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

C. R. Howell

Research plant pathologist, USDA, ARS, National Cotton Pathology Research Laboratory, P.O. Drawer JF, College Station, TX 77841. Accepted for publication 20 July 1981.

ABSTRACT


A strain of Gliocadium 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 sclerotiorum has been reported to be a mycoparasite of Sclerotinia minor (1), and Gliocadium 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 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 dilution 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 (PMNZB) consisting of 50 g of peat moss mixed 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-h 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 587-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 587-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.
<table>
<thead>
<tr>
<th>Treatment at planting</th>
<th>R. solani</th>
<th>P. ultimum</th>
<th>Uninfested</th>
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<tbody>
<tr>
<td></td>
<td>Preem.</td>
<td>Postem.</td>
<td>Total</td>
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<tr>
<td>Peat moss control</td>
<td>55 a</td>
<td>17 a</td>
<td>72 a</td>
</tr>
<tr>
<td>G. virsens</td>
<td>11 b</td>
<td>14 a</td>
<td>25 b</td>
</tr>
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1^PMCZB stands for peat moss Czapek's broth medium.
2^Values in each column followed by different letters are significantly different, P = 0.05, according to Duncan's new multiple range test.
3^Preem. and Postem. = preemergence and postemergence.

virsens 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. virsens 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. virsens 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. virsens revealed the mycoparasite growing from nonviable sclerotia, but not from viable ones. P. ultimum ooospore viability in raw soil was not reduced by treatment with G. virsens.

Examination of the PDA and CSEA cultures in which R. solani was paired with G. virsens did not show any evidence of antibiosis. However, areas where the hyphae of the mycoparasite and phytopathogen conjoined 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. virsens 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 enmeshment and hyphal penetration observed with R. solani did not occur with P. ultimum.

Microscopical observation of dried and ground PMCZB cultures of G. virsens 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.

**DISCUSSION**

Since chlamydospores are resistant to desiccation, the inoculum of G. virsens can be stored, handled, and applied in a dry form without significant loss in viability. When applied with cottonseed in pathogen-infested soil, G. virsens significantly reduced seedling disease initiated by R. solani or P. ultimum. The primary effect of G. virsens on R. solani appears to be parasitism of the host hyphae. The adverse effect of G. virsens 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. virsens (12), the reduction in sclerotia viability in soil, and growth of the parasite from nonviable sclerotia indicate that G. virsens 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. virsens does not attack and reduce the number of viable P. ultimum ooospores in the soil prior to planting, but it may prevent an increase in their numbers by suppressing seedling disease.

**LITERATURE CITED**

and symptomless peach trees and in orchard soils in peach tree decline. 
Phytopathology 61:357-360.
Rhizoctonia solani with a soil-inhabiting basidiomycete. 
Phytopathology 70:655-658.
10. Papavizas, G. C., and Ayers, W. A. 1965. Virulence, host range, and 
pectolytic enzymes of single-basidiospore isolates of Rhizoctonia 
12. Tu, J. C. 1980. Gliocladium virens, a destructive mycoparasite of 