Effects of mycorrhizal fungi on rooting in woody horticultural crops.

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Abstract: Plants with roots colonized by mycorrhizal fungi are potentially more effective at nutrient and water acquisition, less susceptible to disease, and can be more productive under certain stressful environmental growing conditions than plants without mycorrhizae. Although a great deal of research has been performed on seedling responses to inoculation with mycorrhizal fungi and there is a growing body of information describing the benefits of inoculation of tissue culture plantlets, there has been little research on how inoculation influences adventitious root production during cutting propagation, especially in woody perennial crops. This paper reviews concepts associated with using mycorrhizal fungi to influence initiation and growth of adventitious roots and describes the results of several of several studies that assess the influence of mycorrhizal fungi on the adventitious rooting of cuttings from different woody horticultural crops.

1. INTRODUCTION

Several types of mycorrhizal associations occur in woody horticultural crops including vesicular-arbuscular mycorrhizae (VAM), ectomycorrhizae, ericoid mycorrhizae, and arbutoid mycorrhizae (Smith and Read, 1997). Optimal uses for commercially available inoculum of mycorrhizal fungi have not been well defined. One common question is when to apply inoculum of mycorrhizal fungi to obtain maximum benefits from the symbiosis. The benefits from root colonization by mycorrhizal fungi are

thought to be highest when colonization occurs as early as possible during plant growth (Chang, 1994; Sahay and Varma, 2000). In horticultural production systems, this means that inoculum should be present during radicle emergence in seed germination, prior to the weaning or acclimation phase of tissue culture production, or during adventitious root formation in cutting propagation. This paper describes the results of several studies that assess the influence of mycorrhizal fungal inoculum on the adventitious rooting and changes in chemical constituents of cuttings from different woody horticultural crops.

2. ROOTING

In easy-to-root woody perennials, 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 production time. Miniature roses (*Rosa* spp.) and florist azaleas (*Rhododendron* spp.) are commonly propagated by cuttings and, in general, most commercially available cultivars are considered relatively easy to root. We have found that adding VAM fungi (VAMF) into the media of miniature roses increases rooting in several cultivars and even increases rooting for two cultivars that normally take longer to root ('Jolly Cupido' and 'Candy Sunblaze') (*Table 1*). We have also tested the effects of mixing ericoid mycorrhizal fungi into the rooting medium during propagation of florist azaleas and were able to increase rooting of azalea cuttings using two of the three different isolates of ericoid mycorrhizal fungi tested (*Table 2*).

Table 1. Number of primary roots (#) percentage (%) of miniature rose (*Rosa* spp.) cuttings with roots 28 days after cuttings were stuck

Cultivar	Rooting (%)				Number of Primary Roots (#)			
	-HOR[a]		+HOR		-HOR		+HOR	
	-VAMF[b]	+VAMF	-VAMF	+VAMF	-VAMF	+VAMF	-VAMF	+VAMF
'Jolly Cupido'	47a[c]	44a	61b	64b	1.03a	4.37b	4.07b	5.06b
'Candy Sunblaze'	59a	62a	72b	78b	1.91a	3.37b	2.37a	3.78b
'MiniWonder'	86a	87a	84a	96b	4.44a	8.62b	5.34a	14.75c
'Orange Cupido'	87a	100b	85a	95b	2.56a	4.37b	2.43a	5.87b
'Cherry Cupido'	85a	100b	100b	100b	1.85a	6.98b	5.98b	8.54c
'Scarlet Cupido'	45a	67b	100c	100c	0.93a	6.33c	2.50b	9.50d
'Favorite Cupido'	53a	55a	72b	58a	0.66a	1.84a	2.16a	6.16b

[a]-HOR= no hormone; +HOR = 1:10 dilution of Woods Hormone Solution (Earth Science Products Corp., Wilsonville, OR), a commercial mixture of 1.03% IAA and 0.66% NAA.
[b]-VAMF= no VAMF; +VAMF= VAMF (*Glomus intraradices* Schenck & Smith) spores, and colonized root fragments in a clay-based carrier incorporated into rooting media (1:166 (v/v)).
[c] Means based on 3 replicates of 16 cuttings per treatment. Means followed by the same letter within a cultivar are not significantly different ($p<0.05$, Fischer's Protected LSD).

Table 2. Root weight and percentage of florist azalea (*Rhododendron* spp. 'Snowcap') cuttings with roots 54 days after cuttings were stuck

Mycorrhizal Treatment[a]	Rooting (%)		Root Weight (g)	
	-Hormone[b]	+Hormone	-Hormone	+Hormone
-MYC	66a[c]	81b	0.010a	0.062b
OG	83b	87b	0.010a	0.058b
HE I	84b	100c	0.055b	0.187c
HE II	69a	100c	0.029a	0.130c

[a]-MYC= no mycorrhizal treatment; OG= hyphae of *Oidiodendron griseum* and HE=either of two isolates of *Hymenoscyphus ericae* (HE I or HE II) grown on liquid MMN media (Molina and Palmer, 1982), filtered, resuspended in sterile water, and incorporated into the rooting medium (1:18 (v/v); approximately 0.1 g dry weight of hyphae).
[b]-HOR= no hormone; +HOR = 1:10 dilution of Woods Hormone Solution (Earth Science Products Corp., Wilsonville, OR), a commercial mixture of 1.03% IAA and 0.66% NAA.
[c]Means based on 3 replicates of 20 cuttings per treatment. Means followed by the same letter are not significantly different ($p<0.05$, Fischer's Protected LSD).

In more difficult-to-root species, the percentage of cuttings that produce roots can be a limiting factor to production. In response to adding VAMF to a peat-based medium, Douds et al. (1995) reported increased rooting, callus development, and survival of cuttings of *Sciadopitys verticillata*, which can take up to six months to root. Mountain Laurel (*Kalmia latifolia*) can commonly take three to five months to produce roots under commercial propagation systems. We found that cuttings of *K. latifolia* rooted in a shorter period of time when a combination of hormones and ericoid mycorrhizal fungi were used during propagation (*Figure 1*).

3. ROOT INITIATION AND GROWTH

An important consequence of colonization by mycorrhizal fungi is alteration of root system architecture, with increased initiation and branching reported for many species (Hooker et al., 1992). During vegetative propagation, the number of roots initiated influences the length of the production cycle and the quality of the rooted cutting produced. Verkade and Hamilton (1987, 1980) found that the presence of VAMF in the rooting medium increased root development and growth of *Viburnum dentatum* L. and *Ligustrum obtusifolium* var. *regelianum* but not root initiation. In contrast, we have found that application of rooting hormone and adding VAMF into the rooting medium of miniature roses increased the number of roots on several cultivars when compared to controls and cuttings to which only rooting hormone had been applied (*Table 1*).

Root size in terms of root biomass and root length can affect several aspects of root function as well as the quality of rooted cutting produced during propagation. Using florist azalea cuttings, we found that application

of rooting hormone in combination with the addition of ericoid mycorrhizal fungi increased root growth (weight) on cuttings when compared to cutting treated with hormones or untreated controls (*Table 2*). We have also found root length on cuttings of *Leucothoe racemosa* is increased with certain combinations of hormone application and ericoid mycorrhizal fungal isolates (*Table 3*).

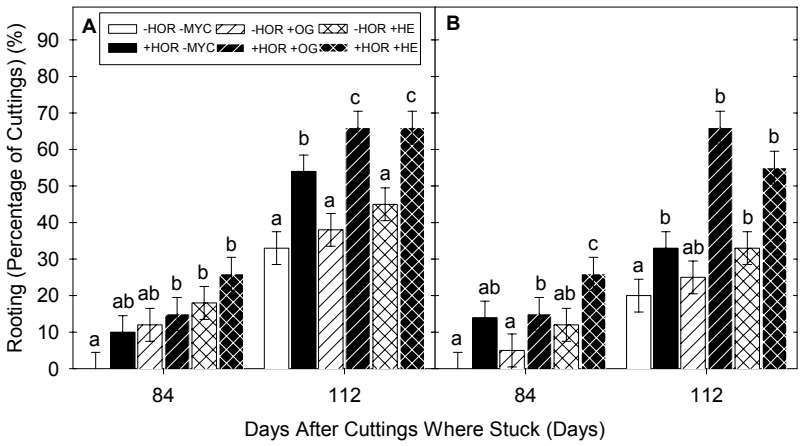

Figure 1. Percentage of *Kalmia latifolia* 'Pink Charm' (A) and 'Olympic Fire' (B) cuttings with roots 84 and 112 days after cuttings were stuck. Means based on 3 replicates of 10 cuttings per treatment. -HOR= no hormone; +HOR = 1:5 dilution of Woods Hormone Solution (Earth Science Products Corp., Wilsonville, OR), a commercial mixture of 1.03% IAA and 0.66% NAA. -MYC= no mycorrhizal treatment; OG= hyphae of *Oidiodendron griseum* and HE= hyphae of *Hymenoscyphus ericae* grown on liquid MMN media (Molina and Palmer, 1982), filtered, resuspended in sterile water, and incorporated into the rooting medium (1:18 (v/v), approximately 0.1 g dry weight of hyphae). The same letter above a column within a measurement date and cultivar are not significantly different ($p<0.05$, Fischer's Protected LSD). Bars on columns represent standard errors.

4. INTERACTION WITH HORMONES

The application of plant growth regulators by themselves and in combination with other substances is commonly used to increase adventitious rooting on cuttings. Mycorrhizal fungi are known to produce many plant hormones (Scagel and Linderman 1998a, b) and polyphenolic compounds which decrease auxin oxidation (Mitchell et al. 1986). Adding mycorrhizal fungi into the rooting medium could result in responses on cuttings similar to that achieved from exogenous application of plant growth regulators, or interact with plant growth regulators in a synergistic or

antagonistic fashion. Using miniature roses, we found that adding VAMF to the rooting medium increased rooting on cuttings from cultivars that did not respond to hormone application ('MiniWonder' and 'Orange Cupido') but did not affect rooting on cuttings from cultivars that responded to hormone application (*Table 1*). With florist azalea cuttings, application of hormone and mixing one of either two isolates of ericoid mycorrhizal fungi into the rooting media increased rooting above the level attained with hormone application alone (*Table 2*). A similar synergistic effect was seen with a combination of hormone application and ericoid mycorrhizal fungi with *K. latifolia* (*Figure 1*) and in early stages of rooting with *L. racemosa* (*Table 3*).

Table 3. Total root length and percentage of *Leucothoe racemosa* cuttings with roots 63, 84, and 109 days after cuttings were stuck.

HOR[a]	MYC[b]	Rooting (%)			Root Length (mm)		
		63 Days	84 Days	109 Days	63 Days	84 Days	109 Days
-HOR	**-MYC**	33a[c]	33a	100a	67a	77a	104a
+HOR	**-MYC**	33a	67b	100a	145b	203b	359b
-HOR	**OG**	33a	67b	92a	46a	50a	61a
+HOR	**OG**	67b	100c	100a	150b	378d	386b
-HOR	**HE I**	67b	100c	100a	88a	238b	391b
+HOR	**HE I**	67b	100c	100a	197c	293c	402b
-HOR	**HE II**	33a	67b	96a	80a	120a	129a
+HOR	**HE II**	67b	100a	100a	220c	233b	395b

[a]-HOR= no hormone; +HOR = 1:10 dilution of Woods Hormone Solution (Earth Science Products Corp., Wilsonville, OR), a commercial mixture of 1.03% IAA and 0.66% NAA.
[b]-MYC= no mycorrhizal treatment; OG= hyphae of *Oidiodendron griseum* and HE=either of two isolates of *Hymenoscyphus ericae* (HE I or HE II) grown on liquid MMN media (Molina and Palmer, 1982), filtered, resuspended in sterile water, and incorporated into the rooting medium (1:18 (v/v); approximately 0.1 g dry weight of hyphae).
[c]Means based on 3 replicates of 20 cuttings per treatment. Means followed by the same letter within a date are not significantly different ($p<0.05$, Fischer's Protected LSD).

5. CULTIVAR- AND FUNGAL-SPECIFIC RESPONSES

When several different cultivars of the same species are commercially propagated, it is financially beneficial to have uniform cultural practices and uniformity in the rooted cuttings produced across cultivars. The level of response of seedlings and tissue culture plantlets to mycorrhizal inoculation has been shown to be specific to the plant cultivar and the isolate of mycorrhizal fungus used. Using several different cultivars of miniature roses (*Table 1*) and *K. latifolia* (*Figure 1*), we found that the degree and type of response cuttings displayed when mycorrhizal inoculum was added to the rooting medium varied with cultivar. When different isolates of ericoid

mycorrhizal fungi were mixed into the rooting media of *K. latifolia* and *L. racemosa* we also found that the degree and type of response of cuttings was dependant on the isolate of ericoid mycorrhizal fungus used for inoculation.

Table 4. Number of primary roots and percentage of kinickinick (*Arctostaphylos uva-ursi* 'Massachusetts') cuttings with roots 35, 56, and 79 days after cuttings were stuck.

HOR[a]	MYC[b]	Rooting (%)			Number of Primary Roots (#)		
		35 Days	56 Days	79 Days	35 Days	56 Days	79 Days
-HOR	**-MYC**	25a[c]	25a	38a	2.9a	21.1a	39.4a
+HOR	**-MYC**	25a	37a	75b	6.0a	16.7a	33.2a
-HOR	**VAMF**	68b	75b	93c	5.1a	19.5a	52.5b
+HOR	**VAMF**	65b	87b	94c	7.6a	39.0b	64.7b
-HOR	**ROOT**	87c	89b	100c	2.2a	22.5a	44.8a
+HOR	**ROOT**	79c	87b	100c	2.1a	68.2c	88.9c

[a]-HOR= no hormone; +HOR = 1:10 dilution of Woods Hormone Solution (Earth Science Products Corp., Wilsonville, OR), a commercial mixture of 1.03% IAA and 0.66% NAA.
[b]-MYC= no mycorrhizal treatment; +VAMF= VAMF (*Glomus intraradices* Schenck & Smith) spores and colonized root fragments in a clay-based carrier incorporated into the rooting media (1:166 (v/v)); +ROOT= ground root fragments from kinickinick, suspended in sterile water, and incorporated into the rooting medium (1:18 (v/v), approximately 0.3 g dry weight of root tissue).
[c] Means based on 3 replicates of 15 cuttings per treatment. Means followed by the same letter within a date are not significantly different ($p<0.05$, Fischer's Protected LSD).

Cultivar-specific responses to different isolates of mycorrhizal fungi have been documented for several factors including, colonization and nutrient uptake. Linderman and Call (1977) reported cultivar-specific differences in rooting of *A. uva-ursi* associated inoculation with different isolates of arbutoid mycorrhizal fungi but there are few reports of cultivar-specific responses to VAM or ericoid mycorrhizal fungi used during the propagation of cuttings. The cultivar-specific responses we have found could be a result of specific interactions between the mycorrhizal fungi, associated bacteria in the inoculum, and traits specific to each cultivar such as environmental, nutritional, or hormonal requirements for optimal rooting.

6. PRE-COLONIZATION RESPONSES

Using cuttings of *Sciadopitys verticillata*, Douds et al. (1995) reported that VAMF exert an influence on plant growth and development prior to colonization. In our studies, increases in root initiation and root growth of cuttings rooted in medium containing mycorrhizal fungi have not always been associated with increased levels of colonization and the response of cuttings to mycorrhizal fungi is sometimes detectable prior to root colonization. It is possible that early changes in root initiation and growth resulting from inoculation with mycorrhizal fungi could be a result of

coincidental inoculation with bacteria associated with the spores, root fragments, and carrier substrates of the inoculum.

The relationship between plant growth promoting rhizobacertia (PGPR) and VAM have been documented. In rooting Douglas-fir cuttings, Parladé et al. (1999) noted that fumigated substrate decreased rooting. The VAMF inoculum used in our experiments consisted of spores and root fragments colonized by the fungi mixed with clay particles. This type of inoculum contains not only the VAMF, but also bacteria associated with the fungal spores, root fragments, and clay particles of the inoculum. We have tested the activity of washings from VAMF inoculum on rooting cuttings from one cultivar of miniature rose and found that although the percentage of rooted cuttings was similar when cuttings were treated with VAMF or washings from the inoculum, root initiation was higher in cuttings treated with VAMF than with only the washings (*Figure 2*). We have also found that mixing VAMF into the rooting medium increases rooting and root initiation on cuttings from plants that do not form VAM (e.g. *Arctostaphylos uva-ursi*, *Table 4*). It is possible that differences in rooting percentages, root initiation, and root growth resulting from adding VAMF into the rooting medium are not solely a response to the VAMF, but could also be attributable to other microorganisms in the inoculum, an effect described by Linderman (1988).

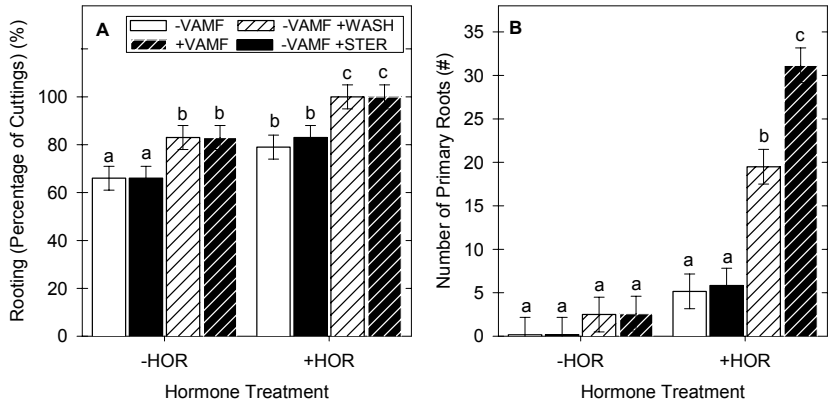

Figure 2. Number of primary roots (B) and percentage (A) of miniature rose (*Rosa* spp. 'Scarlet Cupido') cuttings with roots 28 days after cuttings were stuck. Means based on 3 replicates of 18 cuttings per treatment. .-HOR= no hormone; +HOR = 1:10 dilution of Woods Hormone Solution (Earth Science Products Corp., Wilsonville, OR), a commercial mixture of 1.03% IAA and 0.66% NAA. -VAMF= no VAMF; +VAMF= VAMF (*Glomus intraradices* Schenck & Smith) spores and colonized root fragments in a clay-based carrier incorporated into the rooting media (1:166 (v/v)); +STER= sterilized VAMF inoculum incorporated into the rooting media (1:166 (v/v)); +WASH= VAMF inoculum washed with sterile distilled water, filtered, and incorporated into the rooting media (1:100 (v/v)). The same letter above a column are not significantly different ($p<0.05$, Fischer's Protected LSD). Bars on columns represent standard errors.

7. CHEMICAL CHANGES ASSOCIATED WITH INOCULATION

Many changes in metabolism are known to occur during adventitious root formation including changes in amino acids and proteins important for enzyme function and nitrogen metabolism, and changes in carbohydrates (Hassig, 1986). With miniature roses we have tracked differences in total amino acid, protein, and carbohydrates in cuttings, and compared how mixing VAMF into the rooting medium changes composition during the initial stages of rooting. Differences in protein and amino acids between cuttings exposed to inoculum and cuttings with no inoculum were detectable within two to four days after cutting while differences in carbohydrates were detectable within four to seven days after cutting (*Figure 3*). This ability of mycorrhizal fungi to alter cutting metabolism offers researchers a useful tool to better understand the processes that occur during adventitious root formation.

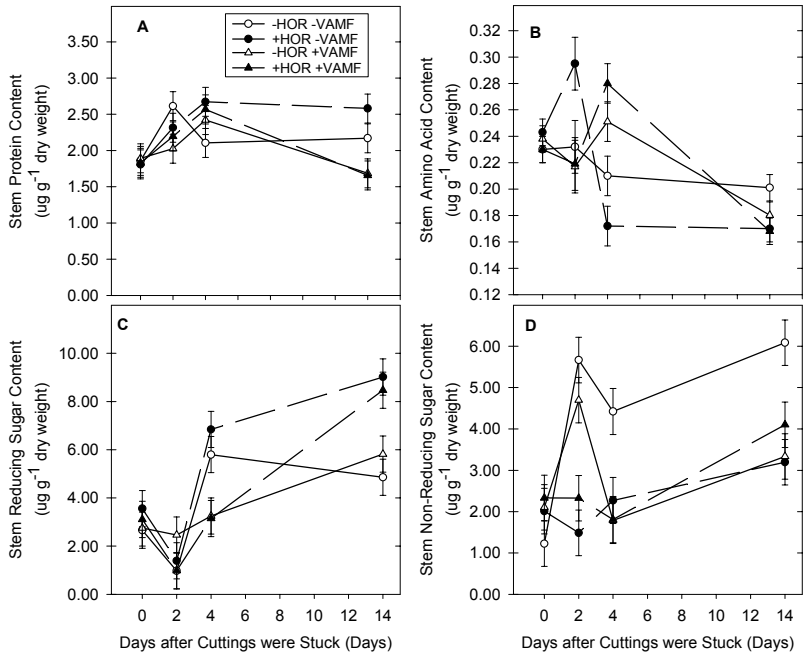

Figure 3. Total stem protein (A), amino acid (B), reducing sugar (C), and non-reducing sugar content of miniature rose (*Rosa* spp. 'Scarlet Cupido') cuttings during the first 14 days after cuttings were stuck. Means based on 3 replicates of 8 cuttings per treatment. -HOR= no hormone; +HOR = 1:10 dilution of Woods Hormone Solution (Earth Science Products Corp., Wilsonville, OR), a commercial mixture of 1.03% IAA and 0.66% NAA. -VAMF= no VAMF; +VAMF= VAMF (*Glomus intraradices* Schenck & Smith) spores and colonized root fragments in a clay-based carrier incorporated into the rooting media (1:166 (v/v)). Bars on symbols represent standard errors.

8. CONCLUSIONS

The degree of response of cuttings to mycorrhizal fungi appears to vary with cultivar and isolate of fungus. Our results and results from other literature suggest that adding VA, ericoid, or arbutoid mycorrhizal fungi into the rooting medium can achieve a rooting response that is equal to or better than the response obtained by using rooting hormone alone. The combination of using rooting hormone and mycorrhizal fungi generally produces a better percentage of rooted cuttings with more roots than cuttings treated only with hormone. Although incorporating mycorrhizal fungi into the rooting medium does not always increase adventitious root growth, inoculation (especially in combination with hormone application) does increase root colonization by mycorrhizal fungi. In soilless substrates lacking indigenous mycorrhizal fungi, mycorrhizal inoculation and colonization has been found to increase crop uniformity, reduce transplant mortality, and increase productivity of geranium, onion (Vosátka, 1995), *Cyclamen persicum*, *Euphorbia pulcherrima*, *Verbena* spp. (Vosátka et al. 1999), *Rosa* spp. (Wilson et al., 1997), strawberry (Chavez and Cerrato, 1990), pineapple (Guillemin et al., 1997), and *Vaccinium corymobsum* (Powel and Bagyaraj, 1984).

The results presented in this paper suggest there are potential ways in which incorporation of mycorrhizal fungi into rooting media during cutting propagation could increase the quantity of rooting and the quality of rooted cuttings. Although adding mycorrhizal fungi into the rooting medium does not always increase adventitious root formation, in some cultivars the combination of mycorrhizal fungi and rooting hormone can increase root initiation and potentially increase the quality of rooted cutting produced.

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