

ESTABLISHMENT OF VEGETATION ON MINED SITES¹ BY MANAGEMENT OF MYCORRHIZAE

by

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Abstract: Plant ecosystems, including those in the tropical, temperate, boreal, and desert zones, began evolving more than 400 million years ago. Trees and other land plants in these environments were faced with many natural stresses including extreme temperature changes, fluctuating levels of available water, soil infertility, catastrophic fires and storms, poor soil physical conditions and competition. Basically, these plants evolved by genetic selection and developed many physical, chemical, and biological requirements necessary to survive these periodically stressed environments. Survivors were those that could form extensive lateral root systems to occupy soil volumes sufficiently large for them to obtain enough essential mineral elements and water to support their above and below ground growth needs. The most competitive plants in these stressed ecosystems were those with the largest root systems. One major biological requirement that evolved was the association of plants with mycorrhizal fungi. This is still true today for land that has been disturbed by mining, construction, and other activities. Successful vegetation establishment on these lands has been achieved by using the biological tools; native tree seedlings, shrubs, forbs, and grasses inoculated with specific, beneficial mycorrhizal fungi. Trees and shrubs are custom grown in nurseries with selected mycorrhizal fungi and forbs and grasses are inoculated in the field during seeding. On these disturbed sites, specific mycorrhizal fungi, such as *Pisolithus tinctorius* (Pt) and other fungi, provide significant benefits to the plants through increased water and mineral absorption, decreased toxin absorption and overall reduction of plant stress. This has resulted in significant increases in plant growth and survival rates, density and sustainable vegetation.

Additional Key Words: reclamation, mycorrhizae.

Introduction

Plant ecosystems, including those in the tropical, temperate, boreal, and desert zones, began evolving more than 300 million years ago.

Trees and other land plants in these environments were faced with many natural stresses including extreme temperature changes, fluctuating levels of available soil water, soil infertility, catastrophic fires and storms, poor soil physical conditions and competition. Basically, these plants evolved by genetic selection and developed many physical, chemical and biological soil requirements necessary to survive these periodically stressed environments. Survivors were those that could form tremendous expanses of lateral roots to occupy soil volumes sufficiently large for them to obtain enough essential mineral elements and water to support their above and below ground growth needs. The most competitive plants in these stressed ecosystems, then and now, were those with the largest expanses of root systems. Competition between plants, especially trees, occurs first between their root systems. The winners of this competition are those that are the first to occupy the largest soil volume with the most functional roots. The physiological function of roots is largely controlled by the presence of diverse soil and root microorganisms.

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Plant Root and Soil Microbiology

An essential attribute developed by forest trees, shrubs, grasses, and other plants was the establishment of partnerships with specific soil and root microorganisms associated with their absorbing roots. In order to survive, plants came to rely on the many benefits provided by a great diversity of these microorganisms, including mycorrhizae and bacteria, in their root zones. See references by Keister and Gregan, 1991, Waisel and others, 1996, and Vancara and Kunc, 1989.

Bacteria

These are single-cell microorganisms, one of the simplest and smallest forms of life known. They are dependent on external supplies of carbon energy. Most species live in soil and are important to soil productivity. Soil bacteria are found in greatest numbers in the upper 12" of soil where their food, i.e. carbon in organic matter and in organic nutrients released from nonwoody absorbing roots, is the most prevalent and where aeration, soil water, inorganic mineral elements, pH and temperature are adequate to satisfy their needs. Bacteria perform many important processes in soil that are essential to soil productivity. Tables 1 - 4 illustrate the biological activities of some of these bacteria.

Table 1

Effect of a Nitrogen-fixing Bacterium on Growth of 3-month-old *Quercus serrata* Seedlings.

Pandey, R.K. et al., 1986

| Treatment | Height (cm) | Shoot wt. (g) | Root wt. (g) |
|--------------------|-------------|---------------|--------------|
| Bacterium (drench) | 46 | 1.8 | 2.3 |
| Control (water) | 14 | 1.3 | 1.6 |

Table 2

Biological control of *Fusarium* root rot of *Pinus strobus* with ectomycorrhizal and bacterial biological control agents.

Ocamb, C.M. et al., 1996

| Treatment | Disease Rating* | % Ecto. Roots |
|---------------------------------|-----------------|---------------|
| <i>Hebeloma</i> ecto + bacteria | 0 | 65 |
| Natural ecto + bacteria | 2.0 | 3 |
| Natural ecto + no bacteria | 3.0 | 3 |

* Disease rating: 0 = no disease to 3.0 = 40 percent of roots rotted.

Table 3

Effects of mycorrhizal-helper bacteria (MHB)* on *Laccaria laccata* ectomycorrhizal development (percent) on seedlings of four tree species.

Garbaye, J. and Duponnois, R., 1992

| Treatment | No MHB | MHB | % Increase |
|---------------|--------|-----|------------|
| Douglas-fir | 61 | 86 | 41 |
| Scotch pine | 47 | 74 | 57 |
| Austrian pine | 41 | 82 | 100 |
| Norway spruce | 62 | 82 | 32 |

* Data is the average of 4 MHB species

Table 4

Response of subterranean clover after 12 weeks to soil inoculation with VAM and a plant-growth-promoting rhizobacterium (PGPR).

Meyer, J.R. and Linderman, R.G., 1986

| Treatment | Shoot wt. (mg) | Root wt. (mg) | No. nodules | VAM % |
|------------|----------------|---------------|-------------|-------|
| VAM | 300 | 230 | 49 | 50 |
| PGPR | 360 | 250 | 49 | 0 |
| VAM + PGPR | 460 | 280 | 66 | 44 |
| Control | 240 | 200 | 33 | 0 |

Some of these bacterial soil processes require free oxygen (aerobic), some require intermediate amounts of free oxygen (microaerophilic) and others require no free oxygen (anaerobic). Soils productive for land plants are aerobic but most also contain microsites which are anaerobic and/or microaerophilic.

Most species of soil bacteria are opportunistic and live freely in soil decomposing organic matter. Other species, called rhizobacteria, have adapted themselves to nonwoody absorbing roots where their food, i.e. organic chemicals and sloughed cells of the growing roots, is present. Rhizobacteria and free-living bacteria can increase mineral element (P, K, Ca, etc.) solubility from insoluble mineral sources, fix atmospheric nitrogen, reduce (by antagonism or competition) many root disease pathogens, and produce plant-growth regulators which contribute to improved root growth. More recently, certain bacteria have been found that increase mycorrhizal development (mycorrhizae helper bacteria) (Smith and Read, 1997). They occupy and function in the rhizosphere of mycorrhizae (mycorrhizosphere). Basically, all of the above bacteria, carrying out different soil and root processes, are collectively referred to as plant growth promoting bacteria (PGPB).

It is important to remember that in a productive soil supporting vigorous plant growth all of the above bacteria, as well as protozoa, fungi, algae, arthropods, worms and mycorrhizae (discussed below) are omnipresent and their activities are in large part responsible for the productivity of the soil and the health of the plants growing there (Keister and Gregan, 1991; Marschner, 1995; Waisel et al., 1996; Glinski and Lipiec, 1990; Vancara and Kunc, 1989).

All of these organisms, including mycorrhizae, working together carry out these various soil processes essential to long-term soil productivity and health of plant ecosystems. The recycling of the essential elements from internal, and from external atmospheric sources, by these organisms is one of the reasons why natural plant ecosystems do not require additional fertilizers to maintain normal growth, development, and function. The main factors that control these natural soil processes and root functions in plant ecosystems during the growing season are adequate soil aeration, a periodic supply of available soil water, stable soil pH, adequate quality and quantity of organic matter, soil temperatures above 50° F, diverse populations of these essential soil and root organisms, functional leaves to produce photosynthate and allocation of carbon energy to the root system.

Mycorrhizae

The term mycorrhiza (fungus-root) is used to describe a structure that results from a mutually beneficial association between the nonwoody roots of plants and species of highly specialized, root-inhabiting fungi. A recently published textbook (Smith and Read, 1997) discusses the plant host range, ecology, physiology and biological significance of mycorrhizal fungi to plant establishment, maintenance, overall health and ecosystem stability. Mycorrhizae are active, living components of the soil and have some properties like those of roots and some like those of microorganisms. The mycorrhizal fungi derive most if not all of their needed organic nutrition (carbohydrates, vitamins, and amino acids) from their symbiotic niche in the primary tissues of roots. Evidence suggests that the mycorrhizal habit evolved as a survival mechanism for both partners of the association, allowing each to survive in the existing environments of low soil fertility, drought, disease, and temperature extremes. Because of this coevolutionary process, mycorrhizae are as common on the root systems of trees and other plants as are

chloroplasts in their leaves. In examining plants in a natural environment, the question is not are the plants mycorrhizal, because most will be, but rather what type of mycorrhiza is present and what is the degree of mycorrhizal development on the roots.

Over 95 percent of land plants form symbiotic relationships with mycorrhizal fungi. (Smith and Read, 1997) Mycorrhizal fungi cannot grow freely in the soil and can only function and reproduce while in a symbiotic root association with their plant host. These unique, root-inhabiting, symbiotic fungi colonize either the outside of fine absorbing roots (ectomycorrhizae) or the inside of the roots (endomycorrhizae).

Mycorrhizal Associations

Ectomycorrhizae

These occur on about 10 percent of the world flora including the Pinaceae (pine, fir, larch, spruce, hemlock), Fagaceae (oak, chestnut, beech), Betulaceae (alder, birch), Salicaceae (poplar, willow), Juglandaceae (hickory, pecan), Myrtaceae (*Eucalyptus*), Ericaceae (*Arbutus*), and a few other trees (Smith and Read, 1997). Most of these tree species have a dependency on ectomycorrhizae for normal growth and development in natural soils; without them they do not survive. Ectomycorrhizae do not occur on nonwoody plants. In most forest situations, as many as 25 species of ectomycorrhizal fungi can exist on roots of a given mature tree. The degree of root colonization may exceed 80 percent of the absorbing roots on trees in high quality soils and can be as low as 10 percent on trees growing in degraded soils.

There are more than 5,000 species of fungi in the world that form ectomycorrhizae with forest trees. Most are ecologically adapted to good quality soils and a few species are adapted to soils of low quality. The majority of ectomycorrhizal fungi produce mushrooms or puffballs as their sexual reproductive stage. Many spores are disseminated by wind from these fruiting bodies, which spread the fungi to new locations. Most ectomycorrhizae have extensive branching habits and usually are large enough to be recognized with the unaided eye. They occur in different shapes, sizes and colors. Many species can be vegetatively propagated in the laboratory in pure culture on artificial media, which is important in their practical application.

Endomycorrhizae

These are the most widespread of all mycorrhizal types and there are three main groups. Ericaceous endomycorrhizae occur on four or five families in the Ericales and include *Rhododendron*, heath, heather, laurel, cranberry, blueberry, and a few others. Orchidaceous endomycorrhizae are a distinct type that occurs only in the plant family Orchidaceae. These two groups will not be discussed here. Vesicular-arbuscular mycorrhizae (VAM) are the third group of endomycorrhizae. Vesicles and/or arbuscules are structures produced by these fungi in the mycorrhizal roots.

VAM have been observed in roots of over 1,000 genera of plants representing some 200-plant families. It has been estimated that over 90 percent of the 300,000 species of vascular plants in the world form VAM (Smith and Read, 1997). These include most agricultural crops, grasses, fruit and nut trees, vines, many desert plants, flowers, ornamentals and most hardwood trees. Nearly all of these plants have a VAM dependency in that they do not grow normally and may die without them in natural soils. Plants that do not have a dependency are still stimulated in growth by VAM especially in stressed environments.

VAM fungi are ubiquitous in nearly all-natural soils that have supported plants. Inoculum density and fungal species diversity, however, vary greatly between soils supporting different species and numbers of plants. Degraded soils contain no or few spores of a limited number of adapted VAM fungal species, whereas, high quality forest soils, especially those supporting hardwood trees, and even desert soils supporting native plants, are rich in them. As with ectomycorrhizae, the degree of root colonization by VAM fungi on plants in a healthy soil can exceed 80 percent of the absorbing roots; in degraded soils the colonization can be as low as 10 percent (Smith and Read, 1997).

There are about 150 species of VAM fungi identified to date, worldwide (Smith and Read, 1997). As with the ectomycorrhizal fungi, a few VAM species are adapted to plants on stressed sites but most species are more effective on plants in good quality soils. VAM fungal colonization of nonwoody roots does not change their size, color or shape, as do ectomycorrhizal fungi. Due to this characteristic, VAM are not recognizable to the unaided eye and, therefore, can only be confirmed microscopically. VAM fungi produce large asexual spores in or on roots in the soil and are disseminated

from one location to another by soil inhabiting animals and insects which ingest the spores and pass them or passively carry the spores on their fur, legs, etc. Because of this characteristic, spread of VAM fungi from areas of their abundance to areas of their deficiency is very slow. Unlike most ectomycorrhizal fungi, the VAM fungi cannot be grown in conventional pure culture on artificial media in the laboratory. In order to produce spore inoculants of these fungi, spores are produced on VAM on specially grown plants, and then separated.

Benefits of Mycorrhizae

The host plants supply mycorrhizal fungi with simple carbohydrates (sugars), derived from photosynthesis, and other essential organic chemicals, such as vitamins and amino acids. A plants' carbohydrate cost to support these mycorrhizal associations can exceed 20 percent of the total amount of carbon fixed annually from photosynthesis by the host plant. In return, the fungi extend vegetative strands, called mycelia, far into the soil, increasing the surface area of the roots to improve absorption of water and essential mineral elements, which are shared with their plant host. Plants with abundant mycorrhizae have a much larger, physiologically active, surface area for absorption of the essential major and minor elements and water than plants with few or no mycorrhizae.

Ectomycorrhizae can increase the absorptive surface area of root systems by more than 700 percent when compared to nonmycorrhizal roots (Smith and Read, 1997). From an energy perspective, a tree would use approximately 100 times more photosynthate (sugars) to form enough nonmycorrhizal absorbing roots with the same absorptive surface area equal to that provided them by their mycorrhizal associations. Most plants, especially trees, are simply not able to produce 100 times more photosynthate, thus, they developed a mutual dependency with mycorrhizal fungi.

Mycorrhizae are able to absorb, accumulate and transfer essential mineral elements and water to trees more rapidly and for longer periods of time than nonmycorrhizal absorbing roots. Unlike nonmycorrhizal roots, mycorrhizae can perform these absorptive functions under extreme soil conditions. Research has shown that mycorrhizae live longer in the soil than nonmycorrhizal absorbing roots (Smith and Read, 1997). Mycorrhizae formed by specific fungi can increase the tolerance of their plant host to drought, compaction, high soil temperatures, heavy metals, soil salinity, organic and inorganic soil toxins,

and extremes of soil pH. Mycorrhizae also depress many root diseases caused by pathogenic fungi, bacteria, and nematodes.

In natural forests, prairies and deserts, many species of mycorrhizal fungi share common plant hosts and form a continuous, interconnecting network of mycelia between plants. The soil is a continuous rhizosphere (and mycorrhizosphere) in closed canopy forests and in other plant ecosystems. Dominant trees in full sunlight will actually transfer photosynthate from the canopy through the network of mycorrhizal fungal mycelia to mycorrhizae on roots of adjacent understory trees that are shaded and thus, produce less photosynthate. The dominant trees can function as nurse trees by supplying carbohydrates to the understory trees and improving their growth and competitive abilities. These trees may or may not even be the same species. More information on the biology of mycorrhizae and their functions can be found in Smith and Read (1997).

Mycorrhizae and Mined Sites

As discussed above, on vegetated low quality stressed sites there are fewer species of mycorrhizal fungi on roots than on plants growing on better quality sites. Obviously, these fungi have adapted to the stressed conditions on these sites.

There is a large body of published scientific research showing the practical significance of the *Pisolithus tinctorius* (Pt) ectomycorrhizae and specific VAM fungi to revegetation of mined lands and other adverse sites in the eastern US and other parts of the world (Smith and Read, 1997). 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. See references by Marx, 1997 and Cordell and others, 1995 and Tables 5 - 8 for specifics on the research.

Table 5

Response of 3-year-old live oak to *Pisolithus tinctorius* (Pt) or natural ectomycorrhizae on a pH7.2 lignite overburden site in Texas.

Davies, F.T and Call, C.A., 1990

| Treatment | Survival % | Height (cm) | Plot Volume (cm ³) |
|-----------|------------|-------------|--------------------------------|
| Pt | 96 | 56 | 263 x 10 ² |
| Natural | 93 | 35 | 59 x 10 ² |

Table 6

Response of a 3 year old loblolly pine to *Pisolithus tinctorius* (Pt) or natural ectomycorrhizae on a severely eroded, air pollution damaged site in Copper Basin, TN

Hatchell, G.E. et al., 1985

| Treatment | % Survival | Height (cm) | Plot Vol. (cm ³) |
|----------------------|------------|-------------|------------------------------|
| Pt | 92 | 67 | 541 |
| Natural | 68 | 51 | 214 |
| Pt + fertilizer | 90 | 92 | 1,240 |
| Natural + fertilizer | 74 | 74 | 612 |

Table 7

Response of one-year-old forage species (from seed) to VAM on an acid coal mine spoil.

Lambert, D.H. and Cole, H. Jr., 1980

| Treatment | % Survival | Foliar wt. (g) |
|-------------------|------------|----------------|
| Birdsfoot trefoil | | |
| VAM | 54 | 34 |
| No VAM | 9 | 3 |
| Crownvetch | | |
| VAM | 54 | 41 |
| No VAM | 7 | 2 |
| Flatpea | | |
| VAM | 44 | 15 |
| No VAM | 3 | 2 |

Table 8

Responses of 5-month-old sweetgum with a VAM fungal "cocktail" in nonfertilized 1:1 phosphate overburden-sand tailings mix.

Sylvia, D.M., 1988

| Treatment | Leaf Area (cm ²) | Root Length (cm) |
|--------------|------------------------------|------------------|
| VAM cocktail | 114 | 960 |
| No VAM | 4 | 128 |

One of the best examples of ecological adaptation by these fungi is the puffball-producing ectomycorrhizal fungus, Pt. Its fruit bodies and ectomycorrhizae have been observed to occur naturally on trees of several species growing on coal mined-lands and other adverse sites worldwide and has been credited with their survival and growth. Only a few other ectomycorrhizal fungal species occasionally occur with Pt on the trees on these sites.

Ectomycorrhizal fungi can not reproduce (i.e. fruiting bodies) unless they are in mycorrhizal association with a tree host from which the fungi get their carbon energy. The occurrence of Pt on the roots of trees originally came from wind-blown spores released from its' fruiting bodies produced in nearby 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 Pt spores. The survival and growth of the trees will be improved only after Pt has colonized a significant quantity of roots. Another reason for its' erratic occurrence is that fruit body 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 spores or mycelia solves the problem resulting from the erratic occurrence of Pt before they are planted on the mined lands. Seedlings with established Pt ectomycorrhizae benefit immediately after outplanting from this unique ectomycorrhizal association and survive and grow better on these difficult stress sites than routine nursery-run seedlings with different native ectomycorrhizae. See references by Cordell, Omdal and Marx, 1991, and Marx, 1996 for techniques and results of nursery inoculations.

After more than two decades of research on field testing, and after the development of industrial methods to mass produce inoculants of Pt and other fungi, several significant operational programs and large scale field demonstrations were initiated in various areas of the US using this technology. The following are two case studies showing the biological and economic significance of this mycorrhizal technology to revegetation of mined land.

Case Studies

Ohio Abandoned Mineland 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 program, the Ohio Division of Mineland Reclamation established criteria in 1982 for the use of tree seedlings with Pt ectomycorrhizae in their coal mined land reforestation program. The program addresses three conditions; (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, and (3) target sites also may have off-site damage, such as sedimentation. 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 minelands that contribute 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. Tables 9 and 10 show field results from 1981 to 1992.

Table 9

Response of 6-year-old Virginia pines to *Pisolithus tinctorius* (Pt) or natural ectomycorrhizae on a pH 2.8, coal mined site in Ohio.

| Caldwell, C. <i>et al.</i> , 1992 | | | |
|-----------------------------------|------------|-------------|--------------------------------|
| Treatment | Survival % | Height (cm) | Plot Volume (cm ³) |
| Pt | 98 | 298 | 74 x 10 ⁴ |
| Natural | 45 | 160 | 3 x 10 ⁴ |

Table 10

Response of pine and oak (1982-1991) to *Pisolithus tinctorius* (Pt) ectomycorrhizae on 137 coal mined sites (averaged) in Ohio.

| Cordell, C.E. and Marx, D.H., 1991 | | |
|------------------------------------|------------|-------------|
| Treatment | % Survival | % Replanted |
| Pt | 85 | <5 |
| Before Pt | <50 | >75 |

On Ohio mineland sites, reclamation costs have been greatly reduced by utilizing seedlings with Pt ectomycorrhizae in their reforestation project. Estimates indicated that it would cost approximately \$7,000 per acre to reclaim strip-mined lands in Ohio using conventional methods (major grading, topsoiling, soil amendments, pH adjustments,

fertilization, and revegetation). In 1980, the cost of reclaiming all of Ohio's abandoned underground and surface mines was estimated to be \$3.5 billion. Since 1981, when they began using seedlings with *Pt* ectomycorrhizae, the cost of reforesting abandoned mineland has averaged only \$300 per acre. This includes the cost of *Pt* inoculum, seedlings, and planting. The *Pt* seedling cost is \$35 per acre. More than 95 percent of the 200 plus sites have been hand planted by local contractors. The typical site is barren, eroded with a mixture of benches and slopes of 2.0H : 1.0V or steeper. 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 (\$20 per 1000 nursery seedlings) of using seedlings with *Pt* ectomycorrhizae in these plantings on a 5 foot by 5 foot tree spacing (1,742 trees per acre) is about 12 percent of the total tree establishment costs of \$300 per acre.

Since its inception in 1981, the Ohio Abandoned Mineland Reforestation Program has planted approximately 5 million seedlings with *Pt* ectomycorrhizae on 3,000 acres of abandoned strip-mines in southern Ohio at a cost of \$900,000. In comparison, traditional reclamation of these same 3,000 acres would have cost \$21 million. The main tree species involved are Virginia, eastern white, and pitch X loblolly (hybrid) pines and Northern red and black oaks. This program continues to expand. About 200 acres are now reclaimed successfully each year with this fungal technology.

Utah Copper Mine Site. This 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 rock dump slopes are 1.5H : 1.0V or steeper with highly acidic conditions. There 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. The high altitude-mining site has low precipitation and freezing winter and hot summer temperatures.

The primary reclamation objectives on the mine waste dumps were to mitigate the production of acidic water, stabilize the dumps, mitigate 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 erosion and to return the land to beneficial use. The

results have been very positive. Tables 11 - 15 show results of this program.

Table 11

Response of 3-year-old pine and oak to *Pisolithus tinctorius* (Pt) or natural (NI) ectomycorrhizae on a copper-mined site in Utah. pH 7.7
Marx, Cordell and Marrs (unpubl.), 1997

| Treatment | Height (in) | Caliper (in) | Root Depth (in) | Root Spread (in) |
|-------------------|-------------|--------------|-----------------|------------------|
| Ponderosa p. + Pt | 12 | 0.63 | 18 | 30 |
| Ponderosa p. +NI | 5 | 0.38 | 10 | 10 |
| Gambel oak + Pt | 20 | 0.56 | 24 | 36 |
| Gambel oak +NI | 6 | 0.25 | 16 | 6 |

Table 12

Root response of pine with specific or natural ectomycorrhizae from nursery after two-years on 18-inch soil and biosolids capped copper-mined waste rock in Utah.
Marx, Cordell and Marrs (unpubl.), 1997

| Treatment | Root Depth (in) | Root Spread (in) | % Ecto. Roots |
|--------------------|-----------------|------------------|---------------|
| Ponderosa Pine | | | |
| <i>Pisolithus</i> | 14 | 16 | 40 |
| <i>Scleroderma</i> | 10 | 10 | 35 |
| Natural | 8 | 8 | 20 |
| Austrian Pine | | | |
| <i>Pisolithus</i> | 12 | 16 | 35 |
| <i>Scleroderma</i> | 12 | 8 | 40 |
| Natural | 6 | 6 | 20 |

Table 13

Root response of pine with specific or natural ectomycorrhizae from nursery after two-years on 18-inch soil and biosolids capped copper-mined waste rock in Utah.
Marx, Cordell and Marrs (unpubl.), 1997

| Treatment | Root Depth (in) | Root Spread (in) | % Ecto. Roots |
|--------------------|-----------------|------------------|---------------|
| Lodgepole pine | | | |
| <i>Pisolithus</i> | 8 | 10 | 45 |
| <i>Scleroderma</i> | 14 | 12 | 75 |
| Natural | ----- | Dead | ----- |
| Jeffrey pine | | | |
| <i>Pisolithus</i> | 10 | 8 | 30 |
| <i>Scleroderma</i> | 12 | 10 | 35 |
| Natural | 4 | 4 | 20 |

Table 14

Root response of native shrubs to VAM after two-years on 5-inch soil and biosolids mix over copper-mined waste rock (pH 2.7) in Utah.

Marx, Cordell and Marrs (unpubl.), 1997

| Treatment | Root Depth (in) | Root Spread (in) | % VAM |
|------------|-----------------|------------------|-------|
| Sagebrush | | | |
| VAM | 10 | 16 | 11 |
| No VAM | 6 | 12 | 0 |
| Sumac | | | |
| VAM | 16 | 36 | 33 |
| No VAM | 12 | 24 | 0 |
| Indigobush | | | |
| VAM | 16 | 24 | 58 |
| No VAM | 10 | 18 | 0 |

The clients' 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 reclaimed areas. Survival and growth rates of preinoculated trees and shrub seedlings and the grasses, flowers and shrubs inoculated at seeding, are significantly higher than the noninoculated plants.

The approach taken to establish vegetation on 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. 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, 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. The reference by Marx, Berry and Kormanik, 1995 provides more information on biosolids.

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 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 both Pt and a similar puffball-producing fungus, *Scleroderma cepa*, which was isolated from an eastern coal mine 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.

By using the natural systems solution to solve their vegetation establishment problems, the client has experienced a reduction in costs ranging from 40 to 80 percent depending on the type of area and condition being reclaimed. Savings have occurred both during the project reclamation work and from the significant reduction in long-term maintenance. Typically reclamation and vegetation establishment costs, not including recontouring, were reduced from a range of \$8,000 to \$13,000 per acre (with soil placement) to less than \$1,000 per acre (without soil placement) to \$5,000 per acre (with soil placement).

Conclusion

Much is known about the biological and ecological value of mycorrhizae and soil/root bacteria to growth and development of plants on sites of different quality. Consistent research and field demonstration results 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 package" for a successful mineland revegetation program. The package includes evaluation of site factors such as pH, available heavy metals, and soil compaction, the use of soil remediation practices such as soil ripping and soil and organic amendments, the use of unique reclamation seedling/inoculating equipment, the selection of site-compatible plant species, and now, the management of specific mycorrhizal fungi and bacteria on the plants. These physical, chemical and biological considerations that must be given to the mineland soils and plants comprise a natural systems approach to successful vegetation establishment.

Literature Cited

- Cordell, C. E., Omdal, D. W. and D. H. Marx. 1991. Identification, management and application of ectomycorrhizal fungi in forest tree nurseries. p. 143-155. In: Diseases and Insects in Forest Nurseries, IUFRO Working Party S2.07-09, Victoria, BC, CANADA (Aug. 1990).
- Cordell, C. E., D. H. Marx, B. Jenkins, C. Caldwell and M. E. Farley. 1995. Mycorrhizal fungi and trees. A practical economical alternative for mineland reclamation. pp. 1-13, 17th Ann. Conf., Nat. Assoc. Abandoned Mineland Program, French Lick, IN (Oct. 1995).
- Glinski, J. and Lipiec, J. (Editors). 1990. Soil physical condition and plant roots. CRC Press, Inc., Boca Raton, FL, 250 pp.
- Keister, D. L. and Gregan, P. B. (Editors). 1991. The rhizosphere and plant growth. Proceedings, Symposium May, 1989. Beltsville Ag. Research Center (BARC), Beltsville, MD. 386 pp.
- Marschner, H. 1995. Mineral nutrition of higher plants (2nd Edition). Academic Press, Harcourt Brace and Co., New York City, NY. 889 pp.
- Marx, D. H., C. R. Berry and P. P. Kormanik. 1995. Application of municipal sewage sludge to forest and degraded land. p. 275-295. in Agricultural Utilization of Urban and Industrial By-Products. American Soc. of Agronomy, Crop Sci. Soc. of America, Soil Sci. Soc. of America, ASA Special Publication No. 58.
- Marx, D. H. 1996. Significance of mycorrhizal management in the production of trees and shrubs. Proceedings International Plant Propagators Soc. 46:538-542.
- Marx, D. H. 1997. Management of mycorrhizae for woody plant production and maintenance. American Nurseryman 185:40-47.
- Smith, S. E. and D. J. Read. 1997. Mycorrhizal Symbiosis. Academic Press, NY, NY. 605 pp.
- Vancara, V. and Kunc, F. (Editors). 1989. Interrelationships between microorganisms and plants in soil. Proc. International Symposium, Czechoslovakia, June, 1987. Elsevier Publishers, New York City, NY. 492 pp.
- Waisel, Y., Eshel, A. and Katkafi, U. (Editors). 1996. Plant roots the hidden half (2nd Edition). Marcel Dekker, Inc., Madison Ave., New York City, NY 1002 pp.