CONTROL OF PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) (LEPIDOPTERA: GELECHIIDAE) LARVAE IN ARIZONA AND TEXAS COTTON FIELDS USING THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA RIOBRAVIS* (CABANILLAS, POINAR, AND RAULSTON) (RHABDITIDA: STEINERNEMATIDAE)

D. H. Gouge, L. L. Reaves,
M. M. Stoltman, J. R. Van Berkum,
R. A. Burke, L. J. Forlow-Jech,
and T. J. Henneberry
USDA-ARS
Western Cotton Research Laboratory
Phoenix, AZ

Abstract

Cotton fields were treated with the entomopathogenic nematode *Steinernema riobravis* (Cabanillas, Poinar and Raulston) for the control of *Pectinophora gossypiella* (Saunders). Pima S-7 cotton situated at V & C farms Queen Creek Arizona, was treated at a rate of 1 billion nematodes per acre. The nematodes were applied using a spray rig with dropped nozzles, immediately after application the field was irrigated. Pima S-6 cotton situated in Texas A & M research center El Paso Texas, was treated at a rate of 1.3 billion nematodes per acre. In this case the nematodes were applied to the irrigation channel during field irrigation.

Both application methods resulted in excellent distribution of the nematodes over the treated areas as indicated by extraction of nematodes from soil samples. Nematodes applied in Arizona persisted for 19 days in large numbers, and could still be recovered from furrows after 75 days. The number of cotton bolls infested with *P. gossypiella* was significantly reduced by the application of *S. riobravis*, and cotton yields from treated plots were 19% higher relative to cotton yields from untreated plots.

In Texas the nematodes persisted for 16 days in large numbers, and could still be recovered from row and furrow bases after 50 days. The nematodes moved rapidly from the furrow soil into the cotton beds. Ten days after application, nematode distribution became fairly even, although the number of nematodes recovered from furrows was significantly higher than bed tops. The mortality of *P. gossypiella* larvae caged in biopsy cassettes was optimized on field irrigation 17 days after nematode application, when the nematodes had redistributed themselves within the rows and furrows. At this point 100% of caged *P. gossypiella* larvae were infected and killed by *S. riobravis*.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1078-1082 (1996)
National Cotton Council, Memphis TN

Introduction

The pink bollworm, *Pectinophora gossypiella* (Saunders) is one of the most serious pests of cotton occurring throughout most of the tropical and subtropical areas of the world (Ingram, 1994). It is considered one of the most damaging cotton pests in Arizona and southern California. Heavy insecticide use often promotes resurgence of secondary pest species such as *Heliothis virescens* (Fabricius), *Helicoverpa zea* (Boddie), and *Bucculatrix thurberiella* (Busck) (University of California, 1984).

Pink bollworm larvae feed on flower buds, flowers, bolls and the seeds within. Damage to developing seeds, and the termination of growth results in boll rotting, premature or partial boll opening, reduction of staple length, strength, and increases trash content in the lint. Estimated yield losses in the U.S. due to pink bollworm range from 9% when chemically controlled to 61% when uncontrolled (Schwartz, 1983), although 100% crop loss can occur with heavy infestations.

Pima cotton is particularly susceptible to pink bollworm attack, due to the long growing season required before harvest.

Existing control programs involve the use of sex pheromones (Flint *et al*., 1985), sterile moth release (Bartlett, 1978), insecticidal use (Watson *et al*., 1988), plant resistance (Wilson, 1987, 1989), and a variety of cultural control practices (Henneberry *et al*., 1980, Ingram, 1980). The introduction of transgenic cotton commercially in 1996 will also offer an alternative short staple variety, resistant to pink bollworm attack (Flint *et al*., 1995).

Entomopathogenic nematodes in the family Steinernematidae are promising biocontrol agents of a broad spectrum of insect pests occupying cryptic habitats (Begley, 1990, Klein, 1990).

Steinernematid nematodes are associated with a bacterial symbiont *Xenorhabdus* spp. The infective juvenile stage of the nematodes seek potential host insects within the soil. Having entered through natural body openings the bacteria is released and causes septicemia and death of the insect.

Recent developments in production through liquid fermentation (Georgis, 1990a) and exemption from registration requirements in most countries (Gaugler, 1988), has led to the introduction of several products commercially (Georgis & Hague, 1991) in citrus, turfgrass, cranberries, ornamentals, and mushrooms (Georgis, 1992).

Steinernematids have been applied using standard agrochemical equipment, in a variety of ways. Nematodes have been applied using aircraft (Lindegren *et al*., 1981), through tractor spray booms (Georgis, 1990a), back-pac sprayers (Gouge & Hague, 1995), soil injection and

shanking equipment (Smith, biosys Columbia MD personal communication), and in furrow irrigation in grapes (Wennemann, Washington State University WA personal communication) and cotton (Forlow-Jech & Henneberry, 1996).

Research has indicated that several cotton pests are susceptible to *Steinernema* spp. including *H. zea*, *Spodoptera frugiperda* (Smith) (Raulston *et al*., 1992), *P. gossypiella*, *Trichoplusia ni* (Hübner), and *Spodoptera exigua* (Hübner) (Henneberry *et al*., 1995). *Steinernema riobravis* (Cabanillas, Poinar and Raulston) was discovered in the Lower Rio Grand Valley of Texas (Cabanillas *et al*., 1994). It appears to be naturally selected for the subtropical, semi-arid environment where it serves as a natural biocontrol agent of *H. zea* and *S. frugiperda*. A nematode isolated from a lepidopteran host inhabiting a semi-arid, subtropical environment, may be considered an ideal candidate for use as a biocontrol agent of *P. gossypiella*.

After the pink bollworm larvae have fed within the cotton boll, they cut out and drop to the soil to pupate (Butler & Henneberry, 1976). It is at this point that the nematodes can infect and kill the larvae. Several generations of pink bollworm occur during the growing season and this paper details mid season application of *S. riobravis* to irrigated cotton crops in Arizona and Texas for *P. gossypiella* control.

Materials and Methods

Initially soil samples from both fields were taken and baited with *Galleria mellonella* (L.). *G. mellonella* was used as a live bait to extract any naturally occurring entomopathogenic nematodes.

Queen Creek, AZ

A 35 acre field of Pima S-7 cotton was divided into 10 plots of 24 rows each (2.64 acres), with 48 row buffer zones between plots. The field had a previous history of heavy pink bollworm infestation.

The crop was sown at 38 inch row centers in sandy loam soil, on March 24th. A single application of *S. riobravis* (biosys, St. 355) was applied July 7th to alternate plots at a rate of 1 billion infective juveniles per acre. The nematodes were applied using a tractor spray boom and sprayed through dropped nozzles at 40psi, in 300 gallons of water per acre. The field was then immediately irrigated.

The nematodes were formulated in water dispersible granules which were added directly into the tractor spray tank, which was then filled to capacity. Nematode viability assessments were made microscopically from samples collected from spray nozzles. Nematode viability never decreased below 98%.

Throughout the experimental period the ambient temperature did not exceed 123^0F (1 foot above the soil surface), the highest soil temperature recorded was 141^0F, one inch below the soil surface. Approximately 10 inches of rain fell.

During the growing season, the entire field received 4 applications of Ovasyn (Amitraz- 1.14 pints per acre) and Thiodan (Endosulfan- 2.14 pints per acre) applied by air, for white fly control.

Sampling

Ten 500cc soil samples were taken from each nematode treated plot at approximately 7-14 day intervals. Five were taken from the row top and 5 from the furrow. Control plots were also sampled periodically, but no transfer of the nematodes into control areas was detected.

The soil samples were baited with 8 late instar *G. mellonella* larvae in large Petri-dishes. Dry soil received distilled water until the sample was moist but not wet. After 4 days incubation at 80.6^0F the larvae were collected from the soil samples and washed in distilled water. The larvae were then dissected in quarter strength Ringers solution under a stereo dissecting microscope and the numbers of infected larvae recorded.

Thirty bolls were also removed from plants in each treated and untreated plot at approximately 7-14 day intervals. The bolls were cracked open by hand and the number of pink bollworm larvae and exit holes counted.

Harvesting

The field was harvested on November 18th. The cotton from the central four rows (0.22 acres) of each plot was weighed on digital trailer scales. 0.5lb samples of cotton were extracted each time. These samples were ginned and the seed x-rayed for damage data. Lint samples were also graded for quality.

El Paso, TX

A 9.2 acre field of Pima S-6 cotton was divided into 6 research plots of 18 rows each (1.5 acres). The field was situated in an area where pink bollworm infestations had been consistently high for many years.

The crop was sown on 38 inch row centers in calcareous soil. The soil was extremely heterogeneous and ranged from sandy to clay loam. The cotton was planted April 4th and the field had previously been laser leveled.

A single application of *S. riobravis* was applied July 5th to the entire field at a rate of 1.3 billion nematodes per acre. The nematodes were unformulated and were received as a 50 liter nematode suspension in water. Samples were withdrawn and microscopic visual viability tests showed that 95% of the infective juveniles were alive and mobile.

The nematodes were applied directly into the irrigation channel as the field was irrigated. Irrigation of the entire field took 6 hours and 4.16 liters of the agitated nematode suspension was added to the channel at the outlet pipe, every 30 minutes.

The irrigation water left the channel through gates at soil level which were opened and closed a section at a time over the 6 hour period.

Throughout the experiment the ambient temperature did not exceed 115^0F (1 foot above the soil surface) and the highest soil temperature recorded was 128^0F one inch below the soil surface.

Sampling

Ten 500cc soil samples were taken from each of the 6 plots at approximately 14 day intervals. Five were taken from the row top and 5 from the furrow as described earlier. The soil samples were baited with *G. mellonella*, incubated, and the number of infected insects recorded as before.

Prior to each irrigation, single laboratory reared, late instar pink bollworm larvae were placed in soil filled biopsy cassettes (1 inch by 2 inch plastic mesh cages). Fresh soil from an untreated field was used to fill the cassettes. The caged insects were then buried 1 inch below the soil surface at 5 points along each plot. At each point a cassette was placed in the row top and in the furrow base. Two days after irrigation the cassettes were recovered and the pink bollworm washed and dissected in quarter strength Ringers solution under a stereo dissecting microscope. The numbers of infected insects was recorded.

Results

No naturally occurring entomopathogenic nematodes were isolated from the soil samples taken prior to application of *S. riobravis*.

Queen Creek, AZ

The number of cotton bolls infested with pink bollworm was significantly ($p<0.001$) reduced by the application of *S. riobravis* to the soil (Fig. 1). Pink bollworm adults usually remain within a localized area throughout the season and only begin to migrate at the end of the year. For this reason we can consider our research plots isolated from one another with regard to pink bollworm population levels in the field. The number of damaged seeds was higher and lint quality was considered to be significantly lower ($p<0.001$) in the samples taken from control areas.

Suppression of the population in the soil in July resulted in an overall reduction of the population in treated plots and reduced damage to the cotton bolls for the rest of the season. Subsequently, at harvest, the mean cotton yield from treated plots was 19% higher relative to the mean yield from untreated plots (Fig. 2) Control plots produced a mean yield of 1574.94 lbs per acre, and *S. riobravis* treated fields produced a mean yield of 1873.69 lbs per acre.

Nematodes applied in July persisted for only 19 days in large numbers (Fig. 3). Severe soil temperatures 1 inch below the surface, and rapid desiccation would be the obvious reasons for this.

El Paso, TX

The mortality of caged pink bollworm larvae immediately after nematode application showed 100% infection in the furrow only (Fig. 4). After 7 days however, the nematodes began to move into the row and some mortality of insects caged in the row top was observed. The greatest insect mortality was observed 17 days after nematode application, when all the larvae irrespective of position in the cotton bed were infected with nematodes (Fig. 4). At this point the nematodes had become distributed throughout the cotton beds (Fig. 5). In the ensuing period both the nematode population and the insect mortality declined. Very low numbers of nematodes could be found up to 90 days after application.

Discussion

Infective juveniles of *S. riobravis* were capable of detecting, infecting and killing larvae of *P. gossypiella* during the time that the fields retained suitable moisture levels after irrigation.

The presence of caged larvae in the row tops in El Paso may have improved redistribution of the nematodes into this area. Georgis and Poinar (1983), described enhanced lateral dispersal of several nematode species due to the presence of a suitable host.

Both application methods employed had no harmful effects upon the nematode infective juveniles, and nematode distribution throughout the fields was found to be uniform.

Nematode persistence was consistently better in the base of furrows. The nematodes will avoid moving into dry areas where their movement is restricted.

The furrow remains moist for longer and the nematodes will be allowed to desiccate gradually as the field dries. Steinernematid nematodes can survive extended periods in an anhydrobiotic state if the drying process is gradual (Simons & Poinar, 1973). Although the nematodes are immobile and non-pathogenic when in the desiccated state, it is a survival strategy that allows the infective juvenile nematodes to persist until more favorable environmental conditions return. When the fields are irrigated, the nematodes become rehydrated and are once again infective and pathogenic to pink bollworm.

Certain nematode species are known to be ecologically adapted with respect to their temperature and humidity requirements. Kung *et al*. (1991), attributed the subtropical origin of *Steinernema glaseri* to be one of the factors allowing it good persistence at high temperatures (15-35^{0}C), but poor persistence at low temperatures (5^{0}C).

Soil flora and fauna will also effect nematode persistence. The pink bollworm larvae which are infected by *S. riobravis* will themselves release juveniles from the cadavers, but many soil micro-organisms and micro arthropods are known to be factors in reducing populations of entomopathogenic nematodes. Nguyen and Smart (1990), demonstrated that nematodes persistence was extended under sterile conditions.

Pectinophora gossypiella larvae are highly susceptible to *S. riobravis* under field conditions. The nematodes may be applied using standard spray equipment or added to the furrow irrigation water during irrigation of laser level fields. As the nematodes are compatible with most chemical pesticides (Georgis, 1990b), and have little effect on beneficial insects (Georgis *et al*., 1991, Poinar, 1989), they can be considered a convenient tool for use in cotton IPM systems.

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.

Acknowledgments

We wish to extend our thanks to our cooperators, James Van Allen and Michael Crigger for their time, land and consideration. Thanks also to Naomi Assidian and her team of workers at Texas A & M Research Center, El Paso, Texas. We thank biosys for supply of *S. riobravis*. Special thanks to Kirk A. Smith, David H. Akey, Hollis E. Flint and Dan Ragsdale for their considerable help and support.

References

Bartlett, A. C. 1978. Radiation Inducing Sterility in the Pink Bollworm. US Department of Agriculture, Science & Education Administration, ARM-W-1.

Begley, J. W. 1990. Efficacy against insects in habitats other than soil. *In*: R. Gaugler & H. K. Kaya. (Eds.). Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL, USA.

Butler, G. D., Jr. and T. J. Henneberry. 1976. Biology, behavior, and effects of larvae of pink bollworm in cotton flowers. Environ. Entomol. 5:970-972.

Cabanillas, H. E., G. O. Poinar, Jr. and J. R. Raulston. 1994. *Steinernema riobravis* n. sp. (Rhabditida: Steinernematidae) from Texas. Fundam. Appl. Nematol. 17:123-131.

Flint, H. M., T. J. Henneberry, F. D. Wilson, E. Holguin, N. Parks, and R. E. Buehler. 1995. The effects of transgenic cotton, *Gossypium hirsutum* L., containing *Bacillus thuringiensis* toxin genes for the control of pink bollworm, *Pectinophora gossypiella* (Saunders) and other arthropods. Southwestern Entomol. 20:281-292.

Flint, H. M., J. R. Merkle, and A. Yamamoto. 1985. Pink bollworm (Lepidoptera: Gelechiidae), field testing a new polyethylene tube dispenser for gossyplure. J. Econ. Entomol. 78:1431-1436.

Forlow-Jech, L. and T. J. Henneberry. 1996. A method of distributing *Steinernema riobravis* in cotton furrow irrigation and pink bollworm larval mortality response. In: D. J. Heber and D. A. Richter Eds.). Proceedings of the Beltwide Cotton Conference, National Cotton Council. In press.

Gaugler, R. 1988. Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agric. Ecosys. and Environ. 24:351-360.

Georgis, R. 1992. Present and future prospects for entomopathogenic nematode products. Bio. Sci. and Tech. 2:83-99.

Georgis, R. 1990a. Commercialization of steinernematid and heterorhabditid entomopathogenic nematodes. Brighton Crop Protection Conference- Pests and Diseases 1:275-280.

Georgis, R. 1990b. Formulation and application technology. *In*: R. Gaugler and H. K. Kaya. (Eds.). Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL, USA.

Georgis, R. and N. G. M. Hague. 1991. A neoaplectanid nematode in the web-spinning larch sawfly, *Cephalcia lariciphila* (Hymenoptera: Pamphiliidae). Ann. App. Biol. 99:171-177.

Georgis, R. and G. O. Poinar, Jr. 1983. Effect of soil texture on the distribution and infectivity of *Neoaplectana glaseri* (Nematoda: Steinernematidae). J. Nematol. 15:329-332.

Georgis, R., H. K. Kaya, and R. Gaugler. 1991. Effect of steinernematid and heterorhabditid nematodes (Rhabditida: Steinernematidae: Heterorhabditidae) on non-target arthropods. Environ. Entomol. 20:815-822.

Gouge, D. H. and N. G. M. Hague. 1995. Glasshouse control of fungus gnats, *Bradysia paupera*, on fuchsias by *Steinernema feltiae*. Fundamental and App. Nematol. 18 (1) :77-80.

Henneberry, T. J., L. A. Bariola, and D. L. Kittock. 1980. Pink bollworm control: potential of cotton crop management to selectively limit host availability. *In*: H. M. Graham. (Ed.). Pink Bollworm Control in the Western United States. US Department of Agriculture, Science and Education Administration, ARM-W-16.

Henneberry, T. J., J. E. Lindegren, L. Forlow-Jech and R. A. Burke. 1995. Pink bollworm (Lepidoptera: Gelechiidae), cabbage looper and beet armyworm (Lepidoptera: Noctuidae) pupal susceptibility to Steinernematid nematodes (Rhabditida: Steinernematidae). J. Econ. Entomol. 88:835-839.

Ingram, W. R. 1994. *Pectinophora* (Lepidoptera: Gelechiidae). In: G. A. Matthews and J. P. Tunstall (Eds.). Insect Pests of Cotton. CAB International.

Ingram, W. R. 1980. Studies of the pink bollworm, *Pectinophora gossypiella*, on Sea Island cotton in Barbados. Trop. Pest Man. 26:118-137.

Klein, M. G. 1990. Field efficacy against soil insects. *In*: R. Gaugler and H. K. Kaya. (Eds.). Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL, USA.

Kung, S. P., R. Gaugler and H. K. Kaya. 1991. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. J. Invert. Pathol. 57:242-249.

Lindegren, J. E., J. E. Dibble, C. E. Curtis, T. T. Yamashita and E. Romero. 1981. Compatibility of NOW parasite with commercial sprayers. Calif. Agric. :35 (3 and 4):16.

Nguyen, K. B. and G. C. Smart. 1990. Preliminary studies on survival of *Steinernema scapterisci* in soil. Soil and Crop Sci. Soc. Fla. Proc. 49:230-233.

Poinar, G. O. Jr. 1989. Non-insect hosts for the entomogenous rhabditoid nematodes *Neoaplectana* (Steinernematidae) and *Heterorhabditis* (Heterorhabditidae). Rev. Nematol. 12:423-428.

Raulston, J. R., S. D. Pair, J. Loera and H. E. Cabanillas. 1992. Prepupal and pupal parasitism of *Helicoverpa zea* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) by *Steinernema* sp. in corn fields in the lower Rio and Valley. J. Econ. Entomol. 85:1666-1670.

Schwartz, P. H. 1983. Losses of yield in cotton due to insects. In: Agricultural Handbook. US Department of Agricultural Research Service. Beltsville, MD.

Simons, W. R. and G. O. Poinar, Jr. 1973. The ability of *Neoaplectana carpocapsae* (Steinernematidae: Nematodea) to survive extended periods of desiccation. J. Invert. Path. 22:228-230.

University of California. 1984. Integrated Pest Management for Cotton in the Western Region of the United States. Division of Agriculture and Natural Resources Publication 3305, CA.

Watson, W. M., A. M. Rashad, and N. M. Hussein. 1988. Potencies of certain insecticides against the pink bollworm, *Pectinophora gossypiella* (Saunders), as influenced by chemical control programs in Egypt. Bull. Entomol. Soc. Egypt (Economic) 15:79-86.

Wilson, F. D. 1989. Yield, earliness, and fiber properties of cotton carrying combined traits for pink bollworm resistance. Crop Sci. 29: 7-12.

Wilson, F. D. 1987. Registration of three cotton germplasm lines. Crop Sci. 27:820-821.

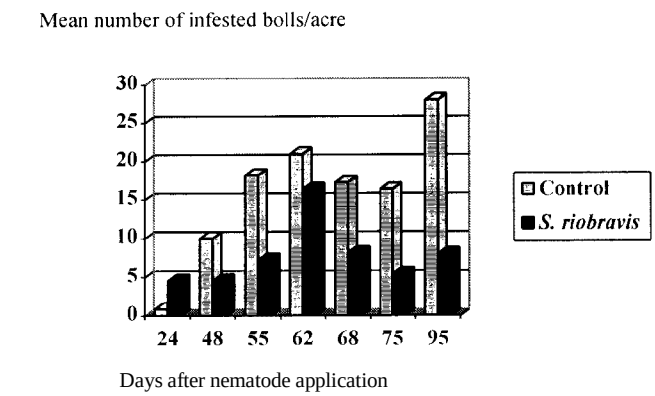

Fig. 1. The mean number of cotton bolls infested with pink bollworm in nematode treated plots, Queen Creek, AZ.

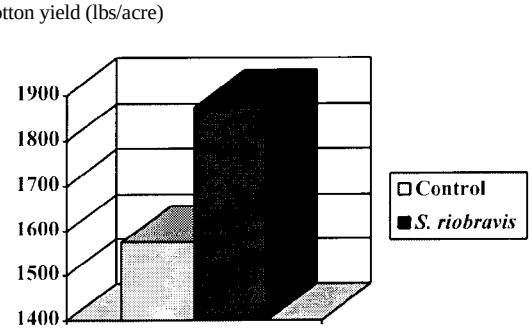

Fig. 2. The mean cotton yield taken from treated plots was 19% greater relative to untreated plots, Queen Creek, AZ.

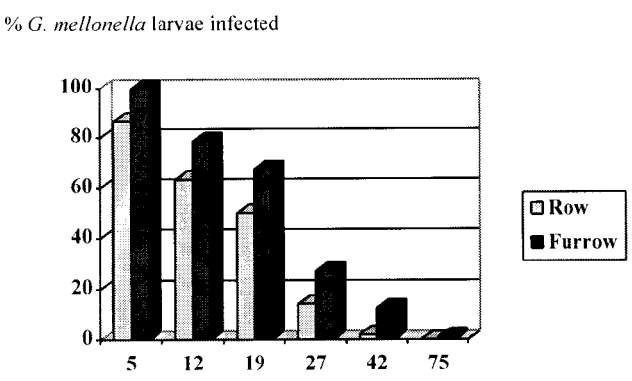

Fig. 3. Persistence of *S. riobravis* in the row top and furrow base, Queen Creek, AZ.

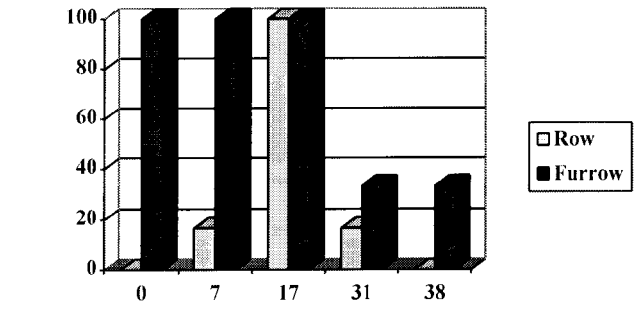

Fig. 4. Mortality of cages pink bollworm larvae due to infection by *S. riobravis*, El Paso, TX.

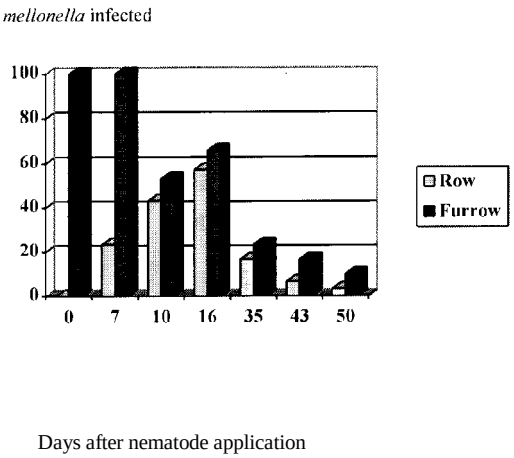

Fig. 5. Persistence of *S. riobravis* in the row top and furrow base, El Paso, TX.