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Suppression of *Rotylenchulus reniformis* on Cotton by the Nematophagous Fungus ARF[1]

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Abstract: The reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, has become a serious threat to cotton (*Gossypium hirsutum* L.) production in the United States during the past decade. The objective of this study is to isolate fungi from eggs of *R. reniformis* and select potential biological control agents for *R. reniformis* on cotton. Soil samples were collected from cotton fields located in Jefferson County, Arkansas. Eight genera of fungi were included in the 128 fungal isolates obtained, and among them were five strains of the nematophagous fungus ARF. The mtDNA RFLP pattern, colony growth characteristics, and pathogenicity indicate the five ARF isolates represent one described strain and one new strain. Light and electron microscopic observations suggest ARF is an active parasite of *R. reniformis,* with parasitism ranging from 48% to 79% in in vitro tests. Three greenhouse experiments demonstrated ARF successfully suppressed the number of reniform nematodes during the first and second generation of the nematode. Reductions in numbers of *R. reniformis* on the roots for the seven application rates of 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% ARF were 87%, 92%, 94%, 96%, 97%, 98%, and and 98%, respectively.

Key words: ARF, biological control, cotton, *Gossypium hirsutum,* reniform nematode, *Rotylenchulus reniformis.*

The reniform nematode, *Rotylenchulus reniformis,* is a serious threat to cotton (*Gossypium hirsutum*) production in the United States, with yield losses ranging from 10% to 70% in infested fields (Kirkpatrick and Lorenz, 1997). In the United States, 11 of the 16 cotton-producing states have infestations of this nematode (Overstreet and McGawley, 1997), and in Mississippi and Louisiana, the extent of the reniform nematode infestation was about 283,400 and 206,500 hectares, respectively, in 1997 (Overstreet and McGawley, 1997). About $13.7 million was lost due to this nematode in 1999 in the United States (Mueller, 2000), but the loss reached $80 million in 2000 (McLean et al., 2001).

Nematicides and crop rotation are currently used to manage the reniform nematode. Nematicides are used extensively because no commercial cotton cultivar has resistance to *R. reniformis* and few alternative crops can be rotated with cotton in practice (Kirkpatrick and Lorenz, 1997). Crops that can be rotated with cotton to control the reniform nematode are corn (*Zea mays*), rice (*Oryza sativa*), grain sorghum (*Sorghum bicolor*), and resistant cultivars of soybean (*Glycine max*) (Kirkpatrick and Lorenz, 1997).

Concern about environmental problems, hazards to human health, and application costs associated with the use of nematicides has led to a sense of urgency in the search for alternative control methods (Kerry, 1990). Biological control methods, if successful, could be integrated with other control methods to help manage this nematode pest. Biological control of *R. reniformis* may shorten rotations and prolong the life of resistant cultivars of soybean that are commonly used in rotation with cotton. However, biological control of the reniform nematode had not been studied in detail before the present study, even though several fungi colonizing the vermiform nematodes of *R. reniformis* had been reported (McLean et al., 2001).

Parasites of eggs and saccate females should be the most effective biocontrol agents of the reniform nematode (Kerry, 1990). Saccate females are partly exposed on the root surface, and eggs are laid into a gelatinous matrix secreted by the female. The eggs in the gelatinous matrix should be conveniently accessible to the egg-parasitic fungi and easily parasitized by these fungi (Stirling, 1991). However, somewhat surprisingly, no egg-parasitic fungi have been reported from eggs of the reniform nematode. Two studies reported that the fungus *Paecilomyces lilacinus* suppressed *R. reniformis* on tomato (*Lycopersicon esculentum* Miller) (Reddy and Khan, 1988; Walters and Barker, 1994), but the fungal isolates used in both studies were from eggs of root-knot nematode, not from the reniform nematode.

An unidentified nematophagous fungus, designated as ARF (Arkansas Fungus), has been isolated from soybean cyst nematode *Heterodera glycines* (Kim and Riggs, 1991). This fungus has shown promising ability to suppress *H. glycines* populations in both sterilized and natural soils under greenhouse conditions (Kim and Riggs, 1995; Timper and Riggs, 1998). Because this fungus does not sporulate, its classification is difficult; however, it is an Ascomycete based on the presence of Woronin bodies around the septum of the hyphae (Kim et al., 1992). Four groups of ARF have been established based on mtDNA RFLP analyses of 17 isolates from soybean cyst nematode, *H. glycines* (Kim et al., 1998).

The objectives of this study were to: (i) isolate and characterize fungi from eggs of *R. reniformis,* (ii) screen potential biocontrol agents by testing the pathogenicity of the isolated fungi in vitro, and (iii) test the efficacy of the selected potential biocontrol agents in greenhouse studies. This is a report of the isolation of the nematophagous fungus ARF from eggs of *R. reniformis,* mtDNA RFLP analysis of the isolated ARF fungal strains, in vitro pathogenicity test of the isolated ARF to *R. reniformis,* visualization of the parasitized *R. reniformis*

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by ARF by light and electron microscopy, and the efficacy tests of ARF in suppressing *R. reniformis* on cotton.

Materials and Methods

Isolation and selection: Soil samples (120) were collected from six cotton fields in Jefferson County, Arkansas, in June 1999. Approximately 500 g soil was collected to a depth of 10-cm from each sampling site, placed in 10-cm-diam. clay pots, and further infested by adding 3,000 eggs/pot of *R. reniformis* obtained from stock cultures maintained on cotton in the greenhouse. Seeds of the cotton cultivar SureGrow 125, susceptible to *R. reniformis,* were planted in each pot, and plants were replaced at 3-month intervals, for about 2 years. The continuous culture on cotton was to build populations of antagonists against *R. reniformis.*

Cotton roots were collected from each pot, and egg masses of *R. reniformis* were harvested with forceps using a stereomicroscope. Each egg mass was rinsed in sterilized water 3 times, placed in a drop of sterilized water on a sterilized glass slide, crushed to release the eggs, and the eggs were examined. Eggs parasitized by fungi were extracted with a fine glass pipet and transferred to petri dishes containing 1.5% water agar supplemented with 12.5 mg of chlortetracycline-HCl and 300 mg of streptomycin sulfate per liter (Kim and Riggs, 1994). The dishes were incubated at 25 °C for 3 days, and eggs were inspected daily. Fungal hyphae that grew from eggs were transferred aseptically to potato dextrose agar (PDA) supplemented with 300 mg/liter of streptomycin sulfate, to establish pure cultures.

Fungi isolated from the eggs of *R. reniformis* were tested in the laboratory for pathogenicity to *R. reniformis* in vitro. A 1-cm-diam. plug was cut at the edge of a fungal colony and placed on 1.5% water agar. Thirty egg masses of *R. reniformis* obtained from stock cultures were surface-disinfested with 0.01% $HgCl_2$ for about 1 minute and rinsed in sterilized water 3 times. Each disinfested egg mass was placed on an agar disk with the fungus, and it was incubated in a growth chamber at about 25 °C for 10 days. An agar plug without fungus was used as control. After 10 days, each egg mass was crushed in a glass tissue grinder to release the eggs and the egg suspension was poured through nested 75-um-pore and 25-um-pore sieves. The eggs retained on the 25-um-pore sieve were washed into a conical centrifuge tube and concentrated by centrifugation (Kim and Riggs, 1994). The percentage of parasitized eggs was determined by examining 100 arbitrarily selected eggs with a compound microscope.

Mitochondrial DNA RFLP analysis of five fungal isolates: Five of the fungal isolates from eggs of *R. reniformis* resembled the nematophagous fungus ARF, which is a common parasite of soybean cyst nematode, *Heterodera glycines* (Kim and Riggs, 1991). Mitochondrial-DNA RFLP analyses were used to determine the relationships among the five fungal isolates and their relationship to the ARF fungus. The procedure for the mtDNA RFLP analyses of the five fungal isolates followed that used in previous research (Kim et al., 1998). Mycelium was produced in a liquid shake culture in pea juice medium (100 ml canned sweat pea juice with 150 ml sterilized distilled water). Total DNA from the mycelium of each isolate was extracted and digested with the restriction enzyme *Hae* III. DNA fragments were separated electrophoretically on 0.81% agarose gels in 0.5× Tris-borate-EDTA buffer. A Southern blot was conducted with two mtDNA probes (2μ18 and 4μ40) obtained from *Colletotrichum orbiculare* (Correll et al., 1993). The mtDNA RFLP patterns of the five fungal isolates were compared with the mtDNA RFLP patterns of the ARF fungus (Kim et al., 1998).

In vivo pathogenicity tests: Isolate ARF-C was tested for pathogenicity to *R. reniformis* eggs in three greenhouse experiments. The fungus was cultured in 250 ml pea juice medium (100 ml canned sweet pea juice with 150 ml distilled water) in 500-ml flasks, which were shaken for 15 days at 25 °C. Mycelium was harvested from the liquid cultures with a vacuum filter, rinsed with sterilized distilled water, and homogenized in sterilized water in a blender. The homogenized mycelium, at 0.1% (w/w) or 0.3% (w/w) wet weight, was mixed into heat-treated silt loam soil (55% silt, 42% sand, and 3% clay; pH 7.6) from the Arkansas River Valley. The infested soil (about 200 g) was placed in 150-cm^3 (7-cm-diam.) foam cups. Nematode-infested soil without ARF was used as a control.

Two seeds of the cotton cultivar SureGrow 125, susceptible to *R. reniformis,* were planted in each cup. After seedlings emerged, 3,000 eggs of *R. reniformis,* obtained from greenhouse stock culture, were added to each cup. Twenty-five days (one generation) later, population levels of *R. reniformis* in the soil and on the cotton roots were determined. Eggs and vermiform nematodes in 100-cm^3 soil samples were extracted using wet-sieving and sucrose centrifugation techniques (Southey, 1986). Eggs on the roots were extracted by the NaOCl method (Byrd et al., 1972). This experiment was conducted as a completely randomized block design with five replications. Statistical analyses were performed using JMP (Version 5.0, SAS Institute, Cary, NC). Where significance was found, differences among treatment means were separated by least significant difference (LSD) at $\alpha = 0.05$ level.

The second experiment was conducted with the same isolate, ARF-C, that was used in the first experiment. Four rates of fungus (0.3%, 0.1%, 0.05%, and 0.01% w/w, wet weight) were used, and the test was run for 50 days (two nematode generations).

The third experiment was similar to the second but seven rates (0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05%, and 0.01% w/w, wet weight) were used to infest the treated soil. The ARF-C isolate used in this experiment was

re-isolated from eggs of *R. reniformis* because it had been in storage for a year. This test was conducted in the spring rather than the summer, when the other two tests were conducted. In addition to the nematode and fungus data collected in the earlier tests, cotton shoot height, fresh shoot, and root weight were measured.

Results

Isolation and selection: A total of 128 fungal isolates, including five fungal isolates that resembled the nematophagous fungus ARF (Kim and Riggs, 1991), were isolated from eggs of *R. reniformis*. Eight genera of fungi were included in the 128 fungal isolates. All fungal isolates were tested in the laboratory for pathogenicity to eggs of *R. reniformis*. Fungal isolates that parasitized less than 15% of the eggs of *R. reniformis* were discarded. Only 5 fungal isolates that resembled the ARF and 12 isolates of *Pochonia chlamydosporia* (syn. *Verticillium chlamydosporium*) were selected for use in further studies (Wang, 2002). Eggs of *R. reniformis* were parasitized by a fungus resembling ARF (Fig. 1).

The five fungal isolates resembling ARF produced sterile white mycelium, sclerotium-like structures on cornmeal agar, and also parasitized eggs of soybean cyst nematode, *H. glycines* (Table 1). Even though most characteristics of the five fungal isolates were similar to those reported for ARF (Kim and Riggs, 1991), their colonies on PDA were different from the colonies described for ARF (Fig. 2), and the colony of ARF-E (one of the five fungal isolates) differed from the colonies of the other four isolates. Colonies of ARF from *H. glycines* were white, aerial mycelium densely woolly or cottony, and sometimes reverse orange (Fig. 2). Colonies of the four isolates in the present study, ARF-A, B, C, and D, were white or pale, moist, aerial mycelia sparse, agar becoming deeply buckled in the center, border even (Fig. 2). However, colonies of ARF-E were pale, concentrically zonate, not moist, aerial mycelium felty, and agar not buckled (Fig. 2).

The ARF fungal isolates ARF-A, -B, -C, and -D infected more eggs than did isolate ARF-E. The parasitism of nematode eggs in vitro was not different among the isolates ARF-A, -B, -C, and -D (Table 1).

Table 1. Levels of in vitro parasitism of eggs of *Rotylenchulus reniformis* and *Heterodera glycines* by five ARF fungal isolates that were isolated from eggs of *R. reniformis*. The five ARF fungal isolates were from a Jefferson county field soil in Arkansas.

	Parasitized eggs (%)	
Isolates	*R. reniformis*	*H. glycines*
ARF-A	74.4 a	93.4 a
ARF-B	77.4 a	90.6 a
ARF-C	79.2 a	94.0 a
ARF-D	76.6 a	92.6 a
ARF-E	47.6 b	73.8 b
Control	2.4 c	1.2 c

Mean values within columns with the same letter are not significantly different of $\alpha = 0.05$ level.

Light microscopic observation revealed that ARF parasitized and destroyed the eggs of *R. reniformis*. The parasitized eggs of *R. reniformis* were engulfed by the mycelium of ARF and subsequently were penetrated by infective hyphae (Fig. 3), and parasitized eggs of *R. reniformis* were packed with hyphae of ARF (Fig. 3).

Mitochondrial DNA RFLP analyses: To determine the similarity of the five ARF isolates from *R. reniformis* to the ARF isolates from *H. glycines* and the relationship

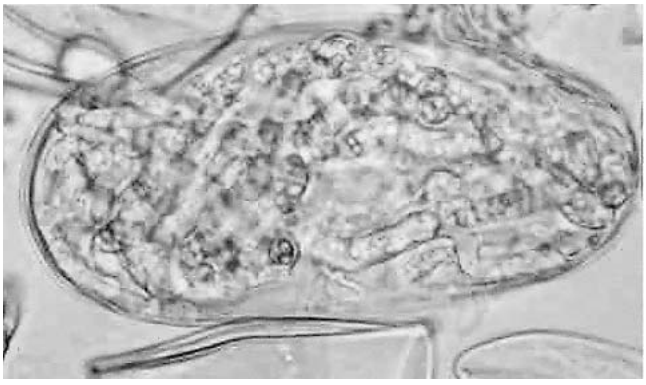

Fig. 1. Egg of *Rotylenchulus reniformis* parasitized by a fungus resembling the sterile nematophagous fungus ARF. This fungus was from a Jefferson County field soil in Arkansas.

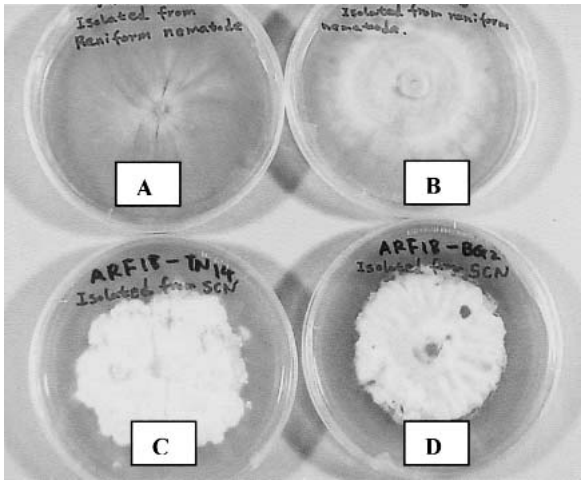

Fig. 2. Colonies of two fungal isolates (A & B) from *Rotylenchulus reniformis* and two isolates (C & D) of ARF from *Heterodera glycines* on potato dextrose agar (PDA) at 25 °C for about 20 days. A) Colony, designated ARF-C, isolated from eggs of *R. reniformis*. B) Colony, designated ARF-E, isolated from eggs of *R. reniformis*. C) Colony of ARF-TN14 (mtDNA RFLP Group IV) isolated from eggs of *H. glycines*. D) Colony of ARF-BG2 (mtDNA RFLP Group I) isolated from eggs of *H. glycines*. ARF-C and ARF-E were from a Jefferson County cotton field soil in Arkansas, ARF-BG2 was from a St. Francis County soybean field soil in Arkansas, and ARF-TN14 was from a Lauderdale County soybean field soil in Tennessee. Colonies of ARF-BG2 and ARF-TN14 are white, aerial mycelium densely wooly or cottony, reverse orange or not. Colony of ARF-C is white or pale, moist, aerial mycelium sparse, agar becoming deeply buckled in center, border even. Colony of ARF-E is pale, concentrically zonate, not moist, aerial mycelium felty, and agar not buckled.

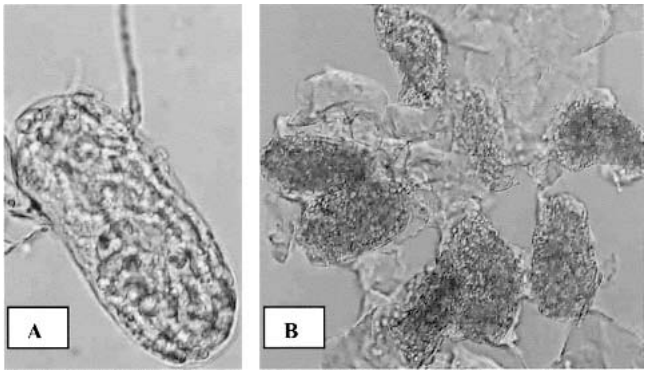

FIG. 3. ARF parasitized and destroyed the eggs of *Rotylenchulus reniformis.* A) The parasitized egg was penetrated by the infective hyphae. B) The parasitized eggs were engulfed by the fungal hyphae of ARF. ARF was from a Jefferson County cotton field soil in Arkansas.

among the five ARF isolates from *R. reniformis,* mtDNA RFLP analyses were conducted. The results revealed that isolates ARF-A, -B, -C, and -D have the same mtDNA RFLP patterns as the Group I of ARF (represented by ARF-BG2), whereas isolate ARF-E was only slightly different when compared with the mtDNA RFLP patterns of the isolates A, B, C, and D (Fig. 4).

In vivo pathogenicity tests: In the first experiment, total number of eggs plus vermiforms of *R. reniformis* on cotton roots was suppressed 13% by the 0.1% (w/w) ARF treatment and 44% by the 0.3% (w/w) ARF treatment (Table 2). Both treatments reduced the numbers of *R. reniformis* in the soil by 47% for the 0.1% (w/w) treatment and 84% for the 0.3% (w/w) treatment compared with the control (Table 2). The percentage of eggs parasitized by ARF on cotton roots was 8.8% for the 0.1% treatment and 12.2% for the 0.3% treatment in this experiment (Table 2); both were different from the level in the control.

Results of the second experiment (50-day, two generations) were consistent with the first experiment (25-day, one generation). The numbers of *R. reniformis* on cotton roots were suppressed by ARF at the 0.05%, 0.1%, and 0.3% application rates (Table 2). The reductions in the numbers of *R. reniformis* on the cotton roots by ARF for the three application rates (05%, 0.1%, and 0.3%) were 59%, 62%, and 93%, respectively. The ARF suppressed nematode numbers in the soil at all four application rates (Table 2). The percentage of eggs parasitized by ARF on cotton roots ranged from 5.8% (at the 0.01% application rate) to 17.0% (at the 0.3% application rate) (Table 2). At the 0.01% application rate, the level of parasitism was not different from the control, but at the other three application rates (0.05%, 0.1%, and 0.3%) the levels of parasitism were different from that in the control.

In the third experiment, numbers of *R. reniformis* on roots and in soil were greatly suppressed by ARF (Table 2). Even at the lowest application rate (0.01% w/w), the numbers of *R. reniformis* on roots and in soil were lower

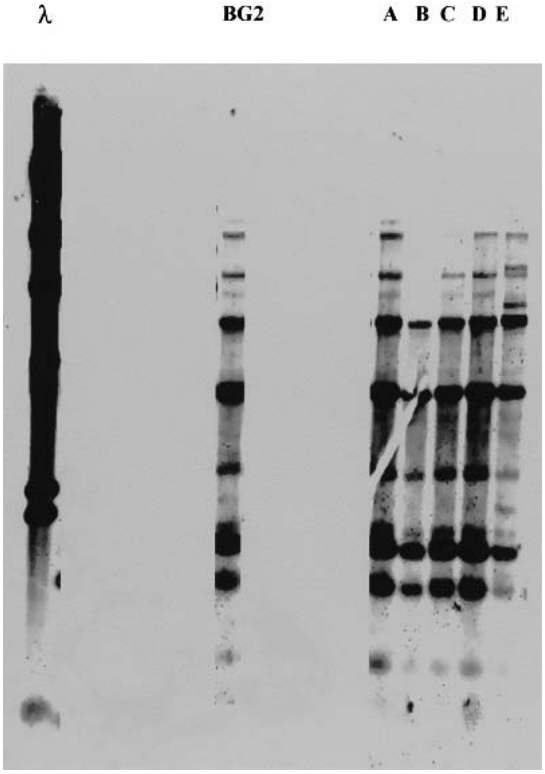

FIG. 4. Mitochondrial DNA RFLP among isolates of ARF. DNA was digested with *Hae* III. Lane 1 was lambda DNA digested with *Hind* III as size markers in kilobases. Lane 2 is ARF-BG2 (mtDNA RFLP Group I) isolated from *Heterodera glycines* that was from a St. Francis County soybean field soil in Arkansas. Lane 3 through Lane 7 are five ARF strains that were isolated from eggs of *Rotylenchulus reniformis* that was from a Jefferson County cotton field soil in Arkansas, and they are ARF-A, ARF-B, ARF-C, ARF-D, and ARF-E, respectively. The mtDNA RFLP patterns of ARF-A, ARF-B, ARF-C, and ARF-D were identical to that of ARF-BG2. The mtDNA RFLP pattern of ARF-E was slightly different compared with the mtDNA RFLP patterns of ARF-A, ARF-B, ARF-C, and ARF-D.

than the nematode numbers in the control (Table 2). The nematode numbers on cotton roots on all higher application rates were not different from the number on the 0.01% rate (Table 2). The percentage of eggs parasitized by ARF was variable (Table 2); however, all of the percentages were higher than the control.

Growth of the cotton plants in the soil treated with ARF was generally better than growth in the control. Growth differences of cotton plants also were observed among the seven ARF treatments. For example, cotton plants grew more vigorously in the pots treated with 0.5% ARF than the cotton plants in the pots treated with 0.01% ARF. Shoot lengths and shoot weights of cotton plants growing in the pots treated with more than 0.1% ARF were different from the control (Table 3).

DISCUSSION

The nematophagous fungus ARF was isolated originally from *H. glycines.* ARF infects several nematode species, including *H. glycines, H. graminophila, H. lespe-*

TABLE 2. Relative numbers of eggs plus vermiforms of *Rotylenchulus reniformis* from cotton roots and soil.[a]

Treatment	Number of eggs and vermiforms/g fresh root (in thousands)	Number of eggs and vermiforms/100 cm^3 soil (in thousands)	Eggs parasitized by ARF (%)
	Greenhouse Experiment 1		
Control	1.05 a	3.30 a	0.0 a
0.1%	0.91 a	1.74 b	8.8 b
0.3%	0.59 b	0.54 c	12.2 b
	Greenhouse Experiment 2		
Control	3.72 a	16.80 a	1.6 a
0.01%	2.48 ab	10.60 bc	5.8 ab
0.05%	1.53 bc	10.80 bc	10.2 b
0.1%	1.42 bc	10.50 bc	10.8 b
0.3%	0.37 c	7.80 c	17.0 c
	Greenhouse Experiment 3		
Control	2.01 a	7.35 a	2.4 a
0.01%	0.26 b	2.58 b	15.4 bc
0.05%	0.17 c	1.20 b	13.2 b
0.1%	0.12 c	0.90 b	20.2 dc
0.2%	0.08 c	0.72 b	22.6 d
0.3%	0.06 c	0.51 b	18.0 dcb
0.4%	0.02 c	0.45 b	23.4 d
0.5%	0.04 c	0.45 b	21.6 dc

For each greenhouse experiment, mean values within columns with the same letter are not significantly different at $\alpha = 0.05$ level.

[a] Measurements were taken 25 days (Experiment 1) and 50 days (Experiments 2 and 3) after infestation with 3,000 eggs of *R. reniformis*. Soil was infested with different rates of ARF (w/w) as indicated in three greenhouse experiments.

dezae, H. leuceilyma, H. schachtii, H. trifolii, Betulodera betulae, and *Meloidogyne incognita* (Kim and Riggs, 1991). However, the strains of ARF originally isolated from *H. glycines* did not infect the reniform nematode, *R. reniformis* (unpubl. data) or *Globodera rostochiensis* (Kim and Riggs, 1991). The five ARF fungal isolates reported in the present study were isolated from eggs of *R. reniformis* and parasitized *R. reniformis* and were anticipated to have mtDNA RFLP patterns different from the four established mtDNA RFLP groups, not only because of the difference of the hosts and pathogenicity but also because of the morphological differences in colony growth. However, ARF-E has an mtDNA RFLP pattern slightly different from the four established groups, but the other four isolates had the same mtDNA RFLP pattern as the mtDNA RFLP Group I from *H. glycines* (BG2 isolate was standard for this group). The similarity between the mtDNA RFLP of the ARF isolates from *R. reniformis* and *H. glycines* suggests that the mtDNA probes used in this study were not tightly linked to the morphological and pathogenicity traits of this fungus.

The ARF-E in this study appears to represent a new mtDNA RFLP group of ARF, and it is designated as mtDNA RFLP group V because its (i) mtDNA RFLP pattern does not match that of any of the four established groups, (ii) colony characteristics are different from those of other ARF fungal isolates, and (iii) virulence was weaker than that of the other four strains isolated from *R. reniformis*.

The mtDNA RFLP that have been conducted do not give enough information to determine the fungal taxon to which ARF belongs. Additional analyses, including ribosomal RNA sequencing, should be done to further define the taxon of ARF and possibly to identify them so they could be given a specific name.

Eggs of *R. reniformis* normally develop and hatch 6 to 7 days after they are laid (Sivakumar and Seshadri, 1971). Compared to the 10 days required for eggs of soybean cyst nematode to develop and hatch (Riggs, 1982), the time required for eggs of *R. reniformis* is a rather short interval for a nematophagous fungus to infect and parasitize the eggs of *R. reniformis*. However, light microscopic observations suggest that ARF is a parasite of *R. reniformis*. ARF may produce natural substances that inhibit the embryonic development and hatching of the eggs of *R. reniformis*. Zaki (1994) reported that the nematophagous fungus *Paecilomyces lilacinus* inhibited the hatching of eggs of *M. javanica*.

The greenhouse experiments demonstrated that ARF successfully suppressed the numbers of reniform nematodes. Because the first greenhouse experiment consisted of only one generation and the only nematode developmental stages to be available for parasitism were saccate females and eggs, the suppression of nematode numbers by ARF probably was mostly through killing the female nematodes. Indeed, females of *R. reniformis* parasitized by ARF were observed in the present study. ARF isolated from *H. glycines* has been reported to infect females and juveniles of *H. glycines* (Kim et al., 1998; Timper et al., 1999). Other nematophagous fungi, such as *P. lilacinus, P. chlamydosporium,* and *P. lecanii,* also infected females of cyst nematodes or root-knot nematodes (Freire and Bridge, 1985; Jatala, 1986; Meyer and Meyer, 1996). Therefore, the suppression of reniform nematode by ARF is not just through parasitizing and destroying the eggs but also through killing sedentary females and juveniles.

The ARF fungus was first reported to be a parasite of *H. glycines,* and it has been found widely distributed in

TABLE 3. Shoot lengths and fresh shoot and root weights of cotton plants grown in soil infested with different rates of the nematophagous fungus ARF.

Treatment[b]	Shoot length[a] (cm)	Fresh shoot weight[a] (g)	Fresh root weight[a] (g)
Control	11.5 a	1.3 a	2.3 ab
0.01%	14.1 b	1.6 ab	2.9 c
0.05%	14.5 bc	1.6 ab	1.8 a
0.1%	17.1 d	2.1 c	1.9 a
0.2%	16.2 cd	2.0 bc	2.2 ab
0.3%	17.3 d	2.3 c	2.4 abc
0.4%	15.7 bcd	2.1 c	2.1 ab
0.5%	17.4 d	2.3 c	2.6 bc

Mean values within columns with the same letter are not significantly different at $\alpha = 0.05$ level.

[a] Measurements were taken 50 days after infestation with 3,000 eggs of *Rotylenchulus reniformis* in Experiment 3, which was conducted in a greenhouse in spring 2002.

[b] Treatments are application rates of ARF as % of the soil (w/w).

the southern United States (Kim and Riggs, 1991; Kim et al., 1998). The wide distribution of ARF suggests it is associated with the long history and wide distribution of *H. glycines* in the southern states (Kim et al., 1998). Rotation of cotton with soybean has become a common practice in Arkansas and other southern states. With the gradual increase in distribution of reniform nematode during the past decade in Arkansas, ARF could easily have shifted its host range to include *R. reniformis*. The five ARF strains isolated from *R. reniformis* also parasitize *H. glycines*. A more extensive survey may be necessary to determine the occurrence and distribution of ARF in cotton fields infested with *R. reniformis*.

ARF parasitizes several nematode species including *R. reniformis*. It can live in diverse agroclimatic conditions (Kim et al., 1998), and mass production of this fungus for field-scale application is possible. Even though biological control of nematodes has been inconsistent, slow, and inadequate to meet the needs of farmers (Kerry et al., 1995), once the introduced nematophagous fungus ARF gets established in a field soil, it may provide sustainable management of target nematodes when combined with other control methods, such as use of resistant cultivars or rotations. The potential of ARF as a biological control agent for both *R. reniformis* on cotton and *H. glycines* on soybean is encouraging. Successful field demonstration trials will be necessary before mass production and widespread application can be considered.

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