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ABSTRACT. Extensive laboratory and field studies by the USDA Forest Service have revealed practical techniques for applying the ectomycorrhizal fungus *P. tinctorius* (Pt) in container and bare-root nurseries and demonstrated the benefits in forestation and mineland reclamation. Benefits 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 seed. A machine has also been developed to apply mycelial inoculum in bare-root nurseries. Considerable interest in use of Pt has been expressed by several **forest and mineland reclamation agencies. Over 1 million seedlings in 1985 and 3 million seedlings in 1986 were inoculated with Pt in operational bare-root and container nurseries in the Southern and Central United States.**

Additional keywords: Ectomycorrhizae, *P. tinctorius*, forest tree nurseries, seedling quality, field forestation, mineland reclamation, alternative inoculation techniques.

Roots of all forest tree seedlings form mutually beneficial structures called mycorrhizae with specific soil fungi. These symbiotic structures are vital to the survival and growth of both the host tree and the fungus. Most

hardwoods and some conifers form endomycorrhizae with species-specific, slow-spreading soil fungi. Most conifers and some hardwoods form ectomycorrhizae with non-species-specific fungi that spread by aerial spore dispersal. Ectomycorrhizal feeder roots are visibly different from nonsymbiotic roots, usually appearing swollen, forked, and/or more prolific (Ruehle and Marx 1979). Ectomycorrhizae benefit host seedlings by increasing root absorption, feeder-root longevity, ability of the roots to assimilate nutrients, and resistance to feeder root pathogens.

For several years, the USDA Forest Service and a large number of cooperating forestry agencies have conducted extensive mycorrhizae research and field studies in forest tree nurseries, in forest plantings, and 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 toxicity, low pH, high temperatures, and drought, that frequently kill other ectomycorrhizal fungi and their host trees (Marx, Cordell, and others 1984).

BENEFITS

Many conifer and some hardwood species on a variety of nursery sites have been artificially inoculated with Pt by treating seedling containers and prefumigated nursery seedbeds. 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. A companion evaluation of container seedlings also demonstrated the effectiveness of commercial Pt vegetative inoculum in 18 nurseries in 9 states and Canada (Marx, Ruehle, and others 1981). Inoculated seedlings have significantly outperformed uninoculated checks that contained natural ectomycorrhizae. 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 increase in the number of plantable 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, negative 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. Currently, over 100 Pt ectomycorrhizal outplantings involving 12 species of conifers are being monitored in 20 states. Over 75 of these outplantings contain southern pine species (primarily loblolly [Pinus taeda L.] and slash pines [P. elliottii var. elliottii Engelm.]) in the Southern United States. Most of these outplantings have been established since 1979 and, consequently, tree survival and growth results are preliminary. However, in outplantings of several conifer species in widespread locations, tree survival and early growth have been significantly improved by Pt inoculations in the nursery. A significant increase (25+%) in tree volume is still being observed on Pt-inoculated eastern white (P. strobus L.), loblolly

(*P. taeda* L.), and Virginia (*P. virginiana* Mill.) pines over check trees after 10 years in western North Carolina. Loblolly pine volume was 31 percent higher, and white pine volume was 151 percent higher than in uninoculated checks (Fig. 2). Outplantings established by the Ohio Division of Mineland Reclamation on mineland reclamation sites in southern Ohio during 1982 and 1983 showed an average survival increase of 23 percent and 24 percent, respectively, for Virginia and eastern white pine seedlings over routine nursery seedlings after 2 years in the field (Fig. 3-a). Treating longleaf pine seedlings with *Pt* inoculum in the nursery increased their survival over uninoculated checks by 17 percent after 3 years in the field in 4 Southern States (Fig. 3-b). Inoculation of longleaf pine with *Pt*, in combination with selected cultural practices **in** the nursery and a benomyl root treatment prior to field planting, has significantly increased the field survival and early growth of bare-root seedlings in several Southern States (Kais, Snow, and Marx 1981; Hatchell and Marx 1986a; Kais, Cordell, and Affeltranger 1986).

NURSERY INOCULATIONS

Practical application of *Pt* or any other ectomycorrhizal fungus has been limited to date because procedures, commercial fungus inoculum, and inoculation equipment necessary to manage the fungus have not been readily available to nurserymen. Over the last 10 years, the USDA Forest Service has been cooperating with several private companies to develop several types of commercial *Pt* inoculum, along with equipment and procedures needed for tailoring bare-root and container-grown seedlings. The types of *Pt* inoculum that are currently available are vegetative inoculum from Sylvan Spawn

Laboratories, Kittanning, Pa., and spore pellets, spore-encapsulated seeds, and bulk spores from either International Tree Seed Co., Odenville, Ala., or South Pine, Inc., Birmingham, Ala. A nursery seedbed applicator has been developed to apply Pt vegetative inoculum prior to sowing in bare-root nurseries. This machine applies the inoculum in bands along seedling rows at desired depths for maximum inoculum effectiveness and efficiency (Cordell, Marx, Lott, and Kenney 1981). **Use of the applicator has reduced the amount of vegetative inoculum** needed by 75% and reduced time and labor requirements as compared to broadcast application. Improvements in technology continue to be made with the various **Pt inocula, methods of application, and the equipment necessary for the different inocula types.**

Procedures for operational nursery use vary among the different commercial Pt inocula 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. In bare-root nurseries, vegetative inoculum can be broadcast on the soil surface and incorporated into the fumigated seedbeds prior to sowing or can be applied with the vegetative inoculum applicator with greater effectiveness and efficiency. For container-grown seedlings, vegetative inoculum can be incorporated into the growing medium before filling the containers or added at selected depths within the container. Bulk spores can be sprayed, drenched, or dusted onto growing medium for containerized seedlings and onto seedbeds in bare-root nurseries, or they can be applied in commercial hydromulch. Spore pellets can either be incorporated into the growing medium or seedbed soil, or they can be broadcast

on the soil surface and be lightly covered. Spore pellets have been applied at several nurseries with a standard fertilizer spreader. Spore-encapsulated seeds are sown by conventional methods. A major disadvantage of the Pt spore inoculum is the absence of a means of determining or controlling spore viability. Consequently, Pt ectomycorrhizal development has been considerably less consistent and effective with spore inoculum than with vegetative inoculum. In the spring of 1986, six nursery tests were established on four pine species to make standardized comparisons of the commercially available Pt inocula types.

COSTS

There is a wide range in cost of commercially available Pt inoculum. Current costs are summarized in Table 1. The vegetative mycelium is sold on a volume basis, while the spore inocula are sold by weight.

Table 1.--Commercial Pt inoculum costs - 1986.

Pt inoculum type	1 <u>Inoculum cost per</u>		
	1,000 seedlings	Hectare of plantation	Acre of plantation
Vegetative mycelium	\$10.00	\$17.93	\$7.26
Spore-encapsulated seed	2.22	3.98	1.61
Spore pellets	2.75	4.93	2.00
Double-sifted bulk spores	0.43	0.77	0.31

¹ Cost estimates are for loblolly and slash pine bare-root nurseries (269 seedlings/sq. meter - 25 seedlings/sq. foot) 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.

² Double sifting is required for even flow through spray nozzles. Standard bulk spores are only sifted once.

OPERATIONAL APPLICATIONS

The demand for Pt-tailored trees is increasing despite the added costs and the financial difficulties that forest industry is experiencing. In 1985, operational Pt inoculations were established in one Central and five Southern United States bare-root nurseries on seven conifer and one hardwood species, and over 1 million seedlings. During the spring of 1986, Pt vegetative mycelium was operationally applied at 10 bare-root nurseries in the United States. Approximately 2 million bare-root seedlings of nine conifer and one hardwood species were inoculated with Pt vegetative mycelium, and over 1 million seedlings were inoculated with spore pellets (Table 2). The Pt-tailored seedling demand is expected to substantially increase during the next 5 years due to the increased emphasis on mineland reclamation and forestation.

Table 2.--Pt ectomycorrhizae inoculations - 1986.

Nursery	Species	Seedlings
<u>Vegetative Inoculum</u>		
Pinson-State-TN	White pine	85,000
Piedmont-State-SC	White pine	15,000
Ashe-Federal-MS	Longleaf pine	20,500
	Slash pine	29,000
	Loblolly pine	29,000
Champion-Industry-FL	Sand pine	85,000
	Longleaf pine	1,500
Taylor-State-SC	Loblolly pine	735,000
Int. Paper Co.-Industry-SC	Loblolly pine	265,000
Weyerhaeuser-Industry-SC	Loblolly pine	70,000
Edwards-State-NC	White pine	60,000
J.P. Rhody-State-KY	Scotch pine	3,000
	Virginia pine	7,500
	Shortleaf pine	4,500
	Loblolly pine	9,000
Vallonia-State-IN	Virginia pine	165,000
	White pine	277,000
	Pitch-Lob hybrid	36,000
	Red oak	3,000
Total		<u>1,900,000</u>
<u>Spore Pellets</u>		
Crescent Land Co.-Industry-NC	Bare-root seedlings	500,000
Int'l Forest Seed Co.-Industry-AL	Container seedlings	750,000

Interest in use of *Pt* ectomycorrhizae in mineland reclamation has increased greatly in the past 5 years (Wolf, Cordell, and Keller 1981). The Ohio Division of Reclamation has requested approximately 200,000 *Pt*-inoculated pine seedlings annually for reclamation sites in southern Ohio. Field tests are underway with *Pt*-inoculated and uninoculated seedlings on surface-mined land with soils that have been amended in various ways. High fertility and intensive site reclamation may reduce the positive effects of *Pt* ectomycorrhizae. However, *Pt* could significantly reduce reclamation costs by limiting site reclamation efforts to initial grass establishment, erosion control, and permanent plantings with trees tailored with *Pt* ectomycorrhizae.

Considerable interest has also been expressed by National Forests, several state forestry agencies, and a number of private companies in use of *Pt* ectomycorrhizae on selected field forestation sites in the Southern and Central United States. A private company with relatively large forest holdings in western North Carolina and northwestern South Carolina has scheduled the annual production of 500,000 eastern white and loblolly pine bare-root nursery seedlings inoculated with *Pt* spore pellets. These seedlings will be used in all the company's forest plantings. National Forests in Ohio and South Carolina have also scheduled the annual production of *Pt*-tailored bare-root seedlings for selected reclamation and forestation sites. The Savannah River Forestry Project, in cooperation with the Department of Energy (DOE) and E. I. Dupont De Nemours & Co., in South Carolina has initiated a 5-year reforestation plan utilizing 1.5 million *Pt*-tailored longleaf and loblolly pines in 1987, with substantial reforestation increases during succeeding years.

TECHNOLOGY TRANSFER

In a special program, the USDA Forest Service is providing mycorrhizae technology to forest tree nurserymen, field foresters, mineland reclamation specialists, and other concerned land managers (Cordell and Webb 1980, Cordell 1985). The present effort emphasizes Pt use on selected forestation sites and in mineland reclamation programs. The use of Pt, and possibly other ectomycorrhizae, in high-value plantings such as Christmas tree farms and the custom-tailoring of southern pine species for forestation also appear promising (Ruehle, Marx, and Cordell 1981; Kais, Snow, and Marx 1981). Recommendations for the successful artificial regeneration of longleaf pine in the Southern United States will include the use of Pt-inoculated seedlings for field plantings.

CONCLUSIONS

The Pt ectomycorrhizal fungus can be used operationally in container and bare-root seedling nurseries for the production of seedlings with significantly improved survival and growth capabilities for field plantings. Several types of effective Pt inoculum can be used, and a machine for mycelium inoculations in bare-root nurseries is commercially available. These recent developments provide nurserymen and land managers with alternatives for using Pt. The need for higher-quality nursery seedlings for successful field forestation and disturbed site reclamation by Federal, State, industry, and private forest land managers is becoming increasingly apparent. The best field planting results have been obtained on adverse sites such as coal spoils and poor reforestation sites. In addition, results are best when planted seedlings have Pt indices >

50 (Pt incidence greater than other natural ectomycorrhizae incidence on seedling feeder roots). Although seedling costs represent a minor portion of forestation expense (less than 10%), seedling quality is the most significant factor in successful forestation and mineland reclamation. Consequently, the benefits of producing Pt-tailored seedlings for selected forestation and reclamation sites should greatly exceed the costs.

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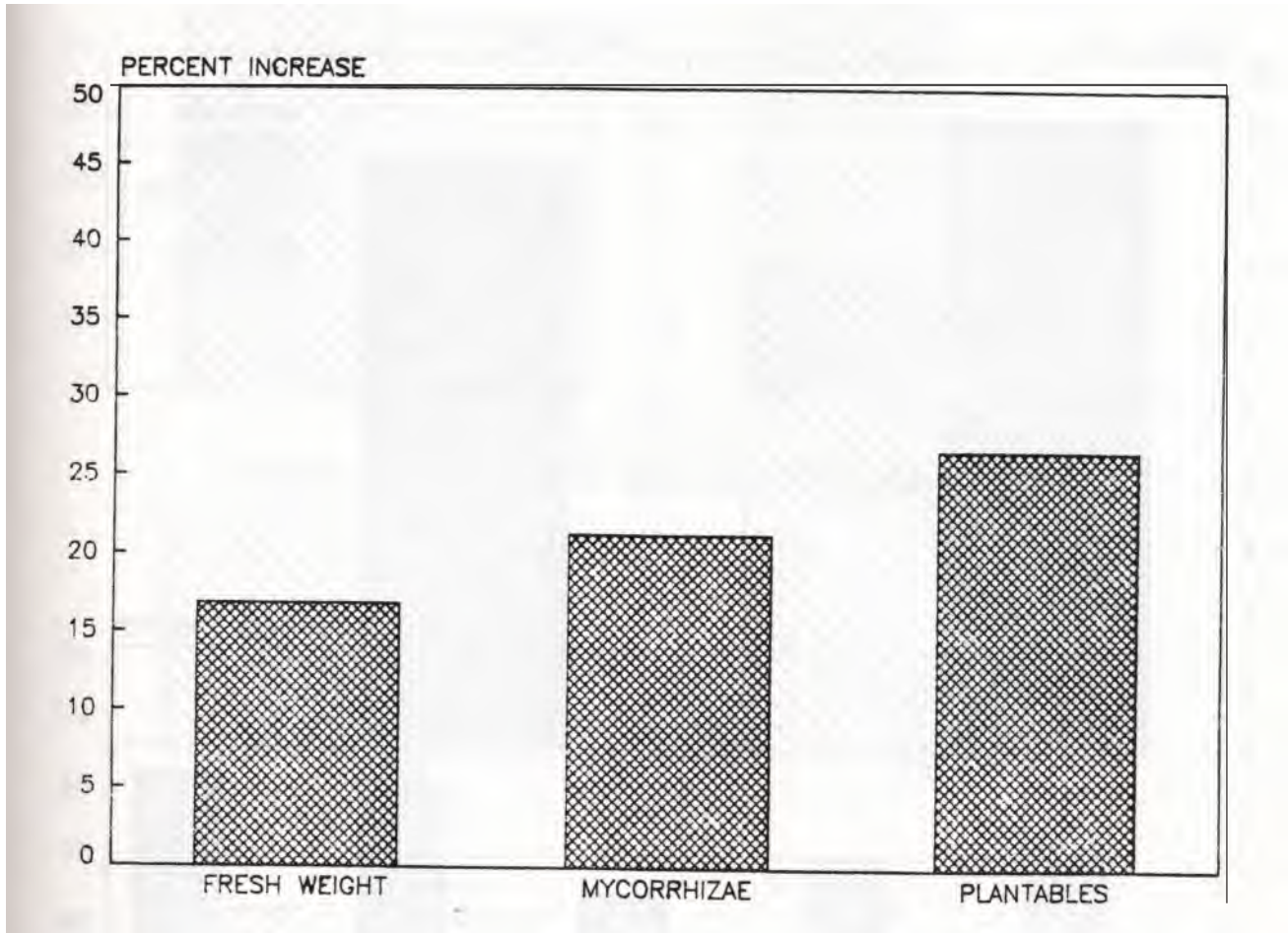


Figure 1.--Increased fresh weights, total ectomycorrhizal development, and plantable seedlings obtained on Pt-inoculated vs. uninoculated southern pine seedlings in 34 bare-root nursery tests, 1978-1980.

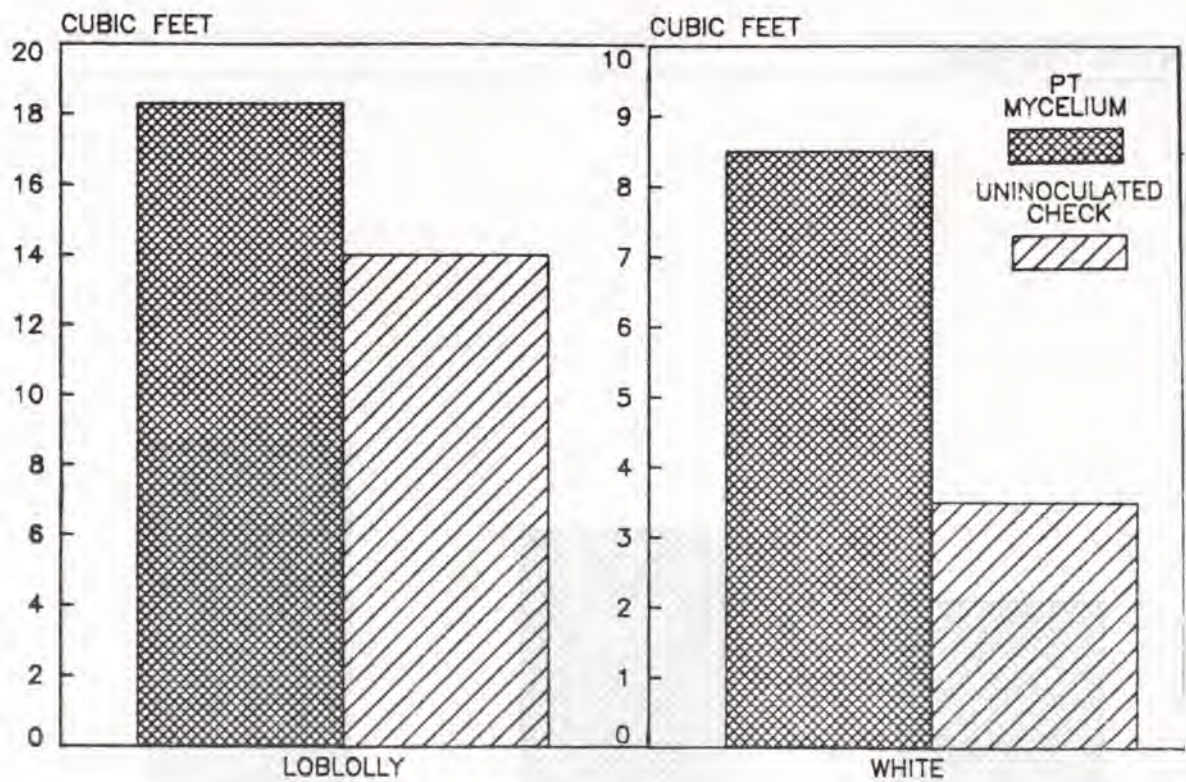
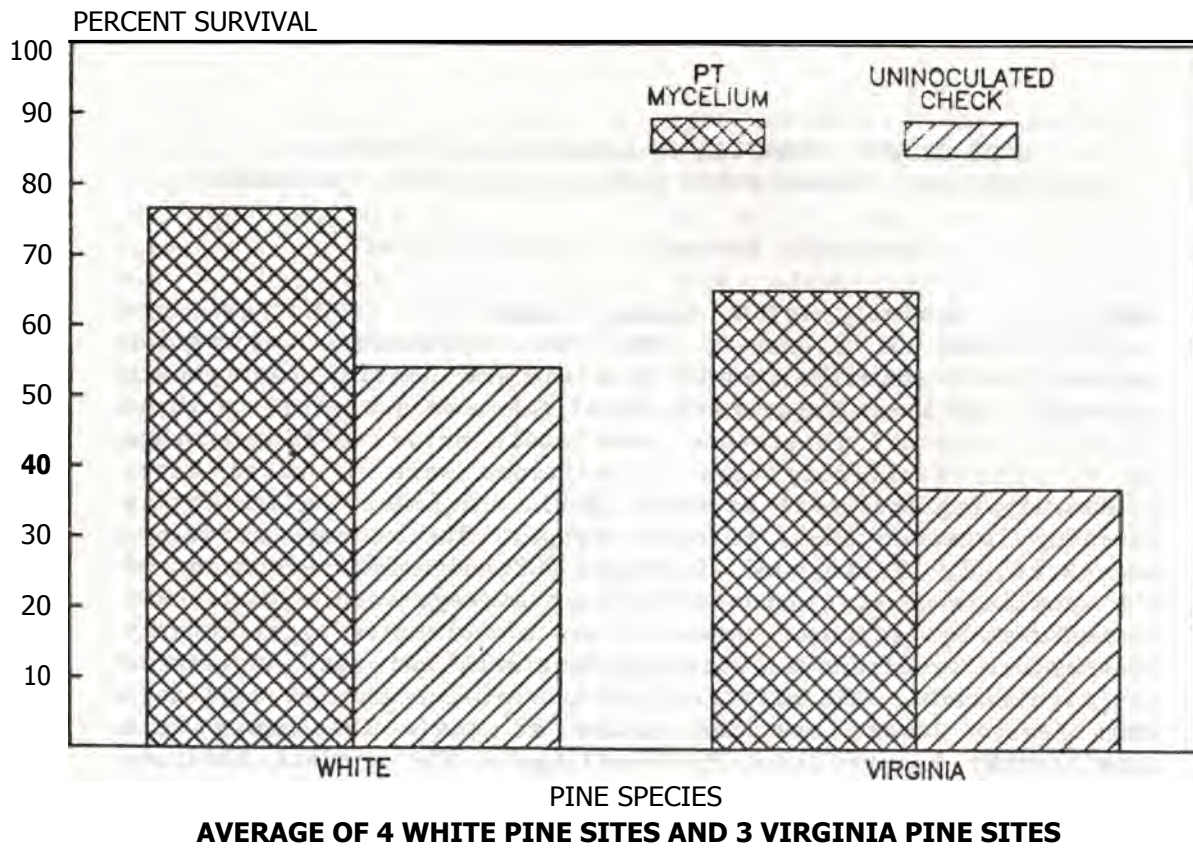
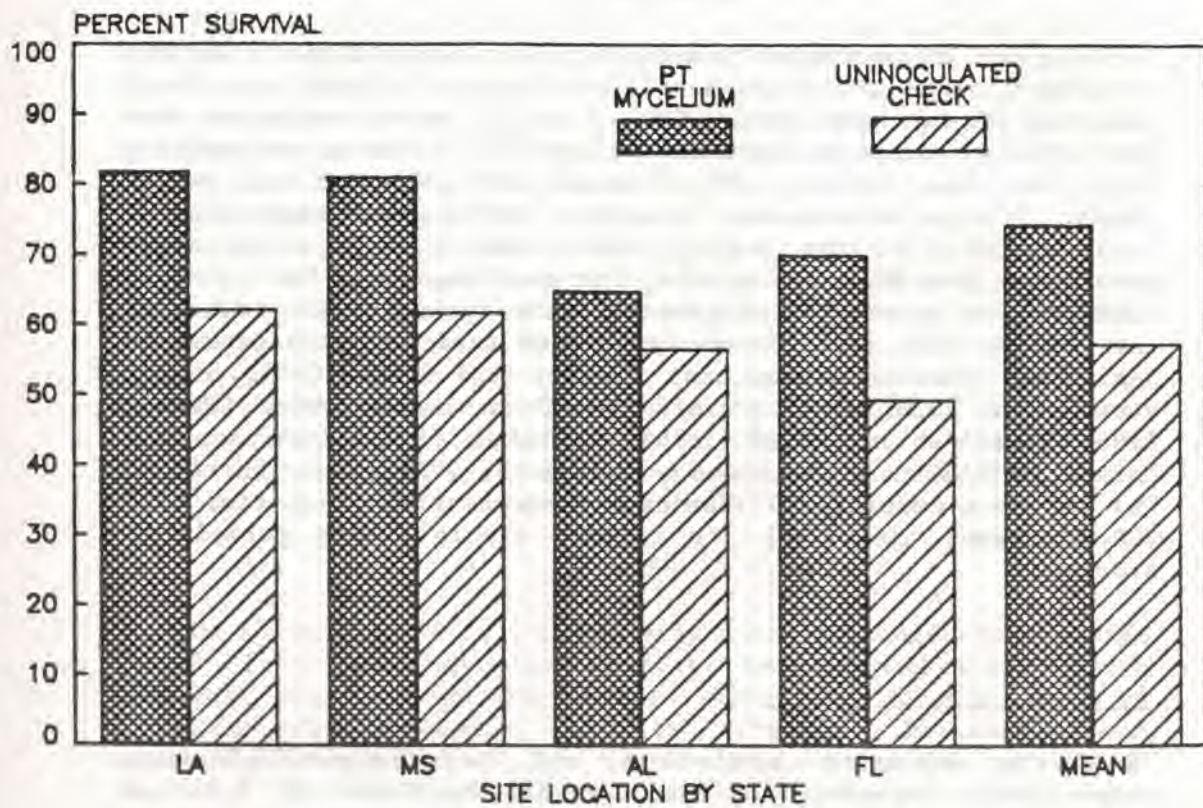


Figure 2.--Positive growth responses obtained on Pt-inoculated vs. uninoculated loblolly pine and eastern white pine after 10 years on a routine forestation site in North Carolina.



a



b

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