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J. AMER. SOC. HORT. SCI. 111(5):810–816. 1986.

# Effect of Peat : Vermiculite Mixes Containing *Trichoderma harzianum* on Increased Growth Response of Radish

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*Additional index words.* *Raphanus sativus, Pythium* spp., *Pisum sativum*

*Abstract.* **The effects of peat : vermiculite mixes on increased growth response of radish (*Raphanus sativus* L. 'Early Scarlet Globe') induced by *Trichoderma harzianum* Rifai (isolate T-12) were investigated. Canadian sphagnum peat and vermiculite were mixed in various ratios to form 0–100% peat mixes. Four levels of T-12 amendment were added to these mixes—0%, 2%, 5%, and 10% (v/v) or 0%, 0.1%, 1%, and 10%. In general, increasing levels of T-12 amendment induced linear increases in radish dry weights after 4 and 5 weeks. Greatest increases were seen in mixes containing 20% peat or 80% peat. The smallest increases were observed when *T. harzianum* was added to 0% peat or 100% peat mixes. There was no effect on the population densities of T-12 after it was introduced into the mixes. No *Pythium* spp. or root disease were detected in the mixes, suggesting that *T. harzianum*, a biological control agent, can increase plant growth independent of any detectable root pathogens.**

The use of *Trichoderma* spp. as biological control agents has been investigated extensively (1, 4, 6, 8). In most instances, increased plant growth and yields were attributed to reductions in plant disease. Recently, *Trichoderma harzianum* induced increased plant growth independent of any plant disease (2, 3). Stimulation of plant growth also was demonstrated in plants grown under gnotobiotic conditions (13). *T. harzianum* amendments hastened germination and flowering, increased the number of blooms per plant, and increased shoot height and dry weight (3). Preliminary investigations established that soil pH, temperature, soil–mix composition, and amendment formulation affect the expression of growth response. The purpose of this research was to examine the effects of 2 soil–mix components, peat and vermiculite, on *T. harzianum* and the enhanced growth of radish. Effects of these and other environmental parameters must be ascertained before consistent and cost-effective results can be achieved with *Trichoderma* spp. in the horticultural industry.

## Materials and Methods

T. harzianum *(T-12) amendment*. *T. harzianum* Ritai isolate T-12 (7) was cultured on a medium of equal parts of wheat bran, peat moss, and water (by volume) (12). The medium was placed in 2-liter Mason jars and autoclaved for 1 hr on 2 successive days. Potato dextrose agar (PDA) plugs (4 mm in diameter) of T-12 were added to the medium, and the cultures were incubated for 14 days at 28°–30°C. This mixture was air-dried, powdered by grinding in a Wiley mill, and screened through a 2-mm sieve.

*Peat : vermiculite mixes*. Nonsterile Canadian sphagnum peat and vermiculite #3 (Grace Horticultural Products, Cambridge, MA 02140) were mixed (v/v) to make 6 combinations as follows: 100% peat : 0% vermiculite, 80% peat : 20% vermiculite, 60% peat : 40% vermiculite, 40% peat : 60% vermiculite, 20% peat : 80% vermiculite, and 0% peat : 100% vermiculite. These mixes were designated 100P, 80P, 60P, 40P, 20P, and 0P, respectively. The pH and water holding capacity (w/w) of the peat and vermiculite were 3.2 and 642%, and 6.0 and 530%, respectively.

T. harzianum *soil–mix treatments*. T-12 peat–bran amendment was mixed with each peat : vermiculite combination at 0%, 2%, 5%, and 10% (v/v). In the 2nd experiment, T-12 peat–bran amendment was added at 0%, 0.1%, 1%, and 10%. Previous work (2) showed that uninfested peat–bran amendment or autoclaved *Trichoderma* peat-bran amendment added to growth media had little effect, or decreased plant growth. In these experiments, peat : vermiculite mixes not treated with T-12 amendments were used as controls to which comparisons were made. Each treatment was placed in 8 plastic pots (7 × 7 × 6 cm). Five radish seeds were planted in each pot, and the plants were thinned to 3 per pot after emergence. Pots were arranged in a completely randomized design on a greenhouse bench, with 8 replicates. Average day and night temperatures were 22° and 12°C, respectively. No additional lighting was used. Plants were irrigated with a fertilizer solution diluted 1:200 through an injector (9) from a stock solution containing 17.1 kg $KNO_3$, 6.1 kg $NH_4NO_3$, 6.2 $MgSO_4$, 2 liters phosphoric acid (80%), 190

Received for publication 16 Oct. 1985. Published with the approval of Director, Colorado Agricultural Experiment Station, Journal Series Paper no. 15-202. Research supported by funds from BARD Research Project no. US-679-830, Science and Education Administration Project 83-CRCR-1-1259, and Western Regional Project W 147. We wish to thank the Dept. of Horticulture, Plant Environmental Research Center, for the use of their facilities. We also thank Mark Juetten for assitance with computer programming, Jim Zumbrunnen and Gary Richardson for statistical analyses, and W.R. Grace and Co. for the sphagnum peat and vermiculite. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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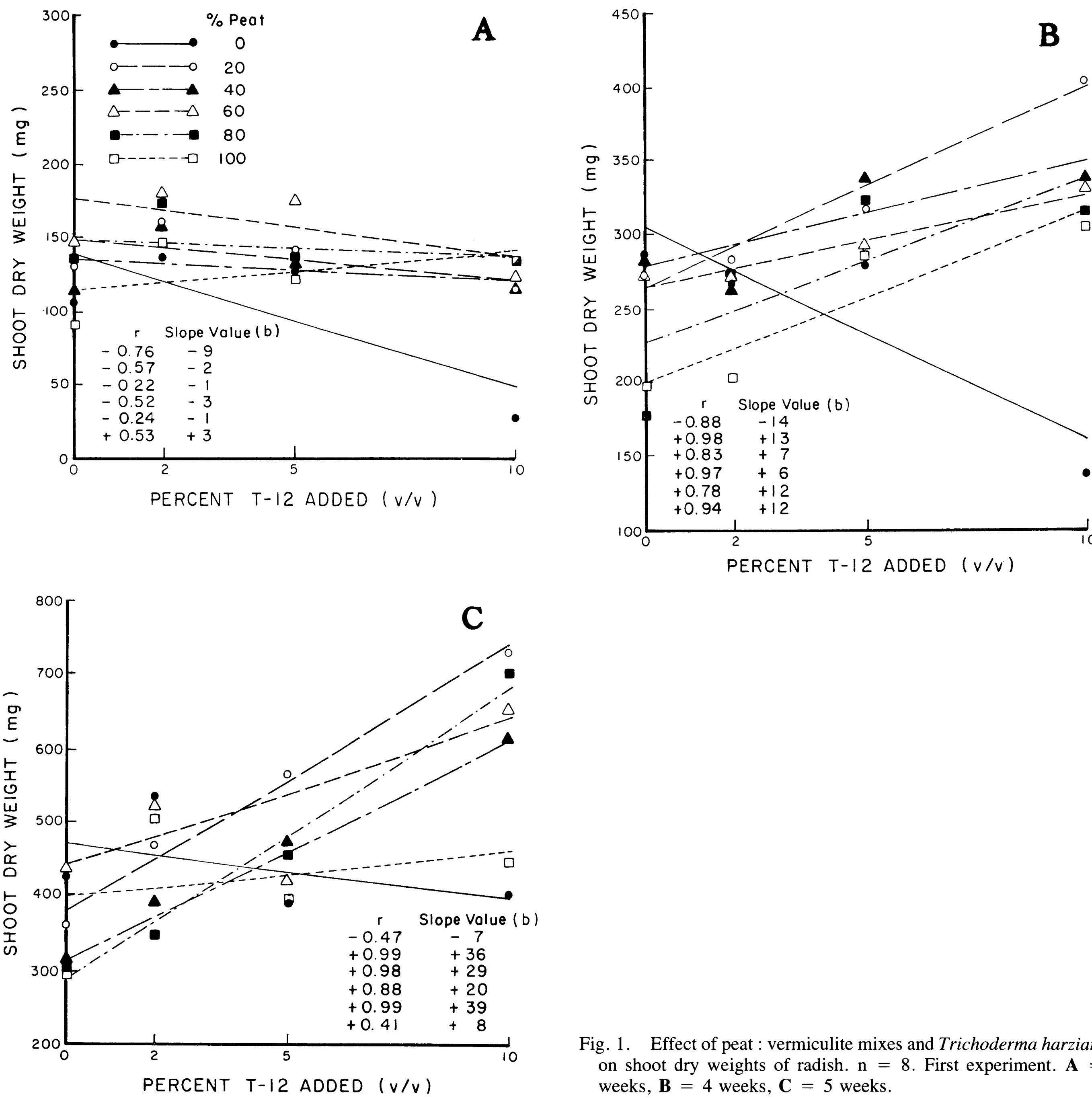
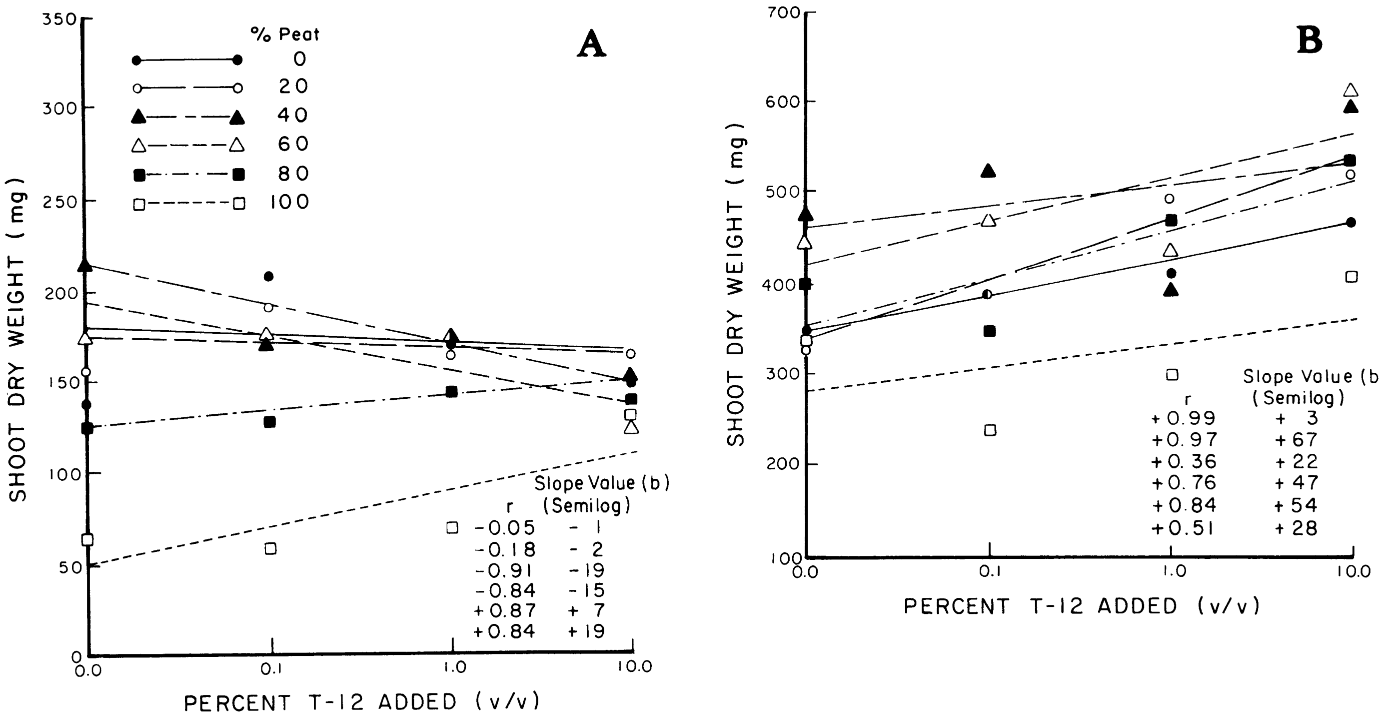



Fig. 1. Effect of peat : vermiculite mixes and *Trichoderma harzianum* on shoot dry weights of radish. n = 8. First experiment. **A** = 3 weeks, **B** = 4 weeks, **C** = 5 weeks.

g $H_3BO_3$, 30 g $ZnSO_4$, 6.3 g ferric ethylenediamine di-o-hydroxyacetic acid, and 16.6 g $CaNO_3$ in 190 liters of water.

One plant per pot was removed after 3, 4, and 5 weeks. Plants were oven-dried at 60°C, and shoot and root weights were determined. Plant dry weight data were transformed to $\log_{10}$ to minimize the increase in the CV resulting from increasing plant size. Shoot and total dry weights ($\log_{10}$) were analyzed by analyses of variance (ANOVA), orthogonal polynomials, and regression analyses. One-way ANOVA was also performed on data at each T-12 level within a peat : vermiculite treatment. Duncan's multiple range test was used for mean separation. Significance was measured at $P = 0.05$. Only shoot dry weight data are presented, but similar results were obtained with total dry weights.

*T. harzianum* (T-12) population densities were quantified on *Trichoderma*-selective medium (5). Samples of each peat : vermiculite combination were oven-dried at 50°C, and the percentage of moisture was determined. All T-12 population densities were expressed as colony-forming units (cfu) per gram of oven-dried peat : vermiculite mix. To assay for *Pythium* spp., a cultivar of pea (*Pisum sativum* L. 'Laxton Progress'), which is susceptible to low population densities (10 propagules/g soil) of *Pythium* spp., was used (10). Five pea seeds were planted in each pot after the last radish plant was removed. Plant emergence percentages were recorded after one week, and plants were examined after 2 weeks of damping-off symptoms. Pea roots were selected at random from each peat : vermiculite combination and placed on *Pythium*-selective medium (11).



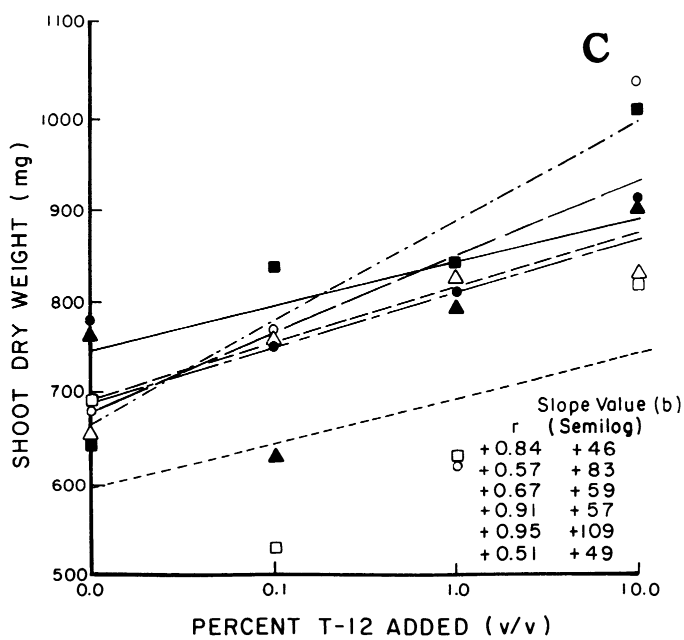




Fig. 2. Effect of peat : vermiculite mixes and *Trichoderma harzianum* on shoot dry weights of radish. n = 8. Second experiment. **A** = 3 weeks, **B** = 4 weeks, **C** = 5 weeks.

## Results

*Effects of peat : vermiculite mixes and T-12 on radish dry weights.* Overall peat : vermiculite (P) × *Trichoderma* (T) treatment interactions were significant in both experiments. P × T × time interactions were observed only in the 2nd experiment. When data at each sampling time were analyzed separately, P × T interactions were significant at 3 and 4 weeks in the first experiment; and at 3 weeks in the 2nd experiment. In general, increasing levels of T-12 caused linear increases in dry weight at 4 and 5 weeks, when T-12 treatments were averaged over peat : vermiculite treatments in both experiments. Exceptions were seen at the 0% and 100% peat combinations in the first experiment. Peat : vermiculite effects showed both quadratic and linear trends when averaged over T-12 treatments.

The following effects of T-12 on plant growth were observed in comparison with appropriate peat : vermiculite combinations not treated with T-12. T-12 exhibited the greatest positive effects (increase in slope) in the 20P and 80P treatments in both experiments at 4 and 5 weeks (Figs. 1 B and C, and 2 B and C). T-12 (10%) significantly increased shoot dry weight (137% and 149%, respectively), after 5 weeks (Fig. 2).

Increases in dry weight induced by T-12 in the 40P and 60P treatments were not as great as in the 20P and 80P treatments. In the first experiment, application of T-12 (10%) significantly increased shoot dry weight in the 40P treatment after 5 weeks (Fig. 2C). Significant increases in total dry weight were observed in both the 40P and 60P treatments. In the 2nd experiment, T-12 did not significantly affect shoot or total dry weight in either the 40P or 60P treatments.

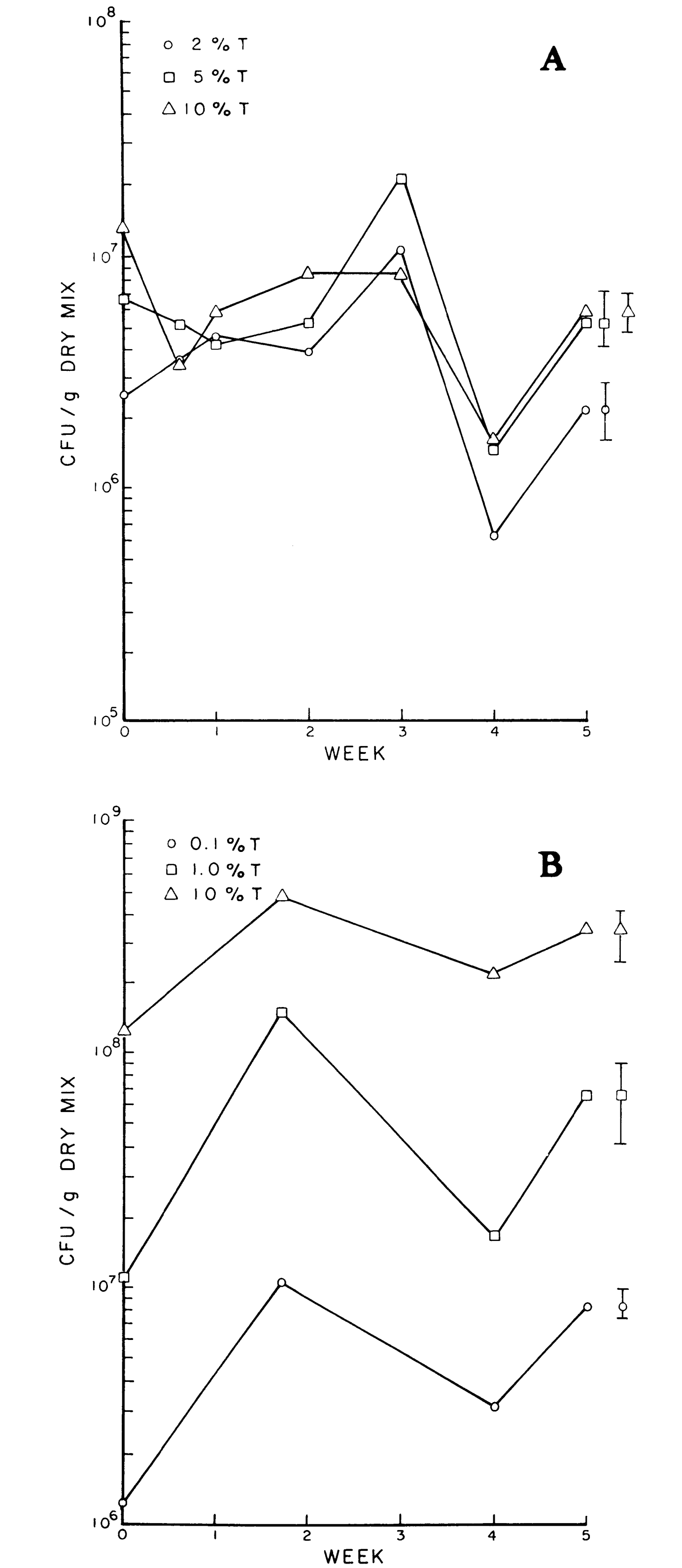



Fig. 3. *Trichoderma harzianum* isolate T-12 population densities over time. Each point represents pooled average population densities of all peat : vermiculite treatments. Bars represent average standard errors of the line (square root of error means squared (ems) divided by n) n = 6. **A** = First experiment, **B** = 2nd experiment.

T-12 caused the smallest increases in dry weight in the 0P and 100P treatments after 4 and 5 weeks. In the first experiment,

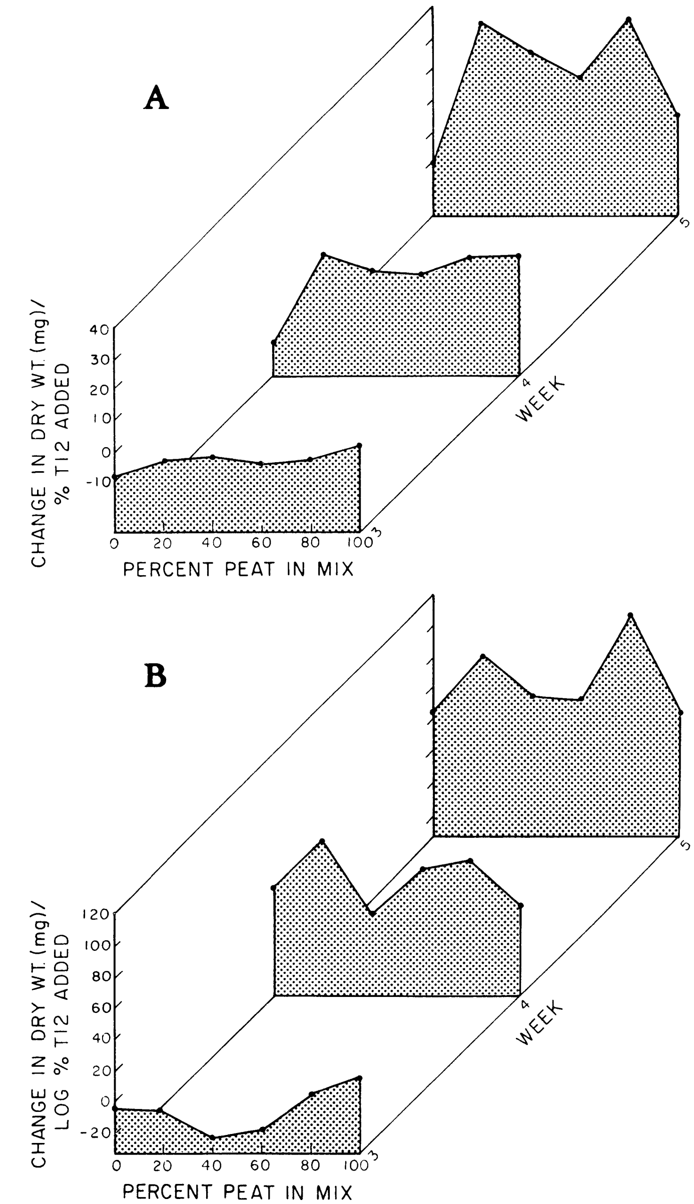



Fig. 4. Effect of *Trichoderma harzianum* isolate T-12 on shoot dry weights of radish grown in various peat : vermiculite mixes. **A** = First experiment, **B** = 2nd experiment.

T-12 reduced shoot dry weight in the 0P treatments at all sampling times. T-12 did not increase shoot dry weight significantly in the 0P or 100P treatments in either experiment.

Under some conditions, however, T-12 decreased radish growth. At 3 weeks, increasing T-12 caused a slight decrease (negative slope) in shoot dry weight (Figs. 1A and 2A). This deleterious effect was not observed in the 100P treatments, where positive slope values were observed.

*Effect of peat : vermiculite mixes on T-12 population densities.* No significant differences in T-12 population densities were observed among peat : vermiculite treatments within each T-12 treatment. Population densities were averaged over all peat treatments and are presented in Fig. 3. In both experiments, the population density of *Trichoderma* spp. in the nonamended mixes ranges from 100 to 20,000 cfu/g. The original population density of T-12 added to the mixes determined the subsequent population densities maintained throughout the experiment. In the first experiment, population densities added to the treatments were within one log unit of each other ($1.4 \times 10^7$, $6.7 \times 10^6$, or $2.4 \times 10^6$ cfu/g). After 5 weeks, average population densities in T-12 treatments were still separated by less than one log unit (Fig 3A). In the 2nd experiment, $1.2 \times 10^8$, $1.2 \times 10^7$, or $1.2 \times 10^6$ cfu/g of T-12 was originally added. After 5 weeks, average population densities of T-12 treatments were still separated by one log unit (Fig. 3B).

*Pythium* spp. were not detected in any treatments, and about 80% of the pea seeds germinated in every treatments. Harvested radish and pea roots showed no root disease symptoms.

## Discussion

*T. harzianum* isolate T-12 increased shoot and total plant dry weight of radishes in most mixes, independent of detectable plant pathogens. The peat : vermiculite ratio of the mix influenced the expression of this growth response, in many cases interacting with the level of T-12 added to the mix. Since peat : vermiculite levels had no effect on T-12 population densities, effects on plant dry weight within each T-12 level cannot be explained by variations in T-12 populations. The different soil environmental conditions (i.e., pH, soil aeration, moisture levels) in each mix might have influenced the production of a secondary metabolite or metabolites by T-12, which caused increased plant growth. This secondary metabolite might be a hormone-like compound that stimulates different plant responses. To date, however, *Trichoderma* spp. have not been reported to produce any plant hormones. *T. harzianum* might also produce enzymes that degrade peat into nutrients available to the plant.

The greatest increases of plant dry weight induced by T-12 were observed in the 20P and 80P treatments (Fig. 4). In the 20P and 80P treatments not receiving T-12, growth of radishes was sub-optimal when compared with the corresponding 40P and 60P treatments. T-12 overcame whatever factors were responsible for sub-optimal growth. T-12 had the least effects in the OP (100% peat) treatments (Fig. 4). Plants without T-12 treatment were stunted and chlorotic. Apparently, T-12 did not overcome these growth-limiting factors. Poor soil aeration might be a growth-limiting factor in the 100P treatments, which were constantly saturated because of the high water-holding capacity of peat. High vermiculite mixes were observed to cause stunting (R. Baker, unpublished data). The 40P and 60P mixes gave the best growth of radishes in the absence of T-12. The rapid and lush growth of radishes in these mixes partially masked the growth-promoting effect of T-12. Time also influenced the expression of *T. harzianum* growth response. The greatest positive effects of T-12 were seen at 4 and 5 weeks. At 3 weeks, T-12 had little or no effect. High levels of T-12 (10%) decreased shoot dry weight after 3 weeks in mixes with high levels of vermiculite. High peat levels protected radishes from this growth depression. It is still unclear whether differences in soil mixes directly affect the receptivity of the plant to the growth-promoting factors produced by *T. harzianum* or whether differences in soil environment directly influence the fungal production of some secondary metabolite. Further research on the nature and identity of this growth-promoting factor is needed.

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