EFFICACY OF *ARTHROBOTRYS DACTYLOIDES, DACTYLARIA BROCHOPAGA, FUSARIUM OXYSPORUM*, AND *PAECILOMYCES LILACINUS* FOR BIOCONTROL OF RENIFORM NEMATODE (*ROTYLENCHULUS RENIFORMIS*).

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Abstract

Six fungal isolates, *Arthrobotrys dactyloides* (isolates *BW-A, GH-A, and HN-A*), *Dactylaria brochopaga*, *Paecilomyces lilacinus*, and *Fusarium oxysporum* were evaluated as potential biocontrol agents for the reniform nematode (*Rotylenchulus reniformis*). The six fungal isolates were increased on wheat, oat, or corn meal and examined for nemaphagous activity in two types of soil (autoclaved, and natural). Cotton ST 5599BR was cultivated in 500 cm³ pots and 1% (v/v) of fungal inoculum was added per pot. Plants were also inoculated with 3,000 vermiform life stages of *R. reniformis*/500 cm³ of soil. The test was placed in a factorial arrangement of a RCBD and allowed to grow in the greenhouse for 60 days. At harvest, plant height, shoot and root mass, number of *R. reniformis* nematodes in the soil, and number of eggs per gram of root were recorded. In autoclaved soil, *GH-A. dactyloides, HN-A. dactyloides, D. brochopaga*, and *P. lilacinus* reduced ($P \leq 0.05$) the total number of *R. reniformins* life stages in soil. *Paecilomyces lilacinus* and *F. oxysporum* reduced the number of eggs in the root. In natural soil, *BW-A. dactyloides* and *F. oxysporum* reduced ($P \leq 0.05$) the number of *R. reniformis* life stages in the soil; however, the number of eggs per gram of root was similar between all isolates and the controls. No phyto toxicity was observed for any fungal isolate. Although the six fungal strains reduced the number of *R. reniformis* life stages in autoclaved soil, our results indicate these isolates are capable of reducing nematode numbers but are not competing adequately in natural soils.

Introduction

The reniform nematode (*Rotylenchulus reniformis*) is a serious economic problem for cotton production in the southeastern United States. Of the total cotton production in Alabama, Mississippi, and Louisiana, 8.5%, 9%, and 4%, respectively, is lost due to this nematode. This loss represents a value of $9,000,000 to $34,200,000 per year (Blasingame et al., 2008). Currently, management is based on the use of chemical nematicides because there are no resistant cotton cultivars to the reniform nematode (Usery et al., 2005; Weaver et al., 2007). Crop rotation with corn and soybeans reduces the reniform populations but the economic cost of this practice is not feasible for a grower (Davis et al., 2003; Gazaway et al., 2007). There is an economic need to reduce the impact of the reniform nematode on cotton crops, hence other management options such as biological control need to be explored. Biological control has not been implemented in cotton crops in the United States but it certainly is an option for management in the future (Robinson, 2007).

Recent studies report that the reniform nematode has been colonized by nematophagous fungi and strains of bacteria at different life stages (Wang et al., 2005; Jayakumar et al., 2003; Walters and Barker, 1994; Kim and Riggs, 1991). *Paecilomyces lilacinus*, *Pochonia chlamydospora* and an unidentified fungus named Arkansas Fungus (ARF) have been documented as parasites of the egg stage of *R. reniformis* (Wang et al., 2004). The vermiform life stages have been colonized by the fungi *Arthrographis* sp., *Pseudorobillarda* sp., and *Fusarium equiseti* and all were found to significantly reduce the nematode population on cotton (McLean et al., 2001).

In Alabama cotton crops, *Arthrobotrys dactyloides* (3 strains), *Dactylaria brochopaga*, *Paecilomyces lilacinus*, and *Fusarium oxysporum* have been isolated from *R. reniformis* (Castillo et al., 2008). *Arthrobotrys dactyloides* and *D. brochopaga* were previously reported reducing populations of *Meloidogyne graminicola* in rice (Singh et al., 2007).

These two fungi produce constricting rings that trap the vermiform nematode. *Paecilomyces lilacinus* has been shown to reduce populations of *R. reniformis* nematodes in tomato plants by up to 36% at harvest season (Walters and Barker, 1994). Isolates of *P. lilacinus* penetrate the egg shell mechanically by production of appressoria (Lopez-Llorca et al., 2008). Toxins produced by *F. oxysporum* have been reported damaging eggs of *Heterodera glycines*, and *Meloidogyne arenaria* (Morgan-Jones & Rodriguez-Kabana, 1981; Freitas et al., 1995; Kiewnick and Sikora, 2006).

The objective of this study is to evaluate the biocontrol potential of *Arthrobotrys dactyloides*, *Dactylaria brochopaga*, *Paecilomyces lilacinus*, and *Fusarium oxysporum* isolated from *R. reniformis* in Alabama against *R. reniformis* in cotton plants, with and without natural competition in autoclaved and natural soil, when increased on three different types of carriers (corn meal, wheat, and oat).

Materials and Methods

Inoculum preparation

Three strains of *Arthrobotrys dactyloides* (*BW-A. dactyloides, GH-A. dactyloides, HN-A. dactyloides*), *Dactylaria brochopaga*, *Paecilomyces lilacinus*, and *Fusarium oxysporum* were isolated from *R. reniformis* nematodes found in cotton crops across Alabama. The fungal isolates were cultured for 7 days on water agar (WA). In a 250 ml conical flask, 150 cm^3 of the carrier (oat, corn meal, and wheat) was added, and moistened with 100 ml of tap water. Embedded seed were autoclaved twice at 121 C and 103.4 kPa for 30 minutes on two consecutive days. Two 5 mm fungal disks cuts from the periphery of 7-day-old WA cultures were aseptically transferred to each flask. Fungal cultures were increased in a growth chamber at 27 °C for 30 days and shaken daily to distribute the fungi evenly.

Inoculation and extraction

The fungal inoculum was adjusted to a 1% (v/v) ratio in the 500 cm^3 polystyrene pots filled with either autoclaved or natural soil. Three cotton ST 5599BR seeds were planted in each pot. Additionally, 3000 *R. reniformis* vermiform life stages and eggs were added to each pot at planting. All pots were grown in the greenhouse bench for 60 days. At harvest, plant height, fresh and dry shoot weight, and fresh and dry root weight were recorded. The *R. reniformis* vermiform life stages were extracted from the soil by combined gravity screening and centrifugation-flotation method. Eggs were extracted from the fresh root systems using the NaOCl method. Vermiform life stages and eggs were counted under an inverted TS100 Nikon microscope.

Statistical analysis

The experimental design was a randomized complete block design (RCBD) with five replications. Treatment design was a factorial arrangement of a RCBD with five replications. The six fungal isolates and two controls were increased on three types of fungal carriers (oat, wheat, or cornmeal), placed in two types of soil (natural or autoclaved), and in the presence or absence of the nematode. One control was the carrier without the fungi, and the absolute control no fungal carrier was used at all. The entire experiment was repeated twice, for a total of 960 experimental units. Data were analyzed in SAS 9.1 (SAS Institute Inc.) using PROC GLIMMIX, and means were compared by a Dunnett's test at the ($P \leq 0.05$) level of significance.

Results

Phytopathogenicity was not observed for any fungal treatment. Plant shoot mass was not affected by the fungal isolates, soil type (autoclaved or natural) the presence or absence of the nematode, or the oat, wheat, or cornmeal carrier ($P<0.0744$) (Figure 1). No interactions were observed between the fungal, carrier, soil, and nematode factors. However, the interaction between soil, nematode and carrier was significant ($P<0.006$) for the root mass produced. The root systems produced were heavier in natural soils than in autoclaved soils, and carrier effect was more pronounced in natural soil. In the presence of the nematode, the root mass of plants exposed to the oat carrier were

greater than the wheat and corn meal treatments in the autoclaved or natural soils (Figure 2). Plants grown in natural soil were taller than the ones from autoclaved soil. There was no carrier effect with soil and nematode combinations (Figure 3).

The total number of *R. reniformis* nematodes in the soil was lower in all six fungal isolate treatments compared to the two controls ($P<0.001$) in the autoclaved soil (Table 1). The total number of *R. reniformis* eggs extracted from the roots was also lower in the six fungal isolate treatments compared with the no carrier control. However, the isolates of *F. oxysporum* ($P<0.003$), and *P. lilacinus* ($P<0.007$) reduced reniform populations when compared to the carrier control (Table 2). Similar responses were observed for the numbers of eggs and vermiform life stages of *R. reniformis* per gram of root. The fungal isolates reduced populations in the autoclaved soil compared to the no carrier control ($P<0.001$) (Table 3).

Reductions in numbers of *R. reniformis* were not observed in the natural soil. The fungal isolates did not reduce the total reniform nematode numbers in the root, nor the eggs and vermiform life stages per gram of root in the natural soil.

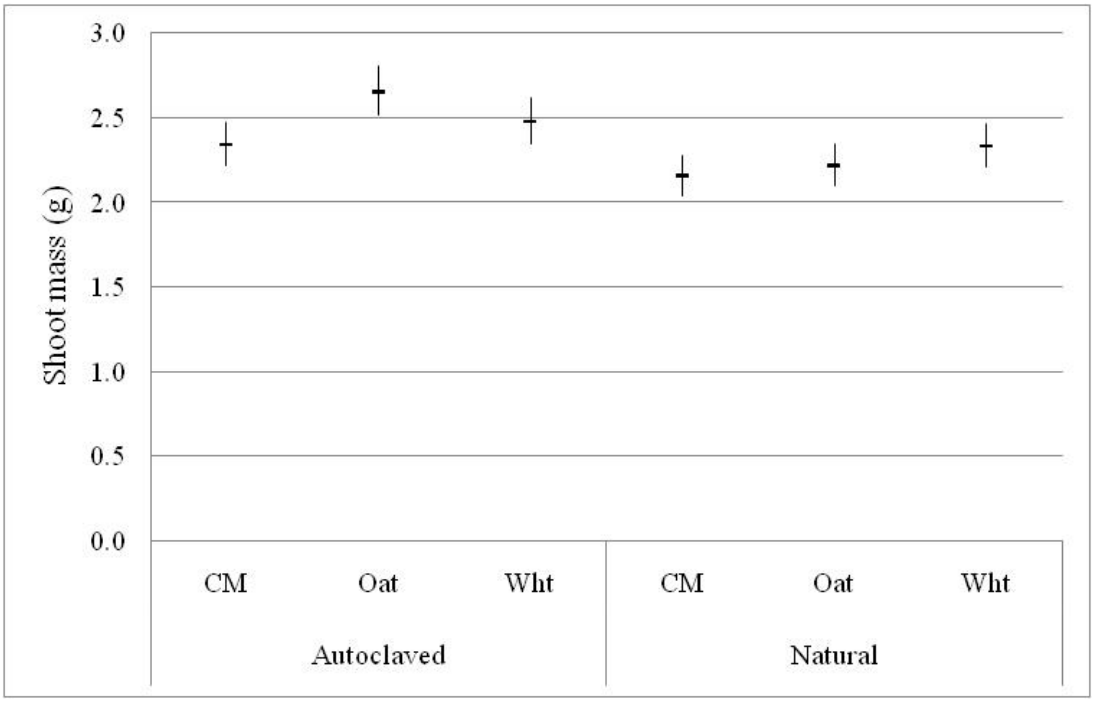

Figure 1. Cotton shoot mass (g) using corn meal, oat, and wheat as fungal carriers with autoclaved and natural soils.

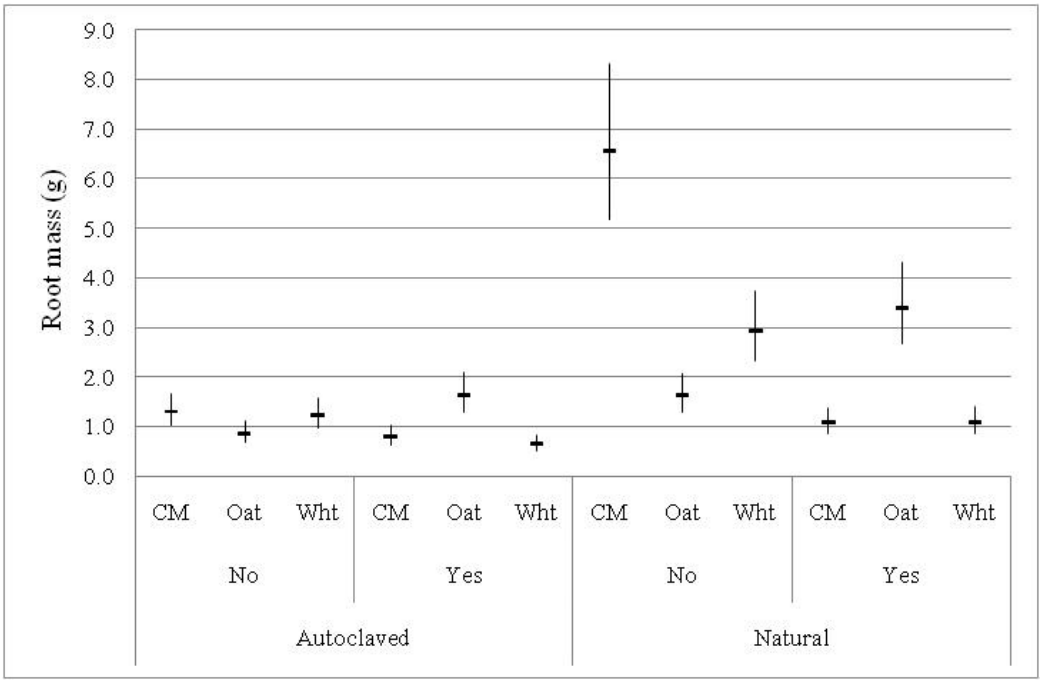

Figure 2. Cotton root mass (g) using corn meal, oat, and wheat as fungal carriers, in autoclaved and natural soils, and with the presence and absence of the nematode.

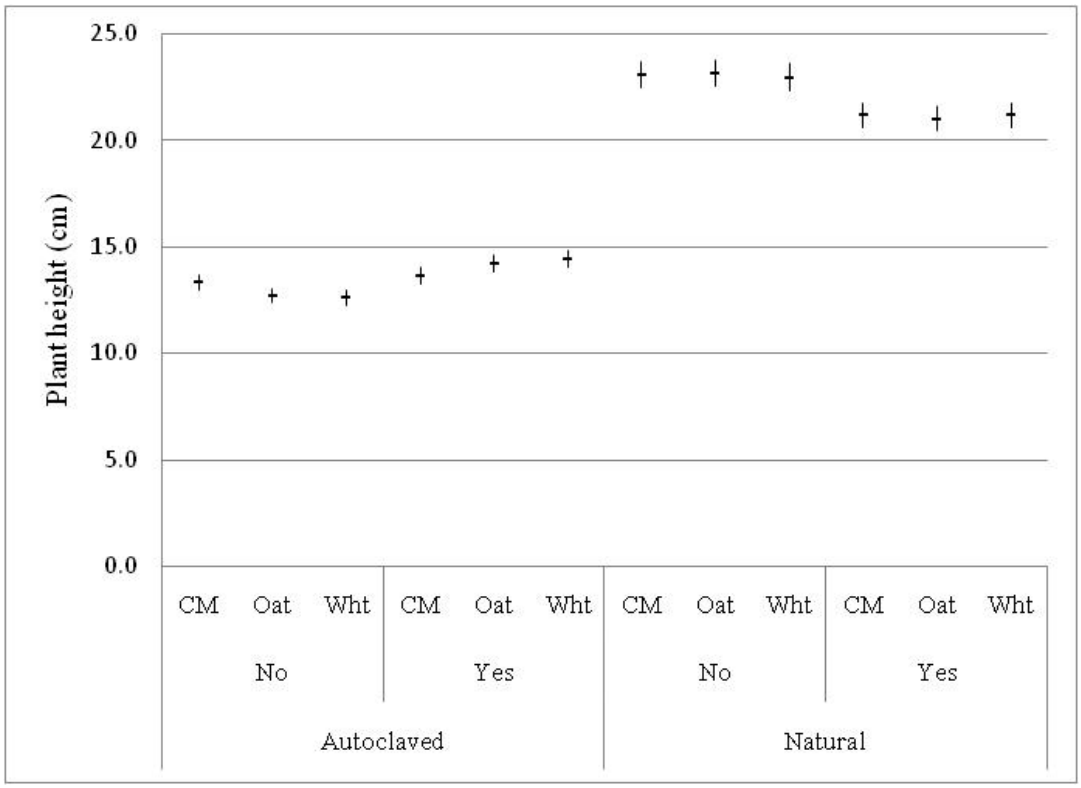

Figure 3. Cotton height (cm) using corn meal, oat, and wheat as fungal carriers, in autoclaved and natural soils, and with the presence and absence of the nematode.

Table 1. Reniform population in natural and autoclaved soils.

Fungi	Autoclaved			Natural		
	R. reniformis/ 150 cm³	Dunnett P vs.		*R. reniformis*/ 150 cm³	Dunnett P vs.	
		No Seed	Seed		No Seed	Seed
No carrier control	4851			2138		
Carrier control	5539			2107		
BW-A. dactyloides	1726	**0.0001**	**0.0001**	1138	**0.0039**	**0.0051**
GH-A.dactyloides	2495	**0.0022**	**0.0002**	1492	0.2001	0.2350
HN-A.dactyloides	1726	**0.0002**	**0.0001**	1138	0.3823	0.4357
D. brochopaga	2495	**0.0018**	**0.0001**	1492	0.9695	0.9845
F.oxysporum	2702	**0.0085**	**0.0008**	1228	**0.0140**	**0.0177**
P. lilacinus	2036	**0.0001**	**0.0001**	1373	0.0721	0.0876

Table 2. Number of eggs and juveniles in cotton roots.

Fungi	Autoclaved			Natural		
	Eggs and Juveniles / Root	Dunnett P vs.		Eggs and Juveniles / Root	Dunnett P vs.	
		No Seed	Seed		No Seed	Seed
No carrier control	5028			1659		
Carrier control	3797			1772		
BW- A. dactyloides	2498	**0.002**	0.131	1388	0.874	0.637
GH - A. dactyloides	2779	**0.013**	0.384	1436	0.950	0.766
HN- A. dactyloides	2683	**0.007**	0.278	1881	0.975	1.000
D. brochopaga	2534	**0.003**	0.153	1192	0.327	0.168
F. oxysporum	1923	**0.001**	**0.003**	1455	0.969	0.814
P. lilacinus	2019	**0.001**	**0.007**	1411	0.916	0.701

Table 3. Number of eggs and juveniles in cotton roots.

Fungi	Autoclaved			Natural		
	Eggs and juveniles / gr of root	Dunnett P vs.		Eggs and juveniles / gr of root	Dunnett P vs.	
		No Seed	Seed		No Seed	Seed
No carrier control	474.9			117.3		
Carrier control	304.2			123.4		
BW- A. dactyloides	178.4	**0.0001**	0.0526	101.6	0.9672	0.8698
GH - A. dactyloides	222.8	**0.0023**	0.4697	91.9	0.7097	0.5258
HN- A. dactyloides	213.7	**0.0012**	0.3400	139.3	0.9224	0.9867
D. brochopaga	182.4	**0.0001**	0.0685	84.0	0.3956	0.2571
F. oxysporum	186.8	**0.0001**	0.0900	92.3	0.7240	0.5400
P. lilacinus	179.8	**0.0001**	0.0576	95.3	0.8316	0.6585

Discussion

The six fungal isolates reduce *R. reniformis* nematode populations by an average of 50% in the autoclaved soil. Elimination of other competitors in the autoclaved soil permit the fungal isolates to established in the soil and reduce *R. reniformis* nematode numbers. In natural soil, the isolates: *GH-A. dactyloides, HN-A. dactyloides, D. brochopaga,* and *P.lilacinus* did not reduce *R. reniformis* populations. This suggests that the fungal isolates attack *R. reniformis*, but are weak competitors in a natural soil environment. In contrast, *F. oxysporum* and *BW-A. dactyloides* reduced reniform populations in both types of soil.

Fusarium oxysporum and *P. lilacinus* reduced the number of vermiform life stages and eggs in the root in autoclaved soil. This confirms previous reports of *P. lilacinus* parasitizing eggs of reniform nematode, and *F. oxysporum* colonoizing eggs of *Heterodera glycines* in Alabama soybean fields (Morgan-Jones & Rodriguez-Kabana, 1988). None of the six isolate tested indicated nematode population reductions in natural soil.

Using wheat, oat, or corn meal as carriers of the fungi does not affect the plant growth in the presence or absence of the nematode. When the nematode is present, oat carrier has an increased effect on root mass in both autoclaved and natural soils. In natural soil, plants were taller, and with a heavier root mass than in autoclaved soil. Plant height and root mass were reduced by the nematode damage in natural soil.

More studies on the biology of these fungi have to be conducted. All fungal strains show to be pathogenic to reniform nematode but at the same time low ability to compete with other microorganism in the soil. It is necessary to improve formulation process, so when the fungi is released into the natural soil the carrier will give it an advantage to be successful in competition with other soil microorganisms. Also, a mix of fungal strains with different modes of action has to be evaluated to improve the control of the reniform nematode during different life stages.

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