

**SUPPRESSION OF PLANT PARASITIC
NEMATODES IN COTTON USING THE
ENTOMOPATHOGENIC NEMATODE
*STEINERNEMA RIOBRAVIS***

**(CABANILLAS, POINAR, AND RAULSTON)
(RHABDITIDA: STEINERNEMATIDAE)**

**D. H. Gouge, L. L. Lee, J. R. Van Berkum
and T. J. Henneberry**

USDA-ARS

Western Cotton Research Laboratory

Columbia, MD

K. A. Smith

biosys Inc.

Abstract

Cotton fields were treated with the entomopathogenic nematode, *Steinernema riobransis*, and Vydate® L for the control of plant parasitic nematodes. Short staple cotton grown near Coolidge, Arizona, was treated at a rate of 1 billion and 2 billion *S. riobransis* nematodes per acre, and 0.5 lb a.i. Vydate® L per acre. Untreated cotton received an application of water only. Treatments were applied through a subterranean drip system with 12 inch spaced outlets. Applications were made in the daily irrigation cycle of 0.33 inches of water, normal irrigation cycles followed.

Products were uniformly distributed over the treated fields. Entomopathogenic nematodes persisted throughout the 6 week experimental period at the 1 billion per acre rate. However, nematodes applied at 2 billion per acre rate disappeared rapidly. Populations of various plant parasitic nematode species were monitored subsequent to treatment application. Nematodes were extracted using a standard sugar flotation technique and counted in 1 ml slide samples. Both *Meloidogyne incognita* and *Tylenchorhynchus* spp. populations were reduced by *S. riobransis* applied at 1 billion per acre rate. Phytoparasitic nematodes were reduced following application of Vydate® L, but control was not sustained beyond one week.

Introduction

Plant parasitic nematodes caused a \$303 million loss from the 1995 cotton crop, according to the Cotton Disease Loss Estimate Committee (CDLEC). A total yield loss of 778,700 bales. When compared to other plant diseases, nematodes are the second largest cause of cotton yield loss (Goodell, 1993).

Entomopathogenic nematodes in the family Steinernematidae are generally used as biocontrol agents

for a broad spectrum of insect pests occupying soil habitats (Begley, 1990; Klein, 1990). However, recent observations have indicated an additional ability to suppress populations of phytoparasitic nematodes (Smith, 1995). Commercial products containing *S. riobransis* (BioVector®, Devour®) are now available for nematode control in turfgrass.

Ishibashi *et al.* (1986) was first to observe the affect of a related entomopathogenic nematode *Steinernema carpocapsae* on *M. incognita*, and reported suppressed galling on tomato roots. Turf studies have also recorded population control of *Tylenchorhynchus* spp. (Smitley *et al.*, 1992).

The only nematode of economic importance found in Arizona cotton is the root-knot nematode (*M. incognita*). This nematode is found throughout the Cotton Belt and it is estimated that more than 75% of cotton fields are infested (Starr, 1993).

M. incognita is sexually dimorphic, although egg production occurs in the absence of males. Upon hatching, the second stage juvenile (J2) invades a host root and induces a trophic system of giant cells. Cortical cells are also induced to multiply and a characteristic gall forms with the female inside. Eggs are retained within a gelatinous matrix outside the swollen females body and appear as golden droplets on the surface of root galls.

Most damage to the cotton plant results from physiological changes caused by nematodes feeding on root tissues. Nematodes stunt the growth of cotton by reducing the normal flow of water and nutrients from the soil to the developing leaves and bolls. Additionally sugars produced by photosynthesis are diverted from root growing points to nematode induced giant cells and used to sustain the developing nematode.

Materials and Methods

Four, 17.2 acre fields of upland cotton were divided into five research plots of 75 rows each. Two plants in each plot were randomly selected and marked with flagging. Initial pre-treatment 500 ml soil samples, were taken from the base of each flagged plant. Plant parasitic nematodes were extracted using a sugar flotation technique (Byrd *et al.*, 1966). One ml slide samples were used from each soil sample to estimate the number of plant parasitic nematodes.

The crop was sown at 40 inch spacing in sandy loam soil, and irrigated with 0.33 inches water daily, through a commercial subterranean drip system.

Root-knot nematode populations are highest prior to or immediately after the crop reaches maturity (Starr, 1993). Mid-season application of two nematicidal agents were applied to the mature crop, via the drip system, incorporating the treatments into the usual irrigation cycle.

Fields received *S. riobraviv* (biosys, Strain 355) at 1 billion and 2 billion infective juvenile nematodes per acre. Nematodes were mixed with water in a 200 gallon steel tank. From here they were injected into the drip system and directed to the application site through a network of pipes.

Drip lines were positioned with one line in the center of each cotton row, and the drip tapes were buried six inches below the surface. Water temperature in the mixing tank never exceeded 99.3°F. A third field received 0.5 lb a.i. Vydate® L per acre (24% Oxamyl by weight, Du Pont). Finally the control field received a water only treatment, this field was used to monitor normal nematode population dynamics over time.

Entomopathogenic nematode viability assessments were made visually with the aid of a microscope as the nematodes were released from drip outlets. Nematode viability never decreased below 88%.

Throughout the experimental period, ambient temperature did not exceed 111°F, one foot above bare ground. Soil temperature measured at the time of treatment was 85°F, one inch below the soil surface, and 63°F at the point of nematode release (drip outlets).

Sampling

Two x 500ml soil samples were taken from each flagged plant (10 from each field) at seven day intervals, for six weeks. Half the soil was used to estimate the number of plant parasitic nematodes which were extracted and counted as before.

The remaining soil was baited with eight late instar *Galleria 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°F, larvae were collected and washed in distilled water. Larvae were then dissected in 1/4 strength Ringers solution under a stereo dissecting microscope and the number of nematode infected insects recorded.

Results

The field treated with *S. riobraviv* at 1 billion per acre rate shows a typical nematode decline profile (Fig 1) with nematodes persisting throughout the six week experimental period. However, the 2 billion per acre treated field shows fewer nematodes initially present and nematode extinction after 2 weeks.

M. incognita and *Tylenchorhynchus* spp. populations increased in the untreated field relative to initial population levels (Fig 2 & 3). Vydate® L caused an immediate reduction in the population of both genera after one week but by week two, populations began increasing.

Both plant parasitic nematode species were reduced by the application of 1 billion per acre *S. riobraviv*. Because of the poor recovery of *S. riobraviv* in the 2 billion per acre field the effects of a 2 billion rate can not be determined. Other genera of plant parasitic nematodes were monitored but were not present in sufficient numbers to establish any impact.

Discussion

Both *M. incognita* and *Tylenchorhynchus* spp. show increased populations relative to initial sampling levels, in untreated fields. Vydate® L caused an immediate reduction in both nematodes one week after application, but control is lost by the second week. Populations of *M. incognita* in both nematode and Vydate® L fields then increased at the same rate as in untreated soil. *Tylenchorhynchus* spp. increases at higher levels, compared to untreated soil.

M. incognita populations were reduced by 83% by 1 billion *S. riobraviv*. *Tylenchorhynchus* spp. was reduced by 85% one week after treatment. No *Tylenchorhynchus* spp. could be found after five weeks. *Tylenchorhynchus* spp. is an ectoparasitic nematode which feeds on surface root cells, possibly continuous exposure to *S. riobraviv* causes increased sensitivity to nematicidal action.

Several mechanisms of nematicidal activity have been offered to explain how entomopathogenic nematodes could have an affect on plant parasitic nematodes. Various predator/prey interactions may affect nematode populations indiscriminately (Ishibashi & Kondo, 1986; 1987). Both entomopathogenic nematodes and plant parasitic nematodes are attracted to CO₂ produced by plant roots (Bird & Bird, 1986). Entomopathogenic nematodes may physically interfere with root invasion and feeding activities of plant parasitics (Bird & Bird, 1986). Recent studies however, indicate that certain substances produced by the symbiotic bacteria (*Xenorhabdus* spp.) associated with entomopathogenic nematodes, have nematicidal activity (biosys, unpublished data).

The entomopathogenic nematode, *S. riobraviv*, can be applied successfully through subterranean drip. Delivering nematodes through a drip line into the root zone exposes invading parasitic nematodes immediately to the beneficial nematodes, and minimizes nematode loss due to certain abiotic factors such as UV and heat induced desiccation.

Currently there are very few commercially available biological control agents for nematode pests. In 1996 *S. riobraviv* (BioVector®) was introduced for plant parasitic nematode control in Turfgrass. Further studies will be undertaken to discover the feasibility of using a beneficial nematode in cotton crops.

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 Howard Wuertz of Sundance Farms, Arizona, for use of his land and consideration. Thanks to biosys, inc. for supplying *Steinernema riobravris*. Many thanks to Paul Bruns for his assistance.

References

Begley, J.W. 1990. Efficacy against insects in habitats other than soil. In *Entomopathogenic nematodes in biological control*. R., Gaugler, and H.K., Kaya, eds. CRC Press, Boca Raton, FL, p. 215-231.

Bird, A.F., and J. Bird. 1986. Observations on the use of insect parasitic nematodes as means of biological control of root-knot nematodes. *Int. J. Parasitol.* 16(5):511-516.

Byrd, D. W., C. J. Nusbaum, and K. R. Barker. 1966. A rapid flotation-sieving technique for extracting nematodes from soil. *Plant Dis. Rep.* 50:945-957.

Goodell, P. B. 1993. Cotton Nematodes. *In: Cotton Nematodes Your Hidden Enemies*. Beltwide Cotton Nematode Survey and Education Committee. pp. 1-3.

Ishibashi, N., and E. Kondo. 1986. *Steinernema feltiae* (DD-136) and *S. glaseri*: Persistence in soil and bark compost and their influence on native nematodes. *J. Nematol.* 18(3):310-316.

Ishibashi, N., and E. Kondo. 1987. Dynamics of the entomogenous nematode *Steinernema feltiae* applied to soil with and without nematicide treatment. *J. Nematol.* 19(4):404-412.

Klein, M.G. 1990. Efficacy against soil-inhabiting insect pests. In *Entomopathogenic nematodes in biological control*. R., Gaugler, and H.K., Kaya, eds. CRC Press, Boca Raton, FL, p. 195-214.

Smith, K. 1995. Host specificity and regulatory issues. In *Abstracts of the second international symposium on entomopathogenic nematodes and their symbiotic bacteria*, p. 46-48. (October 15-17, 1995. Hawaii Imin International Conference Center, The East-West Center, University of Hawaii at Manoa Campus, Honolulu, Hawaii, USA)

Smitley, D.R., F.W. Warner, and G.W. Bird. 1992. Influence of irrigation and *Heterorhabditis bacteriophora* on plant-parasitic nematodes in turf. *Suppl. J. Nematol.* 24(4):637-641.

Starr, J. L. 1993. Root-knot Nematodes. *In: Cotton Nematodes Your Hidden Enemies*. Beltwide Cotton Nematode Survey and Education Committee. pp. 7-12.

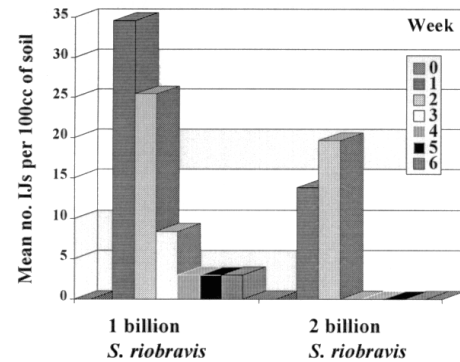


Fig 1. Persistence of *S. riobravris*

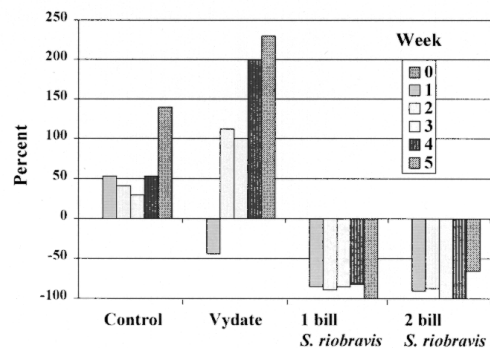


Fig 2. Population of *Tylenchorhynchus* spp. as a percentage increase or decrease relative to the original population level.

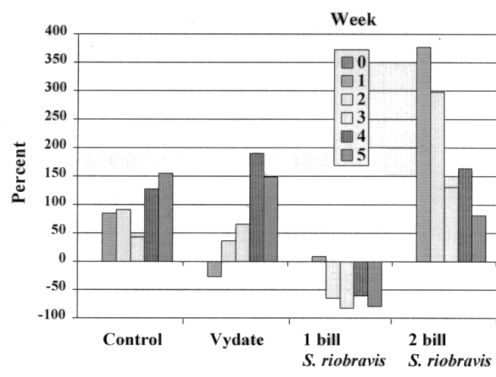


Fig 3. Population of *M. incognita* as a percentage increase or decrease relative to the original population level.