MYCORRHIZAL FUNGI AND TREES—A SUCCESSFUL REFORESTATION ALTERNATIVE FOR MINE LAND RECLAMATION

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Abstract

Successful consistent revegetation of drastically disturbed mine sites (i.e., acid coal spoils and mineral waste dumps) throughout the United States and several foreign countries has been achieved by using the biological "tools"—mycorrhizal fungus inoculated tree seedlings, native shrubs, and grass species. These trees and shrubs are custom-grown in bareroot and container nurseries with selected mycorrhizal fungi and grasses, and forbs are inoculated in the field at the time of planting. On disturbed sites, specific mycorrhizal fungi such as *Pisolithus tinctorius* (PT) or VAM provide significant benefits to the plant symbionts through increased water and nutrient absorption, decreased toxic materials absorption, and overall plant stress reduction.

One of the best examples of the practical application of this symbiotic mycorrhizal fungus-host tree technology is in Ohio. During the past 18 years, the Ohio Division of Mine Land Reclamation—Abandoned Mined Lands (AML) program has utilized the combination of the PT fungus and selected tree species in a successful reforestation project to significantly improve the effectiveness and reduce the cost of AML reclamation projects. Since 1982, over 5 million PT-inoculated pine and oak seedlings have been planted on over 3,000 acres of unreclaimed AML sites. Tree survival has averaged over 85 percent in the PT-inoculated tree plantings with less than 5 percent tree failures as compared with less than 50 percent survival and over 75 percent failures in previous plantings with the same noninoculated tree species. From 1982 to 1998, the Ohio AML reforestation project with PT-inoculated trees has cost approximately $970,000 or $300/acre. In 1998, approximately 340,000 PT-inoculated seedlings were planted on 242 acres at a cost of $195,000 or $805/acre. This represents an approximate 87 percent cost reduction as compared with conventional mineland reclamation methods ($6,000/acre). The PT seedling inoc-ulation cost is only $35/acre or about 4 to 12 percent of the reforestation cost which is relatively minute when compared to the consistent tree survival and early growth benefits obtained on these highly disturbed stressful sites. Interest in the application of this natural environmentally friendly technology to mineland reclamation programs throughout the United States and several foreign countries is expanding. This "total natural systems approach" to successful mineland revegetation is available through a team of scientific and business experts offered by Plant Health Care—Reclamation, Inc.

Introduction

Vast areas of the United States have been rendered nonproductive by over 175 years of intensive, uncontrolled surface and subsurface mining (5). Millions of acres of abandoned mined lands (AML) in the United States along with additional millions in several foreign countries (Asia, Europe, Africa, and South America) are in urgent need of reclamation. The 1990 Abandoned Mined Lands Inventory revealed that it would cost over $3 billion just to eliminate health and safety hazards on AML in the United States. An additional $57.8 billion would be required to reclaim environmental problem AML sites assigned third priority by the Surface Mining Control and Reclamation Act of 1977 (SMCRA). One northcentral state, Ohio, alone contains 200,000 acres of abandoned strip mines. Using conventional AML reclamation techniques (grading, resoiling, fertilizing, and revegetating), the cost of reclaiming these sites is estimated to be $1.5 billion. 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 (5). Mining for natural resources also generates a variety of waste materials that differ significantly in their biological, chemical, and physical characteristics. Factors such as soil pH, organic matter, composition, fertility, moisture, temperature, and microbial composition profoundly influence successful plant (trees, shrubs, and grasses) establishment and growth on these mineland sites (19).

Increasing public awareness and intensified environmental legislation have regulated the mining industry to assure effective reclamation on mined lands. Since the enactment of SMCRA in 1997 (Public Law 95–87), active strip mine reclamation has focused on intensive soil grading, replacing topsoil, and establishing a dense herbaceous cover to quickly control erosion, thus assuring prompt bond release. Although reforestation may not be the se-lected alternative in the reclamation of active mine land operations, it is receiving considerable interest as a viable reclamation "tool" in AML programs. Reforestation, of course, is neither new nor innovative; however, it deserves renewed interest and consideration for mine land reclamation applications. Tree planting on mined land is an excellent reclamation alternative that was deemphasized during the time period in which alternative objectives, regulations, and policies were developed following the enactment of environmental laws in the 1970's (3). 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 and other mine wastes can be capped and sealed away from the environment. However, even with the most intensive procedures to improve soil fertility and structure, efforts to restore previously existing vegetation have all too often failed. Establishment of trees has been especially difficult, often requiring repeated plantings to offset recurrent mortality (8,13).

Mycorrhizae and Mined Sites

The feeder roots of most plant species (trees, shrubs, forbs, flowers, and grasses) are infected by specialized fungi that form beneficial associations called mycorrhizae (fungus roots). The most widespread symbiotic (mutually beneficial) association on plant roots is mycorrhizae. These structures greatly increase root absorption efficiency and are vital to the survival and growth of both the host tree and the fungus. Compared to nonmycorrhizal roots, those roots colonized by mycorrhizal fungi have increased water and nutrient absorptive capacity, nutrient fixation, resistance to root pathogens, and longevity. As the main interface between seedling and soil, mycorrhizae are a key measure of root system quality.

Mycorrhizae are of two primary biological types: endomycorrhizae (which penetrate host cells) and ectomycorrhizae (which grow between the root cells and cover the root surface with a mantle of fungus hyphae).

Endomycorrhizae is the most widespread type and comprises three groups–ericaceous, orchidaceous, and vesicular-arbuscular (VAM) mycorrhizae. The endomycorrhizae also are predominantly found on hardwood tree species along with some conifers. VAM occur on more plant species than all other types of mycorrhizae combined. Over 90 percent of the 300,000 species of vascular plants in the world form VAM in natural soils.

Ectomycorrhizae occur on about ten percent of the world flora, and are predominantly found on conifer species along with some hardwoods (oak, birch, beech, chestnut, hickory, and eucalyptus). Numerous fungi form ectomycorrhizae. In North America alone, at least 2,100 species of fungi form ectomycorrhizae with forest trees. The vast majority of plants in natural environments have mycorrhizae; it is the rule in nature. Therefore, a primary prerequisite of successful mine land reclamation with trees, shrubs, forbs, flowers, and grasses is the most compatible combination involving the mycorrhizal fungi, plant host species, and the soil and environmental conditions on the mine site.

One ectomycorrhizal fungus, *Pisolithus tinctorius* (Pt) has been widely used to improve mine land reclamation tree planting success. Acid coal spoils, kaolin spoils, mineral mine wastes, borrow pits, and other severely disturbed sites have been successfully forested with tree seedlings having Pt ecomycorrhizae. Although the fungus does not directly affect the quality of the mined site, it modifies the tree root system so that it can tolerate adverse soil conditions, such as low pH (3.0), high temperatures, low fertility, mineral toxicity, and drought, that usually kill other ectomycorrhizal fungi along with their host trees (4, 5). Pt offers a practical, economical, effective alter-native to intensive mine land reclamation, particularly on AML projects.

There is a large body of published scientific research showing the practical significance of the Pt ectomycorrhizae and specific VAM fungi to revegetation of mined lands and other adverse sites in the United States and other parts of the world. Most of these field research and demonstrations were done on very acid coal mined lands in the eastern United States that also were droughty with high summer soil temperatures and contained high amounts of Al, S, Mn, and Fe (11). Other research and field demonstrations have been done on kaolin, phosphate, and mineral mines; impoverished eroded soils; and borrow pit sites (10). The results from all sites have been similar. After several years, seedlings with Pt ectomycorrhizae or with selected VAM had significantly greater survival and growth and contained less heavy metals in their foliage than seedlings with ectomycorrhizae or VAM formed by other species of naturally occurring mycorrhizae fungi, (7,12).

Nursery Inoculations and Seeding Production

During the past 20 years, operational programs have been developed for the practical and effective inoculation of bareroot and container nursery seedings with Pt inocula for mine land reclamation programs. Pt was selected for its demonstrated benefits to a variety of host trees and for its adaptability to adverse soil conditions, ease of manipulation, and wide geographic and tree host range. Many conifer and some hardwood tree species on a variety of nursery sites have been artificially inoculated with Pt inocula. Effective Pt vegetative inoculum has consistently improved the quality of bareroot and container nursery seedlings along with subsequent benefits to mined land reclamation and forestation (Figure 1) (15,17). Results obtained from thirty-four bare-root nursery inoculations conducted during the three-year period, 1978 to 1980, 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 seedling harvesting date (Figure 2). Seedlings are the most responsive when at least half or more of their ectomycorrhizae are Pt ectomycorrhizae (Pt index > 50).

Procedures for operational nursery use vary among the different commercial Pt inocula types. With any of the mycelium and spore inocula, the biological requirements of a second living organism are 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. 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. 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 (Figure 3). For container-grown seedlings, vegetative or spore inoculum can be incorporated into the growing medium before filling the containers. Spores also can be sprayed or drenched onto container media for containerized seedlings and onto seedbeds in bare-root nurseries following seed germination and seedling emergence.

In the VAM program, bare-root and container nursery inoculations are continuing, using a multiple-fungal species VAM “cocktail” inoculum on selected hardwood tree seedling, native shrub species, native flowers, and grasses for mine land and forestation applications. Results of VAM inoculations on several hardwood seedling species in several eastern U. S. bare-root nurseries, on selected native shrubs in a western U.S. container nursery, on native shrubs in a western U.S. container nursery, and on native grasses/flowers on a copper mine site in the western United States have all been positive. Research on the consistent positive effects of VAM on eastern hardwood tree species also has been published (9). A variety of ecto and endomycorrhizal fungus species and inocula types targeted for specific applications such as mine land reclamation are presently commercially available from PHC Reclamation, Inc.

Guidelines for MycorTree™ seedling production are designed to maintain healthy root systems with abundant specific mycorrhizae. Development and retention of lateral and feeder roots and mycorrhizae must be considered from seed sowing through seedling harvest and field planting. Nurserymen, field foresters, reclamation specialists, and tree planters must remain aware of the two biological components—the tree seedling and its complement of mycorrhizal fungi.

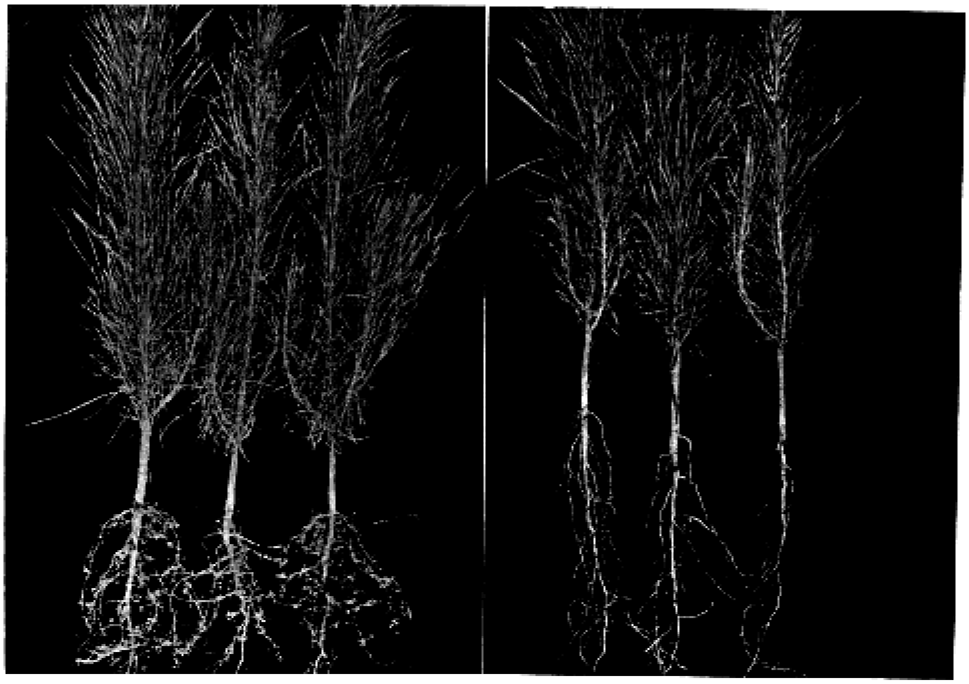

Figure 1. 1-0 loblolly pine seedlings with Pt ectomycorrhizae (left) and with only naturally occurring ectomycorrhizal fungi (right).

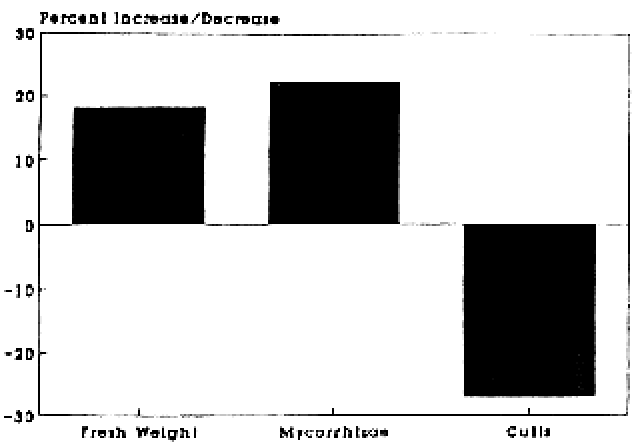

Figure 2. Effects of Pt inoculation with vegetative inoculum on seedling fresh weight, ectomycorrhizal development, and nursery cull reduction in 34 bareroot nursery studies.

Figure 3. Applicator for mycorrhizal fungus inoculations in bare-root nurseries.

Mycorrhizal fungi generally have similar moisture, fertility, and pH requirements as their host plants, but tolerance to extreme or adverse conditions varies among fungus species. Nursery soil and cultural factors that significantly affect mycorrhizal development include pH, drainage and moisture, fertility, fumigation, pesticides, cover crops, shading, seedling spacing and density, and root pruning. Soil and water pH values and water quality are three of the most limiting factors affecting the development of mycorrhizae in most bare-root and container nurseries. Soil fertility and irrigation should be based on the requirements of the seedling. Excessively high levels of phosphorus and nitrogen (particularly in VAM inoculations) along with excessive irrigation (particularly in containers and poorly drained seedbeds) should be avoided. Certain pesticides are particularly detrimental to specific ectomycorrhizae such as Pt, and they must not be used in conjunction with inoculations. Precautions also are warranted when using artificial shade on seedbeds or in greenhouses. Minimum threshold light quantities are required for adequate photosynthate production and subsequent mycorrhizal development. When day length is short, the amount of natural light in greenhouses is often inadequate and must be supplemented with artificial lighting. Proper seedling spacing and density along with custom-applied root pruning practices in bare-root nurseries contribute to high-quality seedlings with maximum lateral roots and mycorrhizae development. These traits are associated with desirable height and basal diameter characteristics (5).

Seedling harvesting, handling, storage, and planting practices also may have significant effects on the retention of lateral roots and mycorrhizae. Ectomycorrhizae are delicate structures and special care must be taken during all stages of seedling handling to retain the feeder root system and mycorrhizae. Feeder roots and their associated mycorrhizae can be ripped off and left behind in seedling beds during harvesting, desiccated in storage, or deliberately cut off prior to field planting. To maintain seedling quality, conventional nursery practices may require modification to minimize damage to feeder roots and mycorrhizae. Stripping of feeder roots has severe negative impacts on seedling field performance (16). During transfer of seedlings from the field to the packing room, and at all other times when seedlings are subject to direct environmental exposures such as wind and direct sunlight, special care is required to avoid drying of the feeder roots and mycorrhizae.

The seedling packing procedure and packing materials also may have significant effects on the ability of the seedling to endure storage, maintain root system quality, and survive field planting. Cold storage is vital to slow seedling respiration and maintain quality particularly for extended storage periods. Numerous studies have documented the effects of storage time on seedling quality. For most tree species and their associated mycorrhizae, proper storage for two to six weeks at 36EF is not detrimental (5).

Successful Mine land Reclamation

Seedlings with Pt ectomycorrhizae have been repeatedly used for successful reclamation of acid coal spoils, mineral mine wastes, kaolin wastes, borrow pits, and other disturbed sites throughout the United States and in several foreign countries. Positive field responses follow successful Pt nursery inoculations, use of suitable mine land reclamation techniques and procedures, and favorable mine land spoil and environmental factors.

Extensive reclamation research has been conducted on custom-grown seedlings with Pt ectomycorrhizae on disturbed and adverse sites of various types in the eastern United States. In 1966, Shramm made the initial observations and report of the widespread association of Pt with pines growing naturally on harsh mine land coal spoils in Pennsylvania (20). Reviews by Marx (11) and Cordell *et al*. (4) discussed improvements in survival and growth of seedlings with ectomycorrhizae on these sites. Maximum benefits were obtained on the coal spoils having higher temperatures, lower pH, and greater moisture stress.

Foliar analyses of seedlings with Pt ectomycorrhizae from mined sites show increases in macronutrients and reduced levels of potentially toxic microelements. Marx and Artman (13) found significantly more N and less S, Fe, Mn, and Al in seedlings with Pt than those with natural ectomycorrhizae on acid coal spoils in Kentucky. On mine spoils in Tennessee and Alabama, Berry (1) measured significantly lower levels of Mn in needles of Pt seedlings than in needles of control seedlings.

Consequently, these and other field planting results strongly suggest the ability of Pt and other selected species of ecto and endomycorrhizal fungi to mediate adverse factors on mine land sites. Research has shown that root systems with abundant ecto or endomycorrhizae also are apparently more effective and efficient in extracting water and essential nutrients from soil during periods of extreme water stress than root systems with either fewer or less adaptable mycorrhizae. In southern Ohio, virginia, eastern white, and loblolly x pitch pines and northern red oak (*Quercus rubra L.*) seedlings with Pt ectomycorrhizae have exhibited significant increases in tree survival and reforestation success when compared with standard nursery seedlings (Table 1) (17).

Table 1. Tree survival and reforestation success following use of Pt pine and hardwood seedlings in 356 reforestation plantings on abandoned mine lands in southern Ohio, 1982 to 1998.

Seedling Treatment	Average Survival %	Planting Success %
Pt-inoculated	85	95+
Noninoculated [1/]	<50	<25

[1/] Reforestation planting efforts using standard nursery seedlings prior to the Pt program

The great diversity of mine land and other severely disturbed sites to planted seedlings with specific mycorrhizae have revealed a number of factors that influence the success or failure of reclamation. Field results continue to demonstrate dramatic increases in survival and growth of MycorTree™ seedlings on sites with adverse pH (acid or alkaline), excessive levels of phytotoxic elements, prolonged moisture stress, high surface temperatures, and low nutrient availability. Exceptions may occur on severely compacted and/or high bulk density soils where increased MycorTree™ survival may be followed by a slowing of growth. Compacted and/or high bulk density soil conditions are most effectively mitigated by subsoiling or deep ripping prior to planting (2). Some ectomycorrhizal fungi, such as Pt, develop well and provide significant tree survival and growth benefits on porous, course, droughty sites but reduced benefits on poorly drained high bulk density soils.

Case Studies

Ohio Abandoned mine land Reforestation Program. One of the best examples of the practical application of this fungal technology is in Ohio. After reviewing the successful results of the field research and demonstration program, the Ohio Division of Mine Land Reclamation established the following criteria in 1982 for the use of tree seedlings with Pt ectomycorrhizae in their abandoned mined land reforestation program (3):

1. The strip-mined sites, gob piles, or industrial mineral sites must have been abandoned since 1972, with no present potential for full-scale reclamation.
2. The sites must presently be barren, eroded, and without adequate stabilizing vegetation.
3. The remaining potential of the site is nonexistent with no full-scale reclamation efforts (grading, topsoiling, fertilizing, and seeding) either proposed or likely to occur on the site.
4. The target sites also must have a history of off-site damage such as sedimentation that has resulted from the site's present condition.

During the past 18 years, the goals and priorities of the Ohio reforestation program have evolved into planting Pt pine and hardwood seedlings to provide a low-cost, low-maintenance, effective reclamation alternative for mined areas that contribute minor quantities of sediment to streams, degrade aesthetics, lack adequate ground cover, and are not eligible for traditional reclamation techniques under federal abandoned mine land guidelines (3). Reclamation plans also must be developed far enough in advance (one to two years) to allow adequate time for seedling production and for administrative planning and decision making.

Since its inception in 1982, the Ohio Abandoned Mine lands Reforestation Program has planted over 5 million PT-inoculated pine and oak seedlings on over 3,000 acres of unreclaimed AML sites (Figure 4). The typical site is barren, eroded with a mixture of bench slopes and out slopes of 2:1 or steeper terrain. Over 95% out of 350 plus sites have been hand-planted by local contractors. The sites also are highly acidic (pH 2.9-3.4), and no soil amendments (ie., lime, fertilizer, or water irrigation) are used. Tree survival has averaged over 85 % in the PT-inoculated tree plantings with less than 5% tree failures as compared with less than 50% survival and over 75% failures in previous plantings with the same noninoculated tree species (17). From 1982 to 1998, the Ohio AML reforestation project with PT-inoculated trees has cost approximately $900,000 or $300/acre. In 1998, approximately 340,000 PT-inoculated seedlings were planted on 242 acres at a cost of $195,000 or $805.00/acre. This represents an approximate 87% cost reduction as compared with conventional mine land reclamation methods ($6,000/acre). The Pt seedling inoculation cost is only $35.00/acre ($.02/seedling) or about 4% of the reforestation cost, which is relatively minute when compared to the consistent tree survival and early growth benefits obtained on these highly disturbed stressful sites.

Utah Copper Mine Site. This Utah copper mine has been active for over 100 years and has disrupted more than 20,000 acres of land. The disturbed areas have extensive erosion, sedimentation of drainages, dust hazards, and little or no satisfactory vegetation. The waste dump slopes are 1.5H:1.0V or steeper with highly acidic conditions. There also are numerous borrow areas with gravelly conditions and several large areas of mill tailings. There is little suitable topsoil readily available and subsoils range from poor to unsuitable quality. This high altitude mining site has low precipitation with freezing winter and hot summer temperatures.

Figure 4. Abandoned mine land (AML) Paxton Site – Perry Co., Ohio, Preplant- 1985 (top photo) and reforested with eastern white and virginia pines – 1991 (bottom photo). The spoil pH is 2.8 with available phosphorus at less than 5 ppm. Tree measurements in 1992 showed a survival of 98%, average basal diameter of 2 inches and average height of 9.5 feet.

The primary reclamation objectives on the mine waste dumps were to mitigate the production of acidic water, stabilize the dumps, mitigate soil erosion and dust, establish vegetation, and return the dumps to wildlife habitat use. The reclamation objectives for the borrow and mill tailings areas were to establish vegetation, eliminate dust hazards, mitigate soil erosion, and return the land to beneficial use (17).

The PHC-Reclamation, Inc. approach to revegetation of this mining area was to use the natural systems solution. It involved the selection of site-suitable plant species based on results from initial test plots. The best combination of site, plant species, and specific mycorrhizal fungi were identified and used in conjunction with other my-corrhizal fungi to provide suitable survival and growth benefits to tree and shrub seedlings and to grasses, forbs, and shrubs started from seed on site. Biosolids were used as a soil amendment to improve the initial adverse physical, chemical, and plant nutrient problems of some of the low quality soils (14).

Unique reclamation equipment for VAM fungal inoculation, seeding, and erosion mitigation also was developed. VAM fungal spores and beneficial bacteria in pelletized form were developed for easy and controlled field inoculation.

A container-grown tree and shrub seedling production program was established in a local tree nursery that included protocols for custom inoculation of trees and shrubs with specific ectomycorrhizal or VAM fungi and bacteria. The ectomycorrhizal fungi included Pt and a similar puffball-producing fungus, *Scleroderma cepa*, isolated from an eastern coal mined site. The VAM fungi included a species isolated from sagebrush growing on undisturbed native soil near the mine site and, also, a "cocktail" of several selected VAM fungal species isolated from other plant species in different physiographic locations.

The results have been very positive. The client's objectives have been met and compliance has been achieved with regulatory agencies. Several thousand custom seedlings have been grown in the nursery and planted on the re-claimed areas. Survival and growth rates of preinoculated trees and shrub seedlings and the grasses, flowers, and shrubs inoculated at seeding, are significantly better than the noninoculated plants.

By using the natural PHC Reclamation, Inc. biological approach to solve their revegetation problems, the client has experienced a reduction in costs ranging from 40 to 80 percent depending on the type of area being reclaimed. Savings in both short-term project reclamation work and long-term maintenance are included. Typically, re-clamation and revegetation establishment costs, minus recontouring, were reduced from a range of $8,000 to $13,000 per acre to less than $1,000 (without soil placement) to $5,000 per acre (with soil placement).

Conclusions

Consistent research and field demonstration results obtained during the past two decades clearly demonstrate the benefits of utilizing selected ecto and endomycorrhizal fungi for the custom production and/or field inoculation of MycorTree™ seedlings, native shrubs, forbs, flowers, and grasses for application in mine land reclamation pro-grams. Reforestation with selected MycorTree™ pine and hardwood seedlings is presently receiving widespread interest as a viable alternative in abandoned mine land reclamation programs in the eastern United States. Positive results have been obtained from the environmental extremes occurring in the moist East to the arid West of the United States. Major scientific breakthroughs in recent years have led to the commercial production of a variety of MycorTree™ products and their practical application in tree seedling nurseries, forestation, and mine land re-clamation sites. Advanced technology discoveries also have revealed the role of a "total intergrated package" in successful mine land reclamation programs. The package includes consideration of site factors such as pH, tox-icity, and compaction. Adopting remediation practices such as subsoiling and soil amendments, using of unique reclamation site seeding/inoculating equipment, and selecting the most compatible plant species and mycorrhizal fungi for the planting site are combined in a holistic approach to effective practical mine land reclamation.

This "natural systems environmentally friendly approach" to successful mine land revegetation is available through a team of scientific and business experts offered by PHC Reclamation, Inc. Reclamation costs vary considerably depending on the products and services requested by the client and the location and complexity of the mine site.

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