

**Observations of Mycorrhizal Inoculation of Pin and Scarlet Oak
Production In Containers**

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(Abstract)

Over the course of the last century mycorrhizal symbiosis has come to be recognized as highly beneficial for the host plant. Increased growth, water relations, nutrient acquisition, amelioration of the effects of metal toxicity, and increased resistance to pathogens are all benefits attributed to plants colonized by mycorrhizal fungi. A large body of literature exists that indicates that mycorrhizal inoculation programs are useful for improving the performance of forest tree seedlings. Commercial mycorrhizal products, many containing the ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker and Couch (Pt), have emerged from this research and are now being marketed for landscape tree growers. In this study, two experiments were conducted to determine the best protocol for inoculation of landscape trees, and to determine if mycorrhizal inoculation is beneficial to trees growing in modern landscape tree container production systems. The first experiment evaluated mycorrhizal inoculation programs utilizing two inocula types employing various substrates for landscape tree production in containers. *Quercus palustris* Muenchh. (pin oak) and *Q. coccinea* Muenchh. (scarlet oak) grown in pine bark, sterilized pine bark, sterilized mineral soil, and sterilized vermiculite-based substrates were inoculated with Pt commercial spore inoculum or Pt vegetative inoculum. The vegetative inoculum was unsuccessful at forming mycorrhizae even though the Pt continued to live in the planting substrates. A higher proportion of pin oak was colonized than scarlet oak in all substrates, and vermiculite was a superior environment for mycorrhizal formation than the other three substrates. The second experiment examined the effectiveness of an indigenous mycorrhizal fungus, *Scleroderma bovista* Fr., to increase growth and resistance to drought stress of scarlet oak grown in containers in the pot-in-pot growing system. *Scleroderma bovista* did not affect tree growth, and mycorrhizal trees in containers proved to be more susceptible to drought stress than nonmycorrhizal trees. Leaf water potential was more negative for mycorrhizal trees, and

conductance was lower for mycorrhizal trees after a 10 day dry down period. A commercial Pt product was also used as an inoculum in this study and again proved completely ineffective at colonizing scarlet oak in pine bark substrate.

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Chapter One

Introduction

In 1885 A.B. Frank coined the term "mycorrhiza" to describe the observed association between fungi and higher plants that did not appear to be deleterious to the plants. Literally translated, mycorrhiza means "fungus-root", and indeed the point of contact between the plant and the fungus is at the root. As the fungus colonizes the host plant root, an exchange system develops that allows for the mycorrhizal relationship to be symbiotic. Mycorrhizal fungi have been shown to improve a plant's ability to acquire mineral nutrients and water from the soil, reduce toxicity from heavy metals and other pollutants, and improve the plants resistance to colonization by pathogens (Burgess et al, 1993; Dixon et al., 1983; Egerton-Warburton and Griffen, 1995; Ross and Marx, 1972). In return the fungus receives carbon in the form of photosynthates from the host plant. Current estimates place the number of plant species capable of forming mycorrhizae in excess of 95% (Sylvia, 1998). Clearly, mycorrhizal symbiosis is an important facet of plant health in established ecosystems.

There are five main types of mycorrhizae: ectomycorrhizae, arbuscular mycorrhizae, ectendomycorrhizae, orchidaceous mycorrhizae, and ericaceous mycorrhizae. Ectomycorrhizae (ECM) are the dominant form of mycorrhizae in boreal and temperate forests. In excess of 4000 species of fungi have been shown to form ECM (Sylvia, 1998). Among others, these associations occur on the roots of members of the Fagaceae, Pinaceae, Betulaceae and Myrtaceae in natural plant communities. This symbiosis is obligitive to the survival of the host plant. Thus, ECM symbiosis is the subject of a considerable body of research which has clearly shown that the mycorrhizal associations enhance tree seedling performance (Burgess et al., 1994; Hatchell and Marx, 1987; Dixon et al., 1984). More recently, 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 has emerged to help support this growing demand for ECM fungi and other mycorrhizal products. Of the wide range of ECM genera only a few have been studied

extensively in controlled experiments. These include species of *Cenococcum*, *Hebeloma*, *Laccaria*, *Paxillus*, *Pisolithus*, *Rhizopogon*, *Suillus*, *Thelophera*, *Scleroderma*, *Piloderma*, *Tomentella*, *Boletus*, *Amanita* and *Tuber* (Cairney & Chambers, 1999). Of this limited selection, *Pisolithus tinctorius* (Pt) is the most widely used and studied ECM fungus, and is the most prevalent ECM ingredient in many commercial mycorrhizal products. *Pisolithus tinctorius* is a desirable fungus to work with because it is globally distributed, easy to grow in vitro, and has been shown to have the ability to form ECM with a wide host range (Marx et al., 1982). Species in at least 20 genera of higher plants have been shown to form mycorrhiza with Pt, including commercially important hard and softwood species in genera like *Quercus*, *Betula*, *Eucalyptus*, *Pinus*, *Tsuga*, and *Pseudotsuga* (Marx, 1977).

Many studies have shown that positive host plant growth responses have been observed for seedlings inoculated with Pt (Marx and Bryan, 1970; Burgess et al., 1994; Dixon et al., 1984). However, isolate specificity may play an important role in the success of Pt inoculation programs. Studies have shown that isolates obtained from one geographical region or genus may be less effective at colonizing plants from other genera or regions, indicating genetic diversity suggesting that Pt may be comprised of more than one species or strain (Junghans et al., 1998; Lei et al., 1990). Burgess et al. (1994) used 20 Pt isolates to inoculate *Eucalyptus grandis* W. Hill ex Maiden. They showed significant differences in colonization rates and level of colonization of Pt on the host tree. These studies suggest that although Pt is an effective colonizer of a wide array of higher plants, a single Pt commercial product may not be effective at colonizing a wide array of tree species.

The vast majority of the controlled experiments utilizing Pt to form ECM have been performed on forest nursery seedlings rather than landscape nursery trees. The possibility exists that the different cultural practices performed at landscape tree nurseries may impact the efficacy of Pt products. In Virginia nurseries, current trends indicate a continued shift from balled and burlapped growing systems to containerized systems (pers. com.). Composted pine bark is the preferred substrate in these systems due to its

water holding characteristics, availability, and low cost. In addition, landscape trees are generally grown to a much larger size than forestry seedlings before outplanting. Trees ranging from 6 to 20 feet in height are quite common. Irrigation and fertilization regimes are typically on a regular schedule in an attempt to produce the largest, most healthy tree in the shortest period of time. This may prove problematic because high water and/or nutrient status may decrease colonization rates and growth responses of trees to Pt inoculation (Marx et al., 1982; Beckjord et al., 1985). However, if mycorrhizal inoculation is able to increase the growth rates of trees in landscape nurseries it may well be worth the added expense of purchasing the product and adapting current production systems.

With these considerations in mind the following research was conducted in an attempt to determine the efficacy of Pt inoculation on *Quercus palustris* Muenchh. and *Q. coccinea* Muenchh. Preliminary research was conducted in an attempt to determine the most appropriate stage in production for mycorrhizal inoculation. Two week old seedlings growing in milled pine bark in 10.2 cm containers were inoculated, and seedlings were also inoculated during potting-up from 10.2 cm containers to 3.8 L containers. No mycorrhizal colonization was observed after either treatment. A second preliminary experiment utilized four different fertilizer regimes; no fertilizer, low fertility, medium fertility, and high fertility. Seedlings growing in milled pine bark in 10.2 cm containers showed no colonization after inoculation under all fertilizer regimes. Because no seedlings were colonized during the preliminary experiments, we determined that substrate type and inoculum type may affect mycorrhizal colonization rates, and these factors were worthy of exploration.

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Chapter Two

Inoculation of Tree Seedlings in Nurseries: Literature Review

Over the course of the past four decades a considerable amount of effort has been put forth to determine the efficacy of inoculating tree seedlings with *Pisolithus tinctorius* (Pt) for the improvement of survival rates and growth of colonized seedlings. Because of promising results in many of these experiments, commercial mycorrhizal products containing Pt as well as other mycorrhizal fungi are readily available. The forest plantation operator and the landscape nursery grower are targeted in the marketing of these products. Some literature does exist evaluating the success of mycorrhizal products with regard to landscape tree production, but the research is by no means complete.

Forest seedling inoculation with Pt:

Inoculation of seedlings for forestry applications with Pt has produced mixed results. In many cases, growth of both angiosperms and gymnosperms has been significantly enhanced. Sharpe and Marx (1986) found that *Carya illinoensis* (Wangenh.) C. Koch seedlings grown in a sandy loam soil showed a significant increase of 6.5% over controls colonized with naturally occurring mycorrhizae. Dixon et al. (1987) showed an increase in dry weight of *Pinus taeda* L. (loblolly pine) seedlings growing in a sphagnum moss-vermiculite mixture inoculated with Pt over controls. *Eucalyptus grandis* W. Hill ex Maiden growth rates were increased by Pt inoculation by up to 45x in a study comparing different Pt isolates (Burgess et al., 1994). Hatchell and Marx (1987) studied *Pinus palustris* Mill., *P. taeda*, and *P. clausa* var. *immuginata* D.B. Ward grown in a mixture of topsoil, sand, and milled pine bark during a long-term experiment. Positive growth responses were seen on all three species when colonized with Pt. However, growth stimulation was limited to the first year on *P. taeda*. In another mycorrhizal study, *Quercus robur* L., *Q. velutina* Lam., and *Q. alba* L. growing in a fumigated vermiculite-based media were inoculated with a variety of ectomycorrhizal fungi including Pt, and an increase in growth resulted in all three species of oak (Dixon et al., 1984).

Clearly the use of Pt can positively influence growth on a wide variety of tree species. However, fungal and tree genotype can play an important role in determining how effective the mycorrhizal symbiosis is at promoting increased growth rates. In the previously mentioned research conducted by Burgess et al. (1994), 20 isolates of Pt were used to inoculate *Eucalyptus grandis* grown in phosphorus deficient pasteurized sand. Isolates were of widespread origin, encompassing a large portion of the Australian continent, Africa, Brazil, North America, and France. Isolate host plants ranged from other Eucalypts, to *Acacia*, *Allocasuarina*, and *Pinus*. Varying levels of mycorrhizae developed on the *E. grandis* seedlings, and isolates obtained from *Pinus* were particularly poor colonizers. In most cases, mycorrhizal development led to corresponding growth increases, encompassing a range of 2 to 45x more growth of *E. grandis* seedlings. This effect may be caused by Pt genetic diversity between North American and Australian isolates. Junghans et al. (1997) used RAPD-PCR analysis to determine that Pt isolates from separate regions had high levels of genetic distance, and suggest that Australian Pt is taxonomically distinct. In addition the authors noted that North American Pt were more successful colonizers of *Pinus* species, while Brazilian isolates of Pt were successful at colonizing *Eucalyptus*. However, some research suggests that the mycorrhizal association of Pt is even more precise than simple regionalism. Interestingly, in a study utilizing three isolates of Pt used to infect *Quercus rubra* L. growing in a forest clay loam/sand mix, Pt spores obtained from basidiocarps associated with *Pinus* were more successful at promoting growth than Pt spores associated with *Q. palustris* Muenchh. and *Q. acutissima* Carruth. (Marx,1979a).

Other studies have indicated that tree genotype is a driving factor in successful inoculation and growth enhancement with Pt. Marx (1979b) treated *Quercus alba* grown from seed collected from four separate mother trees with one isolate of Pt. While all four groups of seedlings formed ECM with Pt, there was variation in the degree of ectomycorrhizal development and growth enhancement. Dixon et al. (1987) noted differences in Pt isolate infectiveness on loblolly pine although all isolates did colonize the seedlings. In addition, the authors observed that growth response varied among trees with genotypic differences. Loblolly pine with different mother trees showed differences

in dry weight, root/shoot ratio, and leaf/total weight ratio when colonized with the same fungal isolate.

Other factors can influence the effectiveness of Pt as a mycobiont. Dry sites with high soil temperatures seem to illicit the greatest host growth benefit from Pt inoculation. Cline et al. (1986) showed that Pt exhibited the greatest growth compared to three other species of mycorrhizal fungi, with the highest mycelial growth rates occurring at temperatures between 21 and 32° C. Field studies support Pt as an effective colonizer in warmer, drier regions. In a previously mentioned study, Hatchell and Marx (1987) observed improved growth of three southern pine species growing in sandy soil. In contrast, a study performed in southwestern Oregon, where climate and soil characteristics differ significantly, showed that Pt provided no enhancement of growth of several conifer species when compared to stock colonized with native nursery fungi (Castellano and Trappe, 1991). In addition to water status, nutrient status can also have an affect on the formation of mycorrhizae by Pt. Beckjord et al. (1985) found that increased phosphorus levels suppressed formation of mycorrhizae by Pt on *Quercus rubra* and *Q. alba*. Ruehle (1980) observed increased Pt colonization of *Q. rubra* grown in milled pine bark with lower nitrogen and phosphorus levels than found in regular applications of standard fertilizers. However, those seedlings with lower foliar nutrient concentrations and higher Pt colonization levels were chlorotic while in production, indicating that they were less healthy.

Finally, the method of inoculation is an important consideration when applying Pt. Research has shown that Pt can effectively be applied with spore inoculations and also by the use of mycelium. Marx et al. (1989) showed that vegetative inoculum was more effective at colonizing *Pinus* than Pt spores. Additional research conducted by Marx (1990) indicated that applying Pt spores at the time of seed sowing can lead to acceptable levels of Pt colonization, classified as a Pt index > 50. However, none of the techniques using spores as an inoculum in this research were as effective at achieving colonization as the mycelium. Mycelium has distinct advantages of higher colonization rates and Pt indices, which leads to increased survival and growth (Marx et al.,1977). However, there

are some advantages to the use of spores when applying Pt. Pt spores can be gathered, stored, and incorporated into an inoculum product more quickly, cheaply, and with greater ease than vegetative inocula (Marx et al., 1979).

Forest seedling inoculation with *Scleroderma* spp.

Species in the genus *Scleroderma* are able to form mycorrhizae with a wide range of host plants. Of the fourteen known genera of host plants that form mycorrhizae with species of *Scleroderma*, there are several common genera to plants that form mycorrhizae with Pt; including *Quercus*, *Pinus*, *Tsuga*, *Pseudotsuga*, *Betula* and *Populus* (Jeffries, 1999). In fact, *Scleroderma* and *Pisolithus tinctorius* are closely related, 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. However, there is far less research addressing the effectiveness of *Scleroderma* as a mycobiont than research studying Pt. That research which does exist shows considerable promise for the use of *Scleroderma* as an inoculum for mycorrhizal formation on trees.

Pinus taeda seedlings showed a greater increase in growth rates when inoculated with *Scleroderma citrinum* versus *Pisolithus tinctorius*, *Rhizopogon roseolus*, and *Thelophora terrestris* (Ford et al., 1985). Over a range of phosphorus levels, seedling growth was increased the most with *Scleroderma*, and mycorrhizal indices were the highest. In a study with *Eucalyptus* spp., *Scleroderma* was a more effective mycobiont at stimulating increased growth than several other fungal species, but was less effective than Pt (Burgess et al., 1993). Beckjord and McIntosh (1983) showed dramatic results with *S. citrinum* inoculation of *Quercus rubra*. Increases of 31 -51% in height and 11-34 % in stem diameter after three years of growth were recorded when compared to trees inoculated with other fungi.

Mycorrhizae and landscape trees:

The body of research regarding the use of ectomycorrhizae, particularly Pt, on forest seedlings is quite extensive. The same can not be said for research regarding

ectomycorrhizae and landscape trees. Garbaye and Churin (1996) noted increases in shoot growth of *Tilia tomentosa* Moench. over a four year period. These trees were 8 years old at the time of planting and were placed in an urban setting. *Quercus virginiana* Mill. was inoculated with a commercial mycorrhizal product containing ECM in another study. After 30 months, there were no differences in growth or survival of the oaks inoculated with mycorrhizal fungi when compared to trees that were not inoculated (Gilman, 2001). In research focusing on the production of landscape trees, Sylvia et al. (1998) inoculated five ECM forming tree species with ECM fungi. *Q. laurifolia* Michx., *Q. myrsinifolia* Blume, *Q. virginiana*, *Taxodium mucronatum* L. Rich., and *Cedrus deodora* Trew. were inoculated while growing in raised in a sandy topsoil, green pine bark, and biosolids compost mix in raised beds or containers. Only the *C. deodora* and *Q. myrsinifolia* were colonized with ECM, and colonization levels were low.

Water relations:

Host plants colonized with ECM have shown increased resistance to stress from water deficit on several occasions. Walker et al. (1989) showed that *P. taeda* seedlings colonized with Pt showed less negative water potentials than those colonized with *Thelephora terrestris* Ehrh.: Fr. when measured during the third growing season after inoculation on a reclaimed coal surface mine site. Amelioration of the effects of drought was observed on *Quercus velutina* seedlings colonized with Pt (Dixon et al., 1983). However, isolate specificity appears to be an important facet of improved plant water relations. Lamhamedi et al (1992) used ten separate dikaryons of Pt to infect *Pinus pinaster* Ait. seedlings. Increased growth and water relations were seen on Pt colonized pines versus controls. However, there were significant differences in the transpiration rate, stomatal conductance and water potential between seedlings colonized with different dikaryons.

Ectomycorrhizal competition:

Although Pt is an effective colonizer of many tree species, it may be ineffective at

promoting growth and survivability of host trees at outplanting due to an inability to compete with indigenous fungi. McAfee and Fortin (1986) showed that *Pinus banksiana* Lamb. seedlings were most effectively colonized with Pt compared to *Laccaria bicolor* L. and *Rhizopogon rubescens* (Tul.) Tulasne. However, when outplanted, Pt root colonization did not continue to increase. In addition, indigenous mycorrhizal fungi were more likely to colonize the roots of Pt associated trees than trees colonized with the other fungi. Castellano and Trappe (1991) showed that Pt was ineffective at improving growth when compared to trees unintentionally colonized with indigenous mycobionts in southwest Oregon. The authors suggest that the moisture levels prevalent in Oregon make it more difficult for Pt to compete successfully with other fungi. Wu et al. (1999) showed that while Pt grew faster than *Suillus luteus* (L.:Fr.) Rouss. in a rhizobox, an unidentified ECM overgrew the Pt mycelia and mycorrhizae and inhibited Pt development over the course of 63 days.

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Chapter 3

Inoculation success rates of *Pisolithus tinctorius* (Pers.) Coker and Couch spores and mycelium when applied to *Quercus coccinea* Muenchh. and *Q. palustris* Muenchh. seedlings grown in various substrates under greenhouse conditions

Abstract

Proper protocol for mycorrhizal inoculation of container grown landscape trees is uncertain. Therefore, vegetative and commercial spore inoculum of *Pisolithus tinctorius* were applied to *Quercus palustris* (pin oak) and *Q. coccinea* (scarlet oak) seedlings. All plants were grown in containers filled with 0.9 liters of 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 completely unsuccessful at infecting seedlings in all substrates. The commercial spore inoculum colonized pin oak at a higher rate than scarlet oak, and vermiculite-based substrate proved superior then the other three substrates for inoculating seedlings with commercial Pt spores.

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 (Burgess et al, 1993; Dixon et al., 1983; Egerton-Warburton and Griffen, 1995; Ross and Marx, 1972). 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, orchidaeous mycorrhizae and ectendomycorrhizae. These symbiotic relationships occur on over 95% of plant species (Sylvia, 1998). Of the five types of mycorrhizae, ECM and AM are by far the most important in most global regions. In the temperate and boreal forests of North America, ECM fungi are the dominant form of mycorrhizae (Sylvia, 1998). Among others, these associations occur on the roots of members of the Fagaceae,

Pinaceae. This symbiosis is obligitive to the survival of the host plant. Thus, ECM symbiosis is the subject of a considerable body of research attempting to determine if fungal - tree associations can enhance tree seedling performance. More recently, 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 has emerged to help support this growing demand for ECM fungi and other mycorrhizal products. Of the wide range of ECM fungal species, only a few have been studied extensively in controlled experiments. These are species of *Cenococcum*, *Hebeloma*, *Laccaria*, *Paxillus*, *Pisolithus*, *Rhizopogon*, *Suillus*, *Thelephera*, *Sclerodemara*, *Amanita*, *Piloderma*, *Tomentella*, *Boletus*, and *Tuber* (Cairney and Chambers, 1999; Miller, 1983).

Pisolithus tinctorius (Pt) is a commonly used ectomycorrhizal fungus in forestry inoculation programs around the world. *Pisolithus tinctorius* has the ability to form ectomycorrhizae with species in at least twenty genera of plants including *Quercus*, *Pinus*, *Tsuga*, *Betula*, *Abies*, *Carya* (Marx, 1977), *Castanea* (Martins et al., 1996), *Populus* (Godbout and Fortin, 1985), and *Larix* (Molina and Trappe, 1982), all important genera for forestry and landscape applications. *Pisolithus tinctorius* has shown some success at improving post-transplant seedling growth and survival rates of many tree species (Marx and Bryan, 1970). Commercial inocula containing ECM in general, and Pt in particular, are being marketed to landscape nursery growers with the claim that inoculation programs can increase growth and survival rates of their plants. However, the vast majority of the research has focused on the growing systems utilized by forest seedling nurseries. The research does not take into account the use of containers for landscape plant production.

Oaks have been shown to derive a host plant benefit with Pt colonization (Dixon et al., 1984, Marx, 1979). Pin oak is generally regarded as an easy oak to grow and transplant successfully (Struve and Moser, 1984), while scarlet oak is considerably more difficult (Harris and Bassuk, 1995). The primary goal of this study was to determine the best mycorrhizal inoculation protocol for container production of these two species. We therefore evaluated two different inoculation methods, utilizing four different substrates.

Materials and Methods

Seedling preparation

Pin oak and scarlet oak acorns were obtained from the Sheffield's Seed Company, Locke, New York. Acorns were soaked in water for 24 hours and cold stratified at 5°C until radicals had emerged from all acorns. The germinated acorns were planted approximately 2.5 cm deep in 10.2 cm, liner containers (4" SVD, T.O. Plastics, Minneapolis, Minn.) filled with 900 cm³ of one of four substrates (see below).

Substrate preparation

Sixty 10.2 cm liner containers were filled with milled pine bark substrate (pH=5.1), which had been sterilized at 220°C for 2 hours. A second group of 60, 10.2 cm containers were filled with non-sterilized pine bark. Mineral soil (Unisom loam, 48.5% sand, 39.4% silt and 12.1% clay, pH=5.8) was screened through a 0.6-cm wire mesh to remove organic debris, sterilized at 220°C for 2 hours, and added to an additional 60, 10.2 cm containers. Vermiculite-based substrate (pH=5.4) was also sterilized at 220°C for 2 hours after finer particles were removed by screening with a 1.4-mm wire mesh, and Canadian sphagnum peat moss passed through the same 1.4-mm wire mesh was mixed into the vermiculite at a proportion of 10% by volume. Sterilization was performed in a closed metal box with heated water to reduce contamination by bacteria or opportunistic fungi such as *Thelophora terrestris* Ehrh.: Fr., which may limit colonization with Pt (Marx and Bryan, 1970).

Inoculum preparation

MycorTree™ Pt spore spray kits were obtained from Plant Health Care, Inc., Pittsburgh Penn. The spore product was mixed with water and applied to seedlings at a rate of 1 g of MycorTree™ Pt spore kit/0.082 m³ of substrate as suggested by the manufacturer. Vegetative inoculum was transferred and grown in liquid Hagem's solution (Van Cotter's

formulation, 1987, pers. com.) 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., on 20 September, 1995. At the time of inoculation, the floating mycelial cultures were placed in a sterilized blender and blended for 10 seconds at low speed. Openings were made into the substrate surface of each pot with a pencil to allow for easy movement of the inoculum into the root profile.

Planting, inoculation, and seedling care

The experiments were carried out at the Greenhouse complex of Virginia Tech. Germinated acorns of scarlet oak and pin oak were planted singly in the containers filled with each substrate on 15 May, 2000 in a completely random statistical design. All seedlings were fertilized with 4 grams of encapsulated slow-release fertilizer (15N-3.96P-9.96K, Osmocote® Plus, the Scotts Co., Maryville, Ohio). On 3 July, 2000 the seedlings were inoculated with either the MycorTree™ Pt spore spray kit mixture, the vegetative Pt inoculum. The control received no inoculum (=10 plants per inoculation x 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 of Virginia Tech throughout the course of the experiment. Seedlings within each substrate treatment were irrigated to container capacity daily or as needed to prevent drought stress until harvest.

Seedling harvest and mycorrhizal assessment

Harvest of seedlings began on 16 September, 2000 and ended on 4 October, 2000. Roots were washed clean of substrate and observed under a dissecting scope at 40x magnification to identify mycorrhizal symbiosis. Roots which showed signs of mantle development were observed and compared with those showing no signs of mantle development. A compound microscope at 100x magnification was used to verify mycorrhizal sheath formation.

Data recording and analysis

Success of inoculation was determined by the presence of mycorrhiza with the morphological characteristics of Pt. Mycorrhizae were determined to be present when the presence of a fungal mantle was observed on the roots of the oak seedlings. Attempts to transfer mycelia from mycorrhizal roots to pure culture to verify that the fungal material was Pt were unsuccessful. Each transfer attempt was overwhelmed by bacteria or other fungi. Olivaceous color of the mantle and the presence of clamp connections in the mantle were therefore used as identification characteristics of Pt. Mycorrhizal symbiosis identified with Pt as a mycobiont were given a "yes" rating, while seedlings with no mycorrhizae or mycorrhizae determined to have arisen from a fungus other than Pt were given a "no" rating. The Genmod procedure of the SAS system (vers. 8.01, SAS Institute Inc., Cary, N.C.) 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 (Stokes and Koch, 2001). When main effects were significant, single degree of freedom contrasts were used to perform pairwise comparisons of all substrates.

Results and Discussion

Spore kit inoculum

Application of the MycorTree™ Pt spore spray kit showed varying percentages of inoculation success (Figure 1). In all substrates, pin oak had a higher proportion of colonization rates than scarlet oak, and no interaction between substrate type and tree species was observed (Table 1 and Figure 1). Because these oaks are closely related, it seems unlikely that fungal isolate source is a primary contributor to the different colonization rates between species; however this possibility is not without support. Dixon et al. (1984) showed varying levels of colonization within oak species inoculated with different Pt isolates. The MycorTree™ Pt spore spray kit is an aggregation of spores obtained from Pt basidiocarps gathered from a variety of sources. As such, the product does not consist of a single Pt isolate, but has been gathered from mycorrhizal

associations from several tree genera. The product, therefore, makes isolate specificity a less likely candidate for explaining variations in colonization rates between tree species. This is especially important in view of the research which suggests that a large percentage of isolate specificity seems to be determined by geographical parameters and Pt phylogeny (Burgess, 1984; Junghans et al., 1998).

Vermiculite incorporated with 10% peat moss is a commonly used substrate for vegetative inoculation of forest seedling programs which have demonstrated improved growth rates (Marx et al., 1982). Landscape tree nurseries in Virginia, and much of the eastern United States, utilize milled pine bark as a growing substrate. However, vermiculite-based substrate had a higher proportion of successful inoculation than all other substrates (Table 2). The use of vermiculite-based substrate for trees grown to landscape size may be limited by problematic plant-substrate-water relations in larger containers as well as prohibitively high costs. Containers with vermiculite-based substrate tend to dry out more quickly than containers with pine bark substrate. A vermiculite-based substrate is not desirable for large trees grown for sale to the landscape industry and retail nurseries under current plant production and holding systems. Mycorrhizae formed on seedlings grown in mineral soils at similar proportions to seedlings grown in pine bark (Figure 1). Mineral soil is a poor choice for growing trees in containers due to its excessive weight and excess water holding capacities (Spomer, 1980). Mineral soil, as a substrate, was included in this research to determine if higher colonization rates could be obtained in a substrate that Pt may be found in naturally, but no benefit of using mineral soil was found.

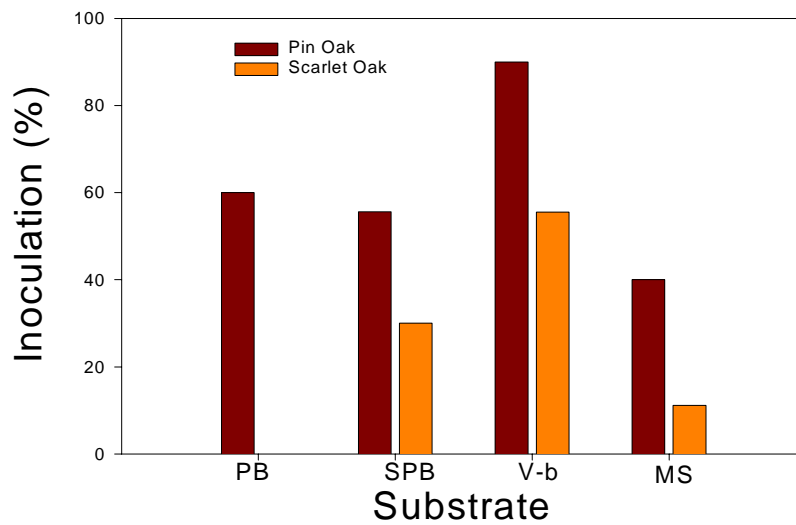
Vegetative inoculum

No mycorrhizal associations were observed with the mycelial treatment. However, living fungal hyphae with the morphological characteristics of Pt were present in all four substrate types. While it is impossible to determine the cause of the failure to form mycorrhizae, several possibilities exist. When mycelium of a mycorrhizal fungus remain in sterile culture for long periods of time, the ability to form mycorrhizae may diminish

(Laiho, 1970; Marx and Daniel, 1976). However, Pt isolate #3303 is currently being used in another experiment at Virginia Tech and has readily formed mycorrhizae with *Pinus virginiana* Mill. (personal consultation). A second possibility that may help to explain the failure of isolate #3303 to form mycorrhizae is isolate specificity. Burgess et al. (1994) showed great variation in effectiveness of Pt to illicit a growth response on *Eucalyptus grandis* W. Hill ex Maiden grown in phosphorus deficient pasteurized sand. However, the variation in positive growth responses observed by Burgess et al. were a result of varying levels of effectiveness of Pt isolates, not varying levels of colonization. In addition, Marx (1979a) showed that Pt inoculation of *Quercus rubra* L. grown in a forest clay loam/sand mix led to greater increased growth when colonized by Pt obtained from *Pinus* than from *Quercus*. This information seems to indicate that at least in some cases isolate source can be a contributing factor to ineffective inoculation. A third factor influencing the success of mycelial Pt inoculum to form mycorrhizae could be tree genotype. A single isolate of Pt has been shown to have varying colonization rates and levels of effectiveness when inoculating same species seedlings obtained from various genotype sources (Marx, 1979b; Dixon et al., 1987). However, as was the case when discussing isolate specificity, tree genotype variation led to different levels of colonization in these cases, not complete failure to infect. Another experiment conducted by Dixon et al. (1984) utilized five isolates of Pt vegetative inoculum obtained from *Quercus* or *Pinus* which all colonized *Q. velutina* Lam., *Q. alba* L. and *Q. robur* L. seedlings at a minimum rate of at least 85 %. Higher fertility levels have also been shown to reduce colonization rates produced by Pt vegetative inoculum (Ruehle, 1980). However, even at fertility levels of 500 µg/ml each of N-P-K, Pt colonization of *Q. rubra* grown in milled pine bark was 89%. Finally, excess heat may reduce fungal growth and mycorrhizal colonization. Cline et al. (1987) showed that optimal growth of Pt isolates varied between 21° and 32°. All isolates studied had reduced growth at 38°C. The authors suggest that the climate of the place of origin of an isolate may impact its ability to grow and form ECMat varying temperatures. All of these studies give possible explanations for the failure of Pt vegetative mycelium to form mycorrhizae with scarlet oak and pin oak; however none of these explanations can wholly explain this failure.

Conclusions

Inoculation of pin oak and scarlet oak with MycorTree™ Pt spore spray kits shows some promise for developing mycorrhizal symbiosis. Although seedlings grown in vermiculite-based substrate showed the highest colonization rates for both species, pine bark also shows promise of being an effective substrate for landscape seedling inoculation programs. With colonization rates approaching 60% for pin oak, inoculation of pin oak is a viable option, but pin oak transplants easily without inoculation.. More work needs to be performed in an attempt to increase successful inoculation rates to those levels enjoyed in forest seedling nurseries utilizing vermiculite-based substrate. In addition, inoculation of trees by mycorrhizal fungi does not always correlate with improved growth and/or survival rates of outplanted trees (Gilman, 2001; Castellano and Trappe, 1991). The lack of improvements in tree growth rates may be attributed to a lack of competitiveness of Pt with indigenous mycorrhizal fungi (McAfee and Fortin, 1985). Finally, scarlet oak was colonized less frequently than pin oak, illustrating the tenuous nature of the symbiosis. Mycorrhizal inoculation will not always garner the same positive results, and isolate source, tree species or genotype, and substrate can all be factors determining the level of success of inoculation programs.



PB = Pine bark, SPB = Sterilized pine bark,
 V-b = Vermiculite-based, MS = Mineral soil

Figure 1. Inoculation rates of pin and scarlet oak grown in various substrates (n=10 for each substrate x species). See table 1 for statistics.

Table 1. Logistic regression comparison of colonization rates of MycorTree™ Pt spore spray kits when applied to pin and scarlet oak in various substrates (n=10 for each species x substrate combination). See Figure 1 for colonization rates.

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

Table 2. Single degree of freedom contrast of pairwise comparisons of colonization rates between substrate types.

Contrast	DF	Chi-Square	P-value
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
Vermiculite-based vs. mineral soil	1	10.09	0.0015

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Chapter 4

The impact of the mycorrhizal fungus *Scleroderma bovista* Fr. on growth and water relations of *Quercus coccinea* Muenchh. during pot-in-pot production

Abstract

The efficacy of mycorrhizal inoculation of landscape trees during pot-in-pot production is unproven. Commercially produced *Pisolithus tinctorius* spore inoculum was therefore used in an attempt to inoculate bare-root *Quercus coccinea* (scarlet oak) trees before transplanting into 51-L containers placed in a pot-in-pot growing system. The commercial Pt product had a colonization rate of zero. An indigenous mycorrhizal fungus, *Scleroderma bovista*, infected many of the trees. Growth and resistance to drought stress of mycorrhizal scarlet oak grown in containers was assessed. Height and tree diameter growth were similar for mycorrhizal and nonmycorrhizal trees. Leaf water potentials (Ψ) were more negative for mycorrhizal trees than nonmycorrhizal trees, while stomatal conductance was reduced for mycorrhizal trees compared to nonmycorrhizal trees after 10 days of a dry-down treatment. Leaf water potential and stomatal conductance measurements indicated that mycorrhizal trees became drought stressed more quickly than nonmycorrhizal trees when grown in containers. Overall, there was no apparent benefit to mycorrhizal colonization of scarlet oak during pot-in-pot production.

Introduction

Mycorrhizae are symbiotic associations between higher plants and fungi that frequently provide the host plant with the benefits of improved nutrition, water relations, and resistance to pathogens and metal toxicity (Burgess et al, 1993; Dixon et al., 1983; Egerton-Warburton and Griffen, 1995; Ross and Marx, 1972). 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, orchidaeous mycorrhizae and ectendomycorrhizae. These symbiotic

relationships occur on over 95% of plant species (Sylvia, 1998). Of the five types of mycorrhizae, ECM and AM are by far the most important in most global regions. The forests of the eastern United States are dominated by trees that form ECM. Of particular importance to forestry researchers are ECM formation of plants in the Pinaceae and Fagaceae families. Thus, ECM symbiosis is the subject of a considerable body of research attempting to determine if fungal - tree associations can enhance tree seedling performance.

In addition to ECM associations of pine and oak species, members of the Betulaceae and Myrtaceae families that form ECM hold interest for landscape plant growers. To achieve ornamental quality, a large number of plant species are produced in landscape nurseries. Recently, landscape tree growers have begun to use ECM on some of these species in an attempt to improve survival and growth rates of trees in the nursery and after outplanting. An industry has emerged to help support this growing demand for ECM fungi and other mycorrhizal products. Of the wide range of ECM genera only a few have been studied extensively in controlled experiments. *Pisolithus tinctorius* (Pers.) Coker and Couch (Pt) is a widely used and studied ECM fungus (Chambers and Cairney, 1999). *Pisolithus tinctorius* is the most prevalent ECM ingredient in many commercial mycorrhizal products. *Pisolithus tinctorius* is a desirable fungus to work with because it is globally distributed, is easy to grow in vitro, and has been shown to have the ability to form ECM with a wide host range (Marx et al., 1982). Species in at least 20 genera of higher plants have been shown to form mycorrhizae with Pt, including commercially important species of hard and softwoods in genera like *Quercus*, *Betula*, *Eucalyptus*, *Pinus*, *Tsuga*, and *Pseudotsuga* (Marx, 1977). *Quercus* species have been shown to enjoy a host plant growth benefit when colonized with Pt (Dixon et al., 1984; Marx, 1979a; Marx, 1979b). Other studies have focused on improved water relations of plants subjected to drought stress (Dixon, et al., 1983; Parke et al., 1983). However, the vast majority of the research has been geared toward the growing systems utilized by forest seedling nurseries, and don't take into account the cultural techniques and practices of the landscape tree grower.

This study began as an attempt to determine if inoculation of scarlet oak with Pt would improve growth and plant water relations for trees grown in the popular pot-in-pot growing system. However, an indigenous fungus was far more successful at colonizing the trees used in this study. Therefore, the primary aim shifted towards assessing the effectiveness of *Scleroderma bovista* Fr. as a mycobiont in association with scarlet oak grown in the pot-in-pot system. *Scleroderma* is another genus which has species that form mycorrhizae with a wide range of host plants. Of the fourteen known genera of host plants, there are several common genera of plants with species that form mycorrhizae with Pt; including *Quercus*, *Pinus*, *Tsuga*, *Pseudotsuga*, *Betula* and *Populus* (Jeffries, 1999). In fact, *Scleroderma* and *Pisolithus tinctorius* are closely related, 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. However, there is far less research addressing the effectiveness of *Scleroderma* as a mycobiont than research studying Pt. That research which does exist shows considerable promise for the use of *Scleroderma* as an inoculum for tree mycorrhizae.

Materials and Methods

Plant material and fungal inoculation

On 20 April, 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 diameters measured 20 cm above the soil surface (standard error of the mean in parenthesis) were 219.5 (4.89) cm and 2.06 (.065) cm, respectively. Roots were washed free of soil, and 15 trees were randomly selected and dipped in MycorTree™ Pt root dip purchased from Plant Health Care, Inc., Pittsburgh, Penn. 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 of water per manufacturer's instructions, and the tree roots were dipped into the inoculum prior to planting into the 51-L containers. All trees were then immediately potted into 51-L containers (B-15, Lerio Inc., Mobile, Ala.) with 100%

milled pine bark (pH 5.1) as a substrate, top-dressed with 240 grams of encapsulated slow-release fertilizer (15N-3.96P-9.96K, Osmocote® Plus, the Scotts Co., Maryville, Ohio), and randomly placed into the pot-in-pot growing system. The remaining 15 trees received identical treatment, but were not dipped into the mycorrhizal inoculum. Height and trunk diameter measurements were taken with a hand caliper three times, on 20 April, 1999, 29 November, 1999 and 18 October, 2000.

Pot-in-pot growing system

The pot-in-pot system consists of 51-L socket containers recessed into the soil on 1.2 m on-center spacing in rows 1.5 m apart. The area between containers is covered with black landscape fabric, and an underground drainage system runs under each row to ensure that containers are not standing in water. Trees were placed in 51-L pots which snugly fit into the recessed socket pots, providing in-ground insulation but allowing for ease of removal. Each pot is equipped with an individual water emitter and was irrigated twice-a-day as needed to ensure that substrate moisture levels remained near container capacity throughout the growing seasons. A second application of 240 grams of encapsulated slow release fertilizer was applied in May, 2000. Trees were grown for two growing seasons under this system.

Mycorrhizal identification

Trees were allowed to grow undisturbed for a period 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. Pisolithus tinctorius* usually develops yellow or olivaceous mycelium (Marx et al., 1970). Sporocarps soon began to form on the surface of the pine bark substrate, allowing for identification of the fungus as *Scleroderma bovista*. This fungus was mycorrhizal with 17 out of the 30 trees

and there was no evidence of the original Pt inoculum treatment on the exterior of any root balls. At the conclusion of all research in October, 2000 the interior of rootballs of five trees colonized with *S. bovista* and five trees with no evidence of mycorrhizal colonization were randomly selected and examined. No other mycorrhizae were identifiable under 40x magnification using a dissecting scope.

Drought stress

On 3 August, 2000 six trees colonized with *S. bovista* and six uncolonized trees were randomly selected and potted up into 78-L containers (EG10000, Nursery Supplies, Inc., Fairless Hills, Penn.) and placed into a completely random statistical design. Trees were allowed to grow for 50 days to reestablish in the 78-L containers. These containers were large to utilize the pot-in-pot system. Therefore, they were left above ground and hand watered once a day or as needed to maintain at container capacity. On 22 September, 2000 three containers in each treatment were covered with plastic to prevent rain from entering the containers. These plants were not irrigated for the remainder of the study. The remaining three trees in each treatment were irrigated daily to container capacity over the course of the experiment. On a preplanned schedule [22, 23, 24, 27, 29 Sept. and 1 Oct. 2000] the trees were measured at two hour intervals, from 700 to approximately 2100 hours, for leaf water potential (Ψ). Leaf water potential was measured with a pressure chamber (Plant Moisture Stress, Corvallis, Or.)(Boyer,1967). Single leaves were removed with a razor blade from the first set of leaves behind the leaf cluster associated with the terminal buds of branches on the lower portion of the trees. Each Ψ value is the mean of two leaf subsamples.

A steady state porometer (LI-1600, LI-COR, Inc. Lincoln, Neb.) was used to measure leaf conductivity on 27, 29 Sept. and 1 Oct. 2000. Measurements were made between 1200 and 1400 hr. when light intensity levels were at least $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Measurement were taken on one sun-facing leaf per plant.

Data analysis

Statistical analysis of mean water potentials and mean conductivity was performed using the one-way analysis of variance procedure of JMP (vers. 4, SAS Institute, Inc. Cary, N.C.) Statistical analysis of trunk diameter and height growth data was performed using the one-way analysis of variance procedure of SAS (vers.8.01, SAS Institute, Inc. Cary, N.C.).

Results and Discussion

Height and trunk diameter growth were similar between mycorrhizal and nonmycorrhizal treatments (Table 1). 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. (1994) inoculated several tree species with isolates of *Scleroderma*. *Eucalyptus grandis* W. Hill ex Maiden showed the greatest increase in shoot dry weight of 32%. Beckjord and McIntosh (1983) inoculated *Quercus rubra* L. seedlings with six species of ECM fungi. Seedlings inoculated with *Scleroderma citrinum* Pers. had greater growth than all other fungi and controls. Up to 34% increases were seen by the third growing season after inoculation. In another mycorrhizal study, *Quercus robur* L., *Q. velutina* Lam., and *Q. alba* L. were inoculated with a variety of ectomycorrhizal fungi including Pt. *Pisolithus tinctorius* elicited increased growth responses in all three species of oak (Dixon et al., 1984). Garbaye and Churin (1997) found that *Paxillus involutus* (Batsch: Fr.) increased the growth rates of *Quercus patraea* (M. Liebl) and *Q. robur* after outplanting in a loamy soil.

Evidence exists that contradicts the theory that mycorrhizae always increase plant growth rates. Colpaert et al. (1996) studied the effect of extensive extramatrical mycelium on the growth of host *Pinus sylvestris* L. seedlings. Nine ECM were studied and all colonized trees had significantly smaller biomass than uncolonized controls. Increased fungal biomass appeared to be directly correlated with decreased plant biomass. Scotch pine colonized with *S. citrinum* had 50% of the biomass of uncolonized trees. While fungal

biomass measurements were not taken in the current study, sporocarp production was extremely high during the entire 2000 growing season (visual obs.) and may have led to an energy drain on the host scarlet oak trees which were grown in a fertile, well watered environment. Beckjord et al., (1985) showed that *Scleroderma auranteum* (Pers.) was less effective than Pt at enhancing growth of *Q. alba* and *Q. rubra*. Overall weight of *Q. rubra* was actually decreased by *S. auranteum*.

Although there was no growth rate increase of tree height or trunk diameter of scarlet oak growing in the pot-in-pot system when colonized with *S. bovista*, potential benefits with regards to water stress amelioration by mycorrhizae may still make mycorrhizal inoculation programs of landscape trees worthwhile. As expected, scarlet oaks that were well watered showed no decrease in water potentials over the course of the 10 day dry down period, regardless of *S. bovista* colonization. Trees that were subject to dry down did show drought stress through more negative water potential measurements (Figures 1-3). By day six of the dry down, trees that were deprived of water had more negative water potentials than wet trees, but mycorrhizal inoculation was not yet a significant factor and there was no significant interaction (Table 2). The same results were observed on the eighth day of the dry down (Table 2). By day 10 of the dry down, mycorrhizae had also become a significant factor, and there was a significant interaction between mycorrhizae and drought stress. Interestingly, the trees colonized with *S. bovista* had more negative Ψ than the nonmycorrhizal trees when comparing trees subject to water deficit stress (Figures 1-3).

Conductance measurements showed a similar trend. Conductance decreased rapidly for plants subject to water deficit stress, and differences were significant by day six. However, no difference was apparent for mycorrhizal versus nonmycorrhizal plants (Table 3). The same results were observed on the eighth day of the dry down. On day 10 the observed decrease in conductivity of mycorrhizal plants compared to nonmycorrhizal plants was also significant, but there was no significant interaction between mycorrhizal treatment and water stress. Lower leaf conductivity is evidence that the mycorrhizal trees are more drought stressed, and have undergone stomatal closure.

Several studies have indicated that mycorrhizal colonization can ameliorate water stress in host plants (Dixon et al., 1983; Duddridge et al., 1980). Walker et al. (1989) studied *Pinus taeda* L. seedlings inoculated with the Pt. After three years of growth on a reclaimed coal surface mine site, seedlings had less negative water potential measurements than controls colonized with *Thelophora terrestris* Ehrh.: Fr. Dry sites with high soil temperatures seem to illicit the greatest host growth benefit from Pt inoculation (Hatchell and Marx, 1987). 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 colonized with native nursery fungi (Castellano and Trappe, 1991). 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 (Newman and Davies, 1988). Dosskey et al. (1990) found that with *Rhizopogon vinicolor* Smith, an ECM fungus, stomatal conductance was enhanced for *Pseudotsuga menziesii* (Mirb.) Franco seedlings even at lower leaf water potential. They hypothesized that the stomatal conductance was correlated to photosynthate export to the fungus. The carbon drain on the host plant may have caused the stomata to remain open. In a study utilizing AM fungal colonization of geranium grown in containers, Sweatt and Davis (1984) 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 enjoyed by mycorrhizal plants. In effect, the increased photosynthetic demand required by mycorrhizal fungi may prove detrimental to trees grown in containers or with restricted root zones by forcing the host plant to use up its water supply more quickly.

Conclusions

This study illustrates the sometimes tenuous nature of mycorrhizal symbiosis. For unknown reasons, the MycorTree™ Pt root dip was ineffective at colonizing landscape size scarlet oak trees. In another study, mycorrhizal inoculation of scarlet oak with Pt spores was ineffective compared to inoculation of pin oak (chapter 3). In contrast, *Scleroderma bovista* proved to be an opportunistic colonizer of scarlet oak. Although mycorrhizae were not evident on the tree roots at the time of digging, *S. bovista* sporocarps were present in the mineral soil bed later in the growing season, indicating many of the trees may have been colonized at the time of digging. However, the mere presence of a mycorrhizal association does not guarantee a benefit to the host plant, particularly in closely monitored systems such as pot-in-pot growing systems. Trunk diameter measurements showed no increases in growth for mycorrhizal trees versus nonmycorrhizal trees. From this perspective scarlet oak trees will not be larger at the time of sale, or sold more quickly, if the mycorrhizal fungus *S. bovista* is utilized. In addition, *S. bovista* showed no benefit in ameliorating the effects of drought stress. In fact, the fungus appeared to exacerbate drought stress of scarlet oaks with a limited water supply and root zone. Colonization with certain ECM fungi does not appear to be beneficial during the production phases of landscape size scarlet oak trees. More 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.

Table 1. P- values for the effects of mycorrhizal colonization on height and stem diameter growth of scarlet oak over two growing seasons analyzed by ANOVA (n=6).			
	Height growth	Stem diameter growth	
	P>F		
<u>Year</u>			
1999	0.2450		0.950
2000	0.7916		0.158
Total	0.4588		0.350

Table 2. P- values for the effects of mycorrhizal colonization and drought stress on mean leaf water potential (Ψ) of scarlet oak analyzed by ANOVA (Ψ = mean of two leaves from each of two randomly selected trees per treatment).

	9/27	9/29	10/1
	P>F		
<u>Source</u>			
Mycorrhizae	0.1282	0.2942	0.0021
Stress	<.0001	<.0001	<.0001
Mycorrhizae*Stress	0.0594	0.3787	<.0001 ^z
z.= Analysis of interaction with ANOVA showed that mycorrhizal trees were significantly different than nonmycorrhizal trees in dry media (p<.0001) but not wet media (p=0.3271).			

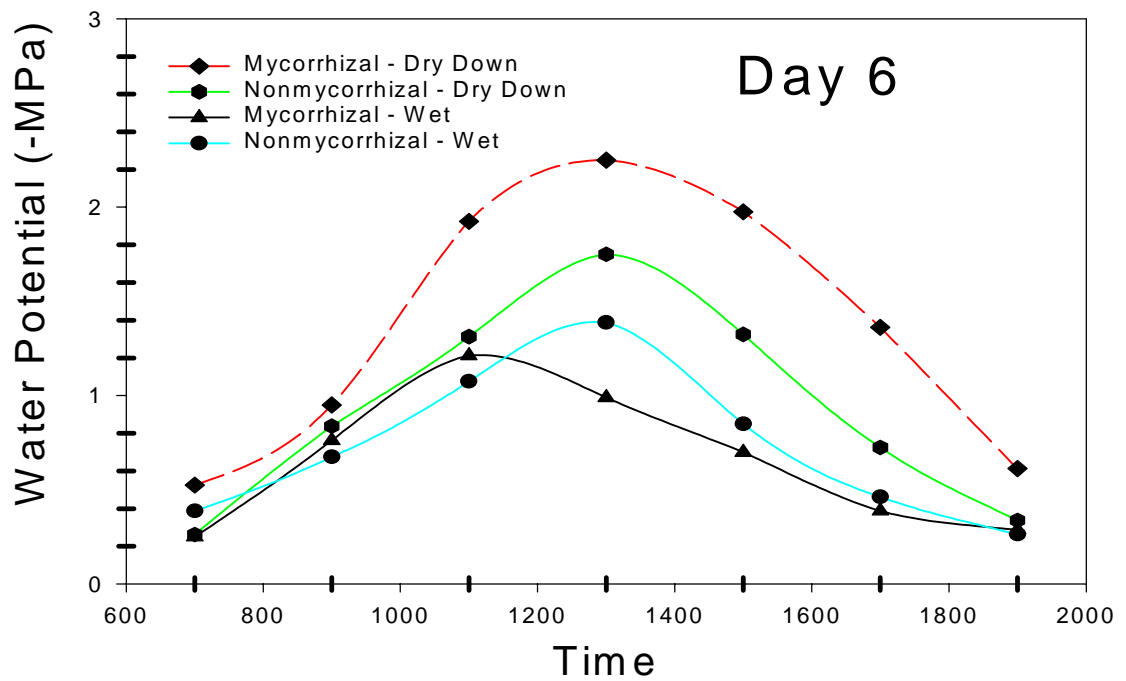


Figure 1. Leaf water potentials of mycorrhizal and nonmycorrhizal trees 6 days after withholding irrigation (dry down) or with daily irrigation (wet). Leaf water potential = mean of two leaves from each of two randomly selected trees per treatment.

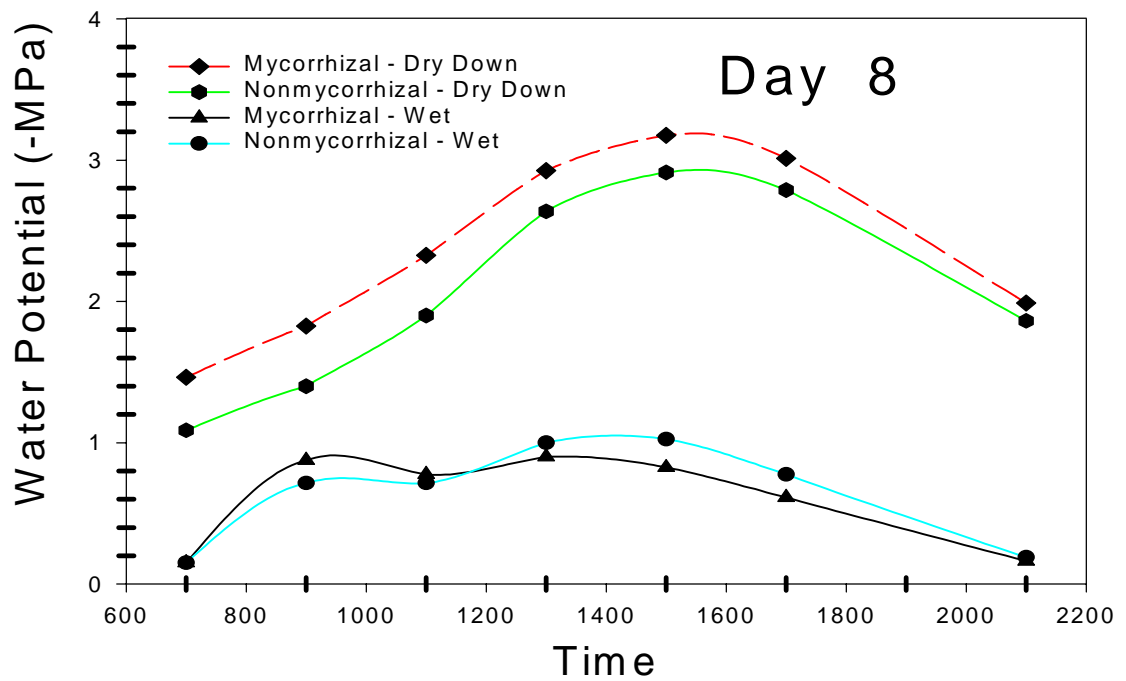


Figure 2. Leaf water potential of mycorrhizal and nonmycorrhizal trees 8 days after withholding irrigation (dry down) or with daily irrigation (wet). Leaf water potential = mean of two leaves from each of two randomly selected trees per treatment.

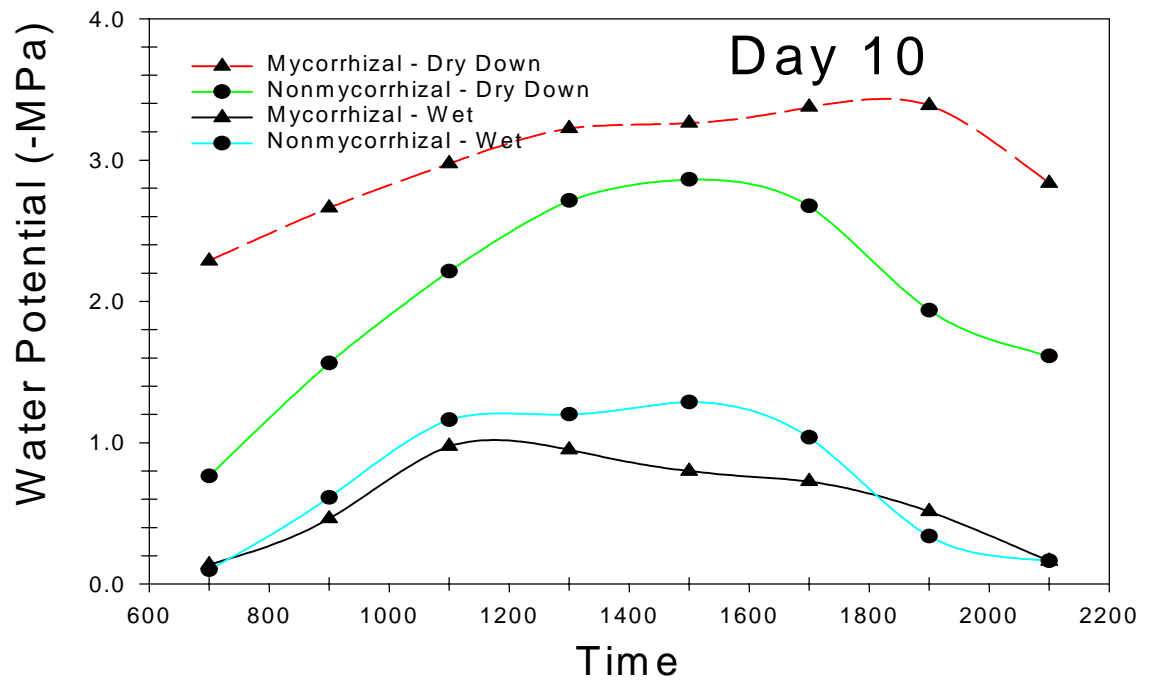


Figure 3. Leaf water potential of mycorrhizal and nonmycorrhizal trees 10 days after withholding irrigation (dry down) or with daily irrigation (wet). Leaf water potential = mean of two leaves from each of two randomly selected trees per treatment.

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Chapter Five

Conclusions

Results of this thesis indicate that mycorrhizal inoculation programs of landscape trees with *Pisolithus tinctorius* (Pt) do not consistently infect all trees where Pt is a known mycorrhizal symbiont. In addition, mycorrhizal symbiosis can decrease leaf water potentials in plants with restricted root zones when they are subject to drought stress. Although Pt is known to form mycorrhizae with both scarlet and pin oak, the commercial product used in this experiment more readily colonized pin oak than scarlet oak (chapter 3). Higher colonization rates may be obtained if more mycorrhizal species of fungi are used for various tree species (chapters 3 and 4). Vermiculite-based substrate is more suited to mycorrhizal inoculation programs that use Pt rather than pine bark, sterilized pine bark, or sterilized mineral soil (chapter 3). Growth of trees in the pot-in-pot growing system was not improved by mycorrhizal colonization (chapter 4). Mycorrhizal trees with confined root zones were more susceptible to drought stress than nonmycorrhizal trees (chapter 4).

There are several possible reasons for the variation of colonization rates of pin and scarlet oak. Isolate specificity (Dixon et al., 1984) and tree genotype (Marx, 1979) may effect compatability between the host plant and the fungus. Mycorrhizal fungi do require carbon from the host plant, and can act as a carbon drain (Dosskey et al. 1990). The rapid growth rate of pin oak compared to scarlet oak may be more suited to supplying the carbon demands of the mycorrhizal fungus, leading to higher colonization rates on pin oak.

Vermiculite-based substrate is the preferred method for applying vegetative inoculum to plants (Marx et al. 1982). Therefore, it is no surprise that the trees growing in vermiculite-based substrate had higher colonization rates than trees growing in other substrates. Unfortunately, the water holding capacity of vermiculite is more limited than pine bark, the current landscape nursery standard, and this characteristic may prove

prohibitive to its large-scale use in landscape tree production. In addition, the increased cost of vermiculite compared to pine bark may also prove prohibitive to the large-scale use of vermiculite-based substrate.

Because no growth benefit was observed for mycorrhizal trees growing in the pot-in-pot system, it is unlikely that mycorrhizal inoculation programs will enable a landscape tree grower utilizing the pot-in-pot system to sell a larger plant in a shorter period of time. In addition, trees with restricted root zones are quite common in landscape and urban environments. The rapid depletion of limited water supplies in restricted root zones may induce higher levels of drought stress in these environments (Sweatt and Davis, 1984), and may limit the survival rates of mycorrhizal trees at some planting sites.

In conclusion, the relationship between trees and mycorrhizal fungi is complex. Mycorrhizal inoculation programs that involve more than one species of tree may benefit from a large number of species of mycorrhizal fungi. In addition, urban and landscape environments are highly variable. Mycorrhizal trees planted in restricted root zone sites may not perform well, while mycorrhizal trees in other sites may outperform nonmycorrhizal trees. Far more research is required to fully assess the advantages and disadvantages of mycorrhizal inoculation programs of trees grown for landscape use.

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Vita

Thomas Paul Martin was born in Fort Belvoir, Va. on September 10, 1969. He was raised in Annandale, Va, and graduated from Bishop Denis J. O'Connell High School in 1987. Tom began at Virginia Tech the following fall, and graduated with a B.S. in psychology in 1992 and added a B.A. in English with a history minor the following year. After working several unfulfilling jobs, Tom and his wife, Lissa, were employed in 1994 by the Association for the Preservation of Virginia Antiquities as curators and site administrators of the Smithfield Plantation House in Blacksburg, Va. At Lissa's urging, the following year Tom also started working at Laurel Creek Nursery and soon fell in love with ornamental plants. While working as the assistant manager of Laurel Creek's retail division, Tom applied to the Graduate school of Virginia Tech to pursue a degree in horticulture. Tom began work on his Master of Science degree in the fall of 1998. He began working as a GTA the following year, where he has assisted in nursery crops, landscape establishment and maintenance, plant propagation, and indoor plants classes. He has also worked as a summer caretaker at the Virginia Tech Urban Horticultural Center. Tom has two wonderful children to keep him busy, Finn is four years old and Riley is six months old.