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Parasitism of Western Corn Rootworm Larvae and Pupae by *Steinernema carpocapsae*

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Abstract: Virulence and development of the insect-parasitic nematode, *Steinernema carpocapsae* (Weiser) (Mexican strain), were evaluated for the immature stages of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Third instar rootworm larvae were five times more susceptible to nematode infection than second instar larvae and 75 times more susceptible than first instar larvae and pupae, based on laboratory bioassays. Rootworm eggs were not susceptible. Nematode development was observed in all susceptible rootworm stages, but a complete life cycle was observed only in second and third instar larvae and pupae. Nematode size was affected by rootworm stage; the smallest infective-stage nematodes were recovered from second instar rootworm larvae. Results of this study suggest that *S. carpocapsae* should be applied when second and third instar rootworm larvae are predominant in the field.

Key words: biological control, corn rootworm, *Diabrotica virgifera virgifera,* entomopathogenic nematode, nematode, *Steinernema carpocapsae.*

Steinernema carpocapsae (Weiser) has good potential as a biological control agent for many insect pests, especially those residing in the soil (4,11). Infective-stage juveniles are capable of locating and rapidly killing insect hosts in soil habitats. Successful control has been achieved for several soil insect pests in potted plants (7,25), citrus (21), and cranberry (22).

Corn rootworms are the most serious insect pest complex of maize in North America. Rootworm immatures reside in the soil; larvae feed on corn roots and disrupt root functions of support and nutrient-water transport. The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, and northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, are the dominant economic species in the complex. Field tests to control rootworm populations with *S. carpocapsae* have yielded mixed results (15,19,23,26). Differences in strain virulence, application techniques, and environmental conditions have been cited as possible reasons for variable field performance (6).

Little is known about the parasitic relationship between *S. carpocapsae* and corn rootworm larvae and pupae. Laboratory studies have documented the susceptibility of first instar northern corn rootworm larvae (23) and third instar western corn rootworm larvae using several strains of *S. carpocapsae* (8). Melanotic encapsulation with several strains of the nematode has been reported in western corn rootworm larvae (8). General patterns of immature susceptibility and nematode development can be inferred from studies on other insects; however, specific information is important for the development of pest-specific control methods. We report on the relative virulence and development of the Mexican strain of *S. carpocapsae* in the larval and pupal stages of the western corn rootworm.

Materials and Methods

Susceptibility of western corn rootworm eggs, larvae, and pupae to *S. carpocapsae* (Mexican strain) was compared using concentration-mortality bioassays. The Mexican strain was chosen because it was the most virulent of four previously tested strains (8). Infective-stage nematodes supplied by biosys (Palo Alto, CA) were produced in *Galleria mellonella* (L.) larvae. Nematodes were stored at 10 C for 0.5–2

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months on moist polyurethane foam before use. Active nematodes were selected for bioassays by collecting those that moved through a nylon screen (28-μm pore size), which was suspended in a separatory funnel filled with distilled water. For each bioassay, a dilution series of 6–8 nematode concentrations, including a control, was prepared. Preliminary bioassays guided the selection of final dilution series for each insect stage. Nematode concentrations per insect ranged from 440 to 8,480 (eggs), 62 to 10,800 (first instar), 20 to 1,984 (second instar), 16 to 185 (third instar), and 393 to 12,560 (pupa). The middle concentration was expected to yield near a 50% mortality response. Nematode concentrations were prepared with 0.15% agar to suspend the nematodes and facilitate the dilution process. Calibration of a series was based on sample counts from the median suspension of the series. Suspensions were prepared one day prior to use in the bioassay and held at 10 C in darkness.

Selected stages of the western corn rootworm from a laboratory colony in its first generation were used. Postdiapause eggs within 4–10 days of eclosion were used for the egg bioassays. Rootworm larvae were reared on germinated corn and segregated into instars based on head capsule size and color; the head capsule of a recently molted larva is a faint brown. Pupae were obtained by allowing individual third instar larvae to form a pupal cell from soil in a bioassay container. Because many larvae use the side of the container as part of the cell, pupal development could be observed. Containers with pupae that were 24–48 hours old were used in bioassays.

Concentration-mortality bioassays were conducted using 70-ml weighing bottles (Nalgene Company, Rochester, NY) with 15 g of a sandy-clay loam soil that had been air dried and sized to particles with a diameter of 0.5–1.0 mm. Egg and larval bioassays were prepared with 1 ml of nematode suspension or 0.15% agar (control) and sufficient water to bring the total soil moisture to 20% (wt/wt). Bioassay containers with larvae received one corn seedling with a primary root length of about 1.5 cm. Containers were incubated for 1–3 hours at 25 C before adding a single egg or larva per container. Pupal bioassays were prepared with 0.5 ml of nematode suspension or 0.15% agar applied to the soil surface. These containers initially received 15 g of soil and 2.5 ml of water to allow the rootworm larva to form a pupal cell. Each bioassay consisted of 10–25 insects per nematode concentration, with each insect exposed to the treatment for 72–78 hours in darkness at 25 C. At the end of the exposure period, the outer surface of each insect was washed with 0.1% sodium lauryl sulfate and rinsed with distilled water to remove any adhering nematodes. Mortality was recorded after holding eggs for 15 days and larvae and pupae for 3 days. Larvae were held with seedling corn.

Nematode development was observed by dissecting and microscopically examining 4–8 insects of each susceptible stage at intervals of 12 hours for first and third instar larvae and 24 hours for second instar larvae and pupae. To synchronize the infection interval, groups of 30–50 insects were exposed for 2 hours to 10,000 infective-stage nematodes. The exposure was terminated by removing the insects from the exposure container and rinsing them with 0.1% sodium lauryl sulfate and distilled water to remove any adhering nematodes. Insects exposed to nematodes were held with fresh corn seedlings at 25 C in darkness. Data were collected only for those insects that contained at least one male and one female nematode. Nematode stage was determined by comparing length and width measurements of representative nematodes (17), comparing internal morphology (17,18), and observing the occurrence of shed cuticles in the insect hemocoel. Estimates of the number of infective-stage nematodes produced per insect were based on the average count from four 0.5-ml samples of a 10-ml suspension prepared from the contents of individual insects with emerging infective-stage nematodes.

Concentration-mortality data were analyzed with the probit program POLO-PC (14). Mean comparisons for nematode numbers per insect stage and nematode size from different insect stages were conducted using SAS-GLM and Tukey's studentized range test (20).

Results

Western corn rootworm larvae and pupae but not eggs were susceptible to infection by the Mexican strain of *S. carpocapsae*. Exposure of rootworm eggs to nematode concentrations of up to 8,480 infective-stage nematodes per egg for 3 days did not influence egg mortality (Table 1). Probit regressions from replicated bioassays on each rootworm stage were not significantly different, so bioassay data were combined by stage. Probit regressions for the rootworm pupal and three larval instars were parallel ($\chi^2 = 6.90$, df = 3, $P = 0.075$) but not equal ($\chi^2 = 171.31$, df = 6, $P < 0.005$). Relative susceptibility, based on probit LC_{50} estimates, increased significantly with succeeding larval instar. Pupal susceptibility was not significantly different from that of first instar larvae.

Infection of rootworm larvae and pupae that were exposed to infective-stage nematodes for a 2-hour period exceeded 60%. During the exposure period, nematodes were observed to attempt entry of the spiracles of rootworm larvae and pupae but were observed successfully entering only the thoracic spiracles of pupae. Based on measurements from 10 insects of each stage, the maximum diameter and standard deviation in microns of the outer opening of the thoracic spiracles were 10.6 ± 1.1 (first instar larvae), 16.4 ± 2.1 (second instar larvae), 26.0 ± 1.7 (third instar larvae), and 52.5 ± 3.3 (pupae). The maximum diameter and standard deviation of the outer opening of the abdominal spiracles were 8.9 ± 1.1 (first instar larvae), 14.3 ± 1.6 (second instar larvae), 21.9 ± 1.7 (third instar larvae), and 37.0 ± 3.7 (pupae). The atria of the thoracic and abdominal spiracles of rootworm larvae and pupae contained internal rings of spines that projected 2–5 μm into the lumen.

Nematode development occurred in all susceptible rootworm stages with a single, complete life cycle in second and third instar larvae and pupae (Table 2). At the incubation temperature of 25 C, host death occurred at 12–24 hours for larvae and 24–48 hours for pupae. A complete life cycle from infective juvenile to infective juvenile occurred in 120–144 hours. Development followed a similar pattern in all rootworm stages, except that it was less synchronized and generally delayed in pupae. For example, first occurrence of ovarian development and egg deposition was 24 and 36 hours later, respectively, for nematode females from rootworm pupae as compared to those from third instar larvae. Nematode development in all rootworm stages was limited by the breakdown of the insect integument and subsequent release of the nematodes.

The number of infective-stage nematodes that were recovered from each rootworm stage increased with insect maturation. The average recovery from 8 second instar larvae was 1,702 (SD 455), from 12 third instar larvae was 3,718 (SD 916), and from 4 pupae was 6,765 (SD 1,960).

Table 1. Concentration-mortality response of western corn rootworm eggs, larvae, and pupae to *Steinernema carpocapsae* (Mexican).

Host stage	LC_{50}†	95% FL	Slope ± SE	Sample‡
First instar	2,904	1,174–5,942	0.96 ± 0.25	197
Second instar	172	86–288	1.45 ± 0.25	359
Third instar	35	11–57	1.72 ± 0.30	220
Pupa	2,408	1,157–3,678	2.06 ± 0.50	140
Egg		Nonsignificant regression		160

† Infective-stage nematodes per host.
‡ Total insects used in the concentration-response analysis.

TABLE 2. Occurrence of nematode stages in rootworm larvae and pupae.

Time (hours)	Rootworm stage: First instar	Second instar	Third instar	Pupa
12	IJ		IJ	
24	IJ, J4	IJ, J4	IJ, J4	IJ, J4
36	J4, A1		J4, A1	
48	J4, A1	J4, A1	J4, A1	J4, A1
60	A1, E		A1, E, J1	
72	A1	A1, E, J1	A1, E, J1, J2	J4, A1
84	A1, J1		A1, E, J1, J2	
96		A1, E, J1, J2	A1, E, J1, J2	A1, E, J1
108			A1, E, J1, J2, J3	
120		A1, J1, J2, IJ, J3, J4	A1, E, J1, J2, J3, J4, A2	A1, E, J1, J2, J3, J4, A2
132			A1, E, J1, J2, IJ, J3	
144		A1, J1, J2, IJ, J3, J4	J2, IJ, J3, J4, A2	A1, E, J1, J2, IJ, J3, J4, A2
156			J2, IJ, J4, A2	
168		J2, IJ, J4, A2		A1, E, J1, J2, IJ, J3, J4, A2
192		J2, IJ		J2, IJ, A2
240				J2, IJ, A2, E, J1

IJ = infective juveniles, J1–4 = 1–4 stage juveniles, A1–2 = first- and second-generation adults, and E = eggs. Dominate nematode stages are double underlined.

The size of first-generation female, male, and infective-stage nematodes varied with insect stage (Tables 3, 4). Except for the length of the male, average nematode size tended to increase with insect maturation. Infective-stage nematodes recovered from second instar rootworm larvae were significantly smaller in length and width than those recovered from the larvae of *Galleria mellonella* (Table 4).

DISCUSSION

Western corn rootworm eggs have a 4-μm-thick chorion that is sculptured with external ridges in the shape of convex polygons (1). Before oviposition, eggs are permeable to sperm, which have a head diameter near 1 μm, and during postdiapause development they are permeable to water (13). Apparently, rootworm eggs are not permeable to or adversely affected by *S. carpocapsae*. There are no reports of steinernematid nematodes causing pathology in insect eggs.

The pattern of progressively higher susceptibility for successive larval rootworm instars and the relatively low susceptibility of the pupal stage is consistent with previous reports for other insect species (2,5,9). Differences in stage susceptibility have been primarily attributed to differences in

TABLE 3. Size of first-generation female and male nematodes from larvae and pupae of the western corn rootworm.

Insect stage	Female size (μm): Length ± SD	Female size (μm): Width ± SD	Male size (μm): Length ± SD	Male size (μm): Width ± SD
Pupa	5,669 ± 949 a	164 ± 25 a	1,128 ± 182 a	89 ± 16 a
Third instar	3,583 ± 1,642 b	166 ± 32 a	988 ± 91 a	84 ± 13 ab
Second instar	2,179 ± 1,048 c	98 ± 29 b	980 ± 125 a	64 ± 15 b
First instar	1,889 ± 476 c	103 ± 12 b	988 ± 32 a	71 ± 5 ab

Measurements (greatest body length and width) were based on 16 females and five males from each insect stage when the females were gravid with separable eggs but no eggs were deposited. Means within columns followed by the same letter are not significantly different ($P = 0.05$; Tukey's studentized range test).

TABLE 4. Size of infective-stage nematodes from larvae and pupae of the western corn rootworm and larvae of *Galleria mellonella.*

Host	Infective length (μm) ± SD	Infective width (μm) ± SD	Sample
Galleria	622 ± 15 a	25 ± 2 a	18
Pupa	559 ± 20 b	24 ± 1 ab	36
Third instar	551 ± 25 b	24 ± 1 ab	59
Second instar	536 ± 40 c	23 ± 2 b	125

Measurements were based on greatest body length and width. Means within columns followed by the same letter are not significantly different ($P = 0.05$; Tukey's studentized range test).

the size or availability of orifices that serve as entrance points for the nematode (i.e., spiracles, anal, oral). For rootworm larvae, size differences in the oral and anal openings are probably most important. The spiracular orifices appear to be too small to serve as points of entrance for infective-stage nematodes that are near 25 μm in diameter. Other factors, like differences in feeding activity, might also influence stage susceptibility (12). However, the similarity of the probit regression slopes supports the premise that the process of infection and pathogenesis is similar for all rootworm stages. For pupae, the only available orifices for nematode entrance are the spiracles. We observed infective-stage nematodes entering the thoracic but not the abdominal spiracles. While the outer diameter of the abdominal spiracles appears to be large enough for nematode entrance, the atrial spines apparently reduce the spiracle lumen and inhibit nematode entrance. Also, access to rootworm pupae is probably restricted by the earthen pupal cell similar to the protection afforded ichneumonid and braconid pupae in silken cocoons (10).

Nematode development followed a pattern previously described in *Aedes aegypti* larvae (24) and in *Galleria mellonella* larvae (17), except that rootworm immatures did not support a second generation of nematodes. Apparently, insufficient host tissues and the fragility of the rootworm integument limited nematode development beyond a single generation. These factors are also assumed to be responsible for the incomplete nematode generation in first instar rootworm larvae.

Poinar (16) conjectured that nematode size could vary due to the parasitized host, associated microorganisms, and physical factors of the environment. We observed differences in nematode size related to insect stage and host species. While our study was not designed to correlate nematode size with the size of the host insect, the trend we observed (i.e., increased nematode size with successive insect stage) supports the hypothesis that nematode size can be influenced by the size of the host. Since nematode size has been demonstrated to be a limiting factor in host susceptibility (3,5), the potential of manipulating nematode size should be pursued. For relatively small insects like corn rootworm larvae, smaller nematodes might improve host invasion.

Our data on western corn rootworm susceptibility and nematode development indicate that control treatments with the Mexican strain of *S. carpocapsae* should be applied when second and third instar rootworm larvae are predominant in the field. This is the period when rootworm immatures are most susceptible to nematode infection and when suppression of the rootworm population would have a significant impact on reducing plant damage. With a nematode generation time of about 6 days and complete reproduction in both second and third instar rootworm larvae, proliferation of the nematode population would be expected within the corn rootworm larval development period. This could help sustain the efficacy of a nematode treatment and provide adequate proliferation of the nematode population to cause significant mortality of rootworm pupae. Thurston and Yule (23) concluded that nematode treatments for northern corn rootworm larvae should be targeted at the first instar before they enter the corn root system. Since northern and western corn rootworm populations are sympatric over most of the U.S. corn belt, additional research on mixed populations is needed to determine the optimum time of application for this important pest complex.

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