HORIZONTAL AND VERTICAL MOVEMENT OF *STEINERNEMA RIOBRAVIS* AND *S. CARPOCAPSAE* (RHABDITIDA: STEINERNEMATIDAE) IN SOIL IN THE LABORATORY

L. Forlow Jech, T.J. Henneberry, M.J. Panter and S. Faulconer
USDA-ARS, Western Cotton Research Laboratory
Phoenix, AZ

Abstract

Steinernema riobravis Cabanillas, Poinar and Raulston, after release on the soil surface in horizontally or vertically oriented bioassay chambers, moved greater distances (in cm) to infect pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) larval hosts compared with *S. carpocapsae* (Weiser) (Kapow selection). Both nematode species induced greater PBW larval mortality in soil with a moisture level of 30% compared with 25% soil moisture.

Introduction

The entomopathogenic nematodes, *Steinernema riobravis*, Cabanillas, Poinar and Raulston and *S. carpocapsae* (Weiser) (Kapow selection) have potential as biological control agents for the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), a major pest of cotton in Arizona. PBW larvae are highly susceptible to invasion by both nematode species but not pupae unless the integument is pierced or incomplete closure of the pupal case occurs (Henneberry et al. 1995). *S. riobravis* is indigenous to the lower Rio Grande Valley of Texas (Cabanillas et al. 1994), and adapted to semi-arid regions. This species of nematode remains viable in damp soil in Arizona for up to 30 days (Lindegren et al. 1994, 1995). Our field studies suggest that *S. riobravis* readily travels through soil in search of PBW larvae but *S. carpocapsae* appears more sedentary (Forlow Jech and Henneberry 1997). This has also been demonstrated in sand bioassays in the laboratory (Lindegren et al. 1993). *S. carpocapsae* has been used successfully for insect control in other crops (Agudelo-Silva et al. 1987, Broadbent and Olthof 1995, Jackson and Brooks 1995). *S. carpocapsae* is used in our studies as a standard for comparison with *S. riobravis*. However, *S. carpocapsae* is adapted to cooler climates and we suggest that it may be used in cotton fields in the early spring or late fall. Use during midseason might be effective after cotton canopies develop to shade the soil (Lindegren et al. 1994).

We developed a successful nematode distribution system for applying nematodes in cotton furrows during irrigation (Forlow Jech and Henneberry 1996, 1997). There is a need for information on nematode mobility and their behavioral characteristics in host searching for further refinement of nematode application technology. In the present studies, we determined the vertical and horizontal movement of *S. riobravis* and *S. carpocapsae* nematodes in the laboratory in Arizona soil.

Materials and Methods

PBW larvae used in the studies were obtained from the laboratory culture at the USDA-ARS, Western Cotton Research Laboratory, Phoenix, AZ. Rearing methods were as described by Bartlett and Wolf (1985). *Steinernema riobravis* and *S. carpocapsae* nematodes were reared on pink bollworm larvae in the laboratory using a modified version of the *in vivo* method of Lindegren et al (1993). All nematodes used had been stored in water at 8ºC for less than 1 week. Petri dish bioassays were run concurrent with the experiments to ensure nematode pathogenicity. Soil used in all larval tests was Avondale loam type (Mesic typic calciorthids 31.6 sand, 46.1 silt, 23.3 clay, pH 6.9) (Lindegren et al. 1993).

Horizontal Movement

Experiment 1. Bioassay cradles were constructed of 7.6 cm polyvinyl chloride (PVC) pipe. Pipe pieces 15.2, 30.5 and 45.7 cm in size were cut in half lengthwise. The bottoms of 100 x 15mm petri dishes were fastened to each end of the pipes with hot glue to form a "cradle" (Fig. 1). Oven-dried and pulverized soil was added to each cradle to a depth of one inch. The soil surface areas were 117.8 (420 g), 232.5 (840 g) and 345 cm^2 (1260 g) for the 15.2, 30.5 and 45.7 cm cradles, respectively. One half of the soil was poured into each cradle and 2 biopsy cassettes (Tissue Path IV, Curtin Matheson Scientific, Inc.), each containing 5 last instar PBW larvae, were placed at one end of each cradle. The remaining soil was poured into the cradles and over the cassettes so that the PBW were buried under 0.64 cm of soil. Water was added by weight to adjust the soil moisture to 25% and allowed to soak in for 30 minutes. The equivalent of 1 billion infective juvenile (IJ) nematodes per acre (25 nematodes per cm^2 of soil surface) were released onto the soil surface at the cradle end opposite to the location of the host larvae. Nematodes were introduced to the soil in less than 1 ml aliquots taken from a stock solution. Treatments were nematode species (*S. riobravis* or *S. carpocapsae* and untreated control) and lengths of the bioassay cradles (15.2, 30.5 and 45.7 cm). Controls were treated with 1 ml of water only. There were 8 replicates for each treatment.

After treatment, cradles were covered with aluminum foil to maintain soil moisture and held at laboratory temperature (26ºC). After 48 hours, cassettes containing PBW larvae were recovered from the soil. PBW were removed from the cassettes and held in clean petri dishes with moist filter paper. After 24 hours, larvae were examined for nematode-induced mortality.

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Experiment 2. Bioassay cradles were as described in experiment 1. However, only the 15.2 and 30.5 cm cradles were used. Each size cradle contained soil moisture levels of 25 or 30% to determine its effect on nematode movement and PBW larval infection. Treatments were nematode species (*S. riobravis*, *S. carpocapsae* and untreated control), length of bioassay cradles (15.2 and 30.5 cm) and soil moisture (25 and 30%). This experiment was replicated 8 times.

Vertical Movement

Experiment 3. Vertical nematode movement was determined using 12.7 cm diameter x 15.2 cm tall polyvinyl chloride (PVC) pipe columns (Fig. 2). Each column consisted of three 5.1 cm stacked sections with a copper screen placed between each section to facilitate column separation and recovery of larvae after treatment. Five biopsy cassettes, each containing 1 PBW larva, were placed at the bottom of each section and covered with soil. Water was added by weight to each individual section so that soil moisture was 29%. The equivalent of 1 billion IJs per acre ($25/cm^2$ of soil surface) were released onto the soil surface of the top ring of each of the columns. Treatments were nematode species (*S. riobravis*, *S. carpocapsae* and untreated control), column section order (top, middle or bottom) and days following nematode release (1, 2 or 3). There were 5 replicates for each treatment for a total of 45 columns.

Experiment 4. Columns were as described in experiment 3. However, nematodes were released onto the surface of either the top section or the bottom section of a column. Treatments were species of nematodes (2) and an untreated control, site of nematode release (column top or bottom section), column section order (top, middle or bottom) and days following nematode release (1, 2 or 3). This experiment was replicated 5 times for a total of 90 columns.

Columns for both experiments were held in the laboratory at 26°C and ambient humidity. Cassettes were removed from each section after 1, 2, or 3 days and larvae placed into clean petri dishes with moist filter paper. After an additional 24 hours, larvae from each section were examined for nematode-induced mortality.

All data were analyzed using factor analysis of variance (MSTAT-C 1988) and means separated contingent upon a significant F test ($P \leq 0.05$) using the method of least significant differences. All percentages were transformed to arcsines before ANOVA procedures. In both soil column tests, PBW larval mortality in the untreated columns was extremely low (0-2.5%) so an Abbott's correction (Abbott 1925) was applied to the treatment data and the untreated control data were not included in the analysis.

Results and Discussion

Horizontal Movement

Experiment 1. *S. riobravis* moved 15.2 and 30.5 cm horizontally, but not 45.7 cm, through soil in 48 hours to infect PBW larval hosts. Mortalities of larvae in cradles treated with *S. riobravis* were significantly greater (87.4, 6.4 and 0.0% for 15.2, 30.5 and 45.7 cm, respectively) than mortalities of larvae in cradles treated with *S. carpocapsae* (6.3, 0.0 and 0.0% for 15.2, 30.5 and 45.7 cm, respectively) ($F = 38.5$, $df = 4,56$) (Fig. 3).

Experiment 2. Both nematode species infected more PBW larvae in soil with 30% moisture compared to 25% soil moisture (Fig. 4). At 15.2 cm cradle lengths, PBW mortality was greater, but not significantly different, at 30% moisture (81.0% mortality) than at 25% moisture (58.7%) with *S. riobravis*. At cradle lengths of 30.5 cm, PBW mortality was significantly greater for a soil moisture of 30% than 25% (51.2 and 2.5%, respectively) ($F = 3.20$, $df = 2,77$). In cradles treated with *S. carpocapsae*, PBW mortality differences were greater between lengths of cradles than between soil moistures (Fig. 4). PBW larval mortality was 24.7 and 41.2% for soil moistures of 25 and 30% in 6 inch cradles, respectively, and 0 and 5% for soil moistures of 25 and 30% in 12 inch cradles ($F = 3.20$, $df = 2,77$). The results of both of these experiments are similar to those in the field following irrigations (Lindegren et al. 1995) and in the laboratory following simulated irrigations (Henneberry et al. 1996a). Larval mortality increases following an irrigation and steadily declines as the soil dries up and nematodes either die, move toward areas of higher soil moisture or develop into a physiological desiccation form that affords protection during adverse moisture conditions (Simons and Poinar 1973).

Vertical Movement

Experiment 3. *S. riobravis* induced significantly greater PBW larval mortality (52.9%) compared with *S. carpocapsae* (18.7%) ($F = 65.72$, $df = 1,68$). PBW mortality was greater, but not significantly different, on Day 2 than on Day 1 or Day 3 after nematode release for both *S. riobravis* and *S. carpocapsae* ($F = 1.26$, $df = 2,68$) (Fig. 5a). *S. riobravis* induced significantly greater percentages of PBW mortality than *S. carpocapsae*, in all three column section levels ($F = 6.67$, $df = 2, 68$) (Fig. 5b) further demonstrating that *S. riobravis* moves further through the soil to find host larvae.

Experiment 4. PBW larval mortalities were significantly greater at the top and middle, but not bottom, column sections when *S. riobravis* was released onto the soil surface of the top section (96.0, 28.0 and 10.7% for top, middle and bottom, respectively) when compared with *S. carpocapsae* (22.7, 5.33, and 9.55% mortalities for top, middle and bottom column sections, respectively) (Fig. 6a). PBW mortality percentages were not significantly different at the bottom section for *S. riobravis* and *S. carpocapsae*

(98.7 and 90.3%, respectively) when nematodes were released onto the soil surface of the bottom column section (Fig. 6b). However at the middle column section, *S. riobravis*-induced larval mortality was significantly greater (88.0%) than *S. carpocapsae*-induced mortality (30.7%). Percent mortalities of PBW larvae recovered from the middle section of columns where nematodes were introduced on the top section were significantly lower (28.0 and 5.3% for *S. riobravis* and *S. carpocapsae*, respectively) than for those recovered from the middle sections of columns where nematodes were introduced on the soil surface of the bottom sections (88.0 and 30.7%, respectively). The data for the middle section results may be a little misleading because the distance nematodes traveled (≤_0.64 cm) from the surface of the bottom column section to reach PBW larval hosts in the bottom of the middle column section was less than the distance required for nematodes to travel (5.1 cm) from the surface of the top column section to the bottom of the middle section. There was a general movement upward by both species of nematodes even though the movement was greater by *S. riobravis* than for *S. carpocapsae*. These results of upward nematode movement are in agreement with those obtained by Moyle and Kaya (1981). In Figure 6b, at the top level, PBW mortality was greater but not significantly different for *S. riobravis* (9.33%) when compared with *S. carpocapsae* (1.33%).

S. riobravis has a higher temperature tolerance and a wider temperature range of activity than *S. carpocapsae* in the laboratory (Henneberry et al. 1996b) and in the field (Lindegren et al. 1995). Our tests were conducted at laboratory temperature (26°C) which should be acceptable for *S. carpocapsae.* However, even under these conditions, *S. riobravis* was consistently more effective in moving and finding PBW larval hosts as evidenced by higher mortalities and therefore may be a preferred candidate for biocontrol of PBW in Arizona.

Disclaimer

Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

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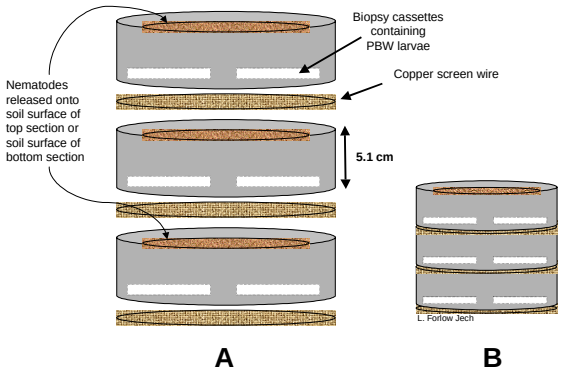
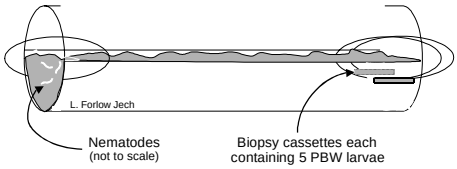


Figure 1. PVC pipe "cradle" with soil. Nematodes were released onto the soil surface at one end of cradle. PBW larvae confined in biopsy cassettes were buried at the opposite end.

Figure 2. A. PVC pipe column shown "exploded". Nematodes were



released onto the soil surface of the top or bottom column section. B. Column sections shown stacked.

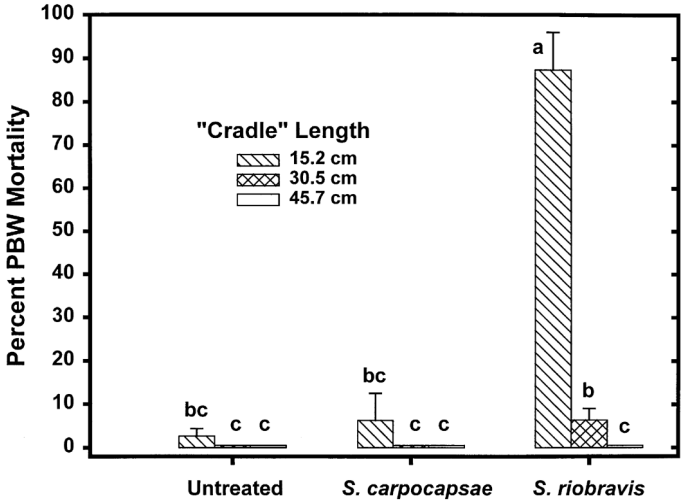


Figure 3. Overall mean ± s.e. of PBW larval mortalities of untreated and nematode-treated soil in 3 different lengths of PVC pipe bioassay "cradles". F = 38.5, df = 4,56; P ≤_0.05.

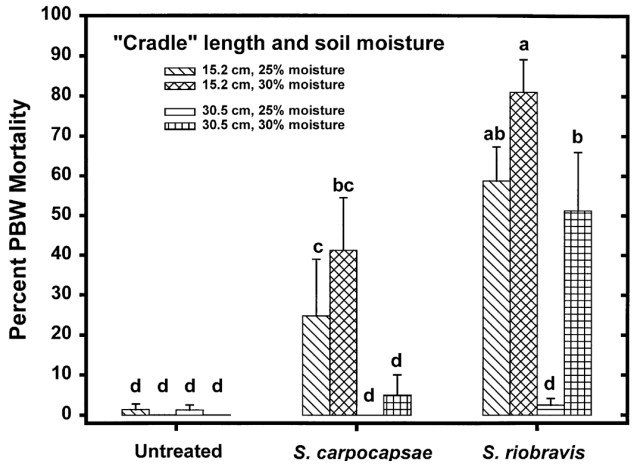


Figure 4. Overall mean ± s.e. of PBW larval mortalities of untreated and nematode-treated soil with 2 different soil moistures in 2 lengths of PVC pipe bioassay "cradles". F = 3.20, df = 2,77; P ≤_0.05.

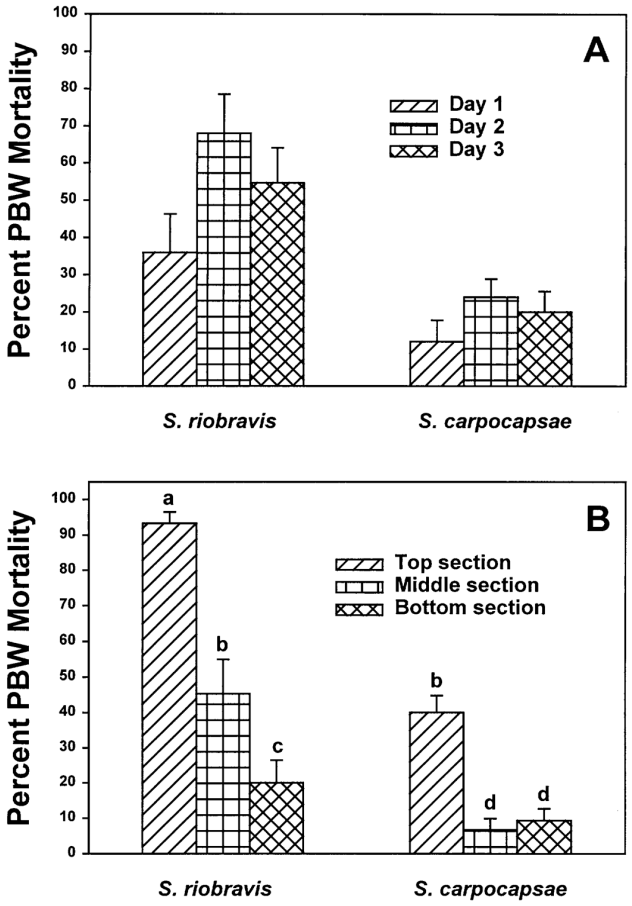


Figure 5. A. Means ± s.e. of PBW larval mortalities of nematode treated soil columns after 1, 2 or 3 days following nematode release. Treatments were not significantly different. F = 1.26, df = 2, 68; P ≤__0.05. B. Means and s.e. of percentage mortalities of PBW larvae recovered from 3 sections of columns treated with nematodes. Nematodes were released onto the soil surface of the top section. F = 6.76, df = 2, 68; P ≤_0.05.

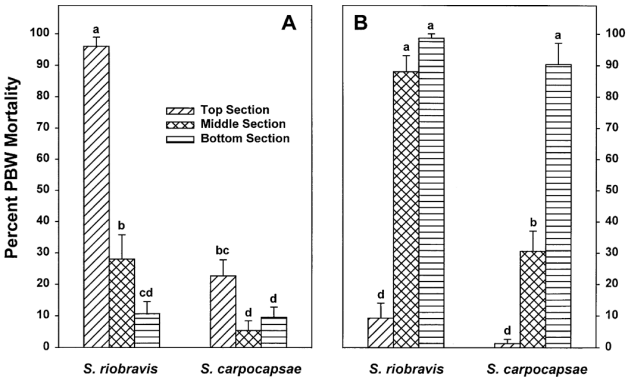


Figure 6. Means ± s.e. of percentage mortalities of PBW larvae recovered from 3 stacked sections of columns treated with nematodes. Nematodes were released onto the soil surface of the top column section (A) or bottom column section (B). Data in both graphs were analyzed together. Graphs were separated for clarity of viewing. F = 23.2, df = 2,154; P ≤_0.05.