# FUNGAL FERMENTATION PRODUCTS FOR CONTROL OF ROOT-KNOT NEMATODES

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## Introduction

Plant-parasitic nematodes are serious pests in most Georgia cotton production areas. Results from a survey of cotton fields in Georgia showed that 69% of the sampled fields had root-knot nematodes (Kemerait, 2005). Other nematode species that attack cotton, such as reniform and Columbia lance nematodes, can be found in other fields in Georgia, so it is safe to say that most growers have to deal with nematode problems at some level. In 2006, according to Georgia Cooperative Extension Service estimates, plant-parasitic nematodes caused crop losses equal to 10% of the crop, for a total of $74.5 million in direct economic losses, and incurred 86% of the cost of pesticides used for disease control (Martinez et al., 2007). Plant-parasitic nematodes typically have a scattered, or patchy distribution across farms and production areas, so the actual losses experienced by growers may vary widely from the overall estimates.

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

## Materials and Methods

Fermentation products from selected fungal cultures were tested for nematicidal compounds through a series of lab, greenhouse, and field trials. Initially, candidate fungi are isolated from various environments by dilution-plating and use of selective growth media. Using this procedure, thousands of isolates of fungi have been obtained. Fungal isolates are then selected from the collection 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 $\mu$m) 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 RR) 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 for further evaluation using additional research protocols. The methods used are similar to the greenhouse screening, but with different treatments applied. During the 2007 project, several fungal isolates were selected for evaluation as dehydrated-powdered products. After fermentation of the fungal isolates, filtrates were allowed to air-dry and the resulting material was applied to greenhouse pots as already described. Liquid media from the same fermentation batches were reserved and applied at the same time as the dehydrated products, for comparison.

During the 2007 growing season, advanced-stage fungal isolates were evaluated in field plots. 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 were fermented in quantities sufficient to treat soil in small field plots at rates equivalent to those used in greenhouse studies. Research plots were located at the Attapulgus Research and Education Center. Plots were planted with cotton DP555 RR on 30 April 2007. The fungal treatments, along with a water control, were applied to 16 replicate plots each. Root-knot nematodes (juveniles+eggs) were assayed during the growing season, and cotton was harvested at maturity.

## Results and Discussion

In cotton field plots located at the Attapulgus Research and Education Center, soil application of culture filtrates from fungal isolate Ga534 significantly decreased the numbers of root-knot nematodes in soil assays over a time period that extended into late August (Table 1). In the first reading, taken on 2 July 2007, root-knot numbers were reduced by 85% in the plots treated with GA534, as compared to the controls. By 30 August 2007, plots treated with Ga534 still had 40% lower root-knot nematode counts than the controls. At the end of the growing season (10 October 2007) there were no treatment differences. Although root-knot nematode population densities were reduced by application of GA534, significant differences in cotton yields were not observed during the 2007 growing season. The extended control of root-knot nematodes late into the growing season was not expected, and such a long-lasting impact from an at-plant application is not typical of nematicides currently on the market. Usually, nematode counts drop soon after application of a nematicide, then resurge to numbers higher than the untreated controls by the end of the season. This longer-lasting impact could 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 scaled up to larger treatment areas. Our program is currently limited in scale by the amount of product that can be fermented in our laboratory.

**Table 1.** Evaluation of fungal culture filtrates for control of root-knot nematodes (*Meloidogyne incognita*) on Cotton (DP 555 RR) in plots located at Attapulgus Research and Education Center .

| Fungal isolate | Number of root-knot nematodes (juveniles+eggs)/ 100 $cm^3$ soil | | | |
|---|---|---|---|---|
| | Nematode assay date | | | |
| | 2 Jul 07 | 30 Jul 07 | 30 Aug 07 | 4 Oct 07 |
| Ga516 | 460 ab* | 6,060 a | 981 a | 489 a |
| Ga534 | 115 b | 3,154 b | 645 b | 523 a |
| Ga630 | 293 ab | 5020 ab | 977 a | 437 a |
| Control | 756 a | 7,511 a | 1,068 a | 500 a |

*Means of 16 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.

Greenhouse studies during 2007 were directed toward evaluation of dehydrated fungal fermentation products for control of root-knot nematodes. Complete factorial design experiments were done to compare liquid and dehydrated-powdered culture filtrates of selected fungal isolates. These studies showed that the culture filtrates from Ga534 and Ga630 performed equally well in control of root-knot nematodes on cotton whether in liquid or powder form. These results are an essential finding in our evaluation of the commercialization potential of the biologically-derived nematicidal products, since marketing and distribution of a product would probably require a dried product.

## Acknowledgments

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

## Literature Cited

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