

# Suppression of Botrytis Blight of Begonia by *Trichoderma hamatum* 382 in Peat and Compost-Amended Potting Mixes

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## ABSTRACT

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Inoculation of an industry standard light sphagnum peat potting mix with *Trichoderma hamatum* 382 (T382) significantly ( $P = 0.05$ ) reduced the severity of Botrytis blight, caused by *Botrytis cinerea*, on begonia plants grown in a greenhouse. In data combined from three experiments, the degree of control provided by T382 did not differ significantly ( $P = 0.05$ ) from that provided by weekly topical sprays with chlorothalonil. In addition, T382 significantly ( $P = 0.05$ ) increased shoot dry weight and salability of flowering plants. Incorporation of composted cow manure (5%, vol/vol) into the light peat mix also significantly ( $P = 0.05$ ) decreased blight severity while shoot dry weight and salability were increased. Blight severity on plants in this compost mix did not differ significantly ( $P = 0.05$ ) from that on those in the light peat mix inoculated with T382. Finally, T382 and chlorothalonil did not significantly ( $P = 0.05$ ) affect blight severity, shoot dry weight, or salability of plants grown in the compost mix. Spatial separation was maintained in begonias between the biocontrol agent T382 and the pathogen. It was concluded, therefore, that the decrease in disease severity provided by inoculation of the peat mix with T382 most likely was due to systemic resistance induced in begonia against Botrytis blight. The suppressive effect of the compost mix against Botrytis blight was unusual because composts typically do not provide such effects unless inoculated with a biocontrol agent capable of inducing systemic resistance in plants to disease.

Additional keywords: *Begonia hiemalis*, biological control, ISR, marginal effects analysis

Botrytis blight, caused by *Botrytis cinerea* L., is an economically important disease of begonia and other greenhouse crops, where it can cause lesions on flowers, leaves, and stems during the entire cropping cycle (12). The inoculum of the pathogen is widely distributed in greenhouses (26). The disease is particularly severe under low light and high humidity greenhouse conditions (21,25). The greatest losses occur during transit and storage (25). Standard control procedures for greenhouse crops depend heavily on cultural practices and humidity control, but also on timely applications of protective fungicides (18,21). Unfortunately, *B. cinerea* has developed resistance to several

fungicides labeled for floricultural crops such as vinclozolin, benzimidazoles, dicarboxamides, and diethofencarb (6,16,21). Recently, biocontrol agents were added to the list of commercial control strategies to improve control of this disease (18).

Bacterial and fungal biocontrol agents have been proposed as topical sprays for control of Botrytis blight on greenhouse crops (6,15,27,29,34). Combinations of biocontrol agents have been proposed as well (17). The disadvantage of topical treatments is that the introduced biocontrol agent most likely will come in direct contact with fungicides applied to the foliage. This implies that it would have to be resistant to such compounds. A solution to this potential problem is the use of rhizosphere microorganisms that induce systemic resistance (ISR) in plants and thus provide control of root as well as foliar diseases (39,41). Fungal as well as bacterial rhizosphere microorganisms may induce this systemic effect in plants (14,20,55). Isolates of several *Trichoderma* spp. can reduce the severity of foliar diseases of plants when applied as seed or transplant treatments, presumably by inducing ISR in plants. They include *Trichoderma asperellum* T-203 (20,55), *T. hamatum* GT3-2 (9), *T. hamatum* T382 (T382) (19,31), *T. har-*

*zianum* T39 (14), *T. harzianum* T22 (30,44), *T. harzianum* T-203 (46,56), and *T. virens* G6 (7,24). To our knowledge, none of these isolates have been tested for activity on begonia against Botrytis blight.

Isolates of the *Trichoderma* spp. listed above can be natural inhabitants of bark, peat, or composts used widely as ingredients in potting mixes in the ornamentals industry (11,22,32,54). However, specific biocontrol agents such as the *Trichoderma* isolates listed above that can induce systemic resistance in plants do not consistently colonize potting mixes before planting of potted crops (28,31). As a result, most compost-amended potting mixes do not naturally reduce the severity of foliar diseases (28,31,38,57). For example, only one of 79 different batches of natural composts tested induced ISR in radish against bacterial leaf spot caused by *Xanthomonas campestris* pv. *armoraciae* (31). Thus, inoculation with an ISR-active biocontrol agent is essential for consistent efficacy against foliar diseases of plants grown in potting mixes.

Several reports show that the microbial carrying capacity of the organic fraction in potting mixes affects suppression of Pythium and Phytophthora root rots (4,22,47,48). For example, slightly decomposed, light fibrous (H<sub>2-3</sub> on the Von Post decomposition scale) sphagnum peat (40) supports the activity of biocontrol agents and suppression of Pythium root rot (3,4). In contrast, highly decomposed, dark sphagnum peat (H<sub>4</sub>) harvested from deeper and older layers in peat bogs does not provide these beneficial effects (3,4). Recent reports indicate that ISR induced by rhizosphere microorganisms in plants also depends on soil organic matter quality. For example, Pharand et al. (38) reported that the degree of protection provided by *Pythium oligandrum* in tomato against crown rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* was improved by amendment of a sphagnum peat mix with composted pulp and paper mill residues. Furthermore, suppression of Phytophthora leaf blight and stem dieback of cucumber provided by T382 in a sphagnum peat mix was enhanced by amendment of the mix with composted dairy manure (28). The severity of the *Phytophthora*-induced disease observed in the latter work did not differ between that provided by a drench

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with the fungicide metalaxyl and that obtained with the fortified compost-amended mix. Apart from biocontrol agents, the quality of organic matter in field soil also seems to play a role in systemic induced resistance (47,52). Thus, expression of such activity in potting mixes may depend on specific microorganisms present in the substrate as well as the chemistry of the organic matter in the mix.

The specific objectives of this work were to determine whether: (i) inoculation of T382 into a light sphagnum peat or a compost-amended potting mix can reduce the severity of Botrytis blight of begonia; (ii) amendment of the light sphagnum peat potting mix with compost affects efficacy of T382 in control of Botrytis blight; and (iii) the effect induced by T382 is systemic in nature.

## MATERIALS AND METHODS

**Preparation of potting mixes.** A light sphagnum peat mix, referred to hereafter as “light peat mix”, and a composted cow manure-amended potting mix, referred to as “compost mix”, were prepared as described by Khan et al. (28). Briefly, the peat mix was prepared by blending light fibrous H<sub>2-3</sub> on the Von Post decomposition scale sphagnum peat (Sungro; Horticulture Canada, Ltd., Lamaque, N.B., Canada) with coarse horticultural grade perlite (Ball Seed Co., West Chicago, IL) at a volumetric ratio of 6:4. The mix was then amended with 0.9 g of gypsum, 0.9 g of potassium nitrate, and 0.9 g of super phosphate as starter fertilizers and 4.3 g of dolomitic lime and 2.8 g of calcium carbonate (<0.15 mm) per liter of mix to adjust the pH to 5.5 to 6.0. Such sphagnum peat mixes do not naturally induce systemic disease resistance in plants (28,31,38,57). The compost mix was prepared by blending the same source of sphagnum peat with coarse horticultural grade perlite and composted cow manure at volumetric ratios of 6:3:1. The compost was prepared in windrows from sawdust-bedded cow manure until it reached a stability level of 0.5 mg CO<sub>2</sub>-C g<sup>-1</sup> dry weight per day (8). At this stage in the composting process, the sawdust in the compost had decomposed adequately to avoid nitrogen immobilization in plants (53). During blending, starter fertilizers other than 0.9 g gypsum liter<sup>-1</sup> mix were not added to this mix because the compost at the amendment rate used provided the concentrations of nutrients recommended at planting (18). The compost mix was amended with 4.3 g dolomitic lime and 1.1 g calcium carbonate (<0.15 mm) liter<sup>-1</sup> mix to adjust the pH to 5.5 to 6.0. The air capacity of both potting mixes was at least 20% (vol/vol) in a 10-cm-tall pot, and the percolation rate exceeded 2 cm min<sup>-1</sup>.

**Inoculation of potting mixes with *Trichoderma hamatum* 382.** Both potting mixes were inoculated with a granular dry powder conidial preparation of T382 (re-

ceived from Sylvan Bioproducts, Inc., Cabot, PA) at a rate of 120 g m<sup>-3</sup> mix to establish an initial population density of 2 × 10<sup>5</sup> CFU T382 g<sup>-1</sup> dry weight mix. Control transplant mixes were not inoculated with T382. All mixes, including the controls, were incubated at 25°C for 7 days before planting. The population of T382 in the control and inoculated potting mixes was monitored on two samples per treatment using a triplicate dilution series on a *Trichoderma* selective medium (10). All treatments were sampled immediately after mix preparation, at planting, and at 30 and 56 days after planting. Colonies of T382 were counted after 10 days of incubation at 24°C. The identity of three presumed T382 colonies per potting mix sample was verified by light microscopy. Phialides produced by T382 examined under a light microscope served to verify the identity of *T. hamatum* according to Bissett (2). Confirmation of three putative isolates of T382 per potting mix sample was performed by polymerase chain reaction (PCR) analysis as described in Abbasi et al. (1) utilizing the T382-specific primers SCE16<sub>347</sub> and SCH19<sub>588</sub>.

**Botrytis blight bioassay.** Rooted cuttings of *Begonia hiemalis* cv. Barbara, received from Oglevee Ltd., Connellsville, PA, were planted in 1,500 ml, 10-cm-tall pots and then incubated in a greenhouse at 17 to 25°C and 40 to 60% relative humidity. Potting mix treatments included the control light peat mix, the control compost mix, and the same mixes inoculated with T382. Foliar treatments included a weekly runoff spray with chlorothalonil (2.3 ml liter<sup>-1</sup>, Daconil, flowable; Monsanto Company, San Ramon, CA) or water. After planting, soluble fertilizer (20:20:20 Peters Fertilizer; Scotts, Marysville, OH) was applied weekly at a rate of 0.125 mg N-P-K liter<sup>-1</sup> to irrigation water to maintain fertility levels within the range recommended for begonia (18). The same concentrations of nutrients were applied to plants in both potting mixes because a preplant compost analysis revealed that nutrients projected to be released during production of the crop would not contribute substantially to fertility of begonia plants. The experiment was a randomized complete block design, with two factors, potting mix and treatment, consisting of three blocks with three replicates per combination of mix and treatment.

Three weeks after transplanting, the relative humidity in the greenhouse was increased with a fogger to between 40 and 85% to enhance symptom development. Plants were inoculated 24 h later with a conidial suspension of *B. cinerea*. In this procedure, inoculum of an isolate of *B. cinerea* originally isolated from begonia was cultured for 7 to 10 days at 25°C on potato dextrose agar (PDA; DIFCO, Sparks, MD). Plates were then flooded with sterilized, distilled water, and conidia

were dislodged with a glass rod. The conidial concentration was determined by using a hemacytometer, and the concentration was adjusted to 2.5 × 10<sup>5</sup> ml<sup>-1</sup> by adding distilled water as needed. All plants were then sprayed to runoff with this inoculum. During preliminary experiments, it was determined that in vitro growth on PDA of the isolate of *B. cinerea* used in this work was inhibited at a concentration of 100 µg a.i. chlorothalonil per ml of PDA medium. The pathogen, therefore, was not resistant to the fungicide used in this work (56). Botrytis blight severity was determined when symptoms developed at 4 to 7 days after inoculation and at 3-day intervals thereafter until 56 days after planting on the basis of percentage of total plant surface displaying symptoms, using a scale of 0 to 100 in 10-unit increments where 0% = symptomless, 10% = 10 percent of total plant displaying symptoms, and 100% = death of the plant.

Three experiments were performed. To avoid interference from disease symptoms caused by *Pythium* inoculum that might inadvertently be introduced with the rooted cuttings or during planting, a drench with metalaxyl (0.08 ml liter<sup>-1</sup>, Subdue Maxx; Novartis Crop Protection, Inc., Greensboro, NC) was applied immediately after planting in each of the experiments. Preliminary experiments showed that this drench with metalaxyl did not affect population development of T382 in potting mixes (H. A. J. Hoitink, unpublished).

The identity of *B. cinerea* in plants showing symptoms of Botrytis blight was verified in each experiment by plating sections of infected leaves and stems on PDA (DIFCO) followed by identification of the pathogen by light microscopy (50). In addition, sections of eight begonia stems and leaves were removed from plants during harvest from each treatment and plated onto the selective *Trichoderma* medium (10) to determine the presence of T382 in that tissue. The identity of T382 was confirmed by PCR as described above.

**Plant salability rating and shoot dry weight.** When flowers had fully developed, individual plants were rated for their salability based on the number of flowering stalks, the number of open blooms, and percentage of the total plant surface showing symptoms of Botrytis blight utilizing an ordinal rating scale: 1 = dead plant; 2 = no flowers, severely diseased and/or severely stunted plant; 3 = no flowers, mild disease severity and stunted plant; 4 = symptomless, with flower buds or flower stalk(s) (but not open flowers); and 5 = symptomless, with one or more flower stalks with at least one flower open. Thereafter, the stem of each plant was cut at the soil surface. The aboveground part of each plant, which included flowers, was then dried for 10 days at 70°C to determine the shoot dry weight.

**Data analysis.** Area under the disease progress curve (AUDPC) was calculated for each plant based on the percent severity values. Analysis of variance (ANOVA) was used to determine the effects of potting mix, treatment, and their interaction on AUDPC and plant dry weight for each experiment. An additional ANOVA was performed for the data pooled across all experiments to determine the effects of potting mix, treatment, and experiment on ANOVA and dry weight. The MIXED procedure of SAS (SAS Inc., Cary, NC) was used for the analysis. Both mix and treatment were considered fixed effects, and the experiment was considered a random effect (43). The Satterthwaite method was used to calculate the denominator degrees of freedom for *F* tests. Multiple comparisons of means based on the least significant difference (LSD) were used to determine differences of means when a main effect or interaction was significant. A significance level of *P* = 0.05 was used for all pairwise comparisons.

Because salability is an ordinal rating, and not a continuous random variable, a nonparametric marginal effects analysis (5,45) was used to determine the effects of potting mix, treatment, and their interaction on salability. As for shoot dry weight and AUDPC, an additional marginal effects analysis was performed on the data pooled across all experiments. The marginal effects analysis is based on ranks of the data, or equivalently, the estimated marginal treatment effects. It was performed using the methodology described in Shah and Madden (45). Multiple comparisons also were used based on the LSD to determine differences in mean ranks (or in marginal effects). A significance level of *P* = 0.05 was used for all pairwise comparisons.

## RESULTS

Symptoms of Botrytis blight developed on the foliage of begonias within the first week after inoculation of plants with *B. cinerea* in each of three experiments. The most severe symptoms developed in the first experiment (Table 1), which was performed during short-day, winter conditions. Blight severity was much lower in experiments 2 and 3, which were performed during longer day, higher light conditions in the spring.

ANOVA indicated a significant effect of the interaction of potting mix and treatment (control, fungicide, or T382) on both AUDPC of disease severity and shoot dry weight for each experiment (*P* < 0.05) and for the data pooled over all experiments (*P* < 0.05). The random effect of experiment also was significant, indicating differences in the disease response for the different experiments. Examination of the ANOVA residuals indicated that no transformations were needed. Moreover, the nonparametric marginal-effects analysis indicated a sig-

nificant mix and treatment interaction for plant salability (*P* < 0.05) for each experiment and for the pooled data.

The significant interaction indicates that the effect of treatment depended on the potting mix, or the effect of potting mix depended on the treatment. For the pooled data, *F* statistics for the interaction were 4.37 (df = 2, 64; *P* = 0.017) for AUDPC, 5.41 (df = 2, 55; *P* = 0.007) for shoot dry weight, and 5.65 (df = 2, 33; *P* = 0.008) for salability. Because of the significant interaction, pairwise comparisons of the six interaction means, rather than the main-effect means, were performed using the LSD to determine which means were different at *P* = 0.05 (Tables 1 to 3). Emphasis is placed on the pooled results.

In all three experiments, the severity of Botrytis blight on plants in the light peat mix was much higher than that observed in the compost-amended mix. Figure 1 illustrates an example (one of three replicates in experiment 1) of the effect of each of

the treatments on the severity of Botrytis blight. Overall Botrytis blight severity on plants grown in the light peat mix that had been sprayed with chlorothalonil was significantly (*P* = 0.05) lower than the severity on control plants (Table 1). However, chlorothalonil did not significantly (*P* = 0.05) increase shoot dry weight of plants grown in the light peat mix (Table 2). It also did not significantly (*P* = 0.05) affect the overall salability of flowering plants in this mix (Table 3). In contrast, inoculation of the light peat mix with T382 significantly (*P* = 0.05) decreased the overall disease severity relative to the control (Table 1), but it significantly (*P* = 0.05) increased overall shoot dry weight and overall salability of flowering plants compared with the control (Tables 2 and 3).

Amendment of the light peat mix with compost significantly (*P* = 0.05) decreased overall Botrytis blight severity compared with the light peat mix control treatment (Table 1). The compost amendment also

**Table 1.** Effects of *Trichoderma hamatum* 382 and chlorothalonil on the area under the disease progress curve (AUDPC) of begonia cv. Barbara plants inoculated with *Botrytis cinerea* and produced in a light peat or a compost-amended potting mix

Potting mix	Pesticide treatment <sup>x</sup>	Disease severity - AUDPC <sup>w</sup>			
		Exp. 1	Exp. 2	Exp. 3	Overall <sup>y</sup>
Peat	Control	1,402.4 a <sup>z</sup>	712.9 a <sup>z</sup>	233.6 a <sup>z</sup>	684.6 a <sup>z</sup>
Peat	Chlorothalonil	844.8 a	229.3 b	181.8 b	398.5 b
Peat	T382	88.1 b	537.0 a	53.9 cd	222.2 bc
Compost	Control	84.9 b	92.1 b	27.0 d	96.8 c
Compost	Chlorothalonil	45.8 b	101.6 b	14.3 d	83.0 c
Compost	T382	55.0 b	149.6 b	74.7 c	127.5 c
					LSD = 236.0

<sup>w</sup>Through 56 days after inoculation, based on a disease severity percent scale.

<sup>x</sup>Mix was either inoculated with *Trichoderma hamatum* 382 during formulation of potting mixes to an initial population density of  $2.0 \times 10^5$  CFU g<sup>-1</sup> dry weight mix, sprayed every 7 days with chlorothalonil, or not treated (control).

<sup>y</sup>Mean of AUDPCs across all replications and all three experiments was determined.

<sup>z</sup>There was a significant interaction of potting mix and treatment. Thus, comparisons of all interaction means were made. Values with the same letter within a column are not significantly different (*P* = 0.05) based on Fisher's least significant difference (LSD). Shown value of LSD is for comparison of overall means only.

**Table 2.** Effects of *Trichoderma hamatum* 382 and chlorothalonil on the shoot dry weight of begonia cv. Barbara plants inoculated with *Botrytis cinerea* and produced in a light peat or a compost-amended potting mix

Potting mix	Treatment <sup>x</sup>	Shoot dry weight (g) <sup>w</sup>			
		Exp. 1	Exp. 2	Exp. 3	Overall <sup>y</sup>
Peat	Control	4.2 c <sup>z</sup>	5.0 ab <sup>z</sup>	9.5 ab <sup>z</sup>	6.7 b <sup>z</sup>
Peat	Chlorothalonil	7.2 b	5.3 ab	7.0 c	6.4 b
Peat	T382	10.3 a	5.8 ab	9.6 ab	8.5 a
Compost	Control	8.5 ab	4.7 b	11.4 a	8.6 a
Compost	Chlorothalonil	8.6 ab	6.1 a	8.9 bc	7.8 ab
Compost	T382	9.5 ab	5.3 ab	7.8 bc	7.2 ab
					LSD = 1.5

<sup>w</sup>Determined 56 days after inoculation.

<sup>x</sup>Mix was either inoculated with *Trichoderma hamatum* 382 during formulation of potting mixes to an initial population density of  $2.0 \times 10^5$  CFU g<sup>-1</sup> dry weight mix, sprayed every 7 days with chlorothalonil, or not treated (control).

<sup>y</sup>Mean of shoot dry weights across all replications and all three experiments was determined.

<sup>z</sup>There was a significant interaction of potting mix and treatment. Thus, comparisons of all interaction means were made. Values with the same letter within a column are not significantly different (*P* = 0.05) based on Fisher's least significant difference (LSD). Shown value of LSD is for comparison of overall means only.

significantly ( $P = 0.05$ ) increased overall shoot dry weight (Table 2) and salability (Table 3). However, topical sprays with chlorothalonil or mix inoculation with T382 did not significantly ( $P = 0.05$ ) reduce *Botrytis* blight severity on plants grown in the compost mix. Furthermore, overall shoot dry weight and salability on plants in the compost mix also were not affected significantly ( $P = 0.05$ ) by either treatment.

The most substantial differences in effects among the three experiments were

observed in the light peat mix. Under high disease pressure in experiment 1, chlorothalonil was significantly ( $P = 0.05$ ) less effective than T382, based on disease severity, shoot dry weight, and salability. In contrast, in experiment 2 under low disease pressure, chlorothalonil provided a significantly ( $P = 0.05$ ) higher degree of *Botrytis* blight control. However, shoot dry weight and salability were not affected. In experiment 3 under low disease pressure, both chlorothalonil and T382 significantly ( $P = 0.05$ ) decreased disease severity, but

salability was not affected even though shoot dry weight was significantly ( $P = 0.05$ ) increased by chlorothalonil.

*B. cinerea* consistently was recovered on acidified PDA from *Botrytis* blight lesions. The population of T382 recovered from the inoculated light peat and compost mixes immediately after potting ranged from 2 to  $4 \times 10^5$  CFU g<sup>-1</sup> dry wt. After 30 days, this population had increased to 2 to  $4 \times 10^6$  CFU g<sup>-1</sup> dry wt potting mix. It remained at this population density until harvest. The identity of these putative T382 isolates was verified by PCR using the T382-specific primers SCE1<sub>6347</sub> and SCH19<sub>588</sub> for each of three colonies per potting mix sample. T382 was not recovered on the selective *Trichoderma* medium from the control light peat or the compost mixes. It also was not isolated from stem sections or leaf tissue removed from plants at harvest for any of the treatments.

## DISCUSSION

The degree of control of *Botrytis* blight provided by inoculation of the light peat mix with T382 was comparable to weekly topical sprays with the fungicide chlorothalonil, a commonly used protectant fungicide for control of this disease in the ornamentals industry (18). An analysis of in vitro sensitivity to chlorothalonil of the isolate of *B. cinerea* used in this work revealed that it was sensitive to this fungicide. Therefore, partial disease control obtained in this work with chlorothalonil was not due to resistance of the isolate of *B. cinerea* to this fungicide. Spatial separation between the pathogen and the biocontrol agent in the host plant was maintained

**Table 3.** Effects of *Trichoderma hamatum* 382 and chlorothalonil on the salability of begonia cv. Barbara plants inoculated with *Botrytis cinerea* and produced in a light peat or a compost-amended potting mix

Potting mix	Treatment <sup>y</sup>	Salability at flowering <sup>w</sup>				
		Overall median	Rank <sup>w,x</sup>			
			Exp. 1	Exp. 2	Exp. 3	Overall <sup>z</sup>
Peat	Control	3.0	15.8 c <sup>x</sup>	13.4 c <sup>x</sup>	42.8 b <sup>x</sup>	69.6 d <sup>x</sup>
Peat	Chlorothalonil	3.0	17.6 bc	26.4 ac	37.4 b	81.6 cd
Peat	T382	3.0	38.2 a	21.0 bc	53.9 b	108.2 bc
Compost	Control	4.0	25.2 ac	40.2 a	80.0 a	140.1 a
Compost	Chlorothalonil	4.0	32.3 ab	32.8 a	70.2 a	130.6 ab
Compost	T382	3.0	35.9 a	31.2 ab	42.8 b	110.5 ac

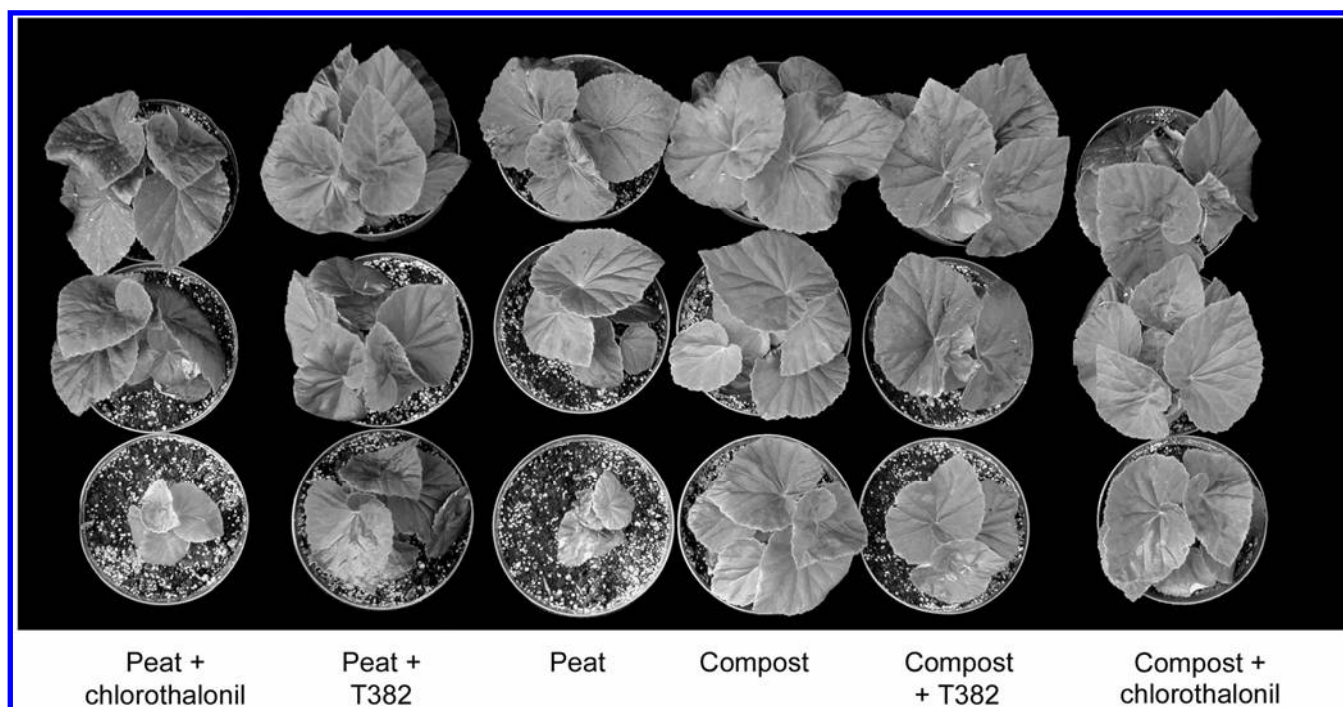
LSD = 29.0

<sup>w</sup> Salability was based on number of flowering stalks, number of open blooms, and percent total plant surface showing symptoms of *Botrytis* blight utilizing a five-point rating scale in which 1 = dead plant and 5 = symptomless with one or more flower stalks with at least one flower open. Mean rank across replications was determined.

<sup>x</sup> There was a significant interaction of potting mix and treatment. Thus, comparisons of all interaction means were made. Values with the same letter within a column are not significantly different ( $P = 0.05$ ) based on pairwise comparisons of mean ranks. For the marginal effects analysis, standard errors of differences are not constant; thus, there is no single LSD. Value shown is mean LSD for overall mean ranks.

<sup>y</sup> Mix was either inoculated with *Trichoderma hamatum* 382 during formulation of potting mixes to an initial population density of  $2.0 \times 10^5$  CFU g<sup>-1</sup> dry weight mix, sprayed every 7 days with chlorothalonil, or not treated (control).

<sup>z</sup> Mean rank of salability was determined across all replications and all three experiments.



**Fig. 1.** Effects of topical applications with chlorothalonil and of *Trichoderma hamatum* 382 on the severity of *Botrytis* blight of begonias produced in a light peat or a compost-amended potting mix. Plants represent one of three replications in experiment 1.

because T382 was not recovered from the stems or foliage of begonias produced in either mix inoculated with this biocontrol agent. Based on criteria proposed for systemic resistance induced by biocontrol agents (39), it may be assumed that ISR played a role in control of Botrytis blight in the light peat mix used in this work.

Control of Botrytis blight with T382 confirms an earlier report on systemic control of this disease by *Trichoderma* (14), which showed that inoculation of *T. harzianum* T39 into a peat potting mix reduced the severity of gray mold on stems and leaves of several plant species, apparently also through root-induced systemic activity. However, efficacy in that work was not compared with a commercial fungicide. Several other reports on biological control of Botrytis blight with *Trichoderma* preparations utilized topical applications of these biocontrol agents (14,15,35,36,49). A disadvantage of such applications to floricultural crops is that the biopesticide carrier leaves residues on the crop, and this reduces its salability. The begonia plants in this work were produced under commercial greenhouse conditions in a light sphagnum peat mix that is used widely in the floriculture industry. Because the salability of the flowering plants was improved through inoculation of the light peat mix with T382, the potential for utilization of *Trichoderma* isolates that induce ISR for control of Botrytis blight in floricultural crops should be significant.

The suppressive effect of the compost mix against Botrytis blight compared with the control light peat mix was unusual because composts typically do not induce systemic resistance in plants without inoculation with a biocontrol agent capable of inducing this effect (23,28,31). The overall disease severity on plants in the compost mix control (mix not inoculated with T382) was significantly ( $P = 0.05$ ) lower than that on plants produced in the nonamended light peat mix or on such plants treated with chlorothalonil. Because the batch of peat used during the preparation of the compost mix was the same as that used in the light peat mix in this work, it may be possible that biocontrol agents in the compost provided the disease control.

The batches of composted cow manure used in the three begonia experiments were prepared from the same raw materials (sawdust and dairy manure) and by the same composting system and composting plant as the composted manure used previously by Khan et al. (28) and Hoitink et al. (23). In their experiments, the natural compost mix consistently failed to induce systemic resistance in: (i) cucumber against *Phytophthora* blight, (ii) *Pieris japonica* against *Phytophthora* leaf blight caused by *Phytophthora parasitica*, and (iii) *Myrica pennsylvanica* against *Botryosphaeria dothidea*, unless the mix was inoculated with T382. The batches of

composts used for the begonia crops in this work were produced during 2002 and 2003, whereas those used in the earlier work on cucumber, *Pieris*, and *Myrica* had been prepared during 2000 and 2001. It appears, therefore, that biocontrol agents that can induce systemic resistance in plants may have colonized the compost at the composting plant naturally, sometime in late 2001 or early 2002. Unique changes in operating procedures at the composting plant that involve the composting as well as the curing process may have contributed to colonization of the compost used in this work by such biocontrol agents.

Factors that contribute to the potential for natural colonization of composts after peak heating by biocontrol agents include process temperatures during composting and curing, environmental factors, and recycling of cured compost into the raw materials (13,22,33,47). The fate of specific biocontrol agents that can induce systemic resistance in plants has not been examined, to our knowledge, although it has been established that they do not survive high composting temperatures (31). Most composting plants that produce composts for incorporation into potting mixes in the United States utilize 3- to 4-m-tall windrows and store composts in tall curing piles (4 to 6 m tall). A large proportion of the material in tall windrows and curing piles typically exceeds 45°C (42). This means that biocontrol agents other than *Bacillus* spp. would not survive the process (22). Furthermore, the moisture content of such stored products typically is lower than 45% on a total weight basis. This implies that the water content of these materials would limit colonization by most bacteria (33), including *Bacillus* spp. that can function as biocontrol agents and tolerate high composting process temperatures (37).

The composted cow manure used in this and earlier work over the 4-year period was prepared in small windrows with a 120-day composting period (6 to 8 turnings per batch) and a windrow height that did not exceed 1.25 m (8,53). A significant proportion of the compost mass in such small windrows remains below 40°C even though the center of such windrows reaches 65 to 70°C over extended periods of time (8,42). In spite of these low edge windrow temperatures, frequent turning of small windrows ensures that all parts of the compost are exposed to high temperatures to meet U.S.-EPA regulations that govern the destruction of fecal pathogens and parasites (51). After composting, the compost used in this work was cured in 2- to 3-m-tall piles where much of the mass also remained at low temperatures. Thus, ample opportunities existed in this entire process for colonization of compost by mesophilic biocontrol agents that cannot survive high process temperatures (22). In 2001, before these experiments with bego-

nia were performed, the operators of the composting plant occasionally blended some dry, cured composted cow manure with the raw manure to reduce sawdust amendment requirements and decrease the total moisture content of the fresh material to within the optimum range for composting (42). Thus, several factors which include low process temperatures and recycling of compost may have contributed over time to colonization of the compost by biocontrol agents that can induce systemic resistance in plants.

In conclusion, opportunities for natural colonization of composts by ISR-active biocontrol agents under commercial conditions are limited. Thus, in order for composts that are widely available on a commercial scale today to induce systemic resistance in plants consistently, compost-amended potting mixes will need to be inoculated with ISR-active biocontrol agents such as T382, particularly because these microorganisms are rare in natural environments (31).

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#### LITERATURE CITED

1. Abbasi, P. A., Miller, S. A., Meulia, T., Hoitink, H. A. J., and Kim, J. 1999. Precise detection and tracing of *Trichoderma hamatum* 382 in compost-amended potting mixes by using molecular markers. *Appl. Environ. Microbiol.* 65:5421-5426.
2. Bissett, J. 1991. A revision of the genus *Trichoderma*. II. Infrageneric classification. *Can. J. Bot.* 69:2357-2372.
3. Boehm, M. J., and Hoitink, H. A. J. 1992. Sustainment of microbial activity in potting mixes and its impact on severity of Pythium root rot of Poinsettia. *Phytopathology* 82:259-264.
4. Boehm, M. J., Wu, T., Stone, A. G., Kraakman, B., Iannotti, D. A., Wilson, G. E., Madden, L. V., and Hoitink, H. A. J. 1997. Cross-polarized magic-angle spinning  $^{13}\text{C}$  nuclear magnetic resonance spectroscopic characterization of soil organic matter relative to culturable bacterial species composition and sustained biological control of Pythium root rot. *Appl. Environ. Microbiol.* 63:162-168.
5. Brunner, E., and Puri, M. L. 2001. Nonparametric methods in factorial designs. *Stat. Pap.* 42:1-52.
6. Buck, J. W., and Jeffers, S. N. 2004. Effect of pathogen aggressiveness and vinclozolin on efficacy of *Rhodoisorula glutinis* PM4 against *Botrytis cinerea* on geranium leaf disks and seedlings. *Plant Dis.* 88:1262-1268.
7. Burns, J. R., and Benson, D. M. 2000. Biocontrol of damping-off of *Catharanthus roseus* caused by *Pythium ultimum* with *Trichoderma virens* and binucleate *Rhizoctonia* fungi. *Plant Dis.* 84:644-648.
8. Changa, C. M., Wang, P., Watson, M. E., Hoitink, H. A. J., and Michel, F. C., Jr. 2003. Assessment of the reliability of a commercial maturity test kit for composted manures. *Compost Sci. Utilization* 11(2):125-143.

9. Chet, I., Harman, G. E., and Baker, R. 1981. *Trichoderma hamatum*: Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. *Microb. Ecol.* 7:29-38.
10. Chung, Y. R., and Hoitink, H. A. J. 1990. Interactions between thermophilic fungi and *Trichoderma hamatum* in suppression of *Rhizoctonia damping-off* in a bark compost-amended container medium. *Phytopathology* 80:73-77.
11. Cotxarrera, L., Trillas-Gay, M. I., Steinberg, C., and Alabouvette, C. 2002. Use of sewage sludge compost and *Trichoderma asperellum* isolates to suppress *Fusarium wilt* of tomato. *Soil Biol. Biochem.* 34:467-476.
12. Daughtrey, M. L., Wick, R. L., and Peterson, J. L. 1995. Compendium of Flowering Potted Plant Diseases. American Phytopathological Society, St. Paul, MN.
13. De Clerq, D., Vandesteene, L., Coosemans, J., and Ryckeboer, J. 2004. Use of compost as suppressor of plant diseases. Pages 317-337 in: *Resource Recovery and Reuse in Organic Solid Waste Management*. P. Lens, B. Hamelers, H. Hoitink, and W. Bidlingmaier, eds. IWA Publishing, London.
14. De Meyer, G., Bigirimana, J., Elad, Y., and Hofte, M. 1998. Induced systemic resistance in *Trichoderma harzianum* T39 and biocontrol of *Botrytis cinerea*. *Eur. J. Plant Pathol.* 104:279-286.
15. Elad, Y. 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Prot.* 19:709-714.
16. Elad, Y., Yunis, H., and Katan, T. 1992. Multiple fungicide resistance to benzimidazoles, dicarboximides and diethofencarb in field isolates of *Botrytis cinerea* in Israel. *Plant Pathol.* 41:41-46.
17. Guetsky, R., Shtienberg, D., Elad, Y., Fischer, E., and Dinor, A. 2002. Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. *Phytopathology* 92:976-985.
18. Hamrick, D. 2003. Rieger Begonias. Pages 253-263 in: *Ball Redbook: Crop Production*. 17th ed. Debbie Hamrick, ed. Ball Publishing, Batavia, IL.
19. Han, D. Y., Coplin, D. L., Bauer, W. D., and Hoitink, H. A. J. 2000. A rapid bioassay for screening rhizosphere microorganisms for their ability to induce systemic resistance. *Phytopathology* 90:327-332.
20. Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., and Lorito, M. 2004. *Trichoderma* species – Opportunistic, avirulent plant symbionts. *Nature Rev.* 2:1-14.
21. Hausbeck, M. K., and Moorman, G. W. 1996. Managing *Botrytis* in greenhouse-grown flower crops. *Plant Dis.* 80:1212-1219.
22. Hoitink, H. A. J., and Boehm, M. J. 1999. Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. *Annu. Rev. Phytopathol.* 37:427-446.
23. Hoitink, H. A. J., Musselman, C. A., Moore, T. L., Horst, L. E., Krause, C. R., Zondag, R. A., and Mathers, H. 2003. Biological suppression of foliar diseases of ornamental plants with composted manures, biosolids, and *Trichoderma hamatum* 382. Pages 50-56 in: *Ornamental Plants, Special Circ.* 189. Ohio Agricultural Research and Development Center, Wooster.
24. Howell, C. R., Hanson, L. E., Stipanovic, R. D., and Puckhaber, L. S. 2000. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. *Phytopathology* 90:248-252.
25. Jarvis, W. R. 1980. Epidemiology. Pages 219-250 in: *The Biology of Botrytis*. J. R. Coley-Smith, K. Verhoeff, and W. R. Jarvis, eds. Academic Press, New York.
26. Keressies, A., Bosker-van Zessen, A. I., Wage-makers, C. A. M., and van Kan, J. A. L. 1997. Variation in pathogenicity and DNA polymorphism among *Botrytis cinerea* isolates sampled inside and outside a glasshouse. *Plant Dis.* 81:781-786.
27. Kessel, G. J. T., de Haas, B. H., Van der Plas Lombaers, C. H., Van den Ende, J. E., Pennock-Vos, M. G., Van der Werf, W., and Kohl, J. 2001. Comparative analysis of the role of substrate specificity in biological control of *Botrytis elliptica* in lily and *B. cinerea* in cyclamen with *Ulocladium atrum*. *Eur. J. Plant Pathol.* 107:273-284.
28. Khan, J., Ooka, J. J., Miller, S. A., Madden, L. V., and Hoitink, H. A. J. 2004. Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against *Phytophthora crown rot* and leaf blight. *Plant Dis.* 88:280-286.
29. Kohl, J., Gerlagh, M., and Grit, G. 2000. Biocontrol of *Botrytis cinerea* by *Ulocladium atrum* in different production systems of cyclamen. *Plant Dis.* 84:569-573.
30. Koike, N., Hyakumachi, M., Kageyama, K., Tsuyumu, S., and Doke, N. 2001. Induction of systemic resistance in cucumber against several diseases by plant growth-promoting fungi: Lignification and superoxide generation. *Eur. J. Plant Pathol.* 107:523-533.
31. Krause, M. S., De Ceuster, T. J. J., Tiquia, S. M., Michel, F. C., Jr., Madden, L. V., and Hoitink, H. A. J. 2003. Isolation and characterization of rhizobacteria from composts that suppress the severity of bacterial leaf spot of radish. *Phytopathology* 93:1292-1300.
32. Kuter, G. A., Nelson, E. B., Hoitink, H. A. J., and Madden, L. V. 1983. Fungal populations in container media amended with composted hardwood bark suppressive and conducive to *Rhizoctonia damping-off*. *Phytopathology* 73:1450-1456.
33. Miller, F. C. 1989. Matric water potential as an ecological determinant in compost, a substrate dense system. *Microb. Ecol.* 18:59-71.
34. Morandi, M. A. B., Maffia, L. A., Mizubuti, E. S. G., Alfenas, A. C., and Barbosa, J. G. 2003. Suppression of *Botrytis cinerea* sporulation by *Clonostachys rosea* on rose debris: A valuable component in *Botrytis* blight management in commercial greenhouses. *Biol. Control* 26:311-317.
35. Nelson, M. E., and Powelson, M. L. 1988. Biological control of grey mold of snap beans by *Trichoderma hamatum*. *Plant Dis.* 72:727-729.
36. O'Neill, T. M., Elad, Y., Shtienberg, D., and Cohen, A. 1996. Control of grapevine grey mould with *Trichoderma harzianum* T39. *Biocontrol Sci. Technol.* 6:139-146.
37. Phae, C. G., Sasaki, M., Shoda, M., and Kubota, H. 1990. Characteristics of *Bacillus subtilis* isolated from composts suppressing phytopathogenic microorganisms. *Soil Sci. Plant Nutr.* 36:575-586.
38. Pharand, B., Carisse, O., and Benhamou, N. 2002. Cytological aspects of compost-mediated induced resistance against *Fusarium crown and root rot* in tomato. *Phytopathology* 92:424-438.
39. Pieterse, C. M. J., Van Pelt, J. A., Verhagen, B. W. M., Ton, J., Van Wees, S. C. M., Leon-Kloosterziel, K. M., and Van Loon, L. C. 2003. Induced systemic resistance by plant growth-promoting Rhizobacteria. *Symbiosis* 35:39-54.
40. Puustjarvi, V., and Robertson, R. A. 1975. Physical and chemical properties. Pages 23-28 in: *Peat in Horticulture*. D. W. Robinson and J. G. D. Lamb, eds. Academic Press, New York.
41. Raupach, G. S., and Kloepper, J. W. 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathology* 88:1158-1164.
42. Rynk, R. 1992. *On-Farm Composting Handbook*. Natural Resource, Agriculture, and Engineering Service, Coop. Ext. NRAES-54, Ithaca, NY.
43. Schabenberger, O., and Pierce, F. J. 2002. *Contemporary Statistical Models for the Plant and Soil Sciences*. CRC Press, Boca Raton, FL.
44. Seaman, A. 2003. Efficacy of OMRI-approved products for tomato foliar disease control. N.Y. State Integrated Pest Management Program Publ. 129, 164-167.
45. Shah, D. A., and Madden, L. V. 2004. Non-parametric analysis of ordinal data in designed factorial experiments. *Phytopathology* 94:33-43.
46. Sid Ahmed, A., Perez, S. C., Egea, G. C., and Candela, C. M. E. 2000. Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. *Eur. J. Plant Pathol.* 106:817-824.
47. Stone, A. G., Scheuerell, Steven, J., and Darby, H. M. 2004. Suppression of soilborne diseases in field agricultural systems: Organic matter management, cover cropping, and other cultural practices. Pages 131-177 in: *Soil Organic Matter in Sustainable Agriculture*. Fred Magdoff and Ray R. Weil, eds. CRC Press LLC, New York.
48. Stone, A. G., Traina, S. J., and Hoitink, H. A. J. 2001. Particulate organic matter composition and *Pythium damping-off* of cucumber. *Soil Sci. Soc. Am. J.* 65:761-770.
49. Sutton, J. C., Li, D. W., Peng, G., Yu, H., Zhang, P., and Valdebenito-Sanhueza, R. M. 1997. *Gliocladium roseum*: A versatile adversary of *Botrytis cinerea* in crops. *Plant Dis.* 81:316-328.
50. Trolinger, J. C., and Strider, D. L. 1985. *Botrytis* Diseases. Pages 20-26 in: *Diseases of Floral Crops*. D.L. Strider, ed. Praeger Publishers, New York.
51. U.S. Environmental Protection Agency. 1993. Standards for the Use or Disposal of Sewage Sludge (40 Code of Federal Regulations Part 503). U.S. EPA, Washington DC.
52. Vallad, G. E., Cooperband, L., and Goodman, R. M. 2003. Plant foliar disease suppression mediated by composted forms of paper mill residuals exhibits molecular features of induced resistance. *Physiol. Mol. Plant Pathol.* 63:65-77.
53. Wang, P., Changa, C. M., Watson, M. E., Dick, W. A., Chen, Y., and Hoitink, H. A. J. 2004. Maturity indices for composted dairy and pig manures. *Soil Biol. Biochem.* 36:767-776.
54. Wolfheckel, H. 1988. The suppressiveness of sphagnum peat to *Pythium* spp. *Acta. Hortic.* 221:217-222.
55. Yedidia, I., Shores, M., Kerem, Z., Benhamou, N., Kapulnik, Y., and Chet, I. 2003. Concomitant induction of systemic resistance to *Pseudomonas syringae* pv. *lachrymans* in cucumber by *Trichoderma asperellum* (T203) and accumulation of phytoalexins. *Appl. Environ. Microbiol.* 69:7343-7353.
56. Yourman, L. F., and Jeffers, S. N. 1999. Resistance to benzimidazole and dicarboximide fungicides in greenhouse isolates of *Botrytis cinerea*. *Plant Dis.* 83:569-575.
57. Zhang, W., Dick, W. A., and Han, D. Y. 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and Arabidopsis. *Phytopathology* 88:450-455.