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Effects of furrow irrigation on the distribution and infectivity of *Steinernema riobravis* against corn earworm in corn

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Summary – The effects of dose, incorporation method, timing of irrigation (before *vs* after), and application via in-furrow irrigation on the distribution, persistence, and infectivity of *Steinernema riobravis* against corn earworm were determined. Prepupae were buried in corn field soils (31-39 °C) 2, 3, 6, 10, 17, 24, and 39 days after nematode application. Within 6 days after nematode treatment, increased infectivity (overall average) resulted when *S. riobravis* was applied via-in furrow irrigation (78 %) compared with spray application on the soil surface before (46 %) or after irrigation (64 %). The overall mean insect mortality was higher when nematodes were incorporated into the subsurface than when they were applied onto the soil surface in those fields that received nematodes before (47.36 %) or after irrigation (63.50 %). At the most effective concentration [200 000 infective juveniles (IJ)/m²] infectivity was 95 % when applied via in-furrow irrigation compared with 56 and 84 % infectivity when applied before and after irrigation, respectively. Parasitism on top of the plant bed was highest with 200 000 IJ/m² (100 %) compared with 100 000 IJ/m² (55 %), 50 000 IJ/m² (53 %) or the control (9 %). No differences in parasitism was observed along the row indicating that nematodes were evenly distributed by irrigation. *Steinernema riobravis* persisted in the field (clay soil) over the 39 - day period. The application of *S. riobravis* via in-furrow irrigation shows great potential for controlling corn earworm in the soil prior to adult emergence.

Résumé – ***Effets de l'irrigation sur la répartition et l'infectivité de* Steinernema riobravis *envers la chenille des épis de maïs*** – La répartition, la rémanence et l'infectivité de *Steinernema riobravis* envers la chenille des épis de maïs ont été étudiées en fonction du dosage, de la méthode d'incorporation, du moment de l'irrigation et du mode d'irrigation utilisé. Les prépupes sont enterrées dans le champ de maïs (31-32 °C) 2, 3, 6, 10, 17, 24 et 39 jours après l'application des nématodes. Six jours après cette application, une augmentation de l'infectivité (moyenne générale) est observée lorsque *S. riobravis* est appliqué par irrigation inter-billons (78 %), en comparaison avec une application par pulvérisation sur le sol avant (46 %) et après (64 %) irrigation. La mortalité moyenne générale des insectes est plus élevée lorsque les nématodes sont incorporés dans le sol, près de la surface, que lorsqu'ils ont appliqués à la surface avant (47, 36 %) ou après (63, 50 %) l'irrigation. A la concentration la plus efficace [200 000 juvéniles infestants (IJ)/m²], l'infectivité est de 95 % avec application par irrigation inter-billons alors qu'elle n'est que de 56 et 84 % respectivement, avec application avant ou après irrigation normale. Le parasitisme sur les sommets des plantes est le plus élevé pour le taux de 200 000 IJ/m² (100 %) en comparaison des taux de 100 000 IJ/m² (55 %), 50 000 IJ/m² (53 %) ou du témoin (9 %). Aucune différence dans le parasitisme n'est observée dans les inter-billons qui pourrait indiquer une répartition irrégulière des nématodes lors de l'irrigation. *S. riobravis* s'est maintenu dans le sol (argileux) des champs pendant une période de 39 jours. L'application de *S. riobravis* par irrigation inter-billons représente donc une potentialité importante de traitement pour contrôler la chenille des épis de maïs avant l'émergence des adultes.

Key-words : biological control, corn earworm, entomopathogenic nematode, irrigation, *Steinernema riobravis*.

The entomopathogenic nematode *Steinernema riobravis* Cabanillas *et al.*, 1994, has shown great potential as a biological control agent of corn earwom, *Helicoverpa (= Heliothis) zea* Boddie, in the Lower Rio Grande Valley of Texas where it is indigenous (Raulston *et al.*, 1992; Cabanillas *et al.*, 1994; Cabanillas & Raulston, 1994 *a*, 1995). The pathogenicity of *S. riobravis* against corn earworm has been established (Cabanillas & Raulston, 1994 *a*), and its efficacy to control prepupae and pupae has been shown in greenhouse and small field plot tests (Cabanillas & Raulston, 1995). However, recent information on its spatial dispersion patterns in its natural habitat showed that augmentation was required to cause a uniform within-field distribution of the nematode (Cabanillas & Raulston, 1994 *b*).

H. zea is a prominent pest of corn, cotton, sorghum, tobacco, and numerous vegetable crops in the United States. Because of the wide variety of crops it attacks, the insect is known by several common names such as the corn earworm, bollworm, tomato fruitworm, and soy-

bean podworm. *Helicoverpa zea* and a closely related species, *Heliothis virescens* (F.), the tobacco budworm, are known as the *Heliothis* complex in the United States. Collectively, the complex reduces American farmers' income by more than $ 1.5 billion/year due to costs of control plus damages incurred. The moths of these species have the capacity for long-range migratory flight which magnifies their status as agricultural pests (Raulston *et al.*, 1986; Sparks *et al.*, 1989).

The current emphasis in agricultural insect pest control research is to reduce dependency on pesticides. Knipling (1978, 1983) provides a theoretical approach to management of *Heliothis* populations on an area wide basis. He indicates the importance of attacking our *Heliothis* problems before they become established as an economic debit in our cropping systems, and advocates the use of combinations of suppression methods including the application of pathogens. The Lower Rio Grande Valley is considered a primary source area for several economically important pests including corn earworm, fall armyworm (*Spodoptera frugiperda* J. E. Smith), and tobacco budworm (Raulston *et al.*, 1986).

The effective use of *S. riobravis* for area-wide suppression of *H. zea* populations will require proper application timing, nematode concentration (Cabanillas & Raulston, 1995), and uniform distribution in the soil. Our interest in nematode application via irrigation water is stimulated by the potential impact it could have on the insect in areas where irrigation is available. Control of corn earworm in large corn growing areas could suppress subsequent moth migrations and establishment of populations in recipient areas. Currently, there is no information on the effects of furrow irrigation on the distribution and efficacy of this nematode. The objectives of our study were to investigate the effects of nematode concentration, incorporation method, timing of irrigation application (before *versus* after spray application) and application via in-furrow irrigation on the distribution, infectivity, and persistence of *S. riobravis* in field corn.

Materials and methods

Research site

The research site was located at the South Farm of the Subtropical Agricultural Research Laboratory, Lower Rio Grande Valley (LRGV), near Weslaco, Texas. The LRGV is an area of intensive agriculture mostly irrigated from the Rio Grande River and associated reservoirs. Approximately 200 000 ha of irrigated corn is planted annually from mid-February to early March. *S. riobravis* was isolated from the research site where it exhibits a patchy distribution (Cabanillas & Raulston 1994 *b*). These experiments were conducted in a corn field that contained a clay soil type (43 % sand, 43 % clay, 14 % silt, 0.89 % organic matter, pH 8.2, 39.9 meq/100 g CEC). Rows were north-south oriented with a 0.1 % slope.

Nematode source

S. riobravis (TX strain) was provided by Biosys Inc. (Columbia, MD) in a gel polymer material contained in an experimental " tea-bag " product. Nematodes were produced in liquid culture (Friedman, 1990) and shipped in insulated containers with Super Ice® cold pack (Super Ice Corp. San Leandro, CA). Infective juveniles (IJ) were suspended in water before their application in corn fields. On the day of application in all experiments, infective juvenile viability and pathogenicity were determined using a Petri dish bioassay. The viability test consisted of counting the number of live and dead IJ from five samples. The pathogenicity test consisted of placing 100 live IJ (in 0.45 ml of distilled water) on filter paper in each of fifty 5 cm-diam Petri dishes. One corn earworm prepupa was placed in each dish. These plates were placed in plastic bags containing moist paper to maintain humidity and incubated in the dark at 28 °C. After 3 days, insects were dissected and examined under a dissecting microscope for the presence of *S. riobravis*. Pathogenicity was evaluated based on the number of dead insects infected by *S. riobravis*. Nematode concentrations applied in all experiments were adjusted according to the viability.

Corn earworm

Corn earworms (CEW) were reared in the laboratory according to the procedures indicated by Raulston and Lingren (1972). Each corn earworm buried in corn field soil was contained in HistoPrep Tissue Capsules (38 × 8 mm; FISHERbrand). Prepupae, 11 days old, were measured (30-40 mm; $\bar{x}$ = 34) and weighed (396-803 mg; $\bar{x}$ = 582) before being buried in the soil.

Experiment 1. Nematodes applied via in-furrow irrigation : Nematode concentration, distance and furrow position

This experiment was conducted in five contiguous sections of a corn field planted on 9 March 1993. Sections were separated by 5 m and consisted of four 120 m - long rows (slope = 0.1 %). Nematode treatments consisting of $0{,}25 \times 10^3$, 50×10^3, 100×10^3, and 200×10^3 IJ/m^2, were randomly assigned to a section. The nematodes were suspended in 60 l of water contained in a 200-l plastic tank. To the nematode suspension, ten drops (0.1 ml) of Triton X-100™ (a wetting and dispersal agent, Beckman Instruments Inc., Fullerton, CA) per liter were added to prevent nematodes from sticking to the side of the container and to aid in the uniform dispersal of the nematodes via the irrigation water. The tank was mounted on a wooden frame (height : 1.32 m, 5 × 10 cm - lumber) attached to a two-wheel trailer. A battery operated (12 VDC) live bait aerator was placed at the bottom of the tank to supply oxygen for the nematodes. Nematode delivery was accomplished by attach-

ing a model " C " Carter Matic Stead – Flow applicator to the tank. Four outlets were used and fitted with No 86 orifices, and flexible PVC tubing (inner diam = 6 mm, outer diam = 9.5 mm) about 3 m long was attached to each outlet. The nematode was delivered through each tube at a flow rate of 333 ml/min. The irrigation water was applied at a flow rate of 2550 ml/s (= 153 000 ml/min/row), it took 45 min to apply the nematodes. The volume of water used per ha was 573 750 liters. The tank was covered with black weed bar to protect nematodes from sun light. The initial temperature in the water tank containing nematodes and the irrigation water were 33 and 32 °C, respectively. Immediately upon arrival from Biosys, *S. riobravis* were applied (June 10, 1993; Lot No. 3Y55AF130) between 14 : 00-20 : 00 CST.

The effects of *S. riobravis* on insect mortality in response to nematode concentration, distance along the row, and position of corn earworm prepupae across the row were determined as follows. Within each section, five 2 m × 1 m plots were arranged down each of the four rows (replicates) at distances of 20, 40, 60, 80 and 100 m from the irrigation source. Six days after nematode application, corn earworm prepupae were buried (5 cm deep, 30 cm apart from each other) in each plot in the furrow bottom (5 CEW), on the side of the bed (5 CEW) and the top of the bed (5 CEW). Five days after burial, the corn earworm were extracted from the soil, transported to the laboratory and dissected to determine parasitism based on the presence of *S. riobravis*.

Nematode efficacy over time was determined at 15 and 39 days after nematode treatment following the previous procedure but using different plots located 1 m from those previously sampled.

To determine numbers of nematodes transported by the irrigation water, five 150-ml water samples were separately collected every 20 m at two separate times. The initial samples were collected when water reached the prescribed distances; the second set of samples were collected when water reached the furrow end. These samples were placed in 275-ml tissue culture flasks (canted neck) and stored at 10 °C for 24 h. The individual samples for each distance were combined into a composite sample. The number of nematodes was estimated from five 1-ml aliquots of water from each composite sample. Following enumeration of nematodes, a Petri plate bioassay procedure was used to determine the pathogenicity of the collected infective juveniles against *H. zea* prepupae. Fifty corn earworm prepupae were transferred individually to Petri dishes containing 100 IJ on filter paper. Insect mortality based on the presence of *S. riobravis* was estimated at 3 days after nematode exposure at 28 °C. The treatments used in this study were arranged in a 5 × 5 × 3 factorial with five nematode concentrations, five distances, and three row positions (bottom, side, and top). This experiment included four replications per treatment.

Experiments 2, 3. Nematodes applied after or before irrigation : nematode concentration, incorporation method

These experiments were conducted separately in two contiguous sections of a corn field planted on 23 March 1993. Each section, separated 5 m from each other, consisted of four 120 m-long rows to correspond with four nematode concentrations including the control (0, 50×10^3, 100×10^3 and 200×10^3 IJ/m^2). Within each row, ten 10 m × 1 m plots were separated into five plots (replicates) for nematode application on soil surface and five plots (replicates) for subsurface application. Plots were set around the corn plants, and each plot was separated from each other by 1 m within each row and 2 m between rows. Each of four nematode concentrations was applied to the soil surface or subsurface in randomly assigned plots. For application to the soil surface, nematodes were suspended in 2 liters of water and applied with a 10-liter Solo Backpack sprayer using a nozzle tip (TEEJET 8001VS) at 20 psi. For the subsurface application, nematodes were applied with the same backpack sprayer to a trench (15 cm wide × 10 cm deep) made with a hoe along both sides of the plant bed. The trench was covered with original soil immediately after nematode application. Each plot was further divided into four quadrats (5 m long × 0.50 m wide). Separate quadrats were used to determine the nematode efficacy at 3, 10, 17, and 24 days after nematode application.

For the experiment on nematode application after irrigation, nematodes (Lot No. 3Y55AF130) were stored at 10 °C for 13 days. These nematodes were applied to the soil on 20 July 1993 (time 17 : 00 CST), 4 days after irrigation. For the experiment on nematode application before irrigation, nematodes (Lot No. 3YOAF194) were used on the same day that they arrived on 27 July 1993 (time 17 : 00 CST). The corn field was irrigated immediately after nematode application (time 19 : 00 CST). Corn earworm prepupae were buried in the plots that received nematodes before and after irrigation, at 2 and 3 days after nematode treatment, respectively. Twenty corn earworm per quadrat were individually buried 5 cm deep at 25-cm intervals along the furrow and 12 cm from the plant base. After 5 days, all buried corn earworms were excavated, transported to the laboratory, and dissected to determine parasitism based on the presence of *S. riobravis*.

The treatments used in each experiment were arranged in a 4 × 2 factorial with four nematode concentrations and two incorporation methods (soil surface and subsurface). Each treatment was replicated five times.

The percent soil moisture was determined gravimetrically from soil samples taken 10 cm deep and 10 cm from the top of the bed. Moisture contents for this soil at 0.33, 1, 5, 10, and 15 bars was 23.6, 19.4, 15.3, 15.0, and 14.7 % respectively.

STATISTICAL ANALYSIS

To compare insect mortality and nematode concentration for each irrigation timing, data were subjected to regression analyses (Anon., 1988), and a comparison of the regression coefficients was performed using the general linear test (Neter & Wasserman, 1974). Insect mortality (dependent variable) was regressed against nematode concentration and incorporation method (independent variables) using linear and quadratic models. The coefficient of determination (r^2) and plots of standardized residuals *vs* predicted values from regression analyses were used to evaluate goodness of fit. The numeric values on nematode concentrations were divided by 1000 before the regression analysis.

Data from the nematode application via in-furrow irrigation were subjected to the SAS GLM procedure (Anon., 1988) to relate insect mortality with distance down row (0, 20, 40, 60, 80, 100 m) and across rows (bottom of furrow, side, and top of bed) for each nematode concentration. Means were separated by protected least significant differences (LSD) at the P d 0.05 level. Data on numbers of infective juveniles transported via in-furrow irrigation were also subjected to the GLM procedure of SAS to relate nematode distribution and distance down rows for each nematode concentration at two separate times (during running water and immediately after water had reached the furrow end). Means were separated by the LSD at the P d 0.05 level.

Results

The average air temperature between 10 June 1993 (begin experiments) and 23 August 1993 (end experiments) was 30 °C (maximum = 38, minimum = 23 °C). The average soil temperature (observed at a depth of 2.5 cm on the side of the plant bed) measured at 8 : 00, 12 : 00, and 16 : 00 CST were 31, 39.5 and 39 °C.

The plot that received nematodes via in-furrow irrigation (Experiment 1) was treated on 10 June and the soil temperature at the time of nematode application was 40 °C. Rainfall occurred 1 (10 mm), 2 (17 mm), 3 (3 mm), 4 (0.5 mm), and 5 (3 mm) days after nematode treatment. Rain also occurred 2 (2 mm), 3 (4 mm), 4 (10 mm), 5 (110 mm, tropical storm " Arlene "), and 6 (4 mm) days after insects were buried. No rain occurred for 3 days before burying insects to determine nematode persistence after 15 days. However, traces of rainfall occurred 1 (3 mm) and 2 (4 mm) days after insects were buried. No rainfall occurred or irrigation was applied for 20 days before burying insects when determining nematode persistence after 39 days.

The plot that received nematodes after irrigation (Experiment 2) was treated on 20 July and the soil temperature at the time of nematode application was 39 °C. Irrigation was applied immediately after burying insects when examining nematode persistence after 17 days (the soil moisture at the time of burial was 11 %). A trace of rainfall (3 mm) occurred 24 h before burying corn earworm when determining nematode persistence after 24 days.

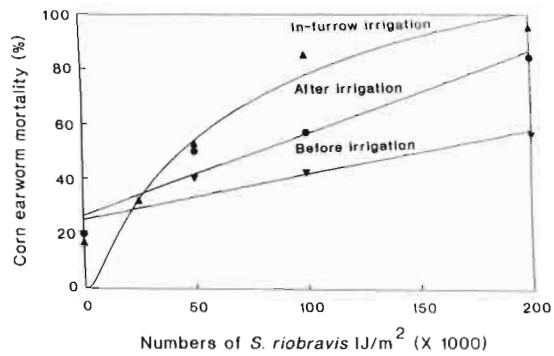

Fig. 1. *Effects of irrigation timing and concentration of* Steinernema riobravis *on mortality of corn earworm, buried within six days after nematode application in corn field.*

The plot that reveived nematodes before irrigation (Experiment 3) was treated on 27 July and the soil temperature at the time of nematode application was 39 °C. Irrigation was applied 2 h after nematode treatment; and this plot did not receive irrigation thereafter to the end of experiment. However, a trace of rainfall (3 mm) occurred 24 h before burying corn earworm when observing nematode persistence after 17 days. The soil moisture at time of nematode application was 5 %.

The pre-application viability and pathogenicity of *S. riobravis* used in the plots that received nematodes via in-furrow irrigation, before irrigation, and after irrigation were 80 and 80 %, 47 and 84 %, 21 and 31 %, respectively, as determined by laboratory bioassay. The effects of irrigation timing and nematode concentration on mortality of corn earworm buried 6 days after nematode application are presented in Fig. 1. The rate of parasitism as a function of nematode concentration was significantly higher for *S. riobravis* applied via in-furrow irrigation (quadratic response) than for nematodes applied before or after irrigation (linear responses) ($P < 0.05$).

The most effective nematode concentration was 200 000 IJ/m^2 when it was applied either via in-furrow irrigation (95 % parasitism), after irrigation (84 % parasitism) or before irrigation (56 % parasitism) (Fig. 1).

EXPERIMENT 1. NEMATODES APPLIED VIA IN-FURROW IRRIGATION : NEMATODE CONCENTRATION, DISTANCE, AND FURROW POSITION

The efficacy of *S. riobravis* applied via in-furrow irrigation on corn earworm buried 6 days after nematode treatment was influenced by nematode concentration ($F = 73.07$; df = 4, 207; $P < 0.0001$) and its distribution across the row (row position) ($F = 10.18$; df = 2, 207; $P < 0.0001$). However, its efficacy did not

change with nematode distribution along the row (distance) (F = 1.38; df = 4, 207; $P = 0.2413$).

The more effective concentrations were 100 000 or 200 000 IJ/m ^{2} which resulted in similar parasitism rate of 85 and 95 %, respectively (Fig. 1). In contrast, concentrations of 25 000 and 50 000 resulted in parasitism rates of 32 and 53 %, respectively. All nematode concentrations applied to the soil via in-furrow irrigation resulted in higher parasitism than the control (17 %). Parasitism of corn earworm in the control plots resulted from an indigenous population of *S. riobravis*. The general response of insect mortality (Y) caused by *S. riobravis* as a function of nematode concentration (C), when nematodes were applied via in-furrow irrigation, was approximated by a quadratic model ($Y = 13.7 + 0.96C - 0.003C^2$; $r^2 = 0.99$). However, the responses were linear when *S. riobravis* was applied before irrigation ($Y = 25.2 + 0.16C$; $r^2 = 0.90$) or after irrigation ($26.6 + 0.30$; $r^2 = 0.94$). Their slopes were significantly different ($P < 0.05$) (Fig. 1).

Nematode distribution across the row significantly influenced the efficacy of *S. riobravis* applied via in-furrow irrigation at 6 days after nematode treatment (Fig. 2). Averaged over all concentrations and distances, the rate of parasitism was higher on the side of the bed (62 %) and bottom of the furrow (60 %) than on the bed top (36 %) (LSD = 8.13; df = 207; MSE = 629.76; $P < 0.05$). Parasitism rates (averaged over concentrations, furrow positions, and replications) were relatively uniform at distances of 20 m (47 %), 40 m (55 %), 60 m (55 %), 80 m (53 %) and 100 m (64 %) (LSD = 16 df = 207; MSE = 629.76; $P < 0.05$).

The distribution of *S. riobravis* across the row had a significant effect on the efficacy of this nematode over time (Fig. 2). Parasitism was higher in the furrow bottom and on the side of the bed than on the top of the bed with the exception of the 6 day post application observation. Parasitism rates at 15 and 39 days post nematode application (averaged over nematode concentrations) were higher at the bottom (62 and 55 %) and side (59 and 42 %) than those on the furrow top (17 and 8 %). Parasitism rates increased with an increase in nematode concentration but decreased with time. For example at 39 days after nematode application, parasitism rates on the bottom and side of the furrow were higher with 200 000 IJ/m^2 (98 and 73 %) and 100 000 IJ/m^2 (94 and 64 %) than those of 50 000 IJ/m^2 (56 and 46 %), and 25 000 IJ/m^2 (20, 20 %) (Fig. 2). In contrast, poor parasitism occurred on the top of the bed with 200 000 IJ/m^2 (15 %), 100 000 IJ/m^2 (14 %), and 50 000 IJ/m^2 (7 %) at 39 days after nematode application. No parasitism occurred on the top of the bed with 25 000 IJ/m^2 (not shown in Fig. 2). The rates of parasitism in the control were higher on the bottom of the furrow and side of the bed (15 and 10 %) than on the bed top (6 %) at 39 days after nematode application (Fig. 2).

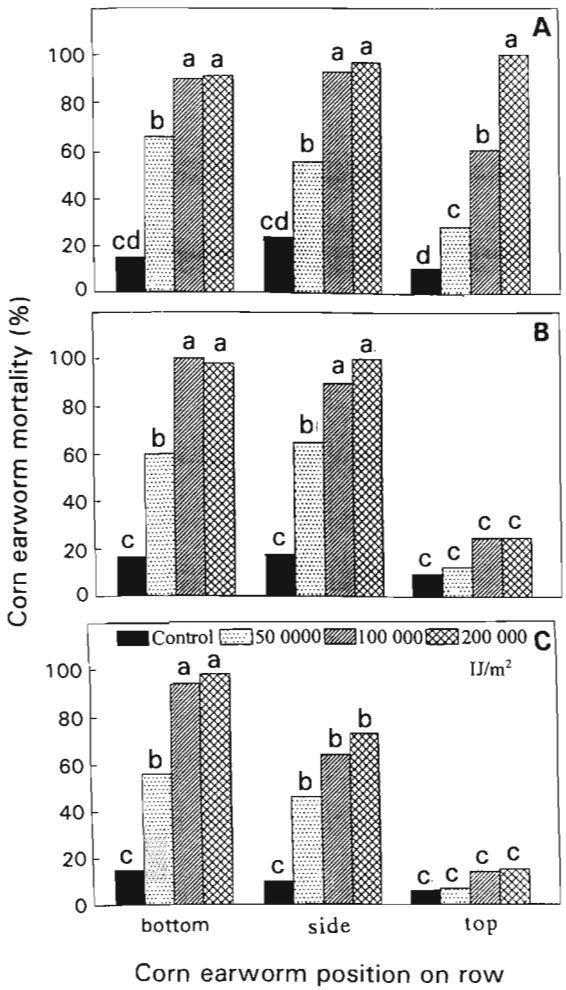

Fig. 2. *Effects of* Steinernema riobravis *applied via in-furrow irrigation on nematode distribution across the furrow and its infectivity against corn earworm over time in corn (Doses are numbers of nematodes per m^2). A, B, C = 6, 15, 39 days after nematode treatment, respectively. Data followed by different letters indicate significant differences (P < 0.05).*

The average numbers of infective juveniles transported by the irrigation water increased with nematode concentration (F = 1266; df = 2,18; $P < 0.0001$) and varied with sampling time (F = 25.58; df = 1, 18; $P < 0.0001$) (Fig. 3). However, they did not change with furrow distance (F = 2.88; df = 4, 18; $P = 0.0525$). Similarly, the numbers of nematodes sampled at different times were consistent and did not change with furrow distance (F = 0.91; df = 4, 18; $P = 0.4803$). Averaged over distances and sampling times, the number of nematodes transported in 100 ml of water was significantly higher for the 200 000 IJ/m^2 concentration (384 IJ) than for the 100 000 IJ/m^2 concentration (196 IJ) or the 50 000 IJ/m^2 concentration (96 IJ)

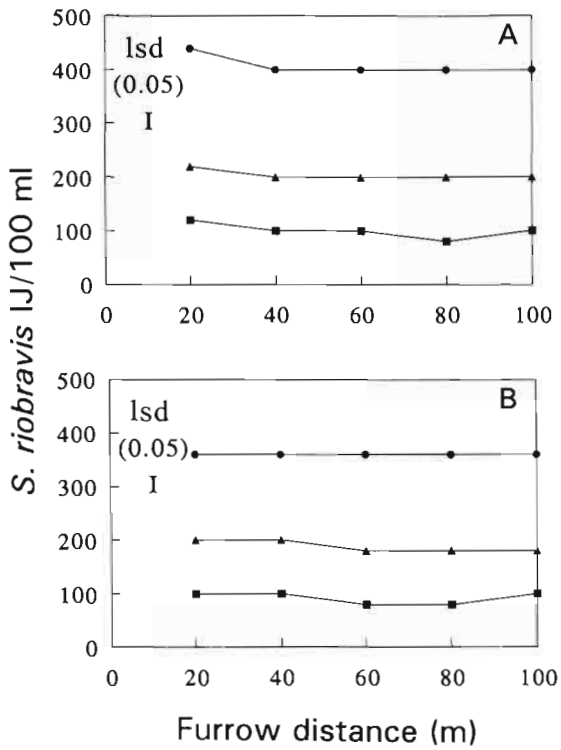

Fig. 3. Distribution of different concentrations of Steinernema riobravis *applied via in-furrow irrigation along the furrow as expressed by the estimated numbers of infective juveniles transported by the irrigation water in corn field. A: During running water; B: Immediately after water had reached the furrow end (Numbers of nematodes are means of five samples/distance/concentration; ■ = 50 000, ▲ = 100 000, ● = 200 000 IJ/m².)*

(LSD = 12,21; df = 18; MSE = 168.89; $P < 0.05$). Also, averaged over all concentrations and distances, more infective juveniles per 100 ml were collected from samples taken while the water was running (237 IJ) than from samples taken after irrigation water had reached the furrow end (213 IJ) (LSD = 9.96; df = 18; MSE = 168.89; $P < 0.05$). The infective juveniles collected from the irrigation water resulted in 100 % corn earworm mortality.

The effects of the application of *S. riobravis* through-furrow irrigation on the mortality of *H. zea* as response to nematode concentration over time are represented in Fig. 4. Corn earworm mortalities (Y) resulting from *S. riobravis* as a function of nematode concentration (C) were approximated by quadratic regression models at 6 ($Y = 13.7 + 0.96C - 0.003C^2$; $r^2 = 0.99$), 15 ($Y = 10.2 + 0.86C - 0.003C^2$; $r^2 = 0.97$), and 39 ($Y = 6.1 + 0.63C - 0.002C^2$; $r^2 = 0.97$) days after nematode application. Although the slopes showed no significant differences ($P < 0.05$), insect mortality at different times (6, 15, and 39 days after nematode treatment) was different (Fig. 4).

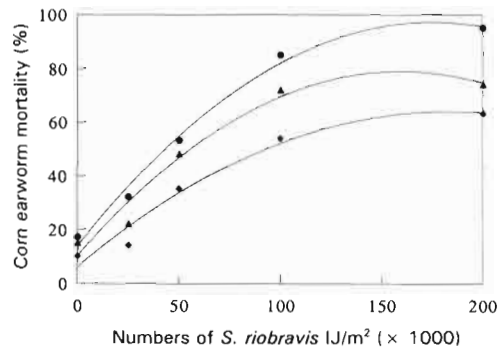

Fig. 4. Effects of the application of Steinernema riobravis *through-furrow irrigation on the control of corn earworm prepupae and pupae as response to nematode concentration over time in corn. (● = 6 days, ▲ = 15 days, ◆ = 39 days after nematode treatment.)*

The most effective nematode concentration applied via in-furrow irrigation against insects buried in soil at 15 and 39 days after nematode treatment was 200 000 IJ/m² (Fig. 4). At this concentration, when averaged over furrow positions and distances, parasitism rates resulted in 74 % and 63 % at 15 and 39 days after nematode application, respectively. Lower parasitism occurred at 15 and 39 days with nematode concentrations of 100 000 IJ/m² (72 and 54 %), 50 000 IJ/m² (48 and 35 %), and 25 000 IJ/m² (22 and 14 %). All nematode concentrations resulted in higher parasitism than the control at 15 and 39 days after nematode application (15 and 10 %, respectively).

Experiments 2, 3. Nematodes applied before or after irrigation: nematode concentration and incorporation method

Parasitism of corn earworm by *S. riobravis* was influenced by both nematode concentration and incorporation method when nematodes were applied before or after irrigation. Mortality caused by *S. riobravis* as a function of nematode concentration for the soil surface or subsurface application before or after irrigation are presented in Table 1, A and B, respectively.

The insect mortality-nematode concentration responses for nematode applications before irrigation was approximated by a quadratic curve when nematodes were incorporated into the subsurface and corn earworms were buried 3 and 10 days after nematode treatment (Table 1 A). However, insect mortality increased linearly with an increase in concentration when nematodes were applied to either the soil surface or below surface at 17 and 24 days post nematode application (Table 1 A).

Similarly, the insect mortality-nematode concentration responses for nematode applications after irrigation was approximated by a quadratic curve when nematodes were incorporated into the subsurface and insects

Table 1. *Parameter estimates and statistics of the field efficacy of* Steinernema riobravis *applied to soil before (A) and after (B) furrow irrigation on the control of corn earworm prepupae and pupae as response to nematode concentration, incorporation method, over time in corn.*

Number of days after nematode application	Incorporation method	Intercept (SE)	Linear Slope (SE)	Quadratic Slope (SE)	*F*	df	*P*	r^2
A 3	surface	22.8 (3.61) *a**	0.15 (0.032) *b*	0 *b*	21.9	1.2	0.04	0.92
3	subsurface	26.8 (6.03) *a*	0.47 (0.153) *a*	−0.002 (0.00070) *a*	7.7	2.1	0.24	0.94
10	surface	22.2 (4.13) *a*	0.10 (0.036) *b*	0 *b*	8.2	1.2	0.10	0.80
10	subsurface	22.0 (6.76) *a*	0.42 (0.172) *a*	−0.001 (0.00079) *a*	10.5	2.1	0.21	0.95
17	surface	21.4 (3.03) *a*	0.09 (0.026) *b*	0 *b*	13.8	1.2	0.06	0.87
17	subsurface	22.0 (0.58) *a*	0.17 (0.005) *b*	0 *b*	112.5	1.2	0.0009	0.99
24	surface	−0.8 (1.36) *b*	0.15 (0.011) *b*	0 *b*	170.0	1.2	0.006	0.98
24	subsurface	7.2 (6.34) *b*	0.21 (0.055) *b*	0 *b*	15.1	1.2	0.06	0.88
B 2	surface	24.6 (4.98) *a**	0.27 (0.043) *a*	0 *b*	41.0	1.2	0.02	0.95
2	subsurface	28.7 (12.43) *a*	0.72 (0.315) *a*	−0.002 (0.0015) *a*	5.5	2.1	0.28	0.92
10	surface	17.4 (2.95) *a*	0.09 (0.025) *b*	0 *b*	11.4	1.2	0.07	0.85
10	subsurface	16.4 (3.17) *a*	0.15 (0.027) *b*	0 *b*	30.3	1.2	0.03	0.94
17	surface	11.2 (1.19) *b*	0.08 (0.010) *b*	0 *b*	64.2	1.2	0.01	0.9
17	subsurface	11.0 (1.13) *b*	0.14 (0.009) *b*	0 *b*	216.8	1.2	0.004	0.99
24	surface	4.8 (4.24) *bc*	0.10 (0.037) *b*	0 *b*	8.0	1.2	0.10	0.80
24	subsurface	4.2 (3.86) *bc*	0.11 (0.033) *b*	0 *b*	12.1	1.2	0.07	0.86

* Parameter estimates followed by different letters indicate significant differences ($P < 0.05$), Neter and Wasserman (1974) general linear test.

were buried 2 days after nematode treatment (Table 1 B). In contrast, linear responses resulted in those plots where *S. riobravis* were applied either to the soil surface or subsurface when insects were buried at 10, 17 and 24 days after nematode application (Table 1 B).

Generally, when corn earworm were buried 2-3 days post nematode application, the highest parasitism occurred when nematodes were applied below the soil surface (72 and 49 % post and pre-irrigation, respectively). Lower parasitism was observed when nematodes were applied to the soil surface (57 and 39 % post and pre-irrigation, respectively). The lowest parasitism rates (25 %) occurred in the control plots.

When comparing the effects of irrigation timing and soil moisture on the efficacy of *S. riobravis* applied at the most effective rate (200 000 IJ/m^2) over time, the delivery of nematodes via in-furrow irrigation resulted more effective (63 % parasitism at 39 days after nematode application) than when it was applied before irrigation (38 % at 24 days) or after irrigation (24 % at 24 days) (Fig. 5). The soil moisture content was higher in corn fields that received *S. riobravis* via in-furrow irrigation (17-21 %) than those treated with nematodes before (15-19 %) or after furrow irrigation (10-19 %) (Fig. 5).

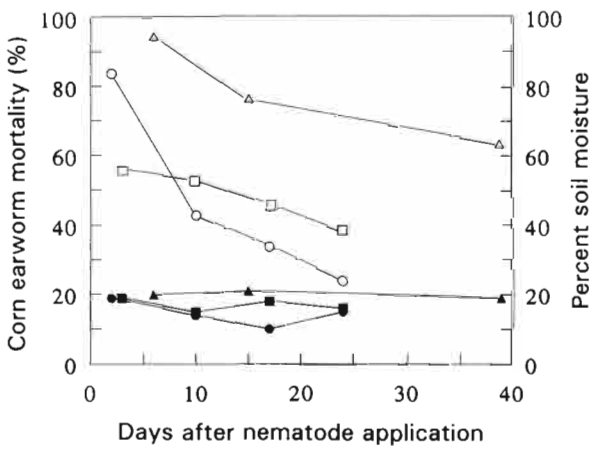

Fig. 5. *Effects of furrow irrigation timings (white symbols) and their soil moisture (black symbols) on the efficacy of* Steinernema riobravis *applied at 200 000 IJ/m² against corn earworm prepupae and pupae in corn field over time. (▲, n, ■, □, ●, ○ represents nematode application via in-furrow irrigation, before irrigation, after irrigation, respectively.)*

Discussion

Moisture is considered the most critical factor for the survival and movement of entomopathogenic nematodes (Kaya, 1990). Our data indicate that application of *S. riobravis* through in-furrow irrigation provided excellent conditions for their distribution, dispersal, and survival. Our data also showed that delivery of the nematodes during irrigation was superior to application either before or after irrigation. Similar results were obtained in the greenhouse and small field plots in 1992 (unpubl.). Furthermore, when corn earworms were buried 2-3 days after nematode treatment, nematode application after irrigation resulted in higher rates of corn earworm parasitism than when nematodes were applied prior to irrigation even though an irrigation was applied immediately after application. This indicates that nematode mortality may have occurred quite rapidly following application to very dry soil (5 % moisture level). Nematode efficacy over time varied directly with soil moisture levels. For example, higher parasitism occurred 10, 17, and 24 days post nematode treatment, in the plots where higher soil moisture was maintained through irrigation.

The application method alone can not account for all observed variation. Possibly factors such as nematode quality and soil moisture at the time of application may have also influenced parasitism. Although nematodes in the in-furrow irrigation experiment and those applied prior to irrigation were used on the day they were received from Biosys, viability differed (80 and 47 % respectively). However, the laboratory bioassay indicated no difference in pathogenicity of these two lots of nematodes (80 and 84 % respectively). Nematodes applied post irrigation had been stored 13 days at 10 °C prior to use and both viability (21 %) and pathogenicity (31 %) were detrimentally impacted. To compensate such differences, nematode densities were adjusted to their corresponding viabilities. Although the nematode quality used for each application method was different, either in age or lot number such comparison among nematode application methods can be established based on their adjusted densities. Such comparisons indicate differences on the overall responses and their regression slopes. The regression models generated from these experiments can be of great value in comparing application methods of *S. riobravis*. These models describe not only the statistical relationships between the insect mortality response and the nematode concentration but they also provide a value for the slope which estimates the change in activity or pathogenicity per unit change in concentration of infective juvenile nematodes. Based on this criterion, the quadratic slopes are indicative parameters of a better response compared to the linear slopes when comparing the rates of parasitism as a function of nematode concentration for each application method. The use of the regression models with their parameters will enable us to optimize release strategies with *S. riobravis*. Considering that the efficacy of this nematode may vary with application method, other factors such as soil moisture and nematode quality should be considered closely.

Nematodes applied below the soil surface resulted in higher corn earworm parasitism than those applied to the soil surface. The quadratic slopes of the subsurface application shows a better response than those linear slopes of the surface application method. Subsurface application probably provided greater protection from drying and adverse sunlight effects. The results obtained in these experiments corroborate recent information on the use of subsurface + surface nematode soil application (Cabanillas & Raulston, 1995). Subsurface application appears to be a viable alternative to in-furrow irrigation application, assuming adequate soil moisture levels. This incorporation method could be used in conjunction with other farming practices such as discing and cultivation.

Although parasitism rates declined over time, *S. riobravis* was able to persist in the soil in the absence of hosts and remain infective for up to 39 days. In similar field studies, this nematode persisted for up to 75 days after nematode application (Cabanillas & Raulston, 1995).

Application of *S. riobravis* via in-furrow irrigation could be a practical system for suppressing corn earworm prior to adult emergence in source areas where corn acts as a nursery crop that produces large numbers of migrants (Sparks *et al.*, 1989). It should be a cost-effective system for growers since irrigation and pest control are combined. These findings could contribute to the commercial application of *S. riobravis* and similar insect pathogens as alternatives to chemicals for suppressing corn earworm and other crop insect pests early in their life cycle - while they are prepupae or pupae in the soil and before they emerge into mobile, reproductive adults.

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