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Enhancement of Snap Bean Emergence by *Gliocladium virens*

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Abstract. **Emergence of snap beans (*Phaseolus vulgaris* L.) in field soil in 1993–95 was enhanced by the biocontrol agent *Gliocladium virens* J.H. Miller, J.E. Giddens, & A.A. Foster. The fungus was applied to each seed at planting as a wheat bran alginate pellet formulation in 1993–95. Preemergence and postemergence damping-off were reduced in plots treated with *G. virens*. Nodulation on the roots of treated plants was numerically increased in 1993 and 1994 compared to nontreated plots. Efficacy of *G. virens* was reduced in 1995, probably due to high ambient temperatures at the time of planting. In plots with reduced stand, leaf area was increased and yield on a per-plant basis was larger than in plots with a better stand. Total yield also was increased in plots with fewer plants, except in 1994. Fungi isolated from failed seedlings included *Fusarium* spp., *Pythium* spp., and *Rhizoctonia solani* Kühn.**

Preemergence and postemergence damping-off are major factors in poor stand establishment in garden and commercial plantings of snap beans. Damping-off is caused by several soilborne fungi, including *Fusarium* spp., *Rhizoctonia solani*, and *Pythium* spp., and is exacerbated by cold, wet soil (Hall, 1991). Damping-off may be reduced, but not controlled, by planting when soils are relatively warm and dry.

Species of *Trichoderma* Pers.:Fr. and *Gliocladium* Corda have exhibited promise in laboratory and greenhouse trials for control of soilborne pathogens (Lumsden and Locke, 1989; Papavizas, 1985). Their applicability for use as biocontrol agents in field situations has not been extensively demonstrated.

One difficulty in using fungi as biocontrol agents on a large scale is the production of inoculum in a convenient, easily applied form (Papavizas, 1985). Recently, a method for encapsulating fungi in alginate pellets has been developed (Lewis and Papavizas, 1984; Walker and Connick, 1983). The objective of my study was to test the efficacy of using an alginate pellet formulation of *Gliocladium virens* for control of snap bean damping-off in the field. Preliminary reports have been published (Smith, 1994, 1995).

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Materials and Methods

An isolate of *G. virens* previously shown to have plant growth-promoting effects on snap bean and biological control ability against *Phytophthora cactorum* (Lebert & Cohn) J. Schröt. (Smith et al., 1990) was used in the present study. *Gliocladium virens* 041 (ATCC 20906) was grown on half-strength malt agar (Difco, Detroit) for 5 to 7 days under constant light to induce production of conidia.

Organic food-grade wheat bran was ground to pass a mesh screen with a 1-mm^2 opening (#20 mesh) in a Wiley mill. Conidia (10^{10}/mL) were added to an aqueous suspension of ground wheat bran, and alginate pellets were made according to the method of Fravel et al. (1984). Pellets were allowed to dry completely in a stream of air at 22 to 25 °C and used within 3 days. If necessary, pellets were stored at 10 °C, sealed in dry metal cans, and used as needed. Pellets also were made without *G. virens* conidia and were used to treat control plots.

Each year before planting, germination tests were conducted on the seed lot to be used that year. In all cases, germination of the seed lots exceeded 99%.

On 17 June 1993, 24 May 1994, and 31 May 1995, 'Bush Blue Lake' snap beans were planted in Cheshire fine sandy loam soil in Hamden, Conn. Initial N levels were 10 to 15 mg·kg^{-1}, and no N fertilizer was added at any time. The area to be planted was cultivated with a rototiller then raked smooth by hand; large stones and clods were removed. Seeds were planted by hand, at a depth of 4 cm and with 4 cm between seeds. Cracked, broken, or discolored seeds were not planted. Between-row spacing was 46 cm, and individual plot rows were 3.1 m long (80 seeds/3.1 m). All spacings were measured and not estimated. All seeds received 3 mL (≈0.2 g) of wheat bran alginate pellets, applied directly atop the seed, and were covered with soil and firmed by hand. Treatments included pellets with or without *G. virens* and treatments were replicated eight times in 1993 (16 plots total), 32 times in 1994 (64 plots total), and 16 times in 1995 (32 plots total). Plots were watered as needed, and weeds were removed by hand. In 1994 and 1995, damage by Mexican bean beetles (*Epilachna varivestis* Mulsant) was reduced by handpicking of larvae and adults and by treating plots with *Bacillus thuringiensis* var. *kurstaki* (Dipel 2X) and malathion (Ortho Malathion 50 Plus; Monsanto Co., St. Louis) according to label recommendations. Stand counts were taken 14 days after planting.

In 1993 and 1994, isolations were made from seedlings that did not emerge. Lesioned hypocotyls were plated onto water agar to detect pathogens and subcultures were made onto acidified potato dextrose agar and Komada's agar (1975) to identify fungi to genus and species, when possible. Isolations for bacteria were not conducted.

Marketable pods were harvested beginning on 5 Aug. 1993, 18 July 1994, and 24 July 1995, and at 3- to 4-day intervals until yields declined sharply. Yield of marketable pods was recorded from the inner 1.8 m of each row; leaving 0.6 m at each end of each row as a buffer between adjoining rows.

After the plants stopped bearing marketable pods, all plots were destructively sampled. Five plants were dug by hand from the middle of the harvested area of each row, and the number of trifoliates, total plant weight, and the number of nodules on the roots of each plant was determined.

Results

In 1993 and 1994, emergence of snap beans, expressed as stand density, was increased significantly by the addition of *G. virens* in bran pellets at the time of planting (Table 1). In 1995, emergence was essentially the same in rows with *G. virens* as in rows without *G. virens.* Daily maximum temperatures were higher than usual in 1995. While the 30-year average daily maximum (°C) for June, July, and August is 25.3, 28.3, and 27.3, respectively, the average daily maximum (°C) for those months in 1995 was 26.4, 30.8, and 30.2.

Average leaf area of plants in rows with reduced stand (no *G. virens*) was increased in all years, except in 1995, when there was no significant difference in stand. Reductions in stand in 1993 and 1994 resulted in somewhat larger plants and a corresponding increase in yield on a per-plant basis. Total yield also was somewhat higher with reductions in plant stand. Nodulation was statistically greater in rows treated with *G. virens* in 1993 and 1994, and while not significant, followed a similar trend in 1995. Fungi recovered from damped-off seedlings included *Fusarium* spp., *Pythuim* spp., *Phytophthora* spp., and *Rhizoctonia solani* (Table 2).

Conclusions

The primary effect of adding *G. virens* to snap bean seeds at planting was an enhancement of seedling emergence. Growth-promoting

Table 1. Stand establishment, leaf area, nodulation, and yield of snap beans treated with wheat bran alginate pellets, with and without *Gliocladium virens* 041, 1993–95.

Criterion	*G. virens*		
	Year (no. plots)	With	Without
Stand density (plants/3.1 m)[z]	1993 (8)	54.4 ± 3.1	31.3 ± 4.4*
	1994 (32)	24.8 ± 1.8	17.8 ± 1.5*
	1995 (16)	18.8 ± 4.1	18.6 ± 2.6NS
Leaf area (dm^2)[z]	1993 (8)	10.9 ± 1.1	10.6 ± 0.8NS
	1994 (32)	16.5 ± 3.3	29.3 ± 3.2*
	1995 (16)	25.3 ± 1.9	22.3 ± 1.4NS
Nodules per plant (no.)[z]	1993 (8)	54.3 ± 11.1	16.9 ± 4.8*
	1994 (32)	30.4 ± 4.7	25.4 ± 3.2**
	1995 (16)	18.6 ± 3.2	23.4 ± 3.5NS
Total yield (kg)[y]	1993 (8)	14.1	18.8
	1994 (32)	54.5	50.5
	1995 (16)	14.9	18.4
Plants harvested (no.)[x]	1993 (8)	435	250
	1994 (32)	370	286
	1995 (16)	139	139
Yield/plant (g)	1993 (8)	32	75
	1994 (32)	147	177
	1995 (16)	107	132

[z]Mean ± standard error of the mean.
[y]Total amount of marketable pods from the inner 1.8 m of all rows in that treatment.
[x]Total number of plants from which harvest data was taken.
NS, *, **Nonsignificant or significant at $P < 0.05$ or 0.1, respectively, by analysis of variance. Comparisons are within years.

Table 2. Frequency of isolation of fungi from damped-off snap bean seedlings. In some instances, more than one fungus was isolated from a seedling.

Fungus species	*Gliocladium virens*		
	Year	With[z]	Without[z]
Fusarium spp.	1993	3	31
	1994	5	20
	1995	2	46
Pythuim spp.	1993	3	12
	1994	0	10
	1995	0	2
Phytophthora spp.	1993	0	0
	1994	0	1
	1995	0	0
Rhizoctonia solani	1993	6	13
	1994	8	18
	1995	8	3
Other fungi	1993	15	18
	1994	18	20
	1995	12	23

[z]Number of damped-off seedlings from which the fungus was isolated.

effects of this isolate reported from previous greenhouse studies (Smith et al., 1990) were absent in the field. Nodulation also was increased on plants that had been treated with *G. virens*. The isolate of *G. virens* used was selected to be tolerant of low temperatures (Smith et al., 1992), and its efficacy may have been reduced by high temperatures at the time of planting in 1995. Increased stand establishment did not result in increased yield; however, in plots with fewer plants, plants were larger (as reflected by a larger leaf area) and more productive than in those plots with more plants.

Reduction in damping-off incidence of snap bean was achieved by addition of *G. virens* at the time of planting. Damage by *Fusarium* and *Rhizoctonia solani* especially were reduced in the present study. Lumsden and Locke (1989) demonstrated control of damping-off in soilless mix with *G. virens*. As damping-off may be caused by many pathogenic organisms (Hall, 1991), this study demonstrates that there is potential for control of or decrease in the number of these pathogens on other host plants in naturally infested field soil. Current control measures for damping-off of many plants are limited to alteration of planting time or use of broad-spectrum fungicides. Use of biological control agents may be a more economical and ecologically acceptable method of controlling damping-off.

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