# PT ECTOMYCORRHIZAL FUNGUS OPERATIONAL INOCULATIONS AND MANAGEMENT IN SOUTHERN FOREST TREE NURSERIES - 1988

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ABSTRACT. Practical techniques have been developed and are being used for applying the ectomycorrhizal fungus P. tinctorius (Pt) in container and bare-root nurseries. Repeatedly demonstrated benefits in forestation and mineland reclamation include significant increases in nursery seedling quality (reduced culls), along with increased survival and growth in field plantings. Types of commercial inoculum include vegetative mycelium, bulk spores, spore pellets, and spore-encapsulated seeds. A commercially available machine efficiently applies mycelial inoculum in bare-root nurseries. The demand for custom-grown, Pt-inoculated seedlings continues to increase. Approximately 5 million seedlings in 1987 and 6 million seedlings in 1988 were inoculated with Pt in operational bare-root and container nurseries in the Southern, Central, and Eastern United States.

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

For several years, the USDA Forest Service and a large number of cooperating forestry agencies have conducted extensive research on mycorrhizae in forest tree nurseries, forest plantings, and plantings on reclaimed mineland. A primary objective has been the practical use of one ectomycorrhizal fungus, *Pisolithus tinctorius* (Pt), in forest land management. This fungus was selected because of its availability, ease of manipulation, wide geographic and host range, and demonstrated benefits to a 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 the controlled nursery plot research phase 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 on a variety of forestation and mineland reclamation applications in several countries. Nurserymen can now get effective Pt inoculum, along with the necessary equipment and technology for operational applications in bare-root and container nurseries. 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, foresters, mineland reclamation specialists, tree planters, and other land managers are challenged to understand and evaluate the benefits of mycorrhizal-tailored seedlings for their operations.

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## BENEFITS

The ectomycorrhizal fungus Pisolithus tinctorius (Pt) provides significant benefits for field forestation and for reclamation projects. Many conifer and some hardwood species on a variety of nursery sites have been artificially inoculated with Pt. Effective Pt vegetative inoculum has consistently improved the quality of nursery seedlings. 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 10 years, over 100 bare-root nursery tests have been conducted in 38 states. Results obtained from 34 nursery tests conducted during 3 years showed that Pt-inoculated southern pine seedlings had a 17 percent increase in fresh weight, a 21 percent increase in ectomycorrhizal development, and a 27 percent decrease in the percent of cull seedlings at lifting time (Fig. 1). In some nurseries, results have been negative, but failures have been correlated with such factors as ineffective Pt inoculum, adverse environment, detrimental cultural practices, and pesticide toxicity (Cordell 1985).

Inoculated seedlings have been planted on a variety of routine forestation sites, strip-mined areas, kaolin wastes, and Christmas tree farms in locations scattered over 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. Over 75 of these outplantings contain southern pine species (primarily loblolly [Pinus taeda L.] and slash pines [P. elliottii Engelm. var. elliottii]) in the Southern United States. Preliminary analyses show significant increases in tree survival and/or growth in over half of the 100+ field studies. Pt-inoculated eastern white (P. strobus L.), loblolly (P. taeda L.), and Virginia (P. virginiana Mill.) pines continue to show significant increases in tree volume growth (25+%) (Fig. 2), as compared with uninoculated check trees on a routine forestation site after 14 years in western North Carolina. Positive field responses are correlated with successful Pt nursery inoculations (Pt index > 50), mineland reclamation sites, and forestation sites with periodic moisture stress. 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 stress factor(s) 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 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 terristris ectomycorrhizae grew less during years of low rainfall than Pt trees. During years with moisture stress, Pt ectomycorrhizae markedly improved diameter growth. In a separate outplanting study in southwest Georgia, loblolly pine seedlings with different amounts of Pt ectomycorrhizae were planted on a good old-field site (site index of 90 ft. at age 25). After 8 years and crown closure, trees with Pt indices of 88 and 68 at planting date had significantly better survival and greater heights, diameters, volumes, and green weights per tree and per acre than T. terrestris seedlings (Pt index of 0). Volume and weight yields per acre were over 50 percent greater, and volume and weight yields per tree were over 20 percent greater for trees in the Pt index 88 treatment than in the control tree treatment. A Pt index of 58 appeared to be the threshold for growth improvements at planting. Increases in tree volumes and annual basal area growth occurred during growing seasons with water deficits of 200 to 325 mm (7.9 to L2.8 inches). Annual growth was not

# Pt NURSERY BENEFITS

## VEGETATIVE INOCULUM VERSUS UNINOCULATED CHECK

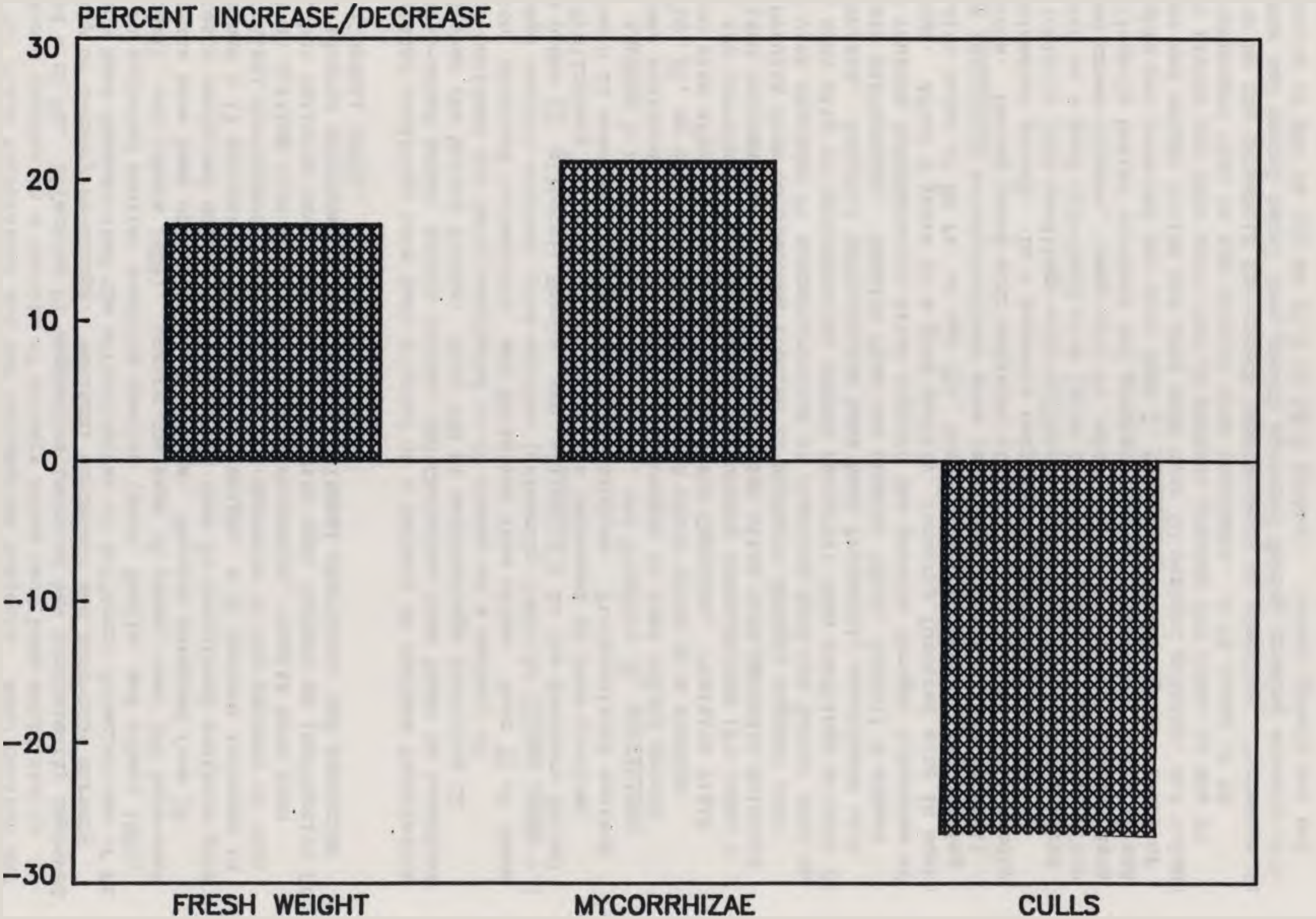



Figure 1.--Effects of Pt inoculation on seedling fresh weight, ectomycorrhizal development, and nursery cull percentage.

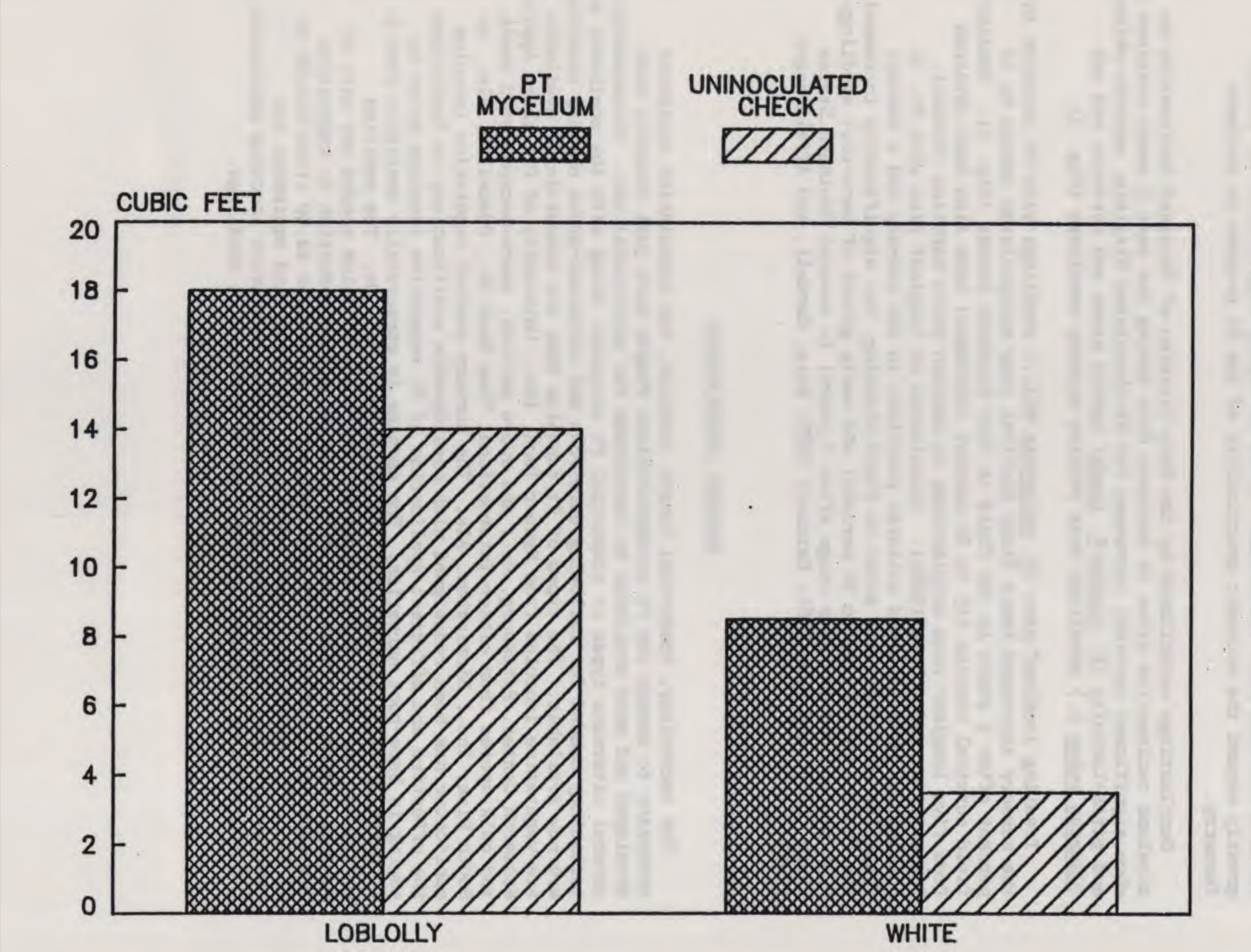



Figure 2.--Positive growth responses obtained on Pt-inoculated vs. uninoculated loblolly pine and eastern white pine after 10 years on a routine forestation site in North Carolina.

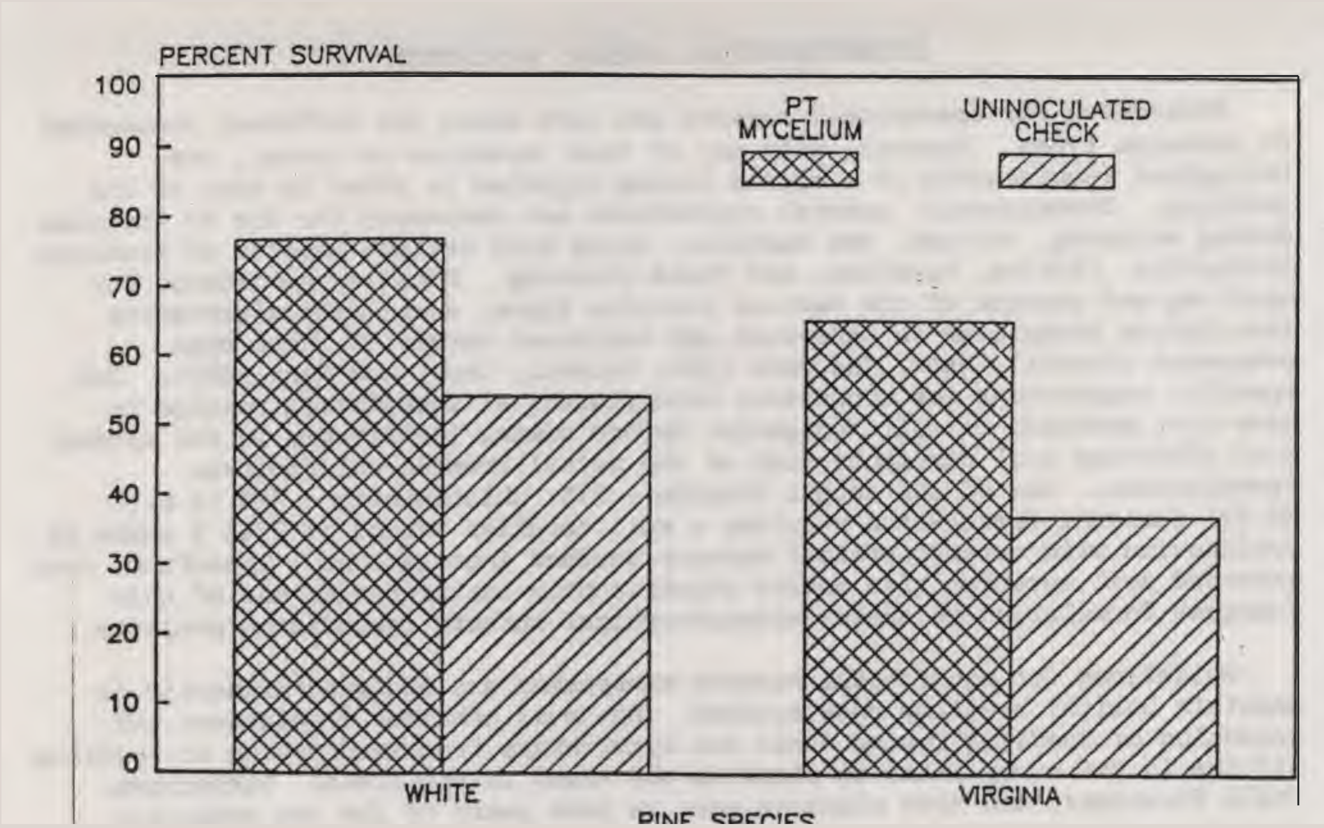
different when water deficits were either greater or less than these amounts. The apparent effectiveness of Pt in alleviating moisture stress conditions on routine southern pine forestation sites is highly significant and should greatly expand the economic practicality of the Pt program in southern forestry.

Outplantings established by the Ohio Division of Mineland Reclamation on mineland reclamation sites in southern Ohio during the past 5 years continue to show significant survival increases for Pt-inoculated Virginia, eastern white, and pitch-loblolly (P. rigida-P. taeda) hybrid pines and northern red oak (Quercus rubra L.) seedlings over routine nursery seedlings (Fig. 3).

Treating longleaf pine (P. palustris Mill.) seedlings with Pt inoculum in the nursery increased their field survival over uninoculated checks by 17 percent after 3 years in the field in four Southern States (Fig. 3). Similar field survival results (15 to 20 percent increases) have also been obtained from five longleaf pine outplantings in eastern North Carolina (Cordell, Summerville, and others 1988a). Inoculation of longleaf pine with Pt, in combination with selected cultural practices in the nursery and a benomyl fungicide root treatment prior to field planting, has significantly increased the field survival (40 to 50 percent) and early growth of bare-root seedlings (75+ percent out of grass stage after 2 years) in several Southern States (Kais, Snow, and Marx 1981; Hatchell 1987; Kais, Cordell, and Affeltranger 1986).

## NURSERY INOCULATIONS

The technology, commercial fungus inoculum, and inoculation equipment necessary to manage the Pt ectomycorrhizal fungus have just recently been developed and made available to nurserymen for operational use. Consequently, several alternate types of commercial Pt inoculum, along with the equipment and technology needed for tailoring bare-root and container-grown nursery seedlings are now available. The types of Pt inoculum that are commercially available are 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, Ala., or South Pine, Inc., Birmingham, Ala. The nursery seedbed vegetative inoculum applicator has been redesigned with considerable modifications to facilitate the effective and efficient application of Pt vegetative inoculum prior to sowing in bare-root nurseries. The machine is commercially available from R. A. Whitfield Manufacturing Co., Mableton (Atlanta), Ga. The modified applicator has reduced the amount of vegetative inoculum needed per unit of seedbed surface by 25 percent with a corresponding reduction in vegetative inoculum costs per thousand seedlings of 25 percent or $2.50 (from $10.00 to $7.50/1,000 seedlings). Improvements in technology and equipment are continuing. They include the development of a vegetative inoculum applicator for side-banding inoculum between rows of established seedlings.




AVERAGE OF 4 Willa. PINE SITES AND 3 VIRGINIA PINE SITES

(a)

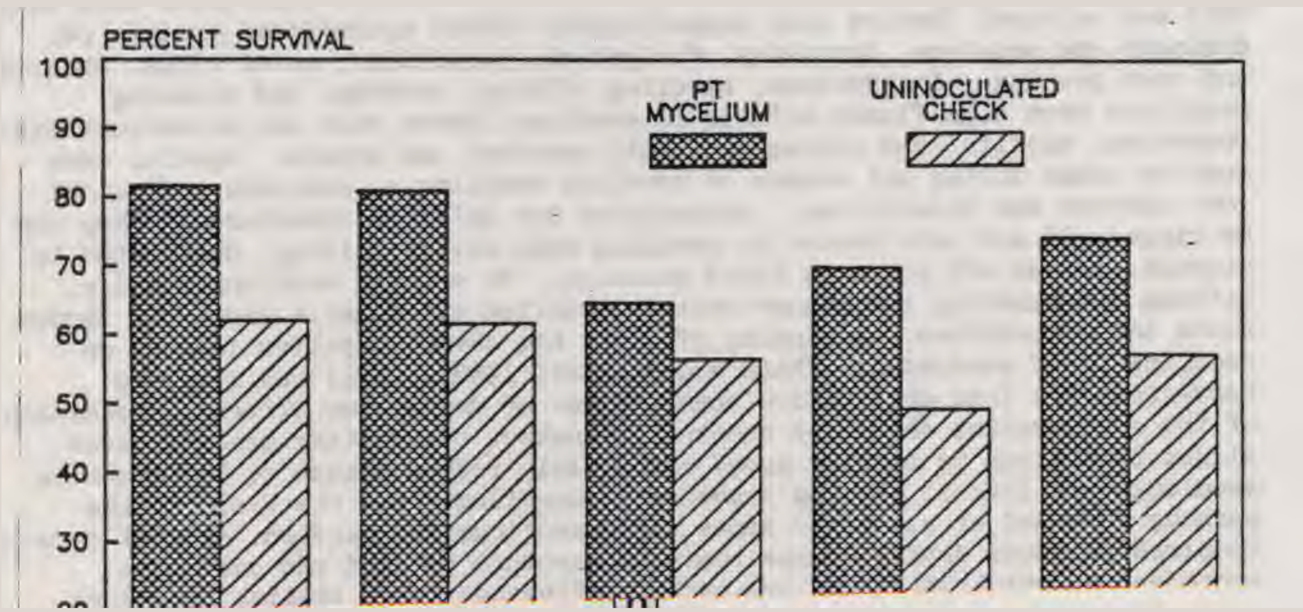



Figure 3.--Increased survival of Pt-inoculated vs. uninoculated (a) eastern white pine and Virginia pine on mineland reclamation sites in Ohio and (b) longleaf pine on routine forestation sites in four southern states.

Procedures for operational nursery use vary among the different commercial Pt inoculum types. However, with any of them (mycelium or spore), the biological requirements of a second living organism is added to that of the seedling. Consequently, special precautions are necessary for the Pt inoculum during shipping, storage, and handling, along with 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). One specific requirement for consistent ectomycorrhizal inoculation results in bare-root seedbeds is soil fumigation before sowing (preferably in the spring) with effective soil fumigants such as the methyl bromide-chloropicrin formulations. Use of the methyl bromide - 67%; chloropicrin - 33% (i.e., MC-33) fumigant formulation requires a soil aeration period of 2 to 3 weeks in conjunction with ectomycorrhizal nursery seedbed inoculations. Therefore, this extended soil aeration time period significantly restricts the use of this fumigant formulation on spring ectomycorrhizal nursery inoculation projects.

Guidelines for mycorrhizal nursery management are designed primarily to maintain healthy seedling root systems. One must consider development and retention of seedling feeder roots and mycorrhizae from seed sowing to seedling lifting in the nursery and to planting the trees in the field. Nurserymen, field foresters, 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 generally require 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. 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 mycorrhizae. Mycorrhizae are delicate structures. They can be ripped off and left behind in seedling beds during lifting, desiccated 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 mycorrhizae. 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 the roots as seedlings are lifted. During transfer of seedlings from the field to the packing room and at all other times when seedlings are 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 material have determined that seedling survival is better when peat moss, clay, or inert super-absorbents are used rather than hydromulch (Cordell, Kais, Barnett, and Affeltranger 1984). Best results are obtained when all root systems are coated or at least in contact with the packing material, so the material should be distributed through the bag, not simply dumped at the bottom or top. Numerous studies have documented the effects of long-term storage on seedling quality. For most tree species and their mycorrhizae, storage for 2 to 6 weeks is not harmful. Longleaf pine is an exception; its seedlings cannot be stored safely for more than a few days.

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 temperature, high winds, and low humidity 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 vegetative mycelium is sold on a volume basis, while the spore inocula are sold by weight. However, 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.

## OPERATIONAL APPLICATIONS

The demand for Pt-inoculated seedlings continues to increase. In 1987, a total of 5.5 million seedlings were inoculated with Pt at 10 nurseries in the Southern and Central United States (Fig. 4). Five conifer and one hardwood species were treated. In 1988, the totals rose to 6 million seedlings at 12 bare-root and container nurseries in the Southern, Central, and Northeastern United States.

During 1987, 1-1/2 million 1-0 loblolly and 1/2 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. The pine seedlings were lifted and planted at the Savannah River Station in January and February 1988. 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 March 1988, 0.75 million longleaf and 1.25 million loblolly pine seedlings were inoculated with Pt at the Taylor, S.C., State Nursery for planting in the winter of 1988-89 at the Savannah River Forest Station. Some 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. Both the longleaf and loblolly pine seedbeds were sown with one of the recently developed precision sowers. This is the first time that longleaf pine seeds were precision sown in seed drill rows and regulated spacings.

Table 1.--Commercial Pt inoculum costs - 1988.

| Pt inoculum type | Inoculum cost per 1,000 seedlings | Inoculum cost per Hectare of plantation | Inoculum cost per Acre of plantation |
|---|---|---|---|
| Vegetative mycelium | $7.50[2] | $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[3] | 0.43 | 0.77 | 0.31 |

[1] Cost estimates are for loblolly and slash pine bare-root nurseries (269 seedlings/m - 25 seedlings/ft ) and forestation plantings (1.8 x 3.0 meter - 6 x 10 foot spacing; 1,794 trees/ha. - 726 trees/ac.) in the Southern United States. Cos for longleaf pine bare-root seedlings (129 seedlings/m - 12 seedlings/ft ) is $15.63/1,000 seedlings, $28.02/ha. of plantation, and $11.35/ac. of plantation.

[2] Inoculation costs for Pt vegetative mycelium inoculum in bare-root nurseries was recently reduced by 25 percent ($10.00 to $7.50) per 1,000 seedlings through the development of more effective and efficient Pt inoculum, inoculation equipment, and techniques.

[3] Double sifting is required for even flow through spray nozzles. Standard bulk spores are only sifted once.

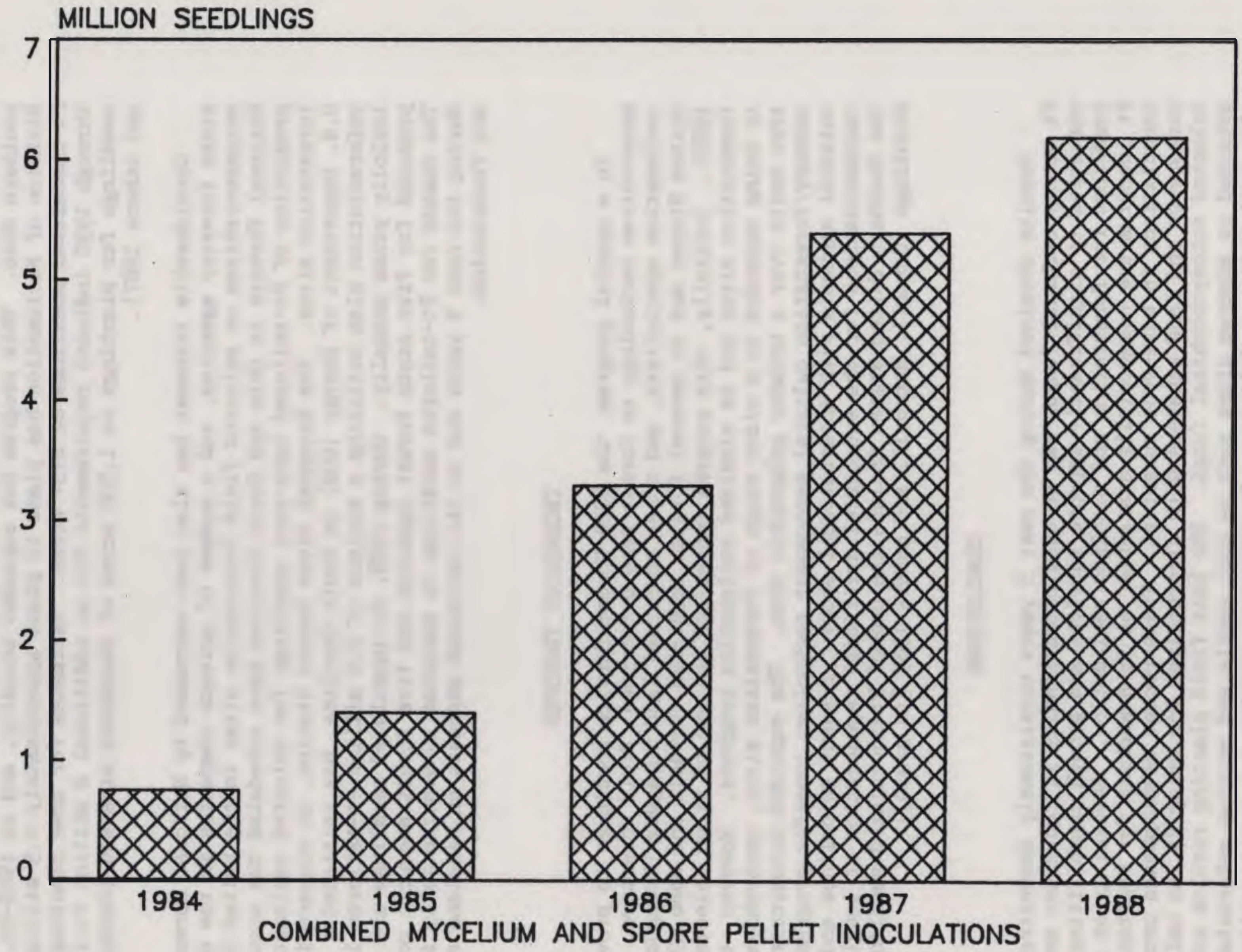



Figure 4.--Operational Pt custom seedling production using commercial vegetative and spore inoculum in bare-root and container seedling nurseries, 1984-88.

Interest in the use of Pt ectomycorrhizae in mineland reclamation has also increased steadily over the past 7 to 10 years. Since its inception in 1981, the Ohio Abandoned Mineland Reforestation Program has planted approximately 1.1 million Pt-inoculated seedlings on 622 acres of abandoned strip mines in southern Ohio. This program has expanded annually, and in 1988-89, the Ohio Division of Reclamation has plans to plant approximately 0.5 million Pt-inoculated seedlings on 275+ acres. Estimates for tree planting in Ohio through 1990 indicate requirements for an additional 2 million Pt-inoculated seedlings for plantings on 1,500 acres of abandoned mineland (Cordell, Owen, and others 1987).

Considerable interest has also been expressed by National Forests, several state forestry agencies, and a number of private companies in the use of Pt ectomycorrhizae on selected field 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 1988, 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 in 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 nursery/forestation cultural management technology transfer program will have national emphasis with international scope. Present guidelines and recommendations for the successful artificial regeneration of longleaf pine in the Southern United States include the use of Pt-inoculated seedlings for field plantings (Cordell, Hatchell, and others 1989 [in press]).

## CONCLUSIONS

Results obtained during the past 5 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 and 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 reforestation 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. 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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