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ENHANCEMENT OF GROWTH OF ORNAMENTALS BY A BIOLOGICAL CONTROL AGENT

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Growth enhancement through the use of biological control agents is reported, showing considerable opportunities for improved profitability in the industry. Estimated costs range from 2.2 to 0.22 cents per unit.

In a recent report to the Secretary of Agriculture (1), Extension Directors from the Northeast, Southern and Western States named profitability in agriculture as the number one priority. North Central Directors rated this research category number two behind farm financial and business management. It is obvious that the chief current concern in agriculture is to increase yield, quality, and profit by use of cost-effective strategies. Of course, this has always been the ultimate goal of floriculture research at Colorado State University. In this article, the latest approach to the attainment of this goal is reviewed. The highlights are derived from four articles currently being published in scientific journals (2-5).

Early Experiments with Bedding Plants

Chang et al. (3) described the stimulation of growth resulting from application of a fungal biocontrol agent to soil in which various ornamentals were planted. For example, petunias growing in soil supplemented with a strain T-95 of *Trichoderma harzianum* grown in a peat-bran culture medium produced significantly more flower buds, side branches, and were higher in fresh and dry weights than non-treated controls grown in conventional bedding plant procedures (Fig. 1). The presence of the fungus was necessary to stimulate growth — peat-bran without T-95, or a medium in which T-95 was grown and then heat-killed, did not induce the response. An experiment confirming this principle was done with radishes (2). The living fungus growing in peat-bran induced up to 270 percent increase in growth over controls (Fig. 2). The fungus applied to soil as spores (conidia) also induced a response of lesser magnitude. Other treatments without, or with killed T-95, actually depressed growth or had little effect.

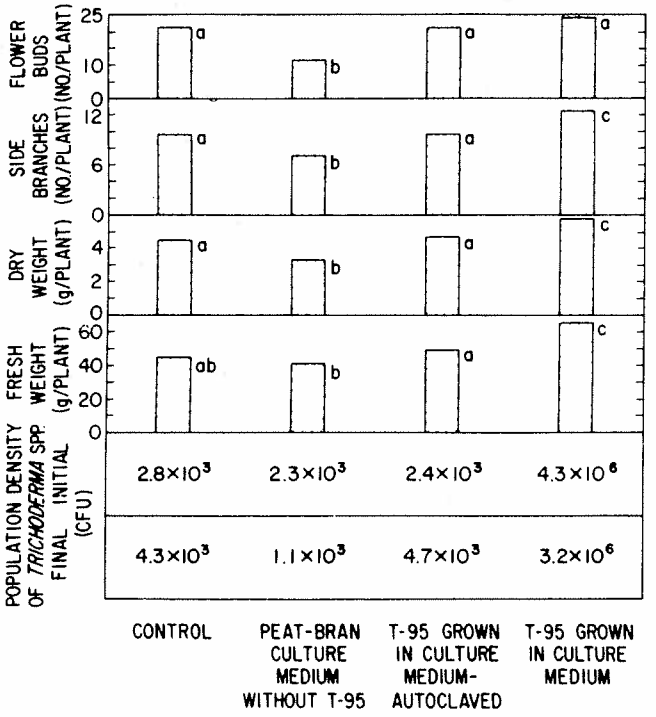

Fig. 1. Growth characteristics of petunias grown 42 days in a greenhouse potting soil when *Trichoderma harzianum* (T-95), grown for 2 weeks in a peat-bran medium, was added to soil (20% v/v). In other treatments, this medium (infested by the fungus) was autoclaved before being added, or the medium with *T. harzianum* was added to soil. Values followed by the same letter did not differ significantly (P = 0.05).

[1]*Professor, and Visiting Scientists, Department of Plant Pathology and Weed Science*

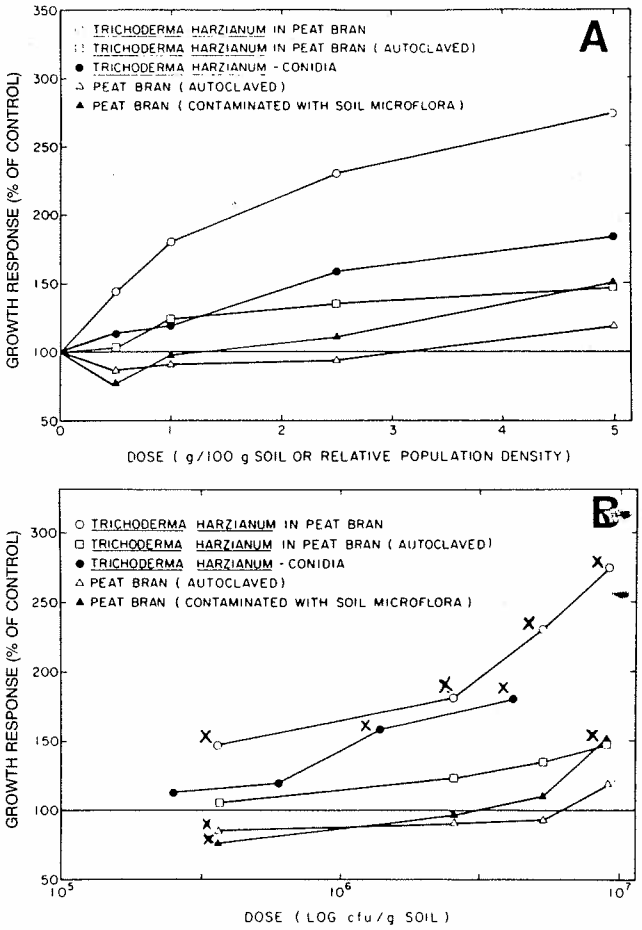

Fig. 2. The effects of *Trichoderma harzianum,* previously grown in peat-bran medium applied at various dosages and various manipulations of this treatment, on the oven dry weight of leaves of radish after 6 weeks of incubation. Each treatment at each dosage contained five replications and there were 10 plants per pot. **A.** Dosages were plotted according to weight of peat-bran, or as relative amounts of conidia added on the abscissa as appropriate. **B.** The same data plotted by matching colony-forming unit dosages on the abscissa. Regression analysis, should no significant differences ($P = 0.05$) in slope among various manipulations. When means of each manipulation were compared, only *T. harzianum* grown in peat bran was significantly different. An "X" identifies points that are significantly different ($P = 0.05$) from the control (the horizontal line at 100%) at each level of application.

In such experiments, the peat-bran culture was mixed into soil up to 20 percent by volume. If plant units require relatively large volumes of soil for growth as, for example, in potted chrysanthemums, such applications might not be cost-effective. Therefore, attempts were made to apply the treatment in propagative media (3). When rooted, cuttings should carry enough of the fungus on their roots to insure increased growth of the transplants. Increased growth from application of T-95 in peat-bran culture to chrysanthemums (later transplanted to pots) was realized with this strategy (Fig. 3, 4). A conidium suspension sprayed on the roots of propagated cuttings was insufficient to induce the response.

No increased growth was noticed when a conidium suspension of strain T-95 was sprayed on cuttings with rooting hormone (3, Fig. 5). Again, however, increases in flower buds, height and weight of plants treated with the peat-bran culture medium in propagative medium in comparison with controls was observed.

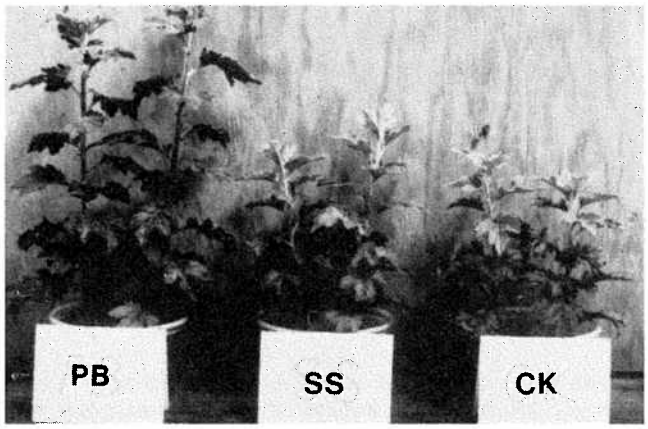

Fig. 3. Growth of chrysanthemums grown 57 days in greenhouse potting soil when *Trichoderma* T-95 was either sprayed (SS) as a conidial suspension (10^8/ml) on roots of cuttings, or grown in peat-bran (PB) culture for 2 weeks and mixed into propagative medium (20% v/v).

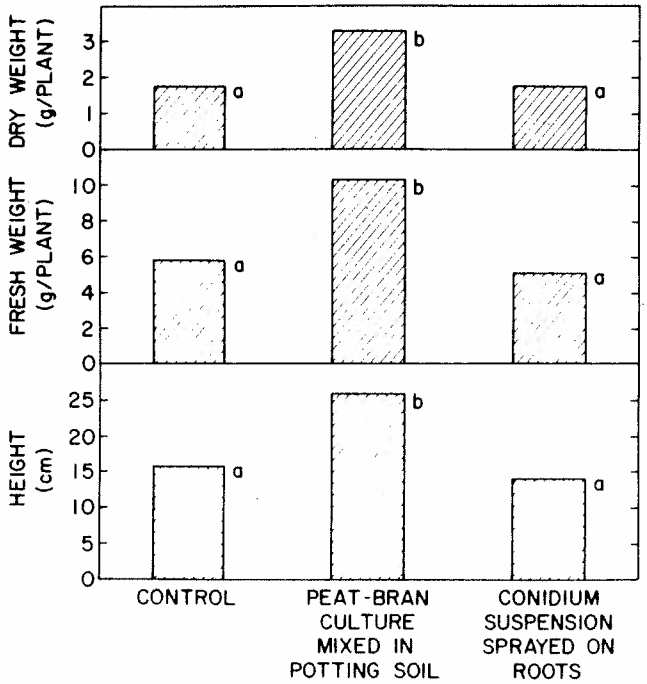

Fig. 4. Growth characteristics of chrysanthemums grown 57 days in greenhouse potting soil when *Trichoderma harzianum,* T-95, was either sprayed as a conidial suspension (10^8/ml) on roots of cuttings or grown in peat-bran culture for 2 weeks and mixed into the propagative medium (20% v/v). Values followed by the same letter do not differ significantly ($P = 0.05$).

Under environmental conditions in Colorado, it is difficult to induce flowering in periwinkle grown from seeds planted in Jan. or Feb. for marketing by May 1. To test whether growth enhancement induced by T-95 could hasten flower development, the peat-bran culture was mixed into steamed container-medium used for seed germination of two cultivars at 10 percent v/v of medium in the Front Range Greenhouses on Jan. 15, 1983. On Feb. 14, 1983, these and other nontreated seedlings were transplanted into soil containing 0, 10, or 20 percent v/v of the peat-bran culture. The 10 percent mix contained approximately 10^6 cfu/g[2] of T-95.

By Apr. 7, 1983, buds were detected only on transplants treated with 10 or 20 percent of the peat-bran culture. Measurements eight days later indicated significant increases in height of plants resulting from these two treatments (Table 1). By May 1, both cultivars in these treatments were in full bloom, whereas controls and those plants treated only in the seed flats with T-95 did not flower completely until another three to four weeks had elapsed.

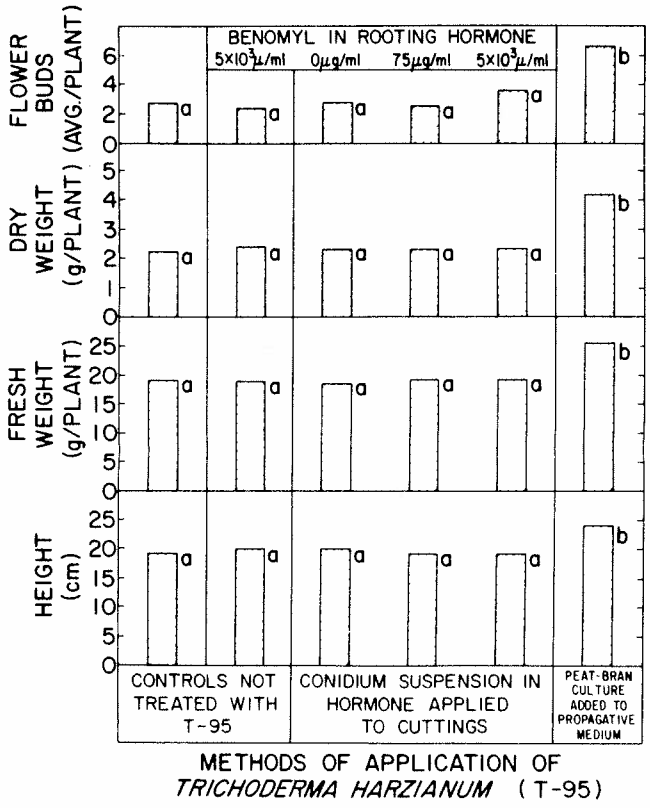

Fig. 5. Growth characteristics of chrysanthemums grown 42 days after transplanting into greenhouse potting soil when *Trichoderma harzianum*, T-95, was applied at propagation as a conidial suspension in rooting hormone (10^8/ml), with or without benomyl, or grown in peat-bran culture for 2 weeks and 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.

[2]*cfu/g = colony forming units per gram*

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.*

In all these experiments, recommended nutrient regimes were applied at each watering. Therefore, the response was not due to nutritional relationships.

The mutant of *T. harzianum*, T-95, has a number of advantages for use in the ornamental industry in contrast to wild-types. If *Trichoderma* spp. are used to promote plant growth and applied as biocontrol agents, they must be compatible with pesticides used in the industry. The unaltered wild-types are tolerant of fungicides commonly used in the ornamental industry such as etheridiazole and pentachloronitrol-benzine. However, they are intolerant of benzimidazoles. T-95 was a mutant selected for tolerance to benomyl. It is also as efficient, or is a better biocontrol agent, against *Rhizoctonia solani*. In a future article, research will be reviewed demonstrating that T-95 can also colonize roots unlike its wild-type parent.

These early experiments, therefore, established that strain T-95 can induce increased growth of ornamental plants and is compatible with fungicides commonly used in the trade. If applied in propagative operations where cuttings are rooted in high density, T-95 can be applied in peat-bran formulation at costs of a fraction of a cent per cutting. The resulting increase in growth pays for the cost of the product many times over.

The Mechanisms Involved in the Increased Growth Response

Candidates for the reason(s) why *Trichoderma* spp. induce increased growth of plants are numerous. The most likely mechanisms, however, are two hypotheses: (1) that such biocontrol agents reduce activity of so-called "minor pathogens" on roots that "nibble" (but do not actually kill), thereby inducing stunting of apparently healthy plants, and/or (2) *Trichoderma* spp. produce growth-regulating factors (chemicals) that stimulate plant growth.

An experimental strategy for testing either of these hypotheses involves the gnotobiotic (= known life) technique. By this method, plants may be grown germ-free and compared with contaminated plants which may be stunted by minor pathogens. If such contaminated plants respond with

increased growth when the *Trichoderma* spp. is placed in the system, and this growth is the same as germ-free plants, this provides evidence for biocontrol of the minor pathogens. On the other hand, if *Trichoderma* spp. are applied to germ-free plants and there is increased growth over germ-free controls, a growth-regulating factor may be implicated.

The extent of influence of *Trichoderma* spp. on minor pathogens has not been tested directly by the gnotobiotic technique although evidence from other experiments suggests that inhibition of such growth-inhibiting microorganisms is accomplished by these biocontrol agents. There is strong evidence for the production of a growth-regulating factor, however. Radishes growing in sterile soil infested with either of two strains of *Trichoderma* spp. had significantly higher dry weights than the control without the fungus (Fig. 6). Further, when cellophane-diffusible metabolites of *Trichoderma* spp. were applied to seed of corn, tomato or tobacco, germination occurred one to two days earlier than in controls (Fig. 7).

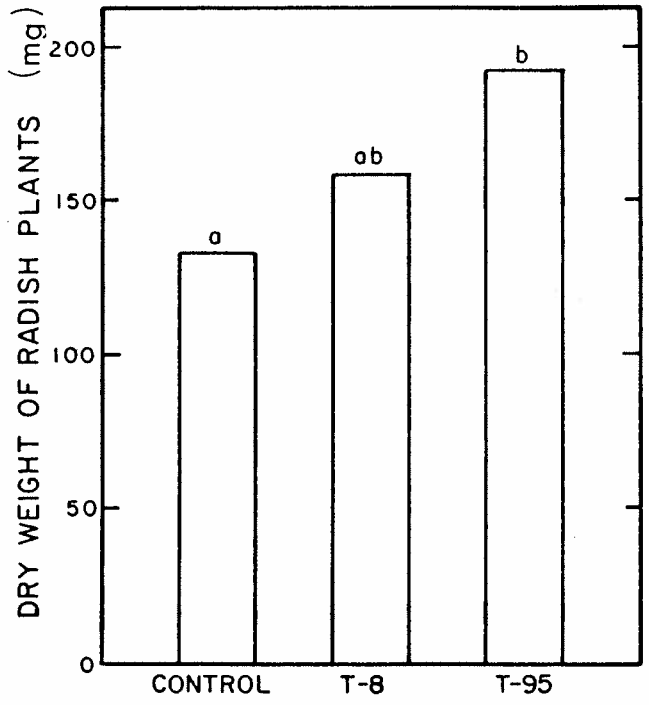

Fig. 6. Effect of *Trichoderma harzianum,* T-95, and *T. koningii,* T-8, on radish dry weight when grown under gnotobiotic conditions. Columns with the same letter do not differ significantly ($\underline{P} = 0.05$). There were six replications.

Some of the implications of production of a growth-stimulating chemical by *Trichoderma* spp. are apparent in propagation. Increases in proportions and rate of seedling emergence of tomato and tobacco were observed (Fig. 8A, B). Strain T-95 applied with the rooting hormone or as a peat-bran culture induced significantly better rooting of carnation cuttings as compared with controls treated only with rooting hormones. Benomyl is often incorporated into rooting hormones to control stem rots. Therefore, the benomyl-tolerant strain T-95 was effective even though this fungicide was present (Fig. 9).

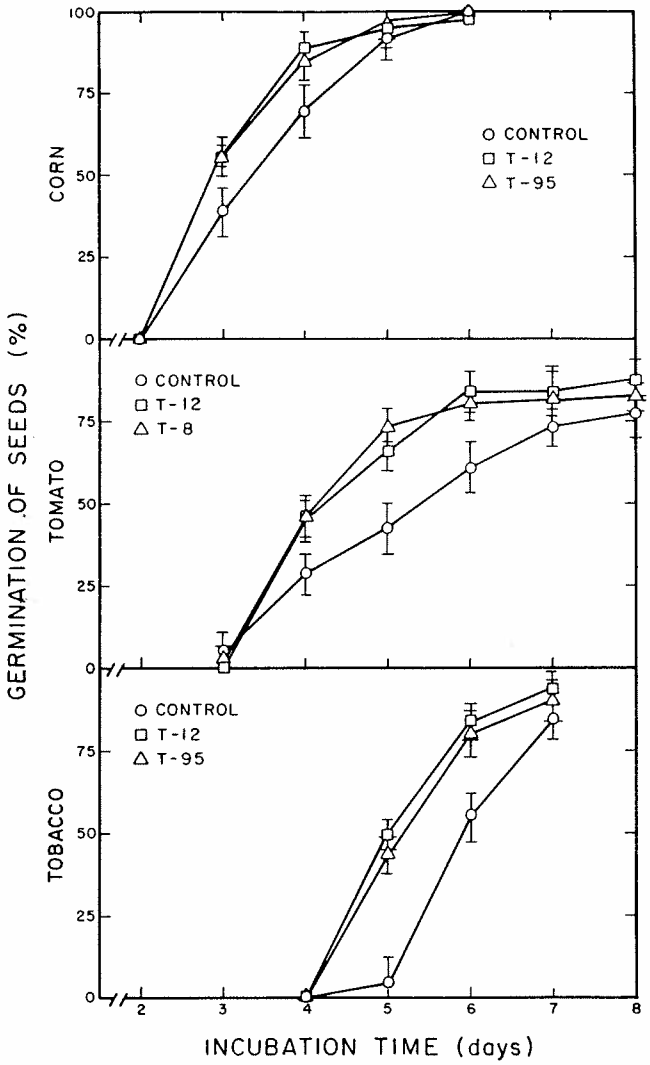

Fig. 7. Effect of cellophane-diffusible metabolites of *Trichoderma harzianum* (T-95) and *T. koningii* (T-8) on seed germination of corn, tomato, and tobacco. Means of six replicates; bars represent one standard deviation from the mean.

Efforts are presently underway to purify and chemically identify the growth-regulating factor. The potential practical applications of the phenomenon are exciting. Here, we have a fungus that not only can perform as a biocontrol agent but can also manufacture and release continuously a growth-stimulating chemical adjacent to germinating seeds and roots of plants.

Factors Influencing Enhanced Plant Growth

Under certain cultural conditions, enhancement of growth of many plant species by *Trichoderma* spp. has been observed in either experimental or commercial facilities. Stimulation of plant growth, however, has not been seen in certain cases. The reasons for this inconsistency are not always apparent but progress towards solving the problem has been achieved and is reported in an article by Windham, et al. (5).

Trichoderma spp. are most active in acid soil. Indeed, amendments of strain T-95 increased tomato growth over

controls at pH 5.5 and 6.2 but not at 7.8 (Table 2); however, the same treatment increased root and shoot weights of radish at soil pH of 6.2 and 7.8. For tomato, there was some evidence that phytotoxicity induced by PCNB was nullified by amendments of strain T-95.

Various species and isolates varied in their ability to induce increases in emergence (Fig. 10) and dry weights (Table 3) of tobacco.

The concentration of the applied agent also may influence plant growth responses. Population densities of 10^5 to 10^6 colony forming units per gram (cfu/g) of soil appear to be optimum for enhanced growth. Little effect was observed when population densities below these values were applied. Stunting occurred when 10^7 to 10^8 cfu/g were added to soil.

Table 2. The effect of growth of tobacco by infestation of soil with conidial suspensions (2.5×10^5 conidia per/g soil) of various isolates of *Trichoderma* spp.

	Dry weights (mg/plant)[a]	
Isolate	Shoot	Whole plant
T. harzianum		
T-12	83^{xy}	561^{wxy}
T-95	145^{w}	758^{wx}
FC	95^{x}	684^{wx}
WT-6	77^{yz}	486^{yz}
WT6-15	121^{wx}	507^{xy}
T. viride		
T-1	131^{w}	656^{wx}
TI-RI	147^{wx}	597^{wxy}
T. koningii (T-8)	107^{wx}	379^{yz}
Trichoderma sp. (T-7)	140^{w}	898^{w}
Control	50^{z}	301^{z}

[a]*Three plants in each of six replications were grown in each treatment. After 6 wk two were harvested and shoot weights determined.*

Table 3. Effects of soil pH on dry weights of root and shoots of tomato and radish treated with *Trichoderma harzianum* (T-95)[a] and pentachloronitrobenzene (PCNB)[b].

	Dry weights (mg)					
	pH 5.5		pH 6.2		pH 7.8	
Treatments	root	shoot	root	shoot	root	shoot
Tomato						
T-95	220^{y}	270^{y}	40^{x}	200^{x}	100^{x}	140^{yx}
T-95 + PCNB	—	—	40^{xy}	130^{y}	60^{xy}	60^{xy}
PCNB	—	—	20^{z}	60^{z}	10^{y}	50^{y}
Control	140^{z}	200^{z}	30^{xy}	100^{y}	50^{xy}	100^{xy}
Radish						
T-95	—	—	170^{x}	260^{x}	230^{x}	200^{x}
T-95 + PCNB	—	—	80^{y}	220^{x}	160^{xy}	180^{x}
PCNB	—	—	80^{y}	130^{y}	60^{y}	120^{y}
Control	—	—	70^{y}	160^{y}	40^{y}	120^{y}

[a]*T-95 was grown in peat-bran culture (12) for 14 days. Added population density in soil was 10^5 cfu/g soil.*
[b]*PCNB was mixed into soil at 5μg a.i./g.*
[c]*For each plant sp., means of each column followed by the same letter did not differ significantly ($P = 0.05$).*

The mix used to support plant growth also profoundly influences the growth response. A future article will treat this subject in detail.

Prospects for Commercialization of the Product

There are problems connected with formulation, consistency of responses, marketing and commercialization of a product containing a growth-enhancing *Trichoderma* spp. Even so, the potential for considerable increase in profits for growers through the use of such agents is intriguing.

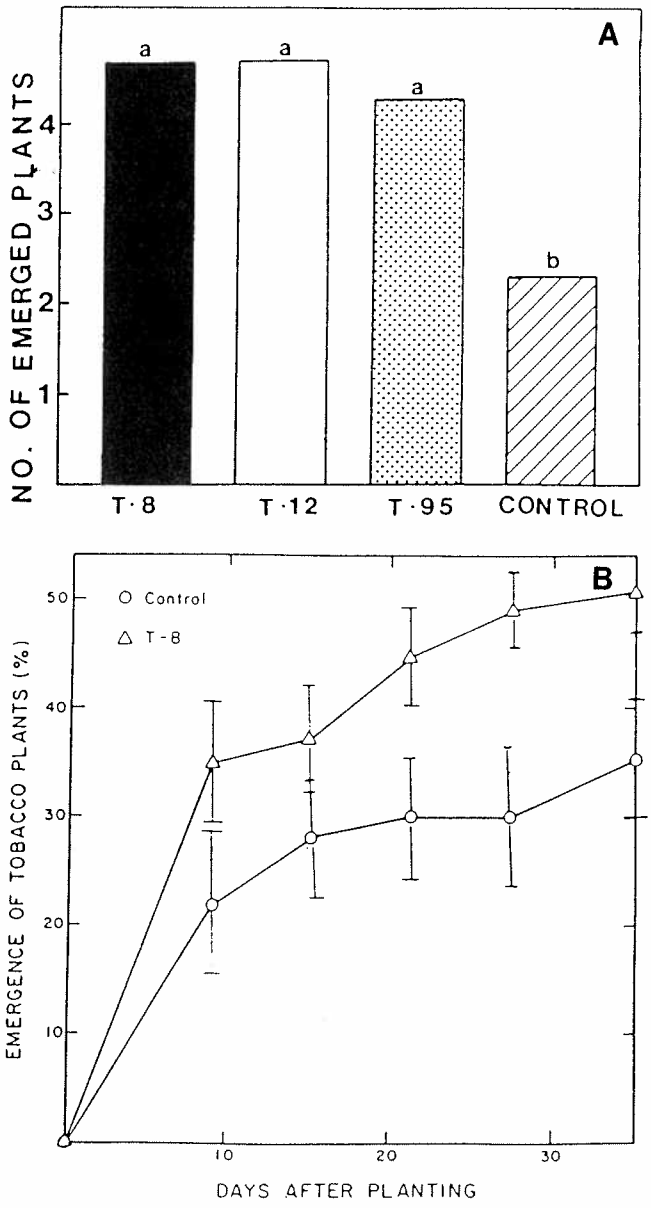

Fig. 8. **A.** Effect of three isolates of *Trichoderma* spp. (*T. harzianum* T-95 and T-12, and *T. koningii* T-8) on seedling emergence of tomato 10 days after planting. *Trichoderma* spp. population density at planting was 10^5 cfu/g autoclaved soil. Columns with lower case "a" are different from the control ($P = 0.05$). **B.** Effect of *T. koningii*, T-8, on emergence of tobacco seedlings. At planting, T-8 population density was 10^5 cfu/g soil. Means of six replicates.

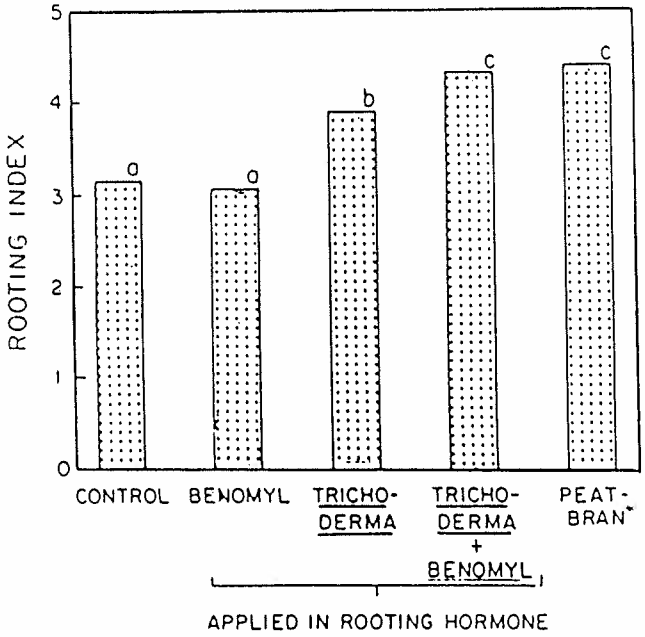

Fig. 9. The effect of rooting of carnations of conidial suspensions (10^8 conidia per/ml) and/or benomyl (25,000 μg/ml) applied in the liquid hormone, or a peat-bran culture of *Trichoderma harzianum*, T-95, mixed into rooting medium (10^6 cfu/cc. Rooting index: 0 = no roots, 5 = abundant roots, 5 cm length after 15 days in the rooting medium. All cuttings were treated with rooting hormone as in conventional propagation. Columns with the same letter do not differ significantly ($P = 0.05$).

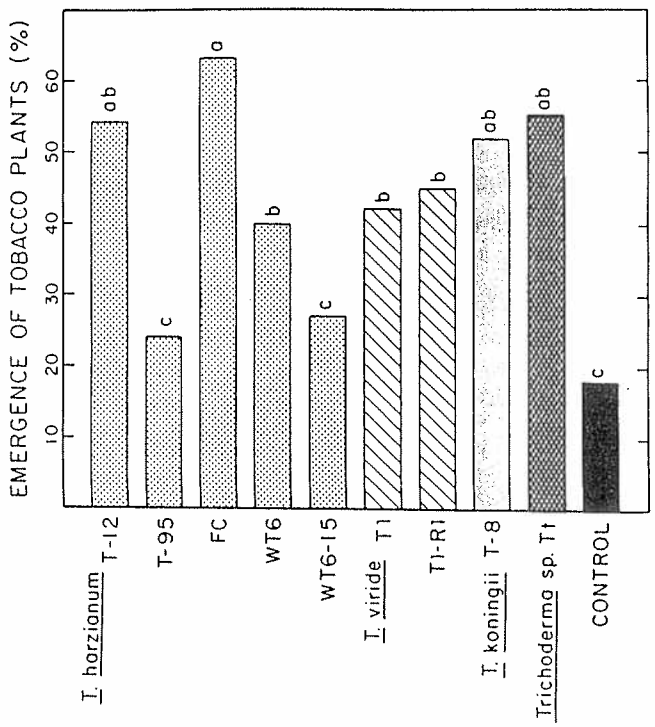

Fig. 10. Variation in effects of conidial suspensions (2.5×10^5 conidia per g soil) of nine *Trichoderma* isolates to increase emergence of tobacco plants. Bars followed by the same letter do not differ significantly ($P = 0.05$).

A major problem remaining for commercialization is formulation. Performance is optimum if the agent is applied to soil associated with a substrate like the peat-bran culture medium at 10^5 to 10^6 cfu/g soil. Production of biologicals is accomplished conventionally by fermentation; however this process has not produced the quantity and quality of product required for responses. Thus, new formulation procedures require development.

Economic parameters were analyzed for successful commercialization. The product should be retailed for $1.00/lb. To develop a reasonable profit, the cost of manufacture should be $0.40. Methodology has been developed within these cost restraints.

What would be the cost to the grower? The peat-bran culture can yield in excess of 10^8 cfu/g substrate. Therefore, a grower could apply the product at a concentration of one percent and induce growth responses. Containers for growing plants vary in capacity from approximately 100 to 1000 g/pot. If the product was applied at one percent concentration, the cost per plant unit ($1.00/lb product) would be $0.0022 to 0.022 per plant unit. A much lower cost per unit would be realized in propagation of cuttings. The product can be applied at the time the planting medium is mixed so no significant additional labor cost would be involved.

Several growers applied the product in the 1985 bedding plant season. Growth enhancement and earlier flowering were observed in petunia, vinca, alyssum, pepper and marigold (Fig. 11).

The potential advantages of such growth enhancement go beyond the desirability of attaining enhanced growth and quality of floricultural crops. For example, flowering may occur two weeks earlier in *Trichoderma*-treated plants. Therefore, planting can be done later resulting in savings in heating costs. Such potential for increased efficiency, quality of crops, and profitability has prompted such research activities at Colorado State University.

The authors wish to thank Spano, Echter, Front Range, Welby and Carr Street Greenhouses for their cooperation in the commercial testing of the Trichoderma*-induced growth response.*

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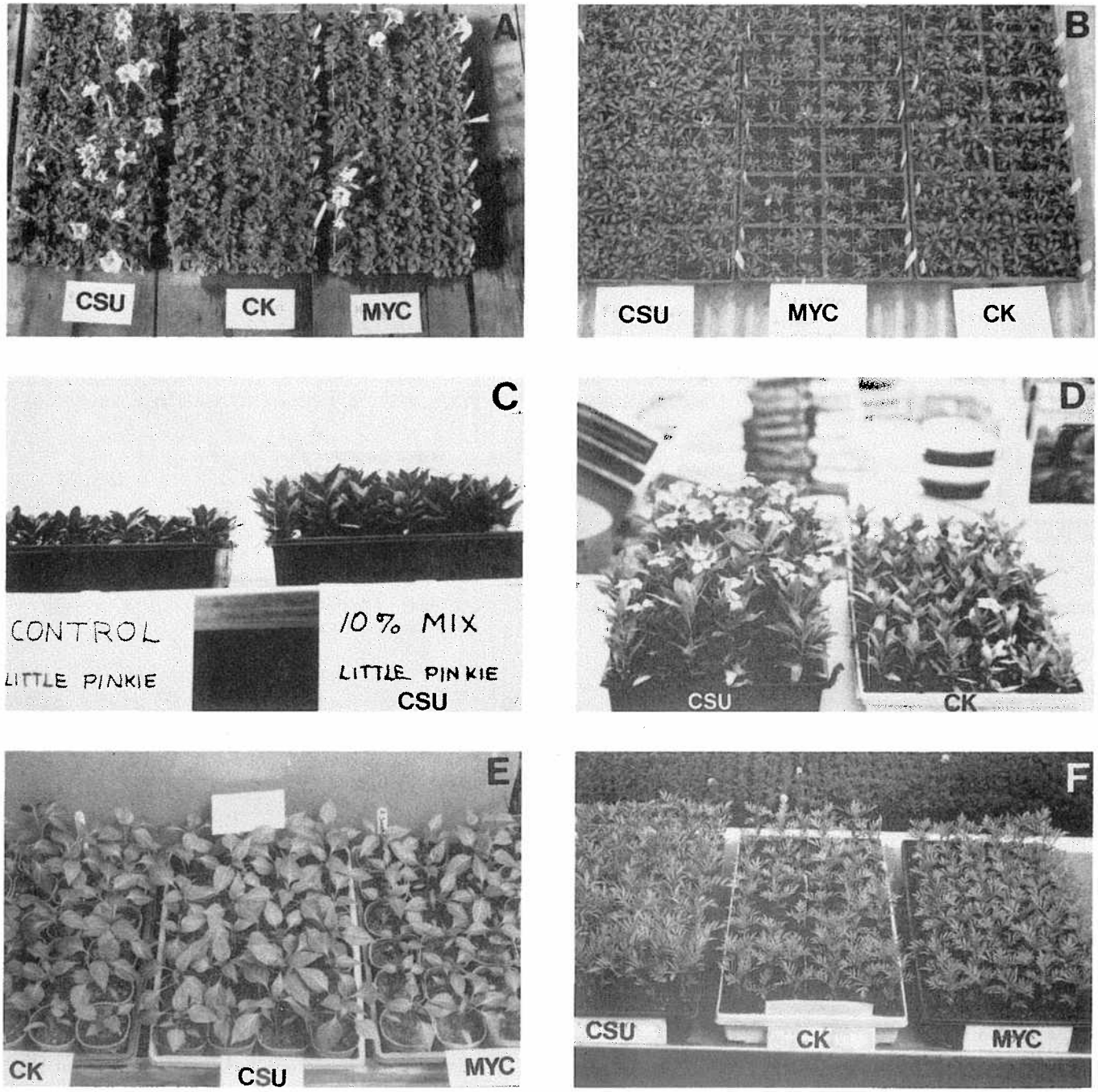

Fig. 11. Growth responses of bedding plants in commercial greenhouse operations treated with peat-bran cultures (CSU, culture formulated at CSU; MYC, a commercial formulation) of *Trichoderma harzianum*. **A.** Petunias and **B.** alyssum grown at Spano Greenhouses. **C.** Periwinkle 60 days old and **D.** 75 days old at Front Range Greenhouses. **E.** Peppers grown at the Carr Street Greenhouses. **F.** Marigolds grown at Front Range Greenhouses.

CORRECTION TO GREENHOUSE CLIMATE SUMMARIES

CGGA Bulletins 427 (January) through 429 (March, 1986).

Gas consumptions reported as an average of double and single covers in the above Bulletins are in error by a factor of ten. The corrected values are given as cubic feet per square foot floor area, natural gas.

Week beginning	Day regime	Night regime
Nov. 3, 1985	25	67
Nov. 10	58	108
Nov. 17	51	121
Nov. 24	51	118
Dec. 1, 1985	59	118
Dec. 8	59	131
Dec. 15	30	97
Dec. 22	30	83
Dec. 29, 1985	28	90
Jan. 5, 1986	21	87
Jan. 12	16	76
Jan. 19	24	84

FORT COLLINS GREENHOUSE CLIMATOLOGICAL SUMMARY FOR FOUR WEEKS, BEGINNING MARCH 2, 1986. (See Bulletin 426 for details.)

	Week beginning							
	Mar. 2		Mar. 9		Mar. 16		Mar. 23	
	Day	Night	Day	Night	Day	Night	Day	Night
Average outside temperature (°F)	55	43	46	41	46	36	60	45
Maximum outside temperature (°F)	74	62	60	66	69	56	80	65
Minimum outside temperature (°F)	34	27	32	26	29	24	40	31
Degree-days of heating	35	77	67	84	67	102	18	70
Average hours in the period	11	14	10	14	12	13	11	13
Accumulated total solar radiation (MJ/sq.m.)	106	0.6	80	1	89	0.8	112	1
Average relative humidity (%)	35	53	50	65	49	72	34	54
Maximum relative humidity (%)	88	93	100	100	87	100	78	89
Minimum relative humidity (%)	7	10	23	22	14	24	9	18
Average absolute vapor pressure (mb)	5	5	5	6	5	5	6	5
Average wind speed (mph)	3	3	4	3	5	2	3	.9
Maximum wind speed (mph)	20	41	32	30	28	31	22	15
Average CO_2 concentration (Pascal)	23	—	23	—	23	—	21	—
Maximum CO_2 concentration (Pascal)	38	—	46	—	46	—	32	—
Accumulated gas consumption (cu.ft./sq.ft.)	7	40	20	60	31	61	8	41

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