Mycorrhizal Fungal Management in Forest Regeneration

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Summary

This paper discusses the importance of mycorrhizae in the reforestation of sites of different quality in the world. Factors affecting mycorrhizal development in nurseries, types of fungal inocula, and the results of field application of the mycorrhizal fungal technology in tree establishment in the US, Mexico, Europe, Africa, Australia and Asia will be discussed. Research and operational programs have shown that the selection, manipulation and management of specific species of mycorrhizal fungi in seedling nurseries can dramatically improve reforestation efforts on a variety of reforestation and mineland reclamation sites throughout the world. Commercially available mycorrhizal fungal inoculants are available for applcation to seedling nurseries in various countries to improve survival and growth of seedlings in the forestation and reclamation programs.

Key words:
mycorrhizal fungi, forestry application, reclamation.

1. Introduction

The term mycorrhizae (fungus-root) is used to describe a mutually beneficial association between the fine nonwoody roots of plants and species of highly specialized, root-inhabiting fungi. The mycorrhizal fungi derive nearly all of their essential organic nutrition (carbohydrates, vitamins, amino acids) from their symbiotic niche in the primary tissues of nonwoody roots. No mycorrhizal fungus has been shown to complete its life cycle without the organic union with a plant host. The evidence suggests that the mycorrhizal habit evolved as a survival mechanism for

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both partners of the association allowing each to survive in the existing environments of low soil fertility, drought, pests, and temperature extremes (1). It has been suggested that the mycorrhizal symbiosis was a prerequisite for the successful colonization of the terrestrial environment by plants some 400 million years ago (2). Because of this co-evolutionary process, mycorrhizae are as common on the root systems of trees and other plants in natural environments as are chloroplasts in their leaves. Allen (3) has described clearly the immense variety of mycorrhizal types and many ecological roles they play in both natural and human-altered plant ecosystems. The two major types of mycorrhizae of importance to reforestation are ectomycorrhizae and the vesicular-arbuscular mycorrhizae (VAM) type of endomycorrhizae.

2. Ectomycorrhizae

Ectomycorrhizal association occurs on about 10% of the world flora. Trees belonging to the Pinaceae, Fagaceae, Betulaceae, Salicaceae, Juglandaceae, Myrtaceae, Ericaceae, and a few others form ectomycorrhizae. Some tree genera, such as *Alnus*, *Eucalyptus, Casuarina, Cupressus, Juniperus, Tilia, Ulmus*, and *Arbutus,* may form both ectomycorrhizae and endomycorrhizae (VAM), depending on soil conditions and tree age.

Numerous fungi have been identified as forming ectomycorrhizae. In North America alone it has been estimated that more than 2,100 species of fungi form ectomycorrhizae with forest trees. Worldwide, there are over 5,000 species of fungi that can form ectomycorrhizae on some 2,000 species of woody plants.

In ectomycorrhizae, intercellular hyphae of the fungi surround cortical cells forming the Hartig net and several hyphal layers cover the outside of the primary root forming the fungus mantle. Ectomycorrhizal fungal colonization normally changes the fine root morphology and color. They may be unforked, bifurcate, nodular, mulit-forked (coralloid), or in other shapes. Their color, which is usually determined by the color of the mycelium of the fungal symbiont, may be jet-black, red, yellow, brown, white, or blends of these colors. Ectomycorrhizal fungus colonization is limited to the primary cortex and does not spread beyond the endodermis or into meristem tissues of the nonwoody root. Many ectomycorrhizal fungi can be grown routinely in pure culture in the lab. An important practical aspect of ectomycorrhizal fungi is that they cannot grow saprophytically in soil. Spores or resistant hyphae may survive long periods in soil without a plant host but these fungi can not grow as saprophytes and complete their life cycles independent of the carbon furnished by their plant host.

Ectomycorrhizae not only aid the growth and development of trees, but they are indispensable for tree survival and growth under natural conditions. The obligate requirement of pine and oak for ectomycorrhizae in natural environment has been clearly shown by numerous workers in tree regeneration trials in former treeless areas and in countries without native ectomycorrhizal trees (4). Mycorrhizae appear to be the first line of biological defense against stress (drought, low fertility, etc.) for trees. Trees with abundant ectomycorrhizae have a much larger physiologically active area for mineral and water absorption than trees with few or no ectomycorrhizae. This increase in surface area comes from both the multi-branching habit of most ectomycorrhizae and from the extensive vegetative growth of hyphae from the ectomycorrhizae into the soil. These extramatrical hyphae function as a secondary root system for mineral and water-absorption and assure maximum water and mineral capture from the soil by the tree. Ectomycorrhizae are able to absorb and accumulate nitrogen, phosphorus, potassium, and calcium and other elements in the fungus mantles more rapidly, and for longer periods of time than nonmycorrhizal fine roots. Ectomycorrhizae provide trees access to both organic and mineral nitrogen. Most nitrogen from organic sources is first decomposed by other microbes before absorption by mycorrhizae. A few ectomycorrhizal fungi may absorb organic nitrogen directly from organic matter in the soil. This is critical in ecosystems where photosynthetic rate is restricted by low inorganic nitrogen availability (5). Ectomycorrhizae also appear to increase the tolerance of trees to drought, high soil temperatures, soil toxins (organic and inorganic), and extremes of soil acidity caused by high levels of sulfur or aluminum. Ectomycorrhizae deter infection of feeder roots by a variety of root pathogens (6,7).

Many species of fungi are normally involved in the ectomycorrhizal associations of a forest stand, a single tree species, an individual tree, or even a short segment of lateral root. As many as three species of fungi have been isolated from an individual ectomycorrhiza (8). Even as a single tree species can have numerous species of fungi capable of forming ectomycorrhizae on its roots at any given time, a single fungus can enter into ectomycorrhizal association with numerous tree species on the same site at the same time. Some fungi are apparently host-specific; others have broad host ranges and form ectomycorrhizae with members of numerous tree genera in diverse families (9).

3. Vesicular-arbuscular mycorrhizae (VAM)

Vesicular- arbuscular mycorrhizae (VAM) form the major group of endomycorrhizae. Vesicles and arbuscules are structures produced by the endomycorrhizal fungus in or on roots. VAM occur on more plant species than all other types of mycorrhizae combined and have been observed in roots of over 1,000 genera of plants representing some 200 families. It has been estimated that over 90% of the 300,000 species of vascular plants in the world form VAM. These include agricultural crops, turfgrass, fruit and nut trees, most hardwoods, vines, desert shrubs, flowers, and woody ornamentals. VAM fungi are ubiquitous in all natural soils except where they have been eliminated by prior land-use practices. Inoculum density and fungal species diversity, however, vary greatly in different soils supporting different plants with different land uses. There are about 150 species of VAM fungi identified to date. None can be grown in pure culture in the laboratory. As with ectomycorrhizal fungi, VAM fungi cannot grow saprophytically in soil and, therefore, can only grow vegetatively in soil from the VAM association with their plant hosts. The plant is the only source of carbon energy for the VAM fungi. They may, however, survive for decades in soil as dormant spores without plant associations. VAM roots are not changed in either color, shape or form as are ectomycorrhizae. Therefore, VAM can only be confirmed microscopically.

VAM increase a plants uptake of water and certain inorganic mineral elements, particularly P, Cu, and Zn. These elements are relatively immobile in soil, and after absorption by the plant, zones of element depletion normally develop near growing nonwoody roots. The extramatrical growth of hyphae from VAM fungi extends beyond the absorbing roots and, thereby, increases the volume of soil from which these mineral elements are absorbed. The additional minerals and water absorption capability due to VAM can result in several-fold growth increases in plants.

There are other significant benefits of VAM to plants. VAM are capable of increasing plant resistance to various fungal root pathogens and parasitic nematodes (10). Plants with VAM have enhanced water uptake, increased tolerance to heavy metals, saline soils and drought, decreased transplant shock, and will bind soil into semistable aggregates (11).

4. Factors affecting mycorrhizal development

Many factors affect both ectomycorrhizal and VA mycorrhizal development. It is necessary, however, to separate those that affect the tree from those that affect the fungal symbionts in soil. Generally, any soil or aboveground condition that influences root growth (i.e., carbon allocation) also influences mycorrhizal development. The first prerequisite to mycorrhizal development is that a susceptible nonwoody root must be preformed by the tree host. There must be a root before it can be colonized and developed into a mycorrhiza. Second, there must be viable inoculum of a mycorrhizal fungus present in the rhizosphere to colonize the root. Third, soil chemical, physical, and biological conditions must be favorable for successful root development and root colonization. The main factors influencing susceptibility of tree roots to mycorrhizal infection appear to be photosynthetic potential, soil fertility, and soil pH (12). High light intensity on leaves and low-to-moderate soil fertility enhance mycorrhizal development. Light intensity below 20% of full sunlight and excessively high soil fertility reduce root susceptibility and mycorrhizal development. However, it normally takes 10 to 50 times the nitrogen and phosphorus normally found in most forest soils to significantly suppress ectomycorrhizal development of forest trees (12,13). Mechanical defoliation reduces mycorrhizal development because of reduced carbon allocation to roots (14). Increased photosynthesis due to CO_2 enrichment of the atmosphere increases mycorrhizal development because of increased carbon allocation to roots (15). Light intensity and fertility appear to influence either the biochemical status of fine roots or the synthesis of new roots, both of which are products of carbon allocation (16). Roots growing rapidly because of high soil fertility contain few simple sugars and they are not highly susceptible to symbiotic fungal colonization (17,18). The supply of photosynthates from the canopy to the fungal symbiont in the roots is of paramount importance to the development, function, and maintenance of mycorrhizae. Photosynthesis not only furnishes the energy and carbon for fungal growth, but is intimately connected with the rate of mineral uptake by mycorrhizal roots (19).

The factors that affect the fungal symbionts directly are those which regulate survival of the fungi in the soil or their growth on roots to form new mycorrhizae. Extremes of soil temperatures, pH, moisture, certain pesticides, etc., and presence of antagonistic soil microorganisms can possibly affect the survival of symbionts and, thereby, influence the mycorrhizal fungus inoculum potential of the soil (20). Unfortunately, with the exception of research on a limited number of fungicides (21) and soil pH (12), many factors that could directly affect survival of inoculum of specific mycorrhizal fungi in soil have not been studied.

5. Practical considerations

There is considerable published research in the world literature proving the biological, physiological, and ecological significance of ectomycorrhizae and VAM to the survival, growth, development and health of many species of agricultural and horticultural plants and of forest trees (22).This information is not only academically interesting, but is critical to our understanding of plant growth and development and their ecology. In the past, the limiting factors in the practical management of mycorrhizae in tree seedling production have been the availability of affordable and good quality inocula of the ectomycorrhizal and VAM fungi and the development of simple and affordable methods to apply these inocula to the soil in bareroot or container seedling nurseries.

In the last decade, many of the problems associated with commercial inoculum production were eliminated allowing the production of quality products containing ectomycorrhizal and/or VAM fungal propagules to diverse plant markets. These fungal products are being commercially applied to plants in diverse green industry markets. The following is a brief review of the research done in support of the practical use of the mycorrhizal fungal technologies in reforestation and mined land reclamation programs around the world. Their applications in arboriculture, agriculture and horticulture were discussed elsewhere (23).

5.1. Forestry

5.1.1. Ectomycorrhizae

The biological requirement of many species of forest trees for ectomycorrhizal associations was initially observed in the early 1900's when attempts to establish plantations of exotic pines in the tropics routinely failed until the necessary symbiotic fungi were introduced. Most tropical areas do not have native ectomycorrhizal trees, therefore, there will be no native ectomycorrhizal fungi. The obligate need of pine and oak seedlings for ectomycorrhizae has also been convincingly demonstrated in the afforestation of former treeless areas, such as the grasslands of Russia and the great plains of the USA (24).

The primary purpose for inoculating with these specialized fungi in world forestry is to provide seedlings with appropriate ectomycorrhizae to improve their survival and growth after planting to create man-made forests. Such treatment has proven essential in forestation of cutover lands and other treeless areas and the introduction of exotic tree species, where native ectomycorrhizal fungi are deficient or reduced in species diversity (25).

Most research on inoculation with ectomycorrhizal fungi has been based on two working premises. First, any amount of ectomycorrhizae formed by any fungus on roots of tree seedlings are essential to seedling survival and growth. Success in alleviating ectomycorrhizal fungal deficiencies has contributed greatly to our understanding of the importance of ectomycorrhizae to trees, especially as they relate to the establishment of plantation forests. Secondly, some species of ectomycorrhizal fungi have proven to be more beneficial to trees, under certain environmental conditions, than others. These fungal species should be deliberately managed for these applications.

The cultural procedures used to produce seedlings in bare-root or container nurseries create environmental conditions that select naturally-occurring ectomycorrhizal fungi adapted to these conditions. These fungi produce mushrooms or puffballs in forests adjacent to nurseries that release many spores that are wind disseminated to the nursery soil. In most parts of the world, the fungus *Thelephora terrestris,* appears to naturally dominate the roots of most pine, oak, birch, beech and spruce seedlings from this natural source of spore inoculum in nursery soils (20) and in containers (26).

Most reports on inoculation techniques with ectomycorrhizal fungi developed in the US, Mexico, Australia, France, China, Africa, and the Philippines involve basidiomycetes (mushrooms and puffballs) on pines, oaks, and eucalypts (24). Techniques were developed mainly in response to the need to improve survival on regular reforestation sites or to grow tree species requiring ectomycorrhizae in tropical areas where ectomycorrhizal fungi were absent. Several types of natural and laboratory-produced inocula and several methods of application have been used over the years. Many of the techniques have proven successful while others have not.

Historically, the most widely used natural inoculum, especially in developing countries, is soil or humus collected from established pine plantations (4,24). In most instances, the original soil inoculum came from mature pine stands on other continents and, therefore, contained a preponderance of exotic fungi adapted to mature trees and not seedlings. The use of exotic forest soil inoculum has major disadvantages. Species composition of ectomycorrhizal fungi in the soil inoculum is usually not known, and the inoculum may also contain harmful microorganisms, including plant parasitic nematodes, and noxious weeds (27). The use of soil inoculum, however, is consistent with the premise that any ectomycorrhizae on seedlings are better than none.

Spores of various puffball-producing fungi have been used as inocula to form specific ectomycorrhizae on tree seedlings. Basidiospore inocula of *Pisolithus tinctorius* (Pt), *Rhizopogon vinicolor*, *R. colossus* and *Scleroderma* spp. have been used on an experimental scale, and, more recently, on an operational scale in the USA, Mexico, China and elsewhere. Spores in puffballs are plentiful and easy to extract, concentrate and store. Pt spores, and those of other fungi, are easily applied. They can be (1) mixed with a carrier before being added to soil, (2) suspended in water and drenched or irrigated into the soil, (3) dusted or sprayed onto soil and, (4) encapsulated or coated onto seeds (28-33). Fortunately, the spores of ectomycorrhizal fungi are small in diameter and can be washed into the root zone by rain or irrigation where they must come in contact with the roots before ectomycorrhizal development can take place. These fungi will not grow any appreciable distance to the roots.

An excellent research and development program to promote the use of ectomycorrhizal and VAM fungi for eucalyptus plantation forestry in China was developed by a group of Australian scientists. An excellent manual (34) has been published that describes in detail necessary methods needed to produce inocula and to form specific mycorrhizae on seedlings in different types of nurseries. Proper field-testing and measurements are emphasized. They reported improvements in survival and early growth of eucalyptus by inoculating the seedlings with both specific ectomycorrhizal and VAM fungi before outplanting.

Pure mycelial or vegetative inoculum of ectomycorrhizal fungi, free of contaminating microbes, is generally recommended as the most biologically sound material for inoculation. Several researchers in various parts of the world have developed procedures for culturing vegetative inocula of a variety of fungi for research purposes. Large-scale nursery applications of pure mycelial cultures have been severely hampered by the inability to produce large quantities of viable and economical inoculum (35,36).

Trotymow and van den Driessche (37) reviewed the published results of outplanting trials with specific ectomycorrhizae on conifer seedlings. Of the 84 reports examined, 49 dealt with Pt ectomycorrhizae. Castellano and Molina (38) also reviewed the world literature on outplanting performance. They found that 66 species of fungi were used experimentally to form ectomycorrhizae on 49 tree species. Over 40 percent were with Pt on 29 different tree species. Pt has a worldwide geographic distribution and is found in forests, pecan orchards, urban settings and on adverse sites, such as severely eroded soils and mined lands. It occurs in both cold and warm climates on a broad range of tree hosts (39). Research has shown that to obtain maximum survival and growth of southern pine seedlings by Pt ectomycorrhizae on normal reforestation sites, a threshold level of at least half of all ectomycorrhizae on seedlings at planting must be those formed by Pt (Pt index >50). On routine reforestation sites, pine seedlings with less than half of all ectomycorrhizae formed by Pt frequently may survive and grow at the same rate as seedlings with the same amount of naturally occurring *Thelephora* ectomycorrhizae (40).

Significant improvements in pine and oak seedling performance have been reported on over 50, physiographically diverse, reforestation sites in the USA (24,41). Pt ectomycorrhizae had only minimal positive effect on survival and growth of seedlings in some cases, but increased growth (plot volumes) by more than 250 percent in others. Where large differences were reported, the control seedlings with naturally-occurring, *Thelephora* ectomycorrhizae at planting survived and grew poorly. Where small differences were reported between treatments, the control seedlings survived and grew considerably better. These observations suggest that seedlings with abundant Pt ectomycorrhizae tolerated environmental stress factors, such as soil water deficits and high temperatures, better than control seedlings. Similar results have been obtained on other hot and dry sites such as in the tropics for establishment of exotic pine plantations. Significant positive field responses of pine to Pt ectomycorrhizae have been reported from the Congo, Nigeria, Brazil, and the Philippines (24).These areas have distinct wet and dry seasons, low soil fertility, and high evaporation rates. In a three-year study in Liberia (42), *Pinus caribaea* seedlings with Pt ectomycorrhizae grew as much during the 13.5 months of dry seasons as trees with other ectomycorrhizae grew during the 22.5 months of rainy seasons. This study demonstrated that Pt ectomycorrhizae could furnish improved drought tolerance to trees. This trait is likely mediated through the large, hyphal strand network of Pt, which exploits larger soil volumes than do fungi without this hyphal network. Similar results were obtained with *P. pseudostrobus* and Pt ectomycorrhizae after three years on an eroded site near Mexico City (43). Other studies on different sites with other tree species showed similar correlations between available soil water and Pt ectomycorrhizae (44-47). Besides Pt, other fungi have been tested for their ability to improve seedling survival and growth on diverse sites (41). LeTacon et al. (48,49) reported that ectomycorrhizae formed by *Laccaria bicolor* on containerized Douglas fir seedlings increased wood volume by 60 percent after eight years on a reforestation site in France compared to trees with only naturally occurring ectomycorrhizae. This fungus persisted on tree roots for the duration of the test, but did not replace any of the native ectomycorrhizal fungi indigenous on the site. It's introduction simply added to the fungal species diversity, as did Pt in all of the aforementioned studies.

Vegetative inocula of *Hebeloma crustuliniforme* and *Laccaria bicolor* are also being used successfully to improve seedling quality of true firs, Douglas-fir and various spruce species for establishment of Christmas tree plantations. Vegetative and spore inocula of Pt are also used to improve the quality of Virginia and eastern white pine seedlings for these special plantations. Nursery bed inoculation with these specific ectomycorrhizal fungi increases the percentage of superior grade seedlings in the nursery, their overall health and their survival and growth in the plantations (50).

It is obvious that in the past many methods were developed to ensure the formation of ectomycorrhizae on tree seedlings used to establish man-made forests. Certain methods have advantages over others. Pure vegetative and spore inocula have the greatest biological advantages. Specific ectomycorrhizal fungi are being actively used in practical reforestation and reclamation programs in the US (51) and in reforestation in China and Australia (34). Most programs involve Pt ectomycorrhizae to create tree plantations on clearcut lands and minelands. Currently about 650 million seedlings are being inoculated annually in the USA. Another 150 million seedlings are being produced in Mexico in Army nurseries with Pt spore inocula.

5.1.2. Vesicular-Arbuscular Mycorrhizae (VAM)

There has also been research published on VAM fungal inoculated hardwood and woody ornamental seedlings in both bareroot and container nurseries. Without exception, all of the researchers used research inoculum of VAM fungi and not commercial sources. A brief review of this research has been published (52,53). Basically, research has shown that many species of woody plants are highly responsive to nursery inoculation with VAM fungi before seeding of fumigated nursery soil. Kormanik (54) and Hay and Rennie (55) showed that sweetgum and yellow poplar seedlings exhibit improved vegetative growth and reproductive responses after field planting following VAM fungal inoculation in nurseries. We are aware of a few applications of commercial VAM fungal inoculum in several forestry and reclamation projects but, other than the information herein, none have been either published or otherwise made available for public use. Unlike ectomycorrhizal fungi, it is important to understand that very few natural soils anywhere in the world that have ever supported or are now supporting plants are deficient in VAM fungi. Intensively tilled soils, adverse mined-lands, fumigated soils and synthetic potting mixes can be expected to be deficient. Since the VAM fungi produce large spores in soil, their dissemination by wind is extremely limited. These large spores are not readily washed into the soil by rain or irrigation. Once deficient in a soil or on a site it may take them decades to reestablish themselves on the site.

5.2. Reclamation

There is a large body of published scientific research showing the practical significance of Pt ectomycorrhizae and VAM formed by specific fungi to revegetation of mined lands and other adverse sites in the eastern US and other parts of the world (51,56). Most of this field research was done on very acid coal mined lands that were also droughty with high summer soil temperatures and contained high amounts of Al, S, Mn, and Fe. Other research was done on kaolin and phosphate mines, impoverished eroded soils and on borrow pit sites. 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 mycorrhizal fungi (57,45,44).

One of the best examples of ecological adaptation by these fungi is Pt. It's fruit bodies and ectomycorrhizae have been observed to occur naturally on several species of trees growing on coal mined-lands and other acidic, adverse sites worldwide. It's presence on these trees has been credited with their survival and growth. Only a few other ectomycorrhizal fungi, such as *Scleroderma*, occasionally occur with Pt under trees on these highly stressed sites. These fungi originate on these sites from spores windblown from adjacent forests.

Unfortunately, the natural occurrence of Pt on these sites is erratic since the site must first support trees whose roots can be colonized from the windblown Pt spores. The survival and growth of the trees are improved only after Pt has colonized a significant quantity of roots. Another reason for its erratic occurrence is that fruit body and spore production in nearby forests varies by season and from year to year due to variable weather conditions.

The artificial inoculation of the tree seedlings in nurseries, either bareroot or container, with Pt spore or vegetative mycelia inocula resolves the problem resulting from the erratic occurrence of Pt on seedling roots. After nursery inoculation, all of the seedlings will have Pt ectomycorrhizae before outplanting. Seedlings with established Pt ectomycorrhizae benefit immediately after outplanting and survive and grow better on acidic and droughty coal mined sites and on droughty, borrow pits and eroded sites than routine nursery-run seedlings with ectomycorrhizae formed by *Thelephora* (51).

The following are two case studies showing the biological and economic significance of this mycorrhizal fungal technology to revegetation of mined land.

5.2.1. Case Studies

Ohio Abandoned Mineland Program. After reviewing the published results of field research, the Ohio Division of Mines and Reclamation established criteria in

1982 for the operational use of tree seedlings with Pt ectomycorrhizae in their coal mined land reforestation program. During the past 18 years, the goals and priorities of the reforestation program have evolved into planting tree seedlings with Pt ectomycorrhizae to provide a low-cost, low-maintenance reclamation method for abandoned minelands that contribute sediment to streams, degrade aesthetics, lack adequate ground cover for wildlife, and are not eligible for traditional reclamation techniques (major grading, resoiling and revegetating) under federal abandoned mineland guidelines.

On Ohio mineland sites, reclamation costs have been greatly reduced by utilizing seedlings with Pt ectomycorrhizae in their reforestation project. Cost to reclaim strip-mined lands in Ohio using conventional methods (major grading, topsoiling, soil amendments, pH adjustments, fertilization, and revegetation) can exceed several thousand US dollars per hectare. Since 1982, when they began using seedlings with Pt ectomycorrhizae, the cost of reforesting abandoned mineland has been reduced by 94% compared to costs of pre-Pt plantings. To date, 356 abandoned mined sites have been reforested with over 5 million Pt seedlings. The typical site is barren, eroded with a mixture of benches and slopes. The sites are highly acidic (pH 2.9 – 3.4) and are not amended, i.e. addition of lime, fertilizer, or water, before planting Pt seedlings. The additional cost of using seedlings with Pt ectomycorrhizae in these plantings on a 1.5 by 1.5 meter tree spacing (4,300 trees per hectare) is about 12 percent of the total tree establishment costs.

Utah Copper Mine Site. This mine has been active for over 100 years and has disrupted more than 9,000 hectares of land. The disturbed areas have extensive erosion, sedimentation of drainages, dust hazards and little or no satisfactory vegetation. The waste rock dump slopes are highly acidic (pH 2.5 to 2.9). There are numerous borrow areas with gravelly soil conditions and several large areas of sandy mill tailings. There is little suitable topsoil readily available and subsoils range from poor to unsuitable quality for capping the waste rock. The high altitude-mining site has low annual precipitation and freezing winter and hot summer temperatures.

The primary reclamation objectives on these three problem soil types were to mitigate the production of acidic water, stabilize the soils, mitigate erosion and dust, establish vegetation and return the area to productive wildlife habitat use. The results to date have been very positive. Several thousand custom-grown mycorrhizal seedlings have been produced in nurseries and planted to reclaim these areas. Survival and growth rates of preinoculated tree and shrub seedlings and the grasses, flowers and shrubs inoculated at the time of seeding in the field, are considerably better than the noninoculated plants. The approach taken to establish vegetation on this mining area was to use the natural systems protocol. It involved the selection of site-suitable plant species based on results from initial test plots. Site and plant species-specific mycorrhizal fungi were identified and used in conjunction with other mycorrhizal fungi to provide optimal survival and growth benefits to tree and shrub seedlings and to grasses, flowers, forbs and shrubs established following direct seeding of the site. Municipal biosolids (58) were used as a soil organic amendment to improve the initial adverse physical, toxic chemical and fertility problems of some of the low quality soils. Unique reclamation equipment for VAM fungal inoculation, seeding and erosion mitigation was also developed. VAM fungal spores and beneficial bacteria in pelletized form were developed for practical and effective 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 beneficial bacteria.

The ectomycorrhizal fungi included Pt and another puffball-producing fungus, *Scleroderma cepa,* isolated from a coal mine site in the eastern US. The VAM fungi included a *Glomus* 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 of the US. The cocktail of VAM fungi were more effective in revegetation of this mining material than was the single, native *Glomus.*

By using the natural systems approach to solve their vegetation establishment problems, the client has realized a reduction in costs ranging from 40 to 80 percent depending on the type of area and condition being reclaimed. Savings have occurred during the project reclamation work along with a significant reduction in long-term maintenance.

Much is known about the biological and ecological value of mycorrhizae and soil/root bacteria to survival, growth and development of plants on sites of different quality. Results from research and field demonstrations obtained during the last two decades clearly show the ecological and economical benefits of managing specific ectomycorrhizal and VAM fungi along with specific bacteria on tree seedlings, shrubs and grasses to improve mineland vegetation establishment.

Positive results have been obtained from the environmental extremes found in the moist East to the arid West of the US. Advanced technology has also revealed the importance of a "total integrated biological package" for a successful mineland revegetation program. The package includes evaluation and consideration of site factors such as pH, available heavy metals, and soil compaction, the use of soil remediation practices, such as soil ripping to remove hardpans and to incorporate soil and organic amendments, the use of unique reclamation equipment for inoculation of soil at the time of seeding, the selection of site-compatible plant species, and now, the management of specific mycorrhizal fungi and bacteria on the plants. These essential physical, chemical and biological considerations comprise a natural systems approach to successful vegetation establishment on minelands.

6. Conclusion

Over the past three decades a tremendous amount of research has been done on the practical use of mycorrhizal fungi demonstrating their effectiveness in improving survival, growth and health of many tree and other plant species on diverse manmade landscapes. Most of the research was done using inocula produced by the researcher in small quantities. In most studies, inoculum was used at excessively high and impractical rates in order to guarantee rapid root colonization by the introduced mycorrhizal fungus. It is reasonably easy to produce small quantities of good quality vegetative inocula of ectomycorrhizal fungi in pure culture, to harvest large quantities of their spores from puffballs collected from the field and to produce effective root:soil inocula of VAM fungi for research studies. However, that does not mean that same techniques can be used to produce large quantities necessary for commercial application. This issue has been discussed (21) and effectively demonstrated with Pt inocula (20,32,33) and by Gianinazzi et al., (59) and Marx et al. (23), for VAM fungal inocula.

Production of good quality inocula is only the first step in the practical use of these fungi. It must have an extended shelf-life to survive the period between production and field application and the storage conditions between these two periods. Simple, but effective, procedures must also be developed for the current application of the inoculants for each different plant market. One formulation of a fungal inoculant cannot be applied to all plant markets. A specific *delivery* system is required for each fungal product and plant situation. The basic procedures are very simple. The inoculants must be placed on the roots or applied to soil where the roots will soon be growing. Susceptible roots must be present before this inoculant can be infective, it must be applied at a rate sufficient to rapidly colonize a significant number of roots and to elicit a positive plant response.

Currently, we estimate that there are about 800 million inoculated tree seedlings produced this year in the world to create manmade forests. There is an obvious and immediate need for rapid expansion of plantation forests worldwide due to increased demand for wood products. Depletion of the natural forest resource and increasing pressure for natural forest conservation have reduced the areas of natural forest available for harvesting. Man-made plantations must make up for the short fall in future timber supply. Data collected nearly a decade ago showed that less than 3 percent of all forests in the world are plantation forests (60). When one considers the millions of hectares of plantations needed on treeless sites throughout the world, the importance of the management of superior species of mycorrhizal fungi as a forest regeneration tool is paramount.

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