

**METHOD OF DISTRIBUTING  
STEINERNEMA RIOBRAVIS  
IN COTTON FURROW IRRIGATION  
AND PINK BOLLWORM  
LARVAL MORTALITY RESPONSE**

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**Abstract**

Entomopathogenic nematodes, *Steinernema riobraviss* Cabanillas, Poinar and Raulston, were released at a rate of 2.6 billion/acre in a cotton field by gradually pumping a nematode slurry through a polyvinyl chloride pipe manifold into furrow irrigation water. Pink bollworm, *Pectinophora gossypiella* (Saunders) (PBW), were buried in wire baskets at 4 soil depths and on the middle and top of row beds. Percent PBW mortalities on Day 0, 14 and 39 following nematode treatment showed that *S. riobraviss* moved 6 inches downward into the furrow and upward into the top of the row bed to find host PBW larvae. Overall mean percent PBW mortalities were significantly higher for larvae buried at 1/2 (33.7%) and 2 (35.8%) inches below the surface than for larvae buried at 4 (24.3%) and 6 (11.5%) inches below the surface. Larvae buried in the middle of the row bed had significantly higher percent mortalities (34.6%) than those buried at the top (11.3%) of the row bed. In all cases, overall mean percent PBW mortalities were significantly higher at Day 0 than at Day 14 and Day 39.

**Introduction**

The pink bollworm, *Pectinophora gossypiella* (Saunders) (PBW), remains one of the most important economic pests of cotton in Arizona and southern California despite several available control methods. Cultural practices (Watson 1980) and early termination of late-season irrigations (Watson et al. 1978) are useful methodology when implemented in PBW management strategies. PBW-resistant transgenic cotton, commercially available for the 1996 season, has the potential to dramatically suppress populations (Flint et al. 1995). However, there is a continuing need for other non-chemical control methods to avoid insecticide resistance and secondary pest outbreaks that result from the overuse of conventional insecticides (Watson 1980).

Biological control of PBW and other lepidopterous cotton pests such as cabbage looper, *Trichoplusia ni* (Hübner) and

beet armyworm, *Spodoptera exigua* (Hübner) with nematodes is in the early stages of development (Lindgren et al. 1994, Henneberry et al. 1995a). Preliminary work by Lindgren et al. (1993a and 1994) and Henneberry et al. (1995b) indicated that the newly described entomopathogenic nematode *Steinernema riobraviss* Cabanillas, Poinar and Raulston (Cabanillas et al. 1994, Raulston et al. 1992) appeared promising for biological control of the soil-associated PBW larval life stage. PBW larvae tunnel out of flower buds or bolls during the growing season to pupate in the soil (Butler and Henneberry 1976) and remain within the top 1.3 cm of soil (Henneberry and Clayton 1979). A properly timed application of insect parasitic nematodes could prove beneficial for control as the larvae drop from the bolls and enter the soil. Although another species of entomopathogenic nematode, *S. carpocapsae* (Weiser) (Kapow selection), locates on or near the soil surface (Kaya 1990), high mid- and late summer soil temperatures in Arizona may limit its effectiveness despite the cooling effects of the plant canopy. Lindgren et al. (1994) showed that in a July field test, 30% of PBW larvae in soil bioassays were infested with nematodes 7 days after soil treatment with *S. carpocapsae* as compared to 76% PBW mortality after soil treatment with *S. riobraviss*. *S. riobraviss* has a higher temperature tolerance and may be efficacious later in the season.

Entomopathogenic nematodes have been distributed in commercial crop growing systems with common agrochemical equipment such as aircraft (Lindgren et al. 1981) and mist sprayers (Lindgren et al. 1987) for navel orangeworm, *Amyelois transitella* (Walker) in almond orchards, drip irrigation on turf for mole crickets, (*Scapteriscus* spp.) (Meredith et al. 1987) and strawberries for 2 species of root weevil, *Otiiorhynchus sulcatus* (F.) and *Phlyctinus callosus* Boh. (Curran and Patel 1988). Foliar applications by backpack sprayer have also been made for leafminers, *Liriomyza trifolii* (Burgess), on chrysanthemums in the greenhouse (Broadbent and Olthof, 1995). Work with insect parasitic nematodes in cotton fields is relatively recent but nematodes have been applied successfully in cotton fields by shanking into the soil (K. Smith, unpublished data, Biosys, Western Regional Office, Vancouver, WA 98686), furrow irrigation and motorized ground spray equipment (D. H. Gouge et al. unpublished data, USDA-ARS, Western Cotton Research Laboratory, Phoenix, AZ 85040). In this paper, we describe a method of distributing *S. riobraviss* into a cotton field during furrow irrigation and examine PBW mortality and nematode movement through the furrow soil profile and plant bed by using a PBW bioassay technique.

**Materials and Methods**

PBW larvae used in these experiments were reared on artificial diet at the USDA-ARS Western Cotton Research Laboratory, Phoenix, AZ, as described by Bartlett and Wolf (1985). To determine movement of *S. riobraviss* in the soil

profile, one pink bollworm larva was placed in each of 500 plastic biopsy cassettes (Tissue Path IV, Curtin Matheson Scientific, Inc.) for placement in the field. Five cassettes with larvae were placed in the bottom of 75 2-inch deep large mesh wire baskets. Five cassettes with larvae were also placed in 25 1/2-inch deep baskets made of the same material. Groups of three deep baskets plus 1 shallow basket were stacked on top of each other so that when the group of baskets was buried in the soil, the cassettes containing larvae were 1/2 inch, 2, 4, or 6 inches under the soil surface (Fig. 1). Twenty-five 6 inch deep holes (8 in. dia.) were dug using a gasoline powered hand-held auger (General 210 Hole Digger, General Equipment Co., Owatonna, MN 55060). Holes were 50 feet apart and located in the bottom of row furrows in a 0.39 acre cotton field that was 315 feet long and planted with 16 rows of Deltapine 5415 cotton on 40 inch centers. One group of baskets was placed in each hole and covered with soil. There were 5 groups of baskets per row placed in every 3rd row (5 rows = 5 reps). Each basket group location was marked with a flag (Fig. 2). The entire procedure was repeated on 18 August and 12 September, 14 and 39 days following nematode application.

On the day of treatment (4 August), soil removed in making burial holes was used to cover the baskets of cassettes since the soil was still untreated with nematodes. On the other two dates, soil taken from untreated areas of the field was used to cover the baskets so that any nematode-induced larval mortality was caused by nematodes persisting in the surrounding soil following treatment. Treatments were distances of 50, 100, 150, 200 and 250 feet from the nematode source, depth buried in the soil (1/2, 2, 4 and 6 inches) and numbers of days following nematode application.

We also buried individual biopsy cassettes containing single PBW, 1/2 inch below the surface, on the top of the row bed or midway down the side of the row bed. On the day of nematode application, 2 cassettes were buried at each location on top and 2 on the sides of the row bed. On days 14 and 39 following nematode application, 10 cassettes were buried on top of the row bed and 10 on the row bed sides at each location (Fig. 2).

Following burial of the larvae in baskets or single cassettes on 4 August, the field was irrigated by using standard siphon tubes to irrigate every row. One quarter hour after irrigation was initiated, we began a gradual release of nematodes into the furrows. Approximately one billion (2.6 billion/ac) encapsulated *S. riobravus* nematodes, supplied by Biosys (Columbia, MD), were mixed with water in a 50 gallon cylindrical plastic tank. Mixing and continuous aeration of the nematode slurry was accomplished using a 1 horsepower air compressor (Pro Air II, DeVilbliss Air Power Company, Jackson, TN 38301) that blew air into a perforated polyvinyl chloride (PVC) tubing ring that was held on the bottom of the tank with

large stones. The nematode slurry was pumped (Series 1, Little Giant Pump Company, Oklahoma City, OK 73112) from the tank through a garden hose to a PVC manifold system. The manifold system was made of Schedule 40 PVC pipes laid across the tops of 16 cotton row beds. PVC pipe lengths were connected with 17 T-joints (for 17 furrows) located at the center of each row furrow. Each T-joint had a 14 inch long 1 cm (inside diameter) black tube that extended into each furrow. Water from irrigation siphons was run into the furrows for 2 hours (approximately 3 acre inches). One and a half hours was required to pump the 50 gallon nematode slurry into the furrows and another half hour to flush the lines. On the day of nematode application (4 August, 1995), the dry soil temperature, taken with a pocket dial thermometer (VWR, Phoenix, AZ) was 90°F at the surface. Irrigation water temperature was 80°F. The field was irrigated on 18 August and 12 September, 14 and 39 days, respectively, following burial of the baskets containing PBW larvae. Less than 2 acre inches of water were applied on 18 August and 12 September due to rainfall of 0.92, 1.28, 0.12 and 0.26 inches which occurred on 15, 20, 23 August and 6 September, respectively.

Three days after burial, the baskets and single cassettes containing larvae for all sample dates were recovered, the mud washed off and the PBW larvae examined for nematode-caused mortality. Soil samples taken before *S. riobravus* application and bioassayed with PBW larvae indicated that there were no indigenous entomopathogenic nematodes present in the soil. There was no PBW larval mortality 3 days after introducing them to untreated and dampened soil.

On all sample dates, ant predation of PBW larvae in the cassettes was extensive. Data were therefore combined for row distances from the nematode source as replicates and depths in the soil (or placement on row bed) and days following application were considered as treatments. PBW mortality caused by unknown bacterial or fungal infections were eliminated and not considered in calculations of percent PBW larval mortality. All percent mortality data were transformed to arcsines before analysis. Data were analyzed using 2 factor factorial ANOVA and means were separated with an LSD test when a significant "F" value was obtained. The 0.05 level of probability was used in all statistical analyses.

## Results

After 3 days exposure in the soil for each sampling date, we found that ant predation on the larvae was extensive, especially for larvae buried 1/2 and 2 inches below the surface. Ants were found inside the biopsy cassettes along with dismembered PBW larvae. Up to 32% of the PBW larvae were removed at 1/2 inches below the surface and 20% at 6 inches below the surface. In some cases, ant predation was so extensive that no statistical analysis could

be conducted. However, analyses of combined data showed percent larval mortality ( $38.3 \pm 10.6$ ) at 50 feet from the nematode release point was significantly higher than  $25.1 \pm 8.3$ ,  $30.0 \pm 8.1$ ,  $18.6 \pm 7.0$  and  $19.6 \pm 8.1$  obtained at 100, 150, 200 and 250 feet from the nematode release point, respectively.

Main effect mean PBW percent mortalities for days following nematode application were 56.7% for Day 0, 19.4% for Day 14 and 2.9% for Day 39 following nematode application. All percentages were significantly different from each other ( $F = 96.2$ ;  $df = 2, 44$ ;  $P \leq 0.01$ , Table 1). There were no significant differences between main effect mean PBW mortalities buried in the soil at 1/2 inch (33.7%) and 2 inches below the surface (35.8%). However, mortalities of PBW larvae buried at 4 and 6 inches below the surface, were 24.3% and 11.5%, respectively. These latter two percentages were significantly different from each other and the mortalities of larvae buried at 1/2 and 2 inches below the surface ( $F = 9.5$ ;  $df = 3, 44$ ;  $P \leq 0.01$ ). On Day 0, 76.7% of the PBW larvae examined were infested with *S. riobravivis* when buried 1/2 inches below the surface as compared with 23.3% mortality when buried 6 inches below the soil surface. Thirty-nine days later 4.5 and 1.0% larval mortality occurred for larvae buried 1/2 and 6 inches below the surface, respectively. Also at Day 39, none of the percent PBW mortalities at each depth were significantly different from each other but all were different from the 4 depths for Day 0 and Day 14 ( $F = 3.2$ ;  $df = 6, 44$ ;  $P \leq 0.05$ , Table 1).

Overall percent PBW mortality was significantly higher for larvae buried in cassettes in the middle of the row bed (34.6%) than at the top of the bed (11.3%) ( $F = 12.8$ ;  $df = 2, 20$ ;  $P \leq 0.01$ , Table 2). Movement of *S. riobravivis* upward through the soil profile to the top of the row bed was shown by non-significant larval mortalities of 17.8, 10.7 and 5.3% for Day 0, 14 and 39 days, respectively, following treatment ( $F = 5.5$ ;  $df = 2, 20$ ;  $P \leq 0.05$ ). However, when means of PBW mortalities for top and middle of the row bed were combined, Day 0, 14 and 39 PBW mortalities were significantly different from each other ( $F = 12.1$ ;  $df = 2, 20$ ;  $P \leq 0.01$ ). By Day 14, numbers of larvae infested with nematodes and buried at the top or middle of the row bed were not significantly different from each other (Table 2).

### Discussion

Entomopathogenic nematodes have been applied in cotton fields using several types of conventional farm equipment. For example, Lindegren et al (1992) applied *S. carpocapsae* All Strain using sub-surface drip lines on a commercial farm. Surface soil sample bioassays taken the day of treatment showed 30% PBW larval mortality after 3 days incubation compared to 76% mortality at the surface in our study using *S. riobravivis*. Bioassays of soil samples following nematode application with a ground sprayer or shanked into the soil in cotton fields showed PBW

mortality of 5 to 35% (K. Smith, unpublished data, Biosys, Western Regional Office, Vancouver, WA 98686). In El Paso, Texas, Gouge et al. (unpublished data, USDA-ARS, Western Cotton Research Laboratory, Phoenix, AZ 85040) applied 1 billion *S. riobravivis*/acre by releasing them directly into irrigation canals that channeled water into furrows through traps located in the bottom of the canals. The action of canal water escaping through the traps kept the water agitated and nematodes suspended, resulting in successful distribution throughout a 30 acre field. In Arizona, most fields are irrigated using siphon tubes set in irrigation ditches with slowly moving water. Nematodes tend to settle out very quickly in unagitated water. In our study, we pumped nematodes directly into the irrigation water that was flowing down the furrows. Our results show that the nematodes were successfully carried to the end of the field by our method of application. The fact that PBW larvae became infected with nematodes when buried 1/2 inches below the soil surface at the top of the row bed and 6 inches below the soil surface in the furrow indicates that *S. riobravivis* will actively migrate through the soil seeking hosts. Lindegren et al. (1993b) found similar results when they showed that *S. riobravivis* moved up or down through a sand column to parasitize *Galleria mellonella* (L.) larvae.

Although *S. riobravivis* persisted in the soil for at least 39 days, larval mortalities at that time were significantly lower (5%) than at 14 days after application (30%). The reason for these low mortalities is unknown since the rows were covered with plant canopy which shaded the soil, preventing high temperature and moisture fluctuations. *S. riobravivis* persistence in the soil, as measured by PBW larval mortality bioassays, was shorter than previously reported by Lindegren et al. (1995). Their data showed that *S. riobravivis* applied in late March persisted at low levels for 90 days in irrigated soil plots exposed to direct sunlight where average midday soil surface temperatures reached 109°F. Studies are continuing to determine nematode application methodology, rates and optimum timing of treatment.

### 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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Table 1. Mean percent nematode-induced PBW larval mortalities following application of 2.6 billion *Steinernema riobravis*/acre during furrow irrigation of a cotton field.

Depth in Soil (inches)	Day Following Treatment <sup>a</sup>			Mean
	0	14	39	
1/2	76.7 a	20.0 cd	4.5 efg	33.7 A
2	73.4 a	30.0 c	4.0 fg	35.8 A
4	53.6 b	17.4 cde	2.0 fg	24.3 B
6	23.3 cd	10.2 def	1.0 g	11.5 C
Mean	56.7 A	19.4 B	2.9 C	

<sup>a</sup> Means of 5 replications not followed by the same lower case letter or overall means not followed by the same capital letter are significantly different. ANOVA, LSD,  $P \leq 0.05$ .

Table 2. Mean percent nematode-induced PBW larval mortalities at the top and middle of a row following application of 2.6 billion *Steinernema riobravis*/acre during furrow irrigation of a cotton field.

Placement on Row Bed	Day Following Treatment <sup>a</sup>			Mean
	0	14	39	
Top	17.8 bc	10.7 bc	5.3 c	11.3 B
Middle	68.0 a	30.1 b	5.6 c	34.6 A
Mean	42.9 A	20.4 B	5.5 C	

<sup>a</sup> Means of 5 replications not followed by the same lower case letter or overall means not followed by the same capital letter are significantly different. ANOVA, LSD,  $P \leq 0.05$ .

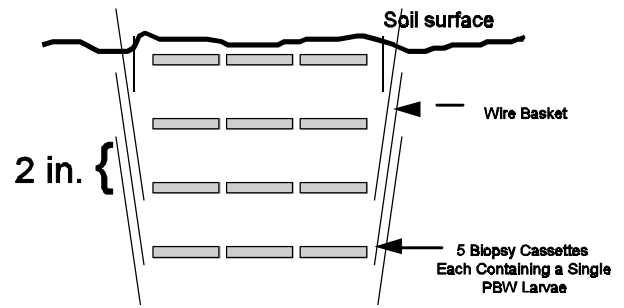


Figure 1. Arrangement of stacked wire baskets containing perforated biopsy cassettes with single PBW larvae for burial in *S. riobravis* treated soil.

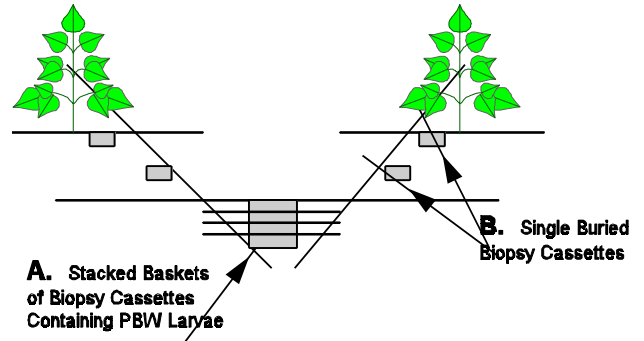


Figure 2. Locations of biopsy cassettes containing PBW larvae in soil treated with *S. riobravis*. "A" is experiment 1 designed to determine the depth in the soil (down to 6 inches) the nematodes would move to parasitize host insects. "B" is experiment 2 designed to determine upward movement of the nematodes in the soil.