Increased Growth of Plants in the Presence of the Biological Control Agent *Trichoderma harzianum*

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ABSTRACT

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The potential of *Trichoderma harzianum* to induce increased growth of various floricultural and horticultural crops was determined. In raw soil containing the fungus, pepper seed germinated 2 days earlier than untreated controls. Steamed or raw soil infested with *T. harzianum* hastened flowering of periwinkle, increased the number of blooms per plant on chrysanthemums, and increased the heights and weights of other plants. Responses occurred consistently at population densities of *T. harzianum* higher than 10^5 colony-forming units per gram of soil when the fungus was applied either in conidial suspensions or in a peat-bran mixture. Such population densities can be achieved economically by applying the agent to propagative beds, where rooted cuttings (e.g., chrysanthemum) may carry over adequate numbers of thallus units to ensure favorable growth responses after transplanting.

Routine use of biocontrol agents for control of plant diseases in agriculture has not been realized (1). There are many reasons for this; however, chief among these is that they may offer no practical advantages and may not be as efficient as conventional, established controls. One feature that could make such agents more attractive is the possibility of enhanced crop growth in addition to disease control. Such enhancement has been achieved with fluorescent pseudomonads (10) and *Trichoderma harzianum* (2).

Growth of radish in response to various preparations of *T. harzianum* Rifai were reported (2). We expanded research to include the responses of other plant species with additional measurements of growth parameters.

MATERIALS AND METHODS

Biological control agents. At Colorado State University, conidia of *T. harzianum* from a culture originally isolated from soil in Colombia (3) were exposed to 100 μg/ml of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Tredom Chemical, Inc., Hauppauge, NY) to obtain an isolate tolerant to greater than 10 μg a.i. of benomyl per milliliter in *Trichoderma*-selective medium (4). A conducive index value (7) was developed for the benomyl-tolerant isolate T-95 selected from more than 100 mutants. This value indicated that it was as efficient a biocontrol agent against *Rhizoctonia solani* as the original wild type.

T-95 was grown on yeast-malt-extract agar (2 g of yeast extract, 20 g of malt extract, and 20 g of agar per liter of distilled water) for 10 days at 25 C under illumination. Conidia were washed from the surface of the culture with sterile distilled water. Density of conidia was adjusted to 10^8/ml with a hemacytometer, and this suspension was sprayed on roots of ornamental plants in certain treatments.

An isolate of *T. harzianum* (T-203) (5) was used at the Hebrew University. To obtain conidial suspensions, the fungus was grown in 500-ml Erlenmeyer flasks containing 50 ml of potato-dextrose agar for 10 days at 30 C under illumination. Conidia were harvested from the substrate, washed several times in water, and suspended in 0.001% Tween 20. This suspension was added to soil at a concentration of 5×10^6 conidia per gram of soil.

In some treatments, a culture medium (11) consisting of equal volumes of wheat bran, peat moss, and water was autoclaved for 1 hr on two successive days, then the fungal agents were added and the culture was incubated for 14 days at 28–30 C. This mixture was air-dried, powdered by grinding and screening, and stored in plastic containers for later use. The population density of this preparation exceeded 10^7 cfu/g and often approached 10^9 cfu/g.

Cultural procedures. In the floricultural experiments at Colorado State University, a peat-perlite mix was used for propagating cuttings treated with rooting hormone containing, in some cases, 25,000 μg a.i. of benomyl per milliliter, duplicating procedures used in commercial floriculture operations (6,8). Cuttings were exposed to mist for 16 sec every 2 min during daylight hours until rooted. Container mix for growing plants was composed of equal portions of soil, peat, and perlite. Both the propagative and container mix were steamed at 83 C for at least 0.5 hr. After cooling, fortuitous reinfestation of the mixtures by indigenous *Trichoderma* spp. occurred. Thus, *Trichoderma* spp. were observed at low levels in controls that were not infested with T-95 on *Trichoderma*-selective medium (4). The pH values of propagative and container media ranged from 6.8 to 7.2 and were not affected by treatments. In the experiments done in commercial greenhouses, approximately the same procedures were employed.

In the floricultural investigations, a fertilizer solution diluted 1:200 through an injector was applied at each irrigation (8) from a stock solution containing 17.1 kg of KNO_3, 6.1 kg of NH_4NO_3, 6.2 kg of $MgSO_4$, 2 L of phosphoric acid (80%), 190 g of H_3BO_3, 30 g of $ZnSO_4$, 6.3 g of ferric ethylenediamine *di-o*-hydroxyacetic acid, and 16.6 g of $CaNO_3$ in 190 L of water. Temperatures averaged 18 C during the day and 13 C at night. Additional lighting usually was not provided. If day-length-sensitive plants were induced to flower, black cloth or additional lighting was applied according to conventional greenhouse management procedures (6).

At the Hebrew University, vegetable plants were grown in raw red-brown sandy loam with the following characteristics: pH 7.3, 78.3% sand, 5.8% silt, 15.5% clay, 0.3% organic matter, 0.02% N, 0.06% K, 0.01% P, and 0.003% extractable Fe. Plants were seeded in plastic boxes 11 × 9 × 6 cm. Ten seeds were sown per box and six replicates were employed. Plants were grown in a greenhouse at 27 ± 3 C.

Plant response measurements. Various measurements were made of plant growth

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responses as appropriate for each crop. These included number and time of seedling emergence, plant length measured from soil to apical bud, fresh or dry weights, and number of branches and flower buds. Mean separations were done by Fisher's least significant difference (FLSD) analysis (as recommended by Madden et al [9]) at $P = 0.05$ in experiments replicated in randomly distributed blocks.

RESULTS

Floricultural investigations. Three seedlings each of petunia (*Petunia hybrida* Vilm. 'Red Flash') were transplanted into soil mix in 14-cm-diameter pots. T-95 grown in the peat-bran medium was added to the mix (20%, v/v). In other treatments, T-95 was grown in the medium, but this was autoclaved before being added to soil, and the medium without T-95 was incorporated into the container mix. There were 15 replicates. After 42 days, plants grown in the mix with the T-95 in peat-bran had significantly greater dry and fresh weights and more side branches than the controls or other treatments (Fig. 1). There was a significant decrease in the number of flowers in plants treated with the raw peat-bran culture without T-95 compared with other treatments. Growth characteristics of petunias grown in pots to which the autoclaved T-95 in peat-bran was added did not differ significantly from those in the control.

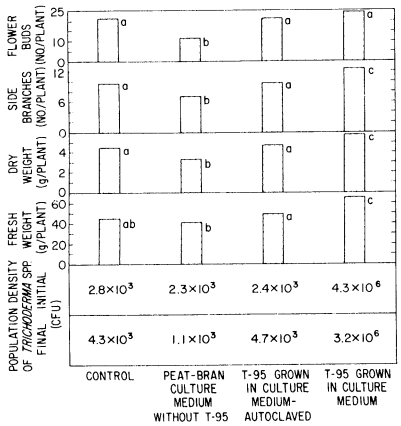

Fig. 1. Growth characteristics of petunias grown 42 days in a greenhouse potting soil when *Trichoderma harzianum* T-95 grown for 2 wk in a peat-bran medium was added to soil (20%, v/v). In other treatments, this medium (infested by the fungus) was autoclaved before being added or the medium without *T. harzianum* was added to soil. Values followed by the same letter do not differ significantly ($P = 0.05$).

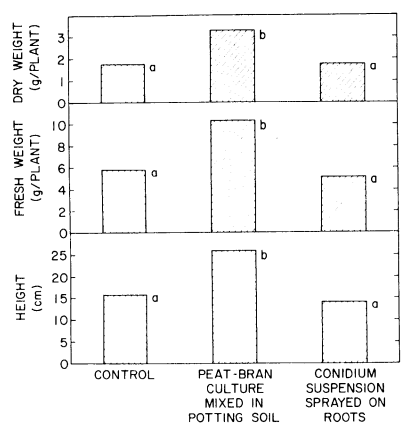

Fig. 2. Growth characteristics of chrysanthemums grown 57 days in greenhouse potting soil when *Trichoderma harzianum* T-95 was either sprayed as a conidial suspension (10^8/ml) on roots of cuttings or grown in peat-bran culture for 2 wk and mixed into the propagative medium (20%, v/v). Values followed by the same letter do not differ significantly ($P = 0.05$).

Chrysanthemums (*Chrysanthemum morifolium* (Ramat.) Hems 'Jade') were rooted and two cuttings were planted per 9-cm-diameter pot. In one treatment, roots of the cuttings were sprayed with a suspension of T-95 (10^8 conidia per milliliter) before transplanting. In another, T-95 grown in peat-bran was added to the soil (20%, v/v). There were seven replicates. After 57 days, plants grown in the container mix amended with T-95 grown in peat-bran were significantly taller and heavier than those in the control or plants whose roots were sprayed with a conidial suspension of T-95 (Fig. 2). At the end of the experiment, the population densities of *Trichoderma* spp. were 4.3×10^2 cfu/g of container mix in the controls, 7.2×10^5 cfu/g in mix to which the peat-bran culture was added, and 9.7×10^3 cfu/g where roots were sprayed with the conidial suspension.

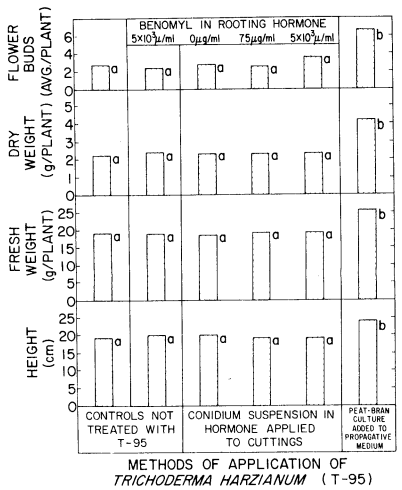

Fig. 3. Growth characteristics of chrysanthemums grown 42 days after transplanting into greenhouse potting soil when *Trichoderma harzianum* T-95 was applied at propagation as a conidial suspension in rooting hormone (10^8/ml) with or without benomyl or grown in peat-bran culture for 2 wk and mixed into the propagative medium (20%, v/v). Values followed by the same letter do not differ significantly ($P = 0.05$); responses to each concentration of benomyl were statistically analyzed separately as compared with the control.

To determine whether growth was enhanced by adding T-95 at the time of propagation, unrooted chrysanthemum cuttings (Jasamine) were sprayed with rooting hormone containing a conidial suspension and/or benomyl at various concentrations. Cuttings also were placed in a propagative medium containing T-95 growing in peat-bran (20%, v/v). After rooting (14 days), the cuttings were transplanted to pots 9 cm in diameter. Regardless of fungicide treatment, there was no increase in growth compared with untreated controls during 42 days in treatments where conidia of T-95 were applied with the rooting hormone with or without benomyl (Fig. 3). There were, however, significant increases in flower bud production, dry and fresh weights, and heights of plants compared with the control when T-95 grown in the peat-bran culture was added to the propagative medium. In the controls, the population density of *Trichoderma* spp. averaged 2.5×10^3 cfu/g of container mix at the end of the experiment. In the treatments in which the conidial suspension was applied with the rooting hormone, densities varied from 0.9 to 1.9×10^3 cfu/g of mix. Where the peat-bran culture of T-95 was added to the propagative medium, there were 2.5×10^5 cfu/g of container mix.

Under conditions in Colorado, it is difficult to flower periwinkle (*Vinca minor* L.) for marketing by 1 May from seeds planted in January or February in covered structures. To test whether growth enhancement induced by T-95 could hasten flower development, the peat-bran culture of T-95 was mixed into steamed container medium used to germinate seeds of two cultivars at 10% (v/v) of medium in a commercial greenhouse on 15 January 1983. On 14

Table 1. Growth responses of two cultivars of periwinkle after treatment with a peat-bran[a] culture of *Trichoderma harzianum* T-95[b]

Treatment	Av. plant ht (cm)[c] Little Pinkie	Little Bright Eyes
T-95 applied in seed flat	14.6 x	17.0 x
T-95 applied to transplants (10% peat-bran culture in soil mix)	35.5 y	38.3 y
T-95 applied to transplants (20% peat-bran culture in soil mix)	40.3 y	42.0 y
Control	14.4 x	15.5 x

[a] T-95 cultured in mixture of 1 part wheat bran, 1 part peat moss, and 1 part water (v/v) for 2 wk.

[b] Within a column, numbers followed by the same letter do not differ significantly ($P = 0.05$) as indicated by Fisher's least significant difference test.

[c] Average heights of 12 plants chosen at random from each of three replicates.

February 1983, seedlings from treated or untreated containers were transplanted into soil containing 0, 10, or 20% (v/v) of the peat-bran culture. The 10% mix contained about 10^6 cfu of T-95 per gram. There were three replicates. By 7 April 1983, buds were detected only on transplants treated with 10 or 20% of the peat-bran culture. Measurements 8 days later indicated significant increases in heights of plants resulting from these two treatments (Table 1). By 1 May, both cultivars in these treatments were in full bloom, whereas the controls and plants treated only in the seed flats with T-95 did not flower completely for another 3–4 wk.

Growth responses in vegetables. A water suspension of conidia of isolate T-203 was applied to the red-brown sandy loam at a rate of 5×10^6/g of soil. Germination of pepper (*Capsicum frutescens* L. var. *grossum* Bailey 'Maor') seeds in treated soil occurred about 2 days earlier than in untreated soil (Fig. 4).

There was a significant increase in dry weights of tomato (*Lycopersicon esculentum* Mill. 'Alma'), pepper, and cucumber (*Cucumis sativus* L. 'Alma') planted in the red-brown sandy loam amended with conidia of T-203 (5×10^6 cfu/g) compared with the controls (Table 2). No significant effects were observed with bean (*Phaseolus vulgaris* L. 'Brittle Wax') or radish (*Raphanus sativus* L. 'Chuma').

Conidial suspensions of T-203 were applied at various rates to the red-brown sandy loam and cucumbers planted.

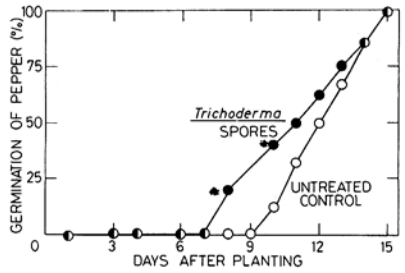

Fig. 4. Germination of pepper seeds when conidia of *Trichoderma harzianum* T-203 were added (5×10^6/g) to red-brown sandy loam. Asterisks indicate a significant difference at each point from the untreated control.

Table 2. Dry weights of plants grown 21 days after planting and treated with conidia of *Trichoderma harzianum* T-203 at 5×10^6 cfu/g of soil

Plant	Dry weights (g/plant)	
	Control	Treated
Bean	2.9	3.2
Radish	1.2	1.3
Tomato	1.9	2.6[a]
Pepper	1.2	1.7[a]
Cucumber	1.5	2.8[a]

[a]Differs significantly from the control (LSD = $P = 0.05$). Means of 10 plants in each of six replicates.

Increases in dry weights and lengths occurred when 10^5 or more conidia per gram of soil were added (Fig. 5).

DISCUSSION

Responses to application of *T. harzianum* to soil were characterized by shorter germination time for pepper seed (Fig. 4), hastening of flowering of periwinkle (Table 1), increase in number of blooms on chrysanthemums (Fig. 3), and increased heights and/or weights of plants (Tables 1 and 2; Figs. 1–3 and 5). Such responses should make the application of biocontrol agents an attractive procedure, especially in covered structures where the cost of heating for environmental control is measured by the time required to produce a crop.

Responses were not observed in all cases: e.g., beans and radish growing in the red-brown sandy loam failed to respond to the presence of the *T. harzianum*. This contrasts with other published results in which increases up to 274% in dry weight of radish were observed after treatment with T-95 (2). This suggests that various unknown factors interact to mediate responses.

Because adequate nutrients (8) were applied in the floricultural investigations, increased growth responses were over and above those typical of nutritional responses.

Responses were observed whether conidia or a peat-bran culture of *T. harzianum* was added to soil or propagative medium. The population density of *T. harzianum* inducing the response usually exceeded 10^5 cfu/g of container medium or soil (Fig. 5). The cost to achieve such a level by applying propagules to container media could be prohibitive. Therefore, various procedures were examined to treat propagative material that could ultimately carry sufficient thallus units of *T. harzianum* into the transplant operation. *T. harzianum* applied to seed flats (Table 1), in rooting hormone (Fig. 3), or sprayed on rooted cuttings (Fig. 2) was not effective in inducing subsequent increases in growth, suggesting that not enough thallus units were carried with transplants to ultimately induce responses. When the agent in peat-bran culture was added to propagative medium used for rooting chrysanthemums, however, increased growth was observed in cuttings transplanted to nonamended potting mix (Fig. 3), where after transplanting, population densities of T-95 were greater than 10^5 cfu/g.

No increased growth in petunias resulted from application of autoclaved peat-bran medium or autoclaved cultures previously supporting growth of *T. harzianum* (Fig. 1). This result was similar to previous observations (2).

If *T. harzianum* is to be used to promote growth in agriculture crops, such treatment must be compatible with pesticides used in the industry. The unaltered agent is tolerant of many fungicides commonly used in the ornamental industry such as etheridiazole and PCNB; however, it is intolerant of the benzimidazoles. This was the rationale for mutation of *T. harzianum* for tolerance to benomyl. If applied in propagative operations where cuttings are rooted in high density (e.g., chrysanthemums), the benomyl-tolerant isolate can be applied in peat-bran formulation at a cost of a fraction of a cent per cutting. The resulting increase in growth more than pays for the cost of the product.

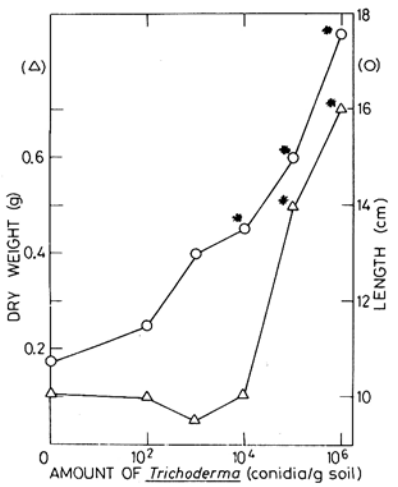

Fig. 5. Length (○) and dry weights (△) of cucumber after 4 wk of growth in red-brown sandy loam to which various population densities of *Trichoderma harzianum* T-203 had been added at seeding. Asterisks indicate a significant difference at each point compared with values observed in soil to which T-203 had not been added.

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