FUNGAL FERMENTATION PRODUCTS FOR CONTROL OF ROOT-KNOT NEMATODES

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Abstract

Nematodes are a continuing threat to cotton production in Georgia, and only a few management tools are available to control crop losses due to nematodes. The primary goal of this project during calendar-year 2004 was to search for new nematode-killing chemicals produced by a group of soil-inhabiting microbes called fungi. Soil samples were collected from cotton fields in Coffee, Colquit, and Mitchell counties, and several thousand species of fungi were isolated from the soil. From these cultures, 250 isolates of different fungal species were selected and evaluated for production of nematode-killing chemicals. Through extensive laboratory and greenhouse screenings, 10-15 fungal isolates were identified that produce nematicidal compounds. Several of these fungal-derived products have consistently reduced root-knot nematode numbers by as much as 68%, compared to untreated controls. The most promising fungal products were also evaluated with different application methods. Of particular importance was the successful use of heat-killed and dried fermentation residues for soil applications, as necessary steps towards the commercialization of new nematode-control products.

Introduction

In 2003, according to Georgia Cooperative Extension Service estimates, plant-parasitic nematodes caused $89 million in crop losses on cotton, and incurred 80% of the cost of pesticides used for disease control (Williams-Woodward, J., et. al., 2004). Nematode problems are increasing yearly in Georgia cotton production areas. A recent nematode survey in Georgia showed that 25 % of the cotton fields sampled were infested with root-knot nematodes at levels that would be damaging to cotton (R. C. Kemerait, unpublished). Several species of nematodes are responsible for losses on cotton, including the Southern root-knot nematode and the reniform nematode. Although average damage levels due to nematodes may be in the 10 % range, these losses are not evenly distributed, and growers with problem fields are experiencing much higher levels of crop loss. Our research on cotton in Georgia has indicated that cotton yield losses due to nematodes may be as high as 60-70% in fields infested with root-knot or reniform (Noe, 1994, 1998), Total crop failures are possible with extreme pest pressures. Populations of these parasitic nematodes may increase 200-300% per year under cotton. Chemical control methods rely primarily on only two nematicides, Telone, and Temik, which are under constant regulatory challenge.

A primary emphasis in our program has been the development of new management tactics for control of nematodes in cotton. Practices being evaluated for nematode control in cotton include applications of soil amendments, such as chicken manures and

litters, more efficient use of nematicides, and use of novel nematicidal compounds derived from microbial culture filtrates. Field and greenhouse studies in our cotton research program have demonstrated that treatment of soil with organic amendments, including poultry manures, may reduce nematode numbers, and limit subsequent crop losses (Kaplan & Noe, 1992, 1993, Riegel & Noe, 1996, Noe, 1998). The suppression of nematodes obtained by treatment with poultry manures may be increased significantly by inoculation the litter or manures with specific fungi that act as biocontrol agents for plant-parasitic nematodes (Kaplan et al., 1991, Riegel & Noe, 2000). We have isolated and identified a number of promising biocontrol fungi that will colonize poultry waste efficiently. Other species of fungi have been isolated from Georgia cotton fields, where there was evidence of natural suppression of root-knot nematodes. Effects of microbial culture factors on the production of nematicidal compounds by selected fungi are also being determined. In this project, we isolated and identified new sources of nematode biocontrol fungi, and investigated application methods to enhance nematode control. Our hypothesis is that the effective use of cultural and biological management practices can reduce much of the economic damage due to nematodes incurred year after year in Georgia.

Materials and Methods

The primary component of this project for the 2004 calendar year was to search for new sources of biologically-based nematicides. Soil samples were collected from cotton fields in Coffee, Colquit, and Mitchell counties. Soilborne fungi were isolated from these samples by dilution-plating and use of selective growth media. Several thousand isolates of fungi were recovered, from which approximately 250 isolates were selected for further evaluation as producers of nematicidal compounds. For evaluation, each fungus was placed in flasks containing nutrient agar and fermented with aeration on platform shakers for 10 days. As an in-vitro assay, liquid cultures were micro-filtered (0.22 μm) and pipetted into sterile microwell plates with freshly-hatched Southern rootknot nematode (*M. incognita*) juveniles. Sterile water was used as a control treatment. Nematode survival rates were determined at 2, 4, 24, and 48 hours after suspension. Liquid fungal-culture filtrates also 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 rootknot nematode (*M. incognita*) eggs were added to the pots, and cotton cv. DPL5415 RR was planted in each pot to serve as a susceptible host. 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 for each fungal-isolate treatment and the controls. After mass screening of the fungal collection, isolates were selected and further evaluated for biocidal production using different evaluation protocols.

Results and Discussion

After preliminary selection from the several thousand fungal isolates that were recovered from soil samples, approximately 250 fungi were further evaluated for

production of nematicidal compounds. Of the 250 that were screened in the laboratory tests, approximately 100 showed some nematicidal activity, and were taken to the second phase of evaluation in greenhouse pots. Of these 100 fungal isolates, 10-15 isolates showed enough root-knot nematode control when applied to soil planted with cotton to warrant their selection for phase 3 evaluation and testing. This final phase of testing includes repeated greenhouse screens, evaluation of rates and application methods for nematode control, and a determination of the efficacy of heat-killed and dried fermentation residues for nematode control in soil. In replicated greenhouse trials with application of autoclaved, filtered fermentation extracts, averaged over 3 separate trials, the most effective fungal fermentation products reduced root-knot nematode numbers by 63-74% compared to untreated controls. Further, the reproductive rates of the nematodes in the most effective treatments were 0.5-0.7(indicating fewer nematodes than were present at planting), 42 days after planting, compared to a nematode reproductive rate of 1.9 in the controls (indicating nearly twice as many nematodes as were present at planting). Different rates of application, with and without serial dilutions, are also being used to determine LD50 rates for biologically active culture filtrates. We continue to observe variability in nematode control results from the soil-treatment evaluations, and development of a final product is slowed by the need to evaluate various protocols for stabilizing the nematicidal activity of selected fungal isolates.

Acknowledgments

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