Biological Control of Damping-Off Caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in Soilless Mix

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ABSTRACT

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Gliocladium virens controlled damping-off of zinnia, cotton, and cabbage caused by *Pythium ultimum* or *Rhizoctonia solani* in nonsterile soilless mix. This antagonist most effectively controlled disease among 50 isolates of bacteria and fungi, including species of *Pseudomonas, Bacillus, Trichoderma*, and *Penicillium*. Twenty isolates of *G. virens* varied in their efficacy in controlling *P. ultimum* and *R. solani*. Some isolates controlled *P. ultimum* but not *R. solani*, and vice versa. This range of activity suggests a complex mechanism of action that might apply to one pathogen but not the other. Inoculant of *G. virens* routinely was preincubated in the soilless mix before contamination of the mix with pathogen inoculum. Control of *P. ultimum* was effective when sporangial inoculum of the pathogen was introduced at the time of planting the host seed; however, control of *R. solani* required prior contact of *G. virens* with inoculum of *R. solani*. Disease control efficacy lasted for at least 2 mo when *G. virens* was introduced with the pathogen inoculum and the mix was planted with zinnia seeds at intervals. The number of colony-forming units of *G. virens* remained high during the testing period, but the number of pathogen propagules was greatly reduced. The efficacy of the isolates tested, however, was not correlated with the number of colony-forming units of *G. virens*. Sodium alginate formulations of isolate G20 of *G. virens*, selected for control of both pathogens, maintained a high population density in a dry formulation when stored for 2 mo at 4 and 20 C, but not at 30 C. Storage of an alginate formulation at these same temperatures in air-dried soilless mix was not successful. Alginate formulations of *G. virens* added to soilless mix before planting seed show promise as a control for damping-off in the greenhouse production of bedding plants.

Additional keywords: biocontrol, soilborne pathogen.

Damping-off diseases in bedding plant production are commonly encountered in the greenhouse and are primarily caused by the ubiquitous pathogen *Pythium ultimum* Trow and *Rhizoctonia solani* Kühn (11,25–27). Among several *Pythium* spp. that cause damping-off, *P. ultimum* is the most consistently virulent and the most frequently isolated (27). Additionally, *R. solani* (anastomosis group 4) is commonly isolated from bedding plants in midwestern greenhouses (25).

Control of damping-off traditionally has emphasized proper sanitation and manipulation of the environment. Disease control has been improved by the recent introduction of soilless potting media (2,28). Despite the improved control of damping-off, losses are significant, and reliance on chemical fungicides is an accepted practice (11,21,28). However, fungicides are not the most desirable means of disease control, for several important reasons. Fungicides are heavily regulated and vary from country to country in their use and registration (13). Additionally, they are expensive, can cause environmental pollution, and may induce pathogen resistance (11,13). Also, fungicides can cause stunting and chlorosis of young seedlings (11). The effectiveness of chemical fungicides may vary if they interact chemically with planting media or are adsorbed, inactivated, or decomposed by components of the media (28).

The objective of this study was to identify potentially useful antagonistic microorganisms that effectively control the two major causes of damping-off disease, *P. ultimum* and *R. solani*, in greenhouse bedding plant production in nonsterile soilless growing media.

MATERIALS AND METHODS

Antagonist isolates and preparation of inoculant. Several antagonists from the collection of the Biocontrol of Plant Diseases Laboratory and from C. Howell, College Station, TX, were tested for their ability to control Pythium and Rhizoctonia damping-off. These included *Gliocladium virens* Miller et al from several sources (Table 1), hereafter referred to as G1 through G20; *G. catenulatum* Gilm. & Abbott (POPPu2); *Trichoderma harzianum* Rifai (POPS2 and TH-15); *T. viride* Pers. ex Gray (T1R9); *Fusarium solani* (Mart.) Sacc. (POPPa26); *Farrowia longicollea* (Krzem. & Bodura) Hawks (POPPu11); *Penicillium* sp. Link ex Fr. (CHC4B); *Humicola fuscoatra* Traaen (TABPu1); and several isolates of *Pseudomonas* spp. and *Bacillus* spp. All were previously reported to suppress Pythium damping-off (18,19).

Cultures were maintained on V-8 juice agar (200 ml of V-8 juice, 800 ml of water, 1 g of glucose, 20 g of agar, and 6.0 ml of 1.0 N NaOH). Antagonist inoculant usually was added to planting media as 3-day-old cultures on autoclaved wheat bran medium (a 1:1 mixture of bran and water) as previously described (14,16), at the rate of 1%, on a dry-weight basis. Other media used for mycelial cultures contained vermiculite (400 g), yeast (80 g), molasses (48 ml), and water (1,600 ml); and peat moss (720 g), yeast (80 g), molasses (48 g), and water (800 ml). All media were sterilized before the addition of 1×10^7 conidia per 100 g of culture. Incubation was for 3 days at room temperature under cool white fluorescent lights.

Alginate pellets (prills) were prepared as previously described (15,17), but instead of fermenter biomass, 1×10^7 conidia of *G. virens* (G20) were added to 100 ml of a suspension containing 1% sodium alginate, 1% vermiculite, or 5% wheat bran in water. The suspension was dripped into 0.25 M $CaCl_2$, and the gelled prills were rinsed in tap water and air-dried. Prills were added to planting mix at the rate of 1%, on a dry-weight basis.

Pathogen inoculum. Isolate PuZ3 of *P. ultimum* was from a damped-off zinnia seedling (*Zinnia elegans* L.) grown in a soil from Beltsville, MD. Other isolates used were PuCNJ from cabbage and PuCTx from cotton. Cultures were maintained on cornmeal agar, and sporangial inoculum was prepared by a modification of a previously described method (3). Cultures (3 days old on cornmeal agar) were flooded with 10% soil extract prepared as described previously (3). One-month-old cultures were harvested by scraping sporangia from the surface of the plate and blending them for 30 sec in a Tissuemiser (Tekmar, Cincinnati, OH). Sporangia were counted with the aid of a hemacytometer and diluted in 100 ml of tap water to provide 300 sporangia per square centimeter when drenched onto the surface of the planting medium in a 16-× 12-cm flat.

The primary isolate of anastomosis group 4 of *R. solani* (R-85) originally was from a Maryland-grown cucumber seedling; the isolate was recultured from a damped-off zinnia seedling grown in greenhouse soil infested with this isolate. Other isolates used were R2 from cabbage in New Jersey and RDB-1 from cotton in Texas. Inoculum was grown in sterile cornmeal sand (240 g of clean quartz sand, 6.0 g of yellow cornmeal, and 75 ml of water) for 2 wk at 25 C. In each of four replicate flats of planting mix, 1.25 g of inoculum was incorporated.

Inoculum of *P. ultimum* and *R. solani* was also prepared with soilless mix previously infested as described above and cropped with zinnia seedlings to simulate inoculum from naturally infested soil (Fig. 1). The infested soilless mix was diluted with noninfested mix to give about 90% disease.

Biological control bioassay. Biocontrol studies were performed in soilless potting mix (Redi-Earth, W. R. Grace & Co., Cambridge, MA), pH 5.5–6.5, moistened with water to a moisture level of approximately 60%, on a dry-weight basis. The ingredients of the mix were as previously reported (2). Preparations of antagonists were incorporated into moistened, nonsterile soilless mix and incubated for 1 wk at 20–30 C in plastic bags before planting, except when amended soilless mix was held for assessing storage ability or for determining the effect on disease after extended incubation.

To simulate contamination of pathogen-free potting mix, we added inoculum of *P. ultimum* or *R. solani* at seeding, using inoculum prepared as described above. In later experiments, we found that inoculum of *R. solani* could be added before seeding for enhanced biocontrol. Zinnia was selected as the host species because of its susceptibility to the damping-off pathogens and for convenience in handling. The hybrid cultivars Gold Sun or State Fair (Park Seed Co., Greenville, SC) were used. After 40 seeds were planted in each flat (four rows of 10 seeds each), flats (12 × 16 cm) were drenched with sporangia of *P. ultimum* in 100 ml of tap water (300 sporangia per square centimeter). All flats were watered thoroughly. Flats infested with *Pythium* were incubated in a growth room at 15–20 C; flats infested with *Rhizoctonia* were incubated in a growth chamber at 25–30 C. Both were supplied with supplemental fluorescent light, to provide about 60 and 50 W/m^2 (400–850 nm), respectively. Seedling stand was determined for *P. ultimum* after 1 wk (primarily preemergence damping-off) and for *R. solani* after 1 and 2 wk (primarily postemergence damping-off). All tests were repeated at least twice and included at least four replicate flats per treatment.

Population density assays of *G. virens* and the pathogens. Samples of soilless mix were taken at different intervals to determine the population density of *G. virens*. Serial dilutions were prepared after 10 min of vigorous stirring in distilled water, and 1.0- and 0.1-ml samples were spread on the surface of TME semiselective medium (24). Fungal colonies on the agar plates were counted 5–7 days after incubation at 25–28 C under continuous fluorescent light, and colony-forming units (cfu) were calculated per gram (dry weight) of soilless mix.

Statistical analyses. The experiments were arranged in a randomized complete block design and were repeated at least twice. Each treatment contained four replicates. Resultant data from repeated experiments were combined, and statistics performed on the combined data, except with repeated experiments in which differences in sampling times were not identical. Analyses of data were usually done by analysis of variance with separation of means by Duncan's multiple range test. Data expressed in percentages were corrected for uneven distribution by arc sine transformation and back-transformed. Repeated measure analyses with general linear model procedures were done on time-dependent experiments and on population density studies with *P. ultimum* and *R. solani*.

Population densities of *R. solani* and *P. ultimum* were determined with a pellet soil-sampling device (8). Fifteen pellets (5 × 5 mm) were deposited on the surface of a medium selective for *P. ultimum* (6) or water agar containing 50 μg each of streptomycin sulfate and Aureomycin per milliliter for *R. solani*. After 24- and 48-hr incubations at 20 and 25 C, respectively, colonies of *P. ultimum* and *R. solani* growing from the pellets were counted. Regression analysis of values corrected for Poisson distribution was used to analyze relative numbers of propagules of the pathogens during the course of an experiment, as indicated elsewhere (8).

TABLE 1. Range of efficacy of *Gliocladium virens* isolates against damping-off of *Zinnia elegans* caused by *Pythium ultimum* and *Rhizoctonia solani*

Isolate	Previous designation	Percent plant stand[x,y]		Colony-forming units of *G. virens* per gram of soilless mix ($\times 10^6$)[x,z]
		P. ultimum	*R. solani*	
Healthy control	—	88.2 a	86.1 a	<0.1 e
Pathogen control	—	9.4 g	26.6 fgh	<0.1 e
G1	GVRJ-1	29.0 efg	23.6 gh	2.7 d
G2	GL2	77.7 abc	30.0 efgh	5.8 d
G3	GL3	24.5 efg	23.8 gh	4.2 e
G4	GL4A2	26.4 efg	27.4 fgh	15.4 b
G5	MTD356-14	33.7 efg	41.2 cdefgh	8.4 c
G6	GV6	74.5 abc	25.1 defgh	9.0 c
G7	MTD29-1	86.8 a	43.9 cdefgh	5.3 d
G8	GV8	15.6 fg	22.0 gh	9.2 c
G9	GVP	40.6 cdefg	24.5 gh	6.8 c
G10	GVMT	89.5 a	25.7 gh	0.2 e
G11	MTD510-1	51.1 bcde	65.5 bc	8.8 c
G12	MTD356-11	34.9 defg	44.4 cdefg	11.3 b
G13	MTD290-18	41.9 bcdefg	39.9 defgh	10.4 c
G14	MTD138-10	32.5 efg	25.1 gh	13.2 b
G15	MTD189-10	79.4 ab	78.6 ab	5.5 d
G16	MTD31-10	33.7 cdefg	58.4 bc	9.7 c
G17	GL17	20.9 efg	36.7 defgh	8.0 c
G18	GV1828	19.7 efg	51.0 cdef	4.5 e
G19	GVA2-3	13.3 g	20.1 h	37.6 a
G20	GL21	56.5 abcde	54.1 cde	7.9 c

[x] Values in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.01$).

[y] Plant stand was determined 2 wk after planting. Data were transformed to arc sines for statistical analyses and back-transformed.

[z] Colony-forming units per gram of dry soilless mix 1 wk after the addition of 1.0% bran culture and incubation at 60% moisture content.

RESULTS

Biocontrol microorganisms tested. In a preliminary survey, over 50 fungal and bacterial isolates were tested for their efficacy against damping-off pathogens of zinnia. Six isolates of *Gliocladium* spp., 17 of *Trichoderma* spp., 18 of *Talaromyces flavus* (Klöcker) Stolk & Sampson, five of miscellaneous fungi, and 11 of bacteria were tested against Pythium and Rhizoctonia damping-off in soilless mix. The bacterial isolates and the isolates of *T. flavus* and *Trichoderma* spp. were ineffective. Only isolates of *G. virens* significantly improved seedling stand in the presence of *P. ultimum* or *R. solani*. Isolate G20 (previously designated GL21) was selected from this preliminary study because of its ability to control diseases caused by *P. ultimum* and *R. solani* and because of past

experience with this isolate against other diseases (4,5,15–17). Later, when 20 isolates of *G. virens* from different sources were compared (Table 1), G20 was not the best for controlling either pathogen alone, but it was among the best overall for both pathogens. The range of abilities of isolates of *G. virens* to control Pythium and Rhizoctonia damping-off was broad, with results varying from no significant improvement in plant stand over that of the pathogen control, to plant stands statistically equal to that of a healthy, noninfested control. Isolates effective against *P. ultimum* were not always effective against *R. solani*, and vice versa. Population densities of *G. virens* in soilless mix were consistently high (10^6–10^7 cfu/g) for all isolates after 1-wk incubation (Table 1), with the exception of isolate G10, which appeared to sporulate poorly even in vitro, and isolates G3 and G18.

Duration of suppressive effect of *G. virens* against P. ultimum damping-off. Five isolates of *G. virens* were examined for the duration of the effect against *P. ultimum* after initial amendment and planting of zinnias (Table 2). In the initial planting, all isolates

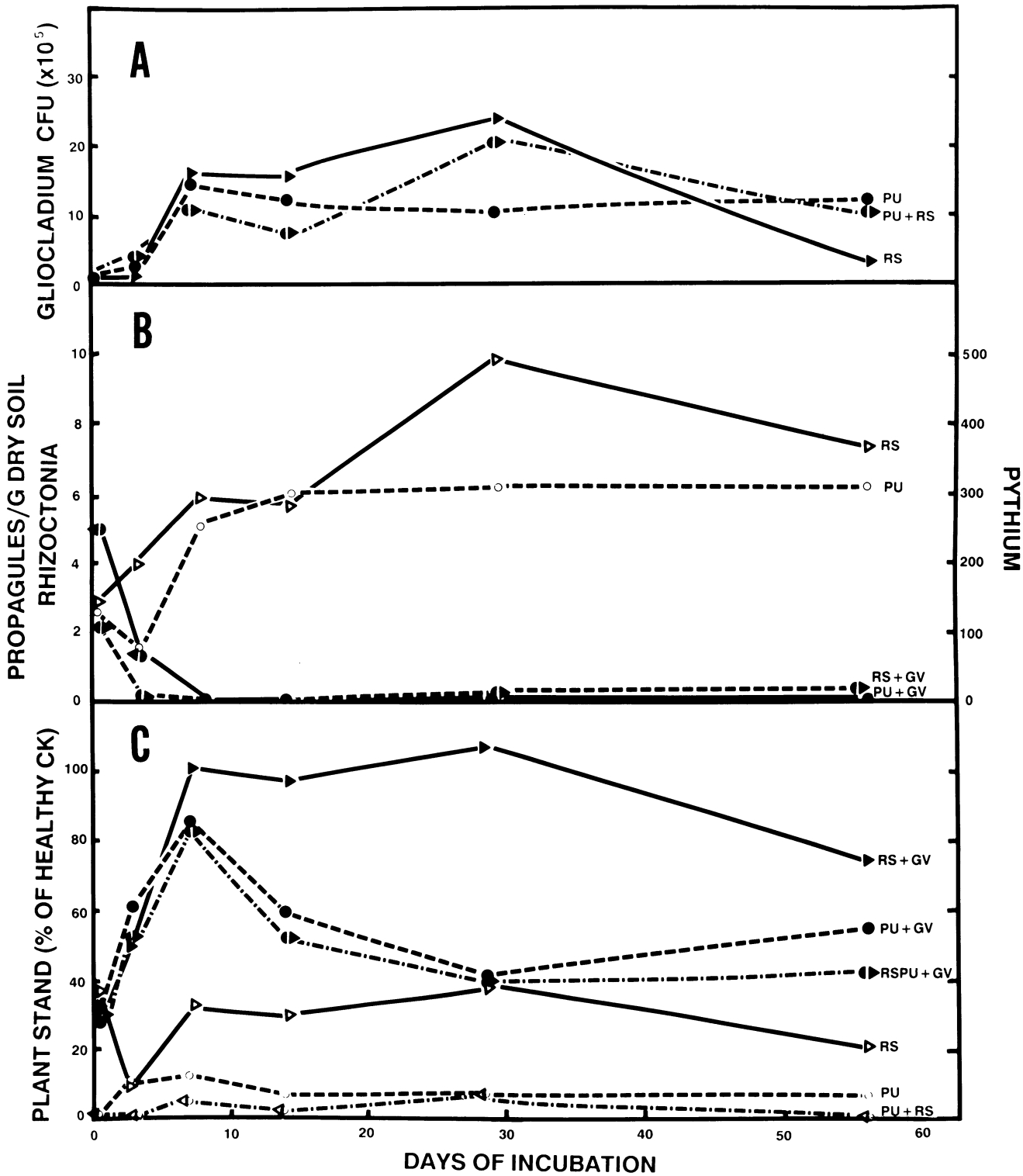

Fig. 1. Colony-forming units (cfu) of *Gliocladium virens* (GV) in a bran culture incubated in soilless mix with *Pythium ultimum* (PU) and *Rhizoctonia solani* (RS) alone and in combination (**A**), effects of the bran culture of *G. virens* on the number of propagules of *P. ultimum* and *R. solani* per gram of soilless mix (**B**), and effects of the bran culture of *G. virens* on damping-off of *Zinnia elegans* in soilless mix caused by *P. ultimum* and *R. solani* alone and in combination (RSPU) (**C**) during a 2-mo incubation. Each pathogen pair with and without *G. virens* was tested for significant differences at zero time and at intervals.

except G3 increased plant stand over that of the pathogen control. Isolates G11 and G20 protected seed and seedlings, resulting in stands statistically equal to that of the healthy control. In subsequent plantings, at which time the remaining plants were removed, the soil of the replicate flats was mixed, and zinnia seed replanted, the effect still was present but diminished. Only G20 retained effectiveness through three replantings, but it no longer was effective in the fourth planting. At that point, however, even the noninfested control had a lower plant stand than in the initial planting, possibly because of cross-contamination.

Effect of timing of infestation with *Rhizoctonia* on biocontrol. Preliminary data indicted that infestation of seed flats at planting often resulted in poorer biocontrol of *R. solani* than of *P. ultimum*. Thus, the time of infestation of soilless mix was tested with two levels of *R. solani* (R-85) after amendment with *G. virens* but before seeding (Table 3). A low level of inoculum of *R. solani* (0.2 g of 2-wk-old cornmeal-sand culture per 700 g of soilless mix, dry weight) was effectively controlled by *G. virens* (G20), at a level statistically equal to that of the healthy control, regardless of the time of infestation. However, when 10 times as much inoculum of *R. solani* was added, disease control was not as effective. Although variable, disease control generally improved with the time allowed for *G. virens* and *R. solani* to interact in the soilless mix before planting. From this, a 3-day preincubation period of *R. solani* inoculum in the antagonist-amended soilless mix was established for maximum treatment efficacy.

Long-term effects of *G. virens* on Pythium and Rhizoctonia damping-off. Bran cultures (3 days old) of *G. virens* added to soilless mix at the rate of 1% (on a dry-weight basis) were effective against damping-off caused by *P. ultimum* and *R. solani* over a period of at least 2 mo (Fig. 1C) when zinnia seed was planted at the time of amendment with *G. virens*. There was no difference between the treatment and the *R. solani* control ($P = 0.64$). However, in the presence of *P. ultimum*, the stand was significantly improved at zero time ($P = 0.005$). After incubation of the amended soilless mix at 20–25 C for 1 wk before planting with zinnia seed, seedling stand was improved significantly with both pathogens, as well as the combination of the two, compared with the stands of the pathogen controls ($P < 0.01$). The effectiveness of *G. virens* against *P. ultimum* and against *P. ultimum* combined with *R. solani* declined after the first week after planting, but plant stand remained significantly higher than in the pathogen controls. After 14 days, the level of disease control with *P. ultimum* remained stable. Disease incidence in the absence of *G. virens* with either pathogen alone or with the pathogens in combination remained high throughout the study period. Detectable pathogen populations remained high in the pathogen controls during the study but were severely depressed in the presence of *G. virens* (Fig. 1B). Pathogen populations were not assessed in the combination treatment. The population density of *G. virens* increased greatly up to 7 days after amendment, remained high ($1–2 \times 10^6$ cfu/g, dry weight) at 28 days, and remained fairly stable thereafter (Fig. 1A).

Effect of alginate formulations on biocontrol. Throughout most of this study, 3-day-old bran cultures of *G. virens* were effective against both pathogens. However, control of Rhizoctonia damping-off was consistently more difficult to achieve than control of Pythium damping-off, and the bran culture was considered to be impractical for ultimate commercial use. Thus, alternative formulations were tested. Several formulations with peat moss and vermiculite were evaluated but had no enhanced effect on control of Rhizoctonia damping-off. Only the formulation containing conidia of *G. virens* (isolate G20) in 2% alginate formed into prills with $CaCl_2$ improved biocontrol of *R. solani* (Table 4). In this case, the alginate prill formulation improved the overall plant stand of seedlings exposed to inoculum of *R. solani*. Also, control of postemergence damping-off caused by *R. solani* was improved substantially by the alginate formulation. An alginate-bran control without added *G. virens* was statistically equivalent to the pathogen control for both pathogens.

Storage ability of *G. virens* in vitro and in soilless mix. Alginate prills containing biomass of *G. virens* were stored at 4, 20, and 30 C in glass jars with screw caps in incubators and at the same temperatures in air-dried soilless mix in plastic bags (30% moisture and about 8×10^4 cfu/g). The population density of *G. virens* (G20) remained relatively high in storage at 4 C in vitro for up to 8 wk (Table 5). However, in storage for 8 wk at 20 and 30 C, approximately 10- and 100-fold reductions occurred, respectively. Storage in semidry soilless mix resulted in variable and seemingly opposite results. Although not statistically different, because of extreme variability, the population density appeared to remain high at 20 and 30 C. *G. virens* was also detected in the autoclaved prill controls. When these semidry soilless mix preparations, with

TABLE 2. Efficacy of *Gliocladium virens* for control of damping-off of *Zinnia elegans* caused by *Pythium ultimum* in repeated plantings

Treatment	Percent plant stand in successive plantings[y,z]			
	1	2	3	4
Healthy control	81.1 a	75.0 a	68.3 a	30.1 a
Pythium control	15.2 d	7.9 e	9.8 cd	6.4 b
Autoclaved bran + *Pythium*	22.1 d	2.5 e	7.9 d	7.5 b
G. virens isolate G3	24.9 d	5.3 e	6.0 d	7.6 b
G. virens isolate G9	46.2 e	7.5 e	12.0 cd	9.8 b
G. virens isolate G11	72.0 ab	19.2 d	22.3 bc	7.2 b
G. virens isolate G17	69.8 b	33.6 c	17.1 cd	5.6 b
G. virens isolate G20	75.7 ab	62.7 b	37.2 b	15.2 b

[y]Values in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.01$). Data were transformed to arc sines for statistical analyses and back-transformed.
[z]After the initial planting, four replicate flats were mixed and replanted for three successive plantings at approximately 2-wk intervals, with 40 seeds per flat.

TABLE 3. Effect of time of infestation with two inoculum levels of *Rhizoctonia solani* in a soilless mix amended with *Gliocladium virens* (isolate G20) on biological control of Rhizoctonia damping-off of *Zinnia elegans*

Time between infestation and planting (days)	Percent plant stand[y]				
	Healthy control	*R. solani* alone[z]		*R. solani* + *G. virens*[z]	
		Low	High	Low	High
0	80.3 a	41.5 b	20.4 b	68.5 a	27.0 b
1	79.7 a	43.0 b	17.8 c	78.5 a	80.7 a
2	86.9 a	29.2 c	11.5 d	79.1 ab	67.3 b
4	83.2 a	21.6 b	15.4 b	69.1 a	81.3 a
7	80.1 a	42.2 b	25.9 c	84.6 a	77.0 a

[y]Values in each row followed by the same letter are not signficantly different according to Duncan's multiple range test ($P = 0.01$). Data were transformed to arc sines for statistical analyses and back-transformed.
[z]*R. solani* (R-85) was added to soilless mix (low = 0.2 g and high = 2.0 g per 700 g of mix) at indicated times before zinnia seed was planted. The mix was amended with a bran culture of *G. virens* at the rate of 1% (on a dry-weight basis) 1 wk before planting.

TABLE 4. Effect of sodium alginate preparations of *Gliocladium virens* isolates on biocontrol of damping-off of *Zinnia elegans* caused by *Pythium ultimum* and *Rhizoctonia solani*

Treatment	Percent healthy plants[z]		Percent postemergence Rhizoctonia damping-off[y]
	P. ultimum	*R. solani*	
Healthy control	82 a	73 a	0 e
Pathogen control	37 d	30 c	59 bc
G20 bran	69 ab	25 cd	61 bc
G17 bran	68 ab	15 d	80 a
G20 alginate-bran	58 bc	44 b	35 d
G17 alginate-bran	53 c	34 bc	48 cd
Alginate-bran control	34 d	20 cd	67 ab

[z]Numbers in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.01$). Data were transformed to arc sines for statistical analyses and back-transformed.

G. virens, were assayed for biocontrol ability, the percent plant stand was improved significantly at zero time. The biocontrol ability of the inoculant was drastically reduced after 2 wk of storage and was eliminated after 8 wk.

Effect of *G. virens* on host-pathogen combinations. Damping-off caused by isolates of *P. ultimum* and *R. solani* from three hosts (cabbage, cotton, and zinnia) was effectively controlled by *G. virens* (Table 6). All three isolates of *G. virens* significantly increased healthy plant stand after 1 and 2 wk of incubation, compared with that of the pathogen control.

DISCUSSION

Of the potential biological control agents tested in this study, *G. virens* most consistently and effectively controlled damping-off caused by *P. ultimum* and *R. solani* in soilless mix. The previous use of *G. virens* for control of several diseases caused by soilborne pathogens is well documented (22). This antagonist has been described as a destructive mycoparasite of *Sclerotinia sclerotiorum* (29), as a biocontrol agent for root rot of beans caused by *R. solani* (30), and as an agent for controlling Pythium and Rhizoctonia damping-off of cotton seedlings (9). In the last-mentioned case, a single isolate of *G. virens* (GVP) effectively controlled both Pythium and Rhizoctonia damping-off of cotton. Although we did not use this isolate for control of cotton damping-off, it was less effective than our isolates for control of zinnia damping-off in soilless mix (Table 1). We selected G20 as an effective isolate for control of diseases caused by both pathogens (Table 1).

The wide range of responses of this collection of *G. virens* isolates to the two pathogens is striking (Table 1). This range of activity suggests a complicated mechanism of action that may involve more than one metabolite, as suggested previously (1,9,10,12). The ability of the various isolates of *G. virens* to grow and sporulate in the soilless mix was similar to its ability to grow in soil as previously reported (14); however, sporulation apparently is not a measure of the ability of individual isolates to control disease. In fact, isolate G10 developed a somewhat lower population density than the other isolates (about 10^5 versus 10^6 cfu/g of soilless mix) and yet was superior for biological control of *P. ultimum*. The ability to control disease is more likely related to the production of specific metabolites or other factors than to the ability to produce fungal reproductive propagules.

The formulation of a fermentation biomass of *G. virens* has been shown to be important in affecting its biocontrol ability (14–17). In this study as well, 3-day-old bran cultures of *G. virens* performed well against damping-off. Because of the impracticality of using freshly prepared cultures in agricultural systems, a formulation better suited for storage and ease of application was needed. The alginate prill formulation previously described (7,15,17) performed well in the soilless mix (Table 4). The viability of *G. virens* in alginate prills in vitro was retained for several weeks in preparations stored at 4 and 20 C. Storage at 30 C, previously reported to be good for *G. virens* (17), was not as good as the lower temperatures for maintaining viability. In contrast to storage in vitro, storage in air-dried soilless mix was not satisfactory, and the preparations did not maintain biocontrol ability (Table 5). In the nonsterile soilless mix, *G. virens* either proliferated and possibly exhausted the food base in the alginate prills or was affected by competition from the resident microflora, including indigenous *G. virens*. It appears that it may not be possible to incorporate alginate prills into soilless mix during manufacture. The formulation can be stored in separate packages and incorporated at planting. However, for commercial application, the viability of

TABLE 5. Longevity and biocontrol ability against *Pythium ultimum* of *Gliocladium virens* (isolate G20) in alginate prills stored in vitro or in soilless mix at several temperatures

Storage time (wk)	Storage temperature or treatment	Percent plant stand[z]	Colony-forming units per gram[y]	
			Storage in vitro	Storage in soilless mix
0[z]	20 C	55.6 b	2.8×10^6 a	1.3×10^6 a
	Autoclaved control	27.3 c	0 b	0 b
	Pathogen control	24.2 c	—	—
	Healthy control	89.1 a	—	—
2	4 C	20.9 b	5.8×10^5 a	8.3×10^3 a
	20 C	30.0 b	5.6×10^5 a	2.2×10^6 a
	30 C	25.3 b	7.1×10^4 b	1.3×10^6 a
	Autoclaved control	17.9 c	—	1.0×10^2 a
	Pathogen control	7.2 c	—	0 b
	Healthy control	74.4 a	—	—
8	4 C	11.8 bc	4.7×10^5 a	1.0×10^5 a
	20 C	17.1 b	1.8×10^5 b	6.2×10^6 a
	30 C	19.9 b	7.8×10^3 b	1.2×10^6 a
	Autoclaved control	7.5 c	—	5.6×10^3 a
	Pathogen control	12.9 bc	—	0 b
	Healthy control	65.1 a	—	—

[y] Values in each column for each time period followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.01$).

[z] Alginate prills at zero time were assayed for colony-forming units before and after being added to soilless mix. Disease control ability was assessed immediately.

TABLE 6. Effect of *Gliocladium virens* isolates on damping-off of cabbage, cotton, and zinnia caused by *Pythium ultimum* and *Rhizoctonia solani* in soilless mix

Pathogen	Host	Treatment	Percent plant stand[z]	
			1 wk after planting	2 wk after planting
P. ultimum	Cabbage	Healthy control	82.7 a	81.6 a
		Pathogen control	10.8 c	9.7 c
		G10	69.9 ab	71.5 a
		G20	65.7 ab	60.9 ab
		G1	56.9 b	48.1 b
	Cotton	Healthy control	84.5 a	85.6 a
		Pathogen control	0.0 c	0.0 d
		G10	80.2 a	74.1 b
		G20	45.6 b	7.4 c
		G1	53.3 b	11.2 c
	Zinnia	Healthy control	64.8 a	61.0 a
		Pathogen control	1.4 d	0.6 d
		G10	46.2 b	46.9 b
		G20	44.3 b	43.1 b
		G1	20.9 c	20.3 c
R. solani	Cabbage	Healthy control	94.6 a	90.4 a
		Pathogen control	82.7 b	81.6 b
		G10	92.8 a	94.9 a
		G20	93.5 a	94.6 a
		G1	93.2 a	93.8 a
	Cotton	Healthy control	84.5 a	85.7 a
		Pathogen control	0.6 c	0.0 c
		G10	65.5 b	17.4 b
		G20	78.3 ab	77.2 a
		G1	74.5 ab	36.4 b
	Zinnia	Healthy control	64.8 ab	51.3 a
		Pathogen control	18.5 c	16.5 c
		G10	56.9 b	42.0 b
		G20	55.0 b	55.0 ab
		G1	70.7 a	63.2 a

[z] Values for each host-pathogen combination for each time period followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.01$). Data were transformed to arc sines for statistical analyses and back-transformed.

G. virens stored at room temperature or above may have to be improved.

The amendment of soilless mix with *G. virens* resulted in an extended protection of seedlings from *P. ultimum* and *R. solani* (Fig. 1 and Table 3). Protection from damping-off diseases is most critical during the early stages of seedling development, because seed and seedlings are most susceptible to disease at this time. *G. virens* protects seedlings for several weeks, at least from an initial infestation. These studies did not address the question of whether seedlings would be protected against inoculum of the pathogens introduced at later times. It was clear that, with *R. solani*, a short period of incubation of the pathogen with the antagonist was required for disease control (Table 3).

Augmentation with biological control agents is recognized as a plausible approach to disease control (23). The approach of introducing *G. virens* into soilless mixes, which are increasingly used in the bedding plant industry, is an excellent application of a biological control measure to reduce or prevent losses caused by damping-off pathogens. Since the greenhouse environment is relatively controlled and soilless mixes are reasonably uniform, the ecological interaction between pathogen-host-antagonist and resident microbiota should be the least complicated. The concept that the more ecologically simple systems are the most amenable to biological control (20) could ideally be applied to the greenhouse production of bedding plants in soilless mixes or pasteurized soil. The applicability of *G. virens* to commercial systems will have to await further evaluation and testing.

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