Effect of *Gliocladium virens* on *Pythium ultimum*, *Rhizoctonia solani*, and Damping-Off of Cotton Seedlings

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ABSTRACT

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A strain of *Gliocladium virens* isolated from the parasitized hyphae of *Rhizoctonia solani*, significantly suppressed damping-off incited in cotton seedlings by this pathogen and by *Pythium ultimum* when the antagonist was placed with cottonseed planted in infested soil. Treatment with *G. virens* more than doubled the number of surviving cotton seedlings grown in soil infested with either pathogen. *G. virens* parasitized *R. solani* by coiling around and penetrating the hyphae. *P. ultimum* was not parasitized by *G. virens*, but was strongly inhibited by antibiosis. Treatment of soil infested with propagules of *R. solani* or *P. ultimum* with *G. virens* resulted in a 63% reduction in the number of viable *R. solani* sclerotia after 3 wk of incubation, whereas oospores of *P. ultimum* were unaffected.

Additional key words: *Gossypium hirsutum*, mycoparasite, biocontrol.

The use of antagonists to control diseases incited by soilborne pathogenic fungi is being intensively studied (11) and may ultimately augment or replace current chemical methods of control. Recently *Trichoderma harzianum* was associated with soil suppressiveness to *Rhizoctonia solani* (3), and *T. harzianum* (4), *T. hamatum* (5), and *Corticium* sp. (9) were reported to successfully protect seedlings from pathogenic fungi. *Sporidesmium sclerotivorum* has been reported to be a mycoparasite of *Sclerotinia minor* (1), and *Gliocladium virens* Miller et al parasitized both *Sclerotinia sclerotiorum* (12) and *R. solani* (13). In the latter case, the severity of white bean root rot was reduced when the antagonist was added to pathogen-infested soil.

In the study reported here, an isolate of *G. virens* found parasitizing a cotton strain of *R. solani* Kühn was used to protect cotton seedlings from damping-off incited by *R. solani* and *Pythium ultimum* Trow. The mode of antagonism toward each plant pathogen is described.

MATERIALS AND METHODS

The mycoparasite used in this work was isolated from the parasitized hyphae of *R. solani* taken from cotton field soil. It was identified as *G. virens* and designated as strain GV-P. The strains of *R. solani* and *P. ultimum* were isolated from field-infected cotton seedlings and designated J_1 and P_1, respectively. Sclerotia of *R. solani* were prepared according to the method of Papavizas and Ayers (10), and oospores of *P. ultimum* were prepared by the method of Ayers and Lumsden (2). The propagules of each fungus were incorporated into unsterile Lufkin fine sandy loam soil with a cement mixer at concentrations of two propagules per gram for *R. solani* and 1,000 propagules per gram for *P. ultimum*. Numbers of propagules were monitored with a multiple pellet soil sampler for *R. solani* (6) and by dilutions on a selective medium for *P. ultimum* (8). Soil was infested 7–10 days before planting.

Preparation of antagonist inoculum. Still cultures of *G. virens* were grown on a sterilized medium (PMCZB) consisting of 50 g of peat moss wetted with 100 ml of Czapek's broth and incubated for 10 days at 25 C. The contents were air-dried, ground to 841-μm (20-mesh) and stored at 5 C until used. Samples were also stored at 25 C in the lab and plated on potato-dextrose agar (PDA) periodically to check viability.

In-furrow treatment with antagonist. Seeds of cotton (*Gossypium hirsutum* L. Stoneville 213') were planted in unsterile soil infested with *R. solani* or *P. ultimum*. *G. virens* inoculum was added as an in-furrow treatment at the rate of 6 g/m of row. Control plantings were treated with ground peat moss. The tests were done in flats of soil in growth chambers with a 14-hr photoperiod and temperatures of 22 C for *R. solani* and 20 C for *P. ultimum*. After 14 days of incubation, counts were made of emerged and surviving seedlings. All treatments were replicated three times and the experiment was done twice.

Effect of antagonist on pathogen propagules. Mature *R. solani* sclerotia in 60-day-old sand-cornmeal cultures (10) were inoculated with PDA plugs of *G. virens* and incubated at 25 C for 30 days. Sclerotia were then washed from the cultures, collected on a 578-μm (35-mesh) sieve and suspended (100 mg/100 ml) in water agar held at 47 C. Ten-milliliter aliquots of the suspension were poured into plates, incubated for 24 and 48 hr, and examined for germinated sclerotia.

Propagules of *R. solani* and of *P. ultimum* were incorporated separately into unsterile soil at the rate of 10 sclerotia and 2,100 oospores per gram, respectively. Infested soil samples were then infested with 1% (w/w) of *G. virens*-PMCZB preparation mixed thoroughly into the soil. The samples were moistened to field capacity (−0.33 bars) at 2-day intervals and incubated for 3 wk at 22 C. The treatments and uninoculated controls were then assayed for numbers of viable sclerotia and oospores. Sclerotia from treated and control soils, recovered by washing the soil over a 578-μm (35-mesh) sieve, were plated on water agar and observed microscopically after 48 hr. Both experiments were conducted with three replicates of each treatment.

In vitro activity of antagonist against *R. solani* and *P. ultimum*. Agar plates of PDA or CSEA medium (7) were inoculated with PDA plugs of *G. virens* and *R. solani* or *G. virens* and *P. ultimum*. The growing cultures were observed macroscopically and microscopically for evidence of antibiosis and mycoparasitism.

RESULTS

The results of in-furrow *G. virens* treatment of cottonseed planted in pathogen-infested and uninfested soil are given in Table 1. Adding *G. virens* to seed planted in soil infested with *R. solani* reduced preemergence damping-off from 55 to 11%. Postemergence damping-off was not significantly different from the control. In soil infested with *P. ultimum* the addition of *G.*

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TABLE 1. Effect of in-furrow treatment with 6 g of *Gliocladium virens* PMCZB[x] per meter of row on damping-off of cotton seedlings in soil infested either with *Rhizoctonia solani* or *Pythium ultimum* or uninfested

Treatment at planting	Damping-off[y] (%)								
	R. solani			*P. ultimum*			Uninfested		
	Preem.[z]	Postem.	Total	Preem.	Postem.	Total	Preem.	Postem.	Total
Peat moss control	55 a	17 a	72 a	50 a	22 a	72 a	17 a	0	17 a
G. virens[x]	11 b	14 a	25 b	19 b	18 a	37 b	13 a	0	13 a

[x] PMCZB stands for peat moss Czapek's broth medium.
[y] Values in each column followed by different letters are significantly different, $P = 0.05$, according to Duncan's new multiple range test.
[z] Preem. and Postem. = preemergence and postemergence.

virens reduced preemergence damping-off from 50 to 19%. Again, postemergence damping-off was not significantly different from that of the untreated controls. Treatment with the antagonist in uninfested soil did not reduce the number of emerged or surviving seedlings compared with the controls.

Introduction of *G. virens* into sand-cornmeal cultures containing mature sclerotia of *R. solani* reduced sclerotial viability from an average of 78 germinated sclerotia per 100 mg of sclerotia in the controls, to eight germinated sclerotia per 100 mg in each of the treated cultures. Viable sclerotia in raw soil infested with *G. virens* was reduced from an average of 9.2/g of soil in the controls to 3.4 germinable sclerotia per gram of soil in the treated samples. Microscopic examination of sclerotia from soil treated with *G. virens* revealed the mycoparasite growing from nonviable sclerotia, but not from viable ones. *P. ultimum* oospore viability in raw soil was not reduced by treatment with *G. virens*.

Examination of the PDA and CSEA cultures in which *R. solani* was paired with *G. virens* did not show any evidence of antibiosis. However, areas where the hyphae of the mycoparasite and phytopathogen comingled contained hyphae of *R. solani* that were empty of cell contents and in various stages of disintegration. As the hyphae of the mycoparasite advanced into areas already occupied by the phytopathogen, numerous sites were observed where the parasite had coiled around and penetrated the host hyphae (Fig. 1A).

On plates where the antagonist was introduced with *P. ultimum*, obvious zones of inhibition occurred around the *G. virens* colonies. When the antagonist overgrew areas already occupied by the phytopathogen, the protoplasm of the *P. ultimum* hyphae became coagulated and disintegrated (Fig. 1B). The envelopment and hyphal penetration observed with *R. solani* did not occur with *P. ultimum*.

Microscopic examination of dried and ground PMCZB cultures of *G. virens* showed that the peat moss particles were heavily infested with chlamydospores. Samples of these cultures have remained in storage at 25 C for 6 mo with no loss in viability.

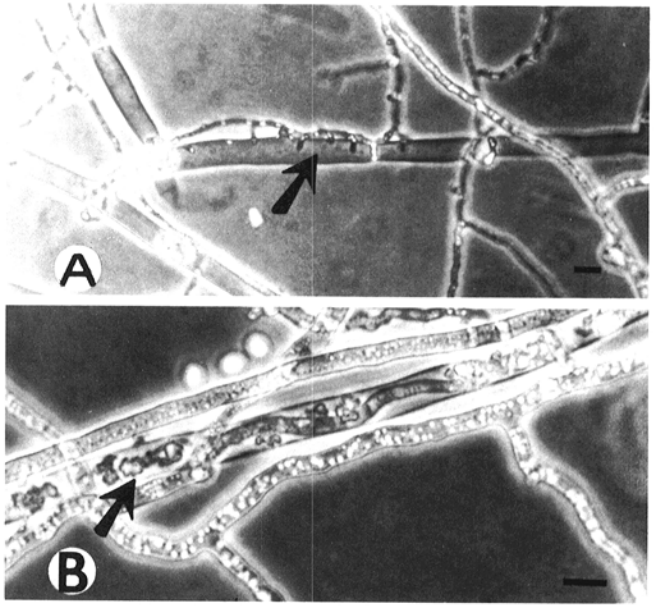

Fig. 1. A, Hyphae of *Rhizoctonia solani* penetrated and parasitized (arrow) by the smaller hyphae of *Gliocladium virens* as they grow along the surface of the host. **B,** Coagulation and disintegration of cytoplasm in a *Pythium ultimum* hypha (arrow) growing in close proximity to *G. virens* hyphae. Bars = 10 μm.

DISCUSSION

Since chlamydospores are resistant to desiccation, the inoculum of *G. virens* can be stored, handled, and applied in a dry form without significant loss in viability. When applied with cottonseed in pathogen-infested soil, *G. virens* significantly reduced seedling disease incited by *R. solani* or *P. ultimum*. The primary effect of *G. virens* on *R. solani* appears to be parasitism of the host hyphae. The adverse effect of *G. virens* on *P. ultimum* appears to be due to antibiosis since no physical parasitization of this pathogen by the mycoparasite was observed.

The colonization of *R. solani* sclerotia by *G. virens* (12), the reduction in sclerotia viability in soil, and growth of the parasite from nonviable sclerotia indicate that *G. virens* is capable of destroying pathogen propagules in field soil. Therefore, treatment with the antagonist not only reduces production of inoculum by reducing disease, but actively reduces the number of preformed pathogen propagules. *G. virens* does not attack and reduce the number of viable *P. ultimum* oospores in the soil prior to planting, but it may prevent an increase in their numbers by suppressing seedling disease.

The characteristics of *G. virens* such as production of resistant chlamydospores that allow dry storage and application, mycoparasitic activity toward *R. solani*, including parasitism of sclerotia in soil, and antibiotic activity toward *P. ultimum* appear to make it an excellent candidate as a treatment for seedling disease prevention. Its use may help facilitate the establishment of uniform stands of healthy cotton seedlings.

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