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I am submitting herewith a thesis written by Amy J. MacKenzie entitled "*Trichoderma harzianum* as a Growth Enhancer of Pot and Cut Floriculture Crops". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Ornamental Horticulture and Landscape Design.

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Trichoderma harzianum as a Growth Enhancer of Pot and Cut Floriculture Crops

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Degree

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Amy J. MacKenzie

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DEDICATION

This thesis is dedicated to my parents

Mr. Harold H. Jones

Mrs. Bonnie M. Jones

who have given me the encouragement
and financial support to meet my educational goals

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ABSTRACT

Trichoderma species have been used extensively for biocontrol of soilborne pathogens. These fungi may also have a growth-enhancing effect independent of mechanisms associated with biocontrol. Although several plants are responsive to growth enhancement by *Trichoderma* spp., cut and potted flower production applications remain largely uninvestigated. Optimization of rate and delivery method is necessary to facilitate commercial use of this fungus. Integrating *Trichoderma* into current greenhouse production schedules could serve to decrease use of fungicides, growth regulators, and labor in the greenhouse, hence lowering costs and environmental impact.

Specific goals of my research were: (i) to optimize rate of media amendment with *Trichoderma harzianum*-isolate T-12 (T-12) as a powdered peat-bran formulation; (ii) to compare amending the rooting medium with T-12 to treating unrooted cuttings with IBA to promote rooting; (iii) to compare the powdered peat-bran formulation to an alginate pellet preparation of the fungus as methods of delivery; (iv) and to investigate T-12 as a growth enhancer of several cut and pot floriculture crops.

Zero, 5, or 25 g of T-12 peat-bran amendment was added per kilogram medium in an effort to enhance the rooting of four chrysanthemum [*Dendranthema X grandiflorum* (Ramat.)

Kitamura] cultivars, two considered easy-to-root ('Davis' and 'White Marble') and two considered hard-to-root ('Dark Bronze Charm' and 'Golden Bounty'). The experiment was repeated with the same rates and cultivars with and without dipping cuttings into a 0 or 7.4mM solution of IBA. We also investigated an alternative method of delivering the fungus to the medium, adding T-12 either as a powdered peat-bran amendment at the rates of 0, 5, or 50g·kg^{-1} or as alginate pellets at 0, 80, or 800g·kg^{-1} produced by reacting a mixture of aqueous sodium alginate containing T-12 powder with Ca^{++}. Finally, we amended the growing medium of several cut flower species and potted poinsettias with T-12 as a powdered peat-bran amendment to investigate enhanced growth.

Addition of the powdered T-12 amendment at 5 and 25 g·kg^{-1} increased root and shoot fresh weights during 21 days of rooting. Midway between transplant to flowering, increases in height, shoot dry weight, and root fresh and dry weight were detected in 'Dark Bronze Charm' with T-12 relative to the control; increases in height, shoot fresh and dry weight, and number of nodes were detected in 'Golden Bounty' with T-12. Amending medium with 5g·kg^{-1} was equivalent to treating 'White Marble' cuttings with 7.4mM IBA with regard to root length, fresh and dry weight, and 'Davis' for root length. Both pellet treatments gave increased shoot height on day 14 and 21, with 800g·kg^{-1} being more effective, over all other treatments, which were similar to controls. Amendment with 50g T-12

powder·kg^{-1} increased root length, 80g T-12 pellets·kg^{-1} medium resulted in decreased root dry weight compared to other treatments, and 800g T-12 pellets·kg^{-1} medium increased shoot dry weight. In the cut flower study, we saw increases in height due to T-12 treatment in zinnia (*Zinnia elegans)* and dill (*Anethum graveolens*) and increased flower number in zinnia. Poinsettias treated with T-12 had greater heights or widths than controls periodically throughout the study, although these differences may not be of commercial interest.

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CHAPTER 1

LITERATURE REVIEW AND INTRODUCTION

Literature Review

Soils naturally suppressive to plant pathogenic fungi have been studied since 1892, when Atkinson reported soils suppressive to *Fusarium* species. Liu and Baker (1980) showed that soil suppressiveness in a Colorado clay loam was correlated with a significant increase in propagule density of *Trichoderma harzianum* Rifai and that inoculum density of *Rhizoctonia solani* Kühn was inversely proportional to the density of *Trichoderma*. A modified Koch's postulate demonstrated that *T. harzianum* was the causal agent responsible for suppressiveness.

Biocontrol mechanisms. Biological control of several plant pathogens by *Trichoderma* spp. has been well-documented. In 1932 Weindling reported that *Trichoderma lignorum* (Tode) Harz parasitized a number of soilborne fungi and suggested that certain pathogenic fungi might be controlled by amendment of soil with *T. lignorum*. Since this time, much research has attempted to describe biocontrol mechanisms of *Trichoderma* spp., which can be categorized as parasitism, antibiosis, or

competition.

Weindling reported parasitism of *Sclerotium rolfsii* Sacc., *R. solani, Phytophthora, Pythium*, and *Rhizopus* spp. by *T. lignorum* (1934). A list of other plant pathogenic fungi parasitized by *Trichoderma* includes the genera *Sclerotinia, Helminthosporium, Fusarium, Armillaria, Colletotrichum, Verticillium, Venturia, Endothia, Diaporthe*, and *Fusicladium* (Wells, 1988).

Specific examples of control by parasitism include *T. harzianum* reducing peanut leafspot caused by *S. rolfsii* (Backman and Rodriguez-Kabana, 1975). In a glasshouse study, wheat bran cultures of the fungus added as a soil amendment effectively reduced damping-off caused by *R. solani* in beans, tomatoes,and eggplants (Hadar, et al., 1979). Elad et al. (1980) were able to duplicate control of *R. solani* as well as *S. rolfsii* in three field experiments with beans, cotton, and tomatoes. Mycoparasitism was thought to be the mechanism responsible for successfully reducing disease incidence in both carnation and strawberry caused by the above two pathogens when *Trichoderma* spp. were applied to infested soils (Elad et al., 1981a, Elad et al., 1981b).

There is much evidence for *Trichoderma* acting as a parasite of soilborne plant pathogens. Elad et al. (1983) used fluorescent microscopy to demonstrate increased enzymatic activity where *T. harzianum* attached to *S. rolfsii* or *R. solani* with mycelial clamps or by coiling around the pathogen.

They also produced scanning electron microscopy photos showing hyphae of the hosts having penetration sites produced by enzymatic action of *Trichoderma*. However, Dennis and Webster (1971a) showed that the mycelium of the plant pathogen sometimes began to lose its cellular contents before coming into contact with a number of *Trichoderma* isolates.

Cell wall degradation by *Trichoderma* spp. of both *R. solani* and *S. rolfsii* is attributed to the release of lytic enzymes such as ß-1,3-glucanase and chitinase by the biocontrol agent, which is then able to utilize the pathogens as a food source (Elad et al., 1982). Rodriguez-Kabana et al. (1978) demonstrated that such proteolytic enzymes are most active at or near a neutral pH, but Dennis and Webster (1971b), found no difference in parasitism between pH 4.0 to 6.5. Addition of cycloheximide, a metabolic poison interrupting protein synthesis, prevented invasion of *R. solani* and *S. rolfsii* colonies on a solid growth medium by *T. harzianum* as well as a reduction in enzymatic activity.

Several compounds having antibiotic activity are produced by *Trichoderma* spp. These antagonistic chemicals released into the soil environment include Trichodermin, a sesquiterpene (Godtfredsen and Vangedal, 1965), Dermadine, an unsaturated monobasic acid (Pyke and Dietz, 1966), Suzukacillin®, and Alamethicine®, peptides (Meyer and Reusser, 1967; Ooka et al.,1966; Reusser, 1967), which have both antifungal and antibacterial properties. Dennis and Webster (1971a) reported

acetaldehyde to be the major volatile antibiotic produced by *Trichoderma*. Lifshitz et al. (1986) reported production of an uncharacterized antibiotic known as "the routing factor" by *T. harzianum* and *T. koningii* Oudem that resulted in plasmolysis of hyphal tip cells of *Pythium* sp. This factor is thought to be responsible for controlling Pythium damping-off of pea, radish, and beet by seed treatment with *Trichoderma* spp. (Harman et al., 1980; Liu and Vaughan, 1965; Wright, 1956).

Competition for space and nutrients is thought to be the primary mechanism of *Trichoderma* spp. in controlling several diseases caused by wood rotting organisms, including *Polyporus* spp. (Hulme and Shields, 1970), *Poria monticola* Muss. (Toole, 1971), *Coriolus versicolor* (L. ex. Fries) Quel (Pottel et al., 1977), *Fomes connatus* (Weinn.) Gill. (Smith et al., 1981), and *Armillaria mellea* (Vahl) Quel. (Bliss, 1951). In addition, Marois and Locke (1985) showed that the proliferation of *F. oxysporum* f. sp. *chrysanthemi*, the pathogen causing chrysanthemum wilt, was reduced by competition following application of *T. viride* (isolate T-1-R9).

The biocontrol potential of *Trichoderma* through competition has been shown to be affected by pH (Weindling, 1934). This is probably due to iron, which is necessary for growth and development of the fungus, being less available at higher pH. In support of this hypothesis, Hubbard et al. (1983) found that fluorescent-type pseudomonads tied up available iron through production of siderophores in soils

with low iron, reducing th ability of *Trichoderma hamatum* (Bon.) Bain to act as a biocontrol agent.

Growth enhancement. Enhanced growth of plants in the presence of *Trichoderma* spp. must be considered both as a result and mechanism of biocontrol. Baker et al. (1984) first reported a 150 to 250% increase in dry weight of radish (*Raphanus sativus* L.) with the addition of *T. harzianum* into the soil either in the form of conidia or a peat-bran culture preparation. Other effects of *Trichoderma* spp. include hastened flowering of periwinkle [*Catharanthus roseus* (L.) G. Don] (Chang et al., 1986; Baker et al., 1985), allysum [Lobularia maritima (L.) Desv.], marigold (*Tagetes erecta* L.), peppers (*Capsicum frutescens* L. var. *grossum* Bailey 'Manor'), and petunia (*Petunia Xhybrida* Hort. Vilm.-Andr.) (Windham et al., 1986)., increased number of flower buds in chrysanthemum [*Dendranthema* x *grandiflorum* (Ramat) Kitamura] and petunia (Chang et al., 1986), hastened seedling emergence of tobacco (*Nicotiana tabacum* L.) (Cole and Zvenyika, 1986; Windham et al., 1986), increased dry weight of tomato, (*Lycopersicum esculentum* Mill. 'Alma') pepper, cucumber (*Cucumis sativas* L. 'Alma'), and chrysanthemum (Chang et al., 1986; Windham et al., 1986), faster rooting of carnation (*Dianthus coryophyllus* L.) (Baker, 1988) and chrysanthemum (Paulitz et al., 1985; MacKenzie et al., 1995), increased height of chrysanthemum (Chang et al., 1986; MacKenzie et al., 1995), and increased number of chrysanthemum nodes (MacKenzie, 1995).

Enhanced growth could have resulted from biological control of minor root pathogens by either parasitism or antibiotic production (Elad et al., 1982; Lifshitz, 1986). However, Windham et al. (1986) reported evidence for a growth-promoting factor being produced by *Trichoderma* spp. When tomato, tobacco, or corn seeds were germinated on a cellophane membrane placed on top of 1% agar medium containing mycelia of *Trichoderma*, germination time was reduced by one to three days although the seeds were in association but not contact with the fungus. Similar to this phenomenon, auxins, cytokinins, and gibberellins are known to be produced by root-colonizing bacteria such as *Azotobacter, Bacillus*, and *Pseudomonas* used in bacterial fertilizers (Brown, 1974).

Enhancement of growth by *Trichoderma* spp. is most likely related to both biocontrol of minor root pathogens and production of a growth-promoting substance. Regardless of the cause, such a growth response could provide the plant with a mechanism of escape from pathogens, thus providing yet another mechanism of biocontrol.

Delivery methods. A primary obstacle in commercial use of *Trichoderma* spp. for both disease control and growth enhancement is delivering the fungus to the plant material. Effective biocontrol delivery methods have included formulation of the fungus in chainsaw oil to inoculate pine stumps during harvest (Hunt et al., 1971) and addition of spores to a liquid fertilizer (Papavizas, 1985) or into

seedling gels used for fluid drilling of small seeds (Fischer et al., 1983).

Mihuta-Grimm and Rowe (1986) found applying *Trichoderma* spp. in a fluid gel drilling matrix containing a 5-day-old liquid culture of the antagonist gave superior control of *Rhizoctonia* damping off of radish than applying as a seed coat, wheat-bran mixture, or liquid culture suspension. Levels of *Trichoderma* spp. in the applications were 5.5 log colony forming units (cfu)$\cdot g^{-1}$ in the peat-bran preparation and 2.9 X 10^8 conidia per milliliter of conidial suspension, which was used to either coat the seed or apply as a liquid to drench the furrow before planting.

Mycelial preparations have been formulated on a peat-bran mixture (Sivan et al., 1984; Baker et al., 1984) and then used as a media amendment. Sivan et al. (1984) added *T. harzianum* using a peat-bran preparation at 5g$\cdot kg^{-1}$ medium or coated seeds with a suspension containing 5 X 10^9 conidia per milliliter and found both methods to be equally effective in controlling *Pythium* damping-off. Paulitz et al. (1986) examined *Trichoderma*-mediated enhanced growth of radish grown in soil mixes containing various ratios of peat : vermiculite amended with 0, .1, 1, or 10% (v/v) *T. harzianum* peat-bran preparation. Population density of *Trichoderma* spp. was 2 to 4.3 log cfu$\cdot g^{-1}$ medium in 0%, 6 to 7 in .1, 7 to 8 in 1, and 8 to 8.4 log cfu$\cdot g^{-1}$ medium in 10% treatment. Because of an interaction between rate of amendment and ratio of peat :

vermiculite, they reported no differences in growth due to rate, but high levels of *Trichoderma* decreased shoot dry weight after 3 weeks if mixes contained a high percentage of vermiculite.

A conidial suspension has also been applied to roots as a spray (Chang et al., 1986). Suspensions containing 10^8 conidia per milliliter resulted in *Trichoderma* levels of 4 log cfu·g^{-1} medium. If this suspension was applied with the rooting hormone, treatments reached 3 to 3.3 log cfu·g^{-1}. They also applied the fungus in a peat-bran preparation, and found amendment at 20% (v/v) of the medium yielded 6 log cfu·g^{-1}. Growth increase occurred at population densities of the fungus at higher than 5 log cfu·g^{-1} medium when the fungus was applied either in a conidial suspension or in a peat-bran mixure.

A more recent development in delivery methods is the encapsulation of fungal material based on the ability of aqueous sodium alginate to react with certain cations, such as Ca^{++}, to form gels (Lewis and Papavizas, 1985). Incorporation of a nutrient carrier, such as bran, into the solution provides a food base for the fungus inside alginate pellets. Lewis and Papavizas (1985) amended a natural soil medium with 0.5% (v/v) pellets containing 5 to 7 log cfu·g^{-1} pellet and obtained 5 to 6 log cfu·g^{-1} medium after 3 weeks.

Viability. Viability of fungal propagules over time is a pertinent commercial issue. Sivan et al. (1984) noted a 91% decrease in population density of a peat-bran preparation from

9.7 to 8.7 log cfu·g^{-1} medium over a one year storage period at 25C. Viability of alginate pellets declined similarly over 24 weeks of storage at 5 or 25C (Lewis and Papavizas, 1985). However, 8.7 log cfu·g^{-1} medium is a more than sufficient population density to provide both biological control (Liu and Baker, 1980) and increased growth response (Baker et al., 1984). Cost of producing the peat-bran formulation may be as low as $0.40 per pound, costing the grower $1.00 per pound, or $0.02 per plant in an average bedding plant operation (Baker, 1988).

Rhizosphere competence. Another factor to be considered in evaluating *Trichoderma* spp. for commercial application is rhizosphere competence, or the ability of the fungus to colonize the outer root surface, an especially important aspect of delivery via seed treatment. Unfortunately, wild types of *Trichoderma* spp. are not rhizosphere competent (Lewis and Papavizas, 1985). Mutation of *T. harzianum* by exposure to *N*-methyl-*N*-nitro-*N*-nitrosguanidine (NTG) produced some strains resistant to benomyl (Chang et al., 1986). Unexpectedly, the mutants were rhizosphere competent both in the presence and absence of benomyl. Subsequently, mutants of *T. viride* and *T. koningii* (Baker, 1989) were also shown to be rhizosphere competent.

Protoplast fusion (Harman et al., 1989) of a rhizosphere-competent mutant of *T. harzianum* (T-95) with a wild type strain (T-12) and selection of potential biocontrol agents

yielded fusants providing biocontrol via seed treatment as effective as a chemical fungicide (thiram) and producing a greater enhancement of growth than either individual parent strain. Applications of this technology may increase the commercial appeal of *Trichoderma* spp. as biocontrol agents and plant-growth stimulants.

Variability. Variability among trials conducted to investigate the action of *Trichoderma* spp. has discouraged development of the fungus as a commercial product. Chang et al. (1986) could not replicate results of increases up to 247% in dry weight of radish propagated in the presence of *T. harzianum* (isolate T-95) reported by Baker et al. (1984). They also saw no significant differences in beans (*Phaseolus vulagaris* L. 'Brittle Wax') growing in media amended with the fungus. Paulitz et al. (1986) saw increased shoot dry weight of radish with *Trichoderma* treatment in one experiment that was not reproduced in a subsequent study.

Introduction

The idea of using a microorganism to provide crop protection and/or growth stimulation is at the present time less than practical. I have investigated enhancement of growth in propagation of unrooted chrysanthemum cuttings by *Trichoderma harzianum* (isolate T-12) with the goal of providing information that in the future may help promote the use of *Trichoderma* spp. as a valuable component of sustainable

agriculture.

Objectives. Specific goals of my research were: (i) to optimize rate of media amendment with T-12 as a powdered peat-bran formulation; (ii) to compare amending the rooting medium with T-12 to treating unrooted cuttings with 1 *H*-indole-3-butanoic acid (IBA) to promote rooting; (iii) to compare the powdered peat-bran formulation to an alginate pellet preparation of the fungus as methods of delivery; and (iv) to investigate *T. harzianum* as a growth enhancer of several cut and pot floriculture crops.

CHAPTER 2

ENHANCED ROOT AND SHOOT GROWTH OF CHRYSANTHEMUM CUTTINGS PROPAGATED WITH THE FUNGUS *TRICHODERMA HARZIANUM*

Abstract

The biological control fungus *Trichoderma harzianum* Rifai can enhance growth of several vegetable and floriculture crops. Zero, 5, or 25 g of *T. harzianum* (isolate T-12) peat-bran amendment was added per kilogram medium in an effort to enhance the rooting of four chrysanthemum [*Dendranthema X grandiflorum* (Ramat.) Kitamura] cultivars, two considered easy-to-root ('Davis' and 'White Marble') and two considered hard-to-root ('Dark Bronze Charm' and 'Golden Bounty'). Addition of the *T. harzianum* amendment at both rates tested increased root and shoot fresh weights during 21 days of rooting. Supplementary treated cuttings were transplanted into untreated growing medium after 21 days. Midway between transplant to flowering, increases in height, shoot dry weight, and root fresh and dry weight were detected in 'Dark

Bronze Charm' with T-12 relative to the control; increases in height, shoot fresh and dry weight, and number of nodes were detected in 'Golden Bounty' with T-12. By this time, there were no detectable differences in 'Davis' or 'White Marble'.

Introduction

Trichoderma species have been used extensively for biocontrol of soilborne pathogens (Chet and Baker, 1981; Sivan et al., 1984). These fungi may also have a growth-enhancing effect independent of mechanisms associated with biocontrol (Chang et al., 1986; Windham et al., 1986). Applications of *Trichoderma* may decrease use of fungicides, growth regulators, and labor in the greenhouse, hence lowering costs and environmental impact. Although several plants are responsive to growth enhancement by *Trichoderma* (Windham et al., 1986), cut and potted flower production applications remain largely uninvestigated. The objective of our study was to determine if amending rooting medium with various rates of *T. harzianum* would enhance root and shoot growth of chrysanthemum cuttings.

Materials and Methods

Two cultivars of chrysanthemum considered hard-to-root ('Dark Bronze Charm' and 'Golden Bounty') and two cultivars considered easy-to-root ('Davis' and 'White Marble') were received as unrooted cuttings (Yoder Brothers, Barberton,

Ohio) on 1 Dec. 1993. 'Dark Bronze Charm' is an 8-week response cultivar with regard to short day requirement for flowering and the others respond in 9 weeks. After being sorted for uniformity, cuttings dipped in 7.4 mM IBA were inserted into a 1 peat: 1 perlite (v/v) medium amended with 0, 5, or 25g T-12 peat-bran amendment/kg medium. The medium was at 24C using EPDM hot-water bottom heat tubing (Biotherm Co., Petaluma, Calif.). *T. harzianum* (isolate T-12) was cultured on peat-bran for 2 weeks, dried, ground to a powder, and mixed thoroughly with the medium (Paulitz et al., 1986). The log number of colony forming units (cfu) of T-12, which was monitored using an agar medium selective for *Trichoderma* spp. (Elad et al., 1981), ranged from 4.9 to 5.5·g^{-1} oven-dried (72 h at 50C) medium during the experiment for both treatments amended with T-12, which were similiar but at least one log unit greater than non-amended controls (Appendix 1).

Cuttings were inserted into 12 53 cm X 27 cm plastic rooting trays with drainage holes, within rows, 10 cuttings per row, and placed immediately under intermittent mist in a glasshouse at 24/18C (venting/night set points). Mist intervals were those recommended by the supplier: 10 sec every 5 min for 4 days, every 20 min days 5-8, and every 30 min throughout the rooting period of experiment. Long day (LD) photoperiod was effected by incandescent night interruption from 2200 HR to 0200 HR. Three 100-watt incandescent bulbs spaced 75 cm apart and placed 85 cm above plant canopy

supplied 5.8 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ measured at plant canopy level. Subsequently, plants received natural short day (SD).

Treatments were arranged as a 3 X 4 X 3 factorial with three rates of T-12 peat-bran amendment, four cultivars, and three harvest dates using 10 single plant replications in a completely randomized design. Shoot height, and shoot and root fresh and dry (72 h at 80C) weights of 10 randomly selected cuttings were measured on each of days 7, 14, and 21. After carefully lifting out each cutting and the surrounding medium with a spatula, we separated shoots from roots 1 to 3 cm above the basal end of the cutting, depending on the cultivar and its tendency to initiate roots along the stem. Shoot height was measured from the base of the cutting to the stem apex.

After 21 days of rooting in the mist bed, 10 separate, intact cuttings were transplanted into untreated growing medium in 0.4-liter pots to be grown to flowering in the greenhouse. Plants were fertilized at each irrigation with Peter's water soluble 20N-4.4P-16.6K fertilizer (W.R. Grace, Cambridge, Mass.). When roots had reached the bottom of the pot, 'Dark Bronze Charm' and 'Davis' were pinched, removing 2.5 cm of the terminal apex, to grow as pot plants. Later we executed center bud removal on these two cultivars and 'White Marble' to grow as sprays. Lateral buds of 'Golden Bounty' were removed to grow the plants as standard cut flowers.

Shoot height, number of nodes ('Golden Bounty' only, because disbudding facilitated quantification), and shoot and

root fresh and dry weights, of 10 randomly selected plants were measured midway from transplanting to expected flowering dates, which were calculated according to the supplier's 1993/94/95 Datefinder. Midpoint for 'Dark Bronze Charm' was 4 weeks from beginning SD and for the others it was 4.5 weeks. Shoot height was measured from the top of the pot. Other characteristics were measured as previously. Analysis of variance was used in data analyses (SAS Proc GLM; SAS Institute, Cary, N.C.) and the means were separated by least significant difference (LSD) at $P \leq 0.05$.

Results

Root fresh weight changes. Amendment with *T. harzianum* increased root fresh weights of the four cultivars of chrysanthemum propagated from cuttings (Fig. 1). An interaction occurred between amendment rate and time during the 21 days of rooting. Root fresh weights were similiar for the three rates of amendment on day 7 for all cultivars, but by day 14, the two *Trichoderma* treatments had increased root fresh weights of all but 'Golden Bounty', a hard-to-root cultivar. For this cultivar, only 25 $g \cdot kg^{-1}$ resulted in increased root fresh weight. By 21 days, the root fresh weight of controls had leveled while that in *Trichoderma*-amended medium had continued to increase. Rooting in medium amended with either amount of *Trichoderma* was similiar for the four cultivars on day 21.

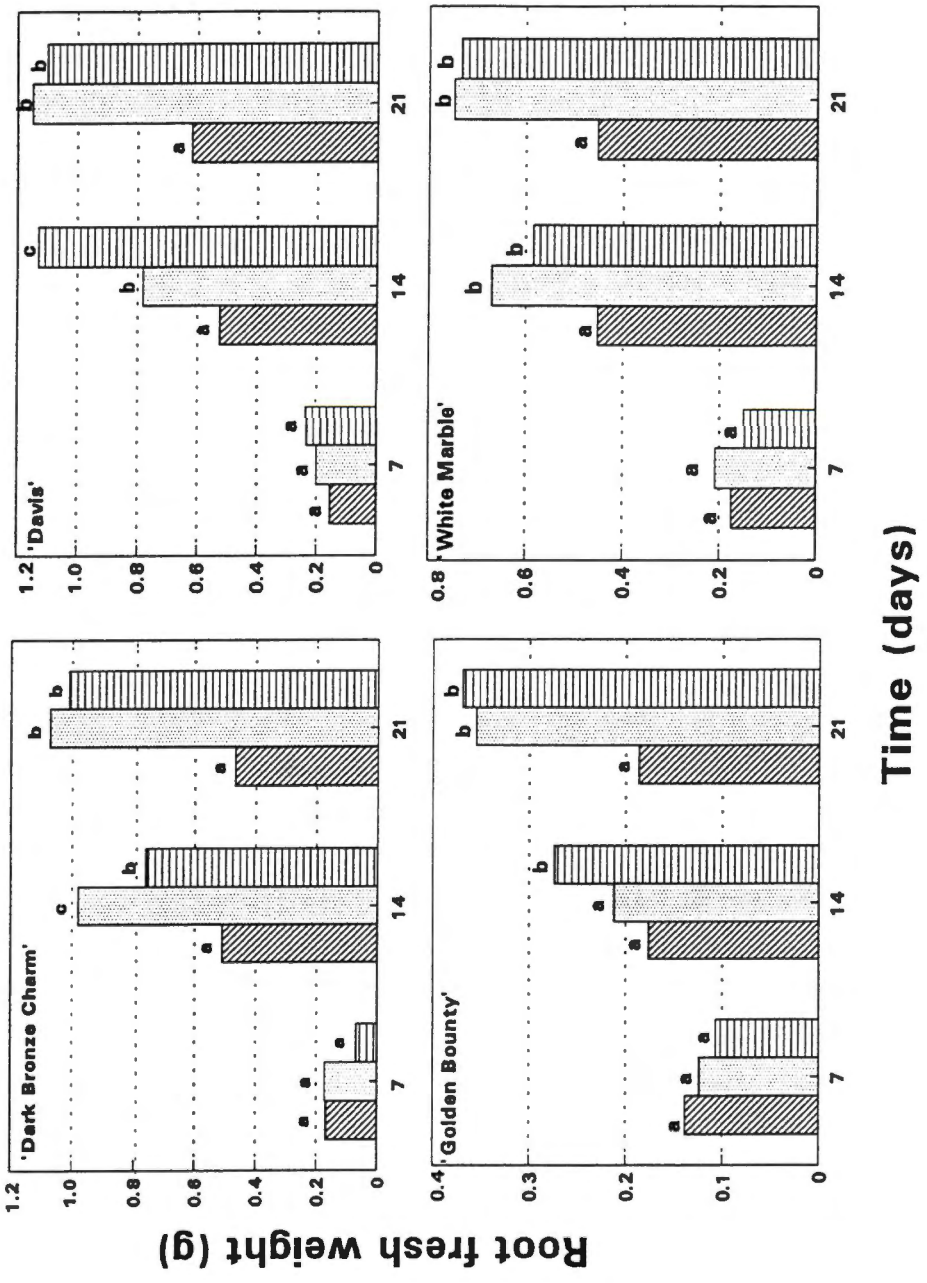


Figure 1. 'Dark Bronze Charm', 'Davis', 'Golden Bounty', and 'White Marble' chrysanthemum root fresh weight following amendment of growing medium with *Trichoderma harzianum* at 0 ▨, 5 ▯, or 25 ▤ $g \cdot kg^{-1}$, and at three sampling times (7, 14, and 21 days). Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

Shoot fresh weight changes. Amendment with *T. harzianum* also increased shoot fresh weight of the four cultivars during the 21 days of rooting (Fig. 2). Again, there was a rate x time interaction. On day 7, all treatments were similar, with the exception of the 25 $g \cdot kg^{-1}$ amendment of 'Dark Bronze Charm', which had a 19% and 24% lower fresh weight than the 5 and 0 $g \cdot kg^{-1}$ treatments, respectively. By day 14, 'Dark Bronze Charm', 'Davis', and 'Golden Bounty' had increased shoot fresh weight with *Trichoderma* treatment. 'Dark Bronze Charm' had the highest shoot fresh weights when the medium was amended with 5 $g \cdot kg^{-1}$ of *Trichoderma*, while 'Davis' and 'Golden Bounty' responded best to 25 $g \cdot kg^{-1}$. By day 21, all cultivars showed increased shoot fresh weight with 25 g *Trichoderma*/kg. 'Dark Bronze Charm' had a 36% increase in shoot fresh weight when treated with 5 $g \cdot kg^{-1}$ of *Trichoderma*, and 18% when treated with 25 $g \cdot kg^{-1}$. 'Davis' had increased shoot fresh weights with both the 5 and 25 $g \cdot kg^{-1}$ treatments, which were statistically the same. 'Golden Bounty' and 'White Marble' had increases only when treated with the 25 $g \cdot kg^{-1}$ rate.

Plant characteristics at midpoint. By the midpoint to flowering, *Trichoderma* treatments affected growth of 'Dark Bronze Charm' and 'Golden Bounty' but not 'Davis' and 'White Marble'. 'Dark Bronze Charm' plants treated with 5 or 25 $g \cdot kg^{-1}$ were similiar and 20% taller than the controls (Fig. 3). At 5 $g \cdot kg^{-1}$, the amendment increased shoot dry weight by 21% over the control, while 25 $g \cdot kg^{-1}$ had no effect. At 5 $g \cdot kg^{-1}$,

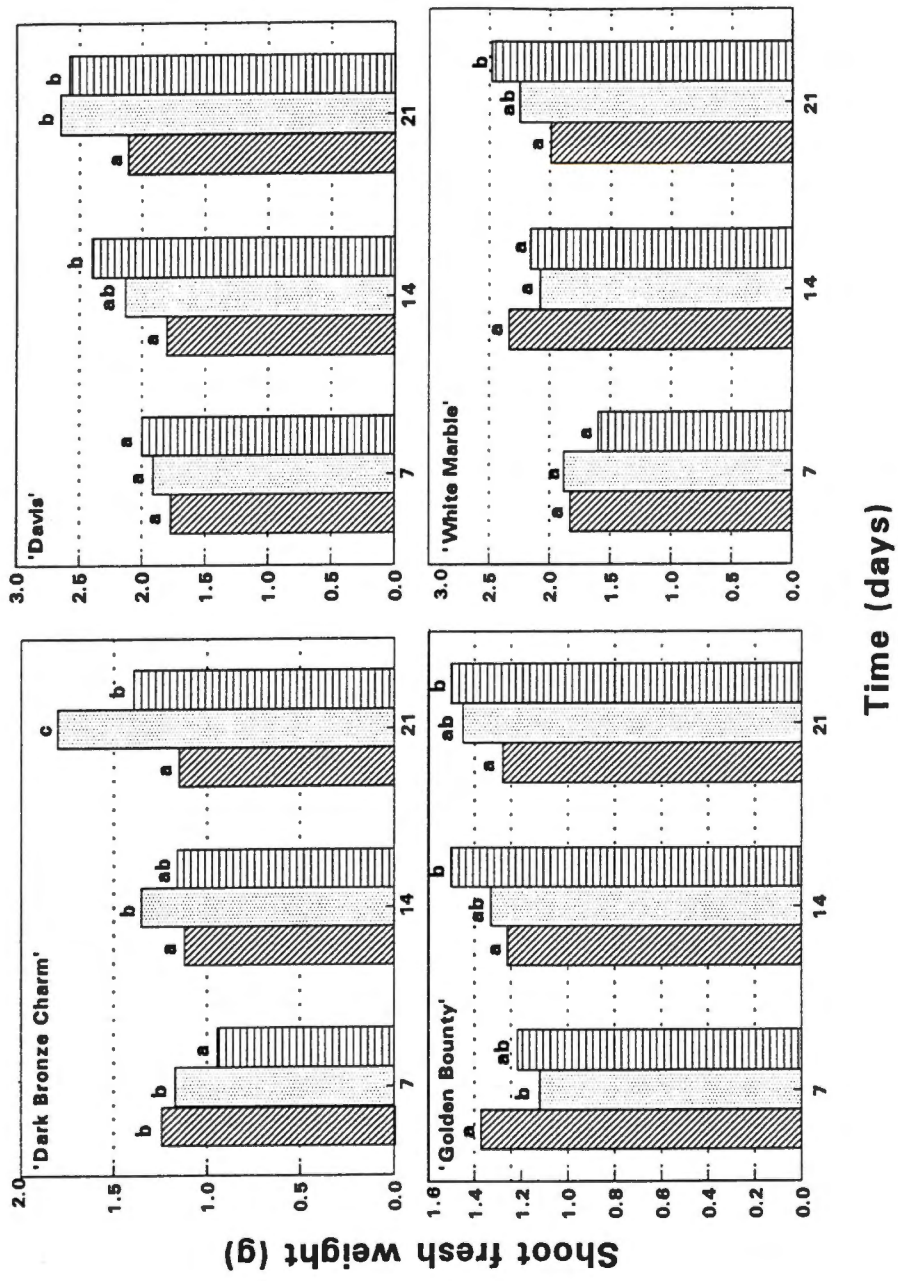


Figure 2. 'Dark Bronze Charm', 'Davis', 'Golden Bounty', and 'White Marble' chrysanthemum shoot fresh weight following amendment of growing medium with *Trichoderma harzianum* at 0 ▨, 5 ▩, or 25 ▤ $g \cdot kg^{-1}$, and at three sampling times (7, 14, and 21 days). Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

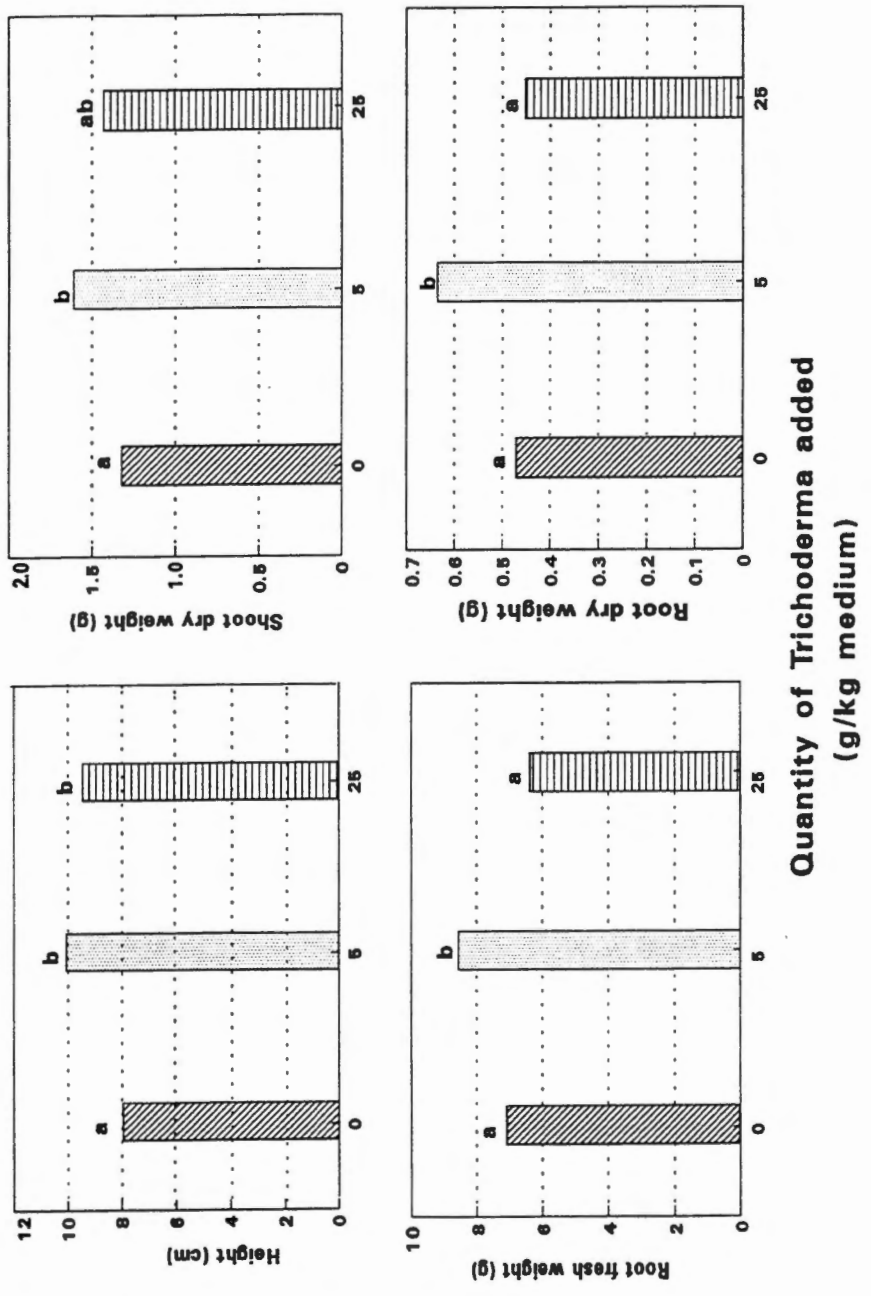


Figure 3. 'Dark Bronze Charm' chrysanthemum height, shoot dry weight, root fresh weight, and root dry weight following amendment of growing medium with *Trichoderma harzianum* at 0 ▨, 5 ▯, or 25 ▤ $g \cdot kg^{-1}$ midway from transplanting cuttings into untreated growing medium to flowering. Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

Trichoderma increased root fresh and dry weights over those of the control and 25 $g \cdot kg^{-1}$, which did not differ significantly. At midpoint, 'Golden Bounty' was 15% taller, had 5 more nodes, a 14% increase in shoot fresh weight, and a 19% increase in shoot dry weight whether treated with *T. harzianum* at 5 or 25 $g \cdot kg^{-1}$ (Fig. 4).

Discussion

Trichoderma spp. have previously enhanced the growth of floriculture crops, increasing shoot and/or root fresh and dry weights, height, and number of flower buds of chrysanthemum and petunia (*Petunia Xhybrida* Hort. Vilm.-Andr.) while hastening flowering of bedding plants, such as periwinkle [*Catharanthus roseus* (L.) G. Don], alyssum [Lobularia maritima (L.) Desv.], and marigold (*Tagetes erecta* L.) (Chang et al., 1986). Optimum rates of application remained uninvestigated. In this study, adding 5 or 25 $g \cdot kg^{-1}$ of a powdered peat-bran amendment of *T. harzianum* increased fresh and dry root and shoot weights, height, and number of nodes of chrysanthemum cuttings. Levels of T-12 ranged from 4.9 to 5.5 cfu/g soilless medium, a density that effectively mediates biocontrol and enhances growth (Liu and Baker, 1980; Windham et al., 1986).

Easy-to-root cultivars responded faster with increased root fresh weight to the treatment than the hard-to-root cultivars. Possibly, the hard-to-root cultivars are less sensitive to a growth regulating compound being produced by

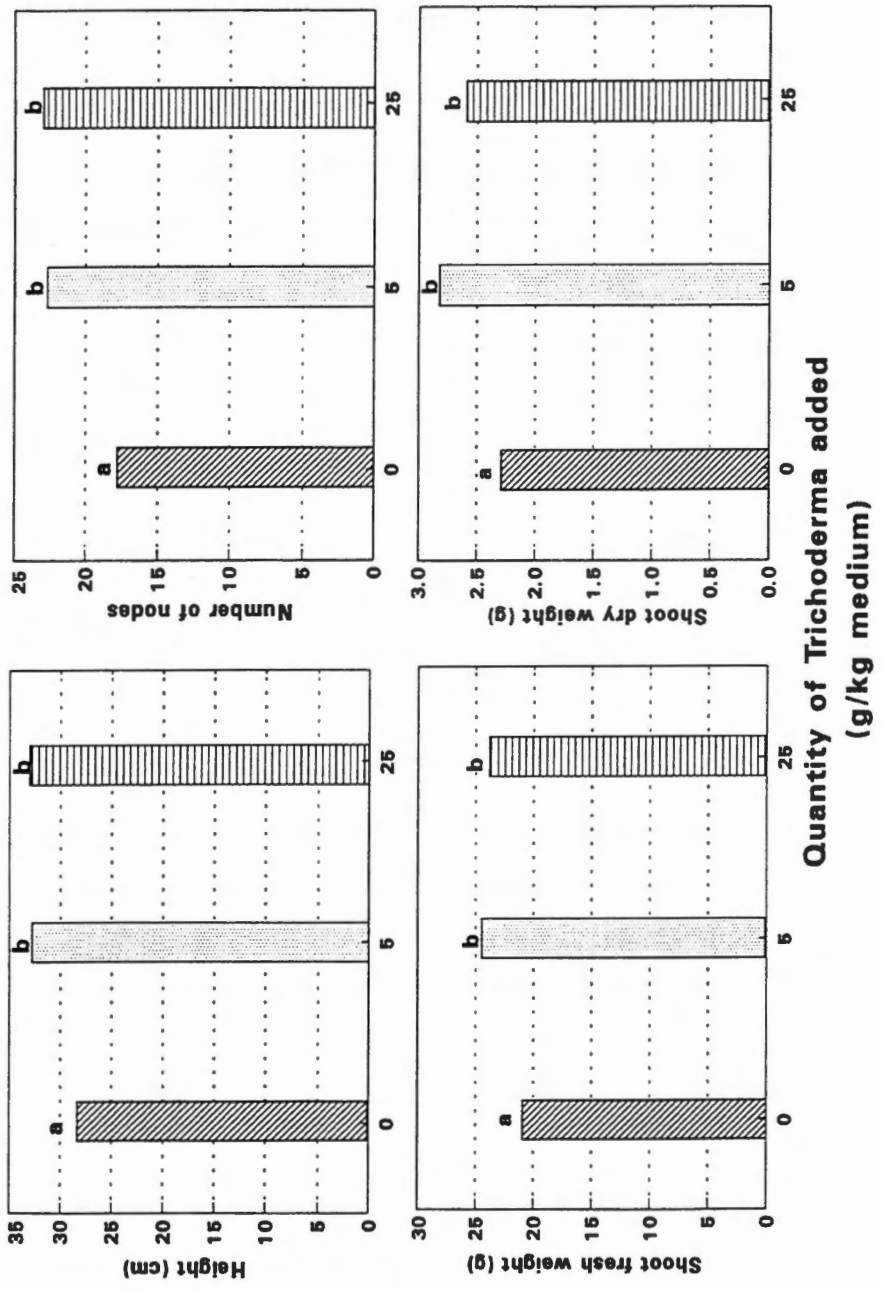


Figure 4. 'Golden Bounty' chrysanthemum height, number of nodes, shoot fresh weight, and shoot dry weight following amendment of growing medium with *Trichoderma harzianum* at 0, 5, or 25 $g \cdot kg^{-1}$ midway from transplanting cuttings into untreated growing medium to flowering. Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

the fungus and, therefore, respond more slowly.

'Dark Bronze Charm', a hard-to-root cultivar, had decreased root and shoot fresh weight in the presence of *Trichoderma* at 25 $g \cdot kg^{-1}$ compared to 5 $g \cdot kg^{-1}$ during the 21 days of rooting. By midpoint to flowering, growth was similar to that of the controls. *Trichoderma* spp. may enhance growth by producing a growth regulating substance (Windham et al., 1986). If such a compound is being introduced by the fungus into the rhizosphere, the inhibition of growth might be due to phytotoxicity at higher concentrations. Rooting hormones applied to excess during rooting have resulted in blackening of the stem, leaf drop, and eventual death of cuttings (Hartmann et al., 1990). The chrysanthemums in our study may have outgrown the inhibition after a few weeks.

By midpoint to flowering, only the hard-to-root cultivars differed due to treatment, indicating that growth stimulation in the early stages of propagation has more long-lasting benefits for hard-to-root than for easy-to-root cultivars. In 'Dark Bronze Charm', 25$g \cdot kg^{-1}$ compromised growth. Root and shoot fresh weights at 25 $g \cdot kg^{-1}$ were less than at 5$g \cdot kg^{-1}$ during the 21 days of rooting, a trend that continued until midpoint to flowering. The increased number of nodes present at midpoint in 'Golden Bounty' could be due to production of a phytohormone, similar to a cytokinin, by the fungus. *Trichoderma* spp. are known to produce growth-regulating substances (Cutler et al., 1989; Cutler et al., 1991).

Increased height, which was observed in both hard-to-root cultivars midway to flowering, would be advantageous for 'Golden Bounty' plants, which are grown as cut flowers, but might decrease the quality of 'Dark Bronze Charm' plants, which are pot mums. We saw no increased flower number despite enhanced shoot growth. As expected, no differences in time to flowering were observed during the experiment because chrysanthemum flowering is controlled by the number of short days received rather than by the amount of vegetative growth.

The practical application of this research to the floriculture industry will be improvement in rooting hard-to-root cuttings and accelerating chrysanthemum pot plant and cut flower production. Future studies should involve developing efficient methods of delivering the fungus to the plant rhizosphere, eliminating the use of a rooting hormone in propagation, and further optimizing application rate.

CHAPTER 3

CHRYSANTHEMUM ROOTING IN A MEDIUM AMENDED WITH *TRICHODERMA HARZIANUM* IN THE PRESENCE AND ABSENCE OF IBA

Abstract

Amending rooting medium of chrysanthemum [*Dendranthema* x *grandiflorum* (Ramat) Kitamura] with *Trichoderma harzianum* Rifai, isolate T-12, a biological control fungus, enhanced rooting of cuttings, sometimes as effectively as treating with IBA. Two cultivars considered easy-to-root ('Davis' and 'White Marble') and two considered hard-to-root ('Dark Bronze Charm' and 'Golden Bounty') were inserted into a rooting medium amended with 0, 5 or 25g T-12·kg^{-1} medium after being dipped into a 0 or 7.4mM solution of IBA. Amending medium with 5g·kg^{-1} was equivalent to treating 'White Marble' cuttings with 7.4mM IBA with regard to root length, fresh and dry weight, and 'Davis' for root length. IBA increased root dimensions in the hard-to-root cultivars without regard to T-12, with the exception of 'Dark Bronze Charm' root length. By day 21,

enhanced rooting was observed in the two hard-to-root cultivars treated with T-12. We saw increases in rooting characteristics of all four cultivars by day 14 or 21 with one or both rates of amendment.

Introduction

Unrooted chrysanthemum cuttings are conventionally treated with auxin-type growth regulator liquid or powder dips to increase number of cuttings forming roots, hasten root initiation, promote uniformity, and to increase the number and quality of roots per cutting (Hartmann et al., 1990). We have previously investigated amending rooting medium with various rates of *Trichoderma harzianum*-isolate T-12 (T-12) and observed increased root fresh weight of chrysanthemum 14 and 21 days after insertion (MacKenzie et al., 1995). Because *Trichoderma* spp. have been used extensively for biological control of the soilborne root rot pathogens *Pythium, Phytophthora,* and *Rhizoctonia* species (Papavizas, 1985; Chet and Baker, 1981; Sivan et al., 1984), media amended with the fungus might provide the added advantage of disease control over using IBA alone. In addition, such a biological method of enhancing rooting might be more environmentally sound than chemical treatment. The objective of this experiment was to compare amending the medium with T-12 to liquid dip treatment with IBA for rooting of chrysanthemuum.

Materials and Methods

T-12 was cultured on peat-bran for 2 weeks, dried, ground to a powder, and mixed thoroughly with the medium (Paulitz et al., 1986). The log number of colony forming units (log cfu) of T-12 was monitored using an agar medium selective for *Trichoderma* spp. (Elad et al., 1981).

Two cultivars of chrysanthemum considered hard-to-root ('Dark Bronze Charm' and 'Golden Bounty') and two cultivars considered easy-to-root ('Davis' and 'White Marble') were received 28 June 1994 as unrooted cuttings (Yoder Brothers, Barberton, Ohio). After being sorted for uniformity, cuttings dipped into 0 or 7.4 mM (1500 mg·liter^{-1}) IBA were inserted into a 1 peat: 1 perlite (v/v) medium amended with 0, 5, or 25 g T-12 peat-bran amendment/kg medium.

Cuttings were inserted into 24 53 cm X 27 cm plastic rooting trays with drainage holes, within rows, 10 cuttings per row, and placed immediately under intermittent mist in a glasshouse at 24/18C (venting/night set points). Mist intervals were those recommended by the supplier: 10 sec every 5 min for 4 days, every 20 min days 5-8, and thereafter every 30 min throughout the rooting period. Long day (LD) photoperiod was natural daylength for the 21 days of rooting.

Treatments were arranged as a 2 X 3 X 4 X 3 factorial with two rates of IBA, three rates of T-12 peat-bran amendment, four cultivars, and three harvest days using 10 single plant replications in a completely randomized design.

Root length and root fresh and dry (72 h at 80C) weights of 10 randomly selected cuttings were measured on each of days 7, 14, and 21. After carefully lifting out each cutting and the surrounding medium with a spatula, we separated shoots from roots 1 to 3 cm above the end of the cutting, depending on the cultivar and its tendency to initiate roots along the stem. Analysis of variance was used in data analyses (SAS Proc GLM; SAS Institute, Cary, N.C.), cultivars were analyzed separately, and the means were separated by least significant difference (LSD) at $P \leq 0.05$.

Results

Number of log cfu ranged from 5.5 to 6.7 $\cdot g^{-1}$ oven-dried (72 h at 50C) medium during the experiment (Appendix 2). Levels of *Trichoderma* spp. were similiar in treatments amended with T-12. Control medium always contained fewer cfu than that of treatments amended with T-12.

A 3-way interaction occurred among IBA, rate of T-12, and day of measurement in 'Dark Bronze Charm' for root length (Table 1, Fig. 5). Root length increased gradually over time in controls (0 mM IBA; 0 $g \cdot kg^{-1}$ T-12). When IBA was applied, root length increased sharply over time regardless of T-12 rate and was greater than controls on days 14 and 21. The presence of T-12 in the rooting medium of IBA-treated plants made no difference at day 7 or 14, but 5$g \cdot kg^{-1}$ increased root length on day 21 compared to IBA alone, which had increased

Table 1. Analysis of variance for effect of T-12 and IBA on root length, root fresh and dry weight of chrysanthemum 'Dark Bronze Charm', 'Davis', 'Golden Bounty', and 'White 'Marble'.

Cultivar	Source	Root length	Root fresh weight	Root dry weight
'Dark Bronze Charm'	T-12	*	*	**
	IBA	***	***	***
	IBA*T-12	***	NS	NS
	IBA*Day	***	***	***
	T-12*Day	**	*	NS
	IBA*T-12*Day	*	NS	NS
'Davis'	T-12	***	***	***
	IBA	NS	***	***
	IBA*T-12	*	NS	NS
	IBA*Day	**	***	**
	T-12*Day	***	**	*
	IBA*T-12*Day	NS	NS	NS
'Golden Bounty'	T-12	***	***	***
	IBA	***	***	***
	IBA*T-12	NS	NS	NS
	IBA*Day	***	***	***
	T-12*Day	***	*	**
	IBA*T-12*Day	NS	NS	NS

Table 1 (cont.)

Cultivar	Source	Root length	Root fresh weight	Root dry weight
White Marble'	T-12	***	***	**
	IBA	NS	***	***
	IBA*T-12	*	*	**
	IBA*Day	*	**	NS
	T-12*Day	**	***	**
	IBA*T-12*Day	NS	NS	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively

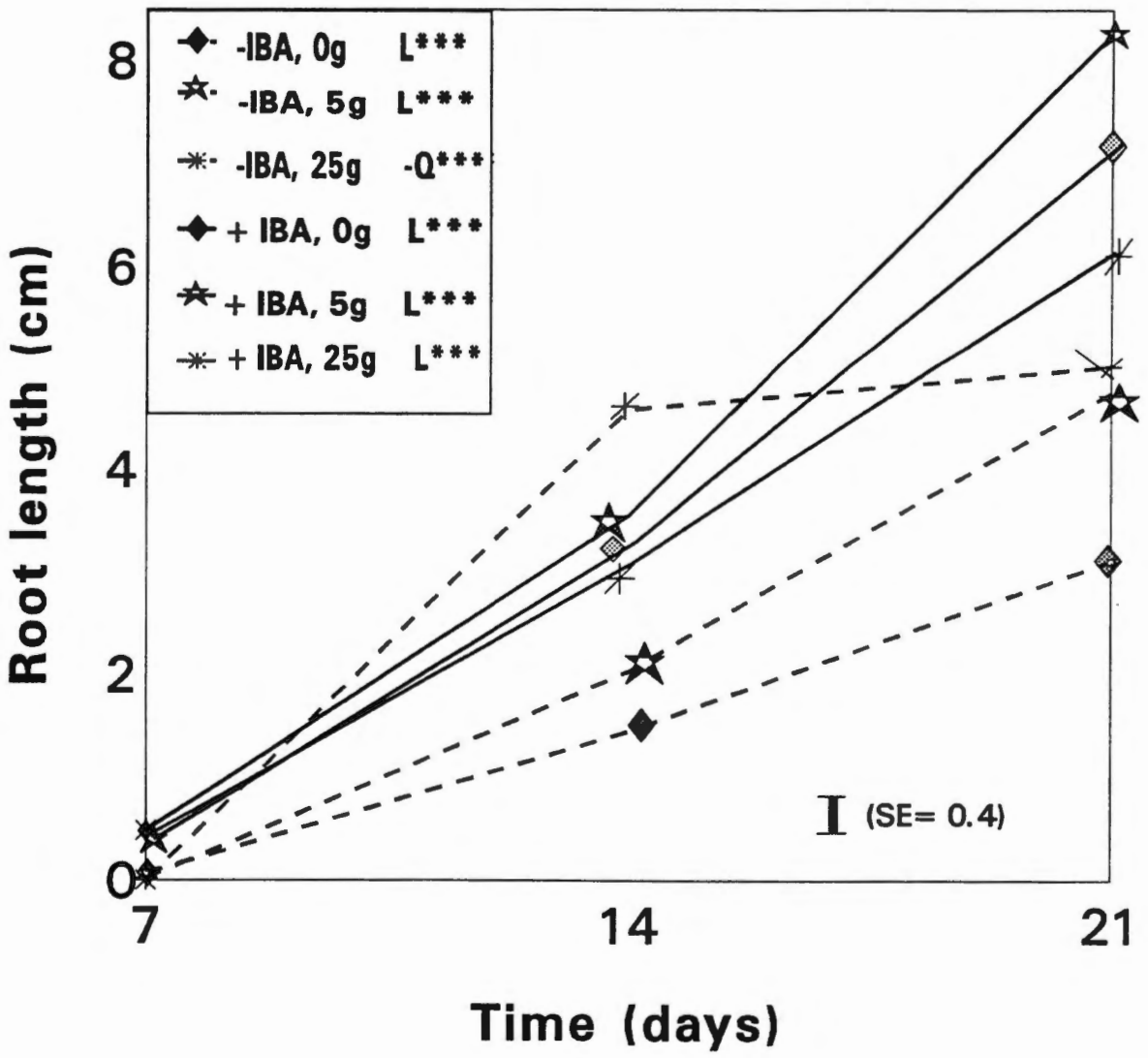


Figure 5. 'Dark Bronze Charm' chrysanthemum root length following amendment of growing medium with *Trichoderma harzianum* at 0 ◆, 5 ☆, or 25 ✳ $g \cdot kg^{-1}$, a liquid dip of 0 (dashed line) or 7.4 (solid line) mM IBA, and at three sampling times (days 7, 14, and 21). Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

length compared to IBA with 25 $g \cdot kg^{-1}$. Without IBA, T-12 at 5 $g \cdot kg^{-1}$ increased root length over time more gradually, and roots were never as long as those treated with IBA. T-12 at 25 $g \cdot kg^{-1}$ without IBA increased root length more than all other treatments at day 14, but no further increase had occurred by day 21.

The T-12 X IBA interaction was significant in the two easy-to-root cultivars for root length (Table 1, Fig. 6). At 0$g \cdot kg^{-1}$ T-12, there was an increase of 31% in 'White Marble' root length with IBA compared to without IBA. At 5 and 25$g \cdot kg^{-1}$ T-12, root length was greater than at 0$g \cdot kg^{-1}$, and the addition of IBA made no difference. Greatest root length occurred when 'Davis' was treated with 5$g \cdot kg^{-1}$ T-12 with or without IBA. At 25 $g \cdot kg^{-1}$, root length was greater than controls only when IBA was applied.

The T-12 X IBA interaction also occurred in 'White Marble' for root fresh and dry weights (Table 1, Fig. 7). In medium amended with 0 or 25 $g \cdot kg^{-1}$ T-12, IBA increased root fresh and dry weight. In medium amended with 5 $g \cdot kg^{-1}$ T-12, root fresh and dry weights were similar regardless of IBA treatment and greater than those propagated in non-amended medium without IBA. Greatest root fresh and dry weight was obtained in 'White Marble' cuttings propagated in medium amended with 25$g \cdot kg^{-1}$ T-12 and treated with IBA. With the exeption of root length in 'Dark Bronze Charm', there was no T-12 X IBA interaction for hard-to-root cultivars (Table 1,

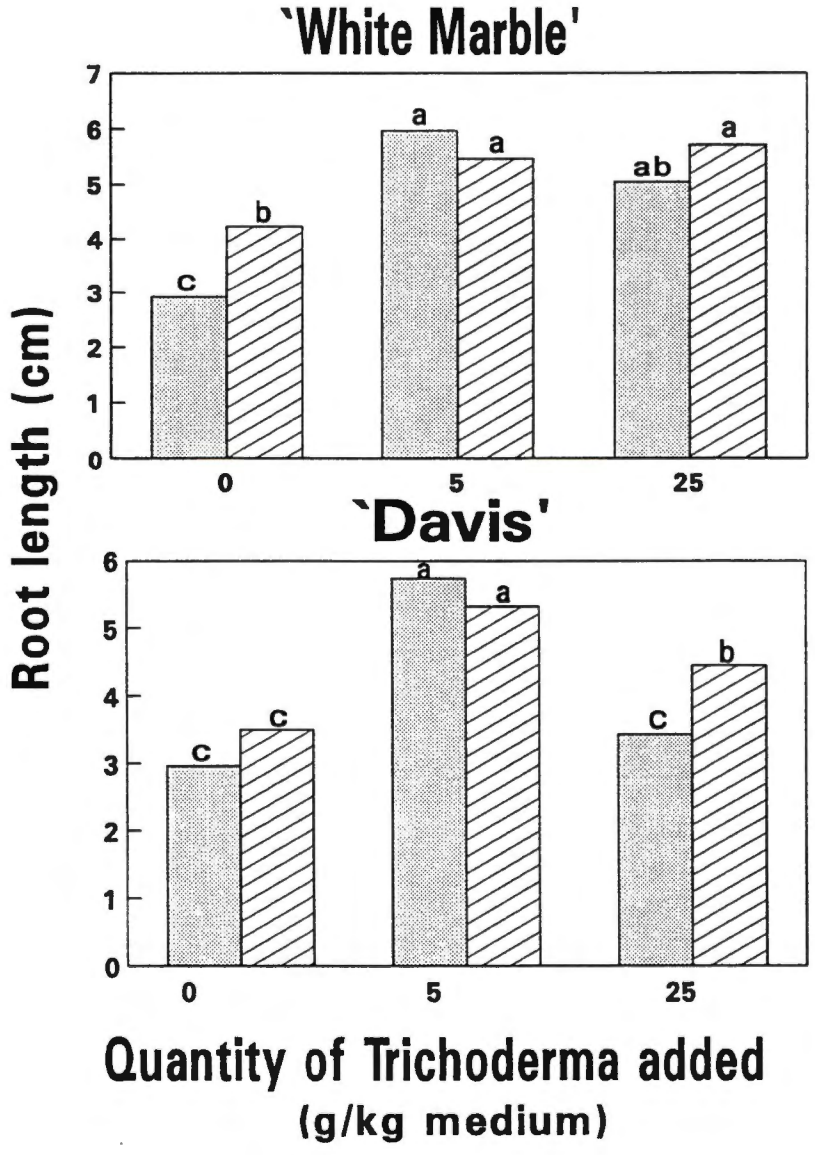


Figure 6. 'White Marble' and 'Davis' chrysanthemum root length following amendment of growing medium with *Trichoderma harzianum* and a liquid dip of 0 ▒ or 7.4 ▨ mM IBA. Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

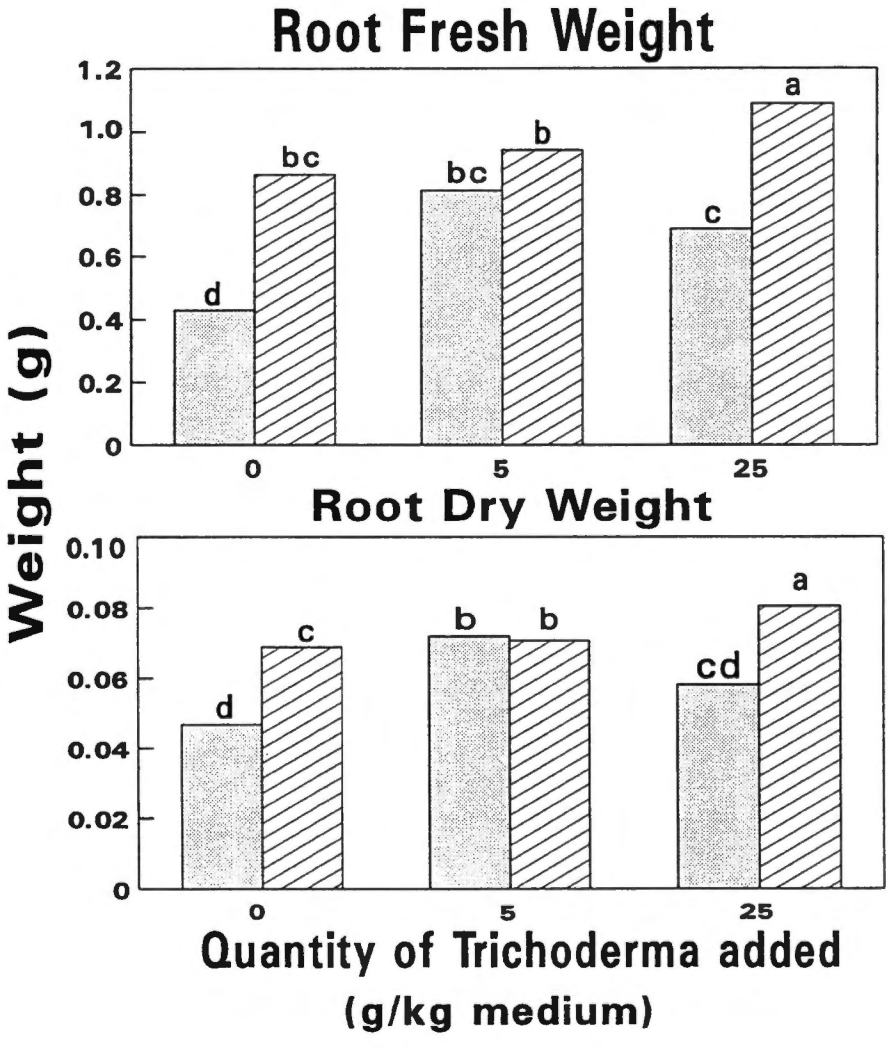


Figure 7. 'White Marble' chrysanthemum root fresh and dry weight following amendment of growing medium with *Trichoderma harzianum* and a liquid dip of 0 ☐ or 7.4 ▨ mM IBA. Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

Fig. 5). However, both rates of T-12 increased all root dimensions compared to non-amended medium, and IBA increased rooting (data not shown).

A rate X day interaction was significant in our previous experiment (MacKenzie et al., 1995) as well as this one (Table 1, Fig. 8). In general, we saw increases in rooting characteristics of all four cultivars by day 14 or 21 with one or both rates of amendment.

Discussion

In earlier work, *Trichoderma* spp. have increased rooting of chrysanthemum (Paulitz et al., 1985; MacKenzie et al., 1995) and other floriculture species (Baker, 1988). Paulitz and colleagues rooted chrysanthemum 'Charisma' and 'White Marble' in a medium amended with peat-bran preparations of *T. harzianum* isolates T-12 and T-95 and reported significant increases in rooting as well as shoot height, fresh and dry weights, and number of flower buds with both isolates. Baker (1988) claimed that unrooted chrysanthemum cuttings usually root at least two days earlier in the presence of *Trichoderma* spp. In the same review, carnations (*Dianthus coryophyllus* L.) were reported to root faster when treated with the fungus than when treated with a recommended rooting hormone alone.

In the two easy-to-root cultivars, using 5 $g \cdot kg^{-1}$ T-12 with or without IBA resulted in the same increased root length compared to controls. This trend was also seen in root fresh

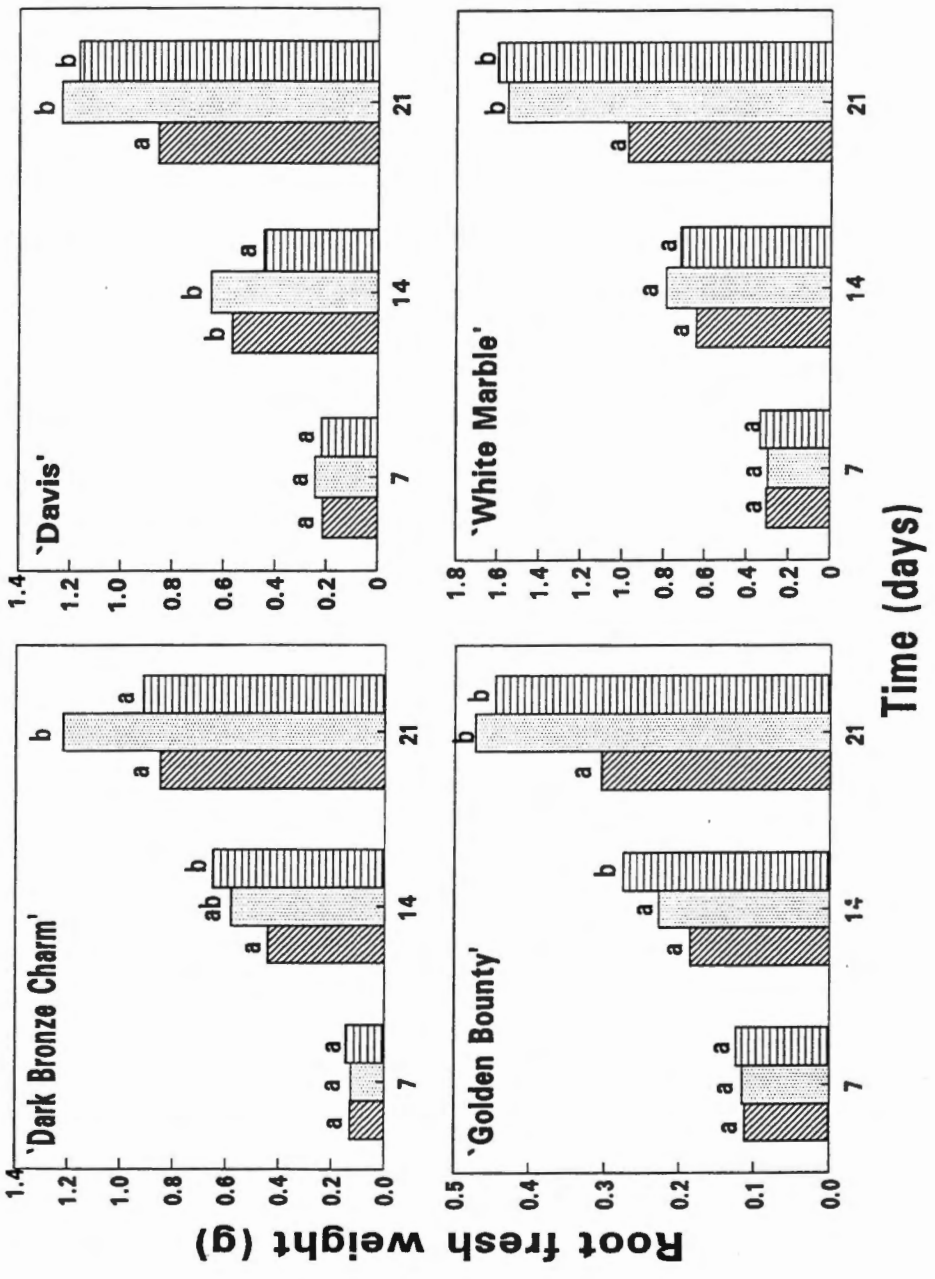


Figure 8. 'Dark Bronze Charm' , 'Davis', 'Golden Bounty', and 'White Marble' chrysanthemum root fresh weight following amendment of growing medium with *Trichoderma harzianum* at 0 ▨ , 5 ▯ , or 25 ▤ $g \cdot kg^{-1}$ and three sampling times (days 7, 14, and 21). Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

and dry weight for 'White Marble'. Using 5 $g \cdot kg^{-1}$ T-12 with IBA resulted in longer roots in 'White Marble' and 'Davis' than did using IBA alone. We hypothesized that at 5$g \cdot kg^{-1}$, the fungus controlled minor root rot pathogens while IBA stimulated root initiation and growth. However, at 25 $g \cdot kg^{-1}$ T-12, the fungus generally could not substitute for IBA. Perhaps a product of *Trichoderma* inhibited root elongation at this rate, and IBA treatment compensated for the constraint.

For the hard-to-root cultivars, IBA and T-12 increased rooting characteristics without an interaction, with the exception of the 3-way interaction involving root length of 'Dark Bronze Charm', and T-12 amendment did not substitute for IBA treatment as was seen in the easy-to-root cultivars.

Rate X day results were similar to our first study (MacKenzie et al., 1995) in that one or both T-12 amendment rates increased root fresh weight of all four cultivars by day 14 or 21. Interactions between IBA and day may have occurred due to differing sensitivity of the plant to the hormone during the course of the experiment. Skoog and Miller (1957) determined that shoot and root organogenesis is controlled by the auxin/cytokinin ratio but Christianson and Warnick (1984) found that timing of explant tissue exposure to these hormones contained in inductive media was also regulative.

Different hypotheses have been proposed to explain enhanced growth of plants, including rooting, in the presence of *Trichoderma* spp. The fungus functions as a biological

control agent both by producing an antibiotic against and mycoparasitizing root rot pathogens (Lifshitz, et al., 1986; Elad, 1986), actions which indirectly provide plants with potential for enhanced growth. Windham et al. (1986) provided evidence for a growth-regulating factor being produced by the fungus. When tomato, tobacco, or corn seeds were germinated on a cellophane membrane placed on top of 1% agar medium containing mycelia of *Trichoderma*, germination time was reduced by one to three days although the seeds were in association but not in contact with the fungus. Similar to this phenomenon, auxins, cytokinins, and gibberellins are known to be produced by root-colonizing bacteria such as *Azotobacter, Bacillus*, and *Pseudomonas* used in bacterial fertilizers (Brown, 1974).

We observed variability among cultivars and treatments when using *Trichoderma,* but we were able to reproduce our results. It seems possible that practical applications of its properties may decrease use of fungicides, growth regulators, and labor in the greenhouse, hence lowering costs and environmental impact. In view of observed variability, our future research will investigate delivery methods of *Trichoderma* to the rhizosphere.

CHAPTER 4

TWO METHODS OF DELIVERING *TRICHODERMA HARZIANUM* INTO PROPAGATION MEDIUM OF UNROOTED CHRYSANTHEMUM CUTTINGS

Abstract

In previous studies we observed increased shoot and root fresh weights in four cultivars of chrysanthemum [*Dendranthema x grandiflorum* (Ramat.) Kitamura] during 21 days of rooting in media amended with *Trichoderma harzianum* Rifai-isolate T-12 (T-12) at 5 or 25 $g \cdot kg^{-1}$ medium. However, we also noted that 25 $g \cdot kg^{-1}$ sometimes decreased growth even though levels of *Trichoderma* spp. in the medium were similar with the two higher rates of amendment throughout the study. Our objectives in this experiment were to investigate higher rates of T-12 medium amendment for possible phytotoxic effects and to examine an alternative method of delivering the fungus to the medium in an effort to reduce variability of growth enhancement among rates with regard to levels of *Trichoderma* in the medium over time. T-12 was added to the medium either as a powdered peat-bran amendment (0, 5, or 50 g T-12

powder/kg medium) or as alginate pellets (80 or 800 g T-12 pellets/kg medium). All treatments were the same on day 7, but by day 21, applications of 800 g T-12 pellets·kg^{-1} medium increased shoot height. Both pellet treatments gave increased shoot height on day 14 and 21 over all other treatments, which were similar to controls. Amendment with 50g T-12 powder·kg^{-1} medium increased root length, and 80g T-12 pellets·kg^{-1} medium resulted in decreased root dry weight compared to controls.

Introduction

A primary obstacle in commercial use of *Trichoderma* spp. for both disease control and growth enhancement is delivering the fungus to the plant material (Lewis, 1991). Several delivery methods for *Trichoderma* spp. have been investigated. Treatment of seeds or seedpieces with dusts or powders from dried biomass of biocontrol agents is one of the more cost-effective methods of delivery and can be efficient, providing that the fungus remains viable and is rhizosphere competent, or has the ability to colonize the outer root surface (Kommedahl and Windels, 1981).

Dusts, powders, and granular formulations of *Trichoderma* spp. can be broadcast, applied in furrows, or incorporated into the soil (Lewis, 1991). Spores have been delivered in liquid fertilizers (Papavizas, 1985) and seedling gels used for fluid drilling of small seeds (Fischer et al., 1983). Conidial suspensions have also been applied to roots as a

spray (Chang et al., 1986). Finally, a more recent development is the encapsulation of fungal material based on the ability of aqueous sodium alginate to react with certain cations, such as Ca^{++}, to form gels (Lewis and Papavizas, 1985). Incorporation of a nutrient carrier, such as bran, into the solution provides a food base for the fungus inside pellets.

Alginate pellets containing fungal material show promise as a delivery system for several reasons. First, this system is one of the few resulting in the introduced fungi increasing to high log cfu levels (6-10) in media not steamed, fumigated, or otherwise treated with biocides (Lewis, 1991). This factor is important when competition is the mechanism of biocontrol, as is the case with control of *Fusarium oxysporum* Schlecht f. sp. *chrysanthemi* Litt., Armst. and Armst. by *Trichoderma viride* Pers. ex. S.F. Gray (Marois and Locke, 1985). Production of pellets is also relatively simple. In addition, the methodology can be adapted to use fermentor biomass (wet biomass of fungi from fermentation vessels) as the fungal material, eliminating the need for drying and pulverizing. Fermentor biomass is a promising manufacturing system due to rapid production of large fungal quantities containing many chlamydospores, the most effective propagules for reproduction (Papavizas et al., 1985).

In a previous study (MacKenzie et al., 1995), we observed increased root and shoot fresh weight during rooting four chrysanthemum cultivars and in some hard-to-root

cultivars during greenhouse croptime. In our earlier study, levels of *Trichoderma* spp. in media amended with 5 or 25 g T-12/kg were the same although greater than the controls, and we noted that 25 $g \cdot kg^{-1}$ sometimes enhanced growth less than 0 or 5 $g \cdot kg^{-1}$. The objectives of this study were (i) to investigate higher rates than previously tested of T-12 media amendment for possible phytotoxic effects and (ii) to examine an alternative method of delivering the fungus to the medium in an effort to reduce variability of both growth enhancement results and *Trichoderma* levels within the medium over time.

Materials and Methods

T-12 was cultured on peat-bran for two weeks, dried, ground to a powder, and mixed thoroughly with the medium (Paulitz et al., 1986). Alginate pellets containing the above powdered T-12 preparation were made by the methods of Connick (1982), Walker and Connick (1983), as modified by Lewis and Papavizas (1985). Medium viscosity (2%) sodium alginate (Sigma, St. Louis, Mo.) was dissolved in distilled water (20g/750ml) at 40C on a stirring hotplate. Wheat bran (Sigma, St. Louis, Mo.) was added to distilled water (50g/250ml). Containers and contents were autoclaved for 30 min and cooled. The powdered T-12 preparation (30g) was added to the sodium alginate and wheat bran solutions and the mixture was comminuted for 30 sec at high speed and then added dropwise to 1 liter of gellant solution (0.25 M $CaCl_2$, pH 5.4), which was

being stirred magnetically on a stirplate. Each droplet formed a distinct, spherical bead upon entering the solution. Beads were separated from the solution and dried for 24 h in a stream of air at 25C.

Serial dilutions of either homogenized T-12 alginate pellets or T-12 powder were prepared and 1-ml aliquots were spread on an agar medium selective for *Trichoderma* spp. (Elad, et al., 1981). We found that 9.2 log colony forming units (cfu) were produced per gram of T-12 pellets and 10.4 log cfu·g^{-1} of T-12 powder, which was 16 times the number of cfu's from the pellets. Using this ratio, we determined that 80 and 800 g T-12 pellets·kg^{-1} medium should be initially comparable to 5 and 50 g T-12 powder·kg^{-1} medium, respectively, two rates of interest due to previous studies. The number of *Trichoderma* spp. cfu in the rooting medium was similarly monitored during the course of the experiment.

Unrooted cuttings of chrysanthemum 'Dark Bronze Charm', a hard-to-root cultivar, were received from Yoder Brothers (Barberton, Ohio) 19 Oct. 1994. After being sorted for uniformity, cuttings dipped in 7.4mM IBA were inserted into a 1 peat: 1 perlite (v/v) medium amended with T-12 either as a powdered peat-bran amendment (T-12 powder) or alginate pellet preparation (T-12 pellets). Cuttings were inserted into media amended with 0, 5, or 50 g T-12 powder or 80 or 800 g T-12 pellets·kg^{-1} medium. The medium was at 24C using EPDM hot-water bottom heat tubing (Biotherm Co., Petaluma, Calif).

Cuttings were inserted into 5 54 cm X 27 cm plastic rooting trays with drainage holes, within rows, 10 cuttings per row, and placed immediately under intermittent mist in a glasshouse at 24/18C (venting/night set points). Mist intervals were those recommended by the supplier: 10 sec every 5 min for 4 days, every 20 min days 5-8, and every 30 min throughout the rooting period of experiment. Long day (LD) photoperiod was effected by incandescent night interruption from 2200 HR to 0200 HR. Three 100-watt incandescent bulbs spaced 75 cm apart and placed 85 cm above plant canopy supplied 5.8 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ measured at plant canopy level.

Treatments were arranged as a 5 X 3 factorial with five rate/delivery methods of T-12 amendment and three harvest dates using 10 single plant replicates in a completely randomized design. Shoot height, root length, and shoot and root fresh and dry (72 h at 80C) weights, of 10 randomly selected cuttings were measured on each of days 7, 14, and 21. After carefully lifting out each cutting and the surrounding medium with a spatula, we separated shoots from roots 1 to 3 cm above the basal end of the cutting, depending on the cultivar and its tendency to initiate roots along the stem. Shoot height was measured from the base of the cutting to the stem apex. Analysis of variance was used in data analyses (SAS Proc GLM; SAS Institute, Cary, N.C.) and the means were separated by least significant difference (LSD) at $P \leq 0.05$.

Results

Number of *Trichoderma* log cfu ranged from 7.2 to 12.9·g^{-1} oven-dried (72 h at 50C) medium during the experiment (Fig. 9). Although all T-12 treatments were equal at the start of the experiment, 800 g T-12 pellets·kg^{-1} provided the highest fungal colonization of medium followed by 80 g T-12 pellets and 50 g T-12 powder·kg^{-1}. Unexplainably and contrary to previous results (MacKenzie et al., 1995), treatment with 5 g T-12 powder·kg^{-1} medium yielded levels of *Trichoderma* spp. similar after 7 days to those of the control. Fortuitous reinfestation of media by indigenous *Trichoderma* spp. may explain why high levels of cfu were observed in controls not inoculated with T-12. We generally observed higher cfu levels with all treatments than observed in previous experiments.

A T-12 X day interaction occurred for shoot fresh weight and height (Table 2, Fig. 10). No differences occurred among treatments on day 7 for any of the variables (data not shown). Shoot fresh weight was the same in all treatments on day 14, but was increased 52% on day 21 by the 800 g T-12 pellets·kg^{-1} medium treatment. Shoot fresh weight did not increase from day 14 to 21, except for in plants treated with 800 g·kg^{-1} T-12 pellets.

On day 14, shoot height was increased 8% by treatment with 80 g T-12 pellets·kg^{-1} medium compared to 0, 5, or 50 g T-12 powder·kg^{-1} medium; treatment with 800 g T-12 pellets·kg^{-1}

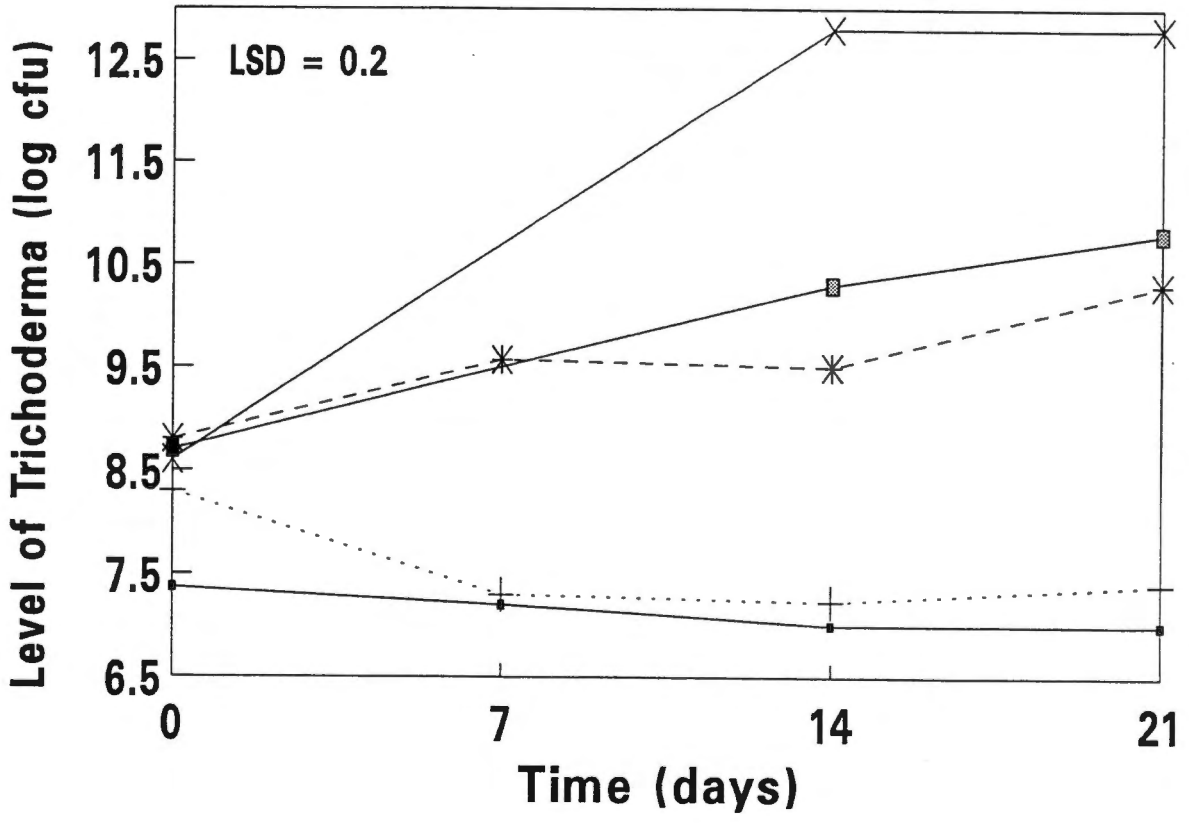


Figure 9. Levels of *Trichoderma* spp. in log colony forming units (cfu) measured at days 0, 7, 14, and 21 during rooting of chrysanthemum 'Dark Bronze Charm' propagated in media amended with 0 —▪— , 5 —+— , or 50 —✳— g T-12 powder or 80 —▩— or 800 —✳— g T-12 pellets·kg^{-1} medium.

Table 2. Analysis of variance for effect of day and T-12 amendment on shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, shoot height, and root length of chrysanthemum 'Dark Bronze Charm'.

Source	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Shoot height	Root length[z]
Day	***	***	***	***	***	***
T-12	***	NS	***	***	***	**
Day * T-12	**	NS	NS	NS	***	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.0001, respectively
[z]Data for days 14 and 21 only

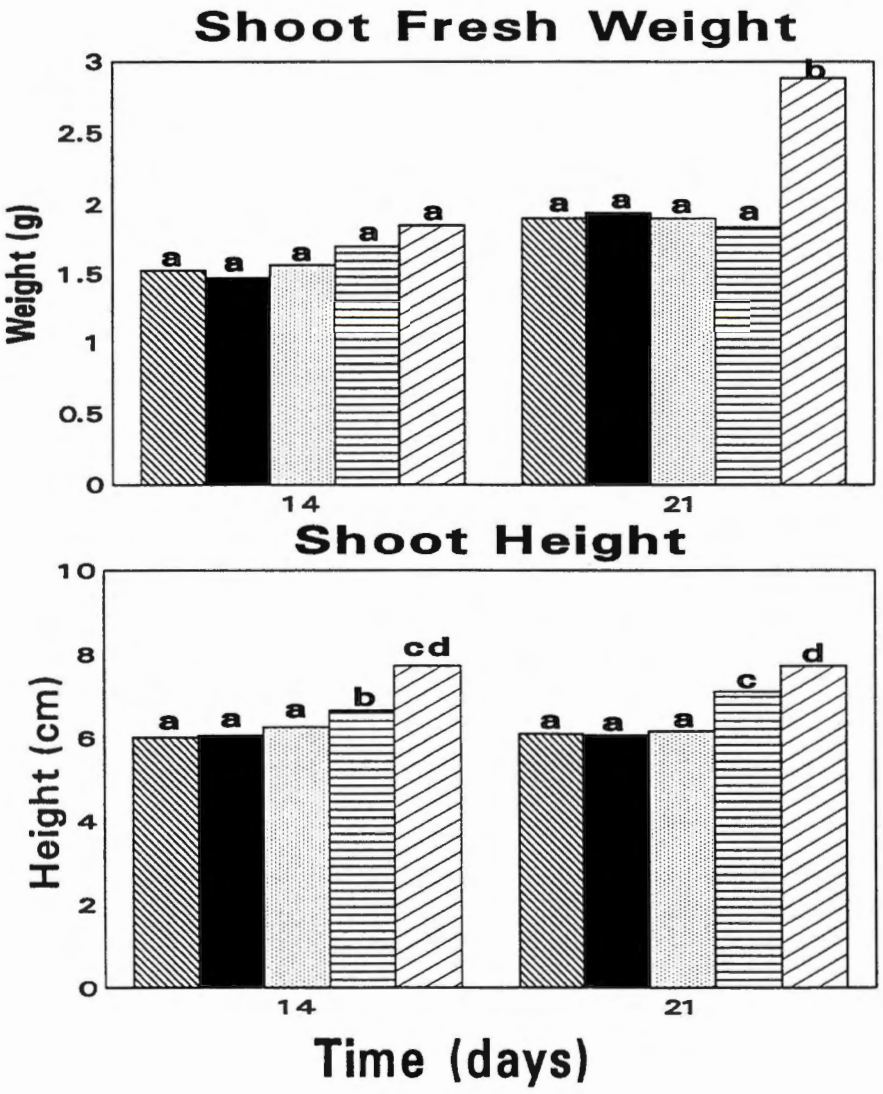


Figure 10. 'Dark Bronze Charm' chrysanthemum shoot fresh weight and shoot height following amendment of growing medium with *Trichoderma harzianum* using five rate/delivery methods of amendment (0 , 5 , or 50 g T-12 powder or 80 or 800 g T-12 pellets·kg^{-1} medium), and three sampling times (7, 14, and 21 days). Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

medium increased shoot height 10% compared to 80 g T-12 pellets·kg^{-1} medium and 18% compared to 0, 5, or 50 g T-12 powder·kg^{-1} medium. The same trend held for day 21, with 80 g T-12 pellets·kg^{-1} medium increasing shoot height by 14% over 0, 5, or 50 g T-12 powder·kg^{-1} medium and 800 g 8% compared to 80 g T-12 pellets·kg^{-1} medium and 21% compared to 0, 5, or 50 g T-12 powder·kg^{-1} medium. Plants treated with 0, 5, or 50 g·kg^{-1} T-12 powder or 800 g·kg^{-1} T-12 pellets did not increase in height from day 14 to 21. The 800 g T-12 pellets·kg^{-1} medium treatment increased shoot dry weight an average of 18% during the study (data not shown).

T-12 powder at 50g·kg^{-1} increased root length 28% over other treatments (Table 2, Fig. 11). Root dry weights in all T-12 treatments were the same as controls, except in 80 g·kg^{-1} T-12 pellets, where a 22% reduction was observed (Table 2, Fig. 11).

Discussion

We found that amending the growing medium of unrooted chrysanthemum cuttings with T-12 as alginate pellets increased shoot characteristics but not rooting. Treatment with 800 g T-12 pellets·kg^{-1} produced plants having the greatest shoot fresh weight and height. Root length was increased by T-12 as a powdered peat-bran amendment at 50g·kg^{-1}. Contrary to a previous study (MacKenzie et al., 1995), we saw no increased

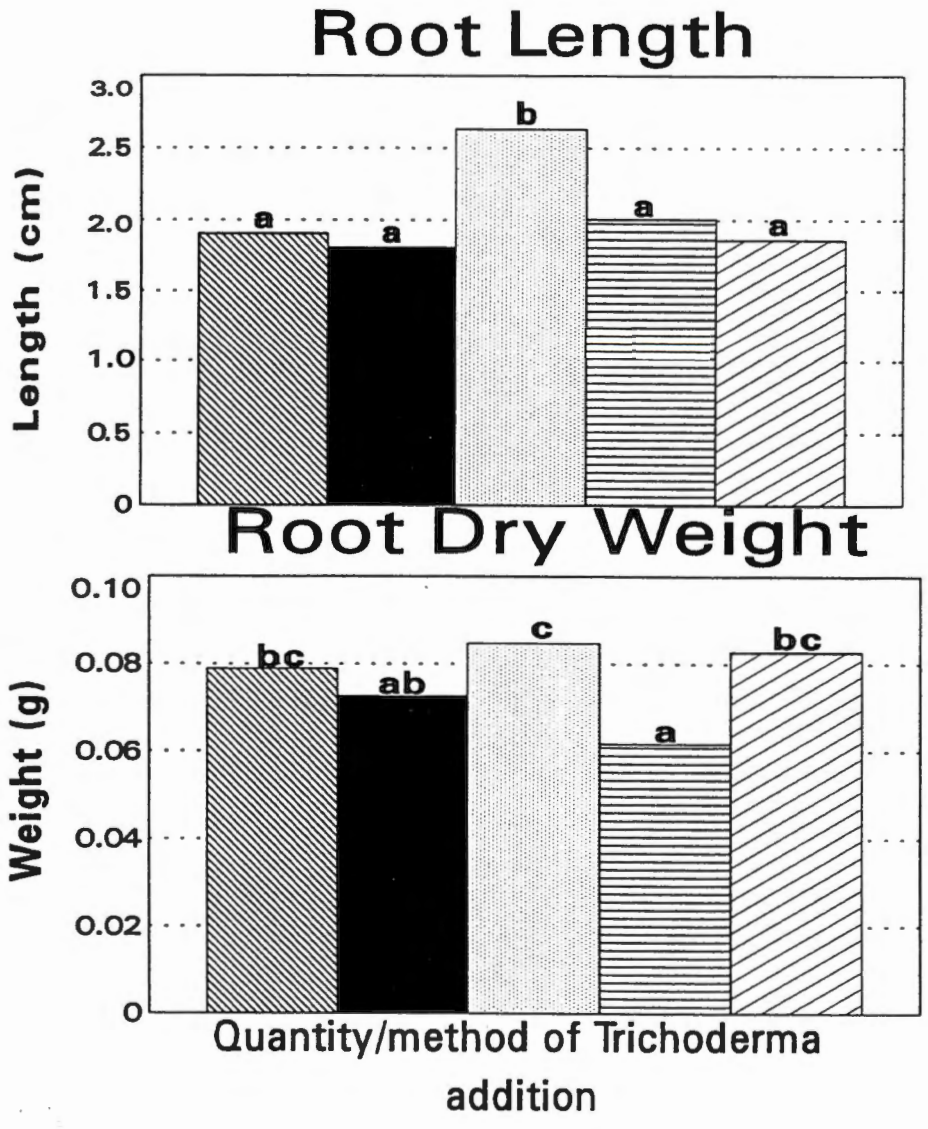


Figure 11. 'Dark Bronze Charm' chrysanthemum root length and root dry weight following amendment of growing medium with *Trichoderma harzianum* using five rate/delivery methods of amendment (0 , 5 , or 50 g T-12 powder or 80 or 800 g T-12 pellets·kg^{-1} medium). Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

root and shoot fresh weights with 5 $g \cdot kg^{-1}$ T-12 powder, but during the present experiment the medium amended with this rate yielded cfu levels similar to those of the control, and consistent with this result, no data indicated differences between the 0 and 5g T-12 powder$\cdot kg^{-1}$ medium. However, variability among trials conducted to investigate the action of *Trichoderma* spp. has occurred previously. Chang et al. (1986) could not replicate results of increases up to 247% in dry weight of radish propagated in the presence of *T. harzianum* (isolate T-95) reported by Baker et al. (1984). They also saw no significant differences in beans (*Phaseolus vulagaris* L. 'Brittle Wax') growing in media amended with the fungus. Paulitz et al. (1986) saw increased shoot dry weight of radish with *Trichoderma* treatment in one experiment that was not reproduced in a subsequent study.

We saw much higher log cfu levels in this experiment than previously, possibly because we inoculated the peat-bran substrate with a fresh culture of T-12. Variations in production of the selective medium used for quantifying levels of *Trichoderma* spp. in the medium may also have caused cfu levels to be greater. In comparison, Lewis and Papavizas (1985) amended a natural soil medium with 0.5% (v/v) pellets containing 5 to 7 log cfu$\cdot g^{-1}$ pellet and obtained 5 to 6 log cfu$\cdot g^{-1}$ medium after 3 weeks. However, Paulitz et al. (1986) noted that different ratios of medium components cause various

results, and they saw decreased shoot dry weight after 3 weeks if mixes contained a high percentage of vermiculite.

No phytotoxic effects to shoot growth were observed at higher rates of amendment, although Baker (1988) reported stunting of eggplant at 8 log cfu·kg^{-1} medium. However, we did note that in the 800 g·kg^{-1} T-12 pellets medium, roots seemed abnormally short and thickened, but neither root length nor root fresh weight differed significantly from controls. The alginate pellets yielded higher log cfu levels than the powdered T-12 preparation, confirming other reports (Lewis and Papavizas, 1985) of this delivery method's efficacy.

CHAPTER 5

AN INVESTIGATION OF *TRICHODERMA HARZIANUM* AS A GROWTH ENHANCER OF CUT AND POT FLORICULTURE CROPS

Abstract

The biological control fungus *Trichoderma harzianum* Rifai has enhanced growth of several floriculture crops, which might improve the efficiency of some greenhouse production schedules while providing the additional benefit of controlling root rot pathogens. We investigated the possibility of enhancing growth and reducing croptime of several specialty cut flower species and poinsettias (*Euphorbia pulcherrima* Willd ex. Klotzch) by applying *T. harzianum*-isolate T-12 (T-12) as a medium amendment. In the specialty cut flower study, levels of *Trichoderma* spp. in medium amended with T-12 stayed consistently higher by two log units than non-amended medium, but negligible growth enhancement was evidenced. We saw increases in height due to treatment in zinnia (*Zinnia elegans* Jacq.) and dill (*Anethum graveolens* L.) and increased flower number in zinnia. We saw fluctuations in levels of *Trichoderma*

spp. in unamended medium during the poinsettia study, which was correlated to fungicidal drench treatments. Poinsettias treated with T-12 had greater heights or widths than controls periodically throughout the study, but these differences may not be of commercial interest.

Introduction

Enhanced growth of plants in the presence of *Trichoderma* spp. has been well-documented. Fungal amendment of the growing medium has resulted in increased fresh and dry weight and hastened flowering of such floriculture crops as periwinkle (*Catharanthus roseus* (L.) G. Don), alyssum (Lobularia maritima (L.) Desv.), petunia (*Petunia Xhybrida* Hort. Vilm.-Andr.), and marigold (*Tagetes erecta* L.) (Chang et al., 1986; Baker et al., 1986). Because *Trichoderma* spp. have been used for biological control of such root rot pathogens as *Sclerotium rolfsii* Sacc., *Rhizoctonia solani* Kühn (Elad et al., 1980), and *Pythium aphanidermatum* (Edson) Fitz. (Sivan et al., 1984), use of this fungus in the greenhouse and field might provide an alternative to fungicides, hence lowering costs and environmental impact. The overall objective of our experiment was to investigate the possibility of enhancing growth and reducing croptime of several floriculture crops during greenhouse production by applying *T. harzianum*-isolate T-12 (T-12) as a media amendment.

Armitage defines specialty cut flowers as "any cut

flowers other than roses, carnations, and chrysanthemums" (1992). The importance of such flowers increased 56% between 1987 and 1990 (USDA 1989, 1991), approaching an estimated wholesale value of $173 million (USDA, 1991). However, efficiency in greenhouse production scheduling is essential to crop profitability. Riall et al. (1988) reported that production of specialty cut flowers was economical in the field but not in the greenhouse.

Poinsettia is the most important flowering pot plant in the United States (Hartley, 1992). Over 41 million pots with a combined wholesale value of more than $147 million were sold in 1988 by 28 states (Hartley, 1992).

Materials and Methods

General. T-12 was cultured on peat-bran for two weeks, dried, ground to a powder, and mixed thoroughly with the medium (Paulitz et al., 1986). The number of colony forming units (cfu) of T-12 was monitored using an agar medium selective for *Trichoderma* spp. (Elad et al., 1981).

Plants were grown in a glasshouse at 24/18C (venting/night set points). Unless otherwise noted, irrigation was as needed by an automatic drip system. Analysis of variance was used in data analyses (SAS Proc GLM; SAS Institute, Cary, N.C.) and the means were separated by least significant difference (LSD) at $P \leq 0.05$.

Specialty cut flowers. Four cultivars of specialty cut flowers and one herb were propagated from seed and transplanted as seedlings (Table 3) into 0.1-liter cell packs containing soilless medium (ProMix BX, Premier Brands, New Rochelle, N.Y.) amended with 0 (control) or 2 $g \cdot kg^{-1}$ medium T-12. Plants were watered as needed by hand and fertilized at each irrigation with 200 $mg \cdot liter^{-1}$ N using Peter's water soluble 20N-4.4P-16.6K fertilizer (W.R. Grace, Cambridge, Mass.). When roots reached the bottom of the cell pack, plants were transplanted into 0.4-liter pots containing unamended ProMix BX and thereafter watered and fertilized automatically.

Each week, number of flowers and internodes and plant height from the top of the container to stem apex were measured. These characteristics were analyzed separately for each species. The experiment was a completely randomized with repeated measures design, with control and T-12 treatments being assigned to individual plants, which were then analyzed seven times over a four week period. A repeated measures model (SAS Institute, Cary, N.C.) was first used to test for sphericity. A split-plot approach was found to be appropriate so this was used since it is not affected by missing data. Least square means were compared using t-tests at $P \leq 0.05$.

Poinsettia. Three cultivars ('Celebrate', 'Freedom', and 'Lemon Drop') of poinsettia were received from Paul Ecke Poinsettias (Encinitas, Calif.) 2 Sept. 1993 as rooted cuttings and transplanted into 0.4-liter pots containing

Table 3. Specialty cut flower and herb species (common name) grown from seed with sow and transplant dates.

Species	Sow date	Transplant date
Anethum graveolens (Dill)	22 Mar. 1993	19 Apr. 1993
Cosmos bipinnatus 'Frühwunder' (Cosmos)	29 Mar. 1993	19 Apr. 1993
Liatris spicata 'Floristan Violet' (Liatris)	1 Feb. 1993	24 Mar. 1993
Platycodon grandiflorus 'Fuji Blue' (Balloon Flower)	8 Feb. 1993	19 Apr. 1993
Zinnia elegans 'Pulcino Mischung' (Zinnia)	22 Mar. 1993	19 Apr. 1993

ProMix BX amended with 0 or 5 $g \cdot kg^{-1}$ T-12.

We examined the effects of various drench treatments on enhanced growth mediated by T-12 in an attempt to define the mechanism of growth enhancement, either biological control of minor root rot pathogens or production of a growth-promoting substance. Plants treated with T-12 were drenched with Subdue 2E (Metalaxyl, Ciba-Geigy Corporation, Greensboro, N.C.) at the rate of 80 $\mu l \cdot liter^{-1}$ water. Controls were drenched with either Subdue 2E (S-control) at the above rate or Banrot 40WP (B-control) (Ethazol and Thiophanatemethyl, Grace Sierra Crop Protection Company, Milpitas, Calif.) at the rate of 600 $mg \cdot liter^{-1}$ water. Drench treatments were applied every three weeks beginning 7 Sept. 1993.

During the first month, plants were fertilized at each irrigation with 400 $mg \cdot liter^{-1}$ N using Peter's water soluble 20N-4.4P-16.6K fertilizer. After one month, the rate was reduced to 200 $mg \cdot liter^{-1}$ N. Plants were pinched 9 Sept. 1993 down to the third internode, leaving five leaves. Plant height (from the top of the pot) and width (across the widest point) were measured every week. We measured the width of the widest bract (across the widest point), number of flowers with and without visible stamens, fresh, and dry (72 h at 80C) weight of the plant material above soil level at harvest on 11 Jan. 1994.

Results

Specialty cut flowers. The log cfu levels in medium amended with 2 $g \cdot kg^{-1}$ T-12 were approximately 6 during the study, with the exception of a peak at 8.5 during week 11 (Fig. 12). Controls ranged between 3 and 4 log cfu and then declined to 0 after week 10.

Few characteristics were enhanced by T-12 amendment in the study but results varied with species (Table 4). We observed an 18% increase in height of dill treated with T-12. Zinnias grown in the presence of the fungus had 48% more flowers and were 18% taller than controls.

Poinsettia. T-12 amended medium contained 5.4 to 6 log cfu while both controls fluctuated between 3 and 5.4 during the study (Fig.12). The peak densities of *Trichoderma* spp. in controls occurred after drench treatments. We saw a two-way interaction between treatment and time for plant height and width (Fig. 13). The two control treatments were the same height on all weeks. On weeks 13, 14, and 17, T-12 treatments were an average of 2.1 cm taller than both controls. T-12 plants were 1.2 cm taller than B-controls on week 3, and 0.5 cm taller than S-controls on week 4.

T-12 and B-control treatments were the same and on average 2.5 cm wider than S-controls on weeks 8 and 13. T-12 was the same as control treatments on week 11, but the B-controls were 3.2 cm wider than S-controls. T-12 plants were 3.0 cm wider than S-controls, and the control treatments were

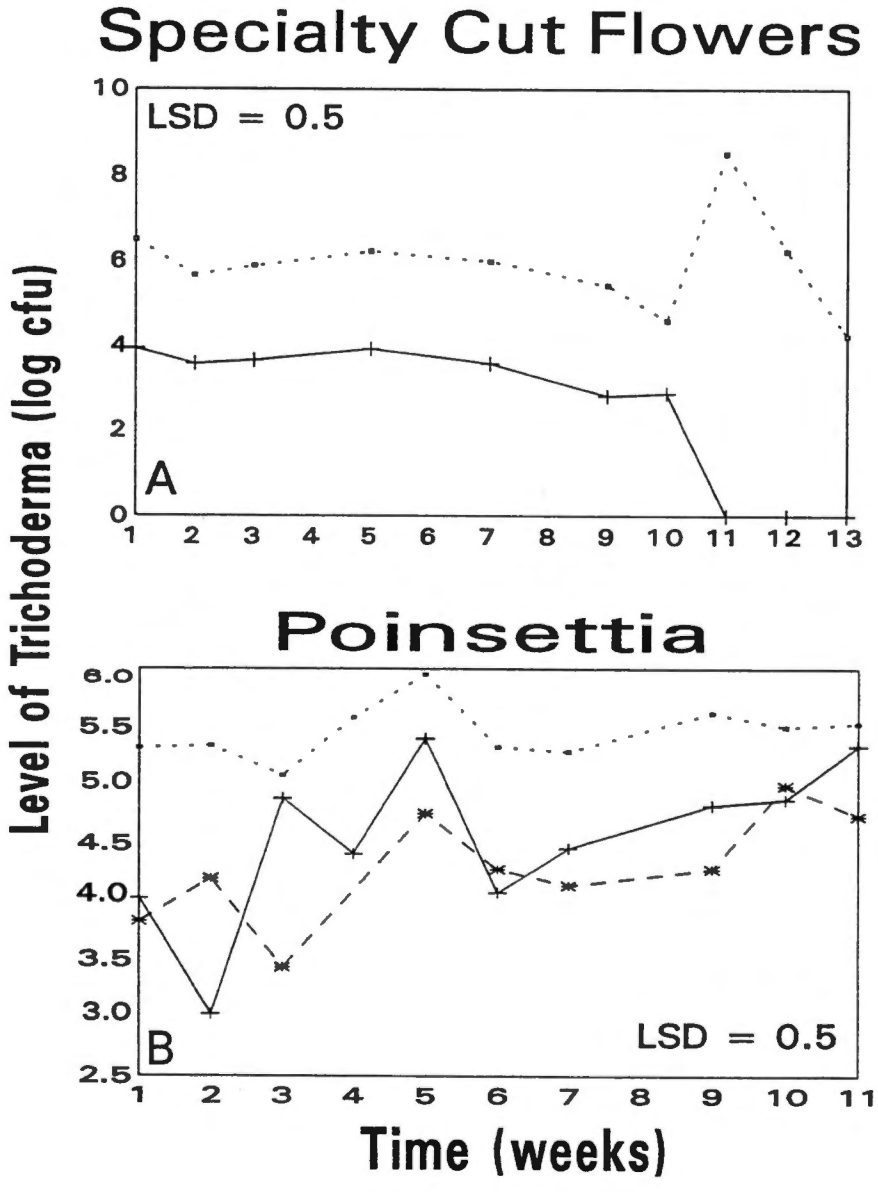


Figure 12. Levels of *Trichoderma* spp. in log colony forming units (cfu) contained in the growing medium (A) amended with 0 -+- or 2 ... g T-12·kg^{-1} during time (weeks) of the specialty cut flower study and (B) amended with 5 g T-12·kg^{-1} and drenched with Subdue ·•· , or untreated controls drenched with Subdue -*- or Banrot -+- for 14 weeks of the poinsettia study.

Table 4. Analysis of variance for effect of *Trichoderma harzianum* (T-12) medium amendment on number of blooms, plant height, number of nodes, and days to flower for five species of specialty cut flower and herb crops.

Plant	Number of blooms	Plant height	Internode number	Days to flower
Balloon flower	·	NS	NS	NS
Cosmos	NS	NS	NS	NS
Dill	·	***	·	·
Liatris	·	NS	·	·
Zinnia	*	*	NS	NS

NS, *, **, Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.
· = Data not applicable to this species.

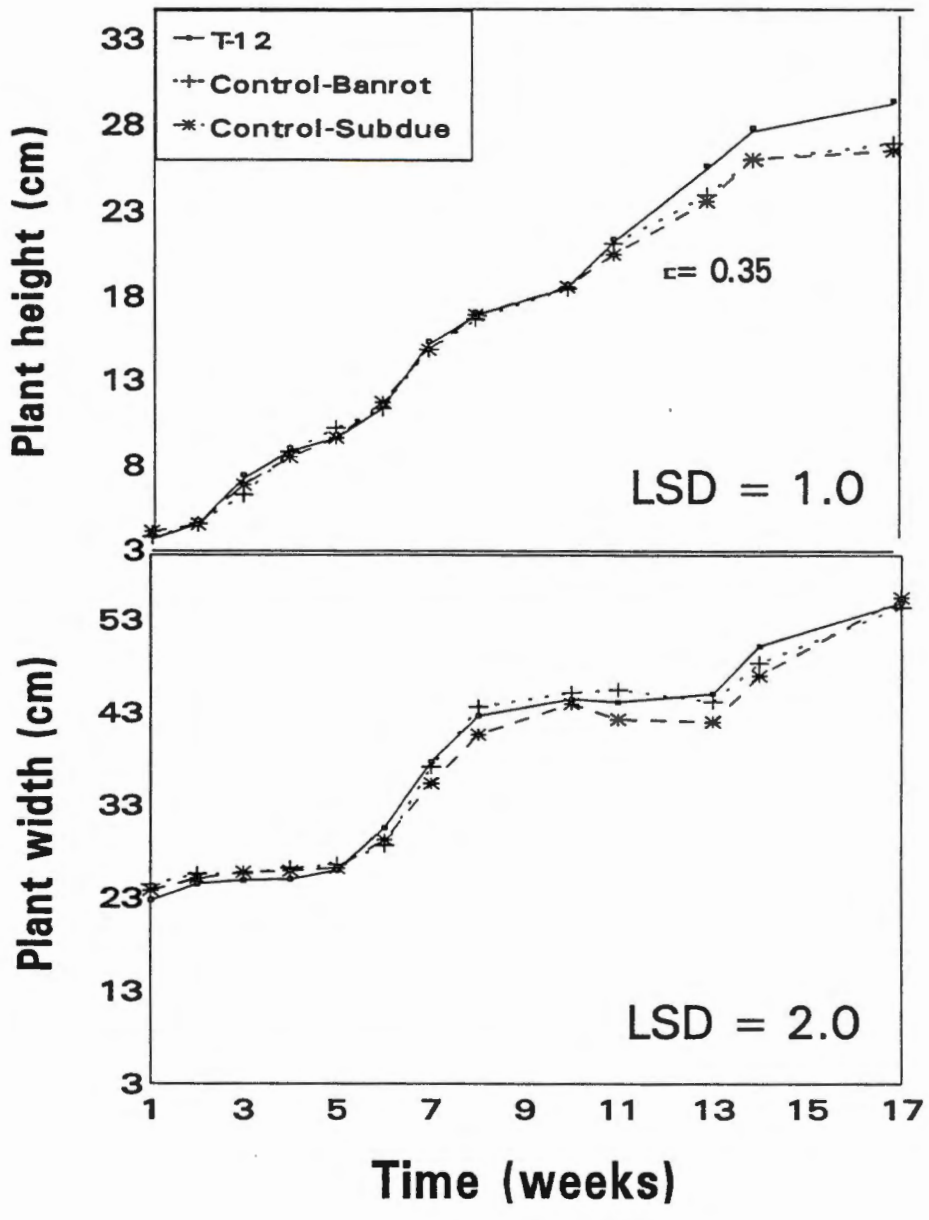


Figure 13. Plant height and width of 3 poinsettia cultivars treated with T-12 and drenched with Subdue -•- , or untreated controls drenched with Subdue -*- or Banrot -+- for 14 weeks.

the same on week 14.

We detected no treatment differences in width of largest bract, number of flowers, fresh, or dry weight at harvest.

Discussion

Levels of *Trichoderma* spp. were always greater in the media amended with T-12. However, we did see low levels of *Trichoderma* in controls due to the presence of indigenous species. We suspected that drench treatments in the poinsettia study caused increased levels of native *Trichoderma* spp. by reducing competition from other microorganisms. Fluctuations were most pronounced in controls drenched with Banrot, a fungicide active *Pythium*, *Phytophthora*, and *Rhizoctonia* as well as *Trichoderma* spp.

Amending the growing medium with T-12 enhanced growth characteristics of zinnia, dill, and poinsettia. Differences between treatments were visible in zinnia and dill, both having an 18% height increase. Several specialty cut flower crops did not respond to the fungus and we did not observe hastened flowering.

The 1 to 3 cm difference in height or width among poinsettia treatments on various weeks was not readily evident, and the lack of treatment differences in harvest data indicated that such increases would not be of commercial importance. Treatment differences were not sufficiently consistent to aid in determining if *T. harzianum* increased

growth by controlling root rot pathogens or producing a growth-promoting substance.

Optimization of rate and delivery methods will be necessary before *Trichoderma* is of commercial value to industry. We have obtained reproducible results using T-12 as a rooting media amendment to enhance rooting and shoot growth of chrysanthemum (MacKenzie, et al., 1995). However, we used higher rates of amendment than were used in the work reported here. Crops may respond differently to various rates of the fungus. Improving delivery of *Trichoderma* spp. to plant material should also be a consideration. With improvement of methodology, the fungus may be a valuable component of sustainable agriculture, both as an alternative to fungicides and as a growth promoter.

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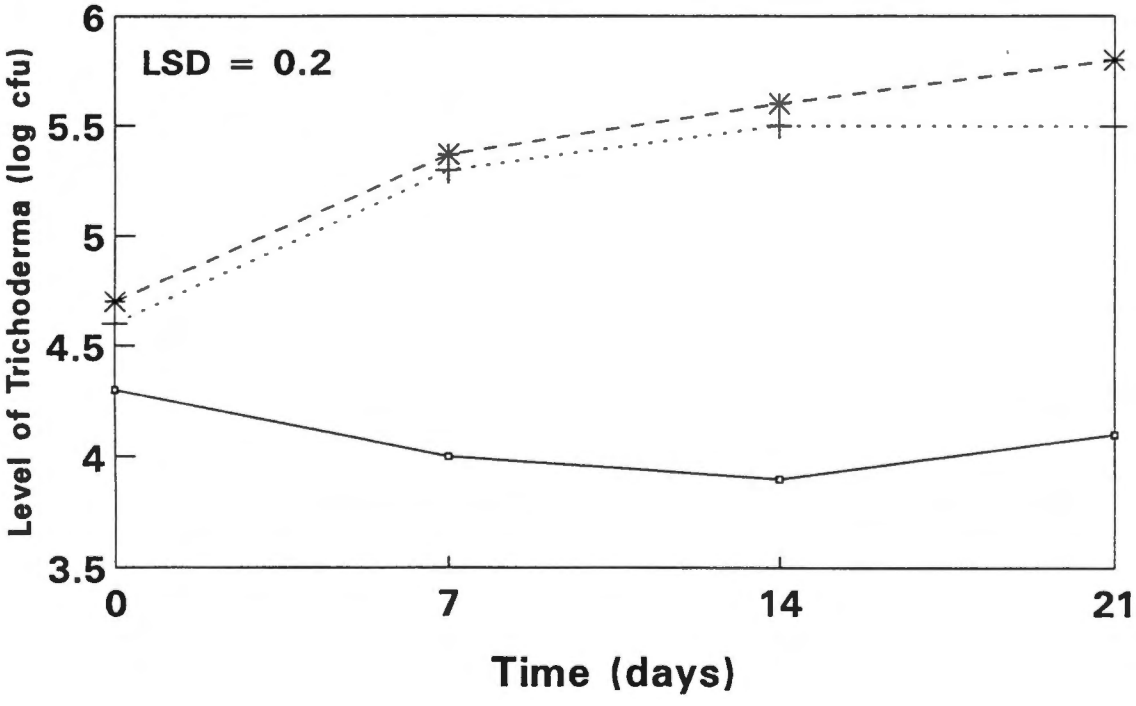
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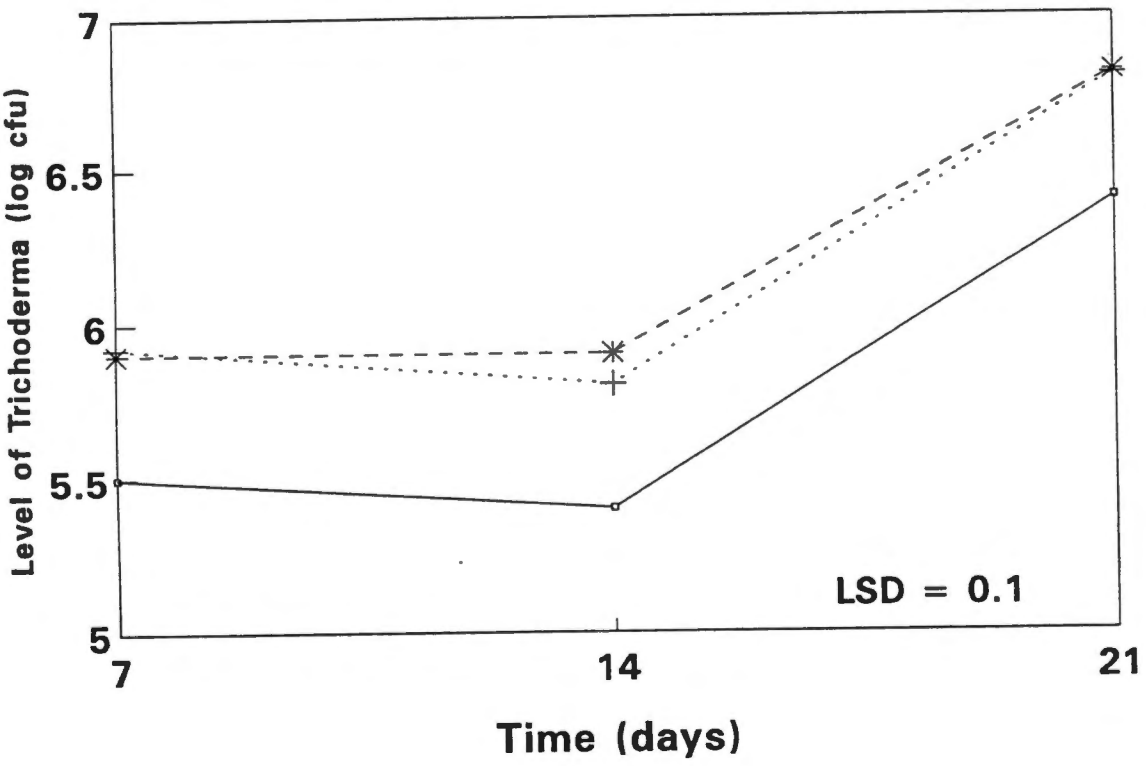
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APPENDIX



Appendix 1. Levels of *Trichoderma* spp. in media amended with 0 -o- , 5 -+- , or 25 -*- g T-12·kg^{-1} measured in log colony forming units (cfu) for Chapter 2 data on days 7, 14, and 21.



Appendix 2. Levels of *Trichoderma* spp. in media amended with 0 -o- , 5 -+- , or 25 -*- $g \cdot kg^{-1}$ measured in log colony forming units (cfu) for Chapter 3 data on days 7, 14, and 21.

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Amy J. MacKenzie was born Amy Kaye Jones on 16 December, 1968 in Kingsport, TN. She graduated from Sullivan South High School 5 June, 1987 and began attending the University of Tennessee the following Fall Quarter. She was a flag corp member of the Pride of the Southland Marching Band Fall, 1987, studied at the University of Bonn, Germany 1989/1990 as an exchange student, was elected Phi Beta Kappa in 1990, and graduated *summa cum laude* with a Bachelor of Science in Biology/German with a minor in Biochemistry 10 May, 1991. During 1991/1992, she was a senior research technician in the Racheff Chair of Excellence of the Department of Ornamental Horticulture and Landscape Design (OHLD) at the University of Tennessee, Knoxville. In January, 1993 she began graduate studies as a graduate research/teaching assistant. In May, 1995 she received a Master of Science degree in OHLD with a minor in Plant Pathology from the University of Tennessee, Knoxville.

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