# PT ECTOMYCORRHIZAL FUNGUS OPERATIONAL INOCULATIONS AND MANAGEMENT IN FOREST TREE NURSERIES -1989

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ABSTRACT. Over the past several years the mycorrhizae research and development program has evolved to the current state whereby pisolithus tinctorius (Pt) is being applied in a practical, efficient and cost effective manner in both container and bare-root nurseries. The benefits of Pt in reforestation and mineland reclamation have been repeatedly demonstrated and include significant increases in nursery seedling quality (reduced culls), and increased survival and growth in field outplantings. Four types of commercial inoculum are currently available, including vegetative mycelium, bulk spores, spore pellets and spore encapsulated seed. Two commercially available machines have been developed to apply mycelium inoculum in bare-root nurseries. The demand for custom-grown, Pt-inoculated seedlings continues to increase. Approximately 6 million seedlings in 1988 and 6.5 million seedlings in 1989 were inoculated with Pt in operational bare-root and container nurseries in the Southern, Central, and Eastern United States.

Additional Keywords: Ectomycorrhizae Pisolithus tinctorius, bare-root nurseries, container nurseries, seedling quality, field forestation, mineland reclamation, commercial inoculum types, inoculation techniques.

For the past several years, the USDA Forest Service, in cooperation with a number of state and private forestry agencies, has been conducting extensive research on mycorrhizae and their applications in forest tree nurseries, forest plantings, and plantings on reclaimed mineland. The primary objective of this venture has been the practical use of one ectomycorrhizal fungus, Pisolithus tinctorius (Pt) in forest land management (Fig. 1). This fungus was selected because of its availability, ease of manipulation, wide geographic and host range, and demonstrated benefits to a wide variety of host trees. Pt is especially tolerant of extreme soil conditions, including low pH, high temperatures, and drought, that frequently kill other ectomycorrhizal fungi and their host trees (Marx, Cordell, and others 1984).

In recent years, the Pt ectomycorrhizal research and development program has evolved from controlled nursery-plot research to relatively large-scale operational applications in both bare-root and container seedling nurseries. Mycorrhizal culture is rapidly expanding to include additional fungi and tree hosts for a variety of forestation and mineland reclamation applications in several countries. Effective Pt inoculum, along with the necessary equipment and technology for successful operational applications in bare-root and container nurseries, is now available to nursery personnel. However, the decision to incorporate ectomycorrhizal fungus inoculations into the nursery management program is shared jointly by the nurseryman and the seedling buyer.

Therefore, nurserymen, tree planters, mineland reclamation specialists, and other land managers are challenged to understand and evaluate the costs and benefits of custom-grown mycorrhizal-tailored seedlings.

Figure 1. Pisolithus tinctorius ectomycorrhizae fungus fruiting body.

### Benefits

The ectomycorrhizal fungus Pt provides significant benefits for field forestation, Christmas tree, and reclamation projects. Numerous conifer and a few hardwood species have been artificially inoculated with Pt. National container and bare-root nursery evaluations have demonstrated the effectiveness of different formulations of Pt inoculum on selected conifer seedling species (Marx, Ruehle, and others 1981; Marx, Cordell and others 1984). During the past 13 years, more than 100 bare-root nursery tests have

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been conducted in 38 states. Results obtained from 34 nursery tests showed that Pt inoculation increased fresh weight of southern pine seedlings by 17 percent, increased ectomycorrhizal development by 21 percent, and decreased the percent of cull seedlings at lifting time by 27 percent (Fig. 2). There have been a few instances where negative results have been incurred, but failures of this kind have been positively correlated with such factors as ineffective Pt inoculum, adverse environment, detrimental cultural practices, and pesticide toxicity.

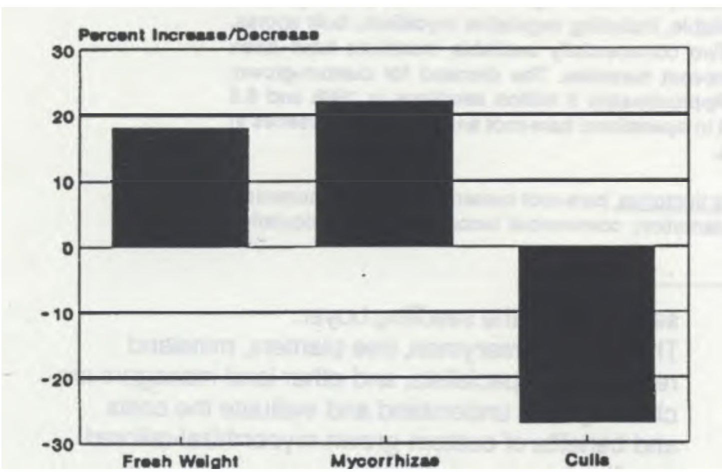
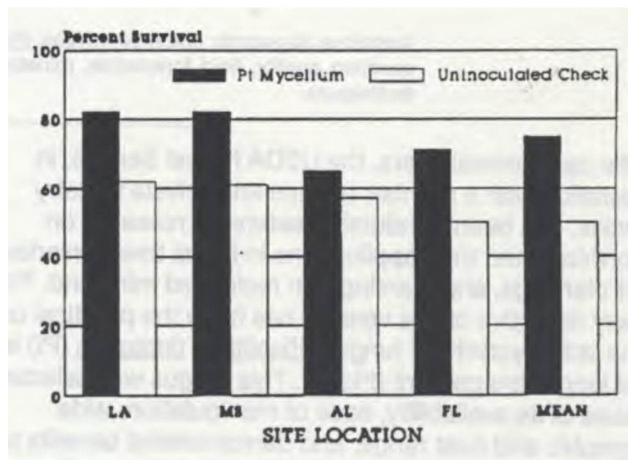



Figure 2. Effects of inoculation with Pt vegetative mycelium on southern pine seedlings in 34 bare-root nurseries.

Inoculated seedlings have been planted on a variety of routine forestation sites, strip-mined areas, kaolin wastes and Christmas tree farms in locations throughout the United States. Over 100 Pt outplantings involving 12 species of conifers are being monitored in 20 states on a variety of forestation, mineland reclamation, and Christmas tree sites. Preliminary analyses show significant increases in tree survival and growth in over half of the 100+ field studies. Pt-inoculation of longleaf pines (Pinus Palustris Mill.) has significantly improved survival after 3 years in the field in 4 Southern States (Fig. 3). Pt-inoculated eastern white (. strobus L) and loblolly(E a L) pines continue to show significant increases in tree volume growth (25 +%) (Fig. 4), when compared with uninoculated check trees on a routine forestation site after 10 years in the field. Positive field responses are correlated with successful Pt nursery inoculations (Pt index 50), with mineland reclamation conditions, and with periodic moisture stress on normal forestation sites. Results from outplanting studies in southern Georgia suggest that seedlings with abundant Pt ectomycorrhizae at planting date are better able to withstand some site or environmental stresses than seedlings without Pt ectomycorrhizae. Rainfall deficiencies have been frequently associated with large growth differences. Results from two studies (Marx, Cordell & others 1988a) on routine reforestation sites support the theory of greater drought tolerance of Pt seedlings. After 4 years on a good quality, formerly forested site in south Georgia (site index of 80 ft. at age 25), trees with only naturally occurring Thelephora terrestris ectomycorrhizae grew less during years of low rainfall than Pt-treated trees. During years with moisture stress, Pt ectomycorrhizae markedly improved diameter growth. The apparent effectiveness of Pt in tolerating moisture stress on routine southern pine forestation sites is highly significant and should greatly expand the economic practicality of the Pt program in forest land management.



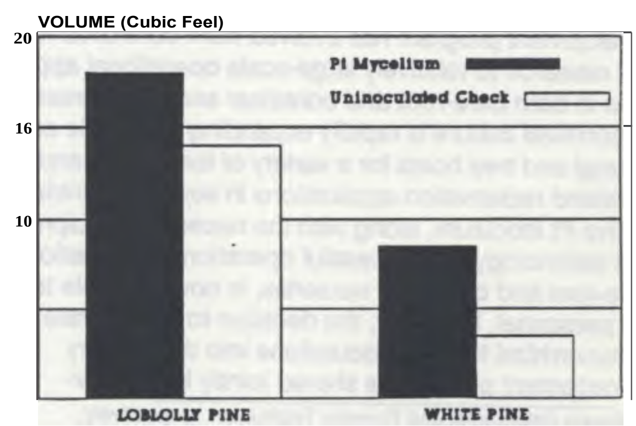




Figure 4. Positive growth responses to Pt inoculation by loblolly and white pines after 10 years on a routine forestation site in western North Carolina.

Extensive reclamation research has been conducted on seedlings custom grown with Pt ectomycorrhizae and outplanted on disturbed and adverse sites of various types in the Eastern U.S. In numerous field tests on coal spoils, annual tree root evaluations have confirmed the ecological adaptation of Pt to these adverse sites. Without exception, seedlings with Pt ectomycorrhizae developed new roots very rapidly, and these roots were quickly colonized by the fungus. Root growth was also routinely followed by the prolific production of Pt fruiting bodies in the vicinity of trees with Pt ectomycorrhizae on their root systems. Outplantings established by the Ohio Division of Mineland Reclamation in southern Ohio during the past 7 years continue to show significant tree survival and growth increases for Pt inoculated Virginia, eastern white, and loblolly x pitch hybrid pines and northern red oak (*Quercus* ultra L.) seedlings over routine nursery seedlings (Fig. 5).

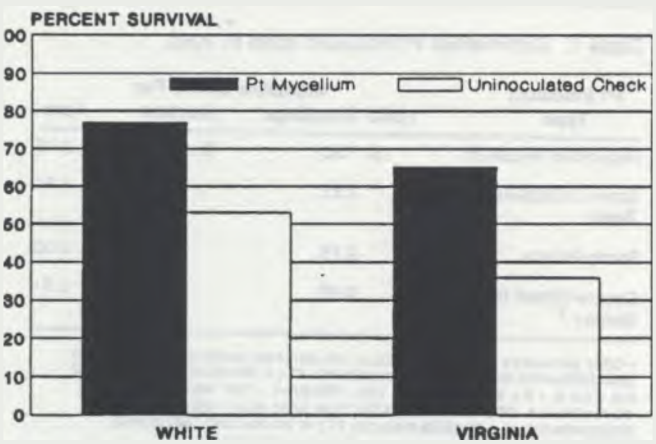



Figure 5. Survival of two pine species on mine reclamation sites in Ohio.

## Nursery Inoculations

The technology, commercial fungus inoculum, and inoculation equipment necessary to manage the Pt ectomycorrhizal fungus have been developed and are available to nurserymen for operational use. The types of Pt inoculum that are commercially available include vegetative inoculum from Mycorr Tech, Inc., University of Pittsburg Applied Research Center, Pittsburg, Pa.; and spore pellets, spore encapsulated seeds, and bulk spores from either International Tree Seed Co., Odenville, Al., or South Pine, Inc., Birmingham, Al. A vegetative inoculum applicator places Pt vegetative inoculum efficiently prior to sowing in bare-root nurseries (Fig. 6). A vegetative inoculum applicator has also been developed for side-banding inoculum between rows of established seedlings. Both applicators are commercially available from R. A. Whitfield Manufacturing Co., Mableton (Atlanta), Ga.

Figure 6. Pt vegetative mycelium being applied to a forest tree nursery seedbed.

Procedures for nursery use vary, depending upon which type of inoculum is used. However, with either mycelial or spore inoculum, the biological requirements of a second living organism are added to those of the seedling. As a result, special precautions are required for shipping, storage, and handling of the Pt inoculum, as well as controlling certain aspects of seedling production, lifting, handling, and field planting. Detailed procedures for handling and storage of the various inoculum types, along with alternative inoculation techniques in bare-root and container nurseries, have been presented (Cordell, Marx, and Owen 1986; Cordell, Owen, and Marx 1987). For successful Pt inoculation in bare-root seedbeds, populations of pathogenic and saprophytic fungi and native ectomycorrhizal fungi that may already be established in the soil must be reduced. Therefore, soil fumigation before sowing (preferably in the spring) with effective soil fumigants such as the methyl bromide-chioropicrin formulations is required.

## Ectomycorrhizae Nursery Management

Guidelines for managing mycorrhizae in nurseries are designed primarily to maintain healthy seedling root systems. One must consider development and retention of seedling feeder roots and mycorrhizae from the time of seed sowing to seedling lifting in the nursery and to planting the trees in the field. Nurserymen, field foresters, reclamation specialists, and tree planters must be made aware of the two symbiotic living organisms they are handling - the tree seedling and its complement of mycorrhizal fungi.

Mycorrhizae require generally the same moisture, fertility, and pH as their host tree seedlings, but tolerance for extreme or adverse conditions does vary. Soil and cultural factors that significantly affect mycorrhizae include pH, drainage and moisture, fertility, fumigation, pesticides, cover crops, shading and root pruning. Soil and water pH values are two of the most limiting factors in the development of ectomycorrhizae in both bare-root and container nurseries. In addition, seedling lifting, storage, and planting practices have significant effects on seedling feeder root and ectomycorrhizae retention, quality, and subsequent field survival and growth. Special care must be taken during all stages of seedling handling to maintain sufficient root systems and ectomycorrhizae. Ectomycorrhizae are delicate structures that can be ripped off and left behind in seedling beds during lifting, dessicated in storage, or cut off prior to field planting. To sustain seedling quality, lifting and handling techniques must be modified to minimize damage to feeder roots and ectomycorrhizae. Stripping of roots has severe negative impacts on seedling field performance (Marx and Hatchell 1986). Full-bed seedling harvesters are less destructive than single- or double-row lifters. Condition of the root systems should be checked throughout the lifting process; even slight reductions in tractor speed can greatly reduce damage to roots and ectomycorrhizae as seedlings are lifted. During transfer of the seedlings from the field to the packing room and at all other times when the seedlings are being handled, special care is required to avoid drying of the roots by exposure to wind and sun.

The procedure by which seedlings are packed influences their ability to endure storage and survive field planting. If extended storage is required, Kraft paper bags with a polyethylene seal will maintain seedling moisture better than seedling bales. Cold storage is vital to slow seedling respiration. Studies comparing packing materials have determined that seedling survival is better when peat moss, clay, or inert super-absorbents are used rather than hydromulch (Cordell, Kais, Barnett, and Affletranger 1984). Best results are obtained when all root systems are coated or at least in contact with the packing material. Numerous studies have documented the effects of storage time on seedling quality. For most tree species and their ectomycorrhizae, storage for 2 to 6 weeks is not harmful.

Improper transportation to the planting site or rough handling during planting can severely reduce seedling vigor. Tree planters should understand proper planting methods and the reasons for them. Where possible, seedlings should be transported under refrigeration. If that is not possible, they should be covered and stacked with spacers to avoid high temperature buildup inside the seedling containers. For machine or hand planting, root pruning at the planting site should be avoided because it eliminates carefully nurtured feeder roots and mycorrhizae. High temperatures, high winds, and low humidity dessicate and kill feeder roots and mycorrhizae very rapidly. The first priority in planting should always be to maintain seedling viability and vigor. The rate at which acres are planted is of no consequence if the seedlings do not survive.

### Costs

There is a wide range in the cost of commercially available Pt inoculum (Table 1). The inoculum cost, however, is only one part of the cost of establishing Pt-inoculated seedlings. Some nurseries purchase the inoculum and include this added cost with the seedling costs to the buyer, while other nurseries prefer that the buyer purchase the inoculum. The Pt vegetative mycelium inoculum is sold on a volume basis, while the spore inocula are all sold by weight. The cost of the most expensive vegetative mycelium inoculum ($7.50/1,000 seedlings, $5.45/acre planted) represents 5 percent or less of the total plantation establishment costs.

Table 1. Commercial Pt inoculum costs in 1989.

| Pt Inoculum Type | Inoculum Costs Per[1] 1,000 Seedlings | Hectare | Acre |
|---|---|---|---|
| Vegetative Mycelium | $ 7.50 | $ 13.45 | $ 5.45 |
| Spore Encapsulated Seed | 2.22 | 3.98 | 1.61 |
| Spore Pellets | 2.75 | 4.93 | 2.00 |
| Double-Sifted Bulk Spores[2] | 0.43 | 0.77 | 0.31 |

1-COST ESTIMATES ARE FOR LOBLOLLY 6 SLASH PINE BARE-ROOT NURSERIES (269 SEEDLINGS/SO. - 26 SEEDLINGS/30. FT.) t FORESTATION PLANTINGS (1 B • 3.0 M. - 6 . 10 FT. SPACING; 1.794 TREES/HA. - 726 TREES/AC.) IN THE SOUTHERN U.S. COSTS FOR LONGLEAF PINE BARE-ROOT SEEDLINGS 1129 SEEDLINGS/80. M. - 12 SEEDLINGS/SO. FT.) IS $1603/100 SEEDLINGS $28.02/HA. OF PLANTATION. & SIL35/AC OF PLANTATION
2-DOUBLE SIFTING IS REQUIRED FOR EVEN FLOW THROUGH SPRAY NOZZLES STANDARD SPORES ARE ONLY SIFTED ONCE

## Operational Applications

The demand for Pt-inoculated seedlings continues to increase. In 1988, 6 million seedlings at 12 bare-root and container nurseries in the Southern, Central, and Northeastern United States were inoculated with Pt. In 1989, the total rose to 6.5 million seedlings, comprising eight conifer and one hardwood species (Fig. 7).

During the spring of 1987, 1.5 million 1-0 loblolly and 0.5 million 1-0 longleaf pine seedlings were successfully inoculated with the Pt ectomycorrhizal fungus and custom grown at the Taylor State Nursery, Trenton, S.C., for forestation plantings at the Savannah River Forest Station, Aiken, S.C. Four field demonstration plantings were established comparing various nursery and field treatments, including seedling quality, Pt inoculation, pine species, tree spacing and site preparation. Preliminary field examinations of these plantings show excellent survival results (95 +%). During the spring of 1988 and 1989, 0.75 million longleaf and 1.25 million loblolly pine seedlings were inoculated with Pt at the nursery for planting in the winter at the Savannah River Forest Station. 3,500 liters of Pt vegetative inoculum were applied in 35,000 linear feet (6.75 miles) of nursery seedbed. This is the largest single artificial ectomycorrhizal inoculation to date.

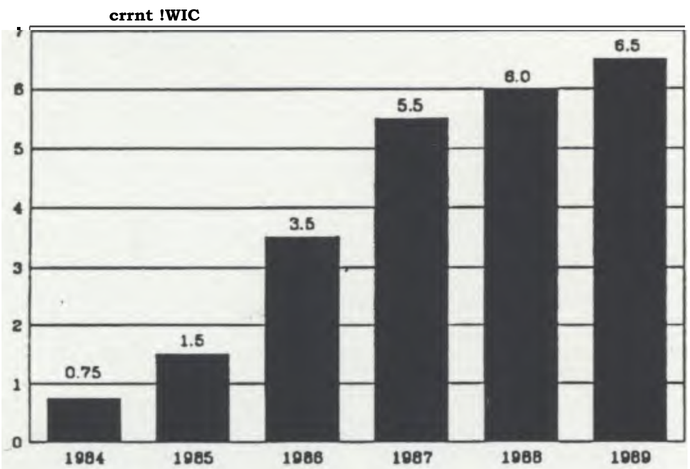



Figure 7. Operational Pt custom seedling production using commercial vegetative and spore inoculum in bare-root and container seedling nurseries, 1984-89.

Interest in the use of Pt ectomycorrhizae in mineland reclamation has increased steadily over the past 7 to 10 years. Since its inception in 1981, the Ohio Abandoned Mineland Reforestation Program has planted approximately 1.4 million Pt-inoculated seedlings on 810 acres of abandoned strip mines in southern Ohio. This program has expanded annually, and in 1988-89, the Ohio Division of Reclamation planted approximately 0.5 million Pt-inoculated seedlings on 285 acres. Estimates for tree planting in Ohio through 1990 indicate requirements for an additional 2.6 million Pt-inoculated seedlings for plantings on 1,850 acres of abandoned mineland (Cordell, Owen, and others 1987).

National Forests, state forestry agencies, and a number of private companies have shown considerable interest in the use of Pt ectomycorrhizae on selected forestation sites in the United States. National Forests in Ohio and South Carolina have scheduled the annual production of Pt-tailored bare-root seedlings for selected reclamation and forestation sites. The Savannah River Forest Station, in cooperation with the U.S. Department of Energy (DOE) in South Carolina, has initiated a 5-year reforestation plan utilizing a minimum of 2.0 million Pt-tailored longleaf and loblolly pines annually. During 1989, Pt-inoculated seedlings are being produced for five state forest agencies and five forest products companies. The demand for Pt-tailored seedlings is expected to substantially increase during the next 5 years due to the increased emphasis on mineland reclamation and forestation.

## Technology Transfer

In a special program, the USDA Forest Service continues to provide mycorrhizae technology to forest tree nurserymen, field foresters, mineland reclamation specialists, and other concerned land managers throughout the United States and several foreign countries (Cordell and Webb 1980, Cordell 1985). Initially, this program emphasized the use of Pt on selected forestation sites and in mineland reclamation programs. However, the program is being expanded to a wider range of forestation sites, mycorrhizal fungi, and tree hosts over a broader geographic area. The expanded ectomycorrhizal technology transfer program will have national emphasis with international scope.

## Conclusions

Results obtained during the past few years consistently demonstrate that the Pt ectomycorrhizal fungus can be used operationally in container and bare-root nurseries to significantly improve survival and growth capabilities of seedlings for forestation and mineland reclamation. Several types of effective Pt inoculum are commercially available, as is a machine for vegetative mycelium inoculations in bare-root nurseries. These recent developments provide nurserymen, foresters, mineland reclamation specialists, Christmas tree growers, and other land managers with alternatives for using Pt, as well as other selected ectomycorrhizal fungi. The best field planting results continue to be obtained on adverse sites such as coal spoils and routine forestation sites with soil moisture deficits. In addition, results are consistently better when planted seedlings have Pt indices 50 (Pt incidence greater than other natural ectomycorrhizae incidence on seedling feeder roots). The cost of Pt seedling inoculation represents only a minor portion of the total forestation expense (5% or less), and high seedling quality is an obvious key to successful forestation and mineland reclamation, and Christmas tree production. Consequently, the benefits of producing custom-grown seedlings with selected ectomycorrhizal fungi for specific forestation and mineland reclamation sites should greatly exceed the costs.

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