

IDENTIFICATION OF FUNGI ASSOCIATED WITH *ROTYLENCHULUS RENIFORMIS*

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Abstract: The objective of this work was to isolate and identify fungi associated with *R. reniformis* in cotton roots. Soil samples were collected in cotton fields naturally infested with *R. reniformis* and from cotton stock plants cultured in the greenhouse. Nematodes extracted from the soil were observed under the stereoscope, and discolored eggs and vermiform stages colonized with mycelia were cultured on 1.5% water agar supplemented with antibiotics, and incubated at 27°C. Identification of the nematophagous fungi was based on the morphological characters, and the ITS regions and 5.8S rDNA amplified by PCR using the primers ITS1 and ITS4. The parasitism percentage on vermiform nematodes from greenhouse samples was 21.2%, and the percentages from cotton fields in Limestone, Henry, and Baldwin counties in Alabama were 3%, 23.2%, and 5.6%, respectively. A total of 12 fungi were identified from *R. reniformis* vermiform stages and eggs. The most frequently isolated fungi were *Arthrobotrys dactyloides* (46%) and *Paecilomyces lilacinus* (14%), followed by *Phoma exigua* (4.8%), *Penicillium waksmanii* and *Dactylaria brochopaga* (3.6%), *Aspergillus glaucus* group (2.4%), *Cladosporium herbarum*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Torula herbarum*, *Aspergillus fumigatus*, and an unidentified basidiomycete were less frequent (1.2%). A high percentage (16.8%) of fungi from colonized nematodes was not cultivable on our media. Out of those 12 fungi, only four have been previously reported as nematophagous fungi: three isolates of *Arthrobotrys dactyloides*, and one isolate of *Dactylaria brochopaga*, *Paecilomyces lilacinus*, and *Fusarium oxysporum*. Molecular identification of *Arthrobotrys dactyloides* and *Dactylaria brochopaga* was consistent with the morphological identification, placing these two fungi in the new genus *Drechlerella* as proposed in the new Orbiliaceae classification.

Key words: *Arthrobotrys dactyloides*, *Dactylaria brochopaga*, *Paecilomyces lilacinus*, reniform nematode, *Rotylenchulus reniformis*.

Rotylenchulus reniformis is a serious problem in cotton production in the southeastern United States, causing an economic loss of \$98 million per year (Blasingame *et al.*, 2007). There are no commercial cotton varieties resistant to *R. reniformis* (Weaver *et al.*, 2007; Starr *et al.*, 2007; Usery *et al.*, 2005); consequently, management is based on the application of nematicides, such as aldicarb, oxamyl, 1,3-dichloropropene, thiodicarb, and metam sodium (Koening *et al.*, 2007; Lawrence *et al.*, 2005; Kinloch and Rich, 2001; Lawrence *et al.*, 1990), and crop rotation with corn, peanut, sorghum, and resistant cultivars of soybean (Davis *et al.*, 2003; Gazaway *et al.*, 2000). The economic cost of managing this nematode pest indicates the need to explore new management options that can be integrated in sustainable systems. Biological control options for *R. reniformis* nematode management are not currently available in the United States (Robinson, 2007).

Numerous studies of fungi colonizing plant-parasitic nematodes have been reported on soybean cyst (*Heterodera glycines*) and root-knot nematodes (*Meloidogyne* spp.) (Kim and Riggs, 1991; Carris and Glawe, 1989; Jatala, 1986). In the case of *R. reniformis*, suppressive soils from five fields in the Lower Rio Grande Valley (LRGV) of Texas and five fields in Louisiana have been reported. In the LRGV the suppression of *R. reniformis* populations was as great as 95%, but the organisms involved in this suppression were not identified (Robinson *et al.*, 2008). In other studies, nematophagous fungi have been isolated and evaluated on different life stages of

R. reniformis. *Paecilomyces lilacinus* significantly reduced numbers of *R. reniformis* eggs under greenhouse conditions (Walters and Barker, 1994). *Pochonia chlamydospora* reduced numbers of *R. reniformis* on cotton roots or in soil after a single application of 5,000 chlamydospores per gram of soil (Wang *et al.*, 2005). Furthermore, a fungus known as Arkansas Fungus (ARF) parasitized 17–21% of *R. reniformis* eggs (Wang *et al.*, 2004). For the juvenile stages, *Arthrographis* sp., *Pseudorobillarda* sp., and *Fusarium equiseti* significantly reduced *R. reniformis* population development on cotton (McLean *et al.*, 2000).

Taxonomy of nematophagous fungi is being redefined with the development of new molecular techniques. In the past, identification of ring-trapping fungi species was based on the morphology of the conidia and conidiophores (Drechsler, 1937), however Scholler *et al.* (1999) reclassified the *Orbiliaceae* genera based on the ITS1, ITS2, and 5.8S rDNA sequences, demonstrating that trapping devices are more informative than other morphological structures.

Based on the life cycle of *R. reniformis* (semi-endoparasitic), we hypothesize that the nematodes are constantly exposed to many antagonists and other organisms in the rhizosphere. The objective of this study was to isolate and identify nematophagous fungi which parasitize *R. reniformis* vermiform life stages and eggs. Fungal isolates were identified by morphological characters and molecular techniques based on the sequence of the ITS and 5.8S rDNA regions.

MATERIALS AND METHODS

Collection of nematode samples: *Rotylenchulus reniformis* nematodes were collected from roots of cotton plants grown to maintain stock cultures of nematodes at the Plant Science Research Center of the Alabama Agricultural

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Experiment Station on the campus of Auburn University. Continuous long-term culture of cotton with *R. reniformis* without repotting increased populations of antagonistic fungi against this nematode in our greenhouse cultures. These nematodes were cultured in 15-cm-diam polystyrene pots which had each been inoculated with 2,000 eggs/500 cm³. The cotton was grown in the greenhouse for 6-18 months. *Rotylenchulus reniformis* nematode samples were also collected from *R. reniformis* infested cotton fields from three cotton production counties of Alabama (Limestone, Henry, and Baldwin). Approximately 1,000 cm³ of soil were collected to a depth of 15 cm in the root zone of the cotton plants during the growing season from June through August. Soils were sealed in plastic bags, labeled, and stored in a cooler for transport to the greenhouse. Vermiform adults and nematode juveniles were extracted from the soil by a modified gravity screening and centrifugation-flotation method (Jenkins, 1964). Eggs were extracted from cotton roots by shaking the roots in 1% NaOCl for 4 minutes at 120 rpm and sieving the solution (Hussey and Baker, 1973).

Nematode samples were observed at 6.3× total magnification under the stereoscope (Nikon SMZ800), where the vermiform stages with trapping-rings and healthy one were counted to determine the fungal parasitism percentage. Discolored eggs of *R. reniformis* and vermiform life stages colonized with fungal mycelia were carefully removed from the counting dish, placed in a syracuse dish, and rinsed with sterile water. Colonized nematodes and eggs were aseptically cultured on 1.5% water agar supplemented with 12.5 mg of chlorotetracycline HCl and 300 mg of streptomycin sulfate per liter (Kim and Riggs, 1994). Cultures were incubated at 27°C and identified after 5 days. Hyphal tips from the fungi growing from the eggs and vermiform life stages of the nematodes were aseptically transferred to a Potato Dextrose Agar (PDA/SA) (Sigma Chemical Co.) culture plates supplemented with 300 mg/liter of streptomycin sulfate (Sigma Chemical Co.) to establish pure cultures.

Morphological identification: Identification of the nematophagous fungi was based on the morphological characteristics of conidiophores and conidia (Nelson *et al.*, 1983, Gerlach and Nirenberg, 1982, Domsch *et al.*, 1980, Barron, 1977 and Drechsler, 1937). For some fungi, it was necessary to induce sporulation on water agar media (WA) by adding 20 *R. reniformis* vermiform stages to each plate. Sporulation was also induced in some cultures by exposing fungal mycelium to a black light lamp (Model X-15B 115 volts 60Hz).

Molecular identification of fungi: Identification of fungi isolated from *R. reniformis* and previously reported as nematophagous fungi was confirmed by sequencing of the internal transcribed spacer (ITS) regions 1 and 2, including the 5.8 rDNA. Fungal isolates were grown on PDA/SA at 27°C for 7 days. After this period, mycelia

were harvested, placed in a mortar, frozen with liquid nitrogen, and transferred to an eppendorf tube without letting the sample thaw. DNA was extracted using the Dneasy Plant Mini Kit® (Quiagen Inc.). PCR reactions of 50 µL were prepared using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1998). PCR conditions included an initial denaturation for 3 min at 95°C, 35 cycles of 1 min at 95°C, annealing for 40 s at 54°C, extension for 40 s at 72°C, and final extension for 10 min at 72°C. PCR amplification products were purified with QIAquick columns (Quiagen Inc.) according to manufacturer's instructions. The resulting amplified products were sequenced at the Auburn University Genomics facility using the same ITS1 and ITS4 primers.

Sequences were manually edited using Chromas Lite 2.01 software (<http://www.technelysum.com.au>). Alignments were done with ClustalW software (Tamura *et al.*, 2007), and then consensus sequences were subjected to Blast analysis in National Center for Biotechnology Information (NCBI) using blastn option. Phylogenetic analyses were performed using Neighbor joining algorithm in Mega 4.1 software (Tamura *et al.*, 2007).

RESULTS

Morphological identification: The percentage of parasitized vermiform nematodes from greenhouse samples was 21.2%, and the percentages from Limestone, Henry, and Baldwin counties were 3%, 23.2%, and 5.6%, respectively. In the greenhouse samples, number of fungi associated with vermiform life stages were *Arthrobotrys dactyloides* (15 isolates), *Paecilomyces lilacinus* (4 isolates), *Penicillium waksmanii* (3 isolates), *Aspergillus glaucus* Group (2 isolates), and *Cladosporium herbarum* (1 isolate) (Fig. 1). No fungal growth was observed from 6 vermiforms visually colonized, and cultured. Numbers of fungi associated with eggs were *Paecilomyces lilacinus* (8 isolates), *Phoma exigua* (2 isolates), *Cladosporium cladosporioides* (1 isolate), *Fusarium oxysporum* (1 isolate), *Torula herbarum* (1 isolate), and an unidentified basidiomycete (1 isolate). No fungal cultures were found in 2 discolored eggs plated (Fig. 1).

From the Limestone field location, only *Phoma exigua* (2 isolates) was associated with vermiform stages of *R. reniformis*; however, three of the colonized vermiform nematodes did not produce any fungal growth in PDA media with antibiotics (Fig. 2). The most frequent fungi founded in the colonized nematodes from Henry field were *Arthrobotrys dactyloides* (23 isolates), and *Dactylaria brochophaga* (3 isolates). In the Baldwin location *Arthrobotrys dactyloides* was also isolated along with *Aspergillus fumigatus* (1 isolate). However, in the Baldwin location, three of the cultured nematodes did not produce fungal growth on culture medium (Fig. 2).

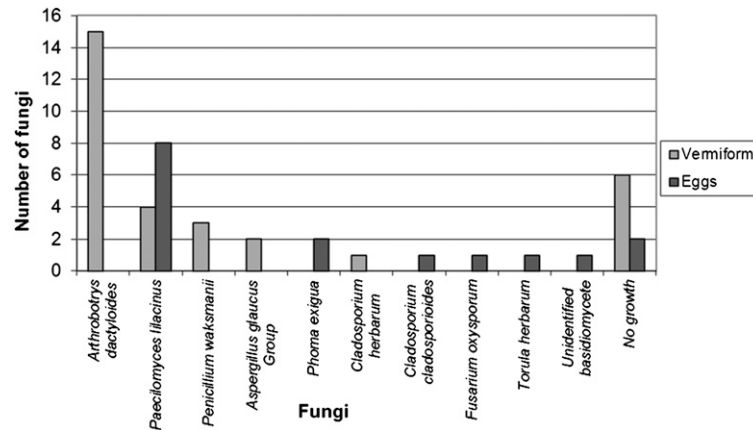


FIG. 1. Number of fungi isolated from symptomatic *Rotylenchulus reniformis* greenhouse samples.

From all the samples taken, the most frequently isolated fungi from *R. reniformis* were *A. dactyloides* (39 isolates) and *P. lilacinus* (12 isolates) (Fig. 3). Followed by *Phoma exigua* (4 isolates), *Penicillium waksmanii* and *D. brachophaga* (3 isolates), *Aspergillus glaucus* group (2 isolates), and *C. cladosporioides*, *C. herbarum*, *F. oxysporum*, *T. herbarum*, *D. brachophaga*, *A. fumigatus*, and unidentified basidiomycete were less frequent (1 isolate). None the less, 14 of the fungal colonized nematodes cultured did not produce fungal culture on the media (Fig. 3).

Morphological characteristics: *Arthrobotrys dactyloides* is a nematode-trapping fungus that produces constricting rings to capture nematodes. The rings were 20 - 32 μm diameter, composed of 3 individual arcuate cells. Conidia are ellipsoidal, 40 to 48 μm long, 8 to 9 μm wide, with 2 cells equally divided by one septum (Fig. 4A), which is the main morphological characteristics that differentiated it from *A. anchonia*. Chlamydospores have been reported on this fungus (Haard, 1968), but they were not observed in any of our strains. *Dactylaria brochophaga* is another nematode-trapping fungus producing constricting rings of 20 to 35 μm in diameter with 3 arcuate cells. Conidia are ellipsoidal, slightly curved, 30 to 40 μm long, 5 to 8 μm wide, and were divided into 4 cells by 3 septa (Fig. 4B).

Molecular identification of fungi: The morphological identification of the fungi reported above as nematophagous fungi was confirmed by sequencing of the ITS1 - 5.8 rDNA - ITS2 region. The three strains of *Arthrobotrys dactyloides* (GH-*A. dactyloides*, HN-*A. dactyloides*, BW-*A. dactyloides*) displayed 99% similarity with *Arthrobotrys anchonia* (AY965753.1) and 97% with *Drechlerella dactyloides* (AY773463.1). *Paecilomyces lilacinus* and *Fusarium oxysporum* isolates presented a 100% coverage of the sequences and a 100% identity with *P. lilacinus* (EU306174.1) and *F. oxysporum* (FJ605247.1) when blast in the GenBank (Table 1).

All of the isolates of *Arthrobotrys dactyloides* group were in a clade in the phylogram, and grouped closely to *Arthrobotrys anchonia*, and *Drechlerella dactyloides*. HN-*Dactylaria brochophaga* grouped with *Drechlerella brochophaga*. The isolates of *P. lilacinus* grouped together in a monophyletic clade. Also, GH-*Fusarium oxysporum* grouped together with a biocontrol strain. *Rhizoctonia solani* was used in this graph as an outgroup (Fig. 5).

DISCUSSION

Long-term cultures of *R. reniformis* maintained in cotton plants in the greenhouse increased the population of fungi antagonistic to reniform nematode.

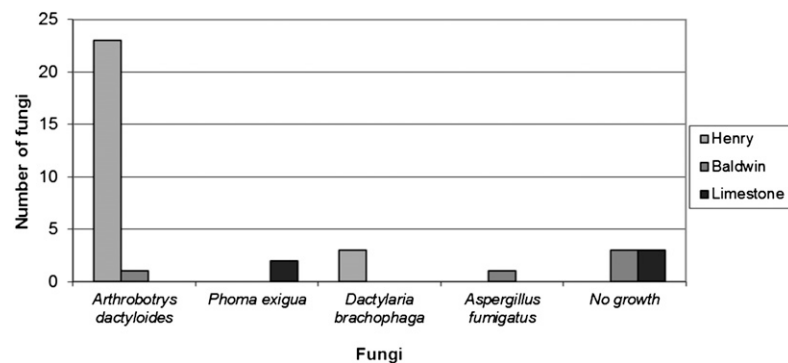


FIG. 2. Fungi isolated from symptomatic *Rotylenchulus reniformis* vermiform life stages from the field locations.

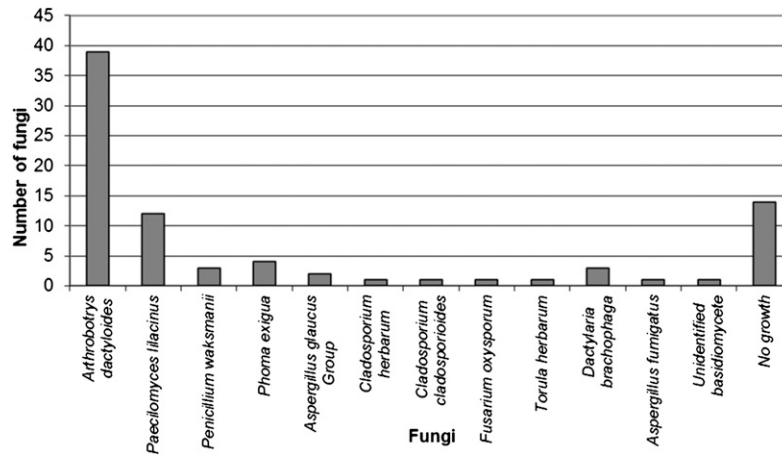


FIG. 3. Percent frequency of occurrence of the total fungi isolated from symptomatic *Rotylenchulus reniformis* from all samples.

Parasitism of the vermiform stages of reniform nematodes was higher on plants in the greenhouse than in the field. Wang *et al.* (2004), also obtained nematode-antagonistic fungal isolates from cotton field soil maintained under greenhouse conditions. Of all the fungi isolated from all locations, only *Arthrobotrys dactyloides*, *D. brachophaga*, *P. lilacinus*, and *F. oxysporum* have been reported as pathogens on other nematode species (Kiewnick and Sikora, 2006; Stirling and Smith, 1998; Freitas *et al.*, 1995). The most predominant nematode-trapping fungus was *A. dactyloides*, which was isolated from the greenhouse and two counties (Henry and Baldwin). *Dactylaria brachophaga* is closely related to *A. dactyloides* and was only identified in Henry County. Both fungi present white mycelium on PDA, however, the most abundant septation in *D. brachophaga* is the feature most decisively distinguishing it as species (Drechsler, 1937; Haard, 1968; Barron, 1977). *Arthrobotrys dactyloides* and *D. brachophaga* have been reported to reduce populations of *Meloidogyne graminicola* on rice (Singh *et al.*, 2007). Additionally, Stirling and Smith (1998a) reported reductions in populations of *Meloidogyne javanica* on tomato roots using isolates of *A. dactyloides*. These two fungi have potential as biological control agents of juvenile stages, and commercial formulations of *A. dactyloides* have been attempted (Stirling *et al.*, 1998b).

Paecilomyces lilacinus is typically a soilborne fungus and is common in the rhizosphere of a number of plants (Domsch *et al.*, 1980). This fungus has been reported on *R. reniformis* on tomato plants, where it reduced the populations of the nematode in greenhouse and microplot trials (Walters and Barker, 1994). This fungus was also very predominant on the cotton plants used for culturing *R. reniformis* in the greenhouse.

Fusarium oxysporum has been previously reported to be destructive on eggs of the soybean cyst nematode in Alabama soybean fields (Morgan-Jones and Rodriguez-Kabana, 1981). There are some biotypes capable of penetrating eggs and causing disorders on the embryonic development through enzymatic and/or toxic effects (Morgan-Jones and Rodriguez-Kabana, 1988). This fungus has never been reported on *R. reniformis* so it is necessary to evaluate the biocontrol potential for this nematode.

Morphological and molecular fungal identification are in agreement for HN-*D. brachophaga*, GH-*P. lilacinus*, and GH-*Fusarium oxysporum*. Only the three strains of *Arthrobotrys dactyloides* showed differences between the two types of identification. Molecular identification indicated 99% of similarity to *A. anthonia* and 97%

TABLE 1. Relationship of fungal isolates colonizing *Rotylenchulus reniformis* with other fungi from the blast analysis in GenBank.

ISOLATE	FRAGMENT SIZE (bp)	HIGHEST MATCH IN THE GENBANK
BW- <i>Arthrobotrys dactyloides</i>	552	99% <i>Arthrobotrys anthonia</i> 97% <i>Drechslerella dactyloides</i>
GH- <i>Arthrobotrys dactyloides</i>	549	99% <i>Arthrobotrys anthonia</i> 97% <i>Drechslerella dactyloides</i>
HN- <i>Arthrobotrys dactyloides</i>	547	99% <i>Arthrobotrys anthonia</i> 97% <i>Drechslerella dactyloides</i>
HN- <i>Dactylaria brachophaga</i>	552	100% <i>Drechslerella brachophaga</i>
GH- <i>Fusarium oxysporum</i>	688	100% <i>Fusarium oxysporum</i>
GH- <i>Paecilomyces lilacinus</i>	543	100% <i>Paecilomyces lilacinus</i>
<i>Paecilomyces lilacinus</i> Strain251	543	100% <i>Paecilomyces lilacinus</i>

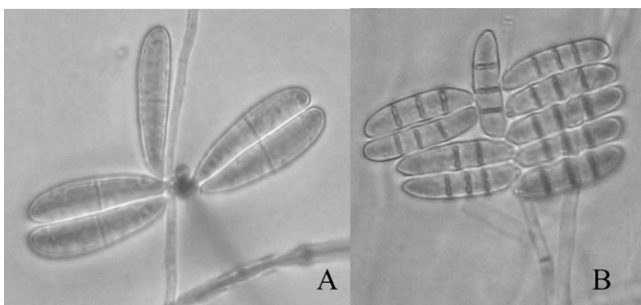


FIG. 4. Conidia of *Arthrobotrys dactyloides* (A), and *Dactylaria brachophaga* (B) (x100).

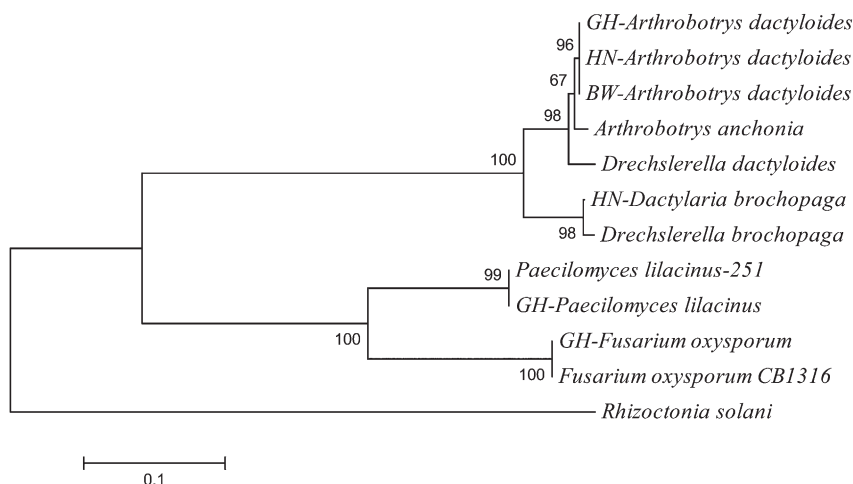


FIG. 5. Phylogram from the fungi isolated from *Rotylenchulus reniformis* nematodes based on ITS and 5.8s rDNA (Neighbor-joining algorithm).

identity with *A. dactyloides*. *Arthrobotrys dactyloides* is closely related to *Arthrobotrys anchonia*, both are ring-trapping fungi, differing in the shape and size of the conidia. *Arthrobotrys anchonia* produce elongated ovoid conidia with unequal cells within each of the conidia, while the *Arthrobotrys dactyloides* conidia are ellipsoid with equal cell lengths (Haard, 1968). Therefore, our morphological observations of the three isolates showed ellipsoid conidia with equal cell size. This allowed us to confirm that the fungi we isolated are *A. dactyloides*. Thus, our isolates will be named *Drechslerella dactyloides*, based on the classification proposed by Scholler et al. 1999, which also renames *A. brochopaga* as *Drechslerella brochopaga*.

Overall, our results suggest that *Arthrobotrys dactyloides*, *D. brochopaga*, *Fusarium oxysporum*, and *Paecilomyces lilacinus* are pathogens of *R. reniformis*. Evaluations of these fungi under *in vitro* and greenhouse conditions will be performed to evaluate their biocontrol potential. Additionally, is it important to develop and evaluate different media and techniques for isolation and identification of fungi associated with colonized nematodes that did not grow on artificial media. There is a possibility that these fungi are endoparasitic fungi that complete their life cycle inside the nematode host, or bacteria parasitized on nematodes. The current research had identified several natural enemies of *R. reniformis* in different life stages of this nematode. The outcome of this research will provide a foundation to develop a biocontrol program that could be added to the actual management practices and improve the nematode control.

LITERATURE CITED

- Barron, G. L. 1977. The nematode-destroying fungi. Canada: Lancaster Press.
- Blasingame, D., Patel, M. V., Gazaway, W., Olsen, M., Kirkpatrick, T., Davis, M., Sprengel, R. K., Kemerait, B., Colyer, P., Wrather, A.,

Goldberg, N., Koenning, S., Banks, J. C., Muller, J., Newman, M., Woodward, J., & Phipps, P. 2007. Cotton disease loss estimate committee report. Proceedings of the Beltwide Cotton Conferences of the National Cotton Council of America. www.cotton.org/beltwide/proceedings/2007. Confirmed on Jan. 4, 2008.

Carris, L. M., and Glawe, D. A. 1989. Fungi colonizing cyst of *Heterodera glycines*. Bulletin 786. University of Illinois at Urbana-Champaign. College of Agriculture. Agricultural Experiment Station. U.S. Department of Agriculture.

Davis, R. F., Koenning, S. R., Kemerait, R. C., Cummings, T. D., and Shurley, W. D. 2003. *Rotylenchulus reniformis* management in cotton with crop rotation. Journal of Nematology 35(1):58–64.

Domsch, K. H., Gams, W., and Traute-Heidi, A. 1980. Compendium of Soil fungi – Volumen I. London: Academic Press.

Drechsler, C. 1937. Some Hyphomycetes that prey on free-living terricolous nematodes. Mycologia 29:447–552.

Freitas, L. G., Ferraz, S., and Muchovej, J. J. 1995. Effectiveness of different isolates of *Paecilomyces lilacinus* and an isolate of *Cylindrocarpum destructans* on the control of *Meloidogyne javanica*. Nematotropa 25:109–115.

Gazaway, W. S., Akridge, J. R., and McLean, K. 2000. Impact of various crop rotations and various winter cover crops on reniform nematode in cotton. Proceedings Beltwide Conference National Cotton Council America pp. 162–163. San Antonio, TX.

Gerlach, W., and Nirenberg, H. 1982. The genus *Fusarium* – A Pictorial Atlas. Biologische Bundesanstalt für Land – und Forstwirtschaft. Institut für Mikrobiologie, Berlin-ahlem.

Haard, K. 1968. Taxonomic studies on the genus *Arthrobotrys* Corda. Mycologia 60:1140–1159.

Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp. including a new technique. Plant Disease Report 57:1025–1028.

Jatala, P. 1986. Biological control of plant parasitic nematodes. Annual Review of Phytopathology 24:453–489.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Kiewnick, S., and Sikora, R. A., 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biological Control 38:179–187.

Kim, D. G., and Rigs, D. 1994. Techniques for isolation and evaluation of fungal parasites of *Heterodera glycines*. Journal of Nematology 26(4S):592–595.

Kim, D. G., and Rigs, D. 1991. Characteristics and efficacy of the sterile hyphomycete (ARF18), a new biocontrol agent for *Heterodera glycines* and other nematodes. Journal of Nematology 23(3):275–282.

- Kinloch, R. A., and Rich, J. R. 2001. Management of Root-knot and reniform nematode in Ultra-narrow row cotton with 1,3-Dichloropropene. Supplement to the *Journal of Nematology* 33(4S):311–313.
- Koenning, S. R., Morrison, D. E., and Edmisten, K. L. 2007. Relative efficacy of selected nematicides for management of *Rotylenchulus reniformis* in cotton. *Nematropica* 37:227–235.
- Lawrence, K. S., Feng, Y., Lawrence, G. W., Burmester, C. H., and Norwood, S. H. 2005. Accelerated degradation of Aldicarb and its metabolites in cotton field soils. *Journal of Nematology* 37(2):190–197.
- Lawrence, G. W., McLean, K. S., Batson, W. E., Miller, D., and Borbon, J. C. 1990. Response of *Rotylenchulus reniformis* to nematicide applications on cotton. Supplement to *Journal of Nematology* 22(4S):707–711.
- McLean, K. S., Palmateer, A. J., and Morgan-Jones, G. 2000. Fungal antagonists of *Rotylenchulus reniformis*. Proceedings of the Beltwide Cotton Conference 1:145–146.
- Morgan-Jones, G., and Rodriguez-Kabana, R. 1988. Fungi colonizing cyst and eggs. Diseases of nematodes Volumen II. 1988. CRC Press. Edited by Poinar, G. O., Jansson, H. B.
- Morgan-Jones, G., and Rodriguez-Kabana, R. 1981. Fungi associated with cyst of *Heterodera glycines* in Alabama soil. *Nematropica* 11:69–74.
- Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* species – An illustrated manual for identification. The Pennsylvania State University Press. University Park and London.
- Robinson, A. F. 2008. Nematode management in cotton. Pp. 149–182 in A. Ciancio and K. Mukerji, eds. Integrated management and biological control of vegetable and grain crops nematodes. Berlin: Springer.
- Robinson, A. F., Westphal, A., Overstreet, C.h., Padgett, G. B., Greenberg, S. M., Wheeler, T. A., and Stetina, S. R. 2008. Detection of suppressiveness against *Rotylenchulus reniformis* in soil from cotton (*Gossypium hirsutum*) fields in Texas and Louisiana. *Journal of Nematology* 40(1):35–38.
- Robinson, A. F. 2007. Reniform in the U.S. cotton: When, where, why, and some remedies. *Annual Review of Phytopathology* 45:263–288.
- Scholler, M., Hagedorn, G., and Rubner, A. 1999. A reevaluation of predatory oriboliceous fungi. II. A new generic concept. *Sydowia* 51(1):89–113.
- Singh, K. P., Jaiswal, R. K., Kumar, K., and Kumar, D. 2007. Nematophagous fungi associated with root galls of rice caused by *Meloidogyne graminicola* and its control by *Arthrobotrys dactyloides* and *Dactylaria brochopaga*. *Journal of Phytopathology* 155:193–197.
- Starr, J. L., Koenning, S. R., Kirkpatrick, T. L., Robinson, A. F., Roberts, P. A., and Nichols, R. L. 2007. The future of nematode management in cotton. *Journal of Nematology* 39(4):283–294.
- Stirling, G. R., and Smith, L. J. 1998a. Field test of formulated products containing either *Verticillium chlamydosporium* or *Arthrobotrys dactyloides* for biological control of the Root-knot nematodes. *Biological Control* 11:231–239.
- Stirling, G. R., Smith, L. J., Licastro, K. A., and Eden, L. M. 1998b. Control of Root-knot nematode with formulations of the nematode-trapping fungus *Arthrobotrys dactyloides*. *Biological Control* 11:224–230.
- Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596–1599.
- Usery, S. R., Lawrence, K. S., Lawrence, G. W., and Burmester, C. H. 2005. Evaluation of cotton cultivars and tolerance to *Rotylenchulus reniformis*. *Nematropica* 35:121–133.
- Walters, S. A., and Barker, K. R. 1994. Efficacy of *Paecilomyces lilacinus* in suppressing *Rotylenchulus reniformis* on tomato. Supplement to *Journal of Nematology* 26(4S):600–605.
- Wang, K., Riggs, R. D., and Crippen, D. 2005. Isolation, selection, and efficacy of *Pochonia chlamydosporia* for control of *Rotylenchulus reniformis* on cotton. *Phytopathology* 95:890–893.
- Wang, K., Riggs, R. D., and Crippen, D. 2004. Suppression of *Rotylenchulus reniformis* on cotton by the nematophagous fungus ARF. *Journal of Nematology* 36(2):186–191.
- Weaver, D. B., Lawrence, K. S., and Van Santen, E. 2007. Reniform nematode in Upland cotton germplasm. *Crop Science* 47:19–24.
- White, T. J., Bruns, T., Lee, S., and Taylor, J. 1998. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J. eds. PCR protocols A guide to methods and applications. Academic Press, Inc.