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The Efficacy of Ectomycorrhizal Colonization of Pin and Scarlet Oak in Nursery Production[1]

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Abstract

Two experiments were conducted to assess the effectiveness of ectomycorrhizae inoculation of pin (*Quercus palustris*) and scarlet oak (*Q. coccinea*) during nursery production. Experiment 1 tested inoculum and substrate protocol. Vegetative and spore inoculum of *Pisolithus tinctorius* (Pt) were applied to pin and scarlet oak seedlings. All plants were grown in 0.9 liter (1 gal) containers filled with milled pine bark, sterilized milled pine bark, sterilized mineral soil, or sterilized vermiculite-based substrate. After two months, mycorrhizal colonization rates were assessed qualitatively. Vegetative inoculum was unsuccessful at infecting seedlings of both species when grown in all of the substrates. Best results were 90% and 60% of plants colonized with spore inoculum for pin and scarlet oak, respectively, grown in vermiculite-based substrate. Sixty percent of pin oaks were colonized by Pt in milled pine bark, whereas no scarlet oaks were colonized. Overall, the spore inoculum colonized pin oak at a higher rate than scarlet oak, and vermiculite-based substrate proved superior to the other three substrates for inoculating seedlings with commercial Pt spores. In experiment 2, Pt spore inoculum was applied as a bare root dip on scarlet oak liners before transplanting into 51 liter (15 gal) containers in a pot-in-pot growing system. Pt had a colonization rate of zero, but an indigenous mycorrhizal fungus, *Scleroderma bovista* (Sb), colonized many of the trees. Height and trunk diameter growth during two years of production were similar for trees colonized and not colonized with Sb. Leaf water potentials were more negative and stomatal conductance was reduced for transplanted colonized compared to not colonized trees during a 10-day dry-down, imposed 50 days after transplanting. Under our conditions, mycorrhizal fungi showed no apparent benefit during production and during initial establishment.

Index words: inoculation, substrate, media, spores, mycelium, transplant, drought.

Species used in this study: pin oak (*Quercus palustris* Muenchh.); scarlet oak (*Quercus coccinea* Muenchh.); *Pisolithus tinctorius* (Pers.) Coker and Couch.

Significance to the Nursery Industry

Mycorrhizal symbiosis is a complex interaction which can be influenced by a wide array of cultural factors and inoculum species. In our experiments, a complete lack of inoculation success with vegetative inoculum, as well as inoculation rates considerably less than 100% with spore inoculum, indicate that growers cannot assume that inoculation will be entirely successful. Pin oak was colonized more frequently than scarlet oak, but pin oak will likely receive less benefit from the inoculation due to its relative ease of culture and transplant ability. Plants in vermiculate-based substrate were colonized most often, but this substrate is probably more suitable for production of mycorrhizal tree liners in small containers than for finished plants in large containers. Only sixty percent of pin oaks had mycorrhizae in milled pine bark, and no scarlet oaks were colonized. *Pisolithus tinctorius* spore inoculum, administered as a bare-rot dip on liners, proved ineffective at colonizing scarlet oak in pine-bark substrate, but local *Scleroderma bovista* naturally colonized over half of the trees. Nonetheless, growth during two years of production in 51 liter (15 gal) containers was not improved, and trees were not protected from early post-transplant drought by the mycorrhizal association. Improvement of colonization rates in pine bark substrate and a more complete evaluation of compatible inoculum:tree species combinations are essential before the wide-scale use of mycorrhizal inocula becomes practical for landscape plant production.

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Introduction

Mycorrhizae are symbiotic associations between higher plants and fungi that frequently provide the host plant with the benefits of improved nutrition, water relations, resistance to pathogens and metal toxicity (5, 12, 15, 32). In return, the mycorrhizal fungi are able to utilize the host plant as a carbon source. There are five main types of mycorrhizal fungi: ectomycorrhizae (ECM), arbuscular mycorrhizae (AM), ericaceous mycorrhizae, orchidaecous mycorrhizae and ectendomycorrhizae. These symbiotic relationships occur on over 95% of plant species (38). Of the five types of mycorrhizae, ECM and AM are by far the most prevalent. In the temperate and boreal forests of North America, ECM fungi are the dominant form of mycorrhizae (38). These associations occur on the roots of members of the Fagaceae and Pinaceae families and many other trees of forest and landscape importance. This symbiosis can be essential to the long-term survival of the host plant in natural ecosystems. Thus, ECM symbiosis is the subject of a considerable body of research attempting to determine if fungal/tree associations can enhance tree seedling performance. Landscape tree growers have begun to use ECM in an attempt to improve survival and growth rates of trees in the nursery and after outplanting. An industry of inoculum production has emerged to help support this growing demand for ECM fungi and other mycorrhizal products.

Pisolithus tinctorius (Pt) is a commonly used ectomycorrhizal fungus in forestry inoculation programs worldwide (22). Pt has the ability to form ectomycorrizae with species in at least twenty plant genera, including *Quercus*, *Pinus*, *Tsuga*, *Betula*, *Abies*, *Carya* (24), *Castanea* (23), and *Populus* (18). Pt has shown some success improving post-transplant seedling growth and survival rates of many

tree species (26). Commercial inocula containing ECM in general, and Pt in particular, are being marketed to nursery growers, claiming that inoculation programs can increase plant growth and survival. However, the vast majority of the research has focused on the growing systems utilized by forest seedling nurseries, and much less research has been conducted on container production of landscape plants.

Inoculation may fail to form mycorrhizae, and successful colonization may result in no measurable benefit to the host plant (6, 18). These phenomena are variously explained by excessive inoculum age and other indices of reduced inoculum vitality (23, 29), isolate specificity and incompatible host plant (5, 28), tree genotype differences in ability to form mycorrhizae (12, 28), high fertility of substrates (33), and high container substrate temperatures (7).

Oaks have been shown to derive benefit from Pt colonization (11, 25). Pin oak is generally regarded as an easy oak to grow and transplant species (36), while scarlet oak is more difficult (19). The primary goal of this study was to determine the best mycorrhizal inoculation protocol for container production of these two species and to evaluate inoculation efficacy for 15 gal pot-in-pot scarlet oaks.

Materials and Methods

Experiment 1. Investigation of inoculation protocol for pin and scarlet oak.

Seedling and substrate preparation. Pin oak and scarlet oak acorns were obtained from Sheffield's Seed Company, Locke, NY. Acorns were soaked in water for 24 hours and cold stratified at 5C (41F) until radicles emerged. The germinated acorns were planted approximately 2.5 cm (1 in) deep in 60, 10.2 cm (4 in) diameter liner containers (4 in SVD, T.O. Plastics, Minneapolis, MN) filled with 900 cu cm (55 cu in) of each of the following of four substrates: (1) sterilized (105C (220F) for 2 hours) milled pine bark substrate (pH = 5.1), (2) non-sterilized pine bark, (3) sterilized mineral soil (Unisom loam, 48.5% sand, 39.4% silt and 12.1% clay, pH = 5.8) screened through a 0.6 cm (0.24 in) wire mesh, and (4) sterilized vermiculite-based substrate (vermiculite:sphagnum peat moss, 9:1 by vol, pH = 5.4) screened through 1.4 mm (0.06 in) mesh. Sterilization was performed in a closed metal box with heated water to reduce contamination by bacteria or opportunistic fungi such as *Thelophera terrestris*, which may limit colonization with Pt (26).

Inoculum preparation. MycorTree™ Pt spore spray kits (Plant Health Care, Inc., Pittsburgh, PA) are dry spore preparations containing approximately 250 million colony forming units (cfu) per gram of product (31). Vegetative inoculum was mycelium in Hagem's solution agar, transferred and grown in liquid Hagem's solution from Pt isolate #3303 of the Virginia Tech culture collection. This isolate was obtained from a sporocarp found under *Pinus strobus* L. in Blacksburg, VA, in 1995. Liquid cultures were grown so that approximately 20 colonies of 10–15 mm (0.4–0.6 in) diameter were present. These colonies were placed in a sterilized blender with distilled water to equal 400 ml total volume and blended for 10 seconds at low speed to produce a homogenous slurry.

Planting, inoculation, and seedling care. The experiments were carried out in a glass greenhouse (day/night temperature = 22/20C (72/68F)) at the Virginia Tech greenhouse complex. Germinated acorns of scarlet oak and pin oak were planted singly in the containers filled with the various substrates on May 15, 2000, using a completely random design. At planting, all seedlings were top-dressed with 4 g of encapsulated slow-release fertilizer (15N–3.96P–9.96K, Osmocote® Plus, the Scotts Co., Maryville, OH). Inoculation treatments were applied on July 3, 2000, and included the (1) MycorTree™ Pt spore spray kit mixture (dry product mixed in water) applied to seedlings at a rate of 1 g of product/0.082 cu m (2.9 cu ft) of substrate as suggested by the manufacturer, resulting in about 275 cfu per pot, (2) the vegetative Pt inoculum administered as five ml aliquots of mycelium, applied with a pipet to dibbled holes in the substrate surface, or (3) controls of each substrate receiving no inoculum (= 10 plants per inoculation × substrate). Immediately after inoculation, the seedlings were irrigated thoroughly to ensure that the inocula had moved into the root profile. The seedlings remained in the greenhouse complex throughout the course of the experiment. Seedlings in each treatment were irrigated to container capacity daily or as needed to prevent water stress until harvest.

Seedling harvest and mycorrhizal assessment. Harvest of seedlings began September 16, 2000, and ended October 4, 2000. Roots were washed clean of substrate and observed under a dissecting scope at 40× magnification to identify mycorrhizal symbiosis. Roots which showed signs of mantle development were observed with a compound microscope at 100× magnification to verify morphological characteristics of Pt mycorrhizae: olive color of the mantle and the presence of clamp connections (40). Mycorrhizae identified with Pt as a mycobiont were given a 'yes' rating, while seedlings with no mycorrhizae were given a 'no' rating. Contamination with other mycorrhizal fungi was rare and was given a 'no' rating.

Data recording and analysis. The Genmod procedure of the SAS system (vers. 8.01, SAS Institute Inc., Cary, NC) was used to fit a Generalized Linear Model to test for effects of species, substrate, and their interaction on the proportion of colonization using logistic transformation (35). Single-degree-of-freedom contrasts were used to perform pairwise comparisons of all substrates.

Experiment 2. Inoculation of pot-in-pot scarlet oak.

This study began as an attempt to determine if inoculation of scarlet oak with Pt would improve growth during production and ameliorate post-transplant plant water relations for trees grown in a pot-in-pot growing system. However, an indigenous fungus, *Scleroderma bovista* (Sb), was far more successful at infecting the trees used in this study. Therefore, our objective shifted towards assessing the effectiveness of Sb as a mycobiont in association with pot-in-pot-grown scarlet oak grown. *Scleroderma* forms mycorrhizae with a wide range of host plants and is closely related to Pt, both belonging to the Sclerodermataceae. *Scleroderma*, like Pt, is common in the hot, dry regions of the southeastern United States, and is adapted to dry sites.

Plant material and fungal inoculation. On April 20, 1999, 30 scarlet oak trees were hand dug from mineral soil beds at the Urban Horticultural Center of Virginia Tech, Blacksburg, VA (USDA hardiness zone 6A). Mean height and trunk di-

ameters, measured 15 cm (6 in) above the soil surface were 2.2 m (86.4 in) [4.9 cm (2 in)] and 2 cm (0.8 in) [0.07 cm (0.03 in)], respectively. Roots were washed free of soil, and 15 trees were randomly selected and dipped in MycorTree™ Pt root dip (Plant Health Care, Inc., Pittsburgh, PA). In addition to spores gathered from an array of Pt sporocarps, MycorTree™ Pt root dip contains a water absorbing polymer and yucca extracts. Inoculum was mixed at a rate of 425 grams inoculum/9.5 liters (15 oz/10 qt) of water per manufacturer's instructions, and the tree roots were dipped into the inoculum prior to planting into 51 liter (15 gal) containers (B-15, Lerio Inc., Mobile, AL), with 100% milled pine bark (pH 5.1) as a substrate. All trees were top-dressed with 240 grams (8.5 oz) of encapsulated slow-release fertilizer (15N–3.96P–9.96K, Osmocote® Plus, the Scotts Co., Maryville, OH) and randomly placed into a pot-in-pot growing system (see below). The remaining 15 trees received identical treatment, but were not dipped into the mycorrhizal inoculum. Height and trunk diameter, taken 15 cm (6 in) from the ground, were measured on April 20, 1999, November 29, 1999, and October 18, 2000.

Pot-in-pot growing system. The pot-in-pot system consisted of 51 liter (15 gal) socket containers recessed into the soil and spaced 1.2 m (4 ft) apart within rows. Rows were 1.5 m (5 ft) apart. The area between containers was covered with black landscape fabric, and an underground drainage system ensured complete drainage. Each pot was equipped with an individual water emitter and irrigated up to twice a day to ensure that substrate moisture levels remained near container capacity throughout the growing seasons. A second application of 240 grams (8.5 oz) of encapsulated slow release fertilizer was applied as a top dress in May 2000. Trees were grown for two growing seasons under this system.

Mycorrhizal identification. Trees were allowed to grow undisturbed in the pot-in-pot system for three months. Starting in July 1999 trees were periodically lifted from their containers to inspect for mycorrhizae on the edges of the root balls. Roots growing out of the bottoms of containers were trimmed as needed to prevent growth into the drainage system. Inspections revealed mycorrhizal colonization of several trees. These mycorrhizae were fluffy and white in appearance to the naked eye, morphological characteristics not associated with Pt. Pt usually develops yellow or olive-colored mycelium (27). Sporocarps soon began to form on the surface of the pine bark substrate, allowing for identification of the fungus as Sb. This fungus was mycorrhizal with 17 out of the 30 trees and, after careful examination of all root balls, there was no evidence of the original Pt inoculum treatment or other mycorrhizal fungi (except Sb) on the exterior of any root balls. In October 2000, the interior of root balls of five trees colonized with Sb and five trees with no evidence of mycorrhizal colonization were randomly selected and examined for mycorrhizal association. No other mycorrhizae were identifiable under 40× magnification using a dissecting microscope.

Drought stress. On August 3, 2000, six trees colonized with Sb and six uncolonized trees were randomly selected and transplanted to 104.5 liter (25 gal) containers (GL10000, Nursery Supplies, Inc., Fairless Hills, PA) in similar substrate as described above and placed into a completely random statistical design. Terminal buds had set on all shoots and no sign of fall coloration was present. Trees were irrigated once a day or as needed to maintain at container capacity. On September 22, 2000 (50 days after transplanting), three containers in each treatment were covered with plastic to exclude rain. These plants were not irrigated for the remainder of the study. The remaining three trees in both treatments were irrigated as before. On a preplanned schedule (September 22, 23, 24, 27, 29 and October 1, 2000) two trees were randomly selected per drought treatment × mycorrhizal status to measure leaf water potential (Ψ). Leaf water potential was measured with a pressure chamber (Plant Moisture Stress, Corvallis, OR) (3). Single leaves were removed with a razor blade from the first set of mature leaves behind the leaf cluster associated with the terminal buds of branches on the lower portion of the trees. There were two samples per replication. Leaf Ψ was measured at two hour intervals, from 700 to approximately 2100 HR.

A steady-state porometer (LI-1600, LI-COR, Inc. Lincoln, NE) was used to measure stomatal conductivity on September 27, 29 and October 1. Measurements were made between 1200 and 1400 HR when light intensity levels were at least 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Measurements were taken on one sun-facing leaf per plant on all trees in random order among plants.

Data analysis. Growth data were subjected to analysis of variance within the GLM procedure of SAS (SAS, vers.8.01, SAS Institute, Inc. Cary, NC). Leaf xylem potential data were graphed for each measurement day and replication and areas under each curve were calculated with SigmaPlot computer software (SigmaPlot 2001 for Windows, vers. 7.0, SPSS Science, Chicago. IL). Areas under curves, representing an integrated daily Ψ (I-Ψ), were subjected to analysis of variance within the GLM procedure of SAS, and mean separation was with Fisher's Protected LSD at $\alpha = 0.05$.

Results and Discussion

Experiment 1. Investigation of inoculation protocol for pin and scarlet oak.

Pt spore treatment application resulted in varying percentages of inoculation success (Fig. 1). In all substrates, a higher proportion of pin oak seedlings were colonized than scarlet oak seedlings, and no interaction between substrate type and tree species was observed (Fig. 1, Table 1). Although Pt is generally regarded as a ubiquitous mycorrhizal fungus and a general colonizer of oaks and pines (Don Marx, personal communication), there was species-specific inoculation success under our conditions. Dixon et al. (11) observed varying levels of colonization between oak species inoculated with different Pt isolates. Also, a large percentage of isolate specificity seems to be determined by geographical parameters and Pt phylogeny (4, 21), and the source(s) of Pt spores in this inoculum product are unknown.

Vermiculite incorporated with 10% peat moss is a commonly used substrate for vegetative inoculation of forest seedlings (28). Landscape tree nurseries in Virginia, and much of the Southern and Eastern United States, normally utilize milled pine bark as a primary growing substrate. In our study vermiculite-based substrate had the most successful inoculation rate (Fig.1, Table 1). However, the use of vermiculite-based substrate for trees grown to landscape size may be limited by problematic plant-substrate water relations in larger containers, a lack of long-term physical integrity, and pro-

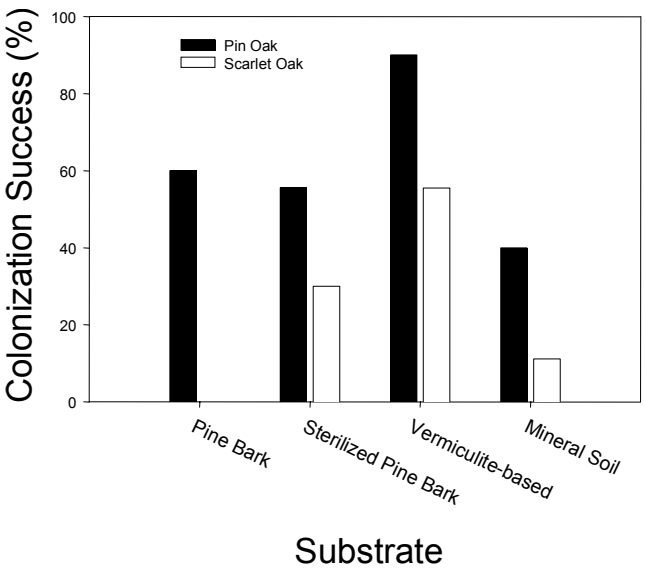

Fig. 1. Colonization success (% of plants that were mycorrhizal) using *Pisolithus tinctorius* spores for mycorrhizal association with roots of pin and scarlet oak grown in various substrates. Inoculation rate for scarlet oak in PB was 0%. n = 10. See Table 1 for statistics.

hibitively high costs. Vermiculite may be a viable substrate for the production of mycorrhizal trees in small containers intended for growing on to landscape size (i.e., liner production) in field or container nurseries. Mycorrhizae formed on seedlings grown in mineral soils at similar proportions to seedlings grown in pine bark (Fig. 1, Table 1). Mineral soil is a poor choice for growing trees in containers due to its excessive weight and excess water holding capacities (34). Although we wanted to determine if higher colonization rates could be obtained in a mineral soil substrate such as where native Pt may be found, no particular benefit of using mineral soil was found.

We were unable to pinpoint the cause of the complete lack of mycorrhizal associations using mycelium as an inoculum. However, Pt isolate #3303 formed mycorrhizae with *Pinus virginiana* in a current study at Virginia Tech (unpublished data, O.K. Miller), attesting to the viability of this isolate.

Although seedlings grown in a vermiculite-based substrate showed the highest colonization rates for both species, pine bark may be an effective substrate for seedling inoculation for some species. With colonization rates approaching 60% for pin oak, inoculation of pin oak is a viable option. However, pin oak transplants easily without inoculation, and inoculation of trees by mycorrhizal fungi does not always correlate with improved growth and/or survival rates of outplanted trees (7, 17). Finally, scarlet oak was colonized less frequently than pin oak, illustrating the inconsistent nature of the symbiosis as a component of production practices. Although inoculation programs are often highly effective (9), mycorrhizal inoculation does not always produce positive results, and isolate source, tree species or genotype, and substrate can all be factors determining the level of success of inoculation programs.

Table 1. Logistic regression comparison of colonization rates of a commercial Pt spore spray kit when applied to pin and scarlet oak in various substrates (n = 10 for each species × substrate combination). See Fig. 1 for colonization rates. n = 10.

Source	DF	Chi-Square	P-value
Species	1	14.63	0.0001
Substrate	3	14.40	0.0024
Species × Substrate	3	3.38	0.3372
Contrasts			
Pine bark vs. sterilized pine bark	1	2.67	0.1022
Pine bark vs. vermiculite-based	1	10.36	0.0013
Pine bark vs. mineral soil	1	0.65	0.4197
Sterilized pine bark vs. vermiculite-based	1	4.23	0.0397
Sterilized pine bark vs. mineral soil	1	1.51	0.2192

Experiment 2. Inoculation of pot-in-pot scarlet oak.

Height and trunk diameter growth were similar between mycorrhizal and nonmycorrhizal treatments (data not shown). A considerable body of research shows that mycorrhizal trees grow more quickly than nonmycorrhizal trees, and several studies utilized species of *Scleroderma*. Dell et al. (10) inoculated several tree species with isolates of *Scleroderma*. *Eucalyptus grandis* showed the greatest increase (32%) in shoot dry weight. Beckjord and McIntosh (1) inoculated *Quercus rubra* seedlings with six species of ECM fungi. Seedlings inoculated with *Scleroderma citrinum* had greater growth than trees inoculated with all other fungi and controls, and up to a 34% increase was seen by the third growing season after inoculation. In another study, Pt inoculation elicited the greatest growth responses of the several inocula types for *Quercus robur*, *Quercus velutina*, and *Quercus alba* (11). Garbaye and Churin (16) found that *Paxillus involutus* increased the growth rates of *Quercus patraea* and *Quercus robur* after outplanting. However, in our study, mycorrhizae did not increase plant growth. Colpaert et al. (8) studied the effect of extensive extramatrical mycelium on the growth of *Pinus sylvestris* seedlings. Nine ECM species were studied, and all colonized trees had significantly smaller biomass than uncolonized controls. Increased fungal biomass was inversely correlated with plant biomass. Trees colonized with *Scleroderma citrinum* had 50% of the biomass of uncolonized trees. Beckjord et al. (2) showed that *Scleroderma auranteum* was less effective than Pt at enhancing growth of *Quercus alba* and *Quercus rubra*. Overall weight of *Quercus rubra* was actually decreased by *Scleroderma auranteum*.

In our study, transplanted trees under drought stress showed lower I-Ψ on the second day (data not shown). By day six, dry trees continued to show more negative I-Ψ than wet trees, and mycorrhizal trees had the most negative I-Ψ (Fig. 2). Eight days after beginning the drought stress treatment, differences due to mycorrhizal colonization were not evident, but increased drought stress on mycorrhizal trees without irrigation was again evident and more pronounced by day 10. The reasons for the lack of evidence for difference between drought-dressed mycorrhizal and nonmycorrhizal trees on day 8 are unclear. Mycorrhizal trees as a group had lower stomatal conductance than nonmycorrhizal trees 6 and 10 days after withholding water, and conductance was particularly low for mycorrhizal trees not irrigated for 10 days (Table 2).

Mycorrhizal colonization can ameliorate water stress in host plants (12, 14). Walker et al. (39) studied *Pinus taeda* seedlings inoculated with the closely related Pt. After three years seedlings had less negative water potential measurements than controls colonized with *Thelophera terrestris*. Dry

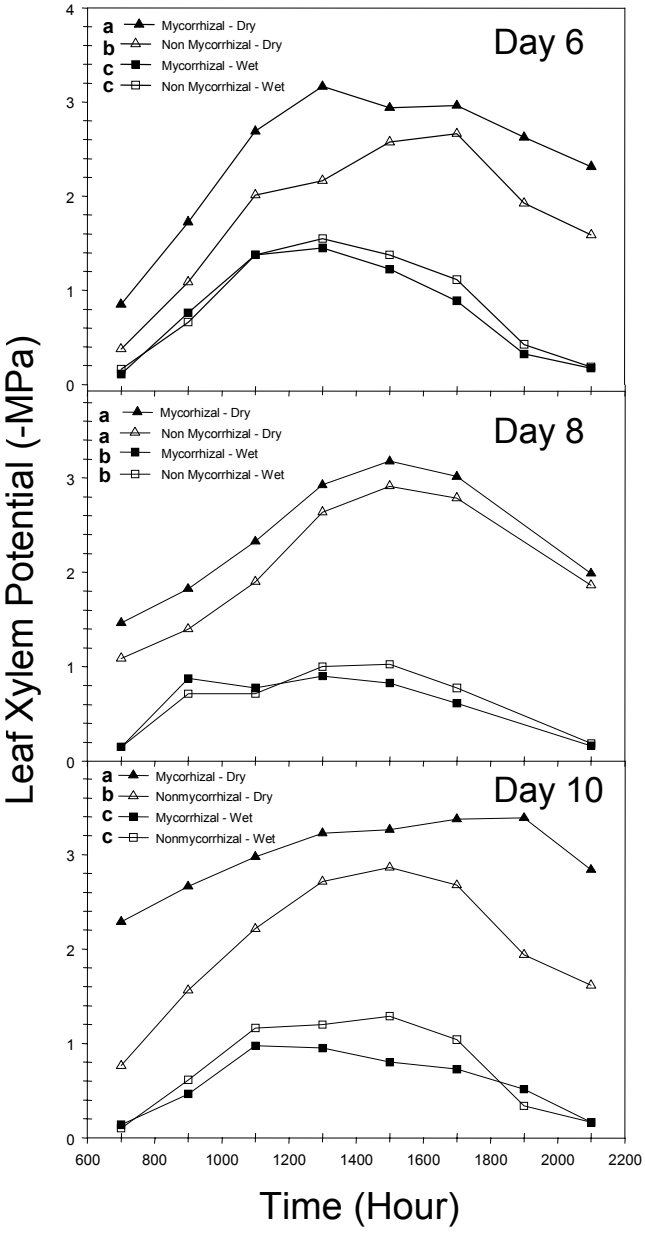

Fig. 2. Mean diurnal pattern of leaf xylem potential for drought stressed (water completely withheld) and well watered mycorrhizal and non mycorrhizal scarlet oak, 6, 8, and 10 days after beginning of drought treatment. Letters by legend indicate differences between areas under curves (2 curves per treatment), according to Fisher's Protected LSD at $\alpha = 0.05$. Each data point is the mean of 2 replications and 2 samples per replication.

sites with high soil temperatures seem to illicit the greatest host growth benefit from Pt inoculation (20). In contrast, a study performed in southwestern Oregon, where climate and soil characteristics differ significantly, showed that for several conifer species Pt provided no enhancement of growth when compared to plants naturally colonized with local nursery fungi (7). Additionally, a study utilizing AM fungi did not show mycorrhizal colonization to be important in raising water potentials when stress was a result of high root-zone temperatures, which are common in southern container nursery production systems (30). In contrast to our scarlet oak data, Dosskey et al. (13) found that with *Rhizopogon vinicolor*, an ECM fungus, stomatal conductance was enhanced for *Pseudotsuga menziesii* seedlings even at lower leaf water potential. They hypothesized that the stomatal conductance was correlated to photosynthate export to the fungus and that the carbon drain on the host plant may have caused the stomata to remain open. Increased photosynthetic demand required by mycorrhizal fungi may prove detrimental to landscape trees in containers or in the ground with restricted root zones by instigating the faster depletion of the water reservoir. In a study utilizing AM fungal colonization of geranium grown in containers, Sweatt and Davis (37) found an increase in water stress of mycorrhizal plants as indicated by a decrease in Ψ. In the restricted system of a container the water supply of a plant with high stomatal conductance will diminish more rapidly than plants with closed stomata. This effect may offset the benefit of the increased absorptive capacity presumably enjoyed by mycorrhizal plants. However, we saw no evidence of increased stomatal conductance for mycorrhizal vs. non mycorrhizal trees in our study (Table 2).

Our study illustrates the sometimes questionable efficacy of mycorrhizal inoculation. In experiment 1, mycorrhizal inoculation with Pt spores of container-grown scarlet oak in pine-bark substrate was completely ineffective compared to inoculation of pin oak, but scarlet oak colonization was successful in other substrates. In experiment 2, Pt root dip was also ineffective at colonizing landscape-size scarlet oak trees in pine-bark substrate. However, colonization by other species of mycorrhizal fungi may be successful in pine-bark substrate. For example, inoculation of several species growing in pine-bark substrate with the VM fungus *Glomus intraradicies* was successful (9), and Sb proved to be an opportunistic colonizer of scarlet oak growing in pine-bark substrate in experiment 2.

Although mycorrhizae were not evident on roots at the time of digging in experiment 2, Sb sporocarps were present in the mineral soil bed later in the growing season, indicating many trees may have been colonized at the time of digging. Trunk diameter growth was similar for mycorrhizal versus nonmycorrhizal trees. From this perspective, scarlet oaks will not be larger at the time of sale, or sold more quickly, if root balls are colonized by Sb. In addition, Sb showed no benefit in ameliorating drought stress after transplanting. In fact, the fungus appeared to exacerbate drought stress of scarlet oaks with a limited water supply and root zone. An increased ability to withdraw water from the limited confines

Table 2. Stomatal conductance and associated statistics for transplanted 15-gal scarlet oak (*Quercus coccinea*), colonized or not colonized with the mycorrhizal fungus *Scleroderma bovista* and water withheld for 6, 8, or 10 days or watered daily. n = 3.

Treatment		Conductance (mmol m^{-2} s^{-1})		
Mycorrhizae	Water status	Day 6	Day 8	Day 10
Colonized	Drought	23.17	26.77	18.97
Colonized	No drought	92.37	141.67	105.33
Not colonized	Drought	54.50	35.43	49.60
Not colonized	No drought	120.60	91.63	136.33
			P > F	
Mycorrhizae		0.080	0.400	0.021
Irrigation		0.002	0.006	0.001
Mycorrhizae × Irrigation		0.920	0.243	0.987

of the original rootball and just beyond for mycorrhizal trees may have resulted in a faster depletion of the limited water reservoir.

Under our conditions, mycorrhizal fungi had no apparent benefit to the host plants during production and during initial establishment. Research in various landscape site environments needs to be conducted to determine if there are reliable benefits to colonized landscape-size trees following outplanting. Outplanting of mycorrhizal landscape trees in areas with unrestricted root zones may prove to be more beneficial than mycorrhizal colonization of trees with restricted root zones.

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