FUNGAL FERMENTATION PRODUCTS FOR CONTROL OF ROOT-KNOT NEMATODES

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Introduction

Nematodes are a major constraint on cotton production in Georgia. As we move into a more competitive global environment for marketing cotton products, the total cost of production per acre becomes an increasingly critical factor the ability of our growers to compete. Plant-parasitic nematodes are a major factor in the net cost of production for cotton grown in Georgia. Results from a recent survey of cotton fields in Georgia showed that 69% of the sampled fields had root-knot nematodes (Kemerait, R., 2005). In 2005, according to Georgia Cooperative Extension Service estimates, plant-parasitic nematodes caused $72 million in crop losses on cotton, and incurred 81% of the cost of pesticides used for disease control (Martinez, A. , et. al., 2005). Although average damage levels due to nematodes may average 10% on cotton, these losses are not evenly distributed, and growers with problem fields are experiencing much higher levels of crop loss.

Options for management of nematodes in cotton are limited. The development of new nematode management options is a key factor in offering more choices to growers, and increasing the competition among nematode-control marketers. Commercially-acceptable cotton cultivars that are resistant to nematodes are not yet available, and breeding of new resistant cultivars is proceeding slowly. Chemical control of nematodes on cotton relies mainly on Temik (aldicarb), and Telone (1-3 dichloropropene). The use of traditional chemical pesticides for control of nematodes is both expensive and hazardous. The primary emphasis of this project is the development of novel nematicidal compounds derived from microbial culture filtrates. These nematicides are more targeted against nematodes and are less hazardous to the environment than traditional pest-control chemicals. Our hypothesis is that the effective use of new biologically-based nematicides can significantly reduce production costs and enhance consumer acceptance of the resulting cotton products, both for fiber and feed.

Materials and Methods

The search for bioactive compounds begins with the collection of soil samples from locations in Georgia with differing soil types and habitats. Soilborne fungi are then isolated from these samples by dilution-plating and use of selective growth media. Using this procedure, thousands of isolates of fungi are obtained. Candidate fungi are then selected from these collections and evaluated for production of nematicidal compounds. For evaluation, each fungus is placed in flasks containing nutrient agar

and fermented with aeration on platform shakers for 10 days. As an in-vitro assay, liquid cultures are micro-filtered (0.22 µm) and pipetted into sterile microwell plates with freshly-hatched Southern root-knot nematode (*Meloidogyne incognita*) juveniles. Sterile water is used as a control treatment. Nematode survival rates are determined at 2, 4, 24, and 48 hours after suspension, with 6 replications per isolate. At the same time that the in-vitro assay is performed, liquid fungal-culture filtrates are 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 are also applied. Southern root-knot nematode (*M. incognita*) eggs are added to the pots, and cotton cv. DP555 is planted in each pot to serve as a susceptible host. Each treatment is applied to 6 replications. Plants are grown on greenhouse benches for 45 days. Plant roots are then removed from the pots and washed, and the nematode eggs are collected and counted. Total numbers of nematode eggs are 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 are selected and further evaluation using additional evaluation protocols. The methods used are similar to the greenhouse screening, but with different soil types, culture media, and fermentation protocols.

During the 2006 project, several advanced-stage fungal isolates were selected for a first trial in field plots. The objective of this study was to evaluate the efficacy of the fungal products over an entire growing season in the field. The four selected fungal isolates were fermented in quantities sufficient to treat the soil in small-scale, containerized field plots at rates equivalent to those used in greenhouse studies. Plots located at the CAES Plant Science Farm in Oconee County were inoculated with root-knot nematodes and planted with cotton DP555. The fungal treatments, along with a water control, were applied to 10 replicate plots each. Root-knot nematodes were assayed 8 times during the growing season, and cotton was harvested at maturity.

Results and Discussion

During 2006, four fungal isolates were selected for evaluation in a replicated field trial, to determine season-long efficacy. Soil applications of two of the isolates, Isolate C, and Isolate D, decreased the numbers of root-knot nematodes in soil assays that extended into early September (Table 1). Although nematode population densities were reduced by several of the treatments, significant increases in cotton yields were not observed for any of the treatments. Further research in the greenhouse has shown that the control obtained with these culture filtrates can be increased by improving the fermentation and application procedures. If a product resulting from this project exhibited extended control of root-knot nematodes in the field, it would be a valuable tool for protecting the current crop, and could also provide carry-over benefits to subsequent crops. These studies need to be repeated, and eventually could be scaled up to larger treatment areas, if large-scale fermentation facilities were available.

Additional work in greenhouse studies during 2006 were directed primarily toward optimization of the fermentation and application protocols for several promising fungal isolates. A complete factorial design experiment was conducted to examine combinations of fermentation duration and application timing for fungal Isolate B. By optimization of these two factors, efficacy of the culture filtrates in controlling root-knot nematodes on cotton was increased by 57%. The effects of specific fermentation media constituents, and fermentation temperatures on the nematicidal activity of culture filtrates were also examined in greenhouse experiments. We continue to observe variability in nematode control results from the soil-treatment evaluations, but the degree of variability has been reduced by continued research on fermentation and application protocols. This is a key area of our research, because the reduction in variability is essential in the commercial acceptance of any nematode control product. The goal of this project is to provide a commercially viable product for use by growers in nematode control.

Acknowledgments

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Table 1. Evaluation of fungal culture filtrates for control of root-knot nematodes (*Meloidogyne incognita*) on Cotton DP 555 in field plots.

Fungal isolate	Number of root-knot nematode juveniles/ 100 cm^3 soil					Yield seed cotton lbs/ A
	Nematode assay date					
	17 Jul	14 Aug	28 Aug	12 Sep	15 Oct	
Isolate A	88 a[a]	249 a	534 ab	895 a	531 a	1,411 ab
Isolate B	63 ab	274 a	221 b	810 a	658 a	1,386 ab
Isolate C	20 b	105 b	242 b	335 b	628 a	1,428 ab
Isolate D	24 b	229 ab	284 b	333 ab	284 a	1,240 ab
Control	146 a	248 a	683 a	727 a	785 a	1,224 b
Nematode control[b]	--	--	--	--	--	1,659 a

[a]Means of 10 replicate plots. Rows with the same letters within a column are not significantly different (P=0.05).

[b]No nematode inoculum added - control for level of nematode damage.

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. , 2006. 2005 Georgia plant disease loss estimates. Cooperative Extension Service, Univ. of Georgia College of Ag. and Env. Sciences. Pub. SB41-08.