult emergence from its peak (10^5 nematodes per 30.5 cm of row).

The second group of trials focuses on the relationship between corn rootworm larval development and stadial susceptibility to nematode infection. Treatments were delayed until second-instar corn rootworms were present (Wright et al. 1993) or predominant (Jackson and Hesler 1995, Ellsbury 1996, Jackson 1996). *S. carpocapsae* significantly reduced root injury and adult corn rootworm emergence in these studies, often matching the performance of insecticide standards. Our 1991 rate trial and 1992 second- (present) and third-instar (predominant) applications targeted similar corn rootworm population age structures, with equal effect. The use of multiple application dates in our 1992 field trial tested the "second- and third-instar" application theory directly, and clearly differentiated between ineffective (egg, first instar) and effective (second- and third-instar) nematode application timing (Figs. 2 and 4). This study also showed that western corn rootworm control is significantly improved by applying nematodes when the vulnerable late second and early third instars are present, rather than applying nematodes to younger second instars in anticipation of host susceptibility. Because only 7 d elapsed between the second- and third-instar treatment dates, these changes in nematode performance raised serious questions about *S. carpocapsae* survival and activity under cornfield conditions.

Soil temperature and moisture are key determinants of nematode survival (Kaya 1990). Neither is considered a significant factor in the timing effects we observed in 1991 and 1992. Overall, weather conditions during both nematode application periods can be characterized as moderate. Daily high and low air temperatures were at or below the 30-yr means on the egg and first-instar (timing trial) application dates in 1991. The rate trial, second-, and third-instar applications fell during a week-long "warm" spell (<4°C above the 30-yr means). The entire application period was cooler than the 30-yr means in 1992 (Table 2). Temperatures were well within the range favorable for nematode activity (Kaya 1990) in both years. There were no extended (>7 d) dry spells during the treatment period in either year, nor were there any flooding rains.

The narrow, late window of opportunity for corn rootworm infection by *S. carpocapsae* presents a logistical challenge to its use in dryland corn, which would be diminished if nematode activity remained high for more than a few days. Unfortunately, reported short-term persistence in cornfields is poor, even with irrigation. In trials against corn rootworm (Wright et al. 1993) and black cutworm [*Agrotis ipsilon* (Hufnagel); Levine and Oloumi-Sadeghi 1992], *S. carpocapsae* basically disappeared within 7 or 8 d of application. Wright et al. (1993) noted that All strain activity fell rapidly in irrigated fields, decreasing up to 95% in the week after application. This precipitous drop may explain why nematodes applied 8 d after western corn rootworm egg infestation failed to prevent severe root injury in a chemigation trial (Wright et al. 1993). A similar decline would substantially account for the poor nematode performance we observed in our 1992 egg stage and first-instar applications.

Although application timing is crucial, our studies also demonstrated that *S. carpocapsae* performance depends on application rate. No treatment of $<10^6$ nematodes per 30.5 cm of row significantly reduced corn root injury in 1991 or 1992. Adult corn rootworm emergence was more sensitive to rate effects. Significant reductions in emergence were achieved by as few as 10^4 nematodes per 30.5 cm of row, in properly timed applications. This level of response to nematode application rate is unusual. Several studies have included nematode rate trials, without generating definitive results (Rohrbach 1969, Munson and Helms 1970, Levine 1984, Oleson and Tollefson 1985, Thurston and Yule 1990, Wright et al. 1993, Ellsbury et al. 1996). It should be noted, however, that most of these investigators applied nematodes well before corn rootworm larvae were susceptible to infection, and that the rates used in their studies were a fraction of our high rates. Jackson and Hesler (1995) did observe significant improvement in root protection with increasing *S. carpocapsae* Mexican strain rate (25,000–200,000 nematodes per plant, applied to second instars), but adult emergence did not exhibit a significant rate response.

The performance measurements used in this study were chosen to provide the broadest basis for comparison with other corn rootworm management strategies (root injury ratings) and the best integration of treatment effects (adult emergence). Corn root injury ratings are, by far, the most frequently reported data in corn rootworm management studies. A damage threshold for root injury of 3.0 on the Iowa 1–6 rating scale is widely used (Mayo 1986), and is considered the benchmark for commercially acceptable corn rootworm control. Three treatments held root injury ratings below 3.0 in 1992, despite heavy corn rootworm pressure: 10^6 nematodes per 30.5 cm of row applied to early third (root injury rating = 2.86) instars, and 10^7 nematodes per 30.5 cm of row applied to second (2.76) or early third (2.58) instars. Adult emergence data can also be presented in terms of a widely recognized benchmark value, in this case, a 50% reduction in adult emergence (LC_{50}). Achieving a 50% adult emergence reduction required the application of at least 10^6 nematodes per 30.5 cm of row in 1991 and 1992 (third-instar application).

Patterns of nematode application timing and rate effects that were not evident in corn root injury data in 1992 were revealed by adult western corn rootworm emergence data. Several nematode treatments enhanced adult emergence relative to the controls, particularly in the egg and first-instar applications (Fig. 4). These data are thought to reflect the interplay between mortality caused by nematode infection and improved survival resulting from reduced competition between late instar larvae for feeding sites on the corn. The interaction of these processes is apparent when peak emergence treatments are examined. The application rate that fostered the best survival decreased progressively (from 10^7 nematodes per 30.5 cm of row to agar control) as nematode application timing improved (from egg stage to third instar). On the other side of the equation, the negative effects of competition between larvae has been documented in previous field trials using western corn rootworm egg infestations. Survival to adulthood declined once densities exceeded 600 western corn rootworm eggs per 30.5 cm of row (Branson et al. 1980, Branson and Sutter 1985, Spike and Tollefson 1988). Adult recovery rates in these studies ranged from 1.6 to 7.5% of the western corn rootworm eggs infested. Our adult recovery rates were similar, and they indicated a possible "crowding effect" in the 1992 study. In 1992, 6.0% of all control eggs reached adulthood (32 beetles per plant, 800 eggs per 30.5 cm of row); but 6.9% of the 1991 control eggs survived (600 eggs per 30.5 cm of row). The best performance of 10^7 nematodes per 30.5 cm of row was remarkably similar in both years. Approximately 1% and 1.1% (third instar applications) of the eggs in this treatment were collected as adults in 1991 and 1992, respectively. In contrast, 8.5% of the eggs treated with 10^7 nematodes per 30.5 cm of row before hatch emerged as adults in 1992.

Although *S. carpocapsae* All strain successfully controlled western corn rootworm larvae in our studies, several obstacles must be overcome before entomopathogenic nematodes can be considered a viable management alternative for this pest. The first obstacle is cost. Effective application rates identified in our studies (10^6 and 10^7 nematodes per 30.5 cm of row, or 43.1 billion and 431 billion nematodes per hectare, respectively) were far higher than those used in other trials (Rohrbach 1969, Munson and Helms 1970, Levine 1984, Oleson and Tollefson 1985, Peters 1986, Thurston and Yule 1990, Wright et al. 1993, Jackson and Hesler 1995, Ellsbury et al. 1996, Jackson 1996), and would be prohibitively expensive. All strain is the least virulent nematode yet assayed against western corn rootworms (Jackson and Brooks 1989). Screening additional strains and species should identify more virulent candidates. Even then, entomopathogenic nematodes may function best when applied against corn rootworm larvae using an innundative approach, as "biological insecticides" rather than "biological control agents" in a strict sense.

The corn rootworm-corn ecosystem is poorly suited to "classical" biological control using entomopathogenic nematodes. It is an annual habitat, frequently disrupted. The availability of alternate hosts to sustain and amplify nematode populations in a timely manner is not ensured. Immature western corn rootworms are poor propagative hosts, whose small body size and fragile integument restrict steinernematid reproduction to a single generation (Jackson and Brooks 1995). Nematode reproduction does not occur in first instars. Therefore, significant alternate host populations (e.g., noctuid, scarabeid, or elaterid larvae) would be needed early in the season to amplify nematode populations while the corn rootworm larvae developed. Nematodes would have to move back into these alternate hosts once the surviving corn rootworms pupated.

The final obstacle is application technology in dryland corn. This may be the most difficult challenge, given the high moisture requirement of many nematodes for soil infiltration, the narrow window of application opportunity, and the height of the corn when corn rootworm larvae reach that window (typically higher than 70 cm). In most years, nematode application may be practical only via chemigation, as demonstrated by Wright et al. (1993) and Ellsbury et al. (1996). Commercial nematode use in dryland corn may, therefore, not be possible until a slow-release, persistent nematode preparation can be incorporated into the soil at planting or during cultivation.

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