Evaluation of Entomopathogenic Nematodes against the Mexican Rice Borer (Lepidoptera: Pyralidae)[1]

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The Mexican rice borer (MRB), *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae), has become the key pest of south Texas sugar cane (*Saccharum* spp.) (2,3), and alternative methods to chemical pesticides are needed to implement integrated pest management programs for this pest. Because information on pathogens attacking the MRB does not exist, we set out to determine the efficacy of three entomopathogenic nematode species, *Heterorhabditis bacteriophora* Poinar 'HP 88' (Rhabditida: Heterorhabditidae), *Steinernema feltiae* (Filipjev) (= *bibionis*) 'SN' (Rhabditida: Steinernematidae), and *S. carpocapsae* (Weiser) 'All', against the MRB and to determine the dose response between MRB and these nematodes.

Larvae of the MRB reared on artificial diet were used in all tests. Third-stage infective juveniles (IJ) of *H. bacteriophora, S. feltiae,* or *S. carpocapsae* were removed from sponges through 425-μm-pore, 45-μm-pore, and 38-μm-pore sieves, diluted to a known volume, and agitated. The number of IJ per milliliter was determined through serial dilutions and by counting live nematodes under a stereomicroscope. Nematodes were dispersed in distilled water to provide desired treatment levels in 3 ml water. Treatments were applied in a geometric progression from 0 to 2,048 IJ/MRB. Diluted suspensions (3 ml), from lowest to highest concentration, were pipetted onto Whatman no. 3 filter paper (9.0 cm d) in each of 20 sterile petri dishes (100 × 15 mm) per treatment for treatments greater than 1 IJ/MRB. Petri dishes containing one IJ/MRB were treated with 3 ml distilled water, and five nematodes were transferred with a Pasteur pipet and the aid of a stereomicroscope. Control petri dishes were treated with 3 ml distilled water alone. Five MRB larvae (third or fourth instar) were placed in each petri dish. Each treatment consisted of five replicates of 20 borer larvae. The susceptibility of early MRB instars was also tested by exposing 20 MRB neonates in a single petri dish to 16 IJ/neonate (LD$_{99}$ for *S. carpocapsae* from dose mortality experiments) of each nematode species. Artificial diet (ca. 1 g) was placed in each petri dish. Petri dishes were held at 26 C, and dead larvae were examined for nematodes at 1–6, 9, and 13 days post-treatment.

Data were analyzed by analysis of variance and contrasts (5) over all doses and time to determine differences in mortality due to nematode species (general linear models procedure [4]). Probit analysis was used to determine the dose, confidence interval and slope at which 50 and 95% of the MRB larvae were killed (probit procedure [4]). Relative toxicities were calculated for each nematode species by dividing the lowest LD$_{95}$ by the LD$_{95}$ for each nematode and multiplying by 100 (1).

Nematode species exhibited significant differences in mortality for MRB third and fourth instars over all doses and sample dates ($F = 23.85; P > F \le 0.0001$). Both steinernematid species exhibited greater mortality of MRB third and fourth instars
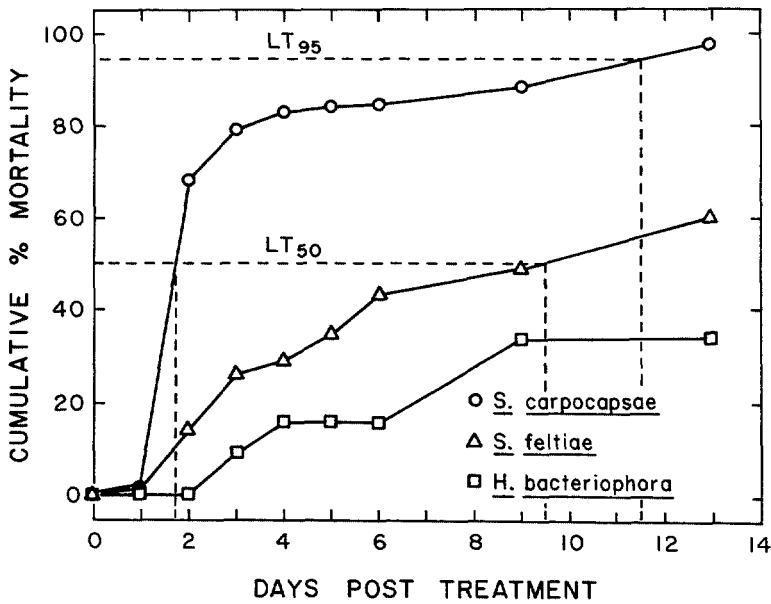
FIG. 1. Cumulative percentage of mortality of Mexican rice borer third and fourth instars exposed to *Heterorhabditis bacteriophora, Steinernema carpocapsae,* and *S. feltiae* (= *bibionis*) at the dosage level of 16 infective juveniles/borer (LD_{99} for *S. carpocapsae*).

than the heterorhabditid ($F \geq 26.8, P > F \leq 0.0001$), but significant differences in mortality of MRB third and fourth instars were not observed among steinernematid species ($F = 1.88, P > F = 0.170$). *Steinernema carpocapsae, S. feltiae,* and *H. bacteriophora* killed 82.5, 74.0, and 44.5%, respectively, of MRB third and fourth instars. Differences in mortality over time were observed for nematode species (Fig. 1). Mortality rates due to steinernematid IJ were much higher and were accomplished much faster than mortality rates due to heterorhabditid IJ (Fig. 1). Within 48 hours after exposure to *S. carpocapsae, S. feltiae,* or *H. bacteriophora* at 16 IJ/MRB larva (LD_{99} for

S. carpocapsae), 67, 15, and 0%, respectively, of the MRB third and fourth instars were dead. The median lethal time of MRB third and fourth instars treated with *S. carpocapsae* IJ was 41.5 hours, with 66% mortality occurring between 24 and 48 hours.

Percentage of mortality due to factors other than nematodes (larval death and nematodes absent) was significantly greater for the heterorhabditid than the steinernematids ($F = 42.88; P > F \leq 0.0001$). Mortalities of MRB third and fourth instars due to other factors were lesser in steinernematid treatments than in heterorhabditid treatments ($F \geq 28.5, P > F \leq 0.0001$), but significant differences in mor-

TABLE 1. Lethal doses (LD), 95% fiducial limits (FL), slopes, standard error of the mean (SE), and relative infectivity (RI) for Mexican rice borer third and fourth instars treated with three species of entomopathogenic nematodes.

	Lethal dose				Slope	
	LD_{50}	95% FL	LD_{95}	95% FL	($\pm$ SE)	RI
Steinernema carpocapsae	1.8	1.2–2.5	8.6	2.5–18.2	2.5 $\pm$ 0.01	100.0
Steinernema feltiae	1.8	1.1–2.7	252.7	151.2–501.9	0.8 $\pm$ 0.01	3.4
Heterorhabditis bacteriophora	37.6	20.1–67.2	5,464.9	1,825.2–32,474.6	0.8 $\pm$ 0.02	0.2

Probit analysis on $\log_{10}$ dose. Relative infectivity as calculated by dividing the lowest LD_{95} by the LD_{95} for each nematode and multiplying by 100. N = 240 borers/treatment.

tality of MRB third and fourth instars due to other factors were not observed among steinernematid species ($F = 0.05$, $P > F = 0.824$). Mortality due to factors other than nematodes was 8.0, 9.0, and 21.0% of MRB third and fourth instars in *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora* treatments, respectively. Mortality in the control treatments (21%) was due to factors other than nematodes.

The steinernematid species exhibited similar LD_{50} and fiducial limits on LD_{50} (95% fiducial limits overlap) (Table 1); however, *S. carpocapsae* exhibited lower LD_{95} and fiducial limits on LD_{95} (95% fiducial limits fail to overlap) and a higher relative infectivity. The slope was greatest in treatments of *S. carpocapsae* (Table 1); LD_{50}, LD_{95}, and fiducial limits were highest (95% fiducial limits fail to overlap) and relative infectivity was lowest in treatments with the heterorhabditid.

Six neonates were killed by *S. feltiae*, six by *S. carpocapsae*, and 10 by *H. bacteriophora*. All other neonates escaped from nematode-treated petri dishes except one neonate that was alive in the *S. feltiae* treatment at 9 days after treatment. Ten neonates were alive and 10 escaped from control petri dishes. Data from experiments on neonates indicated that all three nematode species have the potential to kill MRB larvae infesting leaf sheaths.

The steinernematids *S. carpocapsae* and *S. feltiae* are potentially good biological control agents for MRB larvae in the field.

The sugar cane plant and the MRB are a year around host-pest system. Larvae crawl between the leaf sheath and the stalk, mine the leaf sheath for 2–3 weeks, and then bore into the sugar cane stalk. Water is held in the leaf sheath most of the year, providing a source of moisture for the nematodes. Also, the sheath provides a protected environment for the nematode. Sheltered nematodes may be able to persist in this system for several days or longer, whereas pesticides are active only for short periods (i.e., 3 days). Chemical pesticides are ineffective in the stalk, whereas nematodes may search for and kill borers in the stalk. Further studies are required to determine the feasibility of field applications of the steinernematids for control of the MRB.

LITERATURE CITED

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