

**PINK BOLLWORM LARVAL MORTALITY
FOLLOWING APPLICATION
OF *STEINERNEMA RIOBRAVIS*
ENTOMOPATHOGENIC NEMATODES
IN COTTON FURROW IRRIGATION
L. Forlow Jech and T. J. Henneberry
USDA, ARS, WCRL
Phoenix, AZ**

Abstract

An infective juvenile nematode slurry of 1.3 billion *Steinernema riobrans* Cabanillas, Poinar and Raulston per acre was pumped through a polyvinyl chloride pipe manifold directly into furrow irrigation water in a cotton field. Pink bollworm (*Pectinophora gossypiella*) (PBW) larvae were released onto the soil surface in bottomless buckets 2, 8, 14, 22, 29 and 36 days after nematode application. Prior to each PBW release, the field was either irrigated or had received rain. PBW larvae were recovered 24 hr after each release, held another 24 hr in petri dishes and examined for nematode-induced mortality. Mean percent mortalities ranged from 92.4% at 2 days following treatment to 25.7% at 36 days following treatment.

Introduction

The Pink bollworm, *Pectinophora gossypiella* (Saunders) (PBW), is one of the most important economic pests of cotton in Arizona and southern California. PBW biological control with entomopathogenic nematodes is still in the early stages of development (Lindegren et al. 1994). *Steinernema riobrans*, a newly described (Cabanillas et al. 1994) and high temperature tolerant species, appears promising for control of the soil-associated PBW larval stage (Lindegren et al. 1994, Henneberry et al. 1995).

Nematodes have been applied successfully to cotton fields by furrow irrigation (Forlow Jech and Henneberry 1996, Gouge et al. 1996) and motorized ground equipment (Gouge et al. 1996). Gouge et al. (1996) reported that fields treated with *S. riobrans* in Arizona had a 19% increase in cotton lint yield and fields in Texas had reduced PBW damaged bolls.

In 1995, we showed that PBW larvae became infected with nematodes when buried 1/2 inch below the soil surface at the top of the row bed and 6 inches below the soil surface in the furrow indicating that *S. riobrans* will actively migrate through the soil seeking PBW hosts. In 1996, we further examined nematode distribution by furrow irrigation and the effect on PBW larvae released onto the soil surface.

Materials and Methods

S. riobrans nematodes were applied to a 0.39 acre cotton field during siphon furrow irrigation on August 6, 1996. We used the same PVC pipe manifold system and methods that were previously reported (Forlow Jech and Henneberry 1996). The field was 315 ft long and planted with 16 rows of DPL 5415 cotton.

On the day of nematode application (6 August, 1996), dry soil temperatures, taken 1 inch below the surface with a pocket dial thermometer (VWR, Phoenix, AZ), were 116°F and 90°F in the sun and shade, respectively. Irrigation water temperature was 80°F. The field was irrigated weekly (6, 13, 26 August and 11 September) with approximately 2 acre inches of water except for the weeks of 19 August and 4 September due to 1.1 inch and 0.51 inch rains, respectively.

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). Two days after each irrigation or rain, 5 bottomless plastic buckets with lids were placed at 50 ft. intervals in each of 5 nematode-treated row furrows. The buckets were pushed approximately 1/2 inch into the moist soil, with lids removed, and 50 PBW larvae were introduced onto the soil surface and allowed to acclimate (Fig. 1). Treatments were sampling dates following application and distances of 50, 100, 150, 200 and 250 feet from the points of nematode introduction into the furrows. On the first sample date, ant predation of PBW larvae was extensive. Data for that date therefore were not included in the analysis. Black Flag® insecticide was sprayed on each sampling date thereafter around the periphery of each bucket to reduce ant predation on the larvae. After 24 hours, all PBW larvae that could be located were removed from the soil from each bucket and held in petri dishes containing moist filter paper for another 24 hr. At that time, numbers of nematode-killed larvae were counted. Soil samples taken before *S. riobrans* application and bioassayed with PBW larvae indicated that there were no indigenous entomopathogenic nematodes present in the soil.

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

Nematodes were evenly distributed across the field by the PVC manifold system and furrow irrigation combination (Fig. 2). On day three following treatment, mean PBW mortality for all sampling locations averaged 92.4%. There were no significant differences in PBW mortality among distance from the nematode introduction source on each

sampling date ($F = 0.63$, $df = 16$, 96). PBW mortality was significantly greater 1 week after treatment compared with 2, 3, 4 or 5 weeks after treatment ($F = 26.2$, $df = 4$, 96).

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 and their surface soil sample bioassays taken the day of treatment showed 30% PBW larval mortality after 3 days incubation in the laboratory. In our studies with *S. riobravus* applied during furrow irrigation, 92.4% larval mortality occurred 2 days after treatment when larvae were released on the soil surface. Gouge et al. (1996) in Arizona reported promising PBW control potential after application of 1 billion *S. riobravus* infective juveniles per acre using a tractor spray boom with drop nozzles and in Texas, after application of 1 billion *S. riobravus* per acre by releasing them directly into irrigation canals that channeled water into furrows. The nematodes in Arizona were formulated in water dispersible granules and then dissolved in the tractor tank with water. The field was irrigated immediately following treatment. In Texas, the nematodes were unformulated and delivered directly into the canal water during irrigation. The action of the water escaping through traps located in the bottom of the canals kept nematodes suspended resulting in successful distribution throughout the 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 this and the 1995 study, incorporated nematodes into siphon irrigation water using a PVC pipe manifold system efficiently kept nematodes suspended by water agitation and pumped nematodes directly into irrigation water flowing down the furrows.

Nematode-infected PBW larvae disintegrate rapidly and become difficult to locate in the soil. In our 1995 studies, PBW larvae were exposed to nematodes in the soil for 3 days in small cages (biopsy cassettes) and were easily recovered from the soil. In 1996, larvae were released onto the surface and exposed for only 24 hr to facilitate recovery before extensive disintegration.

PBW larval mortalities were generally higher in 1996 than mortalities for larvae buried at 4 levels in the soil in 1995 studies. For example, our results show more than 25% mortality on day 37 following treatment in 1996 as compared with 5% mortality of PBW larvae after 42 days when larvae were buried 1/2 inches in the soil in 1995 (Forlow Jech and Henneberry 1996). Although *S. riobravus* has been shown to migrate to depths of 12 inches in columns of sand (Gouge et al. unpublished data) and 6 inches into the soil in a cotton field to infect host PBW

larvae, some *S. riobravus*, in the absence of host larvae, appear to stay near the soil surface providing there is enough moisture. It is possible that higher soil moisture levels occurred in 1996 than in 1995 because of weekly irrigations and rain. In 1995, we applied 1 less irrigation and although more rain fell, it did not occur at weekly intervals which resulted in soil drying.

Lindegren et al. (1995) showed that *S. riobravus* 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. In our 1995 studies, nematodes persisted for 42 days in soil shaded by plant canopy where moisture fluctuations were reduced. In 1996 measurable mortality occurred on day 37 following treatment and at that time we terminated the experiment.

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.

Literature Cited

- Bartlett, A.C. and W.W. Wolf. 1985. *Pectinophora gossypiella*. pp. 4415-4430. In P. Singh and R.F. More [eds]. Handbook of Insect Rearing, Elsevier, Amsterdam.
- Cabanillas, H.E., G.O. Poinar, Jr. and J.R. Raulston. 1994. *Steinernema riobravus* n. sp. (Rhabditida: Steinernematidae) from Texas. Fundam. Appl. Nematol. 17: 123-131.
- Forlow Jech, L. and T.J. Henneberry. 1996. A method of distributing *Steinernema riobravus* in cotton furrow irrigation and Pink bollworm larval mortality response. Proc. Beltwide Cotton Prod. and Res. Conf. 2: 707-710.
- Gouge, D.H., L.L. Reaves, M.M. Stoltman, J.R. Van Berkum, R.A. Burke, L. Forlow Jech, and T.J. Henneberry. 1996. Control of Pink Bollworm *Pectinophora gossypiella* (Saunders)(Lepidoptera: Gelechiidae) larvae in Arizona and Texas cotton fields using the entomopathogenic nematode *Steinernema riobravus* (Cabanillas, Poinar, and Raulston) (Rhabditida: Steinernematidae). Proc. Beltwide Cotton Prod. and Res. Conf. 2: 1078-1082.
- Henneberry, T.J., J.E. Lindegren, L. Forlow Jech and R.A. Burke. 1995. Pink bollworm (Lepidoptera: Gelechiidae): Effects of Steinernematid nematodes on larval mortality. Southwestern Entomol. 20: 25-32.

Lindegren, J.E., T.J. Henneberry and L. Forlow Jech. 1992. Mortality response of Pink bollworm to the entomopathogenic nematode *Steinernema carpocapsae*. Proc. Beltwide Cotton Prod. and Res. Conf. 2: 930-931.

Lindegren, J.E., T.J. Henneberry, J.R. Raulston, L. Forlow Jech and K.A. Valero. 1994. Current status of pink bollworm control with entomopathogenic nematodes. Proc. Beltwide Cotton Prod. and Res. Conf. 2: 1242-1243.

Lindegren, J.E., T.J. Henneberry, L. Forlow Jech and R.A. Burke. 1995. Pink bollworm suppression response and field persistence of two insect parasitic nematodes. Proc. Beltwide Cotton Prod. and Res. Conf. 2: 944-945.

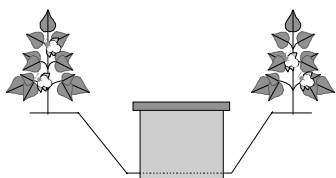


Figure 1. Schematic drawing of placement of Pink bollworm larvae in bottomless buckets in furrows of a cotton field treated with *S. riobravis* nematodes.

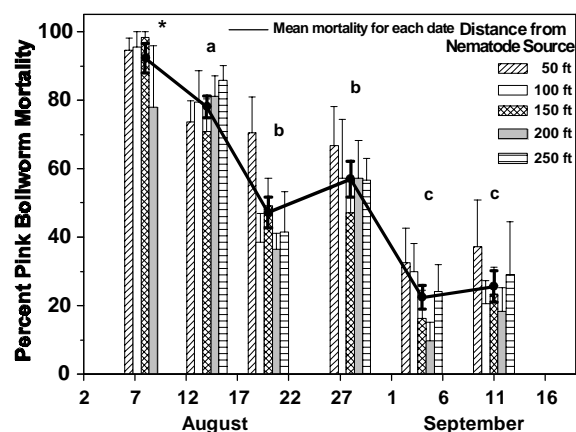


Figure 2. Mean percentages of Pink bollworm larval mortality after being exposed to *S. riobravis* in the soil. PBW were released onto the soil surface at 50, 100, 150, 200 and 250 feet from the point of nematode application into the furrow irrigation water. On the first sampling date (*), ant predation on the larvae was extensive so data were not included in the analysis. Means of 5 replicates not followed by the same letter are significantly different. ANOVA, LSD, $P \leq 0.05$.