

# FUNGAL ANTAGONISTS OF *ROTYLENCHULUS RENIFORMIS*

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## Abstract

The reniform nematode (*Rotylenchulus reniformis*) was collected from a field in continuous cotton production and examined for fungal antagonists. Ten fungi were isolated from the vermiform and adult stages of the reniform nematode. *Acremonium sp.*, *Arthrographis sp.*, *Chaetomium aureum*, *Fusarium equiseti*, *Pseudorobillarda sp.*, and 5 unknown genera were isolated. *Acremonium sp.* and *Arthrographis sp.* occurred in the highest frequency. In greenhouse pathogenicity tests, *Arthrographis sp.*, *Pseudorobillarda sp.*, *Fusarium equiseti*, MSA1 and MSA5 significantly reduced reniform nematode population development on cotton.

## Introduction

The reniform nematode (*Rotylenchulus reniformis*) is the most serious pest to cotton production in the southern United States. This nematode is believed to be spreading and currently considered an economical pathogen of cotton in 10 of the 16 cotton producing states. In 1999, yield losses were estimated to be as high as 8% with an average yield loss of 1.8% over the entire cotton production region. Approximately 350,000 bales of cotton valued at 80 million dollars are considered lost to the reniform nematode.

Reniform nematode management techniques that are available to cotton producers are limited. Without cotton varieties with resistance to the reniform nematode, most producers must rely on the use of crop rotation with non-host crops or nematicides. Nematicides are currently the most widely used method for nematode management. However, the expense of applying nematicides and the market value of non-host crops is not consistently economical. Thus alternative management tactics are being considered.

Fungal antagonists associated with plant-pathogenic nematodes have been reported. Numerous studies of fungi colonizing nematodes including the soybean cyst (*Heterodera glycines*), root-knot (*Meloidogyne spp.*), ring (*Criconebella xenoplex*), and lesion (*Pratylenchus penetrans*) have been conducted. A taxonomically diverse mycoflora has been identified. Information about fungi associated with *Rotylenchulus reniformis* however, is limited. Therefore the objective of this study was to examine the species and frequency of fungi colonizing vermiform stages of the reniform nematode.

## Materials and Methods

Soil samples were collected from a field located at Anguilla, MS in March of 2000. The field had been continuously cropped in cotton. Twenty-five soil cores, 1-inch diameter and 8-inches deep were collected in a randomized systematic sampling pattern across the field. From half the soil, nematodes were extracted using gravity screening and sucrose centrifugation techniques. The remaining soil was placed in the greenhouse and planted with cotton. Reniform were extracted from the greenhouse later in the season in the same manor.

Reniform nematodes were enumerated and examined under the dissecting microscope. Vermiform and female individuals which appeared to be colonized by fungi were placed in a drop of sterile water. These reniform were then surface sterilized in 95% ethanol followed by 0.10% sodium hypochlorite. Surface sterilized reniform were then aseptically plated on Potato Dextrose Agar (PDA) amended with 100 ppm of streptomycin and 50 ppm chlortetracycline. Plates were allowed to incubate at room temperature for 5 to 7 days. Fungi were subcultured for identification. The frequency of colonization of the reniform nematodes was recorded.

## Results and Discussion

An average population density of 12,832 reniform nematodes per 500 cm<sup>3</sup> was recovered from the cotton field.

When viewed under the dissecting microscope, hypha strands were visible within the vermiform and female reniform nematodes. Fungal fruiting structures were observed erupting through the vulva, anus and buccal cavity when examined under the light microscope at 400x (Figure 1).

Fungi isolated from vermiform reniform nematodes included *Acremonium sp.*, *Arthrographis sp.*, *Chaetomium aureum*, *Fusarium equiseti*, *Pseudorobillarda sp.*, and 5 unknown genera (Table 1). *Acremonium sp.*, *Arthrographis sp.* were the fungi isolated at the highest frequency at a rate of 3% from the visibly colonized reniform selected for culturing. Frequency of colonization by the other fungi was at 1%. Of the total reniform population extracted from the soil, approximately 0.015% of reniform were colonized by fungi.

Five of the isolated fungi were then examined for their ability to infect the reniform nematode in greenhouse pathogenicity tests. The fungal isolates were combined with the reniform nematode in sterile soil. Results from these tests indicate that reniform nematode numbers were significantly reduced by all the fungal isolates examined (Table 2). In addition the numbers of free-living nematodes were increased in the reniform plus fungal isolates treatments as compared to the reniform alone treatment.

Preliminary results from the study demonstrate that fungi do exist in nature that are capable of parasitizing the reniform nematode. The ability of these fungi to be used as an alternative management tactic is currently being examined.

Table 1. Fungal antagonists isolated from *Rotylenchulus reniformis*.

Fungus	Isolation frequency
<i>Acremonium sp.</i>	3%
<i>Arthrographis sp.</i>	3%
<i>Chaetomium aureum</i>	1%
<i>Fusarium equiseti</i>	1%
<i>Pseudorabdilla sp.</i>	1%
Unknown species	8%

Table 2. Effect of fungal antagonists of *Rotylenchulus reniformis* on nematode population development in the greenhouse.

Fungus	<i>Rotylenchulus reniformis</i> per 500 cc of soil	Free living nematodes
<i>Pseudorabdilla sp.</i>	8,951 b	17,062 b
<i>Arthrographis sp.</i>	8,934 b	36,739 ab
<i>Fusarium equiseti</i>	8,108 b	36,120 ab
MSA1	14,575 b	41,280 a
MSA5	13,856 b	43,344 a
Control	34,572 a	23,804 ab
LSD (0.05)	3,682	7,158