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Title: Potential of Trichoderma sp. for Inhibition of Botrytis cinerea and Sclerotinia sclerotiorum on Snap Beans.

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Isolates of Trichoderma spp. collected from snap bean foliage were tested for inhibition of grey mold of snap beans caused by Botrytis cinerea. In a detached blossom-pod assay an isolate of Trichoderma hamatum reduced pod rot by 94% compared to the nontreated check. Control was comparable to that obtained with the fungicide vinclozolin. As few as 42 colony forming units (CFU) of T. hamatum per blossom reduced pod rot by 77% compared to the nontreated check. Control was 97% when 233 CFU per blossom were applied. Grey mold was reduced only when spores of T. hamatum were applied to blossoms prior to, or at the same time as, conidia of B. cinerea.

In field studies conditions were very warm and dry. Because B. cinerea is primarily a cool weather pathogen, grey mold incidence was too low to detect meaningful differences among treatments. Differences in the incidence of pod rot caused by Sclerotinia sclerotiorum, however, were observed among treatments in these field

trials. Control of white mold by T. hamatum was comparable to that obtained with the fungicide vinclozolin. Populations of Trichoderma spp. on foliage rapidly decreased following application of a T. hamatum spore suspension but remained higher in treated plots than in nontreated plots up to two weeks following application.

The isolate of T. hamatum used in these trials produces volatile compounds which in vitro inhibits the growth of B. cinerea and S. sclerotiorum. Mean radial growth of B. cinerea in a closed chamber with a T. hamatum colony was 0.6 mm compared to 23.6 mm when enclosed with noninoculated potato dextrose agar (PDA). Mean radial growth of S. sclerotiorum was 0.8 mm when enclosed with a T. hamatum colony compared to 32.3 mm when enclosed with noninoculated PDA.

Potential of *Trichoderma* sp. for Inhibition of *Botrytis cinerea* and *Sclerotinia sclerotiorum* on Snap Beans

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INTRODUCTION

Oregon is the second largest producer of bush snap beans in the United States. Approximately 113,000 metric tons of beans are produced on 9,700 ha annually. In 1985 this crop was worth over 21 million dollars (50).

Grey mold, caused by *Botrytis cinerea* Pers. ex Fr., may be found on all aerial portions of the snap bean (*Phaseolus vulgarus* L.) plant (12). Economic loss is due primarily to pod rot which reduces quality and increases processing costs. When conditions are cool and moist, losses may be substantial.

At the present time grey mold is managed primarily by application of fungicides and to some extent with cultural practices. Cultural practices consist mostly of crop rotation, selection of fields with good air circulation, and modification of irrigation practices to avoid extended periods of leaf wetness (52). When environmental conditions favor the disease, cultural practices alone are not adequate to control grey mold. For this reason, application of a fungicide is necessary to ensure a marketable crop.

In some cases the application of a fungicide may actually increase the severity of *Botrytis*-caused diseases. This is presumably due to the presence of *Botrytis* strains resistant to the fungicide in the fungus

population while natural antagonists are suppressed by the chemical (9,27). Bollen and Scholten (9) reported that benomyl used to control Botrytis rot of cyclamen corms resulted in much more disease on treated than on nontreated plants even though benomyl was originally very effective. In Oregon, the incidence of grey mold of snap beans was greater in plots sprayed with benomyl than in nontreated plots (33,54).

The ability of B. cinerea to develop strains resistant to fungicides (9,20,31,35,36,43,54) coupled with the difficulties encountered with registration of fungicidal compounds has resulted in an interest in biological control strategies.

Organisms which have been shown to have potential for suppression of diseases on the phylloplane include bacteria, yeasts and fungi. In his review of ecological succession on leaf surfaces, Blakeman (8) advised that artificial introduction of organisms to the plant surface is most likely to be successful at the time of the year when those groups form a dominant component of the microflora in the field.

Bacteria often are the first colonists on newly emerging leaves (8) and are frequently considered to be useful biological control agents on young plant tissue. For example, non-ice-nucleating Erwinia herbicola strains became established on leaves of newly emerged corn when colonization by other microbes was limited (34).

Yeasts require an adequate supply of simple sugars to become established on the leaf (8). Increased leakage of sugars as the leaf matures coupled with deposition of pollen and honeydew encourages increases in yeast populations. A supply of sugar is often considered essential when yeasts are applied as biocontrol agents. Sucrose and yeast extracts were applied with yeast cells to control Cochliobolus sativus and Septoria nodorum on field-grown wheat (26).

Filamentous fungi are most numerous at the end of the growing season when spores in the air are most abundant (8). The period of fungal dominance on the phylloplane often coincides with the beginning of leaf senescence. As tissue, senesces plant resistance can be overcome, and fungi may penetrate and colonize internal tissue. For this reason, Blakeman (8) suggested that filamentous fungi are probably the most useful as biological control agents on senescing plant tissue.

Botrytis cinerea requires a saprophytic phase prior to infection of healthy plant tissue (17,30,31,44,46,51). This dependence on an exogenous nutrient source led Wood (53) to suggest control of B. cinerea by "...colonization of dead or moribund tissue by saprophytes so as to preclude subsequent invasion by the pathogen." In the case of beans, senescent blossoms provide an important saprophytic base for infection of pods by B. cinerea (12). Colonization of these blossoms by organisms

antagonistic to B. cinerea should inhibit infection of the developing pods.

Approximately four weeks elapses between first bloom and harvest of snap beans. During this brief period natural populations of inhibitors do not increase rapidly enough to achieve the degree of disease suppression required by commercial agriculture. Artificial culture and application of inhibitory organisms to the blossoms might provide the disease control demanded in commercial bean production. Newly opened blossoms presumably are relatively free of microorganisms resulting in minimal competition for microbes applied as biocontrol agents.

There are examples of biological control of Botrytis-caused diseases by colonization of senescing tissue. Wood (53) inhibited Botrytis rot on detached lettuce leaves in the laboratory when suspensions of antagonists were pipetted on to areas killed by contact with solid carbon dioxide. Almost complete control was obtained with Bacillus dendroides, Penicillium clavariaeforme and Trichoderma viride. Application of a soil suspension also gave a large measure of control. A substantial reduction of Botrytis leaf rot of lettuce in cold frames was obtained with applications of Bacillus dendroides, Pseudomonas sp., Streptomyces sp., Penicillium clavariaeforme, Trichoderma viride, Fusarium sp. and Streptomyces lavendulae applied in a similar manner (53). Pseudomonas sp. and Penicillium clavariaeforme applied to

lettuce seedlings reduced damping-off caused by B. cinerea in cold frames (53).

The inoculation and colonization of dead tomato flowers by Cladosporum herbarum prevented the development of B. cinerea on petals and subsequent fruit infection in glasshouses (41). Grey mold on strawberries was reduced in the field with applications of several species of Trichoderma beginning at early bloom (47). Dry eye rot of apple was reduced in the field by spraying the blossoms with T. harzianum (49). Dubos (23) reduced grey mold on grapes in the field with applications of Trichoderma harzianum to the flowers. These trials were successfully repeated over a period of several years. Other workers (7) inhibited B. cinerea on grapes in the field by applying conidial suspensions of Trichoderma spp., Cladosporum sp. and Aureobasidium sp. beginning at flowering.

The first step in development of a biological control system is to isolate and identify organism(s) with potential for disease control. Because organisms compete most successfully in the environment in which they have evolved and to which they are adapted, the search for biological control agents should be in the same system in which they will be applied (4,19).

The purpose of this study was to examine the potential of isolates of Trichoderma spp. recovered from snap bean foliage to suppress grey mold of snap beans.

MATERIALS AND METHODS

Collection of potential antagonists: In 1985, snap bean plants were collected from 15 commercial fields in Oregon's Willamette Valley. Blossoms and the terminal leaflet of the first trifoliate leaf were removed from each plant. Blossoms and leaflets were placed individually in 250 ml Erlenmeyer flasks containing 5 or 50 ml distilled water, respectively. The wash solution contained 0.1 ml Tween 20 (Sigma Chemical Co., St. Louis, Mo.) per liter as a wetting agent. Flasks were placed on a rotary shaker at 150 rpm for 30 min. The wash solution was serially diluted, and 0.2 ml of each dilution was plated on Trichoderma-selective medium (TSM) (24). After incubation at room temperature for 7 days selected isolates were transferred to malt agar and placed in storage at 4° C.

Greenhouse production of blossoms and pods: Snap beans, cultivar 'Oregon trail' (seed provided by J. R. Baggett, Department of Horticulture, Oregon State University), were seeded in plastic pots (Polycan #1, Anderson Die and Mfg. Co., Portland, Or.) containing the Oregon State University greenhouse soil mix (40% pumice, 20% peat, 20% sand and 20% soil). Prior to planting 1.0 kg calcium nitrate (15.5-0-0, 19% calcium), 1.5 kg sulfomag (0-0-22-22-19), and 2.0 kg superphosphate (0-25-0) were incorporated into each cubic meter of soil. Two weeks after planting seedlings were thinned to three per

container and 6.5 g Osmocote (14-14-14, 2-3 month formulation, Sierra Chemical Company, Milpitas, Ca.) was topdressed on each container. Blossoms and mature pods were harvested for laboratory trials.

Preparation of spore suspensions: Spore suspensions used in laboratory tests were obtained from sporulating 14-day-old cultures of the potential antagonist or of B. cinerea grown on 10-cm-diameter plates of potato dextrose agar (PDA) at room temperature. Ten ml of 0.01 M phosphate buffered saline solution (with 0.01% Tween 20) (PBST) were added to each plate. Spores were removed by gentle agitation with a glass rod. After filtering the suspension through cheesecloth, spore concentrations were determined with a hemacytometer. Concentrations were adjusted by dilution with PBST.

In vivo screening: Thirty-three fungal isolates were tested for their ability to prevent grey mold infection of bean pods. Blossoms and pods were obtained from greenhouse grown bean plants. Blossoms were placed on wire screens in clear plastic boxes (30 x 24 x 10 cm) which contained a thin layer of tap water to maintain high relative humidity, and then covered. Using a chromatography sprayer (Sigma Chemical Co., St. Louis, Mo.) 5 ml of a spore suspension of the potential antagonist were applied to the blossoms in each box. There were two boxes per isolate tested with a minimum of 10 blossoms per box. The blossoms in one box were

treated at 10^4 spores per ml and the blossoms in the other box at 10^5 spores per ml. After incubation in growth chambers at 17° C for 24 hr (16 hr light, 8 hr dark) the blossoms were sprayed with 5 ml of a 10^5 per ml conidial suspension of B. cinerea. Inoculated blossoms were placed in contact with apparently healthy bean pods, and the boxes were returned to the growth chambers. After incubation for 4 days the proportion of blossoms initiating pod rot was determined. Blossoms in check boxes were sprayed with 5 ml of a 10^5 per ml conidial suspension of B. cinerea. No attempt was made to sterilize flowers or pods prior to application of the treatments.

Fourteen isolates which gave some reduction in pod rot in the preliminary test were selected for further screening in replicated trials. Inoculation and incubation procedures were as described above except all potential antagonists were applied at 10^5 spores per ml, and B. cinerea was applied 24 hr later at 10^4 conidia per ml. For comparison, a treatment with B. cinerea alone at 10^4 conidia per ml and a fungicide treatment (vinclozolin, 5 ml at 0.6 g a.i. per liter) were included. Treatments were arranged in a randomized complete block design with three replications blocked over time. An isolate of Trichoderma hamatum (Bonord.) Bain (Is 117) was selected for additional laboratory and field tests.

Spore populations required for disease suppression: The number of T. hamatum, Is 117, spores per bean blossom required to reduce grey mold in vivo was determined. Blossoms were placed on wire screens in plastic boxes as described above. Blossoms were sprayed with 5 ml of either a 10^3, 10^4, 10^5 or 10^6 per ml spore suspension. After a 24 hr incubation period at room temperature the blossoms were sprayed with 5 ml of a 10^4 per ml conidial suspension of B. cinerea. Inoculated blossoms were placed in contact with apparently healthy bean pods. After an additional 4-day-incubation period the proportion of blossoms initiating pod rot was determined. A treatment with B. cinerea applied alone was included as a check. There were four replications in a completely randomized design.

To determine the number of T. hamatum spores applied per blossom, five blossoms were removed from each plastic box immediately after application of the antagonist. The five blossoms from each box were placed together in 25 ml distilled water with Tween 20 (0.1 ml/L) in a 250 ml flask and washed on a rotary shaker for 1 hr at 150 rpm. The wash solution was serially diluted, and 0.2 ml of each dilution was plated on each of two plates of TSM. After 4 days incubation at room temperature colonies characteristic of Trichoderma spp. were counted and the number of colony forming units (CFU) per blossom was determined.

Effect of T. hamatum application time on grey mold: The importance of sequence of T. hamatum and B. cinerea application on incidence of grey mold was tested. Snap bean blossoms were placed in plastic boxes and inoculated as described above. Treatments consisted of: 1) B. cinerea applied 24 hr prior to T. hamatum, 2) T. hamatum applied 24 hr prior to B. cinerea, 3) B. cinerea and T. hamatum applied together, and 4) B. cinerea applied alone. B. cinerea and T. hamatum were applied at 104 and 105 spores per ml, respectively. Inoculated blossoms were placed in contact with apparently healthy bean pods. After incubation at room temperature for 4 days the proportion of blossoms initiating pod rot was determined. Treatments were arranged in a randomized complete block design with four replications blocked over time.

Field trials: T. hamatum was tested for efficacy against grey mold in field trials during the 1986 growing season. Plots of snap beans, cultivar "Romano", were established near Woodburn, Oregon on Amity silt loam (24% clay). Beans were seeded in rows spaced 0.9 m apart at a rate of 13 kg per ha with 450 kg per ha 13-39-0 sidedressed. Two weeks prior to bloom 170 kg per ha ammonium nitrate was sidedressed. Water was applied with solid set sprinklers at a rate of 2.5 cm per wk before bloom and 3.8 cm per wk during and after bloom. Plots were 6 m long and three rows wide. Treatments consisted of: 1) 105 spores per ml applied at early bloom, 2) 105

spores per ml applied at early and full bloom, 3) 10^6 spores per ml applied at early bloom, 4) 10^6 spores per ml applied at early and full bloom, 5) a fungicide treatment (vinclozolin at 0.6 kg a.i. per ha) applied at early bloom and 6) a nontreated check. One half liter of the spore suspension was applied to the center row of each plot. Treatments were arranged in a randomized complete block design with five replications.

Spore suspensions were prepared one day prior to application. Sporulating 14-day-old cultures of T. hamatum grown on PDA were washed with PBST (10 ml per plate) by gentle agitation with a glass rod. The wash solution was filtered through cheesecloth and spore concentrations were determined with a hemacytometer. Tank suspensions of 10^5 and 10^6 spores per ml were prepared by dilution with tap water.

Sprays were applied with a CO_2 pressurized backpack sprayer. Spore suspensions were applied at 280 kPa (40 psi), and the fungicide was applied at 210 kPa (30 psi). The spray boom had two nozzles spaced 46 cm apart and was held 15 cm above the crop canopy.

The number of viable T. hamatum spores on foliage were determined immediatly after application and at weekly intervals for 2 wk following the second application. Three leaves were removed at random from each plot, except those which received the fungicide treatment, and placed together in a 1000 ml Erlenmeyer

flask with 200 ml distilled water. Tween 20 (0.1 ml/L) was included as a wetting agent. Leaves were washed by vigorously shaking the flasks for 30 sec. The wash solution was serially diluted and a 0.2 ml aliquot of each dilution was plated on each of three plates of TSM. Colonies characteristic of Trichoderma spp. were counted after a 4-day-incubation period at room temperature and colony forming units (CFU) per leaf were determined.

At harvest the plots were sampled by randomly pulling eight plants from the center of each plot. All pods over 8 cm in length were examined for symptoms of grey mold and white mold. A minimum of 100 pods per plot were examined.

Effect of volatiles produced by T. hamatum: Inhibition of B. cinerea by volatile metabolites produced by T. hamatum, Is 117, was tested using a modification of Dennis and Webster's enclosed chamber test (21). A 5-mm-diameter plug was transfered from the margin of a 3-day-old T. hamatum colony grown on PDA to the center of a 100-mm-diameter plastic petri dish containing PDA. One of these dishes and one containing noninoculated PDA were sealed together in each of 8 plastic bags and placed in a continually dark 20° C incubator. Fourty-eight hrs later a 5-mm-diameter plug was transferred from the margin of a 3-day-old B. cinerea colony on PDA to a 100-mm-diameter glass petri dish containing PDA. With the tops removed, the glass petri dishes were immediately inverted over the

plastic dishes containing the 48-hr-old T. hamatum colonies or noninoculated PDA. One set of dishes containing B. cinerea inverted over T. hamatum and one set of dishes containing B. cinerea inverted over noninoculated PDA were sealed together in each plastic bag and returned to the 20° C incubator. After an additional 72 hr incubation period radial growth of the B. cinerea colonies was measured. There were 8 replications in a randomized complete block design with plastic bags representing blocks.

An identical test was conducted with S. sclerotiorum in place of B. cinerea.

RESULTS

In vivo screening: Of the 14 isolates tested in the replicated detached blossom-pod assay, 12 significantly ($p = 0.05$) reduced the amount of pod rot (Table 1). Is 117, an isolate of Trichoderma hamatum, reduced the incidence of pod rot by 94%. This was not significantly different from the fungicide treatment which gave 100% control. This isolate was selected for additional laboratory and field tests.

Spore populations required for disease suppression: As few as 42 T. hamatum CFU per blossom significantly reduced ($p = 0.05$) pod rot by 77% (Table 2). With 233 CFU per blossom, control of pod rot was 97%. This was not significantly different from higher populations which gave 100% control of grey mold.

Effect of T. hamatum application time on grey mold: Spores of T. hamatum applied prior to or with conidia of B. cinerea significantly reduced ($p = 0.01$) the amount of pod rot compared to B. cinerea applied alone (Table 3). When conidia of B. cinerea were applied prior to T. hamatum, pod rot was not significantly reduced.

Field trials: The incidence of grey mold in the field was too low to detect meaningful differences among treatments. Differences in white mold pod rot incidence caused by Sclerotinia sclerotiorum (Lib.) deBary, however, were observed among treatments. All four T. hamatum treatments significantly lowered ($p = 0.01$)

TABLE 1. Control of grey mold of snap beans *in vivo* by fungal antagonists

Fungal isolates[1]	Prop. blossoms initiating pod rot[2]	% Control[3]
Control (vinclozolin)	0	100
Is 117 (*Trichoderma hamatum*)[4]	0.06	94
Is 114	0.21	78
Is 118	0.21	78
Is 116	0.28	71
Is 147	0.37	61
Is 160	0.39	59
Is 161	0.39	59
Is 143	0.40	58
Is 144	0.44	54
Is 125	0.48	50
Is 164	0.58	39
Is 126	0.65	32
Is 155	0.84	12
Is 115	0.90	5
Check (*Botrytis cinerea* alone)	0.95	-
FPLSD[5] (p=0.05)	0.20	

[1] Spore suspension (10^5 spores per ml) or fungicide (0.6 g a.i. per L) applied 1 day prior to application of a *B. cinerea* spore suspension (10^4 per ml).
[2] Inoculated blossoms held at 17° C for 4 days.
[3] Percent reduction of pod rot compared to *B. cinerea* applied alone.
[4] Selected for further laboratory and field tests.
[5] Fisher's protected least significant difference.

TABLE 2. Effect of population size of *Trichoderma hamatum* on grey mold of snap beans

Spores/ml applied[1]	CFU[2]/blossom	Prop. blossoms initiating pod rot[3]	%Control[4]
0	0	1.00	-
10^3	42	.23	77
10^4	233	.03	97
10^5	2134	0	100
10^6	11500	0	100
FPLSD[5] (p=0.05)		.17	

1 Spore suspensions of *Trichoderma hamatum* (5 ml) applied 1 day prior to application of 5 ml of *Botrytis cinerea* at 10^4 conidia per ml.

2 Colony forming units (CFU) determined immediately following application of *T. hamatum*.

3 Inoculated blossoms held at room temperature for 4 days.

4 Percent reduction of pod rot compared to *B. cinerea* applied alone (check).

5 Fisher's protected least significant difference.

TABLE 3. Control of grey mold of snap beans with different application times of *Trichoderma hamatum*

Treatment[1]	Prop. blossoms initiating pod rot[2]
Botrytis cinerea first[3]	0.96
B. cinerea alone	0.84
B. cinerea and *T. hamatum* together	0.09
T. hamatum first[4]	0.07
FPLSD[5] (p=0.01)	0.26

1 Five ml suspensions of *T. hamatum* (10^5 spores per ml) or of *B. cinerea* (10^4 conidia per ml) was applied.
2 Inoculated blossoms held at room temperature for 4 days.
3 *T. hamatum* applied 24 hr later.
4 *B. cinerea* applied 24 hr later.
5 Fisher's protected least significant difference.

the incidence of white mold in the field when compared to the nontreated check (Table 4). Differences in the amount of white mold due to application time or spore concentration were not significant. T. hamatum provided control of white mold comparable to the fungicide vinclozolin.

Immediately following application of a T. hamatum spore suspension, populations of Trichoderma spp. on the foliage were higher in treated plots than in nontreated plots (Table 5). Populations of Trichoderma spp. on foliage in treated plots decreased with time but remained higher (1567 compared to 300 CFU per leaf) in plots treated at 10^6 spores per ml than in nontreated plots up to two weeks after the second application.

Effect of volatiles produced by T. hamatum: Radial growth of both B. cinerea and S. sclerotiorum was significantly reduced ($p=0.01$) when these fungi were inverted in a closed chamber over a colony of T. hamatum. After 72 hrs incubation, mean radial growth of B. cinerea enclosed with T. hamatum was 0.6 mm compared to 23.6 mm when enclosed with noninoculated PDA (Table 6). Mean radial growth of S. sclerotiorum was 0.8 mm when enclosed with T. hamatum compared to 32.3 mm when enclosed with noninoculated PDA (Table 7).

TABLE 4. Efficacy of one isolate of *Trichoderma hamatum* for control of white mold pod rot of snap beans (1986)

Treatment	Rate[1]	Timing[2]	% Pod rot	% Control[3]
T. hamatum	10^5 spores/ml	E	2.0a[4]	68
T. hamatum	10^5 spores/ml	E&F	3.4a	45
T. hamatum	10^6 spores/ml	E	2.2a	65
T. hamatum	10^6 spores/ml	E&F	1.2a	81
Vinclozolin	0.6 Kg a.i./ha	E	2.0a	68
Nontreated check	-	-	6.2b	-
Standard Error			1.2	

[1] All spore suspensions applied at 0.5 liter per 6 m row.
[2] E = One application at early bloom.
E&F = Two applications: One at early bloom and one at full bloom.
[3] Percent reduction of pod rot compared to the non treated check.
[4] Means followed by a common letter are not significantly different as determined by linear contrasts.

TABLE 5. Persistence of epiphytic populations of Trichoderma hamatum on snap bean foliage

Conidia/ml[2]	Timing[3]	CFU[1]/leaf 8 Aug[4]	(S.E.)[5]	15 Aug[6]	(S.E.)	22 Aug	(S.E.)	29 Aug	(S.E.)
10^5	E	5600	(3150)	292	(250)	433	(303)	167	(235)
10^5	E&F	-		4067	(3513)	1267	(1037)	167	(204)
10^6	E	53600	(11807)	1500	(913)	1467	(1163)	267	(253)
10^6	E&F	-		65433	(55602)	4567	(3707)	1567	(997)
Nontreated check	-	234	(253)	100	(233)	200	(274)	300	(298)

[1] Colony forming units.
[2] All spore suspensions applied at 0.5 liter per 6 m row.
[3] E = One application at early bloom.
E&F = Two applications: One at early bloom and one at full bloom.
[4] Samples collected immediately following first spray.
[5] Standard error.
[6] Samples collected immediately following second spray.

Table 6. Inhibition of Botrytis cinerea by volatiles produced by Trichoderma hamatum

Treatment[1]	Mean radial colony growth[2] (mm)
B. cinerea with T. hamatum	0.6
Check	23.6
FPLSD[3] (p=0.01)	4.3

[1] A glass petri dish containing a 5-mm-diameter plug of B. cinerea on potato dextrose agar (PDA) was inverted over a plastic petri dish containing noninoculated PDA (check) or a 2-day-old culture of T. hamatum.

[2] Radial growth measured after 72 hrs incubation at 20° C.

[3] Fisher's protected least significant difference.

Table 7. Inhibition of *Sclerotinia sclerotiorum* by volatiles produced by *Trichoderma hamatum*

Treatment[1]	Mean radial colony growth[2] (mm)
S. sclerotiorum with *T. hamatum*	0.8
Check	32.3
FPLSD[3] (p=0.01)	4.1

[1] A glass petri dish containing a 5-mm-diameter plug of *S. sclerotiorum* on potato dextrose agar (PDA) was inverted over a plastic petri dish containing noninoculated PDA (check) or a 2-day-old culture of *T. hamatum*.

[2] Radial growth measured after 72 hrs incubation at 20° C.

[3] Fisher's protected least significant difference.

DISCUSSION

Isolates of Trichoderma were emphasized in this study because the antagonistic action of this fungus against Botrytis (23,47,48,49) and other pathogens (5,13,15,16, 37,38,42) is well known and because these organisms occur naturally on snap bean foliage. Species of Trichoderma rapidly and easily produce large numbers of spores in culture. This is a desirable, if not a mandatory, characteristic of a practical biocontrol organism.

This study suggests that an isolate of Trichoderma hamatum has potential as a biological control agent against grey mold of snap beans. In the detached blossom-pod assay control of grey mold was comparable to that obtained with the fungicide vinclozolin.

As expected, sequence of application and the number of Trichoderma hamatum spores applied to the blossoms were important factors in suppression of grey mold in the detached blossom-pod assay. Grey mold was controlled only when T. hamatum spores were applied to blossoms prior to or with B. cinerea inoculum. Application of 42 CFU or more of T. hamatum per blossom inhibited development of grey mold. With 233 CFU per blossom control was excellent.

A biological control agent must survive a range of environmental conditions in the field. Fluctuations in temperature and moisture on the phylloplane are often extreme (10,11). In these field studies conditions were

very warm and dry immediately following both applications of the antagonist. The average daily high temperature for the month of August during which this trial was conducted was 31^0 C (39). Because B. cinerea is primarily a cool weather pathogen (32), the warm weather experienced during this study was probably responsible for the extremely low incidence of grey mold in the field. Although these population studies were preliminary investigations and statistical significance is not implied, some T. hamatum spores applied to the bean foliage appeared to survive under these conditions. Trichoderma populations decreased rapidly within one week following application. Two weeks after application populations were higher in treated than in nontreated plots.

Field studies indicate that this isolate of T. hamatum is also effective in reducing white mold caused by Sclerotinia sclerotium. The disease cycles of grey mold and white mold are very similar. Like B. cinerea, S. sclerotiorum requires an exogenous nutrient base for infection of healthy plant tissue (1,2,3,22,40,45). As with B.cinerea, senescing flowers provide an important saprophytic base for invasion of bean pods by S. sclerotiorum (1,3,40). Because of the dependence of B. cinerea and S. sclerotiorum on senescing flowers for infection of healthy bean pods it is probable that both pathogens could be suppressed by the same microbe(s).

It is known that some isolates of Trichoderma are capable of producing volatile metabolites which in vitro, inhibit growth of other fungi (21). The isolate of T. hamatum used in these trials (Is 117) also is capable of producing volatile compounds which, in vitro, inhibits the growth of B. cinerea and S. sclerotiorum. The role of volatile compounds on the flowers is not known. Tronsomo and Dennis (47) suggested that because volatile compounds may not accumulate at levels sufficient to effectively inhibit other microbes, non-volatile inhibitors and hyphal interactions may be of greater importance than volatile metabolites on the phylloplane.

Most reports regarding suppression of plant diseases by T. hamatum involve soil-borne pathogens. T. hamatum has been reported to inhibit Rhizoctonia solani on cotton (25), pea (18,28,29) radish (14,28,29,38), bean (14) and potato (6). T. hamatum also protected pea seedlings (14,28,29) and radish seedlings (28,29) from rots caused by Pythium spp.

Reports describing control of foliar pathogens by T. hamatum are less numerous. Field studies in Italy demonstrated that four to six applications of conidial suspensions of T. hamatum beginning at flowering reduced the incidence of Botrytis cinerea on grapevines (7).

It is evident that organisms capable of reducing grey mold and white mold exist on snap bean foliage at populations too low to be effective. If these organisms

were cultured and applied to the blossoms, grey mold and white mold could be reduced. Additional field trials under a wider range of conditions are needed to determine if control of grey mold and white mold of snap beans by T. hamatum or other organism(s) is feasible in commercial production systems.

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