# ROOT RESPONSE OF MATURE LIVE OAKS IN COASTAL SOUTH CAROLINA TO ROOT ZONE INOCULATIONS WITH ECTOMYCORRHIZAL FUNGAL INOCULANTS

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**Abstract.** New fine root growth and ectomycorrhizal development were determined for mature live oak (*Quercus virginiana*) growing in a stressed landscape after soil injection of ectomycorrhizal fungal (*Pisolithus tinctorius* Pt) spores with or without fertilizer (12-48-8) or after vertical mulch application of a mix of Pt spores, soil-enriching bacteria, various organics, and water-managing gels. After 6 months, the injected spores stimulated more ectomycorrhizal development than the fertilizer or water-only control treatments. When spores and fertilizer were applied together, they increased by four times the fine root biomass and by three times the ectomycorrhizal development than observed in the water-only control. The estimated relative root absorbing potential of the roots was 14 times that of the control. After 4 or 6 months, the vertical mulch treatment stimulated six times more fine roots and twice the ectomycorrhizal development found in the control treatment. These differences reflected an 8 to 12 fold increase in the relative root absorbing potential of the fine roots and ectomycorrhizae. In both studies, Pt ectomycorrhizae accounted for over half of the ectomycorrhizal development. Natural ectomycorrhizal development ranged from 18 to 38 % of fine roots in control treatments. The results demonstrated that Pt spore and bacterial inoculants can be successfully introduced into the root system of mature live oaks in a stressed environment, increase fine root growth, and form abundant ectomycorrhizae.

## Introduction

Woody plants growing in natural forest soil form symbiotic associations on their fine absorbing roots with diverse species of mycorrhizal fungi. Mycorrhizae increase plant absorption of essential elements and water, and the plants' tolerance to drought, disease, and other environmental stresses (3). For the past two decades, specific ectomycorrhizal fungal inoculants, in particular those of *Pisolithus tinctorius* (Pt), have been used in forestry on nursery seedlings of a variety of tree species to improve their performance on reforestation and adverse reclamation sites (4).

The ectomycorrhizal technology has only recently been adapted to established large trees growing on low-quality urban soils. On the campus of the University of Michigan, 10 - to 18-inch-diameter (25 to 45 cm) at 4.5 ft. (1.37m) northern red oak (*Quercus rubra*) had significant increases in fine roots and nearly three-times more ectomycorrhizal development following inoculation of the rooting zone with spores of Pt. Root responses were greatest on trees also treated with soil-injected biostimulants and micronutrients. Noninoculated (control) oaks in this study had less than 20% of their fine roots colonized by native ectomycorrhizal fungi (5). Garbaye and Curin (1996) inoculated the backfill of transplanted 8-year-old basswood trees with vegetative inocula of ectomycorrhizal fungi in a Paris suburb. After three years, they reported that inoculation significantly increased height and diameter growth and improved mineral content of leaves. In North Carolina, Smiley et. al. (1997) injected the root zones of mature willow oak (*Quercus phellos*), northern red oak, and pecan (*Carya illinoensis*) in a residential area with spores of Pt with and without a slow-release fertilizer (28-9-9). After 7-months, the fertilizer and Pt spores, applied separately, stimulated increases in fine roots and ectomycorrhizal development. When applied together, however, they stimulated a 4 to 10 fold increase in fine roots and more than doubled the ectomycorrhizal development.

The purpose of the two studies reported here was to determine fine root and ectomycorrhizal response of mature live oak (*Quercus virginiana*) in a stressed urban environment following root zone inoculations with Pt spores applied by soil injection or in a vertical mulch inoculant. These studies were installed as part of a Workshop on Tree Roots and Mycorrhizae held in conjunction with the South Carolina Urban and Community Forestry Council Annual Meeting in Beaufort, SC on September 25 - 27, 1996.

## Materials and Methods

**Site Characterization.** The studies were conducted on 7 native live oaks growing at the Penn Center, St. Helena Island, SC. The trees ranged in diameter from 2 to 5 ft. (0.6 to 1.5 m) and had canopy diameters ranging from 60 to 120 ft. (18 to 36 m), respectively. The larger trees were estimated to be between 150 and 250 years old. For presumably more than 100 years, the soil under these trees has been periodically cleared of leaf litter, exposing mineral soil. For the past decade or more, the soil has been compacted by numerous people nearly every weekend having picnics or other gatherings. During these times it is not unusual to find 8 to 12 trucks and cars parked in the shade of these trees. Other than sparse grass there is no other vegetation under these oaks. The trees had symptoms commonly associated with stress, i.e. many dead large branches, low leaf density, short twig growth, and many fruiting bodies of wood-decay fungi on the main trunks, large branches, and root flares. Soil texture is a sandy loam. Chemical analyses showed soil pH of 5.9 and 1.1 percent organic matter in the upper 8 in (20 cm) of soil. Total soil nitrogen was approximately 2000 mg/kg$^{-1}$ and phosphorus, potassium, calcium and magnesium were 55, 26, 240, and 29 mg/kg$^{-1}$, respectively. Cation exchange capacity was 4.1 cmol/kg$^{-1}$ of soil.

**Root Response Measurements.** Fine roots less than 1/8 in (3 mm) diameter and ectomycorrhizal response to treatment were measured with root-ingrowth cores (RIC) in both studies (6). For these studies, RIC's were 3-in diameter by 7-in (7.6 by 17.8 cm) long plastic screen-tubes which are open-ended and have 1/8 in (3 mm) diameter holes covering 90 percent of the wall surface. A 3-in by 8-in (7.6 by 20.3 cm) diameter soil core was removed from an injection or a vertical mulch location with a special soil extractor tool. The soil was so compacted that many times the soil core extractor had to be driven into the soil with a heavy sledge hammer. The soil from the extractor was screened through a ¼ in (6 mm) mesh screen to remove the roots which were discarded. A treatment-labeled (aluminum tag) RIC was inserted to the bottom of the hole and the root-free, treated soil was used to refill the RIC. Soil was pressed firmly by hand into the RIC. A steel nail was placed on the top of the RIC and then covered with a final inch of treated soil. This soil was then pressed firmly by foot. RICs were buried to minimize vandalism and damage by foot and vehicular traffic. The nail was used so that the RIC could be located later with a metal detector. Normal people activities in the area continued for the duration of the study. Cores were removed after 6 months for the injection study and after 2, 4 and 6 months for the vertical mulch study. A knife was used to cut roots on the outside of the RIC's. The RIC's were removed from the soil and their contents dry screened to collect roots which had grown into them. Roots were washed, cleaned of debris, and the few grass roots discarded. Ectomycorrhizal development was visually assessed on the oak roots at 4X magnification then dried at 70°C for 48 hours and weighed. Pt ectomycorrhizae were easily distinguished from the naturally-occurring ectomycorrhizae by their distinctive mustard-yellow color, multi-branching habit, and yellow hyphal strands. Visual estimates of ectomycorrhizal development have limitations in that they do not consider the total number of lateral roots supporting ectomycorrhizae, and they only estimate the relative proportion of ectomycorrhizal to nonmycorrhizal roots on the lateral roots. The estimates also do not relate to the size or potential absorbing area of the ectomycorrhizae. This limitation can be overcome by using a derived surrogate for surface-area. The relative root absorbing potential parameter for these studies is derived from the simple formula: fine root wt./ft$^3$ X % ectomycorrhizal development ÷ 10. This derived parameter actually underestimates the physical surface area measurements that have been made on ectomycorrhizae (7, 8) but does serve as a surrogate for surface area or absorbing surface and can serve as an index for relative comparison between treatments (6). All data were processed by Analysis of Variance and significant means were identified with Duncans' New Multiple Range Test at P=0.05.

**Soil Injection Study**. Live oaks with 3 to 5 ft. (1.2 to 1.5 m) diameters, each with canopy diameters over 100 ft. (30 m), were used in the injection study. The area under the drip line of each tree was divided into quadrants following the test design used by Smiley *et. al.* (9). Each quadrant received one of the following four treatments chosen at random:

1. Injectable Pt spores with a yucca surfactant at ¼ lb. (114 gm) ($1.1 \times 10^{11}$ spores) per 100 gal. (379 l) of water.
2. Injectable Pt spores at ¼ lb. (114 gm) ($1.1 \times 10^{11}$ spores) plus 5 lb. (2.27 kg) of (12-48-8) water soluble fertilizer with micronutrients per 100 gal. (379 l) of water.
3. Fertilizer alone at the same rate as #2 above.
4. Water only control.

On April 8,1996, injections of 0.5 gal. (1.9 l) of treatment solution to an 8-in (20 cm) soil depth were made on 3 ft. by 3 ft. (0.9 m by 0.9 m) centers in the designated treatment tree quadrant using a motor-driven sprayer with a soil injection nozzle. Injection pressure was 150 psi. Each tree quadrant received 24 injections. Eight injection locations in each quadrant of each tree were selected at random and marked with color-coded flags to designate treatment and to mark the injection spot for placement of RICs. The next day, the 8 RIC's per treatment per tree were installed directly over the injection spot. A total of 96 RICs were installed in this study. All soil cores were removed in mid-September, 1996 and their contents assessed as described earlier. Root data from the 8 RIC's from each quadrant treatment were averaged to represent that treatment replicate. Each of the three trees were statistically analyzed as a block. The study was a randomized complete block design.

**Vertical Mulch Study**. An area under the drip line of four - 2 ft. (0.6m) diameter live oaks, each with canopy diameters of about 60 ft. (18 m) and growing 20 to 25 ft. (6 to 7.6m) apart, was selected. Forty-eight flags spaced 3 to 5 ft. (0.9 to 1.5 m) apart were placed in the area. Twenty-four flag locations were chosen at random for the vertical mulch treatment and the remaining 24 locations were used as controls. The control cores were installed by removing a soil core and inserting a labeled RIC into the hole. The soil in the extractor was screened of roots and, without further treatment, was used to refill the RIC as described in the injection study. The vertical mulch treatment was installed in the remaining locations by removing a soil core, inserting a labeled RIC, and then hand-mixing 3 oz. (85 gm) of a vertical mulch inoculant with the root-free soil from the soil core. The inoculant contains spores of Pt, soil-enriching bacteria, organics, and soil water-managing gels. (It is a product of MycorTree TM Root Saver of Plant Health Care, Inc.) This 10% inoculant to soil mixture was used to refill the RIC as described earlier. A total of 48 RICs were installed in this study. Two, four, and six months after installation, eight cores of each of the two treatments were removed at random and their root contents processed as described. Root data from each RIC was treated as a replicate of that treatment/date. Data were statistically analyzed as a completely randomized design.

## Results

Coastal South Carolina had a significant drought from March to mid-June, 1996. Following study installation in April, the test site did not receive measurable rain until late June. Normal rainfall, approximately 23 in (584 mm), occurred for the remainder of the study period through September. Of the 96 RIC's installed in the soil injection study, all but three were located. Of the 48 RIC's in the vertical mulch study, all but two were located.

**Soil Injection Study**. Injected water (control) and the fertilizer alone induced similar amounts of fine roots and ectomycorrhizae (Table 1).

**Table 1. Root Response of mature live oaks 6 months after injecting spores of *Pisolithus tinctorius* (Pt) and fertilizer.**

| Treatment | Fine Root Wt. (g/ft$^3$ soil)* | % Ectomycorrhizal Roots | Relative Absorbing Potential |
|---|---|---|---|
| Water control | 12.2c[1] | 18b | 22c |
| Fertilizer | 23.5bc | 19b | 45c |
| Pt spores | 39.7ab | 44a[2] | 175b |
| Pt spores + Fert. | 55.1a | 57a[2] | 314a |

1 Means in a column sharing a common letter are not significantly different (P=0.05) according to Duncan's New Multiple Range Test. Variance between blocks (trees) was not significant.

2 Nearly half are *Pisolithus tinctorius* ectomycorrhizae.

* 1 ft$^3$ = 0.028 m$^3$

Naturally-occurring ectomycorrhizae in these treatments was less than 20%. Injected Pt spores alone or combined with the fertilizer stimulated three to four times more fine roots and nearly three times more ectomycorrhizal development than observed in the water control treatment. Fertilizer and Pt spores applied separately induced similar amounts of fine roots but the Pt spore treatment had more than twice the ectomycorrhizal development. About half of the ectomycorrhizae formed in both of the injected spore treatments were formed by Pt. The relative absorbing potential of the fine roots and the ectomycorrhizae produced in response to the injected spore and fertilizer treatments was 7 to 14 times that of the fertilizer alone and the water control treatments, respectively. The combination of spores and fertilizer produced 75% more relative root absorbing potential than Pt spores alone.

**Vertical Mulch Study.** Probably as a result of the drought, there were few fine roots detected in any of the RIC's removed two months after installation. Samples assessed after four and six months, however, showed the effects of the vertical mulch treatment on root growth (Table 2). There were no significant differences in either fine roots or ectomycorrhizal development between the two sampling periods for either of the two soil treatments but there were very large differences between soil treatments. Naturally-occurring ectomycorrhizae ranged from 28 to 38% in the control 4 and 6 month samples, respectively. The vertical mulch treatment had about six times more fine roots and twice the ectomycorrhizal development than detected in the control treatment at both sampling periods. About 60 percent of the ectomycorrhizal development in the vertical mulch treatment were formed by Pt. These differences reflected more than a 12 to 8 fold increase in relative root absorbing potential by the vertical mulch treatment over the control for the 4 and 6 months sampling dates, respectively. Fine roots, ectomycorrhizae and extramatrical mycelia of Pt physically penetrated the hydrated water-managing gels in the vertical mulch treatment.

## Discussion and Conclusions

These results clearly show that inoculation with spores of Pt, either by injection or by vertical mulching, can successfully establish this specific ectomycorrhizal fungus on roots of mature live oak trees growing in a degraded and compacted urban soil. Ectomycorrhizae formed by Pt have been shown to improve survival and growth of trees on various adverse forestation and reclamation sites throughout the world (4).

**Table 2. Root Response of mature live oaks after 4 and 6 months to vertical mulching with *Pisolithus tinctorius* spores, soil enriching bacteria, various organics, and water-managing gels.**

| Sample Period | Treatment | Fine Root Wt. ($g/ft^3$ soil)* | % Ectomycorrhizal Roots | Relative Absorbing Potential |
|---|---|---|---|---|
| 4 months | Control | 10.5b[1] | 28b | 29b |
| | Vertical Mulch | 65.9a | 56a[2] | 369a |
| 6 months | Control | 12.4b | 38b | 46b |
| | Vertical Mulch | 72.6a | 55a[2] | 399a |

1 Means in a column sharing a common letter are not significantly different (P=0.05) according to Duncan's New Multiple Range Test.

2 About 60% are *Pisolithus tinctorius* ectomycorrhizae.

* $1\ ft^3 = 0.028\ m^3$

Inoculation of the oaks in this study in combination with the injection of soluble fertilizer caused considerably more fine root and ectomycorrhizal development than observed in the water-only control. The data show that fine root and ectomycorrhizal development can be increased on mature live oaks in a poor soil with reasonable levels of added fertilizer. In this study, actual elemental concentrations were 0.6 lb. (272 gm) for nitrogen (N), 0.98 lb. (455 gm) for phosphorus (P), and 0.33 lb. (150 gm) for potassium (K) diluted in 100 gal. (379 l) of water. Since 0.5 gal. (1.9 l) of this solution was injected on 3 ft. (0.9 m) centers, the elemental rate applied per 1000 $ft^2$ (93 $m^2$), or 56 injection locations, is only 0.34 lb. (154 gm) of N, 0.55 lb. (250 gm) of P and 0.18 lb. (82 gm) of K. Although it is commonly believed that fertilization depresses ectomycorrhizal development it normally takes much higher amounts of these essential elements to significantly depress new ectomycorrhizal development on trees (1).

The vertical mulch treatment induced more fine root and ectomycorrhizal development than did the Pt spore injection treatment. This is understandable since the vertical mulch product contained considerable amounts of slowly-soluble organic ingredients, different types of bacteria and their "starter" foods, and water-managing gels. Since it is applied dry and contains so many organics and diverse microorganisms, its total effect in soil and on roots would likely last longer than the injectable material containing only Pt spores and a water-soluble surfactant. Many fine roots, ectomycorrhizae, and mycelia of Pt were observed growing into the hydrated water-managing gels in the vertical mulch treatment. Water absorbed by these roots and mycelia should improve water relations for the tree.

The root responses of these oaks are in general agreement with root response measured following similar inoculation and fertilizer treatments on other species of urban trees in different parts of the US (5, 9). The data also show that stressed mature live oak trees will produce significant amounts of fine roots and ectomycorrhizae during the hot summer months as long as adequate inocula of the mycorrhizal fungi, soil moisture, and essential elements are available. As suggested by Smiley et. al. (9), mycorrhizal fungal inoculations should be considered as viable alternatives to soluble fertilizers to stimulate fine root and ectomycorrhizal development on urban trees where natural ectomycorrhizal development is inadequate due to soil compaction, low organic matter and water-holding capacity. As reported here, inoculation either by injection or vertical mulching can improve fine root and ectomycorrhizal development on mature live oaks growing in a stressed man-made environment. This increased root response should increase the trees' ability to mediate soil stresses, (i.e., low soil water and compaction) and, therefore, improve the overall health of the trees. However, only long term monitoring of the symptoms in treated trees will corroborate this speculation.

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**Résumé**. Le taux de croissance en fines radicelles et le développement en ectomycorrhizes ont été évalués chez le chêne vert (*Quercus virginiana*), dans un aménagement aux contraintes difficiles pour le développement, après avoir injecté dans le sol des spores d'ectomycorrhizes (*Pisolithus tinctorius*, Pt) avec ou sans fertilisant (12-48-8) ou après un traitement de *paillis vertical* composé d'un mélange de spores de Pt, de sol enrichi de bactéries, de divers organismes microfauniques et de cristaux de gel hydrophile. Après six mois, l'injection de spores avait stimulé un développement d'ectomycorrhizes plus important que celui du groupe contrôle (eau seulement) ou celui avec fertilisant seul. Lorsque les spores et le fertilisant étaient appliqués ensembles, ils stimulaient une croissance quatre fois supérieure de biomasse en fines radicelles et un développement d'ectomycorrhizes trois fois supérieur par rapport au groupe d'arbres-contrôle (eau seulement). L'estimation du potentiel relatif d'absorption des racines était de 14 fois supérieure à celle du groupe-contrôle. Après quatre à six mois, le traitement par *paillis vertical* a permis de stimuler par six fois la croissance des fines radicelles et du double celui des ectomycorrhizes par rapport au groupe-contrôle. Ces différences reflétaient un accroissement par 8 à 12 fois du potentiel relatif d'absorption racinaire par les fines radicelles et les ectomycorrhizes. Dans les deux études, l'ectomycorrhize Pt comptait pour plus de la moitié du nombre des nouvelles ectomycorrhizes formées. Le développement naturel d'ectomycorrhizes sur les fines radicelles se situait quant à lui entre 18 et 38% chez le groupe-contrôle.

Les résultats démontrent que l'inoculation de spores de Pt et de bactéries peut être réalisée avec succès sur le système racinaire des chênes verts adultes en milieux difficiles, peut augmenter la croissance en fine radicelles, et enfin peut former d'abondantes ectomycorrhizes.

**Zussammenfassung**. Bei einer ausgewachsenen Lebenseiche (*Quercus virginiana*), die an einem schwierigen Standort steht, wurde das Feinwurzelwachstum und die Entwicklung von Ektomycorrhizen nach einer Injektion mit *Ektomycorrhiza-Sporen* (*Pisolithus tinctorius* Pt) mit und ohne Düngergaben (12-48-8), oder vertikaler Mulchung mit einer Mischung aus Pt-Sporen, bodenanreichenrnden Bakterien, verschiedenen organischen Stoffen und Hydrogel bestimmt. Nach sechs Monaten hatten die injezierten Sporen mehr Ektomycorrhiza-Wachstum verursacht, als die Kontrolversuche mit und ohne Dünger. Wenn Sporen und Dünger zusammen gegeben wurden, steigerten sie das Wachstum der Feinwurzeln um das Vierfache und die Entwichlung der Ektomycorrhiza um das Dreifache gegenüber den Kontrollversuchen. Das geschätzte relative Absorbtionspotential der Wurzeln betrug das 14fache der Kontrollbehandlungen. Nach vier oder sechs Monaten stimulierte die Mulchbehandlung das Sechsfache der Feinwurzeln und das Doppelte der Ektomycorrhiza gegenüber der Kontrollen. Diese Differenzen reflektieren eine 8 bis 12fache Steigerung des relativen Absorbtionspotentials der Feinwurzeln und der Ektomycorrhiza. In beiden Studien überwog das Pt-Ektomycorrhiza über die Hälfte der Ektomycorrhiza-Entwicklung. Die natürliche Ektomycorrhiza-Entwicklung rangierte zwischen 18 und 38 % der Feinwurzeln in den Kontrollbehandlungen. Die Ergebnisse demonstrieren, das Pt-Sporen und bakterielle Inokulate erfolgreich in das Wurzelsystem von ausgewachsenen Lebenseichen an Problemstandorten eingeführt werden können, dort das Feinwurzelwachstum steigern und ausreichende Ektomycorrhiza formen können.