

# MYCORRHIZAL FUNGAL INOCULATION OF ESTABLISHED STREET TREES

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**Abstract.** Street trees are frequently subjected to a variety of stressing environmental factors and cultural practices. Those whose roots are colonized by mycorrhizal fungi are reported to better tolerate adverse conditions. However, 6 months after inoculation of 13 cm diameter pin oak (*Quercus palustris*) with a commercial mycorrhizal fungal product, there was no significant response in root dry weight or root colonization. After 1 year, the same trees showed no significant inoculation response as measured by leaf chlorophyll content or trunk diameter. Root dry weight was significantly increased by a commercial mycorrhizal fungal inoculant/fertilizer combination application to 61 cm diameter willow oak (*Quercus phellos*). For 13 cm diameter red maple (*Acer rubrum*), root colonization increased significantly in response to two commercial mycorrhizal fungal inoculant treatments, and root dry weight increased significantly in response to two fertilizer treatments 6 months after application. Our data indicated no apparent measurable growth benefit, under the terms and conditions of this research, to inoculation with a commercial mycorrhizal fungal product unless combined with fertilizer. Pretreatment evaluation of roots for all tree species revealed some colonization by mycorrhizal fungi; therefore, having tree roots analyzed to determine their existing level of colonization may aid in determining whether any benefits might be derived from applying mycorrhizal fungal inoculants.

**Key Words.** *Acer rubrum*; ectomycorrhizal; endomycorrhizal; mycorrhizae; *Quercus palustris*; *Quercus phellos*; vesicular-arbuscular.

Mycorrhizal fungi are naturally occurring or artificially introduced fungi that can colonize the roots of trees, imparting benefits including increased water and nutrient uptake, increased resistance to environmental extremes, and increased resistance to pathogens (Kropp and Langlois 1990; Sylvia and Williams 1992). A majority of the street tree species commonly grown by the nursery industry have the capacity to form mycorrhizal associations with fungi (Smith and Read 1997).

The benefits of applying mycorrhizal fungal inoculants to trees would be expected to be greatest when trees are under stress (Sylvia and Williams 1992). Such stressful times include at transplant (due to root loss), during periods of

environmental extremes (drought, excess heat), when trees are damaged through construction, and as trees begin to mature and decline. Likewise, benefits of inoculation would be expected for street trees due to the limiting soil conditions in which street trees frequently grow: reduced volume, compaction, low organic matter, low nutrient and water reserves, chemical pollution, etc.

There are numerous anecdotal reports of the benefits of introducing mycorrhizal fungal inoculants into soils surrounding the roots of existing street trees. Though practitioners report positive responses to their inoculation trials (Scott-Lifland 2000), conclusive evidence of the efficacy of mycorrhizal inoculants is often lacking. There are a variety of reasons for this, including lack of treatment replication, lack of or inappropriate experimental design, lack of control application, limited evaluation time period, use of inoculant products that include more than mycorrhizal fungal spores or hyphae (i.e., biostimulants, dilute fertilizers, bacteria), and failure to get laboratory confirmation of fungal colonization.

To date, only limited research, using commercially produced inoculants, has been published. Martin and Stutz (1994) reported that pre-transplant inoculation of container-grown Argentine mesquite (*Prosopis alba*) enhanced mycorrhizal root colonization but suppressed post-transplant growth compared to the noninoculated control plants under low soil moisture. Because reduced aboveground growth may be adaptive under adverse conditions, this mycorrhizal response may be ecologically, but not economically, beneficial.

Carlson et al. (2000) reported no survival or growth enhancement 1 year after inoculating willow oak (*Quercus phellos*) at transplant. Gilman (2001) reported no effect on post-transplant stress, growth, or survival after 30 months for live oak (*Q. virginiana*) inoculated at transplant. Morrison and Nicholl (1993) reported some positive growth response to vesicular-arbuscular mycorrhizal inoculation at nursery fertility levels and with competition from indigenous mycorrhizal fungi, but they found little direct response to the inoculum used in their study.

Garbaye and Churin (1996) reported growth stimulation and delayed fall yellowing of leaves when silver linden (*Tilia tomentosa*) were inoculated at transplant with three different mycorrhizal fungi, alone and in combination. This response first appeared 2 years after transplanting. Stabler et al. (2001) reported that tree species native to the desert had greater colonization by arbuscular mycorrhizal fungi (AMF)

than the same species when used as residential landscape trees in more recently disturbed soils. They concluded that AMF might significantly increase landscape tree carbon storage potential depending on tree species, AMF population characteristics, soil water availability, and improved phosphorus uptake.

Smiley et al. (1997) reported a significant and rapid increase in fine root growth and ectomycorrhizal development in response to fertilizer, mycorrhizal inoculant, and a fertilizer/inoculant combination 4 and 7 months after treatment application to existing 56 to 71 cm diameter willow oak, northern red oak (*Q. rubra*), and pecan (*Carya illinoensis*). Marx et al. (1997) reported a similar response to these same treatments 6 months after their application to 0.6 to 1.5 m diameter existing live oaks. Both of these studies involved a small number of trees (five and seven, respectively), with multiple treatments applied to quadrants of roots of the same tree. Both recommended additional, longer-term studies.

The objective of this study was to evaluate the above- and belowground growth and colonization effects of inoculation of soil surrounding established street trees with a commercial mycorrhizal fungal product.

## MATERIALS AND METHODS

Three species of established trees at three different sites were used: 13 cm diameter pin oak (*Q. palustris*) in 3 × 5 m single tree islands, or 3 × 11 m double tree planting islands in an asphalt-covered Lowe's parking lot in Richmond, Virginia, U.S.; 13 cm diameter red maple (*Acer rubrum*) in a 2.4 to 3 m grassy parking lot/street median in Chesapeake, Virginia; and 61 cm diameter willow oak (*Q. phellos*) in street medians of varying dimensions in Chesapeake, Virginia. Tree calipers were measured 10 cm above ground level. The pin oaks had been in the ground for 6 years, the red maples for 4 years, and no data were available for the willow oaks (presumed at least 30 years due to size).

All planting soils were disturbed by infrastructure construction. Soil types and pH were: pin oak—clayey, pH 5.2 to 5.3; red maple—clayey, pH 6.5; willow oak—loamy, pH 6.6 (Virginia Tech Soil Testing Laboratory, Blacksburg, VA). Prior to inoculation at each site, two random soil samples, for analysis for existing mycorrhizal fungi (Soil Foodweb Inc., Corvallis, OR), were removed from within tree drip lines. Roots of weeds and bermudagrass were removed from the samples. Pin and willow oaks are normally ectomycorrhizal (Dixon et al. 1984), and red maples are normally endomycorrhizal (VAM or AMF) (Marx et al. 1989).

Following the method outlined by Marx et al. (1997) and Smiley et al. (1997), a random grid pattern under the drip line of each tree was developed for treatment application and installation of root-ingrowth cores (RICs, Plant Health Care, Frogmore, SC). Four inoculant treatments were applied in May 1998. Using a motor-driven tank and a soil injection nozzle at 150 psi, the following treatments were

delivered to a 20 cm depth: 2 L water (control), 1.4 kg N/378.5 L 9-45-15 water-soluble fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH), 114 g/378.5 L injectable *Pisolithus tinctorius* spores with a yucca surfactant (MycorTree Injectable plus PHC BioPak, Plant Health Care, Pittsburgh, PA), and the above fertilizer and mycorrhizal inoculant combined, each dissolved in 2 L water. Each tree received the following number of injections: pin oak—15 injections on 0.7 m centers; red maple—13 injections on 0.9 m centers; willow oaks—22 injections on 0.9 m centers. Following treatment applications, RICs were installed adjacent to the injection sites for subsequent root harvest and analysis (Marx et al. 1997).

The experimental design was a randomized complete block. In the double pin oak tree islands, only one of the two trees was inoculated. One treatment was applied per tree, with four replications per treatment for pin oaks and red maples, and three for willow oaks.

Root-ingrowth cores were removed in October 1998, 6 months after treatment application, for root colonization analysis and root dry weight determination. Leaf chlorophyll content (Chlorophyll Meter SPAD-502, Spectrum Technologies, Inc., Plainfield, IL) was recorded for pin oak and red maple 1 year after treatment application. Ten mature leaves, randomly spaced in the outer canopy of each tree, were read and averaged per tree. Using a diameter tape, trunk diameter was measured (the average of two measurements taken at right angles) at treatment application and 1 year later for pin oak and red maple. Root colonization (percentage of root sample colonized), root dry weight (roots from all RICs per tree pooled), leaf chlorophyll readings, and change in trunk diameter were subjected to analysis of variance.

## RESULTS AND DISCUSSION

Six months after treatments were applied to the pin oak, there were no significant differences among the treatments in *P. tinctorius* (inoculated fungus) colonization or root dry weight. One year after inoculation, there were no significant increases among treatments in trunk diameter or leaf chlorophyll content. No additional data could be obtained from the pin oak site due to topping of several of the treated trees. Microbial analysis of the pre-inoculation roots of the pin oak revealed an "acceptable" level (Soil FoodWeb rating; no actual numerical quantification given with their quality rating) of pre-inoculation colonization.

Six months after treatments were applied to the red maples, the two mycorrhizal treatments significantly increased the percentage of roots with VAM colonization, and the fertilizer plus mycorrhizal inoculant treatment significantly increased root dry weight over the control and the mycorrhizal inoculant alone treatments (Table 1). One year after inoculation, there were no significant increases in trunk diameter and no significant differences in leaf chlorophyll content. Analysis of the pre-inoculation roots of

the red maple also revealed an “acceptable” level of pre-inoculation colonization that may account for the only significant root growth increase resulting from the two treatments containing fertilizer.

Six months after treatments were applied to the willow oaks, there were no significant differences in *P. tinctorius* (inoculated fungus) or other fungal colonization. As with the pin oak and red maple roots, analysis of the pre-inoculation roots of the willow oaks revealed an “acceptable” level of pre-inoculation colonization.

Pre-inoculation colonization may account for the lack of tree response to the inoculated fungus (*P. tinctorius*), or competition from existing fungi may have prevented the inoculated fungi from colonizing (Smith and Read 1997). It is possible that because the roots had an acceptable level of colonization, nitrogen that was applied in solution was readily absorbed and used by the trees, resulting in increased root growth in response to the fertilizer treatments. The significant increase in root dry weight in response to the fertilizer/inoculant combination (Table 2) may reflect the benefit of increasing colonization of large trees growing in limited soil volumes in order to increase fertilizer absorption.

Analysis of existing tree roots for the presence of native mycorrhizal fungi may be advisable prior to deciding about the application of mycorrhizal fungal inoculants. In addition, the use of other cultural practices, such as fertilization, should be considered relative to the possible effect of these practices on native or inoculated mycorrhizal fungi. Low levels of fertilizer generally do not depress new ectomycorrhizal colonization on trees (Cline and Marx 1995); therefore, on sites where root analysis shows acceptable levels of colonization, treatment with fertilizer alone may be sufficient to stimulate root growth.

## LITERATURE CITED

- Carlson, J., Powell, M.A., and S.L. Warren. 2000. Can mycorrhizae improve tree establishment in the landscape? Proc. SNA Res. Conf. 45:405–407.
- Cline, M.C., and D.H. Marx. 1995. Atmospheric nitrogen deposition and the mycorrhizae of southern commercial forest trees, pp 337–387. In Fox, S., and R.A. Micler (Eds.). Impact of Air Pollutants on Southern Pine Forests. Springer-Verlag, New York, NY.

**Table 1. Root dry weight and percentage of ectomycorrhizal root colonization of red maples 6 months after application of treatments.**

Treatment	Root dry weight (g)	% ectomycorrhizal roots
Water	8 a*	17 a
Fertilizer	18 ab	21 a
Mycorrhizal inoculant	14 a	42 b
Fertilizer + mycorrhizal inoculant	30 b	51 b

\*Means in a column followed by the same letter are not significantly different ( $P = 0.10$ ) using Duncan's Multiple Range Test.

**Table 2. Root dry weight, percentage of ectomycorrhizal root colonization, and percentage of other fungal colonization of willow oaks 6 months after application of treatments.**

Treatment	Root dry weight (g)	% <i>Pisolithus tinctorius</i> root colonization	% other fungi root colonization
Water	7 a <sup>z</sup>	3 <sup>y</sup>	17 <sup>y</sup>
Fertilizer	12 a	5	18
Mycorrhizal inoculant	7 a	5	20
Fertilizer + inoculant	28 b	0	12

\*Means in a column followed by the same letter are not significantly different ( $P = 0.10$ ) using Duncan's Multiple Range Test.

<sup>z</sup>No significant differences.

- Dixon, R.K., H.E. Garrett, G.S. Cox, H.D. Marx, and I.L. Sander. 1984. Inoculation of three *Quercus* species with eleven fungal isolates of ectomycorrhizal fungi. 1. Inoculation success and seedling growth relationships. For. Sci. 30:563–574.
- Garbaye, J., and J.L. Churin. 1996. Effect of ectomycorrhizal inoculation at planting on growth and foliage quality of *Tilia tomentosa*. J. Arboric. 22:29–34.
- Gilman, E.F. 2001. Effect of nursery production method, irrigation, and inoculation with mycorrhizae-forming fungi on establishment of *Quercus virginiana*. J. Arboric. 27:30–38.
- Kropp, B.R., and C.G. Langlois. 1990. Ectomycorrhizae in reforestation. Can. J. For. Res. 20:438–451.
- Martin, C.A., and J.C. Stutz. 1994. Growth of Argentine mesquite inoculated with vesicular-arbuscular mycorrhizal fungi. J. Arboric. 20:134–138.
- Marx, D.H., C.E. Cordell, and P. Kormanik. 1989. Mycorrhizae: Benefits and Practical Application in Forest Tree Nurseries. Forest Nursery Pests (USDA Forest Service Agricultural Handbook) 680:18–21.
- Marx, D.H., M. Murphy, T. Parrish, S. Marx, D. Haigler, and D. Eckard. 1997. Root response of mature live oaks in coastal South Carolina to root zone inoculations with ectomycorrhizal fungal inoculants. J. Arboric. 23:257–263.
- Morrison, S.J., and P.A. Nicholl. 1993. VA mycorrhizal inoculation of landscape trees and shrubs growing under high fertility conditions. J. Environ. Hortic. 11:64–711.
- Scott-Lifland, J. 2000. The great fungi experiment lives up to its name. Tree Care Ind. 11(12):48, 50–51.
- Smiley, E.T., D.H. Marx, and B.R. Fraedrich. 1997. Ectomycorrhizal fungus inoculations of established residential trees. J. Arboric. 23:113–115.

- Smith, S.E., and D.J. Read. 1997. *Mycorrhizal Symbiosis*. Academic Press, New York, NY. 605 pp.
- Stabler, L.B., C.A. Martin, and J.C. Stutz. 2001. Effect of urban expansion on arbuscular mycorrhizal fungal mediation of landscape tree growth. *J. Arboric.* 27:193–202.
- Sylvia, D.M., and S.E. Williams. 1992. Vesicular-arbuscular mycorrhizae and environmental stress, pp 101–124. In Linderman, R.G., and G.J. Bethlenfalvay (Eds.). *Mycorrhizae in Sustainable Agriculture*. Special publication No. 54, American Society of Agronomy, Madison, WI.

**Acknowledgments.** We would like to thank the International Society of Arboriculture's Research Trust for funding, and Don Marx, Selina Marx, Dave Young, and the crews of Arborcare Professional Tree Service and the City of Chesapeake Parks and Recreation for field assistance with this research project.

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**Résumé.** Les arbres de rues sont fréquemment soumis à une variété de stress causés par des facteurs environnementaux et des pratiques culturelles. Ceux dont les racines sont colonisées par des mycorhizes sont reportés comme étant plus tolérants aux conditions adverses. Néanmoins, six mois après l'inoculation de chênes des marais (*Quercus palustris*) de 13 cm de diamètre avec un produit commercial de champignons de mycorhizes, il n'y avait pas de réponse significative quant à la masse sèche en racines ou la colonisation par les racines. Après un an, les mêmes arbres ne montraient aucune réponse significative à l'inoculation, et ce en mesurant le contenu en chlorophylle

foliaire ou le diamètre du tronc. Chez un chêne saule (*Quercus phellos*) de 61 cm de diamètre, la masse sèche en racines était significativement accrue par l'application combinée d'un inoculant commercial de champignons de mycorhizes et d'un engrais. Chez des érables rouges (*Acer rubrum*) de 13 cm de diamètre, la colonisation des racines s'accroissait significativement en réponse à deux traitements avec un inoculant commercial de champignons de mycorhizes, et la masse sèche en racines s'accroissait significativement en réponse à deux traitements avec un engrais six après leur application. Nos données indiquent qu'il n'y a pas de bénéfice apparent et mesurable de la croissance, et ce sous les termes et les conditions de cette recherche, à l'inoculation avec un produit commercial de champignons de mycorhizes, à moins qu'elle ne soit combinée avec un engrais. Une évaluation des racines de toutes les espèces d'arbres, avant le traitement, a révélé une certaine colonisation par des champignons de mycorhizes; par conséquent, avoir analysé au préalable les racines des arbres pour établir le niveau existant de colonisation aurait pu aider à déterminer s'il aurait pu y avoir quelques bénéfices dérivés de l'application d'inoculant de champignons de mycorhizes.

**Resumen.** Los árboles urbanos están frecuentemente sujetos a una variedad de factores ambientales y de prácticas culturales que les llevan a estrés. Aquellos cuyas raíces están colonizadas con hongos micorrízicos se reportan como más tolerantes a condiciones adversas. Sin embargo, seis meses después de la inoculación de pinos (*Quercus palustris*) de 13 cm de diámetro con un producto comercial con micorrizas no hubo respuesta significativa en peso seco de las raíces o colonización de raíces. Después de un año los mismos árboles no mostraron respuesta a la inoculación medida por el contenido de clorofila foliar o diámetro del tronco. El peso seco de las raíces se incrementó significativamente con la aplicación de una combinación comercial de fertilizante/inoculante a encinos de 61 cm de diámetro (*Quercus phellos*). Para maples (*Acer rubrum*) de 13 cm de diámetro, la colonización de las raíces incrementó significativamente en respuesta a dos tratamientos de inoculantes comerciales de micorrizas, y el peso seco de las raíces aumentó significativamente en respuesta a los dos tratamientos seis meses después de las aplicaciones. Nuestros datos no indicaron beneficio aparente en crecimiento, bajo los términos y condiciones de esta investigación, a inoculación con productos comerciales a menos que se combinen con fertilizantes. Las evaluaciones pre-tratamiento de las raíces para todas las especies de árboles revelaron alguna colonización por hongos micorrízicos, por lo que las raíces de los árboles analizados para determinar su nivel de colonización puede ayudar en la determinación de cualquier beneficio derivado de la aplicación de inoculantes.