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Susceptibility of the boll weevil to *Steinernema riobrave* and other entomopathogenic nematodes

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Abstract

The susceptibility of the boll weevil (BW), *Anthonomus grandis* Boheman, to *Steinernema riobrave* and other nematode species in petri dishes, soil (Hidalgo sandy clay loam), and cotton bolls and squares was investigated. Third instar weevils were susceptible to entomopathogenic nematode (EN) species and strains in petri dish bioassays at 30 °C. Lower LC_{50}'s occurred with *S. riobrave TX-355* (2 nematodes per weevil), *S. glaseri* NC (3), *Heterorhabditis indicus* HOM-1 (5), and *H. bacteriophora* HbL (7) than *H. bacteriophora* IN (13), *S. riobrave* TX (14), and *H. bacteriophora* HP88 (21). When infective juveniles (IJs) of *S. riobrave* were applied to weevils on filter paper at 25 °C, the LC_{50} of *S. riobrave* TX for first, second, and third instars, pupae, and 1-day-old and 10-days-old adult weevils were 4, 5, 4, 12, 13, and 11 IJs per weevil, respectively. The mean time to death, from lowest to highest concentration, for the first instar (2.07 and 1.27 days) and second instar (2.55 and 1.39 days) weevils were faster than older weevil stages. But, at concentrations of 50 and 100 IJs/weevil, the mean time to death for the third instar, pupa and adult weevils were similar (1.84 and 2.67 days). One hundred percent weevil mortality (all weevil stages) occurred 3 days after exposure to 100 IJs per weevil. Invasion efficiency rankings for nematode concentration were inconsistent and changed with weevil stage from 15 to 100% when weevils were exposed to 100 and 1 IJs/weevil, respectively. However, there was a consistent relationship between male:female nematode sex ratio (1:1.6) and nematode concentration in all infected weevil stages. Nematode production per weevil cadaver increased with increased nematode concentrations. The overall mean yield of nematodes per weevil was 7680 IJs. In potted soil experiments (30 °C), nematode concentration and soil moisture greatly influenced the nematode efficacy. At the most effective concentrations of 200,000 and 400,000 IJs/m^2 in buried bolls or squares, higher insect mortalities resulted in pots with 20% soil moisture either in bolls (94 and 97% parasitism) or squares (92 and 100% parasitism) than those of 10% soil moisture in bolls (44 and 58% parasitism) or squares (0 and 13% parasitism). Similar results were obtained when nematodes were sprayed on the bolls and squares on the soil surface. This paper presents the first data on the efficacy of *S. riobrave* against the boll weevil, establishes the potential of EN to control the BW inside abscised squares and bolls that lay on the ground or buried in the soil.

Keywords: *Steinernema riobrave*; *S. riobravis*; *Anthonomus grandis*; The cotton boll weevil; Biological control; Entomopathogenic nematodes; Soil moisture; Cotton

1. Introduction

The boll weevil, *Anthonomus grandis* Boheman, has been an economically important cotton pest in the United States since it first arrived from Mexico in 1892 and has caused more than $13 billion in yield losses and control costs to the US cotton industry (National Cotton Council, 1994). It also has been one of the most destructive pests of cotton in other parts of North, Central, and South America where this crop is grown commercially (Ridgway and Lloyd, 1983). It can survive and reproduce only on cotton and a few related plant species. The adults feed on young leaf buds and squares (floral buds). When ready to oviposit, female weevils select squares and young bolls, puncture them with the ovipositor, and lay the egg inside. One or two larvae may complete development in each square or boll. Although infested bolls do not typically abscise, infested squares commonly do, and thus weevil development

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frequently occurs at the soil surface (Cross, 1973; Leigh et al., 1994).

Control of the boll weevil has been dependent largely on the application of synthetic insecticides (Bradley, 1994; Herzog, 1994). Routine applications of these chemicals has been effective in suppression of boll weevils, but also eliminates beneficial predators and parasites, and adversely affect other nontarget organisms, including humans, through contamination of the air, soil, and water (King and Powell, 1992). In view of the economic and environmental problems posed by this pest, a cooperative boll weevil eradication program was implemented in 1971 in southern Mississippi and parts of Louisiana and Alabama. (Brazzel et al., 1994). The boll weevil eradication program in Texas was initiated in 1994 (El-Lissy et al., 1999).

If effective strategies for biological control of the boll weevil could be found, they could benefit the eradication program by providing a means for organic cotton production, even in eradication zones, and a means for controlling weevils in areas that are not easily amenable to aerial pesticide applications, such as near schools or residential areas, or where power lines are prevalent. Although the importance of biological control against the boll weevil has been documented (King et al., 1994), entomopathogenic nematodes have not been investigated for control of this pest. EN are comprised of two classes, the Steinernematidae and the Heterorhabditidae (Grewal and Georgis, 1999; Poinar, 1990). Steinernematid nematodes are effective against soil dwelling insects because of their persistence and searching ability in the soil (Grewal et al., 1994a; Kaya, 1990). Even though the boll weevil is not a soil pest, it does spend a significant portion of its life cycle on the ground. Also, nematodes could be combined with parasitoids, which attack only within the canopy.

Steinernema riobrave (formerly *S. riobravis*), is an entomopathogenic nematode originally isolated from an agricultural field in the Lower Rio Grande Valley (LGRV) of Texas (Cabanillas et al., 1994a). This nematode has shown potential for controlling a variety of insect pests, including the citrus root weevil, *Diaprepes abbreviatus* (Duncan et al., 1996a; Schroeder, 1994), the plum curculio, *Conotrachelus nenuphar* (Shapiro-Ilan et al., 2002), corn earworm, *Helicoverpa zea* (Cabanillas and Raulston, 1995, 1996; Feaster and Steinkraus, 1996), and pink bollworm, *Pectinophora gossypiella* (Gouge et al., 1996). It displays both ambusher and cruiser types of search patterns (Cabanillas et al., 1994a; Grewal et al., 1994a), which means its potential host range could include both mobile and immobile insects. *S. riobrave* also tolerates the high temperatures that occur in cotton fields during peak boll weevil activity (Cabanillas and Raulston, 1996; Grewal et al., 1994b) and is currently available as a commercial product.

The objectives of our study were to investigate: (1) the susceptibility of boll weevils to nematodes, the pathogenicity of *S. riobrave* towards different life stages of the boll weevil in relation to concentration and invasion efficiency of infective juveniles (IJs), sex ratio of invading nematodes, IJ production rates from boll weevils, and (2) the ability of *S. riobrave* to parasitize the boll weevil inside abscised squares and bolls at different nematode concentrations and soil moisture levels.

2. Materials and methods

2.1. Nematode sources

A preliminary test was conducted to compare the pathogenicity of different entomopathogenic nematodes against third instar boll weevils following the same methods used for the filter-paper-substrate petri dish assay at 30 °C as described below. Nematode species *Heterorhabditis bacteriophora* strain IN, *H. bacteriophora* strain HbL, and *H. indicus* strain HOM-1 were obtained from Dr. James Cate, Integrated Biocontrol Systems, *H. bacteriophora* strain HP88 was obtained from Drs. G.C. Smart Jr. and K.B. Nguyen, University of Florida, Gainesville, FL., *Steinernema glaseri* strain NC was obtained from Dr. Patricia Stock, University of California, Davis, CA; *S. riobrave* strain TX was from our laboratory; and *S. riobrave* strain TX as 'BioVector 355,' a commercial product from CERTIS USA (Before: Thermo Trilogy), Columbia, MD.

Steinernema riobrave was originally isolated from soil samples collected in the LRGV of Texas (Cabanillas et al., 1994a), and had been maintained in culture at Kika de la Garza Subtropical Agricultural Research Center, Weslaco, TX, in last-instar corn earworm larvae using techniques developed by Dutky et al. (1964) and modified as follows. One corn earworm larva was placed into each of 30 petri dishes (35 × 12 mm) containing 150 μl of about 100 IJs suspended in water and evenly distributed on a filter paper (Whatman No. 1) in the dish. The dishes were incubated in the dark at 25 °C in a plastic bag containing a moist paper towel to maintain humidity. After 3–5 days, 10 infected corn earworm cadavers were transferred to each three "harvesting" petri dishes (60 × 15 mm) containing plaster of Paris substrate for harvesting IJs. A plaster dish was prepared by adding a mixture of three parts of water with four parts of plaster of Paris (Bondex International, St. Louis, MO) to the bottom of the petri dish up to half of the dish depth. These plaster dishes were air-dried, and moistened with distilled water before transferring the infected insects to the dish. One open plaster dish with infected insects was placed in a larger dish (150 × 25 mm) containing 50 ml distilled water. The larger dish was covered with its lid, and incubated in the dark at 25 °C. The first

harvest of IJs began 9 days after the insects were exposed to nematodes. The IJs were collected in water and stored in a 75 cm^2 (250 ml) culture 'canted neck' flask (Vented Cap, Corning Costar, Cambridge, MA) in the dark at 10 °C. Nematode viability was 100%. Unless otherwise stated, IJs within two weeks of harvest were used in all experiments.

2.2. Susceptibility of the boll weevil to S. riobrave as measured by: pathogenicity, invasion efficiency, sex ratio, and infective juvenile production

Boll weevils were reared in the laboratory on artificial diet at 29 °C and 55% RH until they reached the life stage to be tested. Eggs and diet were obtained from the R.T. Gast Insect Rearing Facility at Starkville, MS (Griffin et al., 1979). The average size and weight for the first instar (3.5 mm, 6 mg), second instar (6.5 mm, 10 mg), third instar (9.5 mm, 44 mg), pupa (7 mm, 34 mg), and adult (6 mm, 22 mg) weevil were estimated.

Pathogenicity of the nematode against first, second, and third-instars, pupae, and 1-day-old and 10-days-old adults was compared at different concentrations in 35 × 12-mm petri dishes each containing one filter paper. IJs were transferred to each dish in 150 µl of water. One insect was placed in each of 25 dishes (35 × 12 mm) containing 0, 1, 5, 10, 25, 50, or 100 IJs. Control insects were treated with water only. Dishes, placed in a plastic bag with moistened paper to maintain moisture, were incubated in the dark at 25 °C and mortality was recorded daily for 5 consecutive days. In each experiment, we tested 25 insects at each nematode concentration, and each experiment was repeated at three different times under similar conditions (for a total of 75 insects at each stage and each concentration). Insect mortality was recorded daily and dead insects were examined for nematode presence by dissection. Any dead insects that had nematodes inside were considered to have died as a result of parasitism by nematodes.

Dead larvae were collected every 48 h, held at 25 °C for another 24 h, and then were dissected to determine the number of nematodes that established in the weevil and sex ratio of invading nematodes.

IJ production of *S. riobrave* was determined in separate test using third instar boll weevils and following the same procedure already described for culturing *S. riobrave*. Three week-old IJs, contained in 150 µl of water, were transferred into each petri dish (35 × 12 mm) containing one filter paper. Twenty insects were placed individually in each of 20 dishes for each nematode concentration (10, 25, 50, and 100 IJ per weevil) and incubated at 25 °C. Five days after nematode exposure, 10 infected cadavers were transferred individually to plaster harvesting dishes (35 × 12 mm) for nematode emergence. Nine days after nematode exposure, the IJs started exiting from the cadavers and the nematode numbers produced daily were counted over an 8-day period.

2.3. Ability of S. riobrave to parasitize the boll weevil inside squares and bolls at different nematode concentrations and soil moisture levels

To determine the efficacy of *S. riobrave* to parasitize boll weevils located inside abscised, infested squares and bolls of cotton, two separate experiments were conducted in potted soil at 30 °C. A Hidalgo sandy clay loam type was used in these experiments as described below. Each experiment included three nematode concentrations and a control (0, 100×10^3, 200×10^3, and 400×10^3 IJs/m^2), two soil moisture levels (10 and 20%) and two square and boll locations (soil surface vs. buried). The first experiment was established with field-infested squares on 2 July 1998, and the second was established with field-infested bolls on 14 July 1998. Boll weevil-infested squares and bolls, and the soil, were collected from a cotton field located at the South Farm research site of the USDA–ARS in Weslaco, TX.

Soil was steam-sterilized (125 °C for 25 min), cooled, and moistened with water to reach the desired moisture level (10 and 20%), then 1750 g of soil per pot was placed in plastic planting pots that were 0.20 m in diameter. Six boll weevil-infested squares were distributed randomly on the soil surface of each four pots, and six infested squares were separately buried 5 cm deep in another four pots for each soil moisture level. Pots were then placed on a lab bench at room temperature overnight. The following day, nematodes (three-weeks old) were suspended in 10 ml of water and applied with a 240-ml spray bottle on the soil surface in each pot. Control pots were treated with water. Two additional pots with soil (10 and 20% moisture) but without nematodes and squares were set apart to monitor the soil moisture gravimetrically to avoid disturbing the treated pots. All pots, covered with clear plastic wrap perforated with 1-mm holes to allow air exchange and reduce evaporation and incubated at 30 °C in a growth chamber with 14:10 h light:dark. Six days after treatment, the squares were collected from each pot, rinsed, and placed in labeled plastic bags to examine insect mortality caused by *S. riobrave* under a dissecting microscope. The second experiment (bolls) followed the same procedure as the first, except that we used 10 boll weevil-infested squares per pot.

The treatments used in each experiment were arranged in a $4 \times 2 \times 2$ factorial with four nematode concentrations, two moisture levels, and two locations of squares/bolls (soil surface and buried). Each treatment was replicated four times (each pot was a replicate unit).

The soil type was a Hidalgo sandy clay loam (50% sand, 24% clay, 26% silt, 1.1% organic matter, pH 8.3,

39.9 meq/100 g cation exchange capacity). The percent soil moisture was adjusted initially to each level tested and monitored daily by the gravimetric method from soil samples taken 5 cm deep in the pot. For this soil, moisture contents of 39.4, 29.3, 24.6, and 18.2% correspond to 0.10, 0.33, 1.0, and 15 bars, respectively (as determined by tensiometer measurements made by Soil and Crop Sciences, Texas A&M University, College Station, Texas). Our soil has a moisture characteristic curve where the 20% moisture level corresponds to about 7 bars (or a pF = 3.85) and the 10% moisture level corresponds to below permanent wilting point (15 bar tension or 18.2% water content). Field capacity of our soil would be at about 0.33 bar tension or 29.3% moisture content (Hillel, 1980).

2.4. Statistical analysis

For the petri dish assays, probit analysis (SAS Institute, 1990) was performed on the concentration response data for each boll weevil stage. Lethal concentrations required to kill 50% of the insects (LC_{50}) were calculated for each weevil stage.

To determine the effects of sex ratio and IJ production of *S. riobrave* in boll weevils, a factorial analysis of variance using PROC GLM (SAS Institute, 1990) was performed and, where appropriate, a pairwise comparison of means was performed with Duncan's multiple range test ($P = 0.05$). A regression analysis (SAS Institute, 1990) was performed to examine the relationships between concentration (the independent variable) and the number of nematodes established in the host, sex ratio, and IJ production of *S. riobrave* (the dependent variables). *S. riobrave* IJ production was studied for each nematode concentration by plotting the cumulative yield of IJs per weevil cadaver over 8 days against harvesting time (days after IJs started to exit from insect cadavers).

The Kaplan–Meier product limit estimate (PROC Lifetest, SAS Institute, 1990) was used to determine the mean time to death for each nematode concentration and for the different stages of the boll weevil exposed to *S. riobrave*. A. Wilcoxon test (PROC Lifetest, SAS Institute, 1990) was used to test the global hypothesis that virulence (time to death) differed between nematode concentrations. A Bonferoni test was used to compare pairs of treatments (Hardin et al., 1996). Time to death often does not have a normal frequency distribution, making these nonparametric tests more suitable than LT_{50} estimates.

For the study where we tested the ability of *S. riobrave* to find and parasitize boll weevil larvae inside squares and bolls, a factorial analysis (SAS Institute, 1990) was used to determine the effect of soil moisture and nematode density on parasitism. Data for treatments with zero means were omitted from the analysis of variance.

3. Results

3.1. Pathogenicity of different nematode species against third-instar boll weevil

All nematode species were pathogenic against third instar boll weevils (Fig. 1A). The lowest LC_{50}'s occurred with *S. riobrave* TX-355, *S. glaseri* NC, *H. indicus* HOM-1, and *H. bacteriophora* HbL. Based on the fiducial limits, these nematodes did not differ from each other in pathogenicity (Table 1), and were more pathogenic than *H. bacteriophora* IN, *H. bacteriophora* HP88, and *S. riobrave* TX (Table 1). The LC_{90}'s were less for *S. glaseri* NC (12 IJs), and *S. riobrave* TX-355 (13 IJs) than those of *H. bacteriophora* HbL (88 IJs), *S. riobrave* TX (97 IJs), *H. indicus* HOM-1 (99 IJs), *H. bacteriophora* IN (148 IJs) and *H. bacteriophora* HP88 (171 IJs).

3.2. Susceptibility of the boll weevil to S. riobrave as measured by: pathogenicity, invasion efficiency, sex ratio, and infective juvenile production

All boll weevil stages were susceptible to *S. riobrave* (Fig. 1). Larvae had the lowest LC_{50}'s and, based on the fiducial limits, the different instars did not differ from each other in susceptibility (Table 2). The LC_{50}'s for pupae and both new and mature adults were also similar to each other, but these stages were significantly less susceptible than the larvae (Table 2). The LC_{90}'s for these instars ranged from 10 to 21 nematodes.

The mean time to death of each boll weevil stage was affected by the number of nematodes applied (Table 3). Weevils generally died more rapidly at higher nematode concentrations than at lower ones. Mortality always occurred more rapidly for early instars than for late instars, for each concentration (Table 3). At concentrations of 50 and 100 IJs per weevil, the mean time to death for the third instar, pupa, and adult weevils were similar (1.84 and 2.67 days).

Both nematode concentration and weevil stage were significant factors in predicting the number of nematodes that established in a weevil ($F_{dose} = 55.42$; $df = 5, 70$; $P = 0.0001$; $F_{stage} = 6.07$; $df = 5, 70$; $P = 0.0001$) (Table 4). These two factors also had a statistically significant interaction ($P < 0.05$), indicating that the effect of concentration on the number of nematodes invading this host depended, in part, on the stage of the host.

Proportionately more females (62%) than males (38%) nematodes penetrated and killed boll weevils, and this difference was statistically significant ($F = 166.03$; $df = 1, 70$; $P = 0.0001$) (Fig. 2). The sex ratio of invading nematodes was not dependent on nematode concentration ($F = 2.017$; $df = 1, 103$; $P = 0.1274$) or weevil stage ($F = 2.017$; $df = 1, 103$; $P = 0.2015$).

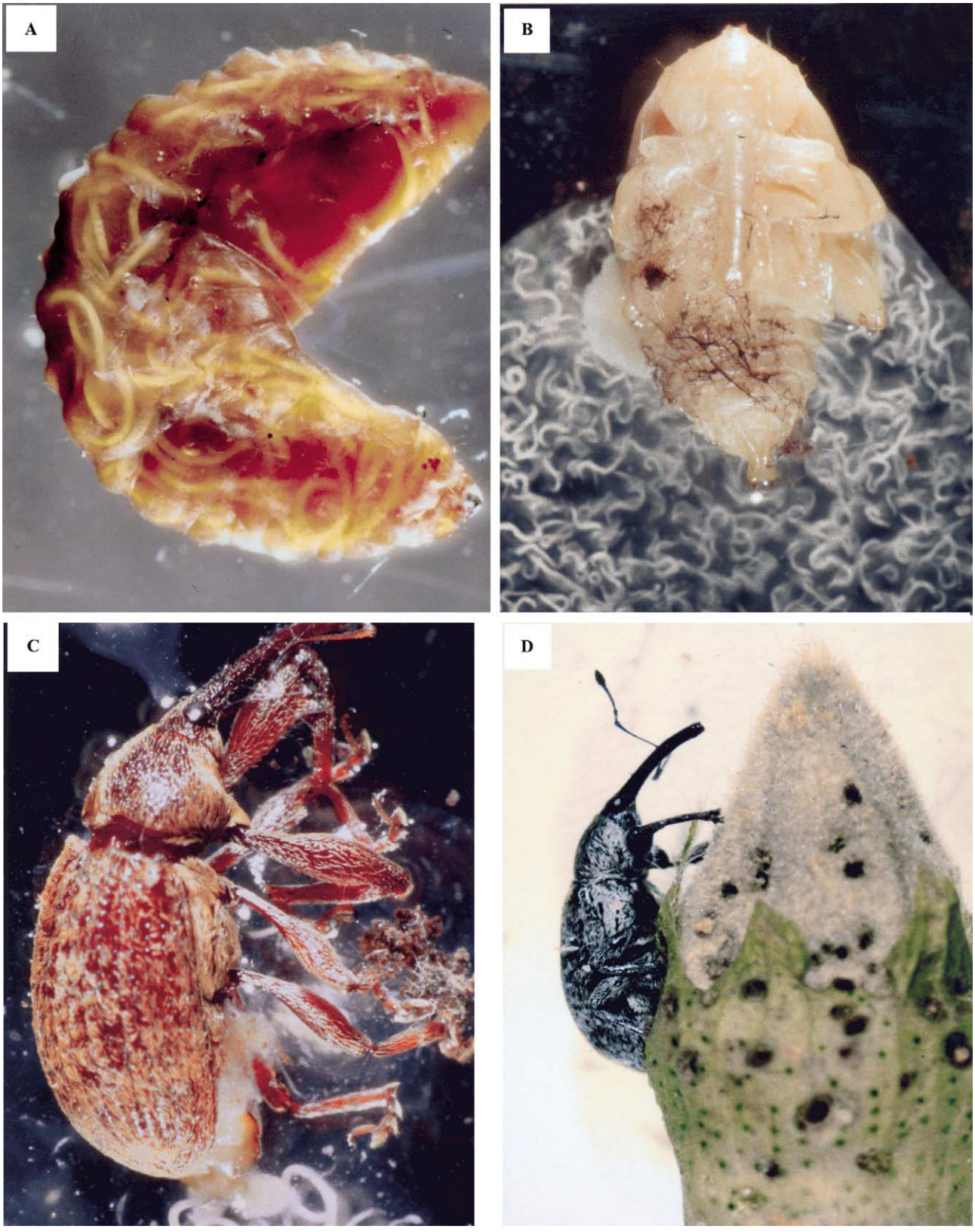

Fig. 1. Susceptibility of the boll weevil to *S. riobrave*. (A) Third instar weevil infected by nematodes. (B) Pupa of the boll weevil parasitized by nematodes found inside abscised cotton bolls, 6 days after nematode application on the soil surface. (C) Adult weevil killed by *S. riobrave* inside abscised cotton bolls, 6 days after spraying nematodes on the soil surface. (D) A healthy adult weevil on the surface of a cotton square. Note the weevil punctures made on the square surface.

Table 1
Pathogenicity of different steinernematid and heterorhabditid nematodes against third instar boll weevil, *A. grandis*, in petri dish bioassays at 30 °C

Nematode species	Strain	LC_{50} (95% *FL*)[a]	χ^2
Heterorhabditis bacteriophora	IN	13 (10–18)[b]	3.42
H. bacteriophora	HbL	7 (2–17)[b]	13.80[c]
H. bacteriophora	HP88	21 (10–45)[b]	11.30[c]
H. indicus	HOM-1	5 (3–7)[b]	4.56
Steinernema glaseri	NC	3 (2–4)[b]	0.55
S. riobrave	TX	14 (7–27)[b]	10.44[c]
S. riobrave	355[d]	2 (1–3)[b]	0.60

[a] Concentration (number of IJs per weevil) required to kill 50% of treated insects and the 95% fiducial limits for the LC_{50}.
[b] Transformed back from a $\log_{10}$ transformation.
[c] Heterogeneity factors of 3.45, 2.83, and 2.61 were used for strains HbL, HP88, and TX, respectively, to make the models fit, and t was adjusted to 2.78.
[d] 355 ('BioVector 355') is the commercial product number for the TX strain.

Table 2
Concentration-mortality responses for the entomopathogenic nematode *S. riobrave* against cotton boll weevil stages in petri dish bioassays

Boll weevil stage	LC_{50} (95% *FL*)[a]	Probit equation[b]	χ^2
First instar	3.9 (2.9–4.8)	$Y = -0.79 + 0.20\,C$	1.36
Second instar	4.9 (2.5–6.9)	$Y = -0.38 + 0.08\,C$	0.79
Third instar	3.8 (3.0–4.8)[c]	$Y = -1.02 + 1.74\log_{10} C$	4.66
Pupa	11.7 (5.7–22.0)[c]	$Y = -1.79 + 1.67\log_{10} C$	18.00
Adult (1-day-old)	12.5 (5.3–26.3)[c]	$Y = -1.98 + 1.80\log_{10} C$	25.70[d]
Adult (10-days-old)	10.8 (8.6–13.3)[c]	$Y = -1.54 + 1.49\log_{10} C$	1.73

[a] Concentration (number of IJs per weevil) required to kill 50% of treated insects and the 95% fiducial limits for the LC_{50}.
[b] General responses of percent insect mortality (Y) as a function of nematode concentration (C).
[c] Transformed back from a $\log_{10}$ transformation.
[d] A heterogeneity factor of 6.42 was used to make the model fit, and t was adjusted to 2.77.

Table 3
Mean number of days to death (±SEM) for each boll weevil life stage after exposure to different concentrations of *S. riobrave* in petri dish bioassays

Boll weevil stage	Nematode concentration (IJs per insect)			
	10	25	50	100
First instar	2.07 (0.13) a[a]	1.75 (0.09) a	1.71 (0.07) a	1.27 (0.05) a
Second instar	2.55 (0.14) abc	2.13 (0.11) ab	1.49 (0.07) a	1.39 (0.07) a
Third instar	2.64 (0.11) b	2.55 (0.11) b	2.34 (0.10) b	1.95 (0.08) b
Pupa	3.16 (0.13) cd	3.01 (0.13) c	2.36 (0.11) b	1.84 (0.09) b
Adult (1-day-old)	3.45 (0.11) d	3.07 (0.10) c	2.27 (0.11) b	2.25 (0.09) b
Adult (10-days-old)	3.70 (0.17) cd	3.04 (0.16) bc	2.67 (0.15) b	2.31 (0.14) b

[a] Values within a column followed by the same letter are not significantly different according to the Bonferoni test ($P \leqslant 0.05$, $\propto = 0.0016$).

Table 4
Mean number (±SEM) of *S. riobrave* found in infected boll weevils for each insect stage after exposure to different concentrations during 48–72 h after death

Boll weevil stage	Nematode concentration (IJs per insect)				
	5	10	25	50	100
First instar	1.53 (0.28)	1.97 (0.06)	3.57 (1.04)	11.20 (3.98)	12.83 (1.07)
Second instar	1.37 (0.03)	2.60 (0.52)	4.07 (1.17)	10.53 (2,63)	19.27 (2.20)
Third instar	2.03 (0.20)	2.50 (0.31)	6.80 (0.95)	15.00 (1.74)	23.57 (7.60)
Pupa	1.23 (0.10)	1.82 (0.10)	2.57 (0.32)	3.00 (0.29)	5.17 (0.52)
Adult (1-day-old)	1.50 (0.28)	1.90 (0.06)	3.50 (1.04)	11.20 (3.98)	12.80 (1.07)
Adult (10-days-old)	1.30 (0.03)	2.60 (0.52)	4.06 (1.17)	10.50 (2.63)	19.20 (2.20)

The number of IJ produced per weevil cadaver increased with nematode concentration (Fig. 3). The highest (8760 IJs) and lowest (6320 IJs) average yields of IJ per insect cadaver occurred at exposure concentrations of 100 and 10 IJs per weevil, respectively. The overall average production of nematodes per infected

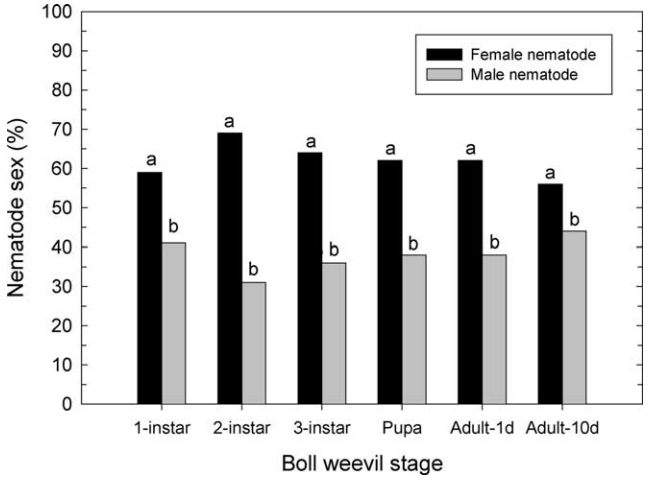

Fig. 2. Sex ratio of *S. riobrave* developed in different infected boll weevil stages. Black and gray bars represent the proportion of male and female adults, respectively. Means followed by different letters indicate significant differences ($P = 0.05$) by Duncan's multiple range test.

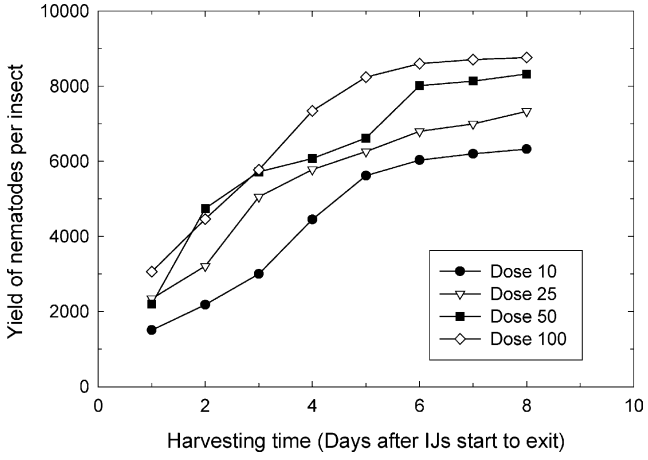

Fig. 3. Cumulative yield of third stage IJs of *S. riobrave* per infected boll weevil over time as response to nematode concentration (numbers of IJs per insect).

weevil was 7680 IJs. IJ production (Y) per insect cadaver as a function of nematode concentration (C) and time (T, days after IJs started to exit from weevil cadavers) as approximated by the quadratic response curve $Y = 343.6 + 68.6\ (C) - 0.39\ (C^2) + 777.8(T)$ ($P = 0.001$; $r^2 = 0.91$).

3.3. Efficacy of S. riobrave to control the boll weevil inside squares and bolls

IJ were able to seek out and parasitize boll weevils that were inside bolls and squares (Figs. 1 and 4). The efficacy of *S. riobrave* against boll weevils inside bolls was influenced by nematode concentration ($F = 52.28$; $df = 3, 7$; $P = 0.0001$) and soil moisture ($F = 13.37$;

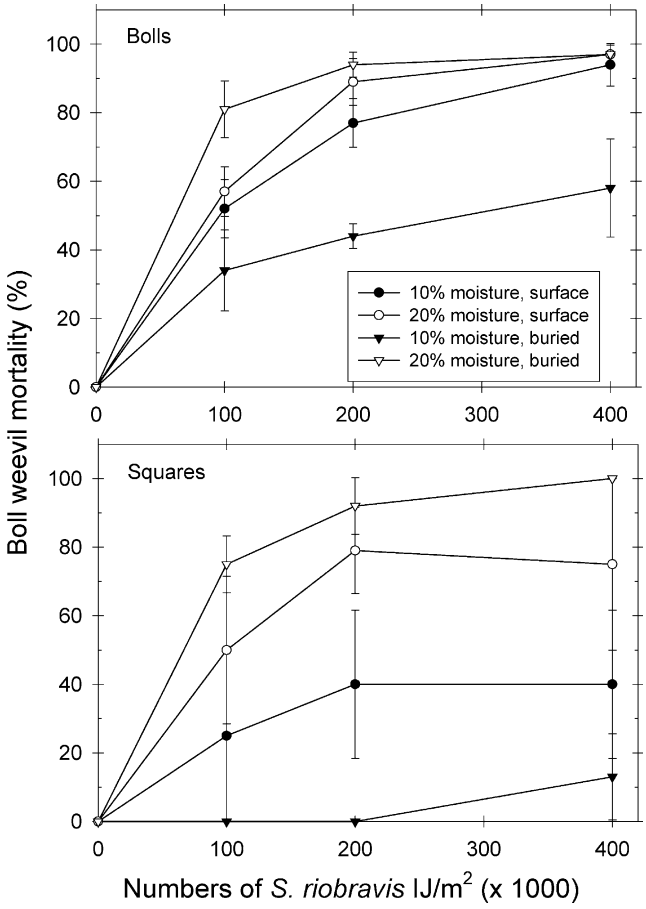

Fig. 4. Effects of *S. riobrave* applied to soil on the control of the boll weevil inside abscised squares and bolls of cotton located on the soil surface or buried, as a response to nematode concentration and soil moisture. Means are statistically different if their standard error confidence intervals do not overlap.

$df = 1, 7$; $P = 0.0081$); however, there was a significant interaction between concentration, moisture, and location ($F = 7, 19$; $df = 1, 7$; $P = 0.0315$). Nematode efficacy against weevils inside squares was influenced by soil moisture ($F = 66.21$; $df = 1, 4$; $P = 0.0012$); however there was a significant interaction between concentration, moisture, and location ($F = 10.06$; $df = 1, 4$; $P = 0.0338$). For both bolls and squares, the highest mortality occurred when the plant parts were buried in soil with high moisture content (20%). The lowest mortality occurred when the plant parts were buried in drier soil (10% moisture). At the most effective concentrations of 200,000 and 400,000 IJs/m^2 in buried bolls or squares, higher insect mortality was observed in pots treated with nematodes at 20% soil moisture in bolls (94 and 97% parasitism) or squares (92 and 100% parasitism) than those of 10% soil moisture in bolls (44 and 58% parasitism) or squares (0 and 13% parasitism). For bolls placed on the soil surface, soil moisture did not have a large impact on the number of fatal parasitisms. Bolls appeared to be more easily invaded than squares.

4. Discussion

This paper presents the first data on the susceptibility of the boll weevil to *S. riobrave* and other entomopathogenic nematodes. We found that *S. riobrave* parasitized both larvae and adult boll weevils. Entomopathogenic nematodes show potential for field control of other weevils (Klein, 1990; Shapiro-Ilan et al., 2002) and the different nematode species and strains differ in their pathogenicity against boll weevil larvae. Our laboratory studies show that third instar weevils, commonly found in abscised squares on the soil surface, are very susceptible to *S. riobrave* TX-355, *S. glaseri* NC, *H. indicus* HOM-1, and *H. bacteriophora* HbL. Although these nematodes are highly pathogenic to this pest, *S. riobrave* tolerates high temperatures (Cabanillas and Raulston, 1996; Grewal et al., 1994b), which makes it a better candidate for controlling boll weevil in warm regions. Under field conditions, *S. riobrave* was superior than *S. carpocapsae*. All in controlling corn earworm in Texas (Cabanillas and Raulston, 1996) and citrus root weevil in Florida (Duncan et al., 1996a; Schroeder, 1994).

Complete mortality of *A. grandis* was achieved with exposure to 100 *S. riobrave* IJs per weevil (all weevils stages). Bedding et al. (1983) suggested that a preliminary scan at a concentration of 100 nematodes per insect may help in selecting nematodes as potential control agents for a particular pest insect. Using this criterion, *S. riobrave* appears to be a promising candidate for controlling boll weevil when applied to larval, pupa, and adult stages. The LC_{50} of *S. riobrave* for boll weevil larvae ranged from 3.8 to 4.9 IJs per larva, as compared to 9 IJs of *S. carpocapsae* on large pine weevil larvae *Hylobius abietis* (Pye and Burman, 1978), and 1000 IJs on *Hylobius pales* larvae (Jackson and Moore, 1969). Boll weevil pupae and adults were much less susceptible than larvae. Larvae required fewer nematodes for fatal infection to occur and mortality was more rapid. At low doses (10–25 nematodes per insect), the number of nematodes that penetrated into and successfully parasitized pupae was not any greater than the number that penetrated and cause fatal infection in larvae or adults. However, far fewer nematodes were found within dead pupae at higher concentrations. This result suggests that the low pupal mortality rates were not due to a large number of nematodes being required to kill pupae, so much as, that nematodes having difficulty penetrating pupae. Low penetration might be attributed to the fact that no feeding occurs during the pupal stage, thus nematodes cannot gain entrance into the insect when it feeds. However, none of these insects were given food during the bioassays, so it is unlikely that active feeding was occurring for any of the life stages tested.

Invasion efficiency, the ratio between the number of nematodes invading a host and the exposure concentration per insect, has been proposed as an alternative to LC_{50} as an assessment of nematode efficacy (Glazer, 1992; Hominick and Reid, 1990). Epsky and Capinera (1994) recommended estimating both nematode invasion and host mortality values. Our invasion efficiencies ranged from about 13 to 30% for all stages except the pupae (5–25%). Although invasion efficiency of *S. riobrave* was dependent on concentration, it was not consistent with the insect stage. For pupae weevils its invasion efficiency was low regardless of the concentration.

From a pest management standpoint, the main goal is to kill large numbers of the target pest to bring it below the economic threshold level; however, if nematode reproduction can occur in the target insect, then longer term management might be achievable. The sex ratio of *S. riobrave* that established in boll weevils was 1:1.6 (male:female). Female biased sex ratios could enhance reproduction rates, but the presence of males is required for reproduction of *S. riobrave* (Cabanillas et al., 1994a; Cabanillas and Raulston, 1994b). Although our study does not fully establish the sex ratio of colonizing nematodes (Grewal et al., 1993), it did show that higher female ratios are established, and this effect is independent of both concentration and insect stage. Male to female ratios were similars for *Steinernema carpocapsae* in *Galleria mellonella* (1:1.6) (Danilov as cited by Bohan and Hominick, 1997), *S. scapterisci* in house crickets *Acheta domesticus* L. (1:2) (Nguyen and Smart, 1992), *S. glaseri* in *G. mellonella* (1:1.4) and *S. anomali* in *G. mellonella* (1:1.6) (Grewal et al., 1993; Lewis and Gaugler, 1994). Bohan and Hominick (1997) indicate that female nematodes have a higher probability of invasion than males; however, sex ratio balances to unity during subsequent reproductive cycles within the host. In any case, *S. riobrave* was able to reproduce in the boll weevil. Higher concentrations led to higher numbers of penetrating nematodes in the host and higher IJ yields. But yield of IJs per penetrating nematode declined with an increase in concentration, to the extent that the maximum total number of IJs that can be produced in boll weevils was estimated to occur at a concentration of about 114 nematodes, based on our quadratic equation. The average yields of IJs (7680 per weevil) was considerably less than for other hosts, such as the corn earworm (311,000 IJs per insect) or *G. mellonella* (200,000 per insect) (Cabanillas and Raulston, 1994b; Dutky et al., 1964).

Our study shows that *S. riobrave* IJs are capable of seeking out and killing developing boll weevils that are well concealed within cotton fruiting structures. This is a critical factor for field efficacy against the boll weevil. Soil moisture is also a critical factor for steinermatid survival and movement (Kaya, 1990). We found low parasitism of weevils within squares when soil moisture was 10% (It corresponds to below permanent wilting

point) but high parasitism when soil moisture was 20% (It corresponds to about 7 bars or a pF of 3.85). Higher soil moisture led to greater infection rates in boll weevil by *S. riobrave*. However, nematode activity and efficacy are severely restricted when moisture levels are insufficient for nematode movement and persistence (Kaya, 1990). Very high moisture levels can also inhibit nematode infectivity by immobilizing the nematodes and can decrease survival by making insufficient oxygen available (Molyneux and Bedding, 1984). It suggests that *S. riobrave* might survive and infect susceptible insects below the wilting point of plants but its efficacy would probably maximize at soil moisture near to field capacity. Similar studies with *S. carpocapsae* show that these nematodes establish at soil moistures below the permanent wilting point of plants (Köppenhofer et al., 1995).

We found that nematode efficacy was greater when plant parts were buried than when they were on the soil surface. This efficacy difference is because below the soil surface, conditions may enhance nematode survival and efficacy (Cabanillas and Raulston, 1994c; Duncan et al., 1996b; Duncan and Mc Coy, 2001; Molyneux and Bedding, 1984; Simons, 1973). The apparent greater infection rates observed in bolls than squares especially at low soil moisture may be attributed to their morphological and structural differences. Infective juveniles of *S. riobrave* have the ability to enter through punctures made by adult weevils for feeding and oviposition (Cabanillas, 1994, unpubl.). Bolls are partially covered by bracts at their base or totally uncovered, but squares are almost completely covered with bracts. Also, bolls are less susceptible to drying due to their greater mass than squares. Because *S. riobrave* IJs (0.03 mm diam.) are smaller than a weevil puncture (~1.0 mm diam) and its high searching ability (Cabanillas et al., 1994a; Duncan et al., 1996a; Grewal et al., 1994a), it makes this nematode a potential candidate to control boll weevils inside these fruiting structures.

Considering that not all boll weevil larvae will occur at the soil surface during the field season; it is not expected that the nematode will attack all weevils in the field. However, such a biocontrol method could be complemented by the introduction of parasitoids that would attack larvae before the fruiting structures abscise. Also, nematodes can be very successful in areas where chemicals are restricted or where fruiting structures are buried in the soil by mechanical cultivation which prevents the effective control of boll weevil by conventional insecticides or parasites such as *Catolaccus grandis* (Guerra et al., 1984; Summy et al., 1994).

Our results show that *S. riobrave* is very pathogenic against larval stages of *A. grandis*, and moderately pathogenic against the pupae and adults. Also, this nematode shows a high searching and killing ability against the well-concealed boll weevil within abscised squares and bolls. The successful results obtained in this study were made under conditions where few of the environmental constraints that limit nematode efficacy in field applications were involved. Further research is necessary, including better application strategies or formulation methods are required before this can become a practical method for boll weevil control.

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