ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH THE RENIFORM NEMATODE (ROTYLENCHULUS RENIFORMIS)

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Abstract

Reniform nematode (*Rotylenchulus reniformis*) from aged cotton greenhouse plants and three different counties in Alabama (Escambia, Limestone, and Baldwin) were extracted and observed under the stereoscope. Nematodes and eggs with dark coloration and hyphal rings and mycelium were cultured on 1.5% of water agar supplemented with antibiotics. Identified fungi associated with reniform nematode and frequencies of isolation were: *Arthrobotrys dactyloides* (49%), *Paecilomyces lilacinus* (15%). *Penicillium waksmanii* (3.8%), *Phoma exigua* (3.8%), *Aspergillus glaucus* group (2.5%), *Cladosporium cladiosporioides* (2.5%), *Cladosporium herbarum* (2.5%), *Fusarium oxysporum* (2.5%), *Torula herbarum* (2.5%), and *Dactylaria brachophaga* (2.5%). *A. fumigatus*, and unidentified basidiomycete were less frequent (1.25%). A high percentage (17.5%) of colonized nematodes did not produce a fungal culture on the media. Only *A. dactyloides*, *P. lilacinus, F. oxysporum,* and *D. brachophaga* have been previously reported as nematophagous fungi in other nematode species.

Introduction

The reniform nematode (*Rotylenchulus reniformis*) is a serious problem on cotton (*Gossypium hirsutum*) in the United States, especially in Alabama, Mississippi and Louisiana, where 8.5% (89,204 bales), 9.0% (225,000 bales), and 5% (66,286 bales) respectively, of the total cotton production is lost to this nematode (Blasingame *et al*., 2007). Currently, there are no resistant cotton cultivars to *R. reniformis* (Weaver *et al*., 2007; Usery *et al.* 2005). Management is based on the use of nematicides and crop rotation with corn (*Zea maydis*), peanut (*Arachis hypogaea*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*) and resistant cultivars of soybean (*Glycine max*) (Wang *et al.,* 2004; Davis *et al.*, 2003). Integrating biological control agents in nematode management strategies is an area of interest (Jatala, 1986). Numerous studies of fungi colonizing nematodes including the soybean cyst (*Heterodera glycines*), root-knot (*Meloidogyne* spp.), ring (*Criconemella xenoplex*), and lesion (*Pratylenchus penetrans*) and reniform nematodes have been conducted (McLean *et al.*, 2001; Kim and Riggs, 1991;Carries and Glawe, 1989).

Biological control of *R. reniformis* in cotton has been studied by Walters and Barker, 1994, they test the efficacy of *Paecilomyces lilacinus* in *R. reniformis* eggs, and found significantly reduction on numbers of *R. reniformis* nematodes, but the fungal isolates of the studies originated from eggs of root knot not from *R. reniformis*. In greenhouse pathogenicity tests, McLean *et al*., 2001 found that *Arthrographis* sp., *Pseudorobillarda* sp., and *Fusarium equiseti* significantly reduced *R. reniformis* population development on cotton. Wang *et al.,* 2004 found between 17 – 21% of eggs parasitized by isolates of fungi named as ARF (Arkansas Fungus). Furthermore, they reported isolates of *Pochonia chlamydospora* reduced the *R. reniformis* numbers on cotton roots or in soil after a single application of 5000 clamydospores per gram of soil (Wang *et al.*, 2005).

We hypothesize that the different developmental stages in the life cycle of *R. reniformis* are sensitive to wide spectrum or specialized antagonists and there are fungal pathogens of this nematode that have not been reported. The objective of this study was to search for nematophagous fungi associated with *R. reniformis*.

Materials and Methods

Rotylenchulus reniformis nematodes were collected from cotton plants cultured at the Plant Science Research Center of the Alabama Agricultural Experiment Station on the campus of Auburn University. The greenhouse samples were stock nematode cultures. We have observed continuous culture of cotton has built populations of antagonists against this nematode in our greenhouse cultures (Lawrence personal observation). These nematodes were cultured in 15 cm diameter polystyrene pot, inoculated with 2000 eggs/pot approximately 17 -18 months. *Rotylenchulus reniformis* nematodes samples were also collected in naturally infested cotton fields across the state to determine what fungal parasite may be naturally colonizing the nematodes. Nematodes from samples of four different counties of Alabama (Escambia, Henry, Limestone, and Baldwin) were collected. In the field sites, approximately 1000 cm^3 of soil between the plants were collected to a depth of 15 cm. Vermiform life stages and eggs were extracted by combined gravity screening a centrifugation-flotation method.

Nematode samples were observed under the stereoscope (Nikon SMZ645). Vermiform nematodes colonized with mycelia or discolored eggs, were tacitly removed from the counting dish and placed in a syracuse dish and rinsed with sterile water. Colonized nematodes were cultured on 1.5% water agar supplemented with 12.5 mg of chlortetracycline HCl and 300 mg of streptomycin sulfate per liter (Kim and Riggs, 1994). Cultures were incubated at 27˚C for identified after 5 days or subcultures for future identification. Fungal growth from the eggs and vermiform nematodes were transferred to a Potato Dextrose Agar (PDAS) culture plates supplemented with 300 mg/liter of streptomycin sulfate to establish pure cultures.

Identification of the nematophagous fungi was based on the morphological characters of conidiophores and conidia. For some fungi it was necessary to induce sporulation on water agar media (WA) plus 20 *R. reniformis* juveniles per dish. Sporulation was also induced by exposing fungal cultures on a black light lamp (Model X-15B 115 volts 60Hz).

Results

The fungi that we found associated with vermiform life stages of *R. reniformis* and frequency of isolation from the greenhouse cultures were *Arthrobotrys dactyloides* (49%), *Paecilomyces lilacinus* (12.5%), *Penicillium waksmanii* (9.7%), *Aspergillus glaucus* Group (6.5%), and *Penicillium herbarum* (3.2%). No fungal growth was observed in 19.4% of the visually colonized, cultured, reniform nematodes. *Paecilomyces lilacinus* (50%), *Phoma exigua* (12.5%), *Cladosporium cladosporioides* (6.3%), *Fusarium oxysporum* (6.3%), *Torula herbarum* (6.3%), and an unidentified basidiomycete (6.3%), were cultured and isolated from eggs of *R. reniformis*. No fungal cultures were found in 12.5% of the discolored eggs (Figure 1).

From the Belle Mina field location, only *Phoma exigua* (25%) was associated with vermiform stages of *R. reniformis,* however, 75 % of the colonized vermiform nematodes did not growth on media. The Henry field was colonized with *Arthrobotrys dactyloides* (95.8%), and *Dactylaria brachophaga* (4.2%). Both are known nematode trapping fungi. Finally, in the Fairhope location, *Arthrobotrys dactyloides* (20%) was also isolated along with *Aspergillus fumigatus* (20%). As seen previously, 60% of the nematodes colonized did not grow on culture medium (Figure 2).

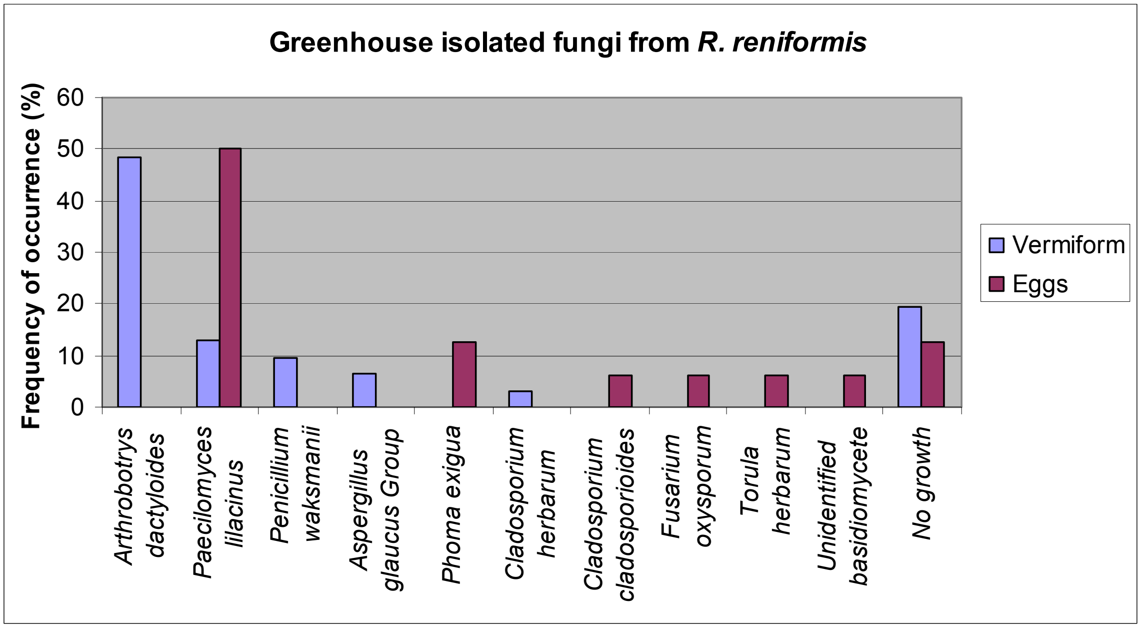

Figure 1. Greenhouse isolated fungi from eggs and vermiform life stages from *R. reniformis*.

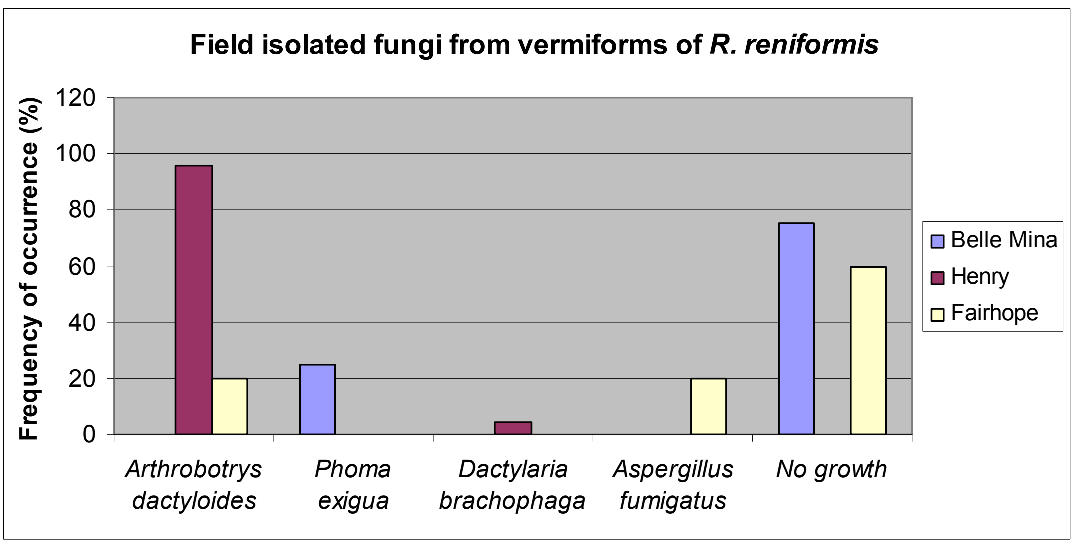

Figure 2. Field isolated fungi from vermiform life stages of *R. reniformis*.

The most frequent isolated fungus from *R. reniformis* was *A. dactyloides* (49%) and *P. lilacinus* (15%). *Penicillium waksmanii* and *P. exigua* were isolated from 3.8% of the nematodes, followed by *Aspergillus glaucus* group (2.5%), and *C. cladiosporioides*, *P. herbarum*, *F. oxysporum*, *T. herbarum*, *D. brachophaga*, *A. fumigatus*, and unidentified basidiomycete were less frequent (1.25%). A high percentage (17.5%) of colonized nematodes did not produce a fungal culture on the media (Figure 3).

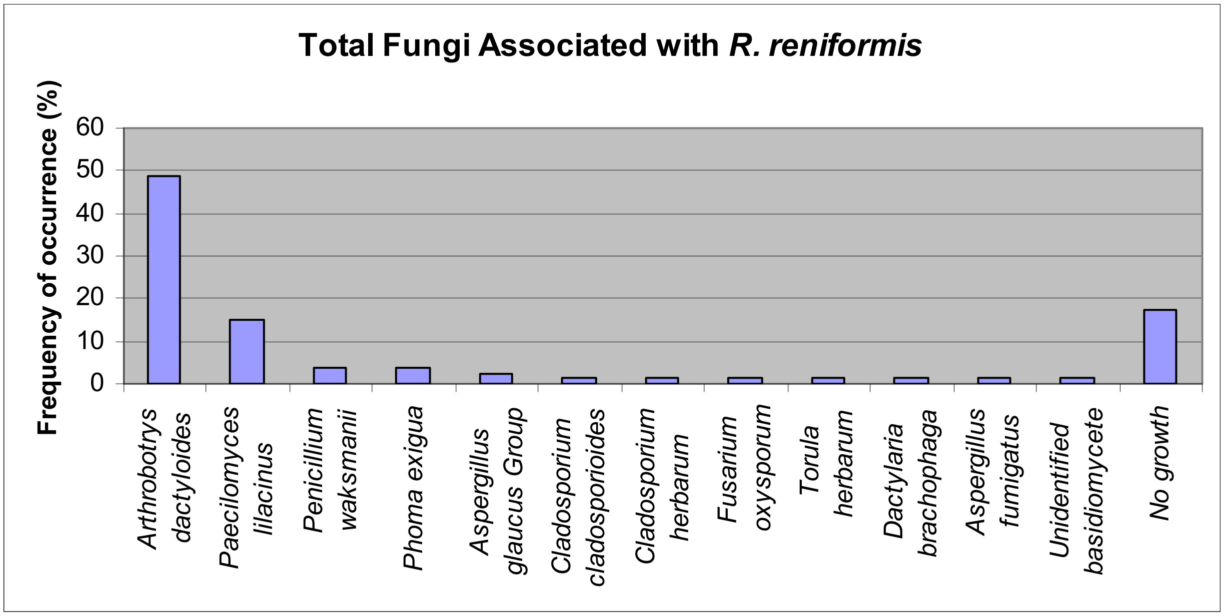

Figure 3. Total fungi associated with *R. reniformis.*

Arthrobotrys dactyloides is a nematode trapping fungus producing organs of capture are rings which were 20 to 32 µm diam, and composed of 3 individual cells (Drechsler, 1937). *Dactylaria brachophag* is also a nematode trapping fungus producing rings of 20 to 35µ in diameter (Drechsler, 1937), however, the conidia are cylindrical and divided in 4 cells by 3 septa. In pure and nematode infested cultures, *D. brachophag* has been intimated and shows a general parallelism with *Arthrobotrys dactyloides*, however the more abundant septation of its conidia is the feature most decisively distinguishing it as a species (Drechsler, 1937). *Paecilomyces lilacinus* produced erect conidiophores with flask shaped phialides and oval to ellipsoidal and smooth shaped conidia (Domsch *et al.*, 1980). *Fusarium oxysporum* produces macro and micro conidia produced on branched or unbranched monophialides. Single celled microconidia are abundant, oval to kidney shapped, and produced in false heads. Macroconidia are sickle shaped with an attenuated apical cell and foot shaped basal cell (Nelson *et al.,* 1983).

Discussion

Arthrobotrys dactyloides, *Dactylaria brachophaga*, *Paecilomyces lilacinus*, and *Fusarium oxysporum* have been reported as pathogens on other nematode species (Kiewnick and Sikora, 2006; Stirling and Smith, 1998; Freitas *et al*., 1995). *Arthrobotrys dactyloides* and *D. brachophaga* has been reported reducing populations of *Meloidogyne graminicola* on rice (Singh *et al.*, 2007). These two fungi have a great potential as a biological control agent of juveniles stages. In the Alabama samples the most predominant was *A. dactyloides.* We isolate this fungus from two counties (Henry and Baldwin) and the greenhouse. *Dactylaria brachophaga* is closely related to *A. dactyloides* and was only identified in Henry County.

Paecilomyces lilacinus is typically a soilborne fungus and it's common in the rhizosphere of a number of plants. This fungus has been reported on *R. reniformis* on tomato plants, reducing the population in greenhouse and microplot trials (Walters and Barker, 1994). This fungus in the field but was very predominant on our greenhouse reniform culture plants.

Fusarium oxysporum has been previously reported to be destructive on eggs of the SCN in Alabama soybean fields (Morgan-Jones & Rodriguez-Kabana, 1981). There are some biotypes capable of penetrating eggs and cause disorders on the embryonic development through enzymatic and/or toxic effects (Morgan-Jones & Rodriguez-Kabana, 1988). It has never been reported on *R. reniformis*, and it is necessary to know if this fungus is pathogenic to the reniform nematode.

It's important to continue with more studies related to *in vitro* pathogenicity tests and greenhouse trials, to evaluate the potential of these fungi against *R. reniformis.* These fungi have good potential of possible biological control agents. They are present in two different stages of the nematode life cycle, eggs and juveniles. Thus these fungi could potentially reduce populations of the nematode eggs and vermiform life stages.

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