OPERATIONAL APPLICATION OF SPECIFIC ECTOMYCORRHIZAL FUNGI IN MINELAND RECLAMATION[1]

by

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Abstract. Revegetation of drastically disturbed lands, such as acid coal spoils, kaolin spoils, borrow pits, and severely eroded sites in the eastern United States, has been significantly improved by planting tree seedlings tailored in the nursery with the ectomycorrhizal fungus, *Pisolithus tinctorius* (Pt). On these disturbed sites, Pt promotes the assimilation and absorption of required nutrients, increases water absorption, reduces the absorption of toxic elements, and, apparently, reduces the overall stress inherent on these sites. Repeatedly demonstrated benefits in forestation and mineland reclamation include significant increases in nursery seedling quality (reduced culls), survival, and growth in field plantings. Extensive research and field evaluations during the past 15 years have provided practical techniques for applying Pt in container and bare-root seedling nurseries. Types of commercial inoculum include vegetative mycelium, bulk spores, spore pellets, and spore-encapsulated seeds. A commercially available machine effectively and efficiently applies mycelial inoculum in bare-root nurseries. The demand for custom-grown, Pt-inoculated seedlings continues to increase. In 1990, 7 million seedlings were inoculated with Pt at 12 bare-root and container nurseries in the southern, central, and northeastern U.S. The Ohio Division of Reclamation planted approximately 0.3 million Pt-inoculated pine and hardwood seedlings on 200 acres of abandoned minelands in southern Ohio during the 1990-91 tree planting season. Additional interest in the initiation of Pt seedling mineland reforestation programs has recently been expressed by state officials, private coal companies, and reclamation contractors in the central and southern U.S. Pt inoculation costs represent less than 5% of the total mineland reclamation forestation expense.

Additional Key Words: *Pisolithus tinctorius*, customized nursery seedlings, seedling quality, field forestation.

Introduction

Vast areas of the United States have been made unproductive by over 175 years of intensive, uncontrolled surface and subsurface mining. Problems associated with abandoned mineland include subsidence, acid or toxic drainage, landslides, sedimentation and flooding, loss of productivity, hazardous impoundments, visual pollution, and abandoned equipment (Ohio Department of Natural Resources 1981). Increasing public awareness and legislation have regulated the mining industry to assure proper reclamation of the affected land. The Surface Mining and Control Act of 1977 requires mine operators to return the land as closely to its original condition as possible. With today's technology, the land can be shaped to its former contour, the soil can be replaced to its approximate previous configuration, and acidic coal spoils can be placed deep in the earth, sealed away from the environment. However, even with the most intensive efforts to improve soil fertility and structure, efforts to restore previously existing vegetation often fail. Establishment of trees has been especially difficult, often requiring repeated plantings to offset recurrent mortality (Marx and Artman 1979, Drake and Woodworth 1990).

An ectomycorrhizal fungus, *Pisolithus tinctorius* (Pers.) Coker & Couch (Pt) has been used to improve mineland-reclamation planting success. Acid coal spoils, kaolin spoils, borrow pits, and other severely eroded sites have been successfully forested with tree seedlings with Pt ectomycorrhizae. Although the fungus does not directly improve the quality of the mined site, it modifies the tree root system so that it can tolerate extreme soil conditions, such as low pH (4.0), high temperatures, low fertility, mineral toxicity, and drought, that usually kill most other ectomycorrhizal fungi and

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subsequently their host trees (Marx et al. 1984). Pt offers a low-cost, effective alternative to intensive mineland reclamation – particularly abandoned minelands (Caldwell 1987).

Pt occurs primarily on conifers, but also on some hardwood trees such as beech, oak, and hickory. The presence of Pt on tree roots is usually accompanied by the production of its fruiting bodies on reclamation sites. Pt ectomycorrhizae are swollen, usually forked or multibranched, and have a distinctive golden or mustard color (Ruehle and Marx 1979). The tree benefits from the ectomycorrhizal relationship with the fungus because water and nutrient absorption is increased, feeder roots live longer, more nutrients are assimilated, and the feeder roots are more resistant to disease (Cordell et al. 1988).

The USDA Forest Service, along with several cooperating forestry agencies, has developed operational programs for the practical, effective inoculation of nursery seedlings with Pt for mineland reclamation. Pt was selected not only for its demonstrated benefits to a variety of host trees, but also for its adaptability to adverse soil conditions, its availability, its ease of manipulation, and its wide geographic and host range (Marx et al. 1984). Successful Pt applications have been obtained in a variety of forest tree nurseries, mineland reclamation sites, and field forestation sites across the United States. Advances in ectomycorrhizal fungus technology continue to improve treatment effectiveness and practicality, to increase inoculum quality, to reduce treatment costs, and to expand to additional ectomycorrhizal fungus and host tree species.

Benefits

Nurseries

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 (see Figure 1).

National container and bare-root nursery evaluations have demonstrated the effectiveness of different formulations of Pt inoculum on selected conifer seedling

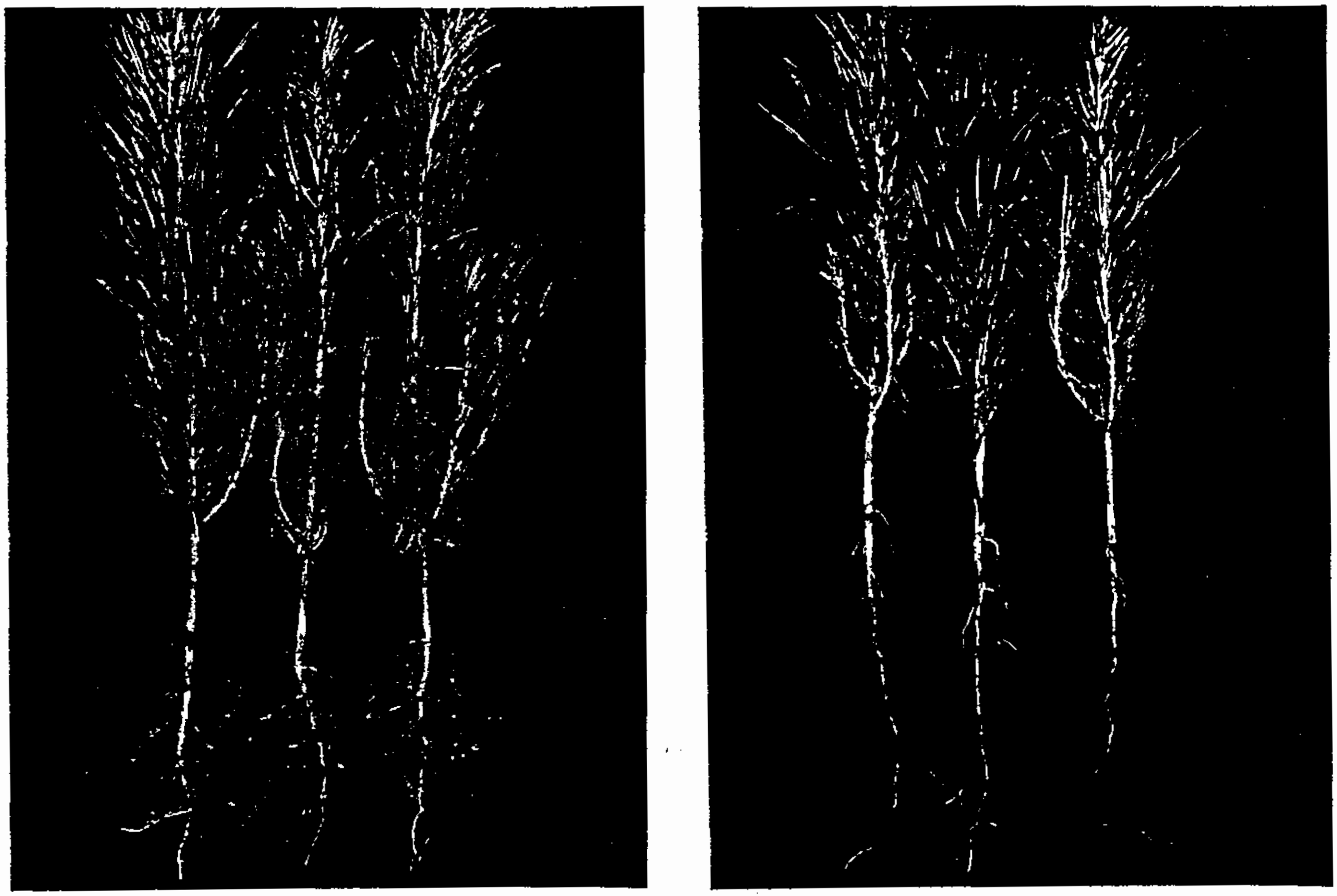
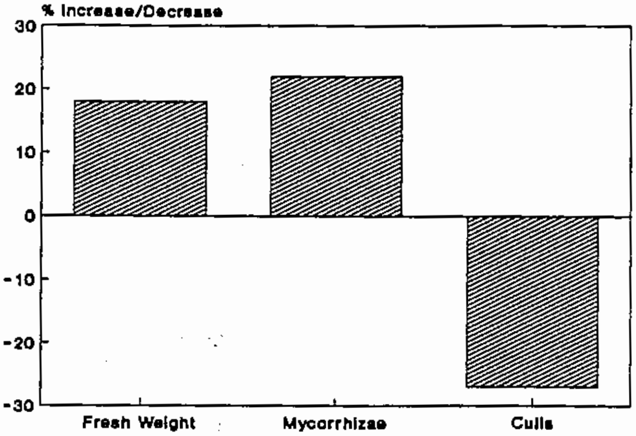


Figure 1. 1-0 loblolly pine seedlings with Pt ectomycorrhizae (left) and with only naturally occurring ectomycorrhizal fungi (right).

species (Marx et al. 1985, Marx et al. 1984). During the past 15 years, over 100 bare-root nursery tests have been conducted in 38 states. Results obtained from 34 nursery tests conducted during the 3-year period, 1978-80, showed that Pt-inoculated Southern pine seedlings had a 17% increase in fresh weight, a 21% increase in ectomycorrhizal development, and a 27% decrease in the percent of cull seedlings at lifting time (see Table 1).

Table 1. Effects of Pt inoculation with vegetative inoculum on seedling fresh weight, ectomycorrhizal development, and nursery cull percentage in 34 bareroot nursery studies.



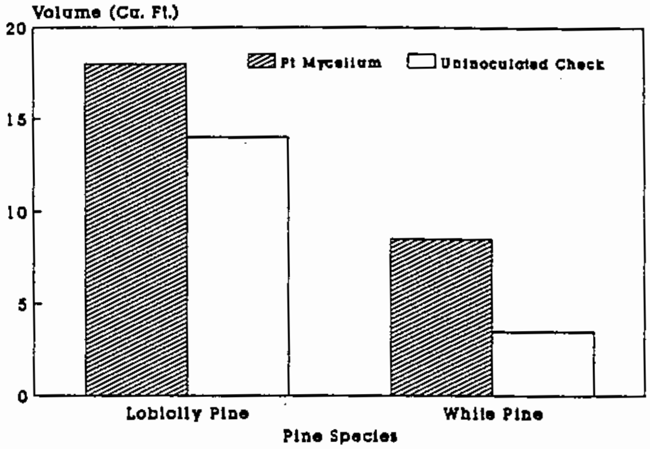
In some nurseries, results have been negative, but failures have been correlated with such factors as ineffective Pt inoculum, adverse environment, detrimental cultural practices, and pesticide toxicity (Cordell 1985).

Field Plantings

Inoculated seedlings have been planted on a variety of routine forestation sites, stripmined areas, and kaolin wastes in locations scattered over the United States. Over 100 Pt outplantings involving 12 species of conifers are currently being monitored in 20 states on a variety of forestation and mineland reclamation sites. Preliminary analyses show significant increases in tree survival and growth in over half of these 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+%) as compared with uninoculated check trees on a routine forestation site after 10 years in western North Carolina (see Table 2).

Positive field responses are correlated with successful Pt nursery inoculations (Pt index > 50), with mineland reclamation conditions, and with mineland site factors.

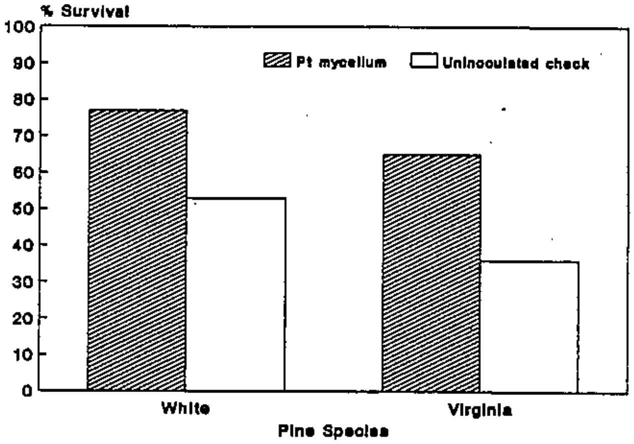
Table 2. Positive growth responses to Pt inoculation by loblolly and eastern white pines after 10 years on a routine forestation site in western North Carolina.



Extensive reclamation research has been conducted on custom-grown seedlings with Pt ectomycorrhizae and outplanted on disturbed and adverse sites of various types in the eastern U.S. Reviews by Marx (1980) and Cordell et al. (1988) discussed improvements in tree survival and growth of Pt-inoculated seedlings on these sites. Marx and Artman (1979) reported that bare-root loblolly pine seedlings with abundant Pt ectomycorrhizae had 40% more plot volume than control seedlings with *Thelephora* ectomycorrhizae after 3 years on an acid coal spoil (pH 4.1) in Kentucky. In addition, shortleaf pine (*P. echinata* Mill.) with Pt ectomycorrhizae were over 400% larger than control seedlings. Berry (1982) outplanted container-grown seedlings of loblolly, pitch (*P. rigida* Mill.) and loblolly x pitch (*P. taeda* x *P. rigida*) pine hybrids with Pt and *Thelephora* ectomycorrhizae on acid coal spoils in Tennessee and Alabama. The results were very impressive after 2-1/2 years. Overall, plot volumes with Pt ectomycorrhizae were over 200% greater on the Tennessee site and over 350% greater on the Alabama site than plot volumes with *Thelephora* ectomycorrhizae. Maximum benefits were obtained on the coal spoils having higher temperatures, lower pH, and greater moisture stress. Walker et al. (1984) outplanted loblolly, shortleaf, and Virginia pine seedlings with Pt and *Thelephora* ectomycorrhizae in a fertility study on a coal spoil in Tennessee. After 6 years, Pt ectomycorrhizae improved survival and growth of pines on stressed sites, regardless of fertility. Volume for outplanted container-grown loblolly pines inoculated with Pt ectomycorrhizae was 75% greater than volume for loblolly pines inoculated with *Thelephora* and 120% greater than volume for loblolly pines without any ectomycorrhizae (Ruehle

1980). On a severely eroded, highly acidic, infertile ridgetop in the Copper Basin in southeastern Tennessee, Berry and Marx (1978) obtained more than a 90% increase in growth at age 2 by inoculating loblolly and Virginia pines with Pt ectomycorrhizae in the nursery rather than planting similar pines with *Thelephora* ectomycorrhizae. In southern Ohio, outplanted Pt-inoculated seedlings of Virginia, eastern white, and loblolly x pitch pines and northern red oak (*Quercus rubra* L.) have exhibited significant increases in survival and growth when compared with standard nursery seedlings (Cordell et al. 1988) (see Table 3).

Table 3. Increased survival of Pt-inoculated vs. uninoculated eastern white pine and Virginia pine on mineland reclamation sites in Ohio.



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.

Nursery Inoculations

The technology, commercial fungus inoculum, and inoculation equipment necessary to manage the Pt ectomycorrhizal fungus have only 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 Forest Tree Seed Co., Odenville, AL, or South Pine, Inc., Birmingham, AL.

Procedures for nursery use vary by inoculum type. With either mycelial or spore inoculum, the biological requirements of a second living organism are added to those of the seedling. Special precautions are necessary for shipping, storage, and handling of Pt inoculum, and certain aspects of seedling production, lifting, handling, and field planting must be closely controlled. 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 by spring soil fumigation. The most effective soil fumigants are methyl bromide-chloropicrin formulations, such as methyl bromide-98%, chloropicrin-2%; and methyl bromide-67%, chloropicrin-33%. After fumigation with the methyl bromide-67%, chloropicrin-33% formulation, seedbeds must be aerated for 2 to 3 weeks prior to ectomycorrhizal inoculations. This extended aeration period significantly restricts the use of this fumigant in spring ectomycorrhizal inoculations in bare-root nurseries. Prior to sowing, vegetative inoculum can be broadcast on the soil surface and incorporated into the fumigated seedbeds, or it can be machine-applied with greater effectiveness and efficiency. For container-grown seedlings, vegetative inoculum can be incorporated into the growing medium before the containers are filled or placed at selected depths in the growing medium in the container. Bulk spores can be sprayed, drenched, or dusted onto growing medium for containerized seedlings and onto seedbeds in bare-root nurseries. Spore pellets can be incorporated into the growing medium or seedbed soil, or they can be broadcast on the soil surface, lightly covered, and irrigated. 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 compared with the vegetative mycelium is the absence of a reliable means of determining or controlling spore viability. Consequently, Pt ectomycorrhizal development, in general, has been considerably less consistent and effective with spore inoculum than with vegetative mycelium inoculum.

A machine has been devised to apply Pt vegetative mycelium inoculum prior to sowing in bare-root nurseries (see Figure 2). The machine is commercially available from R. A. Whitfield Manufacturing Co., Mableton (Atlanta), GA. It reduces the amount of vegetative inoculum needed per unit of seedbed surface by 25% and reduces vegetative inoculum costs from $10.00 to $7.50/1,000 seedlings. Improvements in technology and equipment are continuing.

Figure 2. Machine applies Pt vegetative mycelium inoculum to a forest tree bare-root nursery seedbed.

Ectomycorrhizal Nursery Management

Guidelines for mycorrhizal nursery management are designed primarily to maintain healthy seedling root systems. One must consider development and retention of seedling feeder root and mycorrhizae from 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 aware that they are handling two symbiotic living organisms—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, seedling spacing and density, 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. PH values above 6.5 are extremely detrimental to Pt development. Soil fertility and irrigation should be based on the requirements of the seedling host, but excessively high levels of phosphorous and nitrogen and excessive irrigation should be avoided, particularly in containers and poorly drained seedbeds. Certain pesticides are particularly detrimental to specific ectomycorrhizae such as Pt, and they must not be used in ectomycorrhizal nurseries. Precautions are warranted when using artificial shade materials in nursery seedbeds or greenhouses. Minimum threshold light requirements are necessary for adequate seedling photosynthate production and subsequent ectomycorrhizae development. When day length is short, the amount of natural light in greenhouses is often inadequate. Artificial lighting must be provided in such cases. Optimum seedling spacing and density, and custom-applied root pruning practices help to produce high-quality seedlings with not only desirable heights and basal diameters, but also maximum lateral and feeder root and ectomycorrhizae development.

Seedling lifting, storage, and planting practices have significant effects on seedling feeder roots and ectomycorrhizae. As ectomycorrhizae are delicate structures, special care must be taken during all stages of seedling handling to maintain sufficient root systems and ectomycorrhizae. They can be ripped off and left behind in seedling beds during lifting, desiccated in storage, or cut off prior to field planting. To maintain 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 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 materials have determined that seedling survival is better when peat moss, clay, or inert water-absorbents are used rather than hydromulch (Cordell et al. 1985). 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.

Planting Site Considerations

The great diversity of forestation and reclamation sites planted with Pt-tailored seedlings has defined the specific uses of Pt. Field results continue to demonstrate significant increases in host-tree tolerance of low pH, high levels of soluble phytotoxic microelements, extended low-moisture stress, high soil-surface temperatures, and very low nutrient availability. Except where soils are compacted severely or bulk densities are high, Pt-tailored seedlings have increased tolerance of low pH, high levels of soluble phytotoxic microelements, low levels of moisture, high soil temperatures, and very low nutrient availability. Although Pt may enhance tree survival on highly compacted sites, tree growth soon stagnates. Soil compaction and high bulk density can be mitigated by subsoiling, however. Similarly, Pt will grow profusely and provide survival and growth benefits on porous, coarse, droughty sites but not in poorly drained soil.

Most studies involving Pt on adverse sites have included some form of fertilization or soil amendment to boost fertility, as well as treatments to improve soil structure. However, highly fertile sites, especially those with high phosphorous levels or with fresh and microbially active sludge amendments, tend to mask Pt benefits. Other naturally occurring ectomycorrhizal fungi that are more ecologically adapted to these nutrient-rich conditions may replace Pt. Often these high fertility sites have soil pH above the tolerance level (pH 6.5) of Pt and most conifer seedlings. In addition, competing ground cover vegetation can be so abundant that subsequent establishment of any tree seedlings is difficult without chemical or mechanical suppression. When Pt-inoculated seedlings are used, the phytotoxic effects of any site amendments or pesticides should be determined prior to site selection. Amendments containing sludge and other refuse materials have been used to improve soil fertility and structure, but in some cases these amendments also contained chemicals that are highly toxic to both the trees and their ectomycorrhizal fungal symbionts.

The Ohio Division of Reclamation established a reforestation program for abandoned minelands in 1981 and has since established criteria for the use of Pt-tailored seedlings in this program). (1) The strip-mined areas, gob piles, or industrial mineral sites must have been abandoned since 1972, with no present potential for full-scale reclamation. (2) Currently, the sites must be barren, eroded, or without adequate stabilizing vegetation. (3) Target sites also must have off-site damage, such as sedimentation. During the past 10 years, the goals and priorities of the reforestation program have evolved into planting tree seedlings to provide a low-cost, low-maintenance reclamation method for areas that contribute minor quantities of sediment to streams, degrade aesthetics, lack adequate ground cover, and are not eligible for traditional reclamation techniques (major grading, resoiling, and revegetating) under federal abandoned mineland guidelines (Caldwell 1987).

Planting sites must be selected far enough in advance to allow for adequate seedling production – 1 year for Virginia pine and 2 years for white pine. The reclamation specialist must also allow time for administrative delays. Finally, the biological site requirements of Pt-tailored seedlings must be met: compacted sites must be subsoiled, sites with soil pH of 6.0 or higher must be acidified, and competing vegetation suppressed prior to tree planting (Cordell et al. 1988).

Costs

There is a wide range in the cost of commercially available Pt inoculum (see Table 4). The inoculum cost is only one part of the total cost of establishing Pt-inoculated seedlings on reclamation sites. Some forest tree nurseries purchase the inoculum and include its cost in the price of seedlings, while other nurseries prefer that the buyer purchase the inoculum. The Pt vegetative mycelium inoculum is sold on a volume basis, while spore inocula are sold by weight.

Table 4. Commercial Pt inoculum costs - 1991.

Pt inoculum type	Inoculum cost per[1] 1,000 seedlings	Hectare of reclamation planting	Acre of reclamation planting
Vegetative mycelium	$7.50	$32.27	$13.07
Spore-encapsulated seed	2.22	9.55	3.87
Spore pellets	2.75	11.83	4.79

[1] Cost estimates are for Virginia and eastern white pine bare-root nurseries (269 seedlings/meter^2 - 25 seedlings/ft^2) and mineland reclamation plantings (1.5-m x 1.5-m - 5-ft x 5-ft spacing; 4,303 trees/ha - 1,742 trees/acre) in the central United States.

On many mineland sites, reclamation costs can be greatly reduced by utilizing Pt-inoculated trees. Estimates (1980) suggest that it will cost approximately

$7,000/acre, on the average, to reclaim stripmine spoils in Ohio. The cost of reclaiming all of Ohio's abandoned underground and surface mines has been estimated at $3.5 billion (1980 dollars). Since 1982, the cost of reforesting abandoned mineland has averaged $300/acre. This includes the cost of Pt inoculum, seedlings, site preparation, and planting. The additional cost of using Pt-inoculated seedlings in these plantings at a 5 ft x 5 ft tree spacing (1,742 trees/acre) is less than 5% (maximum inoculum cost = $13.07/acre) of the total tree establishment costs ($300/acre). The additional cost of Pt-inoculated pine seedlings translates to less than $.01/seedling, a minute expense compared to other reclamation costs.

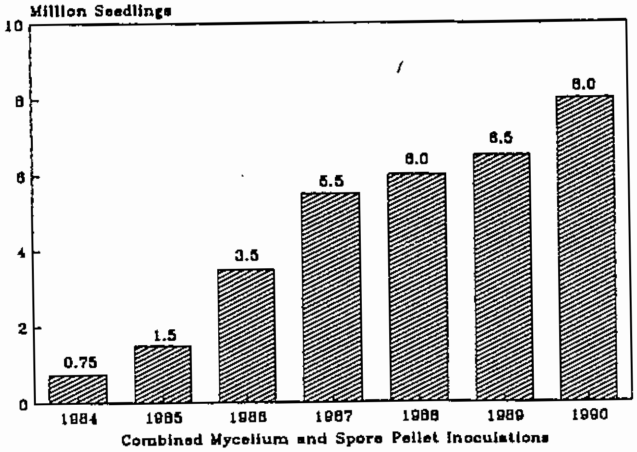
From 1981 to 1990, the 1,400 acres reclaimed through the Ohio reforestation program have cost approximately $420,000. In comparison, traditional reclamation of these same 1,400 acres would have cost approximately $9.8 million.

Operational Applications

The demand for Pt-inoculated seedlings continues to increase. In 1989, a total of 6.5 million seedlings were inoculated with Pt at 12 nurseries in the southern and central United States. Six conifer and 1 hardwood tree species were treated. In 1990, the totals rose to 7.0 million seedlings of 8 conifer and 1 hardwood tree species at 12 bare-root and container nurseries in the southern, central, and northeastern United States (see Table 5). One state nursery in South Carolina is presently producing 1 million longleaf and 1.5 million loblolly pine seedlings inoculated with Pt for reforestation plantings by the USDA Forest Service at the Savannah River Department of Energy (DOE) Site near Aiken, SC. Some 3,500 liters of Pt vegetative inoculum were applied in 35,000 linear feet (6.75 mi) of nursery seedbed in the largest single artificial ectomycorrhizal inoculation to date.

Interest in the use of Pt ectomycorrhizae in mineland reclamation has increased steadily during the past 10 years. Since its inception in 1981, the Ohio Abandoned Mineland Reforestation Program has planted approximately 2.3 million Pt-inoculated seedlings on 1,400 acres of abandoned stripmines in southern Ohio. This program continues to expand and, in 1990-91, the Ohio Division of Reclamation planted 0.3 million Pt-inoculated seedlings on 200 acres. Estimates for tree planting in Ohio through 1995 indicate that an additional 2.0 million Pt-inoculated seedlings will be required for plantings on 1,350 acres of abandoned mineland.

Table 5. Operational Pt custom seedling production using commercial vegetative and spore inculum in bare-root and container seedling nurseries, 1984-90.



Conclusions

Results obtained during the past 10 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 reforestation and mineland reclamation. Therefore, the planting of seedlings with root systems physiologically and ecologically adapted to the adversities of the planting site can be important in mineland reclamation programs. The quality of ectomycorrhizae for a planting site depends on a combination of the host tree and the fungus species. Optimum combinations such as Pt-Virginia pine, Pt-eastern white pine, and Pt-oak species are being produced for specific reforestation applications, such as mineland reclamation.

Several types of effective Pt inocula are commercially available, as is a machine for vegetative mycelium inoculations in bare-root nurseries. These recent developments provide nurserymen, foresters, and reclamation specialists with alternatives for using Pt and other ectomycorrhizal fungi.

The need for high-quality, custom-tailored tree seedlings for successful mineland reclamation by federal, state, and private agencies is becoming increasingly apparent. The greatest benefits from tree seedlings with Pt ectomycorrhizae have been obtained on adverse sites, such as acid coal spoils and borrow pits, that have been subsoiled to reduce soil compaction. Soil compaction, poor drainage, high pH, and competition from other vegetation are primary limiting factors for successful mineland reclamation with Pt seedlings.

Another important factor limiting the success of applications of Pt in nurseries and field plantings is the pH of soil and irrigation water. Although capable of tolerating extremely acidic (pH 3 or lower) conditions, this fungus cannot tolerate pH levels of 6.5 or higher. This means that Pt has limited value in the alkaline soils in the western United States. However, Pt technology can be utilized with other ectomycorrhizal fungi. Recent ectomycorrhizal research objectives include expansion of the Pt technology to other ectomycorrhizal fungi that are tolerant of the alkaline planting sites in the western United States.

The cost of Pt seedling inoculation represents only a minor portion of the total reforestation or reclamation expense (5%), and high seedling quality is an obvious key to successful reforestation and mineland reclamation. Thus, the benefits of producing custom-grown seedlings with selected ectomycorrhizal fungi for specific forestation and mineland reclamation sites are apparently much greater than the costs. Ectomycorrhizal tailoring of tree seedlings for specific applications is one of the most important recent discoveries for improving nursery seedling quality and subsequent tree survival and growth in mineland reclamation reforestation programs.

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