A Formulation of *Trichoderma* and *Gliocladium* to Reduce Damping-off Caused by *Rhizoctonia solani* and Saprophytic Growth of the Pathogen in Soilless Mix

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ABSTRACT

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Commercially manufactured cellulose granules (Biodac) were mixed with a sticker and fermentor-produced biomass of isolates of *Trichoderma* spp. and *Gliocladium virens* to produce a formulation in which chlamydospores in the biomass were "activated" with dilute acid. Activation resulted in the formation of young, actively growing hyphae of the biocontrol fungi within a 2- to 3-day period under no special aseptic conditions. Activated Biodac with biomass of isolates Gl-3, Gl-21, and Gl-32 of *G. virens* and isolate TRI-4 of *T. hamatum* applied to soilless mix at a rate of 1.5% (wt/wt) reduced damping-off of eggplant caused by *Rhizoctonia solani* (R-23) and resulted in stands comparable to that (88%) in noninfested soilless mix. Saprophytic growth of the pathogen was also reduced. The application of either of two activated Biodac formulations to provide the same amount (1.5% with 9.4 mg of biomass per g of Biodac or 0.2% with 75.0 mg of biomass per g of Biodac) reduced preemergence damping-off as well as saprophytic growth of R-23. Also, there was about a 10^3-fold population increase of Gl-3 and TRI-4 in the soilless mix at the time of plant harvest compared with that provided to the soilless mix at the time of formulation addition. Activated Biodac of Gl-3 also reduced the spread of R-23 in soilless mix when the pathogen was applied at specific foci rather than evenly distributed. The inhibition of pathogen spread significantly reduced the postemergence damping-off of cucumber, eggplant, and pepper seedlings.

Additional keywords: biological control

The development of feasible and efficient delivery systems for the application of appropriate microorganisms to the ecosystem is an important component of biocontrol technology. The use of beneficial organisms to combat pests on economic crops is being aggressively pursued worldwide in an attempt to eliminate or reduce the application of pesticides, which can pollute the environment and adversely affect commodity quality (21,22). Soilborne plant pathogens cause seed rot, damping-off, root rot, wilt, and fruit rot, which result in an annual loss of $4 to 5 billion in the United States alone (7). Delivery systems employing various biocontrol agents to control these pests include dusts, alginate pellets, starch granules, extruded granules, and seed coatings (5,9,16,25,26). Invariably, these systems can be prepared quickly under laboratory conditions.

Various organic and inert carriers have also been used, upon which biocontrol agents are grown before addition to the agricultural environment. Such materials include diatomaceous earth, lignite/stillage, wheat bran, and various grains formulated as semisolid fermentation products mainly containing isolates of the biocontrol fungi *Trichoderma* spp. and *Gliocladium* spp. These materials have to be prepared aseptically and kept free of contaminants during relatively long incubation periods. Various systems are discussed in several recent reviews (1,4,6,15,29).

The Biocontrol of Plant Diseases Laboratory (BPDL) developed a technique in which axenic bran formulations containing young, actively growing (2- to 3-day-old) hyphae of isolates of *Trichoderma* spp. and *Gliocladium virens* J.H. Miller, J.E. Giddens, A.A. Foster, & von Arx were added to soil and soilless mix to reduce various diseases (8,12,14). It was suggested that these hyphae, which grew from a food base, attacked the pathogen structure without being inhibited by soil fungistasis, as are conidia (11). A potential formulation based on this principle was subsequently developed and patented (U.S. Patent No. 5,068,105) by the BPDL and W. R. Grace and Co. (13). In the formulation, oven-heated vermiculite and a small amount of bran were mixed with fermentor-produced biomass, abundant in chlamydospores (23), and the mixture was moistened with dilute acid, dried, and stored. When needed for application to soil or soilless mix infested with *R. solani*, the dry vermiculite–bran–biomass was remoistened with dilute acid and incubated for 2 to 3 days. This process was termed "activation." The activated formulation was added to the pathogen-infested milieu. Chlamydospores of *Trichoderma* spp. or *G. virens* in the biomass germinated, and the young hyphae grew on the bran. These hyphae suppressed the spread of the pathogen and significantly reduced its inoculum potential. More recently, a complex cellulose granule with the trade name Biodac has been utilized by the BPDL with the same principle of activation. This report presents data on the preparation of a Biodac formulation, its effect on damping-off of eggplant and pepper caused by *R. solani*, saprophytic growth of the pathogen, the proliferation of the biocontrol agents in soilless mix, and the spread of the pathogen in soilless mix to incite postemergence damping-off of cucumber, eggplant, and pepper seedlings.

MATERIALS AND METHODS

Fungal cultures. Biocontrol fungi used in this study were from the collection of the BPDL and were maintained on potato dextrose agar (PDA; Difco, Detroit, MI) in the light at 23 to 25°C. Isolates included: Gl-3, Gl-21, and Gl-32 of *G. virens* (=*Trichoderma virens*); TRI-4 and 31-3 of *T. hamatum* (Bonord.) Bainier; Th-32 and Th-87 of *T. harzianum* Rifai; and Tv-101 and WT-6 of *T. viride* Pers. ex Gray. Biomass was prepared in a sterilized molasses–brewer's yeast medium seeded with a conidial suspension of a given isolate (23). Biomass was separated from the spent medium by filtration after 9 days of incubation at 25°C, air-dried at 23 to 25°C, and milled to pass a 425-µm screen. The numbers of CFU that developed from clusters of chlamydospores in the biomass were assayed on a semiselective medium (24). Values ranged between 10^7 and 10^8 CFU/g of biomass.

Isolate R-23 of *Rhizoctonia solani* Kühn (AG-4), from the collection of the BPDL, was maintained on PDA and was used in

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the assays for disease and for pathogen growth and spread in soilless mix. Inoculum was prepared as colonized soilless mix and as colonized beet seeds. Colonized mix was prepared by placing actively growing cultures of R-23 on PDA plugs in deep dishes containing 75 cm^3 of moistened soilless mix (Redi-Earth, Scotts, Marysville, OH) supplemented with finely ground cornmeal (0.3% wt/wt, dry weight basis). The inoculum was incubated for 2 weeks at 25°C and used fresh (14). In the other type of inoculum, PDA plugs of R-23 were placed in deep dishes with 50 cm^3 of sterile moist table beet (*Beta vulgaris* L. cv. Detroit Red) seeds. This latter preparation, which simulated natural inoculum of mycelium embedded in organic matter, was incubated for 4 weeks at 25°C and air-dried at 23 to 25°C (12). The dry, colonized beet seed could be stored in the laboratory for several months without appreciable loss in viability.

Production of Biodac formulations. Biodac (Grantek, Inc., Granger, IN) is a product of consistent particle size composed of recycled cellulosic-based paper waste. The granules can be prepared in a variety of sizes and are used as a carrier for agricultural chemicals. Biodac is inexpensive at a cost of $0.17 per kg. To obtain the activated formulations, Biodac granules (size 16/30 mesh) were heat-treated in a convection oven for 3 days at 80°C to reduce microbial contamination. An autoclaved sodium alginate solution (1% wt/vol) also was prepared for use as a sticker. In a 400-ml beaker, 30 g of the Biodac was mixed by spatula with 45 ml of the alginate solution and up to 2.25 g of biomass (75.0 mg of biomass per 1.0 g of Biodac) of the various isolates of *Trichoderma* spp. and *G. virens*. The mixture was spread on aluminum foil and dried overnight with flowing air. The dry formulations were stored at 4°C until use. For activation before addition to the soilless mix, 25 g of Biodac formulation was moistened with 20 ml of 0.05 N HCl, mixed, and placed in a 100-ml beaker covered with punctured plastic film for 2 days. The beakers were incubated in the laboratory at 23 to 25°C, and after the 2-day incubation period, the activated Biodac was observed to be held together by a diffuse network of fungal mycelium. At this time (2 days), the production of conidia and chlamydospores was not evident. Controls contained Biodac without biomass. This system is unique because it appears that the entire activation process can be accomplished in the laboratory with no special precautions to assure sterility or asepsis. Axenic conditions were only required for production of biomass (23).

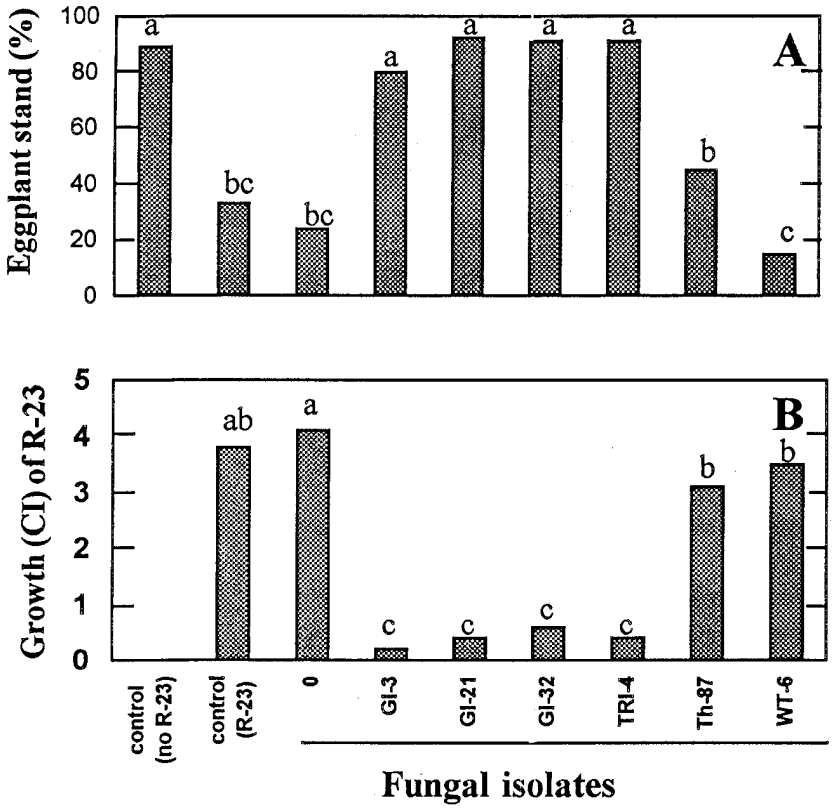

Fig. 1. Effect of activated Biodac containing biomass of isolates of *Gliocladium virens* (Gl-3, Gl-21, Gl-32), *Trichoderma hamatum* (TRI-4), *T. harzianum* (Th-87), and *T. viride* (WT-6) added to soilless mix at a rate of 1.5% (wt/wt, dry weight basis) on **(A)** damping-off of eggplant caused by *Rhizoctonia solani* (R-23) and on **(B)** saprophytic growth of the pathogen after 4 weeks of plant growth. Saprophytic growth index (CI) is from 0 to 5, where 0 = no observable hyphae; 2, 3, 4, and 5 = hyphae on 25, 50, 75, and 100% of the agar surface, respectively. Values for each assay represented by bars with same letter do not significantly differ by Duncan's multiple range test ($P < 0.05$).

Effect of Biodac formulations on *R. solani* damping-off. Redi-Earth was supplemented with a water-soluble N-P-K fertilizer (20-10-10) to provide an additional 50 mg of N/kg and was moistened to –30 kPa. After 1 week, the soilless mix was infested with fresh 2-week-old inoculum of Redi-Earth–*R. solani* and amended with activated Biodac of isolates Gl-3, Gl-21, Gl-32, TRI-4, Th-87, and WT-6. The Biodac was added at a rate of 1.5% (wt/wt, dry weight) containing 75.0 mg of biomass per 1.0 g of Biodac. Controls included noninfested and pathogen-infested soilless mix and soilless mix infested with R-23 and amended with Biodac containing no biomass. After treatment, the soilless mix was incubated in polyethylene bags for 2 weeks at 23 to 25°C in the dark, mixed, and placed in 18 × 12 × 6.6 cm plastic flats. Each flat was planted to three rows of 10 seeds of eggplant (*Solanum melongena* L. cv. Black Beauty) or pepper (*Capsicum annuum* L. cv. California Wonder). For eggplant and pepper, inoculum of Redi-Earth–*R. solani* was amended to the soilless mix at a rate of 0.16 and 0.42% (wt/wt, dry weight), respectively. Adequate moisture was maintained in the flats, which were incubated in a growth chamber at 25 to 30°C supplemented with light for 12-h periods. Plant stand was recorded weekly for 4 weeks.

In one of two similar experiments, activated Biodac with isolate Gl-3 or TRI-4 was added to pathogen-infested soilless mix at a rate of 1.5%, and the formulation of Biodac contained 9.4, 18.8, 37.5, and 75.0 mg of biomass per 1.0 g of Biodac. In the other experiment, activated Biodac with 75.0 mg of biomass per 1.0 g of Biodac was added to the soilless mix at rates of 0.2, 0.4, 0.8, and 1.5%. These rates of activated Biodac provided the same amount of biomass to portions of soilless mix in both experiments. Soilless mix was incubated as described, potted, and planted to eggplant and pepper. Flats were maintained, and plant stand was assayed as described. Controls included noninfested soilless mix without amendment and pathogen-infested soilless mix with Biodac containing no biomass.

To evaluate the effect of Biodac formulations on the spread of *R. solani* from an individual focal point, the following system was used. Fertilizer-supplemented soilless mix was amended with activated Biodac containing isolate Gl-3 at a rate of 1.5%. The activated Biodac contained 75.0 mg of biomass per 1.0 g of Biodac. The soilless mix was immediately placed in flats, and three replicate flats were planted with cucumber (*Cucumis sativus* L. cv. Early Pride hybrid), eggplant, and pepper (three rows of seven seeds). One day after planting, four R-23–infested table beet

seeds were pushed into the medium at each corner of each flat. Appropriate controls were included, and flats were treated as previously indicated.

Disease incidence was recorded weekly for 4 weeks, and the extent of pathogen spread in each flat to cause damping-off was determined from spatial maps (2). Each flat was divided into 21 grids (seven columns of three rows) so that one seed of each crop was planted in each of the grids. The stand in each grid was determined after 4 weeks of growth, and the data for the same grid location in the three replicates were rated on a spatial map based on stand average in that grid across replications in shades of gray so that white indicated no plant stand in the grids, shades of gray indicated stands of 33 and 67%, respectively, and black indicated a stand of 100%. Differences in spread of disease among treatments were determined by analysis of stand by row and column grid locations.

Determination of saprophytic growth of *R. solani* and population of antagonists in soilless mix. The saprophytic growth of R-23 in soilless mix was assayed after harvest of eggplant seedlings at 4 weeks by a method in which autoclaved table beet seeds were used to trap the pathogen (11). A colonization index (CI) of 0 to 5 based on the extent of development of hyphae from the retrieved beet seeds onto a water agar surface was used as a measurement of saprophytic growth: 0 = no observable hyphae; 2, 3, 4, and 5 = hyphae on 25, 50, 75, and 100% of the agar surface surrounding the beet seeds, respectively.

The population numbers (CFU) of the various isolates of *Trichoderma* spp. and *G. virens* were assayed in soilless mixes when the two activated Biodac preparations were added and when eggplant seedlings were harvested after 4 weeks of growth. Dilutions were prepared, and 1-ml aliquots (10^5 to 10^7) were plated on a semiselective medium (24), which allowed for the identification of the introduced isolates because of their characteristic phenotypic growth on the agar.

Statistical analyses. All experiments were repeated once, with three or four replicates per treatment. The treatments were arranged in a randomized complete block design. Data from repeated experiments were combined when the results had homogenous variances, and statistical analyses were performed on the combined data. Analyses of data were done by analysis of variance (ANOVA) with factorial treatment structures and interactions, and separation of means was accomplished by using Duncan's multiple range test.

RESULTS

Influence of activated Biodac with biomass of biocontrol fungi on damping-off caused by *R. solani*, saprophytic growth of the pathogen, and populations of biocontrol fungi in soilless mix. Isolates of *G. virens* and *Trichoderma* spp. in the activated Biodac formulations were variable in the prevention of Rhizoctonia damping-off of eggplant (Fig. 1A). The three isolates (Gl-3, Gl-21, Gl-32) of *G. virens* and isolate TRI-4 of *T. hamatum* formulated as activated Biodac (1.5% wt/wt) reduced disease ($P < 0.01$). With these biocontrol agents, plant stands were comparable to that (88%) in noninfested soilless mix after 4 weeks of growth. However, activated Biodac with isolates of *T. harzianum* (Th-87) and *T. viride* (WT-6) did not suppress disease and yielded eggplant stands equal to those in the pathogen-infested soilless mix not amended with Biodac (32%) or amended with Biodac without biomass (24%).

The isolates effective in disease reduction significantly ($P < 0.05$) limited the saprophytic growth of R-23 in the soilless mix (Fig. 1B). Moreover, there was a significant ($r^2 = -0.91$) inverse correlation between eggplant stand and saprophytic growth of the pathogen. The effectiveness of the isolates was not associated with either the number of CFU of the isolates applied in the activated Biodac or the number of CFU in the soilless mix 6 weeks after amendment addition (data not shown). Depending on the isolate studied, the application of 1.5% activated Biodac provided 5.6 to 10.1×10^5 CFU/g of soilless mix. At the time of eggplant harvest, the populations of isolates ranged between 10^7 and 10^8 CFU/g of soilless mix. There were no correlations between these values

Table 1. Influence of activated Biodac (1.5%, wt/wt) containing various amounts of biomass of *Gliocladium virens* (Gl-3) and *Trichoderma hamatum* (TRI-4) on the damping-off of eggplant caused by *Rhizoctonia solani* (R-23), on the saprophytic growth of the pathogen, and on CFU of the antagonists

Isolate	Biomass Biodac (mg/g)	Stand at 4 weeks (%)	R-23 growth in soilless mix (CI)[w]	Soilless mix CFU/g ($\times 10^8$)[x]
Control (No R-23)	…	92 a[y]	…	…
Control (R-23)	…	18 d	4.1 a	…
Control (R-23/Biodac)	0	10 d	4.0 a	…
Gl-3	9.4	68 bc	1.2 b	1.4[z]
	18.8	65 c	0.4 b	2.6
	37.5	86 a	0.4 b	1.9
	75.0	82 ab	0.2 b	3.1
TRI-4	9.4	86 a	0.8 b	2.4
	18.8	80 abc	0.8 b	1.6
	37.5	90 a	1.0 b	1.1
	75.0	87 a	0.6 b	2.3

[w] Colonization index (CI) for saprophytic growth of R-23 indicates growth of pathogen on agar surface surrounding the beet seeds, rated 0 to 5, where 0 = no observable hyphae; 2, 3, 4, and 5 = hyphae on 25, 50, 75, and 100% of the agar surface, respectively.

[x] CFU/g of soilless mix of indigenous *Trichoderma* and *Gliocladium* was $<10^4$ when amended with 1.5% Biodac not containing biomass.

[y] Numbers in each column followed by the same letter do not significantly differ according to Duncan's multiple range test ($P < 0.05$).

[z] There was no significant difference among values according to Duncan's multiple range test ($P < 0.05$), nor was there any interaction between CFU/g of soilless mix and any other parameter.

Table 2. CFU of *Gliocladium virens* (Gl-3) and *Trichoderma hamatum* (TRI-4) in *Rhizoctonia solani* (R-23)-infested soilless mix at time of amendment and after 6 weeks with various rates (wt/wt, dry weight) of activated Biodac[u]

Biodac (%)[v]	CFU/g of soilless mix: At applic. ($\times 10^6$)[w]	CFU/g of soilless mix: 6 weeks after applic. ($\times 10^8$)[x]	CFU increase
Gl-3			
0.2	0.10	3.8[y]	$3.8 \pm 0.5 \times 10^3$[z]
0.4	0.20	3.1	$1.6 \pm 1.0 \times 10^3$
0.8	0.39	2.9	$7.1 \pm 1.6 \times 10^2$
1.5	0.78	4.1	$5.8 \pm 0.8 \times 10^2$
TRI-4			
0.2	0.07	4.0	$5.1 \pm 1.0 \times 10^3$
0.4	0.15	2.7	$1.8 \pm 0.4 \times 10^3$
0.8	0.30	3.0	$1.0 \pm 0.6 \times 10^3$
1.5	0.60	4.9	$8.4 \pm 1.6 \times 10^2$

[u] Assay performed after harvest of eggplant.

[v] Containing 75.0 mg of biomass of either G1-3 or TRI-4 per 1.0 g of formulation (wt/wt).

[w] Assay immediately after Biodac application.

[x] Assay 6 weeks after Biodac application; CFU of indigenous *Trichoderma* and *Gliocladium* were $<10^3$ and $<10^4$ when amended with 0, 0.2, 0.4, and 0.8, and 1.5% of Biodac not containing biomass, respectively.

[y] There was no significant difference among values according to Duncan's multiple range test ($P < 0.05$), nor was there an interaction between CFU/g of soilless mix and rate of amendment.

[z] Values with standard deviations.

and eggplant stand or saprophytic growth of the pathogen. There was no significant difference ($P > 0.05$) between eggplant stands at 2 and 4 weeks of growth, indicating preemergence damping-off was the most important disease phase.

Because two of the most effective isolates (Gl-3 and TRI-4) represented different species of closely related genera, or possibly even of the same genus, additional experimentation was done with these isolates. In a series of experiments to evaluate the effect of soilless mix amended with a constant rate (1.5% wt/wt, dry weight basis) of activated Biodac containing various amounts of biomass of Gl-3 and TRI-4 on Rhizoctonia damping-off of eggplant and saprophytic growth of R-23, several observations were noted. For example, the formulations prepared with all rates of biomass used (9.4 to 75.0 mg per 1.0 g of Biodac) significantly ($P < 0.01$) reduced Rhizoctonia damping-off and resulted in plant stands greater than those in the pathogen-infested soilless mix controls (18%, 10%) (Table 1). There was no significant ($P > 0.05$) isolate effect on damping-off. However, activated Biodac prepared with as little as 37.5 mg of biomass of isolate Gl-3 and 9.4 mg of biomass of TRI-4 yielded eggplant stands comparable to that (92%) in the noninfested control. In addition, there was a significant ($r^2 = 0.74$) correlation between the amount of biomass (mg) of Gl-3 on activated Biodac and eggplant stand but not between that of TRI-4 and eggplant stand.

With regard to biomass rate and pathogen saprophytic growth, activated Biodac with all rates of biomass of Gl-3 and TRI-4 significantly ($P < 0.01$) reduced saprophytic growth equally compared with that of the pathogen-infested controls (Table 1). Moreover, there was a significant ($r^2 = -0.82$) inverse correlation between eggplant stand and pathogen saprophytic growth. Also, it was shown that amendment to soilless mix with activated Biodac containing all rates of biomass of both isolates Gl-3 and TRI-4 stimulated population proliferation of the isolates to $>10^8$ CFU/g (Table 2). There were no significant differences among population increases, and at the time of eggplant harvest, all treatments increased almost 10^3-fold in soilless mix compared with the amount of propagules (about 7.0×10^5 CFU/g of soilless mix) initially provided by the activated Biodac.

When various rates of activated Biodac (0.2, 0.4, 0.8, 1.5% wt/wt, dry weight basis) formulation containing 75.0 mg of biomass of Gl-3 and TRI-4 per 1.0 g of Biodac were amended to noninfested and pathogen-infested soilless mix, results similar to those of the previous experiment were obtained (Table 3). The rates of activated Biodac added to the soilless mix provided comparable amounts of biomass in both experiments. All rates effectively reduced damping-off of eggplant and pepper as well as reducing saprophytic growth of *R. solani*. There was a highly significant ($r^2 = 0.89$) correlation between eggplant and pepper stands. Analysis also indicated that activated Biodac of either isolate was similar ($P > 0.05$) in effectiveness to reduce damping-off and saprophytic growth of the pathogen; whereas various rates of Biodac containing no biomass were ineffective on the processes. Generally, all rates of the activated Biodac with Gl-3 and TRI-4, except the 0.2% rate, yielded crop stands comparable to those of the noninfested controls. Both antagonists proliferated significantly in the soilless mix (Table 2). Regardless of the rate of activated Biodac applied, the CFU provided by both Gl-3 and TRI-4 at the time of application increased to $>10^8$ CFU/g of soilless mix 6 weeks after amendment (2 weeks of incubation + 4 weeks of plant growth). This represented an increase of approximately 10^3-fold.

Influence of activated Biodac with biomass of *G. virens* (Gl-3) on spatial spread of *R. solani* and its influence on damping-off diseases in soilless mix. The Biodac Gl-3 formulation suppressed pathogen spread from specific foci and inhibited seedling damping-off, depending on the crop tested. Over all treatments, disease was significantly ($P < 0.05$) less severe in pepper than in cucumber and eggplant seedlings (Fig. 2). The average stands of pepper, cucumber, and eggplant were 75, 58, and 52%, respectively (data not shown). In all three crops, activated Biodac of Gl-3 resulted in stands comparable to those in noninfested controls (81 versus 86%, 76 versus 84%, and 95 versus 94%, for cucumber, eggplant and pepper, respectively).

Spatial analysis of the spread of disease in column and row grid locations indicated that activated Biodac of Gl-3 significantly ($P < 0.05$) reduced damping-off of seedlings of all three crops and resulted in stands comparable to those in noninfested controls at all column and row locations (Fig. 2). Stand differences among the treatments were most evident in the outer columns (first, second, sixth, and seventh), which demonstrated that the spread of the pathogen from its infestation foci was inhibited by the Biodac formulation. However, in the various control treatments, R-23 actively spread from the tray corners toward the center (third, fourth, and fifth) columns.

Table 3. Influence of various rates (wt/wt, dry weight) of activated Biodac of *Gliocladium virens* (Gl-3) and *Trichoderma hamatum* (TRI-4) on damping-off of eggplant and pepper caused by *Rhizoctonia solani* (R-23) and on saprophytic growth of the pathogen

Isolate	Biodac (%)[x]	Biomass with Biodac (mg)	Plant stand at 4 weeks (%)[y]		R-23 growth in soilless mix (CI)[z]
			Eggplant	Pepper	
Control (no R-23)			90	92	…
Control (R-23)			34	48	5.0
0	0.20	0	28	50	5.0
Gl-3		9.4	75**	68*	0.3*
TRI-4		9.4	92**	72*	0.6*
0	0.40	0	16	20	4.7
Gl-3		18.8	89**	80**	0.2*
TRI-4		18.8	90**	80**	0.1*
0	0.80	0	10	26	4.9
Gl-3		37.5	92**	87**	0.4*
TRI-4		37.5	91**	90**	0.5*
0	1.5	0	12	18	4.4
Gl-3		75.0	90**	93*	0.2*
TRI-4		75.0	88**	85**	0.2*

[x] Containing 75.0 mg of biomass of Gl-3 and TRI-4 per 1.0 g of formulation.

[y] Stand percentages of each crop for each amendment rate with an asterisk (*) are less than that of noninfested control (90%) but greater than those of pathogen-infested control (34%) without added formulation or appropriate controls ($P < 0.05$). Stand values with two asterisks (**) are comparable to those of the noninfested control (90%) ($P < 0.01$). Stand percentages are inversely related to the amount of damping-off caused by R-23.

[z] Saprophytic growth values from soilless mix planted to eggplant with an asterisk (*) are highly significantly less that the values for the appropriate controls. Colonization index (CI) indicates the growth of R-23 on the agar surface surrounding beet seeds, rated 0 to 5, where 0 = no observable hyphae; 2, 3, 4, and 5 = hyphae on 25, 50, 75, and 100% of the agar surface, respectively.

DISCUSSION

For the most part, the beneficial results with the Biodac formulations of the biocontrol fungi were similar to data obtained in other studies in horticultural greenhouses and in the field using alginate prills, pregelatinized starch–flour granules, and extruded pellets to reduce various soilborne diseases (3,9,10,14,16,28). Activated Biodac with various fungi was shown in this study to reduce damping-off diseases of various crops caused by *R. solani* and the saprophytic growth of the pathogen in soilless mix. Also, significant population proliferation was obtained. The application of Biodac is of special interest since it is an extension of the concept in which actively growing hyphae of biocontrol fungi were

used in two approaches to reduce soilborne plant pathogens such as *R. solani* and *P. ultimum*. In the first system (11,14), an axenic bran preparation containing 3-day-old inoculum of isolates of *Trichoderma* spp. and *G. virens* was incorporated into soil or soilless mix. That method may be disadvantageous because axenic growth conditions were required as well as a bulk preparation. In the second procedure (13), which uses vermiculite, axenic conditions were not necessary except for biomass production. Although the production of actively growing hyphae was achieved within 3 days without special precautions, the use of vermiculite as a carrier may present problems of excessive bulk as well the generation of dust.

The application of Biodac with an activation system may be significantly advantageous for the development of a commercial biocontrol product. The Biodac granules are homogeneous and can be manufactured in a variety of sizes. In addition, the material is composed entirely of cellulose, a natural substrate for the growth of *Trichoderma* and *Gliocladium* (17). Data in the present report suggested that either low rates of activated Biodac with a given amount of biomass or somewhat higher rates of activated Biodac with less biomass were effective in the reduction of damping-off. The appropriate system for application would depend upon a requirement for adequate spatial dispersion of biocontrol agent inoculum and/or cost of the formulation.

As a corollary to formulation effectiveness, isolate specificity and propagule type in the biomass are important considerations. It was recognized that chlamydospores are the major components from which actively growing hyphae of isolates of *Trichoderma* and *Gliocladium* develop (23) and that isolates of *G. virens* and *T. hamatum* were generally better biocontrol candidates than isolates of *T. harzianum* and *T. viride*, an observation noted in this report as well as in others (9,10,18). In addition to disease reduction to evaluate formulation effectiveness, the ability of various preparations to reduce the saprophytic growth of *R. solani* appears to be a reliable and more easily performed technique than setting up disease assays. This was demonstrated in this report and elsewhere (9,10).

The ability of a biocontrol agent in a formulation to inhibit the spread of the pathogen is perhaps more important than the effectiveness of the formulation to eliminate pathogen propagules evenly distributed in the soil or soilless mix. Spread of *R. solani* is particularly important because of its high competitive saprophytic growth. In natural ecosystems, pathogen inoculum is generally aggregated so that subsequent spread can occur under appropriate conditions (19). The data in Figure 2 addressed the problem of pathogen spread. Application of an effective formulation to enable establishment of the biocontrol fungus before a pathogen is introduced into the ecosystem might be an appropriate approach. It remains to be determined whether preincubation of activated Biodac of Gl-3 in soilless mix for several days or weeks to allow proliferation of Gl-3 would be more effective in preventing pathogen spread than addition of the Biodac preparation only 1 day before the R-23–infested beet seeds. It was previously shown that microorganisms added to a fumigated or steamed soil before reinvasion by a pathogen induced stress that would inhibit the spread of the pathogen (19,20).

The exploitation of Biodac formulations to control soilborne plant pathogens, plant nematodes, and soil-inhabiting insects affords a productive opportunity to evaluate this material in biocontrol. The requirements necessary for the formulation of biocontrol agents have been discussed previously (27). Research is in progress in the BPDL to study Biodac preparations with regard to storage and shelf-life, effectiveness against diseases caused by other isolates of *R. solani* and other pathogens, and pathogen–crop specificity.

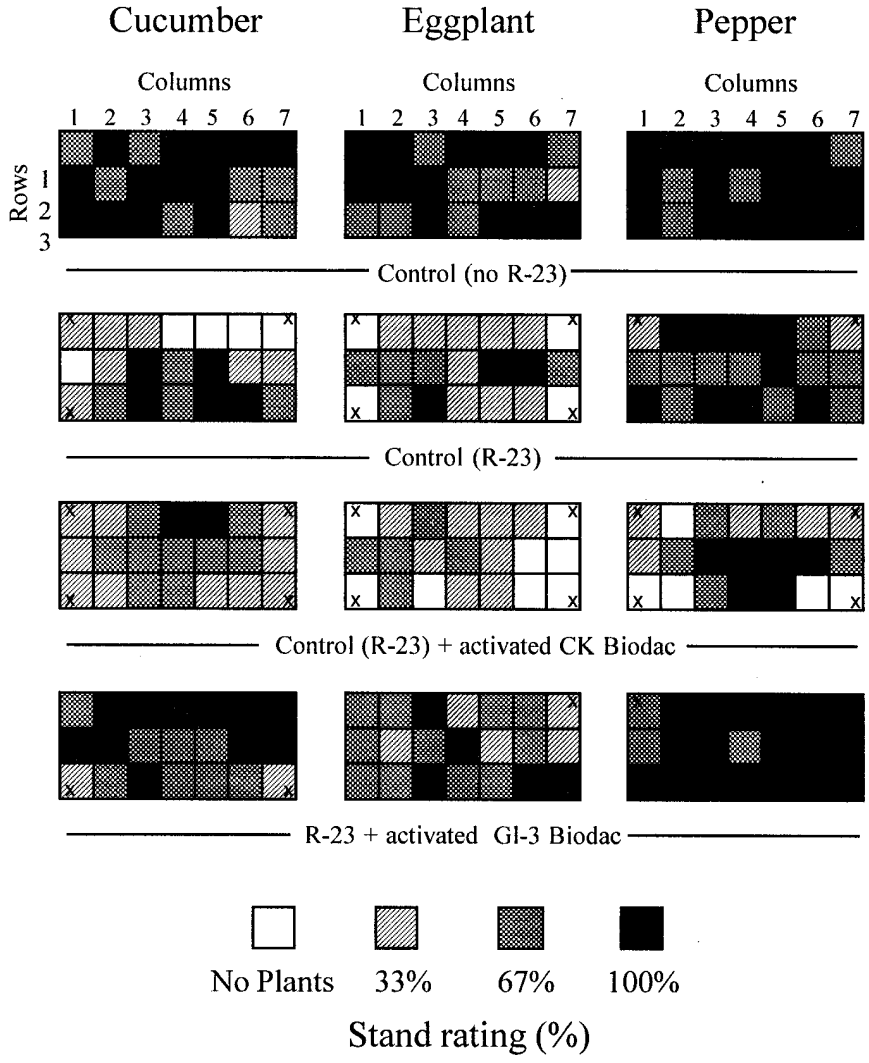

Fig. 2. Effect of activated Biodac containing biomass of *Gliocladium virens* and Biodac containing no biomass on the spatial distribution of stands of cucumber, eggplant, and pepper seedlings indicating spread of *Rhizoctonia solani* (R-23) from infestation foci (×) through soilless mix to incite postemergence damping-off.

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