Influence of Pyrax/Biomass of Biocontrol Fungi on Snap Bean Damping-off Caused by *Sclerotium rolfsii* in the Field and on Germination of Sclerotia

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ABSTRACT

Lewis, J. A., and Fravel, D. R. 1996. Influence of Pyrax/biomass of biocontrol fungi on snap bean damping-off caused by *Sclerotium rolfsii* in the field and on germination of sclerotia. Plant Dis. 80:655-659.

A 2-year field study at Beltsville, MD, of soil artificially infested with sclerotia of *Sclerotium rolfsii* strain Sr-1 demonstrated the ability of fermentor-produced biomass of *Gliocladium virens* isolate Gl-3 in a powder formulation to prevent damping-off of snap beans caused by this pathogen. Plant stands were counted 11 and 35 days after planting. In addition, the CFU of Gl-3 per g of soil in the treatment plots were determined. Pyrax/biomass amended at rates of 15, 30, 60, and 120 g/1.1 m^2 plots to provide 0.6 to 6.6 × 10^4 CFU of Gl-3 per g of soil significantly increased plant stands after 35 days, compared with 7 and 19% stands in the pathogen-infested control soils for 1992 and 1993, respectively. In 1992, the stand increase was correlated (r^2 = 0.92) with increased rates of the preparation, such that 60 and 120 g of the Pyrax/biomass per plot resulted in stands comparable to those (>85%) in the noninfested control plots. In 1993, although there was no significant correlation (r^2 = 0.601) between rate of amendment and plant stand, all rates gave stands greater than that in the infested control but not as great as that in the noninfested control. Generally, soil populations of Gl-3 increased by 11 days with higher, but not lower, rates of Pyrax/biomass to about 10^5 CFU/g soil during both years. Population levels tended to decline after 35 days of plant growth, but generally remained higher than the amounts added. This population increase suggested establishment of Gl-3 in the soil. A study to determine the influence of Pyrax and biomass of various isolates of *Trichoderma* spp. and *G. virens* on the germination of sclerotia of two *S. rolfsii* isolates (Sr-1 and Sr-3) indicated considerable specificity. *G. virens* isolates were more effective in reducing sclerotial germination than were isolates of *T. viride, T. hamatum,* and *T. harzianum.* Moreover, isolate Gl-3 was more effective that the other *G. virens* isolates. In addition, *S. rolfsii* isolate Sr-3, which produces larger and darker sclerotia than those of Sr-1, was less affected by the various isolates of *Trichoderma* spp. and *G. virens* than was Sr-1.

Sclerotium rolfsii Sacc. is a ubiquitous, endemic soilborne plant pathogen that can attack a wide variety of crops and wild plants in semi-tropical and tropical areas of the world (3,29). The pathogen causes seedling damping-off, blight, and stem rot, which results from infection of plants by germinating sclerotia on or near the soil surface. These sclerotia, produced abundantly on the infected plant, are the resistant, over-wintering survival structures of the pathogen.

Current chemical control approaches are often ineffective, uneconomical, or unavailable (13,29). In addition, the use of various pesticides, including fungicides, may adversely affect the quality of the crop as well as the environment. Consequently, disease control alternatives, especially those that utilize biological approaches, either alone or within an integrated management system, are being actively investigated. Various preparations of the biocontrol fungi *Trichoderma* spp. and *G. virens* and bacteria have been used individually or with soil solarization or seed treatment in field tests to reduce vegetable diseases caused by *S. rolfsii* (5,8,14,17,23, 31,32,34). Several reports (5,17) discuss the fact that biocontrol ability was specific with regard to not only the biocontrol agent, but also the isolate of *S. rolfsii.* Preliminary work in the Biocontrol of Plant Diseases Laboratory (BPDL) determined that *G. virens* isolate Gl-3 was one of the most effective biocontrol fungi against *S. rolfsii* isolate Sr-1 (26).

Successful biocontrol depends largely on the use of appropriate formulation and delivery systems of efficacious agents (22). Recent reports have reviewed the technological advances in these systems, including the use of microbial biomass for seed treatments, granules or pellets, powders, and solid matrices containing activated biomass (6,18,19). One of the easiest and most effective formulations to prepare is a mixture of inert clay carrier, such as Pyrax, with a fermentor-produced powdered biomass (25). These preparations, made with isolates of *Trichoderma* spp. and *G. virens,* have reduced Rhizoctonia diseases of potatoes (4) and cotton (16) and damping-off and blight of snap bean caused by *S. rolfsii* (17,26).

This present study describes the effect of various rates of Pyrax/biomass of *G. virens* isolate Gl-3 on the damping-off and blight of snap beans in the field caused by *S. rolfsii* isolate Sr-1 during a 2-year period, and on the inoculum density of *G. virens* isolate Gl-3. Data are also presented on the influence of soil amendment with Pyrax and biomass of various isolates of *Trichoderma* spp. and *G. virens* on the germination of sclerotia of *S. rolfsii* isolates Sr-1 and Sr-3. A preliminary report has been presented (15).

MATERIALS AND METHODS

Soils and fungal inocula. Soil for both greenhouse and field tests was a loamy sand from Beltsville, MD: pH 6.4, 0.5% organic matter, 84% sand, 8% silt, and 8% clay. For the germination studies, soil was brought to the greenhouse, passed through a 3-mm mesh screen, moistened to about –0.3 mPa, and kept moist in a greenhouse bench (18 to 24°C) for at least 1 week.

Two strains of *S. rolfsii* Sacc. were used in these studies. Strain Sr-1, originally isolated from bean, was from the collection of the BPDL and strain Sr-3, originally isolated from peanut, was from R. Rodriguez-Kábana, Auburn University, Auburn, AL. Sclerotia of Sr-1 are tan in color and about 1.0 mm in diameter, whereas sclerotia of Sr-3 are black and measure 2.0 to 3.0 mm in diameter (9,15,17,24,26). Cultures were incubated on 9-cm-diameter petri plates of commercial potato dextrose agar (PDA) and in the light at 22 to 25°C. After 30 days, sclerotia were dislodged from the surface of the PDA plates with a soft brush and used immediately (26).

The biocontrol fungi used in this study were from the collection of the BPDL and included the following: *T. hamatum* (Bonord.) Bainier, isolates Tm-34, TRI-4; *T. viride* Pers.:Fr., isolates WT-6, Tv-1; *T. harzianum* Rifai, isolates Th-31, Th-77, Th-87; and, *G. virens* J. H. Miller, J. E. Giddens, & A. A. Foster (= *T. virens*), isolates Gl-3, Gl-21, and Gl-32. Cultures were

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Accepted for publication 1 March 1996.

Publication no. D-1996-0401-04R

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maintained on PDA and incubated in light at 22 to 25°C. Production of biomass abundant in chlamydospores of the isolates was accomplished in a molasses/brewer's yeast medium with subsequent drying and milling of the fungal biomass, as described in detail by Papavizas et al. (25). CFU per g of milled biomass were as follows: Tm-34, 5.1×10^3 (3.71 $\log_{10}$); TRI-4, 8.2×10^4 (4.91 $\log_{10}$); Th-31, 3.3×10^4 (4.52 $\log_{10}$); Th-77, 1.1×10^4 (4.04 $\log_{10}$); Th-87, 1.6×10^5 (5.2 $\log_{10}$); WT-6, 7.1×10^4 (4.85 $\log_{10}$); Tv-1, 3.5×10^6 (6.54 $\log_{10}$); Gl-3, 4.4×10^6 (6.64 $\log_{10}$); Gl-21, 3.0×10^6 (6.48 $\log_{10}$); and Gl-32, 7.1×10^3 (3.85 $\log_{10}$). CFU were determined on a semiselective medium (27).

Pyrax/biomass formulations were prepared by mixing Pyrax ABB (R. T. Vanderbilt, Norwood, CT), a pyrophyllite silicate of 6.5, with biomass of the fungus (9:1, wt/wt) on a ball mill to blend the biomass with the carrier. (4,25). This provided 100 mg of biomass per g of formulation.

Biological control in the field. The effect of various rates of Pyrax/biomass of Gl-3 on damping-off of snap beans caused by *S. rolfsii* isolate Sr-1 was studied in field trials in 1992 and 1993. The field was seeded with rye (*Secale cereale* L.) in the autumn and this cover crop was incorporated into the soil to a depth of 15 cm in the spring. Other than metalaxyl as a seed treatment, no pesticides were used. Soil treatments were established in a completely randomized design with four replications.

During the last week of July, 0.9 × 1.2 m plots (1.1 m^2) were fertilized with 10-10-10 fertilizer (60 kg N/ha) and infested with a mixture of 10 g of *S. rolfsii* (Sr-1) sclerotia in 120 g of fine quartz sand moistened with 10 ml of water by broadcasting inoculum onto all plots except the four noninfested control plots. All plots were then hand-raked to a depth of 5 cm. At the same time, biocontrol preparations of Pyrax/biomass were applied broadcast at rates of 15, 30, 60, or 120 g per plot and then raked into the soil to a depth of 5 cm. These rates provided 0.6×10^4, 1.0×10^4, 2.9×10^4, and 6.6×10^4 CFU of Gl-3 per g of soil in 1992, and 1.0×10^4, 1.4×10^4, 4.5×10^4, and 6.3×10^4 CFU of Gl-3 per g of soil in 1993. Controls consisted of noninfested plots and pathogen-infested plots with Pyrax (120 g per plot) without fungal biomass. One week later, metalaxyl-treated snap bean (*Phaseolus vulgaris* L.) seeds (cv. Bush Blue Lake 274) were planted in plots with three rows of 25 seeds per row. The plots were irrigated with overhead sprinklers following the addition of Sr-1 inoculum and every 4 to 5 days thereafter as necessary. Plant stand was counted 11 and 35 days after planting and the extent of pre- and postemergence damping-off was determined. Plots were hand-cultivated as needed to remove weeds.

At the time of soil infestation, and 11 and 35 days after planting, 5-g (dry weight equivalent) portions of sieved soil were collected to determine population densities of *G. virens*. Serial dilutions of soil in water were prepared and 1-ml samples were immediately spread on the semiselective medium (27). Characteristic colonies of *G. virens* were counted after 7 to 10 days of incubation at 22 to 24°C under continuous fluorescent light.

Germination of *S. rolfsii* sclerotia. Moist soil (200-g dry weight equivalent) was infested with fresh Sr-1 or Sr-3 sclerotia to give 25 to 35 sclerotia per 40 g of dry soil. Portions of this soil were used for subsequent assays. Concurrently, soils were amended with Pyrax/biomass preparations of 10 isolates of *Trichoderma* spp. and *G. virens* at rates of 1.0 g and 4.0 g of preparations for 100 g of soil (dry weight equivalent). Controls consisted of sclerotia-infested soils amended with Pyrax containing no biomass. The amended soils were mixed and placed in glass beakers covered with polyethylene film that was punctured with a needle to permit gas exchange and incubated in the laboratory in a randomized block design at 22 to 24°C for 2 weeks. The experiment was performed twice, each with four replicates.

The sclerotial germination assay used was a modification of two methods based on the ability of methanol to stimulate sclerotial germination (32,33). After the 2-week incubation period, soils were air-dried for 1 day and four 40-g portions of each replicate were spread evenly in 15-cm-diameter plastic petri plates. Soils were misted with 1% methanol until moist and the plates were placed in plastic bags. After 5 days of incubation at 22 to 24°C, the number of sclerotia of *S. rolfsii* isolates Sr-1 and Sr-3 that germinated in the soil was counted.

Statistical analyses. Data were analyzed after arcsine transformation by analysis of variance using the Statistical

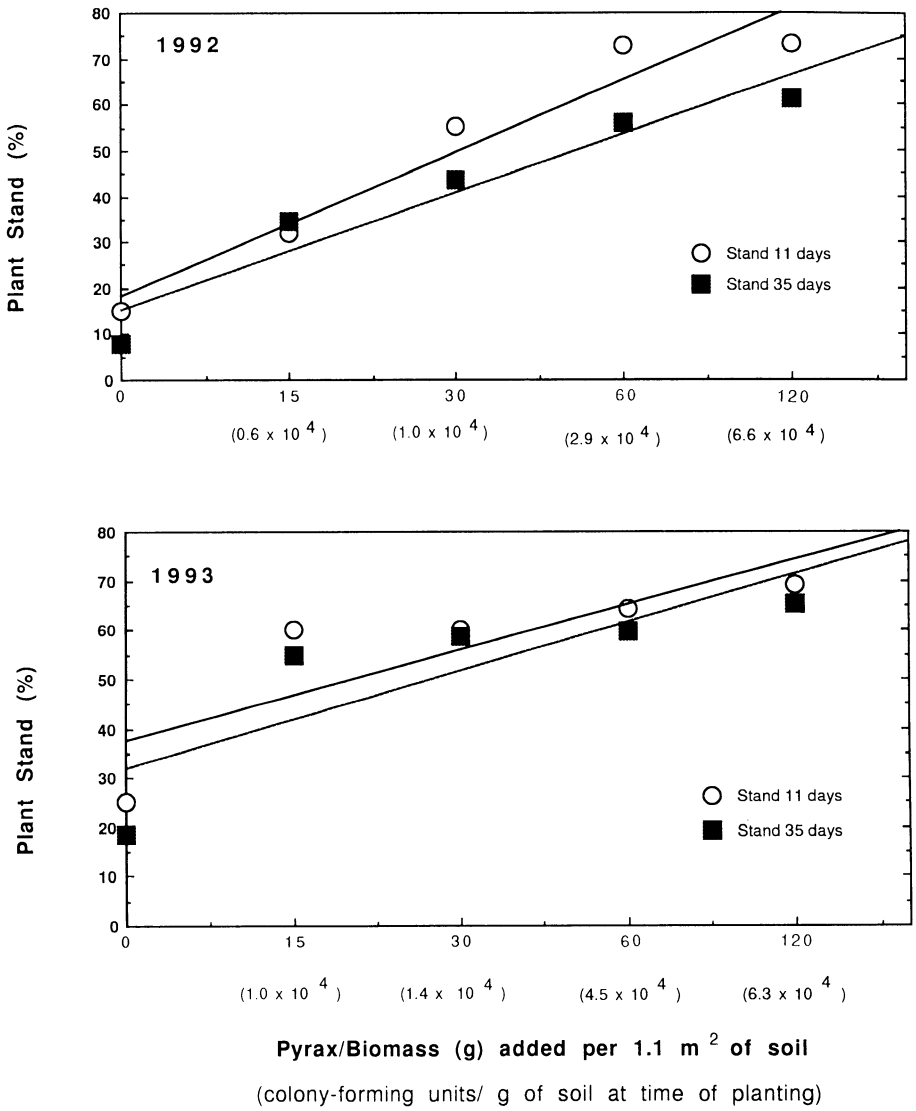

Fig. 1. Relationship between rate of soil amendment by Pyrax and biomass of *Gliocladium virens* (isolate Gl-3) and snap bean stand in the field during 1992 and 1993. Regression equations for 11 and 35 day stands in 1992: $y = 18.15 + 1.05x$, $r^2 = 0.88$; $y = 15.09 + 1.85x$, $r^2 = 0.85$. Regression equations for 11 and 35 day stands in 1993: $y = 37.43 + 0.61x$, $r^2 = 0.35$; $y = 31.79 + 0.66x$, $r^2 = 0.35$.

Analysis System (SAS) (SAS Institute, Cary, NC). Means were with Duncan's multiple range test and regression analyses were used to establish relationships when necessary.

RESULTS

Effects of Pyrax/biomass of Gl-3 on snap bean damping-off in the field. The stands of beans after 11 days of growth in noninfested, control field plots in 1992 and 1993 were 88 and 89% of the seed planted, respectively. Since these values were similar to those obtained from agar-dish and paper-towel germination assays, little of the seed was nonviable or affected by preemergence damping-off caused by native soilborne plant pathogens. However, after 35 days of growth, the stands in 1992 and 1993 in these control plots were 60 and 58%, respectively. The 35-day stands were significantly less ($P < 0.01$) than the stands at 11 days of growth. This 30% stand reduction was due to microorganisms in the soil, other than *S. rolfsii*, capable of causing postemergence damping-off. In plots infested with *S. rolfsii* and amended with Pyrax without biomass of Gl-3, the plant stands were 16 and 27%, respectively, after 11 days of growth in 1992 and 1993. After 35 days of growth, the stands in the 2 years were 17 and 19%, respectively. The stands at 35 days were not different from the stands at 11 days ($P > 0.05$). Consequently, damping-off of beans in infested plots was due to the preemergent activity of *S. rolfsii*. Stands for both years were similar for each sampling time ($P < 0.05$).

In 1992, plant stand was significantly increased by incorporation of Gl-3 ($P < 0.001$). At both 11 and 35 days after planting, amending soils with increasing rates of Pyrax/Gl-3 biomass increased plant stand ($r^2 = 0.88$ and $r^2 = 0.85$, respectively) (Fig. 1). In addition, at both dates, Pyrax/biomass at rates of 60 g and 120 g per plot resulted in plant stands comparable to those in noninfested control plots ($P < 0.05$). Even the lowest rate of 15 g of Pyrax/biomass per plot gave plant stands greater than those in the pathogen-infested soils at both days of growth ($P < 0.05$).

Results in 1993 (Fig. 1) were similar to results in 1992. Incorporation of Gl-3 significantly increased plant stand compared with the treatment in which Gl-3 was not incorporated ($P < 0.001$) but there was no difference between stands at 11 and 35 days of growth. In 1993, at both dates of assessment of plant stand, all rates of Pyrax/biomass amended to the plots resulted in bean stands greater than those in the pathogen-infested plots, but there was no relationship between amendment rate and plant stand ($r^2 = 0.35$ and $r^2 = 0.35$ for 11 and 35 days, respectively). Moreover, although rates of 15 g and 120 g Pyrax/biomass per plot resulted in similar stands, no treatment ever gave stands comparable to those in the noninfested control plots for the two dates. In both years, there was no significant postemergence damping-off at either sampling time. This observation further substantiates that the disease incidence was due primarily to preemergence damping-off caused by the pathogen.

Population dynamics of Gl-3 in plots amended with Pyrax/biomass. Population levels (CFU/g of soil) of Gl-3 in field soil in 1992 and 1993 amended with the four rates of Pyrax/biomass (15, 30, 60, 120 g per plot) during the 35-day period after planting ranged from approximately 10^3 to 10^5 CFU/g of soil (Table 1). For each year, there were significantly higher populations of Gl-3 with higher amounts of added Pyrax/biomass ($P < 0.01$). In addition, populations during both years varied according to the time of assay ($P < 0.001$). Generally, populations increased when assayed 11 days after planting compared with populations at the time of infestation. In most cases, populations at the 35-day assay period were less than those at the 11-day assay period, although the higher rates of Pyrax/biomass (60 and 120 g/1.1 m^2) resulted in populations at 35 days that were higher than those at infestation. With respect to populations of Gl-3, there was a significant interaction between rate of Pyrax/biomass amendment and day of assay in 1992 ($P < 0.03$) and 1993 ($P < 0.02$). There was no effect of year ($P = 0.76$) on the results.

Effect of Pyrax/biomass of various isolates of *Trichoderma* spp. and *G. virens* on germination of *S. rolfsii* sclerotia. There were highly significant effects ($P < 0.0001$) between isolates of *S. rolfsii*, the antagonists studied, CFU per g of soil (population) of the antagonists, and the interactions between *S. rolfsii* isolate and antagonists on sclerotial germination of the pathogen. There also was a significant interaction between *S. rolfsii* isolate and antagonist population on sclerotial germination. This indicated that the importance of population levels of the antagonists differed among *S. rolfsii* isolates. As populations of Gl-3, Gl-21, and Gl-32 increased, germination of Sr-1 sclerotia decreased (Table 2). For the other isolates, within the population range tested, increasing populations of the antagonists had no effect on Sr-1 germination. With Sr-3, only Gl-3 and TRI-4 showed any decrease in sclerotial germination with increasing amounts of the antagonist. For both isolates of *S. rolfsii*, isolates of *G. virens* were more effective than isolates of *Trichoderma* spp. in reducing germination of sclerotia of the pathogen.

DISCUSSION

Although this study confirmed a previous field trial in which a Pyrax/biomass formulation of *G. virens* isolate Gl-3 prevented snap bean damping-off caused by *S. rolfsii* (17), it also demonstrated that rates less than those previously used could significantly reduce disease incidence. Pyrax is a suitable carrier for the biomass because it is inexpensive and is the result of neutralized clay (talc), which makes it compatible with a biocontrol fungus. The biomass contains insoluble nutrients trapped with the biomass during filtration. These nutrients are sufficient to stimulate the proliferation of populations of Gl-3 in the soil.

The 1992 data indicated that increasing antagonist inoculum resulted in increased plant stands. For example, the rates of Pyrax/biomass that provided 2.9×10^4 CFU of Gl-3 per g of soil (60 g of product per plot), and 6.6×10^4 CFU of Gl-3 per g of soil (120 g of product per plot), respectively, resulted in stands comparable to those in the noninfested control plots (88%). In the 1993 trial, the data differed from the 1992 results in that no rate response was found. A rate of product (15 g per plot) that provided 1.0×10^4 CFU of Gl-3 per g of soil resulted in a plant stand comparable to that achieved by 6.3×10^4 CFU of Gl-3 per g of soil (120 g of product per plot). Although the stands with these rates ranged between 65 and 75%, these

Table 1. CFU of *Gliocladium virens* (isolate Gl-3) at day of planting (0) and 11 and 35 days after planting in field plots amended with four rates of Pyrax/biomass formulations in 1992 and 1993

		CFU (log_{10})	
Pyrax/biomass (g/1.1m2 of soil)	DAP[y]	1992	1993
15	0	3.81 a[z]	4.04 a
	11	3.80 a	3.97 a
	35	3.91 a	3.50 b
30	0	4.01 b	4.16 b
	11	4.32 a	4.48 a
	35	4.16 b	4.05 b
60	0	4.46 c	4.65 b
	11	5.12 a	4.74 a
	35	4.79 b	4.40 b
120	0	4.82 c	4.80 c
	11	5.54 a	5.71 a
	35	5.19 b	5.12 b

[y] Days after planting.
[z] Numbers within each column for each rate followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

stands were about 25% less than the 90% stand in the control plots not infested with the pathogen. It is of interest to speculate why plant stands in 1993 were greater than those in 1992 in control infested plots and when 15 g of product per plot was used. Perhaps indigenous antagonists to *S. rolfsii* developed in 1993 as a result of the management practices in 1992. A similar phenomenon was observed with the antagonist *Sporidesmium sclerotivorum* in plots continually planted with lettuce and infested with *Sclerotinia minor* (1).

The rates of product used in 1992 and 1993 provided about 10 times more inoculum than the rates provided in a previous study (17). In that study, Pyrax/biomass preparations that provided 1.2×10^3 CFU of Gl-3 per g of soil effectively prevented disease under both greenhouse and field conditions. In addition, Pyrax/biomass of Gl-3 added to soil in the greenhouse to provide 6.0×10^3 CFU of the biocontrol fungus per g of soil controlled *S. rolfsii,* resulting in snap bean stands that were comparable to those in the noninfested soil (26). This may further suggest the presence of indigenous antagonists that compete with Gl-3 in the present study. Nevertheless, the data from all the years of field study suggested the effective biocontrol potential of Pyrax/biomass of Gl-3.

With regard to the field dynamics of Gl-3 populations after the addition of Pyrax/biomass, the significant increase in CFU of the biocontrol fungus per g of soil after 11 days of plant growth, especially with the higher rates of the amendment, was consistent with a previous field observation (17). It was also shown, as previously (17), that although the CFU of Gl-3 per g of soil declined after 35 days of growth, the CFU remained similar to those of the biocontrol fungus when it was initially added or remained significantly higher than these levels. This would suggest the ability of the biocontrol fungus to establish itself in the soil. Again, this is similar to the long-term effect observed with the establishment of the mycoparasite *S. sclerotivorum* against *S. minor* (1).

Due to the difficulty of assessing field populations of *S. rolfsii*, our investigation did not involve assay of pathogen inoculum in the field as a result of biocontrol (30). However, this area should be explored. Recently, populations of *S. rolfsii* were shown to decrease in soybean fields after application of *T. harzianum* and *T. koningii;* this was correlated with an increase in soybean yield (7). In tomato fields, *G. virens* reduced numbers of *S. rolfsii* sclerotia by up to 100% at a 30-cm depth in a 3-year study (32). Decreased *S. rolfsii* populations were also associated with decreased disease on peanuts and increased yields in field plots treated with *T. harzianum*, fungicides, and an onion crop rotation (2).

Our results also demonstrated the importance of specificity in biocontrol. For example, Pyrax/biomass preparations with isolates of *G. virens* were more effective than those with *Trichoderma* spp. in reducing germination of sclerotia of *S. rolfsii* isolates Sr-1 and Sr-3. This is consistent with other observations in which isolates of *T. viride* and *T. hamatum* were ineffective in suppressing disease of snap beans caused by *S. rolfsii* in the greenhouse. Also, although isolates of *T. harzianum* were less effective than isolates of *G. virens* (26), some *T. harzianum* isolates were more effective than others. Other studies have shown the specificity of isolates of *Trichoderma* spp. and *G. virens* on the Rhizoctonia disease of cotton (16), potatoes (4), and ornamentals (20). Of 15 isolates of *T. harzianum* and *G. virens*, one isolate of *T. harzianum* and three of *G. virens* were highly effective against *S. rolfsii* on peanut (21). Moreover, the present report also shows that *G. virens* isolate Gl-3 was generally more effective than the other isolates of *G. virens* studied.

To further complicate the problem of specificity, germination of isolate Sr-1 sclerotia was more affected by *G. virens* isolates than was germination of isolate Sr-3 sclerotia. It has been postulated that the ability of antagonists to destroy sclerotia depends on the size and melanin content of the sclerotia (11,12,24). For example, as in the present study, the larger and darker the sclerotia (e.g., Sr-3), the less invasion and reduction in germination of sclerotia, compared with those that are smaller and tan in color (e.g., Sr-1). Germination of sclerotia may also not be entirely related to their colonization and penetration by isolates of *Trichoderma* spp. and *G. virens* (9, 10,11). Although large sclerotia could be infected by these isolates, infection did not always result in destruction of the sclerotia.

Since formulation technology is critical in the implementation of biocontrol, the use of the powdered clay Pyrax with milled biomass of a biocontrol agent represents a feasible approach for the development of a product that can be easily delivered. The product can be prepared by standardized methods (28) with no aseptic technique required except for preparation of the biomass (25). With the expansion of formulation technology, other suitable systems used for reduction of various diseases caused by *S. rolfsii* include alginate prill, activated mycelium on vermiculite/ bran, infested grain products, and mycelial powders of *Trichoderma* spp. and *G. virens* (14,17,21,31,32). The entire area of formulation technology requires additional research efforts. The use of Pyrax/biomass as a broadcast, in-furrow, or seed or tuber application should be particularly exploited, especially with diseases of high value crops caused by sclerotial pathogens such as *S. rolfsii* that appear quite amenable to biocontrol.

Table 2. Effect of Pyrax/biomass formulations of isolates of *Trichoderma* spp. and *Gliocladium virens* on germination of sclerotia of two isolates of *Sclerotium rolfsii* (Sr-1 and Sr-3)

	CFU[x]		Germinated sclerotia (% of control)[y]	
Isolate	**1.0 g**	**4.0 g**	**Sr-1**	**Sr-3**
Control	0		100 abcd[z]	100 ab
		0	100 abcd	100 ab
T. hamatum	Tm-34	2.71	85 abcde	93 abc
		3.04	82 bcde	79 cdef
	TRI-4	3.91	77 cde	87 bcd
		4.44	72 de	68 efgh
T. harzianum	Th-31	3.52	84 bcde	92 abcd
		4.12	82 bcde	80 cde
	Th-77	3.04	96 abc	86 bcd
		3.65	105 a	100 ab
	Th-87	4.20	94 de	105 a
		4.81	76 cde	80 bcd
T. viride	WT-6	3.85	73 de	82 cde
		4.45	76 cde	88 bcd
	Tv-1	5.54	92 abcd	93 abc
		6.15	76 cde	82 cd
G. virens	Gl-3	5.64	24 g	57 h
		6.25	5 g	8 i
	Gl-21	5.48	47 f	84 cd
		6.09	23 g	63 gh
	Gl-32	2.85	46 f	66 fgh
		3.48	50 f	77 defg

[x] Of isolates provided by 1.0 g and 4.0 g of formulation per 100 g of soil, respectively. Control formulations contained 0.80 g and 3.3 g of Pyrax without biomass per 100 g of soil, respectively.

[y] In Sr-1 control soil, amended with 0.80 g and 3.3 g of Pyrax per 100 g of soil, 21 and 26 sclerotia out of 30 germinated, respectively; in Sr-3 control soil, amended with the same amounts of Pyrax, 23 and 22 sclerotia out of 30 germinated, respectively.

[z] Numbers within each column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

ACKNOWLEDGMENTS

We thank Karen Stanley, Martha Hollenbeck, Dawn Rogers, and Cara McCormack for their expert laboratory and field assistance.

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