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CLADORRHINUM FOECUNDISSIMUM: A POTENTIAL BIOLOGICAL CONTROL AGENT FOR THE REDUCTION OF *RHIZOCTONIA SOLANI*

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Summary—The effect of bran preparations of the potential biocontrol fungus *Cladorrhinum foecundissimum* on the survival and saprophytic growth of the soilborne plant pathogen *Rhizoctonia solani* in soils and soilless potting mix and on its ability to reduce incidence of damping-off of sugar beet, eggplant and pepper caused by this pathogen was investigated. Bran inoculum of isolate Cf-1 of *C. foecundissimum* incubated for as long as 17 days did not reduce survival of the pathogen on *R. solani*-infested sugar beet seed in a loamy sand (LS) or sandy clay loam (SCL), but bran inoculum of Cf-1 older than 6 days prevented growth of the pathogen from the beet seed into soil. The saprophytic growth of *R. solani* applied as a soilless mix–cornmeal inoculum was also prevented by isolate Cf-1 in 4 different soil types. All 4 isolates (Cf-1, ATCC 62373, CBS 181.66 and CBS 182.66) of *C. foecundissimum* also prevented saprophytic growth of *R. solani* in LS when inoculum of the pathogen consisted of a soilless mix supplemented with cornmeal. 10-Day-old bran inoculum of isolates Cf-1 and ATCC 62373 prevented sugar beet damping-off caused by *R. solani* in LS after 4 wk of growth. Use of Cf-1 resulted in stands (>90%) greater than those in non-infested control soils. On the contrary, use of isolates CBS 181.66 and CBS 182.66 resulted in stands less than those in pathogen-infested soil. Bran preparations of isolates Cf-1 and ATCC 62373 prevented damping-off of eggplant and pepper in soilless mix and, depending on the rate of inoculum used, resulted in stands comparable to those in pathogen-free soilless mix.

INTRODUCTION

Advances in the research of biological control of soilborne plant pathogens are accelerating at a rapid rate. This phenomenon is partly due to increased knowledge in the production, formulation and delivery of various biocontrol agents, which include fungi, bacteria and actinomycetes (Baker and Dunn, 1990; Hornby, 1990; Tjamos *et al.*, 1992; Lumsden and Vaughn, 1993). In addition to the two most commonly studied biocontrol fungal genera, *Trichoderma* and *Gliocladium* (Papavizas, 1992), members of other less commonly known genera are receiving increased attention. These include *Sporidesmium sclerotivorum* (Fravel *et al.*, 1992), *Laetisaria arvalis* (Lewis and Papavizas, 1992; Murdoch and Leach, 1993), *Stilbella aciculosa* (Lewis and Papavizas, 1993), *Talaromyces flavus* (Fravel and Adams, 1986), non-pathogenic *Fusarium oxysporum* (Alabouvette *et al.*, 1993) and *Pythium oligandrum* and *Coniothyrium minitans* (Whipps *et al.*, 1993).

The soilborne pathogen *Rhizoctonia solani* is ubiquitous and, under the appropriate environmental conditions, can severely damage more than 200 crops of economic importance. The destruction includes seed and seedling rot, root and hypocotyl damage, aerial blight and fruit rots. Several years ago, scientists in the Biocontrol of Plant Diseases Laboratory (BPDL) observed that a white, actively-growing fungus grew on water agar plus antibiotics from beet seed placed for 3 wk in various soils infested with *R. solani*. Placement of beet seed in *R. solani*-infested soil is routinely done to assay inoculum density of the pathogen (Papavizas and Lewis, 1986). The unknown fungus grew from beet seed onto agar within 24 h, displaced *R. solani* from the beet seed and prevented saprophytic growth of the pathogen in the soil. The colonies spread broadly with floccose, grayish to pinkish ochraceous aerial mycelium, which occasionally formed clusters of swollen hyaline vegetative hyphae. The inconspicuous conidia were dacryoid to globose and 3–3.5 × 2.5–3 μm in size. The culture, designated Cf-1, was identified by Dr W. Gams [Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands] as *Cladorrhinum foecundissimum* Sacc. and Marchal. It is of interest that the fungus has been recovered abundantly from washed soil particles (Gams *et al.*, 1969), as well as in buckwheat baits placed in soil (von Arx and Gams, 1966), a similar observation to that which we observed in beet seed.

The fungus is described as a common soil

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inhabitant, but is hardly ever recovered with soil dilution plates since conidia are not capable of germination (Domsch *et al.*, 1980; Mouchacca and Gams, 1993). It has not been investigated as a major biocontrol fungus. In culture, it has shown significant antibiotic activity against *R. solani* and *P. ultimum* (Domsch and Gams, 1968). Another species of *Cladorrhinum*, *C. brunnescens*, significantly inhibited the white rot fungi *Trametes versicolor* and *Stereum rugosum* (Antheunisse and Burema, 1983). *Cladorrhinum* has also been shown to decompose cellulose, pectin and xylan (Domsch and Gams, 1969).

Our investigation was initiated in an attempt to exploit the ability of *C. foecundissimum* to inhibit saprophytic growth of *R. solani* in soil and soilless mix and as a potential biocontrol agent to prevent damping-off of eggplant, pepper and sugar beet seedlings caused by the pathogen. A preliminary report has appeared (Lewis and Papavizas, 1988).

MATERIALS AND METHODS

Fungal cultures, soils and soilless mix

Isolates of *C. foecundissimum* Sacc. and Marchal, with potential for biocontrol, were maintained on potato-dextrose agar (PDA). The 4 isolates used in various parts of this study were: Cf-1 from the culture collection of the BPDL; 181.66 and 182.66 from the CBS isolated from Dutch soil; and 62373 from the American Type Culture Collection (ATCC), Rockville, MD. ATCC 62373 was isolated from cocoa fibers buried in Dutch soils. Inoculum of these fungi consisted of wheat bran preparations made by comminuting a 2-wk-old PDA culture plate of each isolate in 50 ml of sterile water and adding 5 ml of the suspension to 50 g of sterile wheat bran and water (1:1, w/w) in 1 liter Erlenmeyer flasks (Lewis and Papavizas, 1987a). The bran preparations were incubated in the dark for various periods at 21–23°C.

The pathogen used in the studies was isolate R-23 (AG-4) of *R. solani* Kuehn from the collection of the BPDL. Cultures were maintained on PDA. Inoculum for experiments consisted of either pathogen-infested table beet (*Beta vulgaris* L. cv Detroit Red) seed or Redi-Earth® inoculum (W. R. Grace and Co., Cambridge, MA), according to the particular experiment (see below). Since *R. solani* is usually found in soil fractions as mycelium embedded in organic debris, pathogen-infested beet seed was used as inoculum to simulate the natural inoculum. Beet seed was mixed with an equal volume of water, placed in flasks, autoclaved and seeded with an agar plug of R-23. Preparations were incubated in the laboratory at 25–26°C for 28 days and then air-dried. This inoculum was used within 1 wk of drying, but it could be kept under normal laboratory conditions for several months without appreciable loss in viability. Inoculum for the soilless mix was prepared by placing PDA plugs of actively-growing *R. solani* in twice autoclaved, moistened soilless mix supplemented with finely-ground cornmeal at a rate of 0.3% (w/w) on a dry wt basis (Lumsden and Locke, 1989). The inoculum was grown for 12 days at 25°C and used fresh.

For most experiments to determine the effect of *C. foecundissimum* on the survival and saprophytic growth of *R. solani*, a natural loamy sand (LS) of pH 6.4, 0.5% organic matter (OM), 84% sand (S), 8% silt (Si) and 8% clay (C) was used. Where indicated, other natural soils used included: a sandy loam (SL) of pH 5.1, 0.2% OM, 77% S, 12% Si and 11% C; a loam (L) of pH 6.8, 2.3% OM, 35% S, 40% Si and 25% C; and a sandy clay loam (SCL) of pH 4.5, 3.2% OM, 46% S, 21% Si and 33% C. The soils were screened (<2.0 mm) and maintained at about −30 kPa for 3 wk in the greenhouse before use. Granular NPK fertilizer (10:10:10) was added to soils at a rate of 0.05% (w/w) on a dry wt basis and powdered limestone was added to the SCL to adjust the pH to 6.2–6.4. For some planting trials, a soilless potting mix (Redi-Earth®) was used, the ingredients of which have already been reported (Arendt, 1987). The potting mix was used directly from the bag and was not steamed, autoclaved or fumigated. Before use, the mix was moistened with a solution of NPK fertilizer (20:10:10) at a rate of 0.05% (w/w) to obtain a moisture content of −30 kPa.

Effect of C. foecundissimum on survival and saprophytic growth of R. solani

Moist LS and SCL were amended with *R. solani*-infested table beet seed (1.2 g 200 g soil^{-1}, dry wt basis) and maintained at −30 kPa for 1 wk. Bran, infested with isolate Cf-1 of *C. foecundissimum* and incubated for 0, 3, 6, 10 or 17 days was then mixed with the soils at a rate of 1.0% (w/w). Pathogen-infested soils with or without non-infested bran were used as controls. After an additional 3 wk of incubation, seed was retrieved, washed and placed on water agar with antibiotics to determine pathogen survival (Papavizas and Lewis, 1986). The saprophytic growth of *R. solani* in the soil from which the seed was removed was determined by the table beet seed colonization method (Papavizas and Lewis, 1986). A colonization index (CI) of 0–5 based on the extent of hyphal growth on agar surface from each beet seed was used to express survival and saprophytic growth of *R. solani* in soil: 0 = no observable hyphae of *R. solani* from seed; 1 = several threads or strands of hyphae; 2, 3, 4, 5 = hyphae on 25, 50, 75 and 100% of the agar surface surrounding the beet seed, respectively (Lewis and Papavizas, 1993).

An experiment was also performed with four soil types to determine the effect of a 10-day-old bran preparation of isolate Cf-1 at a rate of 1.0% (w/w) on the saprophytic growth of *R. solani* in these soils. Infested soils with or without non-infested bran was used as the controls. In this experiment, the pathogen was added as a culture grown on soilless mix amended with cornmeal. A 12-day-old fresh *R. solani* inoculum

was added at a rate of 1.0% (w/w) to moist soil 1 wk before addition of the bran preparation of Cf-1. The table beet seed colonization method (Papavizas and Lewis, 1986) was used to assay saprophytic growth after 3 wk of incubation.

In order to establish the effect of various isolates of *C. foecundissimum* on the saprophytic growth of *R. solani* in soil, the pathogen was added as a soilless mix–cornmeal inoculum at a rate of 1.0% (w/w) to moist LS. A week later, 10-day-old bran preparations of *C. foecundissimum* isolates Cf-1, CBS 181.66, CBS 182.66 and ATCC 62373 were added to the soil at a rate of 1.0%. Soils with or without non-infested bran were used as the controls. The table beet seed colonization method was used to assay the saprophytic growth after 3 wk of incubation.

Effect of C. foecundissimum on damping-off

The effect of *C. foecundissimum* isolates on Rhizoctonia damping-off of sugar beets was studied in LS and of eggplant and pepper in soilless mix. Inoculum of *R. solani* consisted of soilless mix–cornmeal infested with the pathogen and inoculum of the antagonists in the form of 10-day-old infested bran preparations. Moist 1 kg portions of fertilizer-supplemented LS (dry wt equivalent) were amended with *R. solani* inoculum at a rate of 0.16% (w/w), incubated for 1 wk in plastic bags at 22–25°C and treated with bran preparations of isolates Cf-1, CBS 181.66, CBS 182.66 and ATCC 62373 at a rate of 2.0% (w/w). After an additional week of incubation, soils were placed in plastic flats (18 × 12 × 6.5 cm) and planted with metalaxyl-treated seed (0.4 g a.i. kg^{-1} seed) of sugar beet (*Beta vulgaris* L. USH-20). After 24 seeds (3 rows of 8 seeds each) were planted in each flat, flats were watered thoroughly and incubated in a growth chamber at 25–30°C supplemented with fluorescent light for 12 h periods to provide ~700 $\mu E\ m^{-2}\ s^{-1}$. Seedling stand was determined after 1, 2 and 4 wk of growth to establish the incidence of damping-off.

To evaluate the influence of various amounts of bran preparations of isolates Cf-1 and ATCC 62373 on eggplant and pepper damping-off caused by *R. solani*, NPK(20:10:10)-supplemented moist soilless mix was amended with *R. solani* preparations at rates of 0.16% (w/w) and 0.3% (w/w) on a dry wt basis, respectively. After incubation for 1 wk as above with soil, pathogen-infested soilless mix was treated with non-infested or infested bran of 10-day-old bran preparations of Cf-1 and ATCC 62373 at rates of 0.30 and 0.70% (w/w, dry wt basis) for eggplants and 0.3, 0.7, 1.3 and 2.6% (w/w) for peppers. After an additional week, soilless mixes were placed in plastic flats, planted and treated as were soil flats planted with sugar beets. Soilless mixes were planted with non-treated eggplant (*Solanum melongea* L. cv. Black Beauty) seed and pepper (*Capsicum annuum* L. cv. California Wonder) seed, 30 seeds (3 rows of 10 seeds each) per flat.

Statistical analysis

All tests were repeated twice and included at least 4 replicates per treatment. The experiments were arranged in a randomized block design. Resulting data from repeated experiments were combined and statistical analyses 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 ANOVA separation of means by Tukey's Studentized range (HSD). Data expressed in percentages were corrected for uneven distribution by arc sine transformation and back-transformed.

RESULTS

Effect of C. foecundissimum on survival and saprophytic growth of R. solani in soils

Antagonist-free bran (control) as well as bran inoculum of Cf-1 incubated from 0 to 17 days did not reduce survival of *R. solani* in LS or SCL (Table 1). However, the ability of *R. solani* to grow from beet seed to colonize both soils (saprophytic growth) was significantly reduced by bran with 3- to 17-day-old inoculum of Cf-1, but not freshly prepared (0 day) inoculum of the antagonist. Moreover, the magnitude of *R. solani* reduction caused by the 6-, 10- and 17-day-old inoculum was greater than that by the 3-day-old inoculum. There was no interaction between soil type and age of inoculum ($P = 0.76$). The extent of reduction of saprophytic growth by the various aged bran preparations was similar in both soils ($P < 0.001$).

In determining the effect of Cf-1 on the saprophytic growth of *R. solani* from soilless mix–cornmeal inoculum into 4 soil types, there was no interaction between antagonist inoculum and soil type ($P = 0.69$). Saprophytic growth was significantly reduced ($P < 0.001$) by bran preparations of Cf-1 in all 4 soils (Table 2). There was a slight, but significant, reduction in saprophytic growth ($P < 0.05$) in L and SCL soils compared to that in L and SCL in pathogen-infested soil amended with an antagonist-free preparation of bran.

Application of each of 4 isolates of *C. foecundissimum* also significantly ($P < 0.05$) reduced saprophytic growth of *R. solani* from soilless mix–cornmeal inoculum into LS (Table 3). Isolate ATCC 62373 was as effective as Cf-1 from the BPDL in this respect and confirmed the data with isolate Cf-1 of the previous experiment (Table 2). Although the isolates (181.66 and 182.66) from the CBS also significantly reduced saprophytic growth of the pathogen in LS, they were not as effective as the other isolates.

Effect of C. foecundissimum on damping-off

The application of bran infested with isolates Cf-1 and ATCC 62373 of *C. foecundissimum* for 10 days significantly prevented ($P < 0.05$) sugar beet damp-

Table 1. Survival and saprophytic growth of *R. solani* (R-23) in LS and SCL with various aged bran preparations of *C. foecundissimum* (Cf-1)*

Soil	Inoculum age of Cf-1 (days)	Survival of *R. solani* in infested beet seed (CI)†	Saprophytic growth of *R. solani* from infested beet seed into soil (CI)
LS	Control (*R. solani* only)	4.4a‡	3.6a
	Control (antagonist-free bran)	4.3a	3.2a
	0	4.2a	3.5a
	3	4.3a	2.3b
	6	3.8a	0.6c
	10	4.4a	0.4c
	17	3.8a	0.1c
SCL	Control (*R. solani* only)	4.3a	4.1a
	Control (antagonist-free bran)	4.1a	4.0a
	0	3.7a	4.0a
	3	3.6a	2.6b
	6	4.3a	1.4c
	10	4.4a	0.8c
	17	4.6a	0.8c

*Assayed 3 wk after addition of bran preparations (1%, w/w) to *R. solani*-infested soils.
†Colonization index (CI) indicates growth of *R. solani* on agar surface surrounding beet seed; 0 = no growth; 1 = few threads; 2, 3, 4, 5 = 25, 50, 75 and 100% of surrounding surface covered by hyphae, respectively.
‡Values in each column for each soil followed by the same letter are not significantly different according to Tukey's Studentized range (HSD).

ing-off caused by *R. solani* in LS compared to that incited by the pathogen in *R. solani*-infested soil or in infested soil amended with bran not containing any biocontrol fungus (Fig. 1). Statistical analysis of the interaction between weeks of incubation and biocontrol isolate indicated that plant stands did not vary significantly due to application of the biocontrol preparations during the 4-wk plant growth period. However, between 1–2 wk of incubation, there was a significant reduction in plant stands in the pathogen-infested systems. During the 4-wk incubation, isolate Cf-1 resulted in sugar beet stands (>90%) consistently greater ($P < 0.05$) than that obtained in the non-infested-pathogen control soil (77%). Although isolate ATCC 62373 also resulted in stands (60%) significantly greater than those in pathogen-infested soils (<20%), the stands were not as good as those in non-infested control soil. Unfortunately, bran preparations of isolates CBS 181.66 and CBS 182.66 did not perform well and resulted in stands less than (<25%), or similar to, those of pathogen-infested soil (Fig. 1).

The application of bran preparations of isolates Cf-1 and ATCC 62373 to establish their effect on damping-off of eggplant and pepper was chosen because these isolates were most effective in the reduction of saprophytic growth of *R. solani* in soilless mix (Table 3). To determine interactions among isolates, the rate of bran application and plant stand after 2 and 4 wk of growth, an ANOVA was performed for each crop. With eggplant there was no antagonist effect (Table 4). Both Cf-1 and ATCC 62373 performed similarly ($P = 0.67$) and rates resulted in stands comparable to those in non-infested control soilless mix and there were no replicate effects ($P = 0.97$). However, the high rate (0.7%) of Cf-1 more effectively prevented disease than a similar rate of ATCC 62373 ($P < 0.05$). The incidence of damping-off of eggplant increased with continued incubation so that there was more disease after 4 wk of growth compared to that after 2 wk of growth ($P < 0.0001$). As observed, there was significant interaction between the antagonist and duration of

Table 2. Saprophytic growth of *R. solani* (R-23) from soilless mix–cornmeal inoculum in LS, SL, L and SCL amended with a 10-day-old bran preparation of *C. foecundissimum* (Cf-1)*

Soil	Inoculum of Cf-1	Saprophytic growth of *R. solani* from soilless mix-cornmeal into soils (CI)†
LS	Control (*R. solani* only)	4.4a‡
	Antagonist-free bran	4.6a
	Cf-1 (1%)	0.7c
SL	Control (*R. solani* only)	4.2ab
	Antagonist-free bran	4.0ab
	Cf-1 (1%)	1.0c
L	Control (*R. solani* only)	4.1a
	Antagonist-free bran	3.2b
	Cf-1 (1%)	0.2c
SCL	Control (*R. solani* only)	4.5a
	Antagonist-free bran	3.0b
	Cf-1 (1%)	0.2c

*Assayed 3 wk after addition of bran preparations (1%, w/w) to *R. solani*-infested soils.
†, ‡See Table 1.

Table 3. Saprophytic growth of *R. solani* (R-23) from soilless mix–cornmeal inoculum in LS amended with 10-day-old bran preparations of 4 isolates of *C. foecundissimum**

Isolate and inoculum	Saprophytic growth of *R. solani* from soilless mix–cornmeal into soil (CI)†
Control (*R. solani* only)	3.8a‡
Control (antagonist-free bran)	3.2a
C. foecundissimum	
Cf-1	0.1c
ATCC 62373	0.1c
CBS 182.66	1.5b
CBS 181.66	1.6b

*Assayed 3 wk after addition of bran preparations (1%, w/w) to pathogen-infested soils.
†, ‡See Table 1.

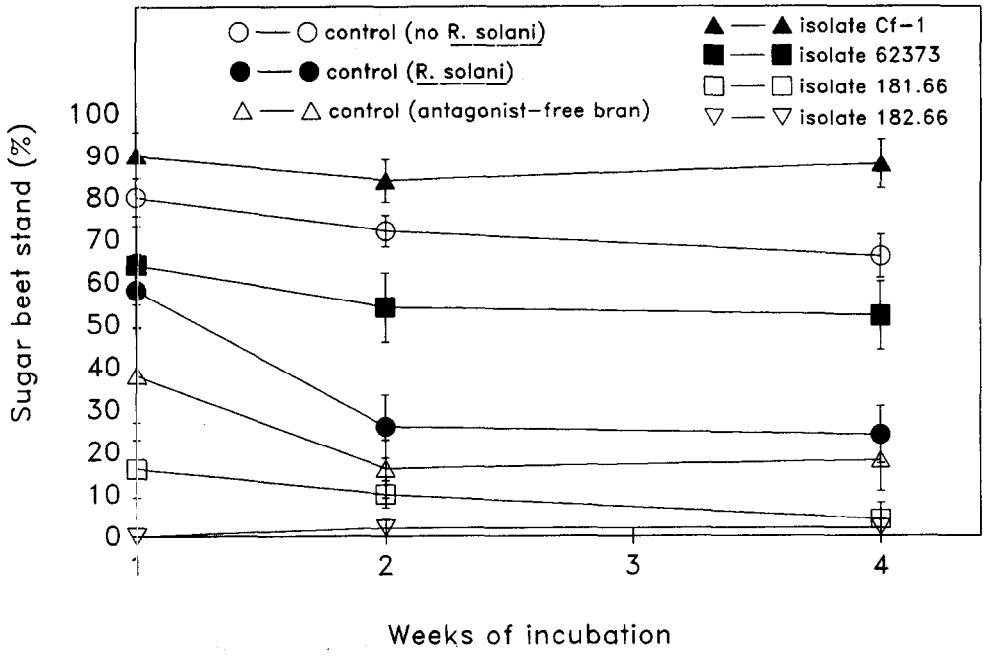

Fig. 1. The effect of 10-day-old bran preparations of isolates Cf-1, ATCC 62373, CBS 181.66 and CBS 182.66 of *C. foecundissimum* added at a rate of 2.0% (w/w) to a LS soil infested with *R. solani* supplied as soilless mix–cornmeal inoculum on sugar beet damping-off over a 4-wk incubation. Controls consisted of pathogen-free soil, pathogen-infested soil and pathogen-infested soil amended with bran with no biocontrol fungi. Lines at points represent the SD.

plant growth ($P < 0.0001$), but no interaction between *C. foecundissimum* isolate and the rate of inoculum application ($P = 0.65$). With pepper damping-off, bran preparations of isolate Cf-1 were slightly better for control than was isolate ATCC 62373 ($P < 0.05$) (Table 4); and, as with eggplant damping-off, there were no replicate effects ($P = 0.82$), but high rates of the antagonist inoculum were more effective in disease control than low rates ($P < 0.0001$). High rates of biocontrol inoculum gave stands comparable to those in pathogen-free control soilless mix. There was also less disease control with increased duration of plant growth ($P < 0.0001$), and a significant interaction between the antagonist and duration of plant growth ($P < 0.001$).

DISCUSSION

It appears that the rapid development of *C. foecundissimum* from beet seed used as a bait to trap *R. solani* in soil is not entirely unusual. von Arx and Gams (1966) also reported an abundant occurrence of

Table 4. Eggplant and pepper stands in soilless mix infested with *R. solani* (R-23) amended with several rates of 10-day-old preparations of isolates Cf-1 and ATCC 62373 of *C. foecundissimum* after 2 and 4 wk of growth

Isolate	Rate of inoculum (%, w/w)	Eggplant stand (%)*		Pepper stand (%)	
		2 wk	4 wk	2 wk	4 wk
Control (no *R. solani*)	0	95a‡	89a	92a	89a
Control (*R. solani* only)	0	57b	10c	42cd	17d
Control (antagonist-free bran)†	0	43b	7c	29d	7d
Cf-1	0.3	90a	85a	69b	20d
	0.7	87a	73a	73ab	21d
	1.3	ND	ND	92a	89a
	2.6	ND	ND	92a	79ab
ATCC 62373	0.3	94a	79a	47cd	18d
	0.7	83a	57b	49c	18d
	1.3	ND	ND	83ab	56c
	2.6	ND	ND	87ab	69bc

*Eggplant stand amended with Cf-1 and ATCC 62373 at rates of 1.3 and 2.6% were not determined (ND).

†Antagonist-free bran preparations were added to *R. solani*-infested soilless mix at a rate of 0.7% (w/w) for eggplant trials and 2.6% (w/w) for pepper trials.

‡See Table 1.

C. foecundissimum in buckwheat baits placed in soil. What is unusual is the ability of *C. foecundissimum* to be a potent inhibitor of saprophytic growth of the pathogen in soil and soilless mix (Tables 2 and 3), which may be related to its capacity to produce antibiotics against *R. solani* (Domsch and Gams, 1968). However, the inability of *C. foecundissimum* to reduce survival of *R. solani* in beet seed is not uncommon with biocontrol fungi. It is difficult for these organisms to displace an existing resident population in a food base or piece of organic debris (Bruehl, 1975). Similar results were observed with bran preparations of *L. arvalis* (Lewis and Papavizas, 1992), *S. aciculosa* (Lewis and Papavizas, 1993) and some formulations of *Trichoderma* spp and *G. virens* (=*T. virens*) (Lewis and Papavizas, 1987a; Lewis *et al.*, 1991). In contrast, bran preparations of selected antagonists such as *T. hamatum* (Tm-23, TRI-4) and *G. virens* (Gl-21) successfully eliminated *R. solani* from a food base (Lewis and Papavizas, 1985). All of the previous reports indicated that it was as common for a wide variety of biocontrol fungi as well as for *Cladorrhinum* to prevent saprophytic growth of *R. solani* from a food base into soil, but not to eliminate the pathogen within the colonized debris.

Our results also confirm the effectiveness of a variety of *C. foecundissimum* in reducing *R. solani* in several soil types. However, this fungus is not the only antagonist effective in reducing the inoculum denisty of *R. solani* in several soil types (Tables 1 and 2). Such effectiveness has also been reported for other biocontrol fungi such as *T. hamatum* (TRI-4), *S. aciculosa* and *L. arvalis* (Lewis and Papavizas, 1987a,b, 1992, 1993). Other factors to consider in assessing biocontrol effectiveness are isolate specificity and age of the applied inoculum. Also, the initial source of inoculum, such as mycelia grown on PDA, may be a factor in isolate effectiveness. For example, a growth medium less rich than PDA in soluble carbohydrates may result in a more effective inoculum (W. Gams, pers. commun.). It is also well known that species as well as isolates within a given fungal species have different biocontrol capabilities against specific pathogens of individual host plants. In our study, 2 isolates of *C. foecundissimum* were more effective against *R. solani* than 2 other isolates of the same species. It was of interest that generally, isolate Cf-1 isolated in Beltsville, performed better than the other 3 cultures isolated from Dutch soils. Does this suggest some sort of regional specificity? In other studies, some isolates of binucleate *R. solani* were more effective in controlling *R. solani* of sugar beet in the field than other isolates (Herr, 1988). Cotton damping-off was also controlled more effectively with *T. hamatum* than with *T. harzianum* (Elad *et al.*, 1992) and Rhizoctonia diseases of potato were controlled more effectively with preparations of *T. viride* than *T. hamatum* (Beagle-Ristaino and Papavizas, 1985).

For potential commercial use, the age of biocontrol inoculum is also critical. In our study 10- and 17-day-old bran formulations were more effective in the reduction of *R. solani* inoculum than younger preparations. Although this was also true for preparations of *S. aciculosa* (Lewis and Papavizas, 1992), 10-day-old bran preparations of *L. arvalis* were as effective as 5-day-old preparations in reducing pathogen inoculum (Lewis and Papavizas, 1993). On the contrary, it has routinely been established in the BPDL that young, actively-growing preparations (3-day-old) of *Trichoderma* spp and *G. virens* reduced inoculum density and disease caused by several pathogens better than older preparations (Lewis and Papavizas, 1987a; Lumsden and Locke, 1989; Papavizas and Lewis, 1989). Although our study was performed with bran preparations, research remains to be done using alginate prill and starch–flour formulations (Dunkle and Shasha, 1988; Lumsden and Locke, 1989) of *C. foecundissimum* for their effect on disease control.

The mechanism responsible for pathogen control has not been elucidated, but the possibility for antibiotic production exists (Domsch and Gams, 1968; Antheunisse and Burema, 1983). Moreover, the demonstration of *R. solani* growth inhibition on PDA plates by isolates of *C. foecundissimum* has been observed in the BPDL (data not reported). The observation by Domsch and Gams (1969) that *C. foecundissimum* produces cellulases and pectinases may also be implicated in the destruction or inhibition of selected pathogens containing these constituent materials.

The baseline for biocontrol capability is a demonstration of disease control and this has been adequately established in this study. However, considerable research remains to be done with formulation, timing and rates of application. A major obstacle in the establishment of isolate efficacy has been our inability to adequately determine biomass production in the bran. It is possible that the ineffectiveness of isolates CBS 181.66 and CBS 182.66 in disease control compared to Cf-1 and ATCC 62373 may be due to lack of substantial biomass formation by the former isolates in bran. This possibility also requires investigation.

The observation that bran formulations of isolates CBS 181.66 and CBS 182.66 inhibited sugar beet stand more than that in pathogen-infested soil (Fig. 1) was also unexpected. Generally, when reported, biocontrol metabolites may actually stimulate plant growth (Harman and Taylor, 1988). However, occasionally, growth-altering substances such as herbicides may be produced which retard plant growth (Jones and Hancock, 1987). This may have occurred in our study. Different effects on disease and pathogen inoculum were also observed in soils compared to soilless mix. A comparison of the data is obviously difficult because of the inherent differences in texture and microbial activity between the two milieux.

Nevertheless, the results indicate the potential value of further evaluating *C. foecundissimum* as a biological

control fungus to suppress damping-off diseases of economic crops caused by *R. solani* as well as other soilborne plant pathogens.

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REFERENCES

Alabouvette C., Lemanceau P. and Steinberg C. (1993) Recent advances in the biological control of Fusarium wilts. *Pesticide Science* **37**, 365–373.

Antheunisse J. and Burema J. (1983) Biological and chemical inhibition of ligno-cellulose decay by white rot basidiomycetes. *Journal of General and Applied Microbiology* **29**, 257–269.

Arendt G. L. (1987) Mixing peat-like mixes. *Bedding Plants News* **18**, 7.

von Arx J. A. and Gams W. (1966) Über *Pleurage verruculose* und die zugehörige *Cladorrhinum*-Konidienform. *Nova Hedwiga* **13**, 199–208.

Baker R. R. and Dunn P. E. (Eds) (1990) *Pest Management: Biologically Based Technologies*. American Chemical Society, Washington, DC.

Beagle-Ristaino J. and Papavizas G. C. (1985) Biological control of Rhizoctonia stem canker and black scurf of potato. *Phytopathology* **75**, 560–564.

Bruehl G. W. (1975) Systems and mechanisms of residue possession by pioneer fungal colonists. In *Biology and Control of Soil-borne Plant Pathogens* (G. W. Bruehl, Ed.), pp. 77–83. American Phytopathological Society, St Paul, MN.

Domsch K. H. and Gams W. (1968) Die Bedeutung vorfruchtabhängiger Verschiedungen in der Bodenmikroflora. *Phytopathplogische Zeitschrift* **63**, 165–176.

Domsch K. H. and Gams W. (1969) Variability and potential of a soil fungus population to decompose cellulose, pectin, xylan, and carboxymethylcellulose. *Soil Biology & Biochemistry* **1**, 29–36.

Domsch K. H., Gams W. and Anderson T.-H. (1980) *Compendium of Soil Fungi*. Academic Press, London.

Dunkle R. L. and Shasha B. S. (1988) Starch encapsulation of *Bacillus thuringiensis*: a potential new method for increasing environmental stability of entomopathogens. *Environmental Entomology* **17**, 120–126.

Elad Y., Kalfon A. and Chet I. (1992) Control of *Rhizoctonia solani* of cotton by seed-coating with *Trichoderma* spp spores. *Plant and Soil* **66**, 279–281.

Fravel D. R. and Adams P. B. (1986) Estimation of United States and world distribution of *Talaromyces flavus*. *Mycologia* **78**, 684–686.

Fravel D. R., Adams P. B. and Potts W. E. (1992) Use of disease progress curves to study the effects of the biocontrol agent *Sporidesmium sclerotivorum* on lettuce drop. *Biocontrol Science and Technology* **2**, 341–348.

Gams W., Domsch K. H. and Weber E. (1969) Nachweis signifikant verschiedener Pilzpopulationen bei gleicher Bodennutzung. *Plant and Soil* **31**, 439–450.

Harman G. E. and Taylor A. G. (1988) Improved seedling performance by integration of biological control agents at favorable pH levels with solid matrix priming. *Phytopathology* **78**, 520–525.

Herr L. (1988) Biocontrol of Rhizoctonia crown and root rot of sugar beet by binucleate *Rhizoctonia* spp and *Laetisaria arvalis*. *Annals of Applied Biology* **113**, 107–118.

Hornby D. (Ed.) (1990) *Biological Control of Soil-borne Plant Pathogens*. CAB International, Wallingford.

Jones R. W. and Hancock J. G. (1987) Conversion of viridin to viridiol by viridin-producing fungi. *Canadian Journal of Microbiology* **33**, 963–966.

Lewis J. A. and Papavizas G. C. (1985) Effect of mycelial preparations of *Trichoderma* and *Gliocladium* on populations of *Rhizoctonia solani* and the incidence of damping-off. *Phytopathology* **75**, 812–817.

Lewis J. A. and Papavizas G. C. (1987a) Reduction of inoculum of *Rhizoctonia solani* in soil by germlings of *Trichoderma hamatum*. *Soil Biology & Biochemistry* **19**, 195–201.

Lewis J. A. and Papavizas G. C. (1987b) Application of *Trichoderma* and *Gliocladium* in alginate pellets for control of Rhizoctonia damping-off. *Plant Pathology* **36**, 438–446.

Lewis J. A. and Papavizas G. C. (1988) Biocontrol of *Rhizoctonia solani* (Rs) by some novel soil fungi. *Phytopathology* **78**, 862.

Lewis J. A. and Papavizas G. C. (1992) Potential of *Laetisaria arvalis* for the biocontrol of *Rhizoctonia solani*. *Soil Biology & Biochemistry* **24**, 1075–1079.

Lewis J. A. and Papavizas G. C. (1993) *Stilbella aciculosa*: A potential biocontrol fungus against *Rhizoctonia solani*. *Biocontrol Science and Technology* **3**, 3–11.

Lewis J. A., Papavizas G. C. and Lumsden R. D. (1991) A new formualtion system for the application of biocontrol fungi to soil. *Biocontrol Science and Technology* **1**, 59–69.

Lumsden R. D. and Locke J. C. (1989) Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens*. *Phytopathology* **79**, 361–366.

Lumsden R. D. and Vaughn J. L. (Eds) (1993) *Pest Management: Biologically Based Technologies*. American Chemical Society, Washington, DC.

Mouchacca J. and Gams W. (1993) The hyphomycete genus *Cladorrhinum* and its teleomorph connections. *Mycotaxon* **48**, 415–440.

Murdoch C. W. and Leach S. S. (1993) Evaluation of *Laetisaria arvalis* as a biological control agent of *Rhizoctonia solani* on white potato. *American Potato Journal* **70**, 625–634.

Papavizas G. C. (1992) Biological control of selected soilborne plant pathogens with *Gliocladium* and *Trichoderma*. In *Biological Control of Plant Diseases* (E. C. Tjamos, G. C. Papavizas and R. J. Cook, Eds), pp. 223–230. Plenum Press, New York.

Papavizas G. C. and Lewis J. A. (1986) Methods for isolating and identifying *Rhizoctonia solani* and for reducing inoculum. In *Methods for Evaluating Pesticides for Control of Plant Pathogens* (K. D. Hickey, Ed.), pp. 50–53. American Phytopathological Society, St Paul, MN.

Papavizas G. C. and Lewis J. A. (1989) Effect of *Gliocladium* and *Trichoderma* on damping-off and blight of snapbean caused by *Sclerotium rolfsii*. *Plant Pathology* **38**, 277–286.

Tjamos E. C., Papavizas G. C. and Cook R. J. (Eds) (1992) *Biological Control of Plant Diseases*. Plenum Press, New York.

Whipps J. M., McQuilken M. P. and Budge S. P. (1993) Use of fungal antagonists for biocontrol of damping-off of Sclerotinia disease. *Pesticide Science* **37**, 309–313.