

BIOLOGICAL NEMATOCIDES FOR COTTON PRODUCTION

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Introduction

The goal of this project was to conduct an extensive evaluation of a newly-developed bionematicide for control of root-knot nematodes on cotton. Nematodes are the number one cotton disease problem in Georgia. In 2008, according to Georgia Cooperative Extension Service estimates, plant-parasitic nematodes caused cotton losses equal to 10% of the crop, for a total of \$50 million in direct economic losses, and incurred 82% (\$9.5 million) of the cost of pesticides used for disease control (Langston, D , et. al., 2009). Approximately 69% of the cotton fields in Georgia have root-knot nematodes (Kemerait, R., 2005). Plant-parasitic nematodes typically have a scattered, or patchy, distribution across farms and production areas, so the actual losses experienced by growers vary widely from the overall estimates.

Available control methods for nematodes are limited, and current management practices rely heavily on one or two nematicides that were developed in the 1960's. These older products present serious challenges in terms of cost and issues with human and environmental health concerns. There is a critical need for new nematicides with entirely new chemistries for control of plant-parasitic nematodes. Several new nematode-killing products have been derived in my lab from a group of soil-inhabiting microbes called fungi. This project was designed to demonstrate efficacy of one of the new products on a more extensive basis, using replicated field trials across the major cotton producing areas in Georgia.

Materials and Methods

As part of our ongoing effort to develop new nematicidal chemistries, fermentation products from selected fungal cultures have been tested for the presence of nematicidal compounds through a series of lab, greenhouse, and field trials. In this process, fungal cultures were isolated from various environments by dilution-plating and use of selective growth media. Using this procedure, thousands of isolates of fungi have been obtained from different fields and environments. The resulting fungi were then evaluated for production of nematicidal compounds. To obtain the products for testing, each fungal isolate was placed in flasks containing nutrient agar and fermented with aeration on platform shakers for 21 days. To test for evidence of nematicidal activity, the liquid cultures were micro-filtered (0.22 μm) and pipetted into sterile microwell plates with freshly-hatched Southern root-knot nematode (*Meloidogyne incognita*) juveniles. The micro-filtering removed all viable stages of the fungus, and left only the products of the fermentation. Sterile water was used as a control treatment. Nematode survival rates

were determined at 2, 4, 24, and 48 hours after suspension, with 6 replications per isolate. During the same time the in-vitro assay was performed, liquid fungal-culture filtrates were also applied to a sterile soil mix in 6" greenhouse pots. Control treatments of sterile water, and a filtrate of the nutrient agar used for fermentation were also applied. Southern root-knot nematode (*M. incognita*) eggs were added to the pots, and cotton cv. DP555 was planted in each pot to serve as a susceptible host. Each treatment was applied to 6 replications. Plants were grown on greenhouse benches for 45 days. Plant roots were then removed from the pots and washed, and the nematode eggs were collected and counted. Total numbers of nematode eggs were compared using ANOVA followed by mean separation (LSD) for each fungal-isolate treatment and the controls. After mass screening of the fungal collection, a few isolates were selected for further evaluation using additional research protocols. The methods used to prepare products for field trials were similar to the methods used for greenhouse screening, but with much larger quantities required. Using these methods, we identified a group of promising fungal products for nematode control.

During the 2009 growing season, an advanced-stage bionematicide (GA534) from our lab was selected for an extensive field evaluation in four different locations across Georgia. Research sites were located at UGA research facilities in Decatur, Tift, Burke, and Oconee counties (Fig.1). These sites were chosen to be broadly representative of soils and production areas for cotton in Georgia. Identical research designs were implemented at all of the sites, with 10 replications per treatment at each of the sites. More than 250 plots were used in the studies. The large extent of the research design required large inputs of the experimental fermentation product. The rates applied to the field plots were upscaled from rates that were shown to be effective in greenhouse pot studies. In excess of 300 gallons of the experimental product were fermented in our limited lab facilities during the first 3 months of 2009 in order to complete the project as planned. At the beginning of the experiment, the research plots were inoculated with root-knot nematodes and planted with cotton DP555 B/RR. The experimental treatments, consisting of the experimental bionematicide (GA534), a media control that was fermented with a fungus that does not produce any nematicidal compounds, and a water control, were applied to 10 replicate plots for each treatment at each site. Root-knot nematodes (juveniles+eggs) were assayed 5 times during the growing season, including a pre-planting assay to determine existing levels of nematode infestation. At each nematode assay date, 10-15 soil cores were removed from the root zones of cotton plants in each plot. Individual soil cores from each plot were mixed before processing. Nematodes were collected from the soil samples by elutriation and centrifugation. Nematodes were then counted under a stereomicroscope. Root fragments from each of the soil samples were caught on a sieve during elutriation. Root-knot nematode eggs were collected from the root fragments using a sodium hypochlorite extraction method. Data were reported as combined juvenile + egg counts. Nematode counts from each assay date were also averaged across dates for a growing season estimate of root-knot nematode population densities in each plot. All of the research plots were managed as appropriate for the growing area and were irrigated as

needed. Cotton was harvested from individual plots at maturity. Total numbers of nematodes at each assay date and cotton yields were compared using ANOVA followed by mean separation (LSD, $p=0.05$) for each treatment and the controls.

Results and Discussion

There were no significant interactions between experiment location and treatments during the 2009 growing season, so data from all 4 sites were combined for analysis. In combined data, the experimental bionematicide from fungal isolate GA534 significantly ($p<0.05$) decreased the numbers of root-knot nematodes in soil assays over a time period that extended from approximately 40 days after planting (mid to late June) until 100 days after planting (late August to early September) (Table 1). In the first reading, taken 40 days after planting, root-knot numbers were reduced by 55% in the plots treated with GA534, as compared to the water controls. By 60 days after planting, plots treated with GA534 had 40% lower root-knot nematode counts than the controls. At 100 days after planting, the reduction was still significant at 25%. Control of root-knot nematodes is most important during the early phase of cotton growth, and that is when the product was most effective. However, the extended control of root-knot nematodes late into the growing season was a bonus for this experimental product. Long-term reductions in nematode population densities from an at-plant application are historically not typical of nematicides currently on the market. Often, nematode counts drop soon after application of a nematicide then resurge to numbers higher than the untreated controls by the end of the season. At harvest, there were no treatment differences in the nematode population densities. This lack of treatment effects at harvest is often observed after the cotton plants have been defoliated and the nematodes have ceased feeding. The root-knot nematode population densities averaged across all assay dates were significantly reduced by 24% after application of GA534, as compared to water controls. Cotton yields were increased by 8% (approximately 55 lbs lint/acre) in plots treated with the experimental bionematicide GA534. The results of the 2009 growing season were very encouraging for commercial development of GA534 as a new bionematicide for cotton. The product was effective across all four of the research sites in Georgia, which had different soils and weather conditions. The long-term nematode control and increased yields observed in this research after application of a relatively unimproved fungal culture derivative demonstrated a high potential for this product. Increased efficacy can be anticipated when production is further refined in a commercial environment.

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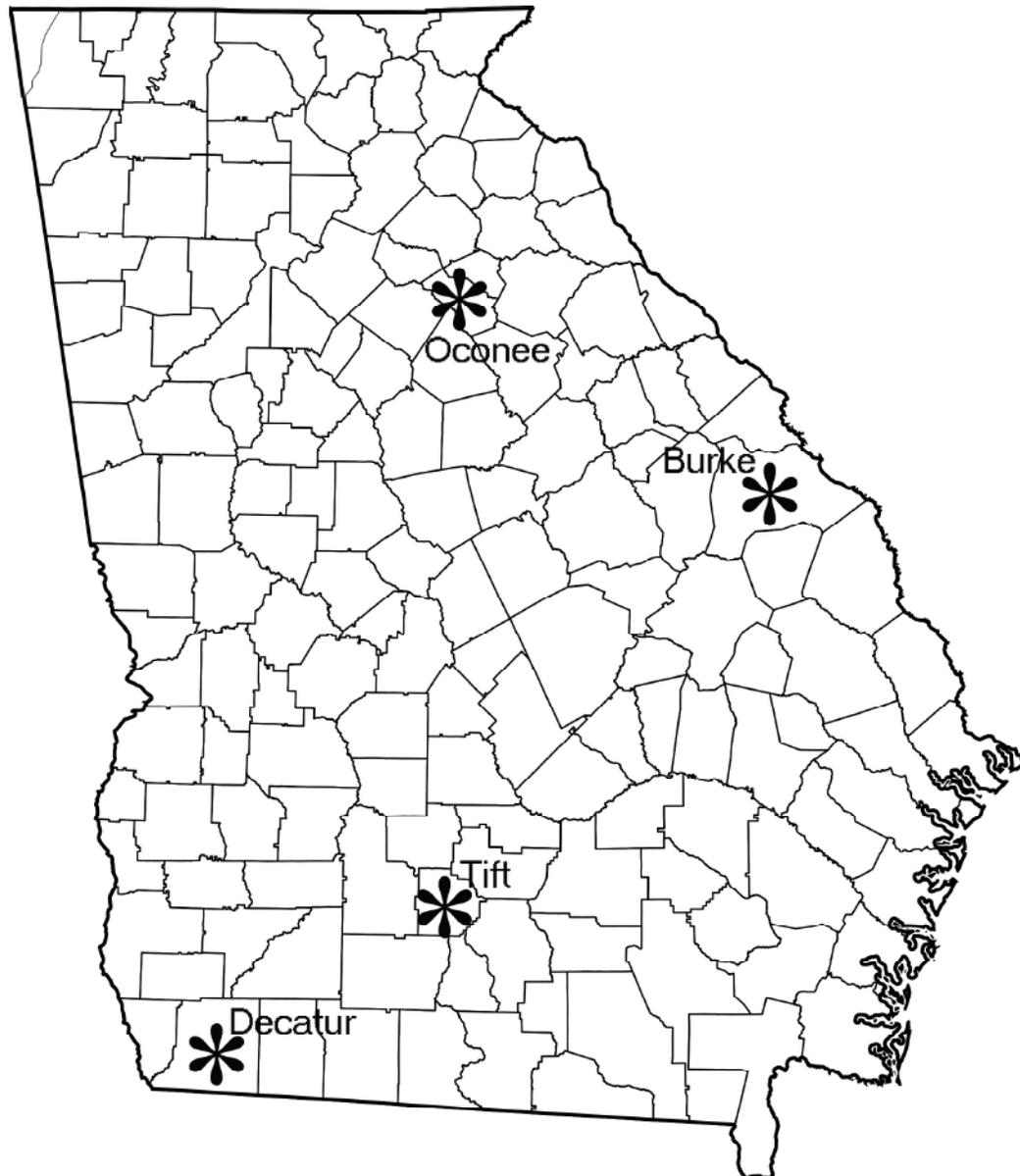


Figure 1. Location of research sites for 2009 evaluation of GA534 bionematicide. Research plots were located at the Attapulgus Research & Education Center in Decatur County, on a research facility operated by the Tifton campus, CAES-UGA, in Tift County, at the Southeast Georgia Research & Education Center in Burke County, and at the CAES Plant Sciences Farm in Oconee County. These sites were chosen to be broadly representative of soils and production areas for cotton in Georgia.

Table 1. Evaluation of an experimental bionematicide (GA534) for control of root-knot nematodes (*Meloidogyne incognita*) on Cotton DP 555 B/RR using plots combined across four research sites in Georgia. Research sites were located at UGA research stations in Decatur, Tift, Burke, and Oconee counties.

Treatment	Number of root-knot nematodes (juveniles+eggs)/ 100 cm ³ soil Nematode assay date				2009 Season average	Cotton yield lbs lint/acre
	40 DAP**	60 DAP	100 DAP	Harvest		
GA534	534 b*	1,110 b	2,636 b	827 a	1,650 b	732 a
Media*** control	1,106 a	1,436 ab	3,878 a	822 a	2,277 a	695 ab
Water control	1,185 a	1,856 a	3,530 a	822 a	2,162 a	677 b

* Means of 40 replicate plots combined for 4 research sites. Rows with different letters are significantly different (P=0.05). Nematode count data were transformed log₁₀(x+1) for analysis.

** Days after planting.

*** Fungal fermentation media control. A fungal isolate that does not produce nematicidal compounds incubated in the same fermentation media as GA534.

Literature Cited

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