FUNGAL FERMENTATION PRODUCTS FOR CONTROL OF ROOT-KNOT NEMATODES

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Introduction

Nematodes are the number one disease problem in Georgia on cotton. In 2007, according to the University of Georgia Cooperative Extension estimates, plant-parasitic nematodes caused crop losses equal to 8% of the crop, for a total of $50.2 million in direct economic losses, and incurred 86% of the cost of pesticides used for disease control (Martinez, A., et. al., 2008). Approximately 69% of cotton fields in Georgia have root-knot nematodes (Kemerait, R., 2005) and there are several other species of parasitic nematodes that have been found in other fields. Plant-parasitic nematodes typically have a scattered or patchy distribution across farms and production areas, so actual losses experienced by growers are likely to vary widely from overall estimates.

The goal of this project is to identify and develop biologically-based nematicidal products. At this time, growers rely mainly on Temik (aldicarb) and Telone (1-3 dichloropropene) for pesticide control of nematodes in cotton. More options, that are both cost-effective and more environmentally acceptable, are needed for growers. Biologically-based nematicides are more targeted against nematodes and are less hazardous to the environment than traditional chemistries. We anticipate that the use of new biologically-based nematicides may also enhance consumer acceptance of the resulting cotton products for both fiber and feed.

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 evaluated for production of nematicidal compounds.

To obtain the products to be tested, 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 (*M. 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. At the same time that the in-vitro assay was performed, liquid fungal-culture filtrates were 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. 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.

During the 2008 project, we continued the field evaluations of several fungal isolates. Additionally, promising products were selected from the results of greenhouse trials done in 2007 for field evaluation as dehydrated-powdered products. After fermentation of the fungal isolates, filtrates were allowed to air-dry and the resulting material was applied to field plots. Two advanced-stage fungal isolates were evaluated for a second year in field plots during the 2008 growing season. The tests were identical for the two year span of this trial and data were combined for presentation in this report.

The objective of this study was to evaluate the practical effectiveness of fungal products that had shown activity in the greenhouse by studying them over an entire growing season in the field. Three fungal isolates (two nematicidal candidates, and a fungal control with no nematicidal activity) were fermented in quantities sufficient to treat soil in small field plots at rates equivalent to those used in greenhouse studies. The research plots were located at the Attapulgus Research and Education Center. At the beginning of the experiment, the plots were inoculated with root-knot nematodes and planted with cotton DP555. The fungal treatments, along with a water control, were applied to 12 replicate plots for each treatment in 2008, and 16 plots for each treatment in 2007. Root-knot nematodes (juveniles+eggs) were assayed during the growing season, and cotton was harvested at maturity. The same trial that was conducted at Attapulgus for two years was replicated at the Plant Science Farm in Oconee County for the first time in 2008 using the methods already described. This same protocol was also used to evaluate the dry-formulations of the fungal products with 10 replicate plots for each treatment at the Attapulgus Research and Education Center.

Results and Discussion

In combined data from the 2007 and 2008 growing seasons collected from field plots located at the Attapulgus Research and Education Center, soil application of culture filtrates from fungal isolate GA534 significantly ($p<0.05$) decreased the numbers of root-knot nematodes in soil assays over a time period that extended into late August (Table 1). Root-knot nematode numbers were reduced by 74% in the plots treated with GA534, as compared to the water controls, 60 days after planting. By 120 days after planting, plots treated with GA534 had 44% lower root-knot nematode counts than the controls. There were no treatment differences at harvest. This lack of treatment effect

at harvest is often observed after defoliation of the cotton plants when the nematodes have ceased feeding. The isolates GA630 and GA516 did not provide a significant reduction in nematode counts at any of the assay dates when compared to the water control. Although root-knot nematode population densities were reduced by application of GA534, significant differences in cotton yields were not observed among the treatments during the 2007 or 2008 growing season. However, the extended control of root-knot nematodes late into the growing season was a bonus for this experimental product. Long-term reduction of nematode population densities from at-plant application is not typical of nematicides currently on the market. Oftentimes, nematode counts drop soon after application of a nematicide and then resurge to numbers similar to or higher than the untreated controls by the end of the season.

Also during the 2008 growing season, a similar test was conducted at the Plant Science Farm in Oconee County for the first time. The root-knot nematode numbers were very low in the newly-developed test site and a significant difference among treatments was observed only at 120 days after planting (Table 2). The very low nematode population densities at the beginning of the season provided little information, but as the season progressed and root-knot nematodes increased in number, GA534 again proved effective in lowering the nematode counts in cotton. No differences in cotton yields were observed among the treatments. Even so efficacy for GA534 in the different soil environment found in the Piedmont area was observed, but this trial will need to be repeated with higher population densities of root-knot nematodes.

A third experiment was conducted at the Attapulgus Research and Education Center during 2008 to evaluate dry-formulations of the same products that were used in the liquid-fermentation studies. There were no significant differences among the treatments at any of the nematode assay dates in this study (data not shown). Greenhouse studies had shown efficacy for GA534 and GA630 when applied after drying, but effective rate and application methods in the field studies for the dry formulations have not been developed. This is an essential component for the commercialization potential of biologically-derived nematicidal products since marketing and distribution of a product would probably require a dried product. We will continue to develop and test methods for dry formulations in future field tests.

Acknowledgments

This work was supported in part by the Georgia Cotton Commission and Cotton Incorporated.

Table 1. Evaluation of fungal culture filtrates for control of root-knot nematodes (*M. incognita*) on Cotton DP 555 in plots located at Attapulgus Research and Education Center for 2007 and 2008 growing seasons combined.

Fungal isolate	Number of root-knot nematodes (juveniles+eggs)/100 cm^3 soil			
	Nematode assay date			
	60 DAP*	90 DAP	120 DAP	Harvest
Ga516	1150 ab**	5562 ab	742 ab	418 a
Ga534	417 b	4122 b	516 b	401 a
Ga630	697 ab	5292 ab	760 ab	348 a
Control	1593 a	7676 a	914 a	440 a

* Days after planting.

** Means of 28 replicate plots over 2 years. Rows with different letters are significantly different (P=0.05). Data were transformed log10(x+1) for analysis. Antilogs are presented for comparison.

Table 2. Evaluation of fungal culture filtrates for control of root-knot nematodes (*M. incognita*) on Cotton DP 555 in plots located at the Plant Science Research Farm, Oconee County, GA for the 2008 growing season.

Fungal isolate	Number of root-knot nematodes (juveniles+eggs)/ 100 cm^3 soil			
	Nematode assay date			
	60 DAP*	90 DAP	120 DAP	Harvest
Ga516	76 a**	31 a	35 a	146 a
Ga534	14 a	5 ab	3 b	10 a
Ga630	11 a	3 b	130 a	75 a
Control	9 a	8 ab	66 a	40 a

* Days after planting.
** Means of 10 replicate plots. Rows with different letters are significantly different (P=0.05). Data were transformed log10(x+1) for analysis. Antilogs are presented for comparison.

Literature Cited

Kemerait, R. 2005. Cotton Disease and Nematode Management. Pp. 30-39 in *2005 Georgia Cotton Production Guide*, Cooperative Extension Service, University of Georgia. Pub. CSS-05-01.

Martinez, A. , 2008. 2007 Georgia plant disease loss estimates. Cooperative Extension Service, Univ. of Georgia College of Ag. and Env. Sciences. Pub. SB41-10.