A Mechanism for Increased Plant Growth Induced by *Trichoderma* spp.

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ABSTRACT

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Enhanced plant growth resulting from amendments of soil with *Trichoderma harzianum* and *T. koningii* was investigated to determine if increased growth could be attributed to a direct effect of these *Trichoderma* spp. on the plant or a secondary effect due to control of minor plant pathogens. Addition of *Trichoderma* spp. to autoclaved soil increased rate of emergence of tomato and tobacco seedlings over that of the controls. Eight weeks after planting, root and shoot dry weights of tomato and tobacco were increased 213–275 and 259–318%, respectively, over the controls. When population densities of soil microflora (other than *Trichoderma* spp.) were determined, no quantitative and qualitative differences were observed between soils infested with *Trichoderma* spp. and the controls. When soil fertility was increased, the level of increased tomato growth induced by *Trichoderma* spp. was enhanced. Radish plants grown in gnotobiotic conditions with *T. harzianum* T-95 were larger than radish plants grown under similar conditions without the agent. The rate of seed germination was increased compared with controls where *Trichoderma* spp. were not present when seeds were separated from *Trichoderma* spp. by a cellophane membrane. We conclude that these *Trichoderma* spp. produced a growth-regulating factor that increases the rate of seed germination and dry weight of shoots and stems.

Various disease control measures including the use of biological control agents (8,9,13,20), fumigation (5,14,21), and fungicides (16) induced increased plant growth. This response usually was attributed to an indirect effect associated with control of plant pathogens. For example, Kloepper and Schroth (8,9) reported that radish seeds treated with plant growth-promoting rhizobacteria and grown under gnotobiotic conditions, did not produce larger plants. In raw soil, however, increased growth resulting from a similar treatment suggested that the response resulted from reduction in activity of minor pathogens (17) in the rhizosphere.

The use of *Trichoderma* spp. as biological control agents is clearly established (e.g., 2,3,6,15,19). However, except for increases in plant growth induced by *T. harzianum* Rifai or *T. viride* Rifai in our laboratory (1,12), this type of response has not been reported in the literature. Experiments to characterize enhanced plant growth associated with treatments involving *Trichoderma* spp. tested the null hypotheses that such treatments did not have a direct effect on plant growth and that increased growth was due to control of minor plant pathogens.

MATERIALS AND METHODS

Soil used in these experiments, unless otherwise stated, was an Ascalon sandy loam collected near Nunn, CO (11). Soil was adjusted to 10% moisture before autoclaving at 1 kg/cm^2 for 1 hr and 15% (−0.7 bars) after cooling. Unless stated otherwise, plants were watered with autoclaved nutrient solution containing 11.34 kg of KNO_3, 5.44 kg of $MgSO_4$, 2.27 kg of NH_4NO_3, 110 g of $NaBO_3$, 15 g of K_2SO_4, and 400 ml of H_3PO_4 in 95 L of water.

Thalli of *T. harzianum* and *T. koningii* Rifai were grown in autoclaved peat-bran (1) for 14 days in mason jars. After drying, the fungus preparation was passed through a 1-mm-mesh soil sieve, and the number of colony-forming units (cfu) per gram of soil were quantified on media selective for *Trichoderma* (4). Conidia of *Trichoderma* spp. were grown on malt-yeast extract medium for 7 days, and conidia were harvested and washed three times in sterile distilled water. A final population density of 10^5 cfu/g of soil was used in all experiments unless stated otherwise. In all experiments, seeds were surface disinfested in a 0.5% sodium hypochlorite and 5% ethanol mixture for 1 min. Unless otherwise stated, plants were grown in 20-cm-diameter pots containing approximately 400 g of soil. All experiments were designed with an appropriate raw soil control as described by Baker et al (1). Plant weights were obtained for oven-dried plants.

Tomato (*Lycopersicon esculentum* L. 'Big Boy') or tobacco (*Nicotiana tabacum* L. 'Speight G-28') were used as indicator plants to measure the effects of *Trichoderma* spp. (cultured in peat-bran) on plant growth. Five tomato seeds were planted in each pot and plants were thinned to one per pot after emergence. Plants were harvested and weighed 9 wk after planting. Tobacco seeds were planted 10 per pot, thinned to one seedling per pot, and weighed 10 wk after planting. There were six replications.

To determine if the agent was interfering with the reinfestation of autclaved soil by microorganisms that could decrease plant growth, *T. harzianum* (cultured in peat-bran) was added to soil which was divided equally among five pots (10-cm diameter); five pots of autoclaved soil served as controls. Five tomato seeds were planted in each pot. After 6 wk, soil dilutions were plated on acidified potato-dextrose agar, acidifed water agar, King's B agar, and a medium selective for *Trichoderma*. After 2- and 5-day incubations, plates from the two treatments were examined for both quantitative and qualitative differences in soil microflora. These microorganisms were grown individually in 100 ml of potato-dextrose broth: the fungi in still culture for 4 days and bacteria for 48 hr in shake culture. Mats of the fungi or suspensions of the bacteria were introduced into autoclaved soils to test for their ability to affect plant growth. Tomato seeds were planted in each of five pots of soil infested with one of the isolated microorganisms.

Isolates T-12 and T-95 of *T. harzianum* were added to two soils (Ascalon sandy loam, and a potting mix consisting of 32% Colorado peat, 17% soil, 17% vermiculite, 17% perlite, and 17% sand). Soils to which T-12 and T-95 were not added served as controls. There were six replications. Tomato seeds were planted, seedlings were thinned, and watered with either distilled water or

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nutrient solution to determine if the effects of *Trichoderma* spp. on plant growth were similar to plant growth effects associated with mycorrhizal fungi at high or low nutrient regimes (18). Plants were harvested at 6 wk after planting.

Contaminant-free radish (*Raphanus sativus* L. 'Early Scarlet Globe') seedlings were grown in an axenic environment (10) in 2-L mason jars containing 300 g of sterilized soil. Four-day-old mycelial mats of *T. harzianum* T-95 and *T. koningii* T-8 were added to each of six jars to assess the ability of *Trichoderma* spp. to stimulate plant growth under axenic conditions. Six jars with radish seedlings without *Trichoderma* spp. served as controls. After 6 wk, plants were harvested and weighed. Soil from the jars was assayed to assure that gnotobiotic conditions had been maintained.

Four-day-old mycelial mats of *T. koningii* T-8, *T. harzianum* T-95, or T-12 were homogenized, added to cooled 1% water agar, and poured into petri dishes. A sterile cellophane disk was placed on the surface of the medium and a sterile mason jar ring placed on

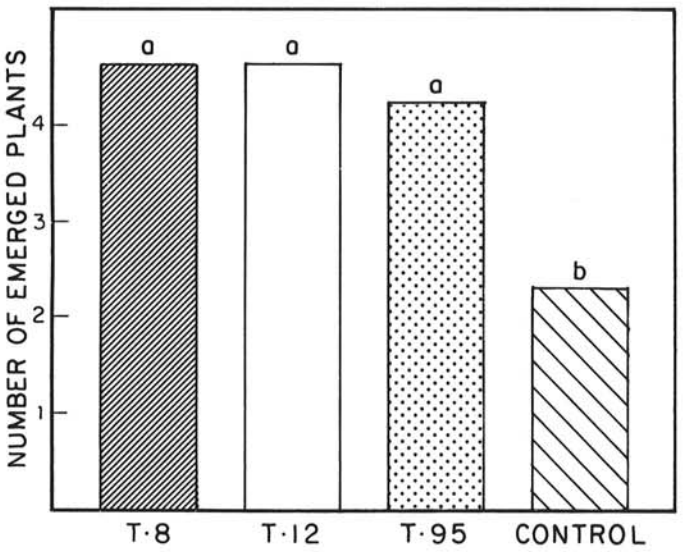

Fig. 1. Effect of three isolates of *Trichoderma* spp. (*T. harzianum* T-95 and T-12, and *T. koningii* T-8) on seedling emergence of tomato 10 days after planting in soil. Population density of *Trichoderma* spp. at planting was 10^5 cfu/g of autoclaved soil. Columns with an asterisk are different from the control (Fisher's least significant difference, $P = 0.05$). The data are the means of six replications.

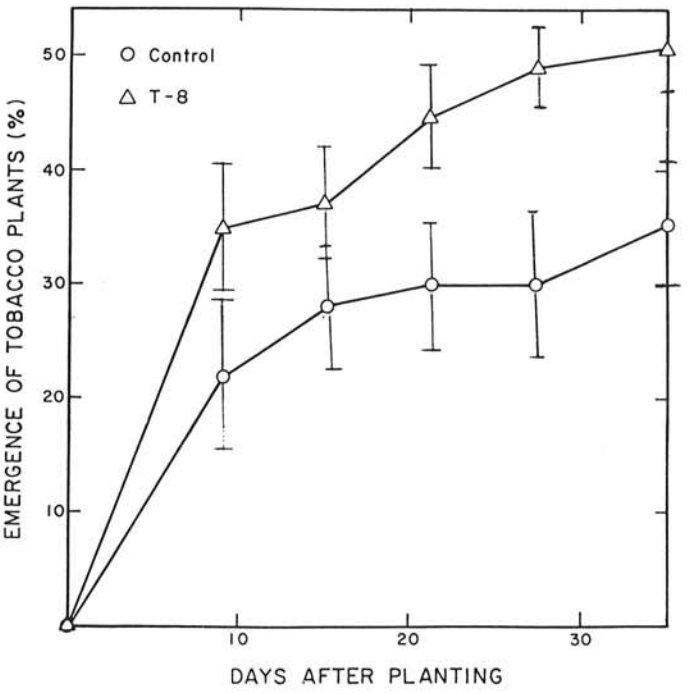

Fig. 2. Effect of *Trichoderma koningii* T-8 on emergence of tobacco seedlings. At planting, the population of T-8 density was 10^5 cfu/g of soil. The data are the means of six replicates; the bars represent one standard deviation from the means.

TABLE 1. Effect of addition of *Trichoderma* spp. on dry weights of roots and shoots of tomato and tobacco[a]

Treatment	Dry weights (g)[b]	
	Root	Shoot
Tomato[c]		
T. harzianum (T-12)	0.20*	0.44*
T. harzianum (T-95)	0.17*	0.44*
T. koningii (T-8)	0.22*	0.54*
Control	0.08	0.17
Tobacco[d]		
T. koningii (T-8)	1.82*	2.31*
Control	0.23	0.87

[a] *Trichoderma* spp. grown in peat-bran culture (1) for 14 days. Population density was 10^5 cfu/g soil at planting.

[b] For each species, means followed by an asterisk within a column are significantly different from the respective control (LSD, $P = 0.05$). There were six replications.

[c] Five tomato seeds planted in each pot and plants thinned to one per pot after emergence. Plants harvested and weighed 9 wk after planting.

[d] Tobacco seeds were planted 10 per pot and seedlings were thinned to one per pot after emergence. Tops were weighed 10 wk after planting.

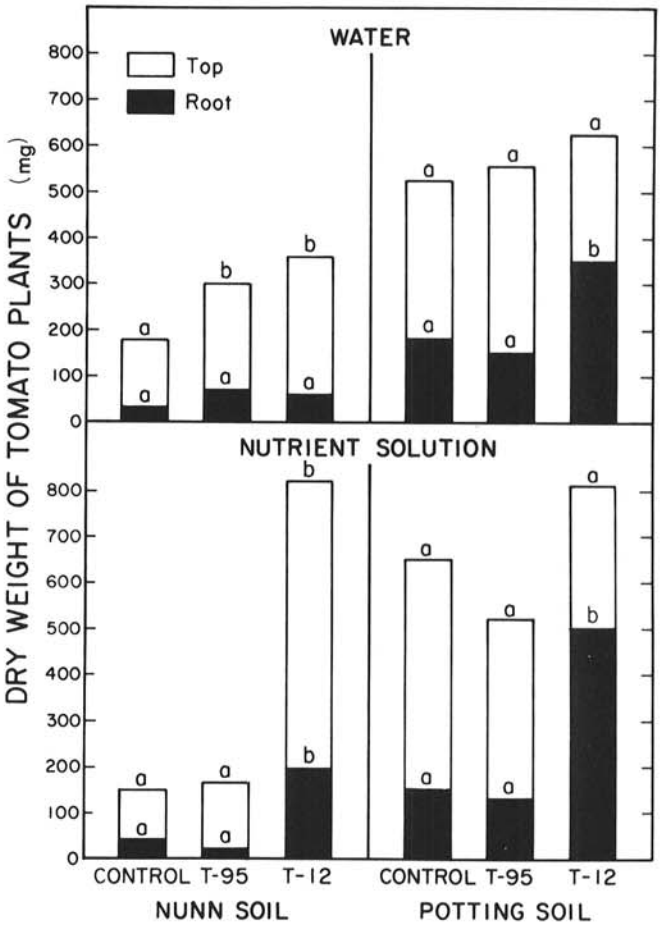

Fig. 3. Enhancement of tomato growth by two isolates of *Trichoderma harzianum* (10^5 cfu/g soil) in two soils irrigated with either sterilized nutrient solution or sterilized distilled water. For each plant part, root or shoot, and each watering regime, distilled water or nutrient solution, columns with the same letter do not differ according to Fisher's least significant difference ($P = 0.05$). The data are the means of six replicates.

the disk to hold the membrane flush with the surface of the medium. Ten caryopses of maize (*Zea mays* L.), and seed of tobacco and tomato were each placed on top of the membranes of five plates to determine if *Trichoderma* spp. could stimulate seed germination without being in contact with the caryopses or seed. Dishes without *Trichoderma* spp. served as controls. Seed or caryopses were incubated at 28 C, and rates of germination determined from 2 to 8 days.

Mean separations were done by Fisher's least significant difference (FLSD) analysis at $P = 0.05$ in experiments replicated in randomly distributed blocks.

RESULTS

Enhanced plant growth response. Significant increases in rate and/or total emergence of tobacco and tomato seedlings in soil treated with *Trichoderma* spp. were observed (Figs. 1 and 2). Sizes of emerged plants in soil infested with *Trichoderma* spp. were more uniform than those of control plants. Tomato root and shoot dry weights in soil treated with *Trichoderma* spp. increased 213–275% and 259–318%, respectively, over the controls (Table 1). Tobacco root and top weights were increased 266–291%, respectively, by addition of *T. koningii* T-8.

Reinfestation of steamed soil by microorganism. To determine whether microorganisms reinfesting steamed soil might induce adverse effects on plant growth, microorganisms were isolated from autoclaved soil that had been exposed to the ambient environment for 6 wk. Fungi isolated included *Aspergillus* sp., *Fusarium* spp., *Mucor* sp., *Penicillium* spp., and *Rhizopus* sp. Bacteria isolated were not identified. None of these organisms reduced or influenced tomato growth significantly when introduced into steamed soil.

Effect of fertilization on enhanced plant growth induced by *T. harzianum* T-95 and T-12. Tomato plants grown in Nunn sandy loam, inoculated with isolate T-12, and supplied with nutrients showed a significant increase in dry weight of shoots and roots compared to the controls; however, only shoot weights were increased by treatments with T-12 or T-95 when plants were not fertilized (Fig. 3). In potting soil, T-95 had no influence on shoot or root weight whether nutrients were supplied or not. Significant differences in root weight were observed in the same soil when T-12 was added.

Effect of *Trichoderma* spp. on radish plants growing in gnotobiotic conditions. Both T-95 and T-8 increased the dry weight of radish plants grown in gnotobiotic conditions as compared with uninfested controls; however, only the increase in the T-95 treatment was significant (Fig. 4).

Effect of metabolites produced by *Trichoderma* spp. on seed germination. The effects of filterable metabolites produced by isolates T-8, T-12, and T-95 of *Trichoderma* spp. were tested by separating seeds or caryopses from cultures of the isolates with a membrane. The time required for germination of tomato, tobacco, or corn was shortened significantly by 1–3 days by such treatment in comparison with untreated controls (Fig. 5). There was no effect on the ultimate level of germination.

DISCUSSION

Two mechanisms have been advanced most frequently to explain the nature of the increased growth response induced by certain members of the soil microflora. The first hypothesis is that enhanced growth of plants induced by pseudomonads is thought to be due predominantly to biological control of minor pathogens

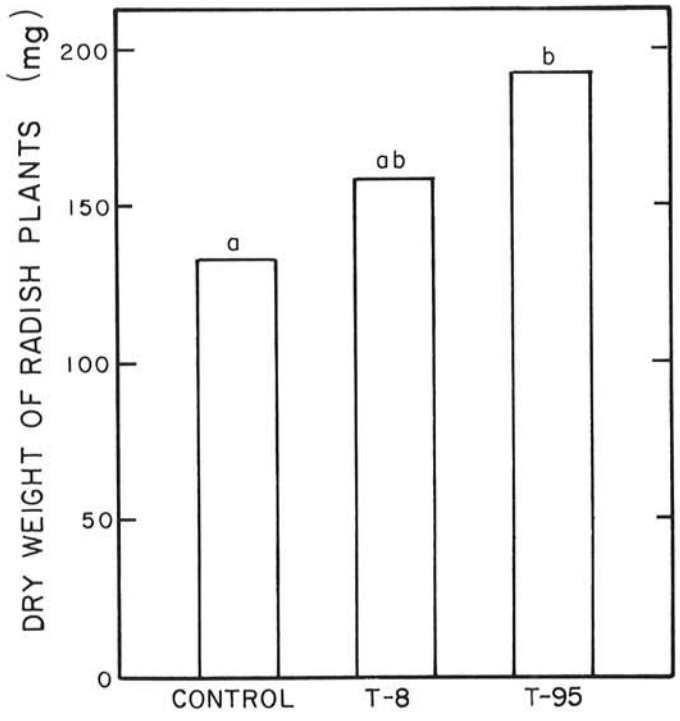

Fig. 4. Effect of *Trichoderma harzianum* T-95 and *T. koningii* T-8 on dry weights of radish plants grown under gnotobiotic conditions. Columns with the same letter do not differ according to Fisher's least significant difference ($P = 0.05$). The data are the means of six replicates.

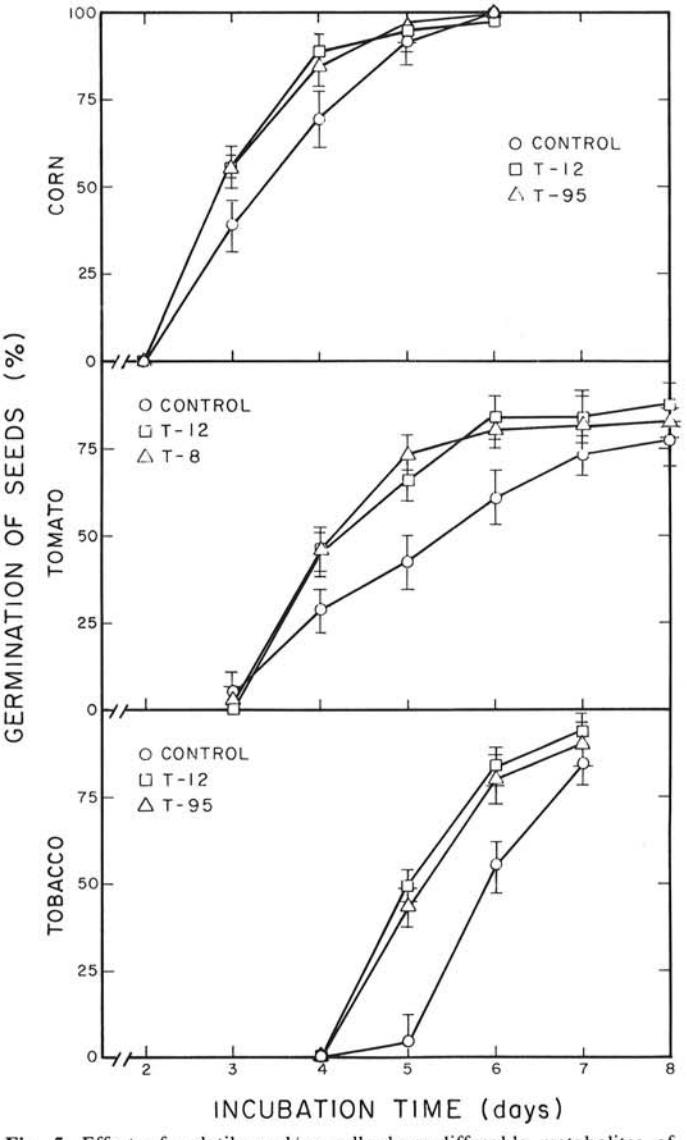

Fig. 5. Effect of volatile and/or cellophane-diffusable metabolites of *Trichoderma harzianum* (T-95) and *T. koningii* (T-8) on seed germination of corn, tomato, and tobacco. The data are the means of five replicates; the bars represent one standard deviation from the mean.

(9,17,20). The other hypothesis, so far not demonstrated clearly for any biological system, is that a microbial agent produces growth-regulating metabolites.

The experiments described here were performed with autoclaved soil, sterilized nutrient solutions, or under axenic conditions (10) to test the impact of potential minor pathogens. None of the microorganisms reinfesting autoclaved soil decreased plant growth when added to autoclaved soil. However, *T. harzianum* (T-95) induced increased growth of radish grown under gnotobiotic conditions in comparison with axenic, uninfested controls (Fig. 4). This suggests that, unlike pseudomonads (9), *T. harzianum* may increase growth by production of a growth-stimulating factor.

In previous work (1), plants involved in growth-stimulating experiments were watered with nutrient solution to preclude the complicating effect of a nutrient response from introduction of *Trichoderma* spp. In experiments reported here, except for top growth of tomatoes in the presence of T-95, growth was the same or greater when plants were watered with nutrient solutions than with water alone (Fig. 3). This effect is the opposite of what would be expected if the agents were acting as mycorrhizal fungi (18). Also, microscopic examination revealed no evidence of such a relationship.

Additional evidence for a growth-stimulating factor produced by *T. harzianum* spp. was obtained by germinating seeds in association with, but separated from the agent by a membrane. In contrast to controls, stimulation of germination occurred even though isolates of *T. harzianum* were not in contact with the seed (Fig. 5). In this case, the fungi could have influenced the pH of the (germination) environment; however, Hora and Baker (7) demonstrated that wide differences in hydrogen ion concentrations, which commonly occur in soil, had no significant effect on seed germination of tomato.

These results support the report of Lindsey and Baker (12) that a fungus can induce growth-regulative effects on plants. Our results warrant further description of the phenomenon, extraction and identification of growth factors, and, perhaps, commercialization of the product.

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