Evaluation of *Steinernema riobravis* (Nematoda: Steinernematidae) Against the Mexican Rice Borer (Lepidoptera: Pyralidae)[1]

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Abstract The virulency of an endemic nematode, *Steinernema riobravis* Cabanillas, Poinar and Raulston (Nematoda: Steinernematidae), was tested against the Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae), in the laboratory and field. *Steinernema riobravis* caused 100% mortality in *E. loftini* larvae at all concentrations of 20 to 240 nematodes per larva 2 d post treatment. Numbers of juvenile progeny increased significantly with inoculum dosage of nematodes. Average juvenile progeny ranged from 2,000 per borer larva at 10 nematodes per larva to over 4,000 per larva at 120 per larva. A field experiment on sugarcane (*Saccharum* spp. cv 'NCo 310') was performed using three treatments: (1) control (no nematodes); (2) low application rate (1.24×10^9 nematodes/ha); and, (3) high application rate (2.47×10^9 nematodes/ha). At weekly intervals, the field was sampled for numbers of internodes per stalk, numbers of internodes damaged by borer larvae, and parasitoids reared from larvae collected. The field results showed the nematode treatments were ineffective in reducing borer incidence or damage. Percentage of bored internodes and numbers of borers collected significantly increased with time, but treatment effects were not significant. Numbers of parasitoids emerging from the larvae collected were too low to be analyzed statistically. Plant height, aboveground biomass, and juice quality were not significantly affected by treatment. The ineffectiveness of the nematode applications may be due to desiccation caused by exposure to sunlight and inadequate humidity, as well as poor contact with the target insect.

Key Words *Steinernema riobravis, Eoreuma loftini,* Mexican rice borer, sugarcane, nematode, biological control

The sugarcane industry generates gross annual income of $40 to $65 million for the Lower Rio Grande Valley of Texas (Cowley 1998). The key insect pest is the Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae). Recent field surveys commonly record about 20% of cane stalks damaged by the borer (Legaspi et al. 1997). Despite substantial crop damage due to the rice borer, sugarcane farmers currently do not treat their crop with insecticides, apparently under the assumption that current levels of borer damage do not result in measurable economic loss and that the cryptic lifestyle of the pest restricts contact with control agents (Legaspi et al. 1999). By boring into the stalks and packing its tunnels with frass, the pest is naturally protected within its host plant. The recent determination of loss-injury relationships by

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Legaspi et al. (1999) has renewed grower interest in developing control methods against the borer. Using regression analysis, Legaspi et al. (1999) estimated that the injury level of 20% bored internodes actually results in an annual loss of about $1,181.04 / ha, and a total loss of $10 to $20 million to the local industry. Furthermore, the rice borer is continuing its northeast migration along the Gulf Coast and further threatens sugarcane and other gramineous crops such as corn, rice, sorghum, wheat and forage grasses throughout the South (Legaspi et al. 1997).

The sugarcane system in south Texas presents an ideal opportunity to evaluate biological control agents against *E. loftini.* An important consequence of the abandonment of chemical controls is that farmers currently do not view insecticides as a viable alternative against which biological methods will be compared. Biological control agents also will not themselves be adversely affected by insecticides. Entomophagous nematodes are relatively untested against pests of sugarcane. In preliminary field trials in Madero, TX, *Steinernema feltiae* (Filipjev) was applied against the rice borer in sugarcane fields at the rate of 5.7 billion/ha (Pfannenstiel and Browning 1989). Although nematodes were recovered after application, rice borer populations were not affected. In order of decreasing virulency, laboratory bioassays have shown that *S. carpocapsae* (Wieser) 'All', *S. feltiae* 'SN', and *Heterorhabditis bacteriophora* Poinar 'HP88' (Rhabditida: Heterorhabditidae) were all capable of killing rice borer larvae (Ring and Browning 1990).

The relatively recent discovery of an entomopathogenic nematode native to the Lower Rio Grande Valley suggests a promising control agent already acclimated to its subtropical semi-arid environment. *Steinernema riobravis* Cabanillas, Poinar and Raulston was isolated from soil samples from corn fields in Weslaco in 1990 (Cabanillas et al. 1994). The nematode appears to be endemic to the area, where it was found parasitizing prepupae and pupae of the corn earworm [*Helicoverpa zea* (Boddie)] and fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] (Lepidoptera: Noctuidae) (Cabanillas and Raulston 1994). Soil applications of *S. riobravis* were also effective against *H. zea* (Cabanillas and Raulston 1995). Laboratory studies on the pink bollworm [*Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)] have also shown that *S. riobravis* is more heat tolerant than *S. carpocapsae* (Cabanillas and Raulston 1996, Henneberry et al. 1996b). Field survival of this nematode species may be enhanced by temporary moisture deficits that trigger survival mechanisms (Henneberry et al. 1996a, Duncan et al. 1996). *Steinernema riobravis* has also been evaluated as a control agent against the cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae) (Schroeder et al. 1996) and citrus root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) (Duncan and McCoy 1996). We studied the efficacy of *S. riobravis* as a biological control agent of *E. loftini* in the laboratory and field.

Materials and Methods

Laboratory virulency. *Eoreuma loftini* were obtained from a colony reared at the Texas Agricultural Experiment Station, in Weslaco. *Steinernema riobravis* was obtained from the USDA, ARS Cotton Insects Research Unit (now with the Integrated Farming and Natural Resources Research Unit), Weslaco, TX. *Eoreuma loftini* fourth

instars were isolated individually in Petri dishes (5 cm diam) lined with filter paper. Infective juvenile *S. riobravis* nematodes were suspended in distilled water at the following concentrations: 0 (control), 20, 30, 40, 80, 160, 200, and 240 nematodes per borer larva. The suspension of nematodes was applied at the rate of 0.5 ml per Petri dish. Four insects were tested at each concentration of nematodes. The Petri dishes were kept in a sealed plastic bag at ambient temperatures. The numbers of nematode juvenile progeny were measured using White's trap protocol (White 1927). Mortality was assessed 2 d post treatment.

Field evaluation. The field test was performed at the Annex Farm of the Texas Agricultural Experiment Station in Mercedes. Weather data (air temperature and relative humidity) were recorded using an evapotranspiration monitoring station (model ET106; Campbell Scientific, Logan, UT) fitted with an air temperature and relative humidity probe (CS500-LC5; Vaisala, Woburn, MA). The test field measured 90.8 × 24.4 m and consisted of 15 rows of sugarcane (*Saccharum* spp., var. 'NCo 310') planted 1.5 m apart. The field was divided into 12 blocks (randomized complete block design: 3 treatments × 4 replicates). Each block consisted of 3 rows of cane measuring 21.3 × 4.6 m. On the longitudinal (N-S) axis, blocks were separated by 1.9 m of cane; crosswise (E-W) blocks were separated by 3 rows of cane. There were three treatments: (1) control, no nematodes; (2) low rate, *S. riobravis* was applied in the form of a commercial product (BioVector 355®, Biosys, Palo Alto, CA; acquired by ThermoTrilogy, Columbia, MD) at the rate of 1.24×10^9/ha; (3) high rate, *S. riobravis* applied at 2.47×10^9/ha. Spraying was performed on 2 June 1997 using a tractor-mounted sprayer (3.2 kph speed) at a pressure of 7.04 kg/cm^2.

In each 3-row block, the two outer rows were sampled for presence and damage caused by *E. loftini*. The middle row was used to measure yield. At each sampling date, 10 stalks were collected and the following information was collected: numbers of internodes, numbers of damaged internodes, and parasitoids emerged. All borer larvae recovered were recorded and placed in 18.5-ml diet cups (Fill-Rite, Newark, NJ) filled with artificial diet and covered with a 37.5-mm polycoated pull tab cap (StanPac, Tonawanda, NY). The larvae were then placed in a growth chamber (Percival, Boone, IA) and held until emergence of adult moths or parasitoids at 27 ± 3°C, 50-60% RH and 14:10 (L:D) h photoperiod. Sampling dates were: 21, 28 May, 10, 17, 24, 30 June, 4, 7, 23, 30 July, 4, 13, 19, 27 August, 1, 10, 15 September, 2, 22, and 28 October 1997. Plant height was measured on 1 December 1997 using a sample of 20 stalks selected from the outer rows. The field was harvested on 11 December 1997. Yield was estimated by harvesting all above-ground biomass of the middle rows (tops were removed) in each block and obtaining the fresh plant weight. Juice quality was determined in a sample of 15 stalks through the following measures: ash content (mhos), commercially recoverable sugar content (lbs sugar per ton of cane; g/2 kg), pol content (polarimeter reading, %), and juice purity (%). Terminology and methods are described in Chen (1985) and Legaspi et al. (1999).

Statistical analysis. Statistical analysis was performed using the Systat Statistical Package (SPSS 1998; SPSS, Chicago, IL). Regression analysis was used to determine the effects of nematode inoculum dosage on juvenile production. Mean plant heights, yield and juice quality were analyzed for treatment effects (1-Way ANOVA). Percentages of bored internodes, numbers of borers and parasitoids recorded were analyzed for effects of time and treatment (2-Way ANOVA). Percentage data were transformed (square root-arc sine method) prior to analysis, but are presented as untransformed means.

Results and Discussion

Laboratory virulency. The numbers of juvenile progeny were significantly affected by inoculum dosage ($Y = 2048.1 + 17.3x$; $F = 19.5$; df = 1, 23; $P < 0.01$; $R^2 = 0.46$; SE constant = 284.9; SE slope = 3.9) (Fig. 1). Average juvenile progeny ranged from 2,000 per borer larva at the lowest nematode concentration tested to over 4,000 per larva at the highest. Two days post treatment, *S. riobravis* caused 100% mortality in fourth instar *E. loftini* larvae at all nematode concentrations tested.

Field evaluation. During mid-July to late-October, 1997, mean air temperature was 26.8 ± 0.4°C (mean ± SE) (Fig. 2); RH was 76.2 ± 0.8%. The percentage of bored internodes was significantly affected by sampling date ($F = 38.6$; df = 19, 2327; $P < 0.01$) but not treatment ($F = 1.8$; df = 2, 2327; $P = 0.2$) (date × treatment: $F = 0.8$; df = 38, 2327; $P = 0.9$). The percentages of bored internodes generally increased with time for all treatments (Fig. 3). The numbers of *E. loftini* larvae collected in the stalks

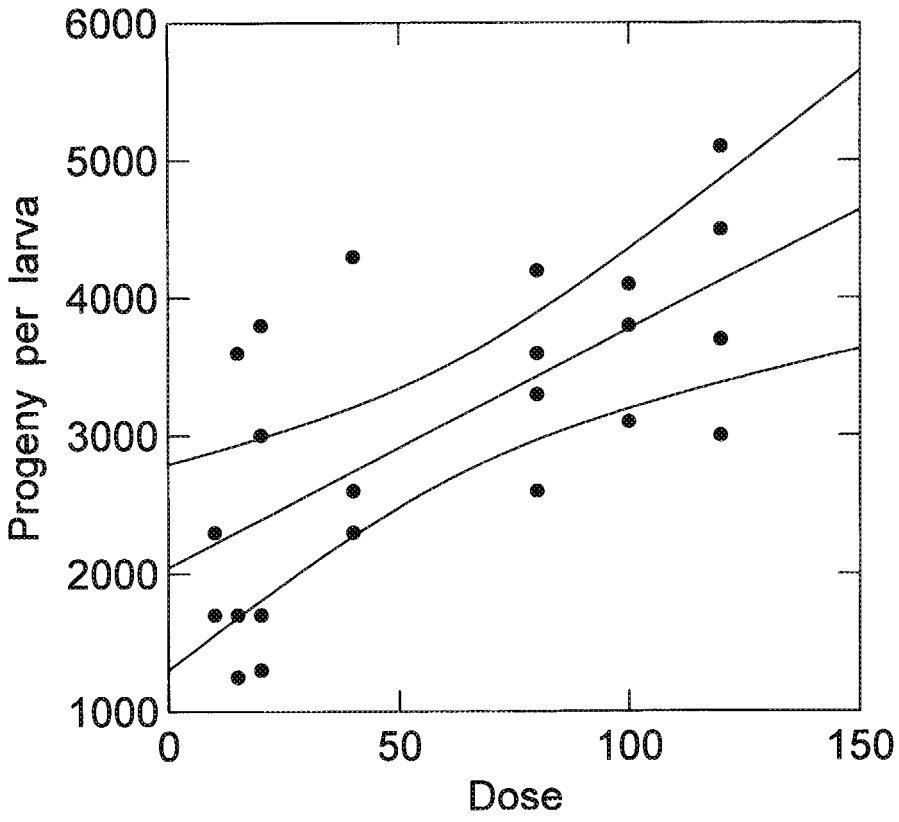

Fig. 1. Numbers of juvenile progeny per host larva as a function of inoculum dosage using White's (1927) trap protocol. The line drawn was fitted by linear regression ($Y = 2048.1 + 17.3x$; $F = 19.5$; df = 1, 23; $P < 0.01$; $R^2 = 0.46$) and includes 95% confidence limits.

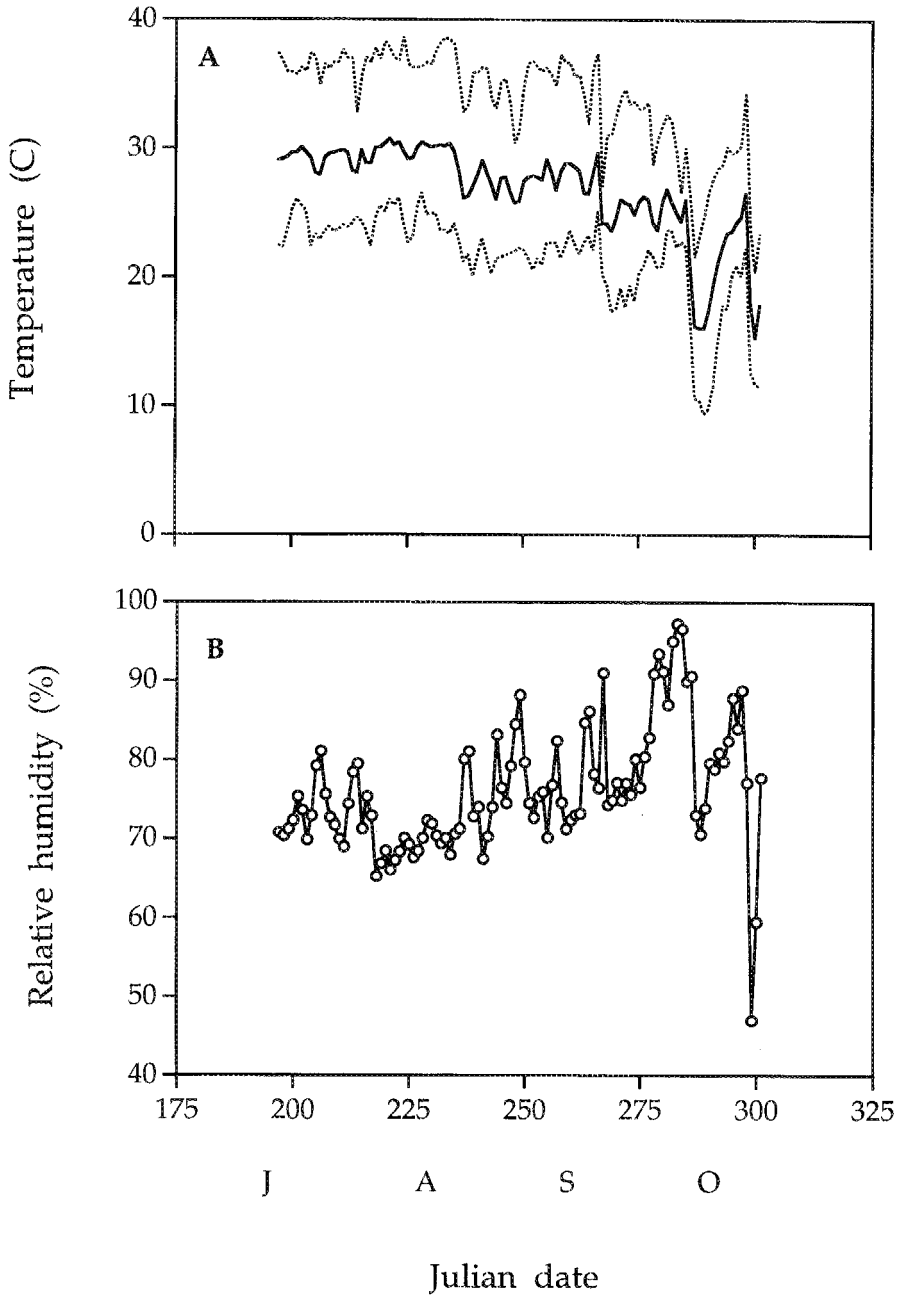

Fig. 2. Air temperature (°C) and relative humidity (%) in Weslaco, TX (1997). (A.) Mean air temperature is indicated by the solid line, daily minima and maxima by the dashed lines. (B.) Mean relative humidity is shown for mid-July to late-October.

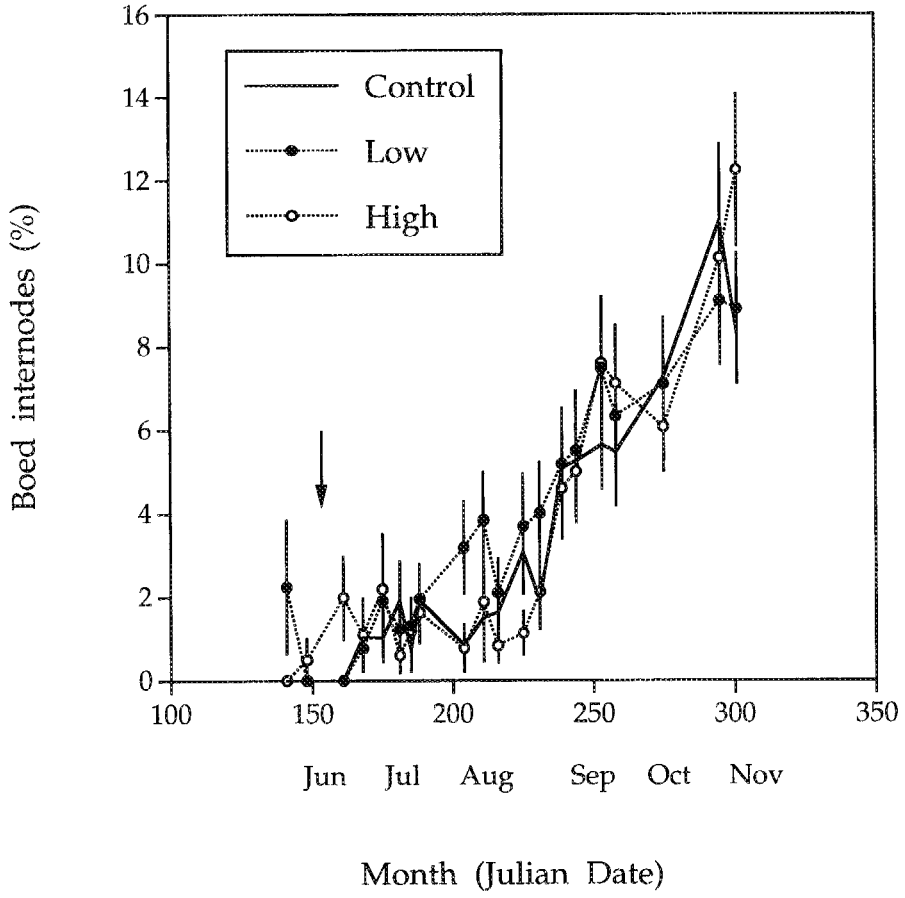

Fig. 3. Percentages of bored internodes (mean ± SE) as affected by low and high rates of nematode applications in 1997 (arrow indicates application).

also was significantly affected by date ($F = 20.9$; df = 19, 2338; $P < 0.01$) but not by treatment ($F = 1.6$; df = 2, 2338; $P = 0.2$) (date × treatment: $F = 1.6$; df = 38, 2338; $P < 0.05$). As in the case of percentages of bored internodes, numbers of *E. loftini* larvae recovered generally increased with time (Fig. 4). The numbers of parasitoids emerging from the larvae collected were too low to be analyzed statistically. Mean plant heights were not affected by treatment: control, 1.9 ± 0.08 m (mean ± SE); low rate, 1.89 ± 0.03 m; and, high rate, 1.95 ± 0.05 m ($F = 0.34$; df = 2, 8; $P = 0.7$). Aboveground sugarcane harvest was not significantly affected by treatment: control, 242.9 ± 13.2 kg; low rate, 246.3 ± 10.9 kg; and, high rate, 252.0 ± 28.1 kg ($F = 0.06$; df = 2, 9; $P = 0.9$). Measures of juice quality were not affected by treatment: ash content, 8.9 ± 0.3 mhos ($F = 0.3$; df = 2, 9; $P = 0.7$; $R^2 = 0.1$); recoverable sugar, 167.8 ± 7.5 g/ 2 kg ($F = 1.1$; df = 2, 9; $P = 0.4$; $R^2 = 0.2$); pol, 11.7 ± 0.3% ($F = 1.0$; df = 2, 9; $P = 0.4$; $R^2 = 0.2$); and juice purity, 80.7 ± 2.8% ($F = 1.2$; df = 2, 9; $P = 0.4$; $R^2 = 0.2$).

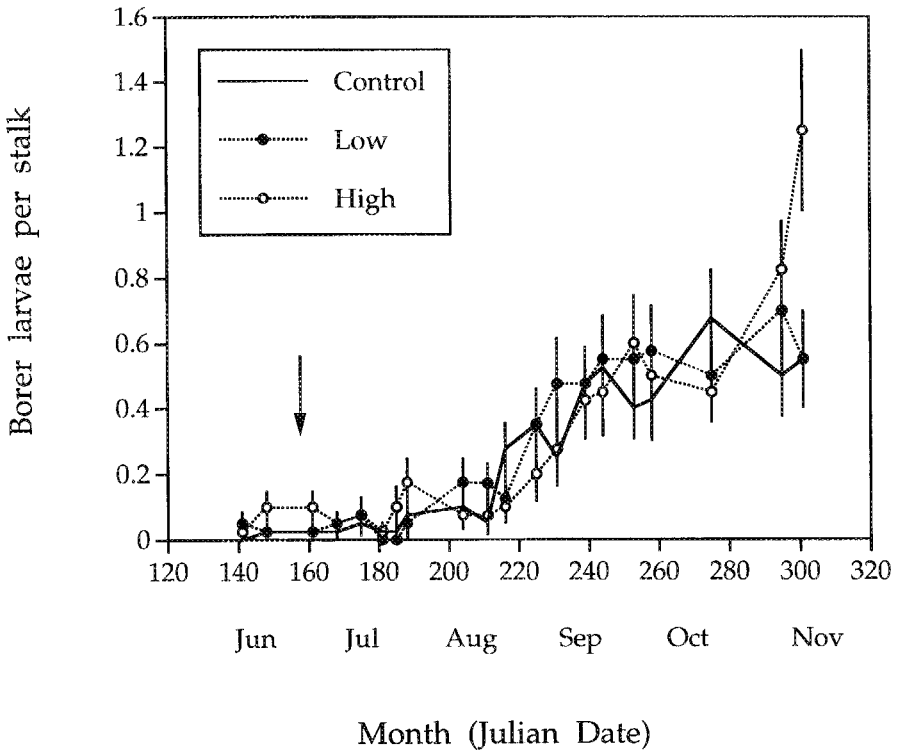

Fig. 4. Number of *E. loftini* larvae per stalk in 1997 (mean ± SE) as affected by nematode applications (arrow indicates application).

Nematodes in the family Steinernematidae are among the most promising biological control agents against agricultural and horticultural insect pests (Cabanillas et al. 1994). The commercial product we used, BioVector 355, is labelled for use against three species of citrus weevils (Coleoptera: Curculionidae): *Diaprepes abbreviatus* (L.), *Pachnaeus litus* Germar and *P. opalus* Oliv. The nematode *S. riobravis* possesses many attributes that make it especially promising in the Lower Rio Grande Valley. Optimal growth and development occurs at the high temperatures characteristic of the region (about 30°C). The infective juvenile stage is also highly mobile and displays both strategies of waiting for potential hosts and actively seeking them (Cabanillas et al. 1994). Soil is the natural habitat of *S. riobravis* (Cabanillas and Raulston 1995), whereas *E. loftini* larvae are often located within the cane stalks. Egg masses of the rice borer are laid in dried leaves on the ground, and the first instars move up to the whorl to tunnel inside the stalk. Nevertheless, we performed an early application of nematodes because of the possibility of infecting borer larvae prior to their tunneling into the stalks. We also were interested in the possibility that *S. riobravis* might possess the mobility to attack the host larvae even within the stalk.

In our laboratory virulency tests, *S. riobravis* killed *E. loftini* larvae, as has been found in similar tests using *S. carpocapsae, S. feltiae,* and *H. bacteriophora.* Numbers

of juvenile progeny increased with dosage of nematode inoculum. However, laboratory studies are poor predictors of field efficacy. The effectiveness of microbial pesticides in the field depends on several factors, including the innate susceptibility of the target pest, degree of exposure to the pesticide, prevailing environmental conditions and physiological interactions between the host and pathogen (Fuxa and Tanada 1987, Tanada and Kaya 1993). Exposure of the target depends largely on the probability that the pest will come in contact with the biopesticide, which in turn depends on factors, such as the persistence of the microbial agent in the field, the distribution of the microbial within the crop canopy, and the behavior and morphology of the target pest. Achieving effective spray coverage in the dense sugarcane canopy is always problematic, regardless of the control agent applied.

The ineffectiveness of *S. riobravis* in the field is likely due to insufficient contact with the target pest and nematode desiccation caused by exposure to sunlight and inadequate humidity. The host finding ability of the nematode is apparently insufficient to cause measurable effects in host suppression or prevention of damage to sugarcane. Despite the heat tolerance and persistence in soil documented for *S. riobravis* (Cabanillas and Raulston 1995, Henneberry et al. 1996b), mortality in the aboveground sugarcane system may be too high. Without significant improvements in technology to deliver the nematode to the target insect, the use of *S. riobravis* against *E. loftini* in the field is unlikely to produce satisfactory control.

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