Evaluation of Steinernema riobravis, S. carpocapsae, and Irrigation Timing for the Control of Corn Earworm, Helicoverpa zea

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Abstract: Two entomopathogenic nematodes, Steinernema riobravis and Steinernema carpocapsae, were compared for their ability to parasitize corn earworm, Helicoverpa zea (Boddie) prepupae and pupae in corn plots at the Lower Rio Grande Valley of Texas. The most effective S. riobravis concentration was 200,000 infective juveniles (IJ)/m² (95% parasitism), as compared with 100,000 IJ/m² (81%), 50,000 IJ/m² (50%), 25,000 IJ/m² (31%), and the control (13%). No parasitism occurred in plots receiving S. carpocapsae. The ineffectiveness of S. carpocapsae was attributed to high (>38°C) soil temperatures. Parasitism was higher when S. riobravis was applied at 200,000 IJ/m² through furrow irrigation (97%) or post-irrigation (95%) than when nematodes were sprayed onto the soil before irrigation (82%). Parasitism of corn earworm prepupae by S. riobravis persisted up to 36 days after application and was higher in the treated plots (80%) than the natural parasitism of the control plots (14%). These results show that at high field soil temperatures S. riobravis is more effective against corn earworm than S. carpocapsae.

Key words: biological control, corn earworm, entomopathogenic nematode, irrigation, soil temperature, Steinernema carpocapsae, Steinernema riobravis.

The corn earworm, Helicoverpa (=Heliothis) zea (Boddie) (Lepidoptera: Noctuidae), a major insect pest of the New World, attacks corn and other cultivated and wild host plants (7). This insect feeds primarily on the fruit of its hosts and, in corn, usually feeds first on the silks and then channels downward into the ear. Once larvae enter the silk channel of the corn fruit, they are well protected, allowing high survival. Thus, corn serves as a nursery crop to produce large adult populations that can migrate long distances to infest other susceptible crops such as cotton, sorghum, tobacco, and many vegetable crops.

In July 1990, Steinernema riobravis Cabanillas et al. was isolated from soil samples in corn fields near Weslaco, Texas (2). Since then, successful results have been obtained with S. riobravis for control of corn earworm (3,5), pink bollworm Pectinophora gossypiella Saunders (13), and the root weevil Diaprepes abbreviatus L. (22). Under a cooperative research and development agreement between the Agricultural Research Service and biosys Inc., a biological control company, S. riobravis is being developed as a commercial insecticide.

Our control strategy is focused on the prepupal and pupal stages of corn earworm populations in the soil, thus preventing adult emergence and subsequent migration. Timing soil applications of S. riobravis with the life cycle of the target insect is a key efficacy factor (5). Our previous laboratory studies showed that Steinernema carpocapsae (All strain) was highly pathogenic, thus suggesting its potential to control corn earworm (unpubl. data).

About 200,000 ha of corn planted annually in the region of the Lower Rio Grande Valley of Texas and northern Tamaulipas, Mexico, depends on irrigation. Delivering nematodes through irrigation could be a potential system for suppressing corn earworm populations. Objectives of the present studies were to: i) compare the field effectiveness of S. riobravis and S. carpocapsae against corn earworm prepupae and pupae in response to nematode concentration; ii) determine the influence of timing furrow irrigation application (before, after, and through furrow irrigation) and nematode distribution on the greenhouse efficacy of S. riobravis; iii) evaluate...
the effects of nematode application before and after furrow irrigation on the field efficacy of *S. riobravis*; and iv) assess the influence of nematode concentration and distribution when applied via in-furrow irrigation on *S. riobravis* field efficacy.

MATERIALS AND METHODS

**Research site:** The study was conducted at the South Farm of the Subtropical Agricultural Research Laboratory in the Lower Rio Grande Valley near Weslaco, Texas. *Steinernema riobravis* occurs naturally in this area, parasitizing prepupae and pupae of corn earworm and fall armyworm. Field plots were planted with yellow field corn var. Pioneer 3192 in a sandy clay soil (44.8% sand, 37.1% clay, 18.1% silt; 1.2% organic matter; pH 8.3; row slope 0.1%). The hydrometer method (1) was used for soil texture analysis.

Soil temperatures were obtained via copper-constantan thermocouples at three soil depths (2.5, 5, and 10 cm on the side of the plant bed). Thermocouples were connected to an Omnidata Easy Logger Model EL 824-GP (Omnidata International, Logan, UT), and temperature data were scanned at 15-minute intervals and then averaged hourly. Soil moisture was determined gravimetrically from samples taken 10 cm deep and 10 cm on the side of the plant bed. Moisture contents for this soil at 0.33, 1, 5, 13, and 15 bars was 21.8, 19.0, 14.4, 11.2, and 12.7%, respectively.

**Cultures:** The nematodes used were *S. riobravis* (Texas strain) and *S. carpocapsae* Weiser (All strain). *Steinernema riobravis* was reared in vivo in our laboratory, using corn earworm prepupae as a host. *Steinernema carpocapsae* was produced in vitro by biosys Inc. (Columbia, MD). The infective juveniles (IJ) of *S. riobravis* and *S. carpocapsae* were stored at 10 C for 28 and 13 days, respectively. Before application, nematode viability and pathogenicity were determined in the laboratory at 28 C. Pathogenicity was tested with 100 IJ/corn earworm prepupa in 50 dishes (5-cm diam) containing filter paper. Insect mortality was recorded 3 days after nematode exposure.

Corn earworms (CEW) were reared on artificial media at 29.5 C (20). Prepupae collected 11 days after eclosion averaged 30 mm in length (25–34 mm) and weighed 500 mg (400–600 mg) before being buried.

**Experiment 1. Field evaluation of *S. riobravis* and *S. carpocapsae:** This experiment was conducted in a corn field, planted on 6 March 1992. Treatment plots (1 by 4 m) were established in four rows (replicates) of corn. Plots were separated by 10 m within each row and 2 m between rows. The treatments were arranged in a 5 x 2 factorial with five nematode concentrations including the control (0, 25 x 10^3, 50 x 10^3, 100 x 10^3, and 200 x 10^3 IJ/m^2) and two nematode species. The experimental design was a randomized complete block with ten treatments and four replications.

Before application, *S. riobravis* and *S. carpocapsae* viability and pathogenicity were 95 and 100%, respectively. Nematodes were suspended in 8 liters of water and applied with a sprinkling can at 1700 hours CST on 11 June 1992. One drop of Triton X-100 (a wetting agent, Beckman Instruments, Fullerton, CA) per liter was added to prevent nematodes from adhering to the container. One day after treatment, 10 prepupae per plot (five prepupae on each side of the plant bed) were individually buried 5 cm deep at 80-cm intervals along the bed and 12 cm from the plant base. After 5 days, exposed corn earworms were excavated and dissected to determine nematode presence (2,18).

Nematode populations were estimated 20 days after application by using the modified Baermann funnel extraction method and a laboratory soil bioassay, as described in a similar study (5). Soil samples were taken systematically to a depth of 10 cm in the row from plots treated with 200,000 IJ/m^2 of *S. riobravis* or *S. carpocapsae* and their controls. Ten borings per plot were taken separately with a 5-cm sampling tube and mixed to obtain composite samples (1,000 cm^3/sample).

Rainfall (3.6 mm) occurred 24 hours before application. The mean air tempera-
tured was 31°C (24–41°C). The average soil temperature (5 cm deep) at the time of treatment, burial, and digging of corn earworms was 37, 36, and 39°C, respectively. Soil temperatures at three soil depths are presented in Fig. 1A. Soil moisture at the time of application, burial, and digging of *H. zea* were 23, 19, and 17%, respectively.

Insect mortality (dependent variable) was regressed against nematode concentration (independent variable) using linear and quadratic models through PROC REG of SAS (25). The significance of the regression coefficients, residual mean square, and the coefficient of determination ($R^2$) were used to evaluate goodness of fit to a model.

**Experiment 2. Irrigation timing and nematode distribution on *S. riobravis* efficacy in greenhouse:** The effects of one concentration of *S. riobravis* (135,000 IJ/m$^2$) and three timings of irrigation application (nematodes applied before, after, and through irrigation) on parasitism were compared in the greenhouse in February 1992. Nematodes were applied to steam-sterilized soil obtained from the research site. Soil was air-dried, passed through a 9-mesh (2-mm) sieve, and placed in three wooden boxes (1.44-m length × 1.03-m width × 0.38-m height) to a depth of 0.23 m. To simulate a field prepared for furrow irrigation, the soil was formed into a 0.12-m deep furrow in the center of the box with two 0.25-m half beds—one bed on each side of the center. The furrows were irrigated with tap water (64 liters/box) at application time.

For nematode application before irrigation, nematodes in 8 liters of water were applied to dry soil with a sprinkling can, followed immediately by irrigation. For nematode application after irrigation, nematodes were applied 2 hours after irrigation. For nematode application through furrow irrigation, a hydroponic siphon mixer (Hyponex ¾-inch) was attached to a water meter (Master Meter ¾-inch). The nematodes were applied by siphoning from a 500-ml nematode suspension into the water stream. A buffer made of PVC pipe limited the soil impact of running water. All boxes were capped with brown wrapping paper to minimize evaporation.

To determine nematode distribution along (distance effect) and across the row (position effect), corn earworm larvae were allowed to burrow into the soil and pupate. Larvae were placed 5 cm apart in the furrow bottom (40 larvae), and on the sides and tops of the bed (40 larvae on each side and top). The effect of *S. riobravis* on insect mortality was determined at four distances (0–30, 30–60, 60–90, and 90–120 cm). Five days after burial, larvae were collected, washed, and transferred individually to “White” trap dishes (26) for an additional 7 days at 25°C in the dark. Parasitism was based on *S. riobravis* progeny exiting from the cadavers.

Air and soil temperatures averaged 24°C (21–24°C). Soil moisture at the time of
burial and digging *H. zea* prepupae was 35 and 27%, respectively.

Data from this experiment were analyzed by factorial analysis of variance using PROC GLM of SAS (25), which was followed by protected least significant differences (LSD) at the \( P = 0.05 \) level to separate means.

**Experiments 3 and 4. Field application of *S. riobravis* before or after furrow irrigation:** These experiments were conducted in a corn field planted 23 March 1992. Each experiment, separated by 5 m, consisted of 12 rows (60-m long, 0.1% slope). Treatment plots (1 by 4 m) were established in four rows (replicates). Plots were separated by 10 m within each row and 2 m between rows. Treatments were two nematode concentrations (100 \( \times 10^3 \) and 200 \( \times 10^3 \) IJ/m\(^2\)) and a control, arranged in a randomized complete block design with four replications.

Nematodes were stored for 13 days before application. Nematodes were suspended in 8 liters of tap water and applied to each plot (4 m\(^2\)) with a sprinkling can at 1800 hours CST on 13 July 1992.

Plots that received nematodes before irrigation were irrigated 24 hours after application. Plots that received nematodes after irrigation were irrigated 3 days before nematode application. Twenty prepupae per plot (10 prepupae on each side of the plant bed) were buried (5 cm deep and 12 cm from the plant base) evenly through the plot two days after nematode treatment. After 5 days, all corn earworms were collected and dissected to determine parasitism. One-meter sections of each plot were left undisturbed to evaluate nematode persistence. Thirty days after application, nematode populations were estimated by using the modified Baermann funnel extraction method and a laboratory soil bioassay previously indicated (5).

Rainfall (38 mm) occurred 5 and 6 days after application. The mean air temperature was 33 C (26–38 C). The average soil temperature (5 cm deep) at the time of application, burial, and digging of corn earworms was 34, 37, and 37 C, respectively. Soil temperatures are presented in Fig. 1B. Soil moisture in fields treated before and after irrigation was measured at the time of application (18%, 18%), burial (23%, 22%), and digging (28%, 27%) of *H. zea*.

**Experiment 5. Field application of *S. riobravis* via in-furrow irrigation:** This experiment was conducted in a corn field planted 4 August 1992. Nematode treatments consisting of 0, 100 \( \times 10^3 \), and 200 \( \times 10^3 \) IJ/m\(^2\) were randomly assigned each to two rows (20 m long). Rows with different treatments were 5 m apart. Nematodes in 10 liters of water were applied into the irrigation water to cover the rows in 20 minutes. Nematodes were applied at 1700 CST on 9 September 1992.

The effects of nematode concentration, distance from application source, and position of corn earworm prepupae across the row were determined as follows. Five prepupae/m were buried (5 cm deep) along the length of the rows on top of the plant bed (100 prepupae), on the two sides of the bed (200 prepupae, 12 cm from plant base), and the two furrow bottoms (200 prepupae). The efficacy of *S. riobravis* was determined at 0–5, 5–10, 10–15, and 15–20 m from the irrigation source. Five days after burial, the corn earworms were collected and dissected to determine parasitism. Nematode persistence was determined 36 days after application, following the procedures indicated in Experiment 1.

Rainfall occurred 2 (1.5 mm), 7 (1.3 mm), 14 (8.4 mm), 15 (0.5 mm), and 19 (0.5 mm) days after application. The mean air temperature and soil temperature (5 cm deep) were 29 (22–37 C) and 30 C, respectively. Soil moisture at the time of application, burial, and digging of *H. zea* were 16, 26, and 24%, respectively.

To compare the effects of *S. riobravis* concentration in response to irrigation timing on *H. zea* parasitism, insect mortality data (from experiments 3–5) were subjected to factorial analysis of variance using PROC GLM of SAS (25); means were separated by Fisher's protected least significant difference (LSD) test \( (P = 0.05) \). The same statistical procedure examined the
effects of distance and position across the row on *H. zea* parasitism when *S. riobravis* was applied via in-furrow irrigation, and compared the mean numbers of nema-
todes extracted from soil after application in all treatments.

**RESULTS**

Experiment 1. Field evaluation of *S. riobravis* and *S. carpocapsae*: Parasitism rate as a function of nematode concentration was higher (*P = 0.0001*) for *S. riobravis* (quadratic response) than for *S. carpocapsae* (no response). The general response of corn earworm mortality (*Y*) as a function of concentration of *S. riobravis* (*X*) was approximated by a quadratic model (Fig. 2).

The most effective *S. riobravis* concentration was 200,000 IJ/m² (95% parasitism) as compared with 100,000 IJ/m² (81% parasitism), 50,000 IJ/m² (50% parasitism), and 25,000 IJ/m² (31%). Natural *S. riobravis* parasitism of 13% was observed in control plots (Fig. 2).

The mean numbers of *S. riobravis* and *S. carpocapsae* infective juveniles extracted from soil 20 days after nematode applica-
tion were 60 and 0 IJ/100 cm³, respectively. This population of *S. riobravis* affected 90% corn earworm parasitism in laboratory bioassays (Table I).

Experiment 2. Irrigation timing and nematode distribution on *S. riobravis* efficacy in greenhouse: The efficacy of *S. riobravis* against *H. zea* was influenced by nematode concentration (*F = 109.03*; *df = 2, 27*; *P = 0.0001*). No differences in *H. zea* parasitism were found due to timing of furrow irrigation (before, during, or after irrigation) (*F = 1.78*; *df = 2, 27*; *P = 0.1887*) or because of the interaction between irrigation timing and concentration (*F = 1.34*; *df = 4, 27*; *P = 0.2815*).

The most effective concentration (averaged over application methods) was 200,000 IJ/m², which resulted in 92% parasitism; this was higher (*P < 0.05*) than parasitism in plots receiving 100,000 IJ/m² (78%) or the control plots (16%) (LSD = 11; *df = 27*; MSE = 180.15; *P = 0.05*). Application of 200,000 IJ/m² resulted in...
Table 1. Extraction of Steinernema riobravis and S. carpocapsae infective juveniles (IJ) and their infectivity on corn earworm prepupae after treatment in soil naturally infested with S. riobravis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. days after trt.</th>
<th>No. of IJ per 100 cm³</th>
<th>Insect mortality lab bioassay (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. riobravis</td>
<td>20</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>S. carpocapsae</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Before irrigation</td>
<td>30</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>After irrigation</td>
<td>30</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Through irrigation</td>
<td>36</td>
<td>56</td>
<td>80</td>
</tr>
<tr>
<td>Control</td>
<td>36</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>LSD (P = 0.05)</td>
<td></td>
<td>4.2</td>
<td>6.8</td>
</tr>
</tbody>
</table>

*S. riobravis* applied:

- Before irrigation: 30 days, 48 IJ/100 cm³, 50% mortality
- Control: 30 days, 10 IJ/100 cm³, 11% mortality
- After irrigation: 30 days, 50 IJ/100 cm³, 55% mortality
- Control: 30 days, 11 IJ/100 cm³, 12% mortality
- Through irrigation: 36 days, 56 IJ/100 cm³, 80% mortality
- Control: 36 days, 12 IJ/100 cm³, 14% mortality
- LSD (P = 0.05) = 4.2

Based on 20 corn earworm prepupae exposed to soil samples per each time, in plots treated with 200,000 IJ/m².

Parasitism of 82, 96, and 97%, when nematodes were applied to the soil before, after, and through irrigation, respectively.

Experiment 5. Field application of *S. riobravis* via in-furrow irrigation: The efficacy of *S. riobravis* applied via in-furrow irrigation was influenced by the interaction between nematode concentration and corn earworm position across the row (position) (F = 7.44; df = 4, 24; P = 0.0005) (Fig. 3). The highest parasitism on the bottom, side, and top of the plant bed was obtained with 200,000 IJ/m² (98, 98, and 93%, respectively) as compared with 100,000 IJ/m² (92, 91, and 80%, respectively). Parasitism was significantly lower in control plots (bottom 24%, side 20%, and top 0%; LSD = 3.20; P < 0.05). The results of the greenhouse experiments were consistent with the field experiment in that no differences were observed down the row (F = 2.49; df = 3, 24; P = 0.0845).

The average number of *S. riobravis* infective juveniles extracted from soil 30–36 days after application (experiments 3–5) were 48–56 IJ/100 cm³, resulting in 50–80% *H. zea* parasitism in the laboratory bioassay (Table 1).

Discussion

Soil moisture and temperature affect field efficacy of entomopathogenic nematodes (8,12,15,17,24). In our field tests, failure of *S. carpocapsae* to parasitize corn earworm was attributed primarily to high soil temperatures. The optimum temperature range for *S. carpocapsae* is 23–30 C, with significant mortality occurring at >35 C (10,14,16). Although laboratory results show that *S. carpocapsae* (All strain) is an effective corn earworm parasite at 24 C (unpubl. data), its effectiveness was limited under the hot field conditions of our experiment. Successful parasitism by *S. riobravis* under field conditions may be attributed in part to its subtropical origin and adaptation to high temperatures (2,4,19). In our tests, *S. riobravis* killed corn earworm at soil temperatures >38 C. Its ability to withstand high temperatures make this nematode an attractive biological control agent in high-temperature zones.
Steinernema riobravis and S. carpocapsae: Cabanillas, Raulston

Knowledge of the temperature limits and optima of each nematode species can be important for effective field application. Grewal et al. (11) reported that the optimum temperature for nematode penetration and establishment in Galleria mellonella (wax moth) larvae was 24°C for S. carpocapsae and 32°C for S. scapterisci. Research on thermal adaptation shows that S. riobravis infected G. mellonella over the widest temperature range (10–39°C) (12). At 37°C, only S. glaseri, S. riobravis, and Steinernema sp. infected hosts, and S. riobravis was the quickest to cause insect mortality (LT50 18 hours) (12).

Soil moisture may be even more important than temperature for reliable biocontrol of insects. Irrigation and application method appear to influence the effectiveness of S. riobravis. Unsatisfactory control was attained where nematodes were applied to dry soils (before irrigation). Considering the application method (sprinkling on the soil surface), desiccation and exposure to sunlight may have been nematode mortality factors (9). The nematodes will be most effective when applied to moist soils after irrigation (6,23) or via in-furrow irrigation.

Application of S. riobravis via in-furrow irrigation may have great potential for controlling the soil-inhabiting stages of corn earworm and other susceptible insect pests. Although the influence of irrigation and application techniques on entomopathogenic nematodes has been previously reported (6,23), most soil applications have been by methods (6,21,23,27) other than furrow irrigation. In greenhouse and field studies, parasitism was higher when S. riobravis was applied via or after irrigation compared to before irrigation. Application via in-furrow irrigation produced the highest parasitism rates, probably because of a homogeneous nematode distribution. Although consistent results were obtained here, comparison of these methods on a larger scale will be necessary to determine the possibilities of using irrigation water as an application method for entomopathogenic nematodes.

Variation of nematode efficacy in response to insect position in the bed is an area that has received little attention. Generally, row crops accentuate the patchy distribution of nematodes. Although low levels of corn earworm mortality and the spatial pattern of S. riobravis in the field have been reported (4,19), there is no information on nematode efficacy in response to host position in the bed. Our study indicates that S. riobravis causes corn earworm mortality on prepupae and pupae located either on the bed bottom or side; mortality is limited when the insect is positioned on top of the plant bed as observed in the control plots. This suggests that a successful application of S. riobravis will depend on a system that provides a uniform nematode distribution in the bed.

Other factors affecting the efficacy include nematode concentration over time. Application of 200,000 IJ/m² (2 billion IJ/ha) resulted in >93% parasitism. These results confirm previous findings that this concentration, when applied at the critical time relative to corn earworm exit from the corn ear, causes high parasitism (5). The nematode population data show that S. riobravis remains active in the soil for up to 36 days, suggesting that optimal soil moisture may improve nematode survival to enhance nematode efficacy.

Our results show that S. riobravis is effective for suppressing corn earworm under field conditions of high soil temperature with irrigation. Considering its tolerance of high temperatures and ability to survive long periods in the soil, S. riobravis shows promise as an effective biocontrol agent.

LITERATURE CITED


