Cultivar Specific Effects of Mycorrhizal Fungi on the Rooting of Miniature Rose Cuttings[1]

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Abstract

The benefits from root colonization by mycorrhizal fungi are thought to be highest when colonization occurs as early as possible during plant growth. We assessed whether addition of VA mycorrhizal fungi (VAMF) inoculum into rooting medium during cutting propagation would increase the quantity of rooting and the quality of rooted cuttings for five different cultivars of miniature roses (*Rosa* spp.). Four weeks after cuttings were stuck, the number of cuttings with roots for two cultivars that normally take longer to root, increased with addition of VAMF inoculum into the rooting medium. The combination of hormone treatment (IBA and NAA) and VAMF inoculum in the rooting medium increased the number of rooted cuttings and the number of roots per cutting for three cultivars when compared to cuttings that only received hormone treatment. Increases in root initiation and root growth of cuttings rooted in medium containing VAMF inoculum were not always associated with increased levels of root colonization by VAM fungi. Our results indicate that although adding VAMF inoculum into the rooting medium does not always increase root initiation, in some cultivars the combination of VAMF inoculum and rooting hormones can increase root initiation and potentially increase the quality of rooted cutting produced.

Index words: vesicular-arbuscular mycorrhizal fungi, VAM, *Glomus intraradices*, rooted cuttings.

Species used in this study: miniature roses (*Rosa* spp. L 'Jolly Cupido', 'Candy Sunblaze', 'White Miniwonder', 'Cherry Cupido', 'Orange Cupido').

Chemicals used in this study: Wood's Hormone Solution (1.03% indol-3-butyric acid and 0.66% 1-napthalene acetic acid).

Significance to the Nursery Industry

Although commercially produced inoculum of VA mycorrhizal fungi (VAMF) is readily available to horticulturists, cultivar specific responses to inoculation, and the optimal time for using inoculum during vegetative propagation, are unclear. Our results showed that addition of VAMF inoculum into the rooting medium of miniature roses increased the amount of rooted cuttings for cultivars that normally take longer to root and increased the number of roots and root growth on several cultivars. Increases in root initiation and growth in response to adding VAMF inoculum into the rooting medium can decrease the amount of time for cuttings to attain an adequate amount of roots for transplanting and increase the quality of rooted cutting obtained. Our results also showed that increases in root initiation and root growth on cuttings rooted in medium containing VAMF inoculum were not always associated with increased levels of root colonization by VAM fungi. The response of cuttings to VAMF inoculum may not solely be a result of mycorrhizal fungi in the inoculum, but could be a result of coincidental inoculation with bacteria associated with the spores, root fragments, and carrier substrates in the VAMF inoculum. Cultivar specific responses to adding VAMF inoculum into the rooting media suggests that combinations of cultivars, hormone applications, and VAMF inocula should be tested before VAMF inoculum is used on a large scale across all cultivars of a crop.

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Introduction

Mycorrhizae are symbiotic associations between plant roots and certain soil fungi (25). The potential for root colonization by mycorrhizal fungi to enhance plant productivity is well recognized (5, 10). Plants with mycorrhizae are potentially more effective at nutrient and water acquisition (1, 2, 3, 14, 24), less susceptible to disease (15), and can be more productive under certain stressful environmental growing conditions (17) than plants without mycorrhizae.

Vesicular-arbuscular mycorrhizal fungi (VAMF) are one type of mycorrhizal fungi that are commonly associated with the roots of horticultural crops. Optimal uses for commercially available VAMF inoculum have not been well defined. One common question is when to apply inoculum to obtain maximum benefits from the VAMF. The benefits from VAM root colonization are thought to be highest when colonization occurs as early as possible during plant growth (9, 19). In horticultural production systems, this means that to obtain maximum benefits from VAM colonization, inoculum should be present during radicle emergence in seed germination, during adventitious root formation in cutting propagation, or prior to the acclimation phase of tissue culture production.

Successful adventitious rooting during cutting propagation depends on several factors including the physiological and environmental conditions of the propagation stock plants and the environmental conditions during the formation of adventitious rooting (13). Miniature roses (*Rosa* spp.) are commonly propagated by cuttings and, in general, most commercially available cultivars are considered relatively easy to root. The objective of this study was to determine whether addition of VAMF inoculum into the rooting medium during cutting propagation would increase the quantity of rooting and the quality of rooted cuttings for different cultivars of miniature roses.

Materials and methods

Five different cultivars of miniature roses (*Rosa* spp. L.) were used in this study: 'Jolly Cupido' (PP7734), 'Candy Sunblaze' (PP9621), 'White Miniwonder' (PP7276), 'Cherry Cupido' (PP9635), and 'Orange Cupido' (PP10820). Cultivars were obtained as cuttings from Yoder Brothers, Inc., Barberton, OH. Cuttings were rooted in 50 mm^3-cell flats (126 cells per flat) of wedge shaped Oasis blocks (SO#5640) under intermittent mist. Each rooted cutting was then transplanted into a 10 cm (4 in) pot (Gage Dura Pot #GDP400) containing a mix of 80% peat (Sunshine Grower Grade White, SunGro, Hubbard, OR) and 20% perlite (Coarse Horticultural Grade, Supreme Perlite, Portland, OR) amended with 20 ml of a slow release fertilizer (SRF) (Osmocote 14–14–14, Scotts Company, Marysville, OH) per pot. Plants were maintained in a glasshouse with supplemental light (16/8 hr light/dark), average day/night temperatures of 21/16C (75/65F), fertilized once a week with 50 ml of a liquid fertilizer (LF) (approximately 10% K, 10% P, 40% N, 20% Ca, 7% Mg, 8% S, 4% Na, and less than 0.05% of Mn, Cu, Zn, B, and Mo) and watered as needed. After six weeks plants were transplanted into cylindrical 1 g pots (Lerio 7 5/8 in × 7 1/8 in) containing a mix of 30% composted Douglas-fir bark (Whitney Farms, Independence, OR), 60% peat, and 10% perlite amended with 50 ml of SLR per pot. Plants were maintained in a glasshouse with supplemental light (16/8 hr light/dark), average day/night temperatures of 21/16C (75/65F), fertilized once per week with LF and watered as needed. Periodic pest and pathogen control measures were performed as needed and included foliar application of 10% sodium bicarbonate solution, DMI fungicides and Benzimidazole for powdery mildew (*Sphaerotheca pannosa* var. *rosae*), Imidacloprid for aphids, Diflubenzuron for fungus gnats (*Bradysia* spp.), *Neoseiulus fallacis* predators for spider mites (*Tetranychus* spp.), and *Neoseiulus cucumeris* predators for thrips (*Frankiniella* spp). Flower heads were pruned from plants weekly.

For mycorrhizal treatments, a commercial inoculum (Tree of Life Nursery, San Juan Capistrano, CA) of the VAM fungus (*Glomus intraradices* Schenck & Smith) containing spores, colonized root fragments and other propagules in a clay-based carrier was incorporated into the rooting medium at a rate of 1:166 (v/v). For controls, sterilized inoculum was added into the rooting media at the same rate. Hormone treatment consisted of a two-minute dip in 1:10 dilution of Woods Hormone Solution (Earth Science Products Corp., Wilsonville, OR), a commercial mixture of 1.03% indol-3-butyric acid and 0.66% 1-napthalene acetic acid.

Two-node cuttings of each cultivar were taken four weeks after stock plants had been transplanted into 1-g pots. Cuttings were sorted for uniformity (based on fresh weight), and then were sanitized by dipping the end of each cutting into 10% bleach solution for 20 minutes then rinsing with water. Cuttings of each cultivar were randomly placed into four treatment groups: no hormone and no VAM inoculum (CON); hormone treatment and no VAM inoculum (HOR); no hormone treatment and VAM inoculum (VAM); hormone treatment and VAM inoculum (HOR-VAM). Cuttings for the VAM treatment and the controls (CON) were stuck directly into 10 cm (4 in) pots (Gage Dura Pot #GDP400) containing 80% perlite, 20% peat, and either 3 ml of sterilized inoculum (CON treatment) or 3 ml of VAM inoculum (VAM treatment) placed directly in the region where the cutting was stuck. Cuttings in the HOR and the HOR-VAM treatment were dipped into Woods Hormone Solution then stuck into 4" pots containing the same perlite-peat mix and either 3 ml of sterilized inoculum (HOR treatment) or 3 ml of VAM inoculum (HOR-VAM treatment). For controls and treatments, two cuttings were stuck per pot and each pot treatment was replicated 16 times. Pots were randomized among four flats and placed in a glasshouse under intermittent mist with supplemental light (16/8 hr, light/dark) and average day/night temperatures of 21/16C (75/65F).

Aboveground and belowground measurements. Cuttings were harvested 28 days after the start of the experiment. At this time, the condition of the cutting was rated as rooted, callused, or dead. The number of primary and secondary lateral roots on each cutting was counted and removed from the cutting. Fresh weights were obtained for stem and root portions of each cutting, then all samples were dried to a constant weight at 60C (152F) and dried weights were obtained. VAM colonization of fresh roots was assessed on 1-cm sections after clearing and staining by modified procedures of Phillips and Hayman (22), replacing lacto-phenol with lacto-glycerin. Percentage of root length with signs of VAM colonization was estimated by the method of Biermann and Linderman (6).

Experimental design and statistical analyses. The experiment was set up in a randomized block design with each treatment unit (pot) replicated four times in each of four flats (blocks). Blocking was necessary to account for possible variations in temperature and moisture in the mist bed. Within-block treatment replication was done to provide adequate replication for analysis of survival data. Data for each cultivar were subjected to two-factor Analysis of Variance (ANOVA) using the Statistica statistical package (26). Shoot and root dry weights were square-root transformed, and percentage rooting and root colonization were arcsin transformed prior to analysis to correct for unequal variance and best model fit. Fisher's protected LSD test was used to separate treatment means within each cultivar (21). Actual data are reported in tables and figures. The relationship between percentage root colonization and other variables was assessed using Pearson's Correlation (r).

Results and Discussion

In easy-to-root cultivars of miniature roses, the percentage of cuttings that produce roots is not a limiting factor to production, but the time it takes for cuttings to grow an adequate amount of roots may increase the production time for a specific cultivar. Cuttings developed an adequate amount of roots for transplanting on all five cultivars of miniature roses in approximately 4–6 weeks after application of a 1:10 dilution of Woods Hormone Solution. In general, 'Jolly Cupido' and 'Candy Sunblaze' cultivars took longer to root than the other cultivars used in this experiment (data not shown).

Four weeks after cuttings were stuck, cultivar and treatment differences in rooting (percentage of cuttings with roots) were detectable (Table 1). Without VAMF inoculum, application of hormone increased rooting of 'Orange Cupido' and 'Cherry Cupido'. Adding VAMF inoculum into the rooting medium increased rooting of 'Jolly Cupido' and 'White Miniwonder' when compared to cuttings with no VAMF in-

Table 1. Influence of hormone treatment and inoculation with the VAM fungus *Glomus intraradices* on rooting of cuttings (percentage of cuttings with roots) from five cultivars of miniature roses four weeks after cuttings were stuck.

	Rooting (%)				
Treatments[z]	**'Jolly Cupido'**	**'Candy Sunblaze'**	**'White MiniWonder'**	**'Orange Cupido'**	**'Cherry Cupido'**
Control	47a[y]	59a	87a	87a	85a
Hormone	44a	62a	87a	100b	100b
VAMF	64b	56a	97b	85a	100b
Hormone + VAMF	61b	72b	100b	100b	100b
ANOVA effects	**ANOVA P-values**				
Hormone	0.987	0.089	0.803	0.000	0.057
Inoculation	0.026	0.076	0.048	0.548	0.040
Hormone × Inoculation	0.984	0.045	0.831	0.687	0.005

[z]None = sterilized VAMF inoculum, Hormone = 1:10 dilution of 1.03% IBA and 0.66% NAA and sterilized VAM inoculum, VAMF = VAMF inoculum (1:166 v/v), Hormone + VAMF = VAMF inoculum (1:166 v/v) and 1:10 dilution of 1.03% IBA and 0.66% NAA.

[y]Means followed by the same letter or letters within a cultivar are not significantly different from each other ($p < 0.05$, Fischer's Protected LSD).

oculum in the rooting medium. The combination of hormone treatment and VAMF inoculum in the rooting medium increased rooting of the 'Candy Sunblaze', 'Jolly Cupido', and 'White Miniwonder' cultivars compared to cuttings that only received hormone treatment.

Douds et al. (11) increased rooting of cuttings in response to adding VAM fungi in the rooting medium. Using a VAM fungus in a peat-based medium, they reported significantly increased survival, callus development, and rooting percentages for *Sciadopitys verticillata* cuttings, which usually take up to six months to root. In our experiment, adding VAMF inoculum to the rooting medium increased rooting on cuttings from cultivars that did not respond to hormone application but did not affect rooting on cuttings from cultivars that responded to hormone application. Our results demonstrate that adding VAMF inoculum into the media increased rooting for two cultivars that normally take longer to root. 'Jolly Cupido' rooted better with VAMF inoculum in the rooting medium, with or without hormone treatment, while 'Candy Sunblaze' rooted better with VAMF inoculum in the rooting medium when hormone was applied.

Root initiation. During vegetative propagation, the number of roots initiated influences the length of the production

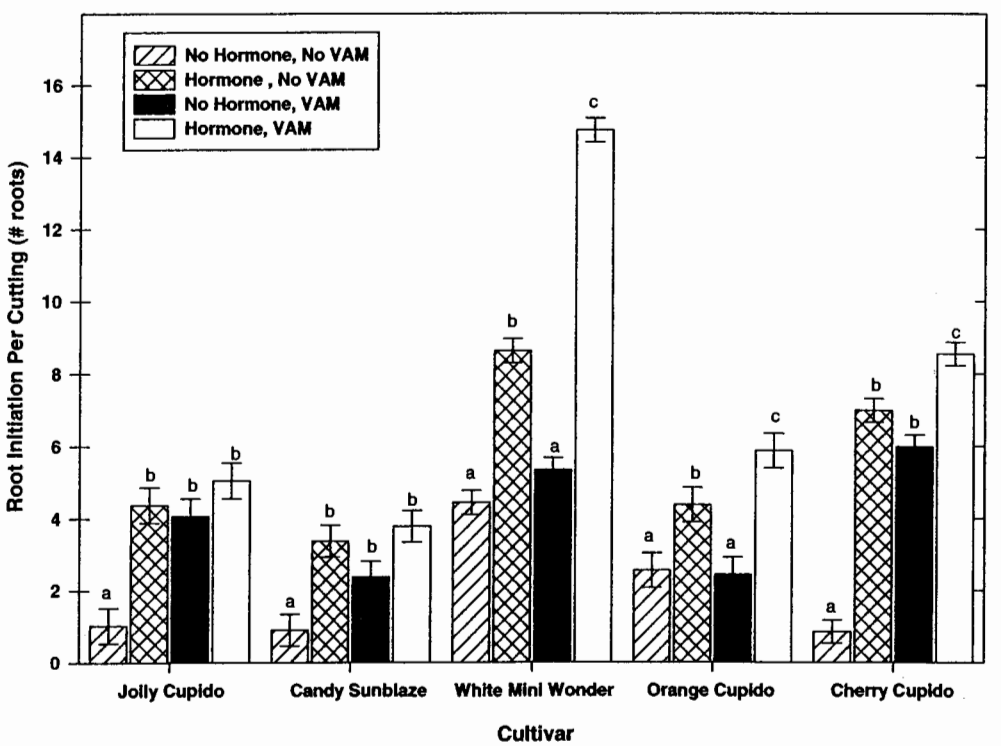

Fig. 1. Influence of hormone treatment and inoculation with the VAM fungus *Glomus intraradices* on root initiation on cuttings from five cultivars of miniature roses four weeks after cuttings were stuck. Different letters above bars represent treatment means significantly different from each other within a cultivar ($p < 0.05$, Fischer's Protected LSD).

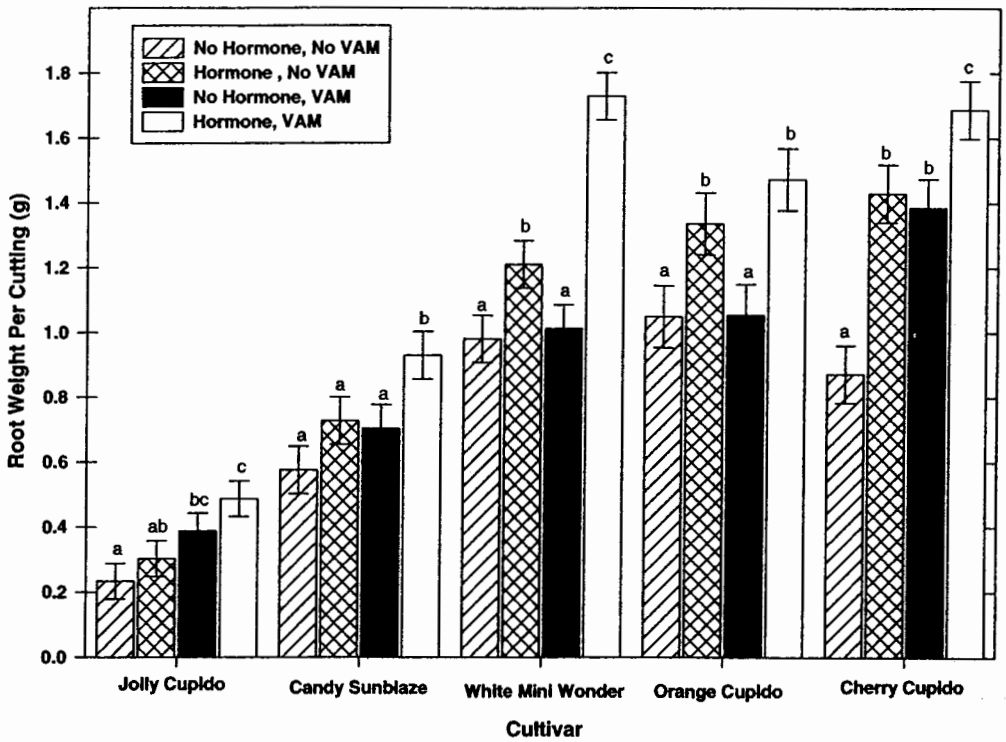

Fig. 2. Influence of hormone treatment and inoculation with the VAM fungus *Glomus intraradices* on the dry weight of roots on cuttings from five cultivars of miniature roses four weeks after cuttings were stuck. Different letters above bars represent treatment means significantly different from each other within a cultivar ($p < 0.05$, Fischer's Protected LSD).

cycle and the quality of the rooted cutting produced. Four weeks after cuttings were stuck, cultivar and treatment specific differences in root initiation (number of primary roots) were detectable (Fig. 1). When compared to controls, application of hormones significantly increased the number of primary roots for all five cultivars. Addition of VAMF inoculum into the rooting medium increased root initiation of 'Jolly Cupido', 'Candy Sunblaze' and 'Cherry Cupido' cultivars to levels equal to that found on cuttings to which hormone had been applied. Application of rooting hormone and adding VAMF inoculum into the rooting medium increased the number of roots on 'Orange Cupido', 'Cherry Cupido', and 'White Miniwonder' cultivars when compared to controls and cuttings to which only rooting hormone had been applied. In contrast to our results, Verkade and Hamilton (28) found that the presence of VAM fungi in the rooting medium increased root development and growth of *Viburnum dentatum* L. but not root initiation (28). Our results indicate that although adding VAM inoculum into the rooting medium does not always increase root initiation, in some cultivars the combination of VAMF inoculum and rooting hormone can increase root initiation and potentially increase the quality of rooted cutting produced.

Root growth. Growth of roots after initiation can also influence the length of a production cycle and the quality of the cutting produced. Four weeks after cuttings were stuck, cultivar and treatment specific differences in root growth (root weight per cuttings) were detectable (Fig. 2). Application of hormone to cuttings significantly increased root growth on 'White Miniwonder', 'Orange Cupido', and 'Cherry Cupido' cuttings compared to untreated controls. Addition of VAMF inoculum increased root growth on cuttings from 'Jolly Cupido' and 'Cherry Cupido' cultivars when compared to untreated controls. For all five cultivars, cuttings treated with the combination of hormone and VAM inoculum had the most root growth when compared to cuttings from other treatments. Increases in root growth and root initiation in response to adding VAMF inoculum into the rooting medium can potentially decrease the amount of time for cuttings to attain an adequate amount of roots for transplanting.

Root size. Root size can affect several aspects of root function. Four weeks after cuttings were stuck, cultivar and treatment specific differences in root size (average dry weight per root) were detectable (Table 2). Roots on cuttings treated with rooting hormone were smaller (shorter and/or thinner) than roots on cuttings from untreated controls. With most cultivars, addition of VAM inoculum into the rooting medium had little influence on root size when compared to cuttings from untreated controls. Since root length and diameter were not measured in this study, it is impossible to state whether the smaller roots on the hormone-treated cuttings were shorter or thinner than the cuttings from other treatments. Further study is needed to determine how root size and anatomy on hormone-treated cuttings differ from roots on cuttings from rooting media containing VAMF inoculum and on untreated controls. A detailed study on the anatomical differences between these roots could aid in determining the mechanism through which VAMF inoculum increases root initiation and growth in cuttings.

Colonization. Four weeks after cuttings were taken, cultivar and treatment-specific differences in root colonization

Table 2. Influence of hormone treatment and inoculation with the VAM fungus *Glomus intraradices* on the average weight per primary root on cuttings from five cultivars of miniature roses four weeks after cuttings were stuck.

Treatments[z]	Weight per root (g/root)				
	'Jolly Cupido'	'Candy Sunblaze'	'White MiniWonder'	'Orange Cupido'	'Cherry Cupido'
Control	0.493b[y]	0.918c	0.256b	0.770c	1.021b
Hormone	0.315a	0.574b	0.174ab	0.438b	0.204a
VAMF	0.242a	0.900c	0.284b	1.241d	0.231a
Hormone + VAMF	0.326a	0.407a	0.126a	0.346a	0.197a
ANOVA effects	**ANOVA P-values**				
Hormone	0.073	0.006	0.021	0.000	0.000
Inoculation	0.391	0.063	0.842	0.058	0.132
Hormone*Inoculation	0.035	0.059	0.457	0.049	0.027

[z]None = sterilized VAMF inoculum, Hormone = 1:10 dilution of 1.03% IBA and 0.66% NAA and sterilized VAM inoculum, VAMF = VAMF inoculum (1:166 v/v), Hormone + VAMF = VAMF inoculum (1:166 v/v) and 1:10 dilution of 1.03% IBA and 0.66% NAA.

[y]Means followed by the same letter or letters within a cultivar are not significantly different from each other ($p < 0.05$, Fischer's Protected LSD).

by VAM fungi were detectable (Table 3). Untreated controls and cuttings that received only hormone treatment showed no sign of VAMF colonization. Colonization of cuttings rooted in medium containing VAMF inoculum varied with cultivar and hormone treatment. Hormone application to cuttings from three cultivars ('Jolly Cupido', 'Candy Sunblaze', and 'White Miniwonder') increased root colonization over that found in cuttings receiving no hormone treatment. Root colonization by VAM fungi significantly correlated ($p < 0.05$) with increases in rooting on 'Candy Sunblaze' cuttings ($r = 0.85$), increases in root initiation on 'White Miniwonder' cuttings ($r = 0.72$), increases in root growth on 'Candy Sunblaze' ($r = 0.65$) and 'White Miniwonder' ($r = 0.73$) cultivars. Verkade and Hamilton (29) found that extensive mycorrhizal development occurred on roots of *Ligustrum obtusifolium* var. *regelianum* after six weeks of rooting in a medium inoculated with *Glomus mosseae*, and this coincided with substantial increases in root development but no effects on root initiation were found. In our experiment, increases in root initiation and root growth of cuttings rooted in medium containing VAMF inoculum were not always associated with increased levels of colonization. Verkade and Hamilton (28) found that inoculation of *Viburnum dentatum* cuttings increased the number of root initials emerging from stem cuttings five weeks after sticking, but they concluded that this effect occurred only after infection and was mediated through an effect of the fungus on plant metabolism, rather than an effect of fungal exudates prior to infection.

We have used the same fungal isolate on different cultivars of miniature roses and found that the response of cuttings to VAMF inoculum is detectable prior to root colonization (data not shown). Using cuttings of *Sciadopitys verticillata*, Douds et al. (11) also reported that VAM fungi exert an influence on plant growth and development prior to colonization. It is possible that early changes in root initiation and growth resulting from inoculation with VAM fungi could be a result of coincidental inoculation with bacteria associated with the spores, root fragments, and carrier substrates of the VAMF inoculum. The relationship between plant growth promoting rhizobacertia (PGPR) and VAM have been documented (4, 18). In rooting Douglas-fir cuttings, Parladé et al. (20) noted that fumigated substrate decreased rooting. The VAMF inoculum used in our experiment consisted of spores and root fragments colonized by the VAM fungus mixed with clay particles. This type of inoculum contains not only the VAM fungus, but also bacteria associated with the fungal spores, root fragments, and clay particles of the inoculum. It is possible that differences in rooting percentages, root initiation, and root growth resulting from adding VAMF inoculum into the rooting medium are not solely a response to the VAM fungi, but could also be attributable to the associated microorganisms in the inoculum an effect described by Linderman (16).

Cultivar variation. The degree and type of response miniature rose cuttings displayed when VAM inoculum was added to the rooting medium varied with cultivar. Cultivar specific responses to VAM fungi have been documented for colonization and P uptake (12), but there are no reports of cultivar-specific responses to VAM fungi used during the propagation of cuttings. The cultivar-specific responses presented in this paper could be a result of specific interactions between the VAM fungi, associated bacteria in the inoculum, and traits specific to each cultivar such as environmental, nutritional, or hormonal requirements for optimal rooting.

Potential long-term benefits. Although the degree of response of miniature rose cuttings to VAMF inoculum varied with cultivar, for most cultivars our results indicate that adding VAMF inoculum into the rooting medium is equal to or

Table 3. Influence of hormone treatment on colonization by the VAM fungus *Glomus intraradices* on roots from cuttings of five cultivars of miniature roses grown in medium containing VAMF inoculum.

Cultivar	VAM Colonization (% of root length)	
	No hormone	Hormone
'Jolly Cupido'	1.78a[z]	16.67b
'Candy Sunblaze'	3.75a	16.87b
'White MiniWonder'	10.62a	17.50b
'Orange Cupido'	13.52a	17.19a
'Cherry Cupido'	19.43a	20.20a

[z]Means followed by the same letter or letters within a cultivar are not significantly different from each other ($p < 0.05$, Fischer's Protected LSD).

better than the rooting response obtained by using rooting hormone under the conditions tested. The combination of using rooting hormone and VAMF inoculum generally produced a better percentage of rooted cuttings with more roots than cuttings treated only with hormone. Although VAMF inoculation did not increase the percentage rooting and root growth for all cultivars tested, inoculation (especially in combination with hormone application) did increase root colonization by VAM fungi. VAMF colonization has been shown to increase the survival and growth of rose explants (32) and strawberry transplants (8). Verkade et al. (27) found that inoculation of *Cornus sericea* cuttings with *Glomus fasciculatum* substantially increased plant growth during later stages of development. In comparison, inoculation of seedlings of *C. sericea* with the same VAM fungus resulted less growth differences in plants due to inoculation. In soilless substrates lacking indigenous mycorrhizal fungi, mycorrhizal inoculation has been found to increase crop uniformity, reduce transplant mortality, and increase productivity of geranium (7) onion (30), *Cyclamen persicum*, *Euphorbia pulcherrima*, *Verbena* spp. (31), and *Vaccinium corymobsum* (23). In our experiment, although VAMF inoculum did not increase rooting in some of the cultivars tested, the root colonization resulting from inoculation could result in a higher quality cutting that is better able to withstand the stress of transplanting and increase growth during later stages of plant development.

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