

Enhanced Root and Shoot Growth of Chrysanthemum Cuttings Propagated with the Fungus *Trichoderma harzianum*

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Abstract. *Trichoderma harzianum* Rifai, a fungus that controls soilborne pathogens, 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 ×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'). Adding the *T. harzianum* amendment at both rates tested increased root and shoot fresh weights during 21 days of rooting, relative to the control. Supplementary treated cuttings were transplanted into nontreated 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'.

Trichoderma species have been used extensively for biocontrol of soilborne pathogens (Chet and Baker, 1981; Sivan et al., 1984). These fungi also may 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 considered easy to root ('Davis' and 'White Marble') were received as nonrooted 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 1*H*-indole-3-butanoic acid (IBA) were inserted into a 1 peat : 1 perlite (v/v) medium amended with 0, 5, or 25 g *T. harzianum* (isolate T-12) peat-bran amendment/kg medium. The medium was at 24C using EPDM hot-water, bottom-heat tubing (Biotherm Co., Petaluma, Calif.). *Trichoderma 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 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 10⁵ to 10⁶/g oven-dried (72 h at 50C) medium during the experiment.

Cuttings were inserted into 12 53 × 27-cm plastic rooting trays with drainage holes, within five 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 the experi-

ment. Long day (LD) photoperiod was effected by incandescent night interruption from 2200 to 0200 HR. Three 100-W incandescent bulbs spaced 75 cm apart and placed 85 cm above the plant canopy supplied 5.8 μmol·m⁻²·s⁻¹ measured at plant canopy level. Subsequently, plants received natural short day (SD).

Treatments were arranged as a 3 × 4 × 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 nontreated 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 Inst., Cary, N.C.), and the means were separated by least significant difference 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 amendment rate × time interaction occurred during the 21 days of rooting. Root fresh weights were similar 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·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.

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Rooting in medium amended with either amount of *Trichoderma* was similar for the four cultivars on day 21.

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 \times time interaction. On day 7, all treatments were similar, with the exception of the 25 g·kg⁻¹ amendment of 'Dark Bronze Charm', which had a 19% and 24% lower fresh weight than the 5 and 0 g·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·kg⁻¹ of *Trichoderma*, while 'Davis' and 'Golden Bounty' responded best to 25 g·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·kg⁻¹ of *Trichoderma*, and 18% when treated with 25 g·kg⁻¹. 'Davis' had increased shoot fresh weights with both the 5 and 25 g·kg⁻¹ treatments, which were statistically the same. 'Golden Bounty' and 'White Marble' had increases only when treated with the 25 g·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·kg⁻¹ were similar and 20% taller than the controls (Fig. 3). At 5 g·kg⁻¹, the amendment increased shoot dry weight by 21% over the control, while 25 g·kg⁻¹ had no effect. At 5 g·kg⁻¹, *Trichoderma* increased root fresh and dry weights over those of the control and 25 g·kg⁻¹, which did not differ significantly. At midpoint, 'Golden Bounty' was 15% taller, had five 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·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* × *hybrida* 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·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. T-12 levels ranged from 10⁵ to 10⁶ cfu/g soilless medium, a density that effectively mediates biocontrol and enhances growth (Liu and Baker, 1980; Windham et al., 1986).

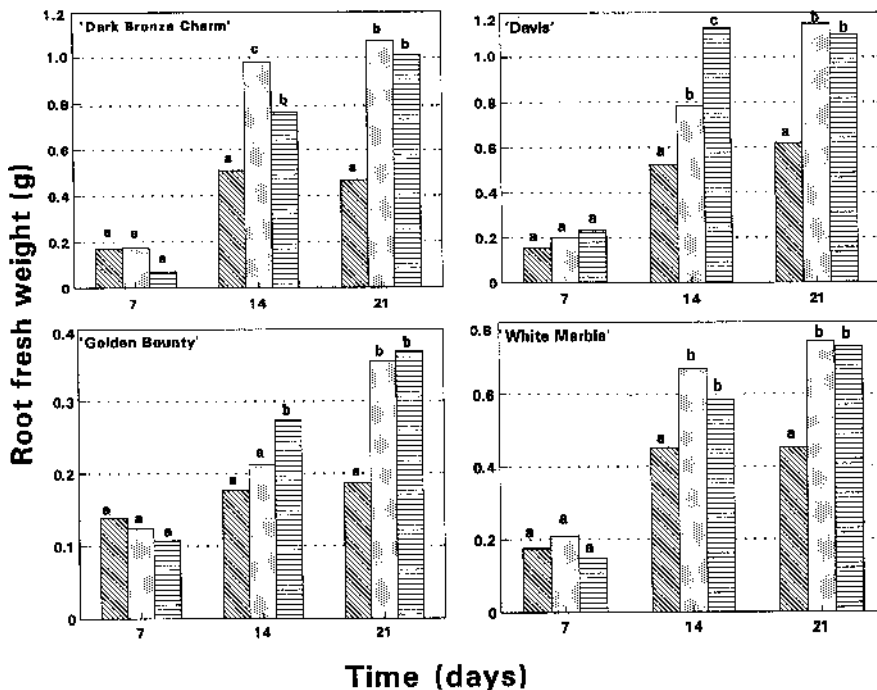


Fig. 1. 'Dark Bronze Charm', 'Davis', 'Golden Bounty', and 'White Marble' chrysanthemum root fresh weight following amendment of soilless growing medium with *Trichoderma harzianum* at 0, 5, or 25 g·kg⁻¹, and at three sampling times (7, 14, and 21 days). Mean separation using LSD test ($P \leq 0.05$).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

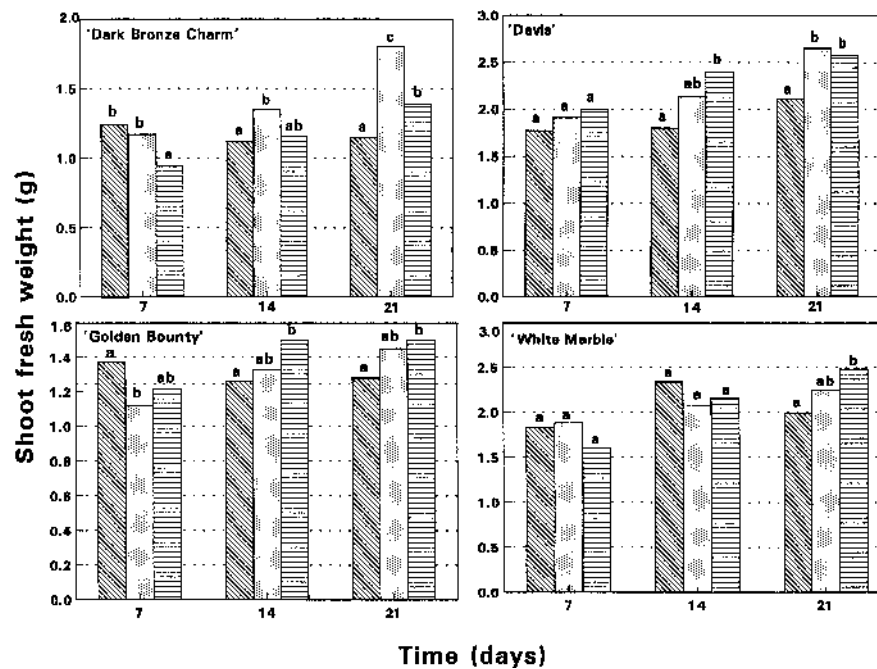


Fig. 2. 'Dark Bronze Charm', 'Davis', 'Golden Bounty', and 'White Marble' chrysanthemum shoot fresh weight following amendment of soilless growing medium with *Trichoderma harzianum* at 0, 5, or 25 g·kg⁻¹, and at three sampling times (7, 14, and 21 days). Mean separation using LSD test ($P \leq 0.05$).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

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 the fungus, and therefore respond more slowly.

'Dark Bronze Charm', a hard-to-root cultivar, had lower root and shoot fresh weight in

the presence of *Trichoderma* at 25 g·kg⁻¹ than at 5 g·kg⁻¹ during the 21 days of rooting. By midpoint to flowering, growth was similar to that of the controls. The mechanism of enhanced growth by *T. harzianum* may be production of 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

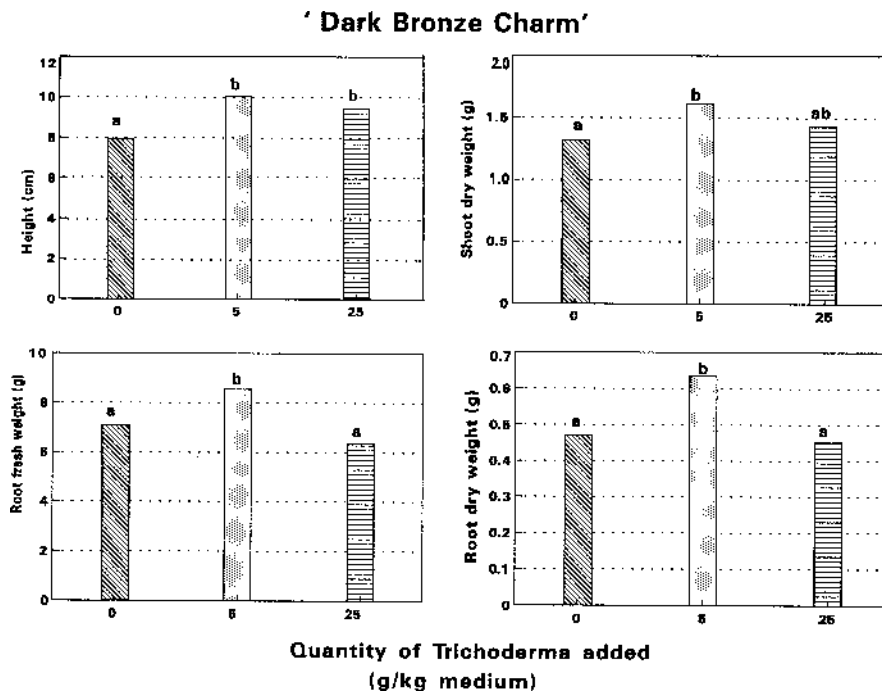


Fig. 3. 'Dark Bronze Charm' chrysanthemum height, shoot dry weight, root fresh weight, and root dry weight following amendment of soilless growing medium with *Trichoderma harzianum* at 0, 5, or 25 g·kg⁻¹ midway from transplanting cuttings into nontreated growing medium to flowering. Mean separation using LSD test ($P \leq 0.05$). The data are the means of 10 replications.

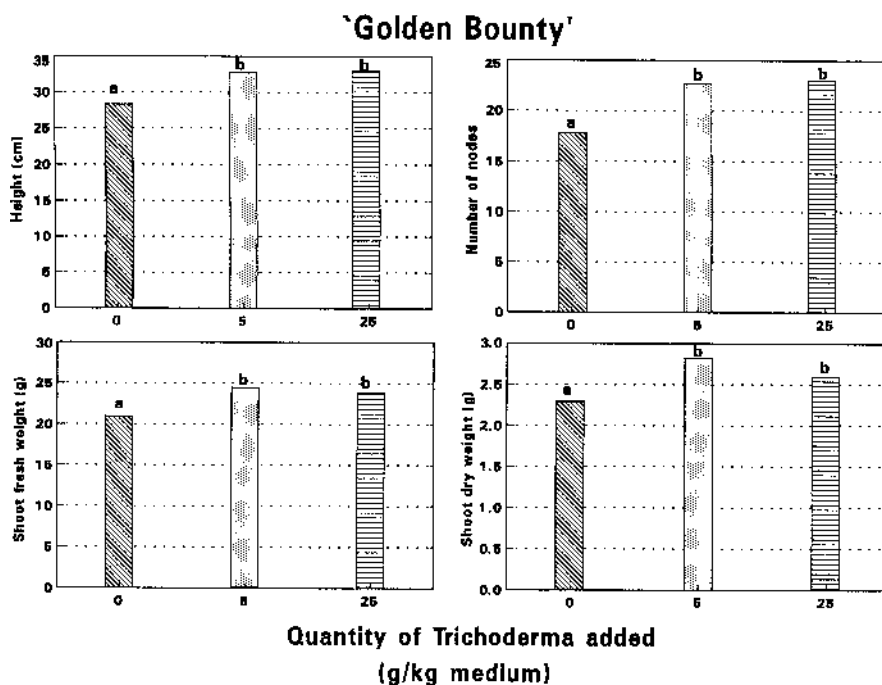


Fig. 4. 'Golden Bounty' chrysanthemum height, number of internodes, shoot fresh weight, and shoot dry weight following amendment of soilless growing medium with *Trichoderma harzianum* at 0, 5, or 25 g·kg⁻¹ midway from transplanting cuttings into nontreated growing medium to flowering. Mean separation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

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·kg⁻¹ compromised growth. Root and shoot fresh weights at 25 g·kg⁻¹ were less than at 5 g·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. One species of *Trichoderma* (*T. koningii* Oudem) produces growth-regulating substances (Cutler et al., 1989, 1991). 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.

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