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Soil Factors Affecting Mycorrhizal Use in Surface Mine Reclamation

By Michael R. Norland

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UNIT OF MEASURE ABBREVIATIONS USED IN THIS REPORT

°C	degree Celsius	mm	millimeter
cm	centimeter	MPa	megapascal
g	gram	$\mu g\ g^{-1}$	microgram per gram
M	molar,	μm	micrometer
m	meter	μM	micromolar
$mg\ kg^{-1}$	milligram per kilogram	pct	percent
$mg\ L^{-1}$	milligram per liter	$st\ ha^{-1}$	short ton per hectare

SOIL FACTORS AFFECTING MYCORRHIZAL USE IN SURFACE MINE RECLAMATION

By Michael R. Norland[1]

ABSTRACT

Surface and subsurface stabilization of mining-related wastes through revegetation depends upon the physical, chemical, and biological condition of the waste following mining. Mining disturbances can significantly alter the soil physical, chemical, and biological characteristics of a site, reducing or eliminating mycorrhizal fungi from the soil. Mycorrhizae are economically and ecologically important because they can alleviate environmental stresses caused by lack of proper soil condition and because they are vital to stabilization of mining waste by increasing plant survival and biomass through increased nutrient and water uptake. This report discusses some of the ecological factors that may affect mycorrhizae-plant associations on mining-related wastes and provides general information on mycorrhizae inoculation technology.

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INTRODUCTION

Mining wastes vary widely in their physical, chemical, and biological characteristics, which affect plant establishment, survival, and growth. Site factors that affect plant establishment include soil moisture, temperature, fertility, and microbial activity. Stabilization of mining wastes through revegetation usually requires the use of soil amendments to ameliorate the physical and chemical properties of the waste and to provide a source of energy for the reestablishment of a microbial community. These conditions are necessary to establish a vegetative cover on and to stabilize the mining waste. The primary objectives of mine land reclamation are to minimize environmental degradation, to facilitate the reestablishment of a functional plant-soil system, and to develop an aesthetically pleasing landscape (*113*).[2] Tate (*113*) states that long-term plant community stability relies upon the development of a functional soil microbial community. The microbial community of a given site is composed of numerous species and genotypes that differ in their environmental tolerances, physical requirements, and habitat preferences (*87*). The rehabilitation of a disturbed site requires that a tolerant microbial community be reestablished.

Soil microorganisms are responsible for the decomposition of plant litter, the mineralization of essential plant nutrients, nutrient cycling, the accumulation of organic matter, and beneficial changes in soil physical characteristics such as the formation and maintenance of stable soil structure. One group of soil microorganisms important to the development of long-term plant community structure is mycorrhizal fungi. "Mycorrhizae" literally means "fungus-root" and is used to describe the symbiotic association between roots and fungi. These common soil fungi form symbiotic associations with almost all higher plants and usually improve plant survival and growth, especially in nutrient-poor soils. It is a symbiotic association because the plant acquires nutrients through the fungus (mycotrophy); the fungus cannot complete its life cycle independently of the host plant, being a physiological obligate symbiont (fungal dependency); and the plant is mycorrhizal dependent, as expressed by its morphological and physiological characteristics (*10*).

Mycorrhizae have been used as an amendment to alleviate stresses encountered by plants established on mine wastes. In highly disturbed areas, such as mine waste sites, the absence of mycorrhizal fungi may account for the poor survival of plants used for stabilization.

Plants allocate a high proportion of photosynthate to roots, and a large amount of that photosynthate is diverted to mycorrhizal symbiots or exuded into the surrounding rhizosphere (*86*). The beneficial aspects that mycorrhizae impart to higher plants include the regulation of nutrient and water uptake and the alteration of nutrient availability and the competitive interactions between plants. Levels of infection are usually less on disturbed sites than on sites with native vegetation (*5*, *93*), but many plant species colonizing coal spoils contain mycorrhizae (*30*). Application of cultured[3] or on-site (*7*) field mycorrhizae inoculum has frequently improved plant growth. Improvements in plant growth and yield by inoculation with efficient mycorrhizae strains can be expected in soils that have a low number of indigenous mycorrhizae or contain less effective mycorrhizae than those selected (*36*).

Five types of mycorrhizae are recognized; however, only two types of mycorrhizae are used in mine waste stabilization: ectomycorrhizae and vesicular-arbuscular mycorrhizae (VAM). The other types of mycorrhizae are "ericoid," restricted to some species in the Ericaceae family; "orchid," restricted to some species in the Orchidaceae family; and ectendomycorrhizae, formed by species in families other than Ericaceae, but in the Ericales.

Ectomycorrhizae are characterized by the formation of a sheath or mantle, which surrounds the roots (fig. 1) (*64*). Inside the sheath is the Hartig net, a network of hyphae extending into the root. The fungal hyphae penetrate and occur in the intercellular spaces of the root cortical cells, but do not enter the cells. Outside the sheath is a network of external hyphae that extend into the soil. Ectomycorrhizae associations are generally formed only on primary tissues; roots with secondary thickening remain uninfected. The presence or absence of the Hartig net remains the most reliable index of ectomycorrhizal development and an indication of ectomycorrhizae presence (*124*).

VAM do not form a fungal sheath. There is little change in the gross morphology of infected roots, and colonization is not recognizable without staining. Colonization originates from hyphae arising from soil-borne propagules (spores or mycorrhizal root fragments) or from a VAM plant growing nearby (*10*). The fungus may gain entry either through a root hair or by penetrating the epidermis of the root. Jeanmougin suggests (*56*) that when hyphae of VAM enter host cells, they penetrate the cell wall through a combination of enzymatic processes and physical pressure. The hyphae of VAM occur both intercellularly and extracellularly (fig. 2) (*127*). The hyphae grow within and between the epidermal and cortical cells and extend into the soil. VAM are usually found in the primary cortex of roots. The formation of very finely branched intracellular structures known as arbuscules and

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[2] Italic numbers in parentheses refer to items in the list of references at the end of this report.

[3] Work done by the University of Minnesota Plant Pathology Department under Bureau contract JO22500.

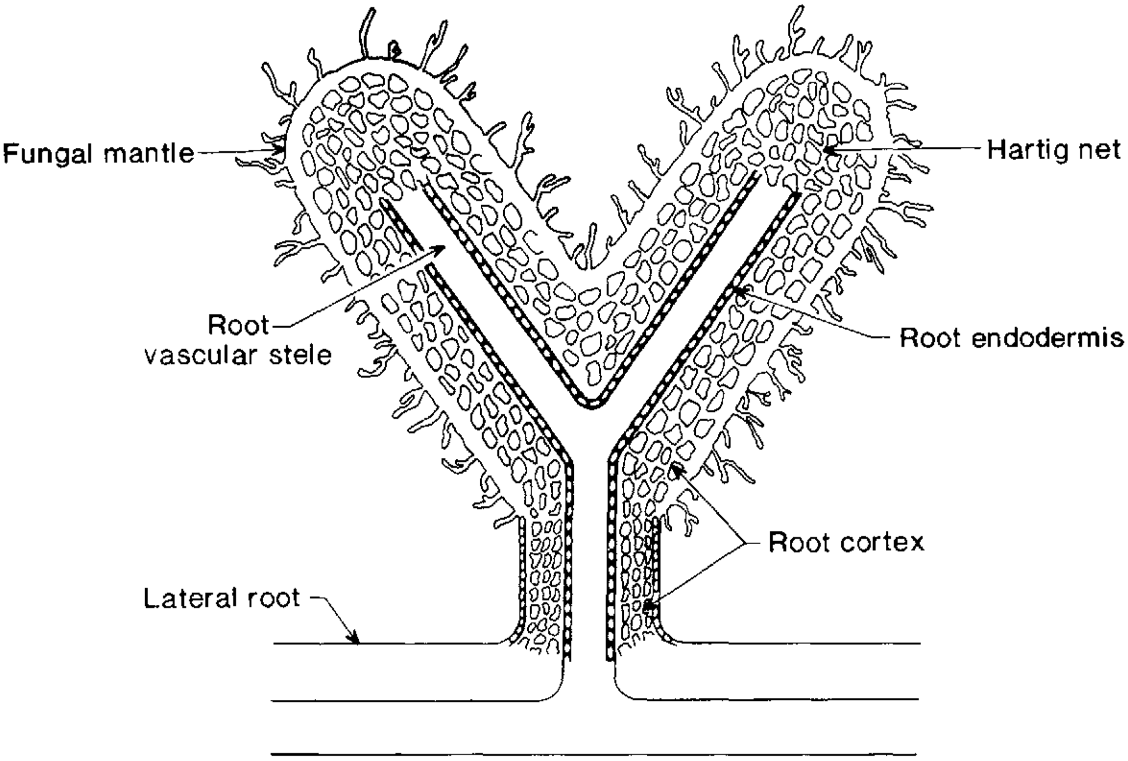

Figure 1.—Representative cross section of an ectomycorrhizae. Adapted from Marx (*64*); used with permission of D. H. Marx, USDA Forest Service, Athens, GA..

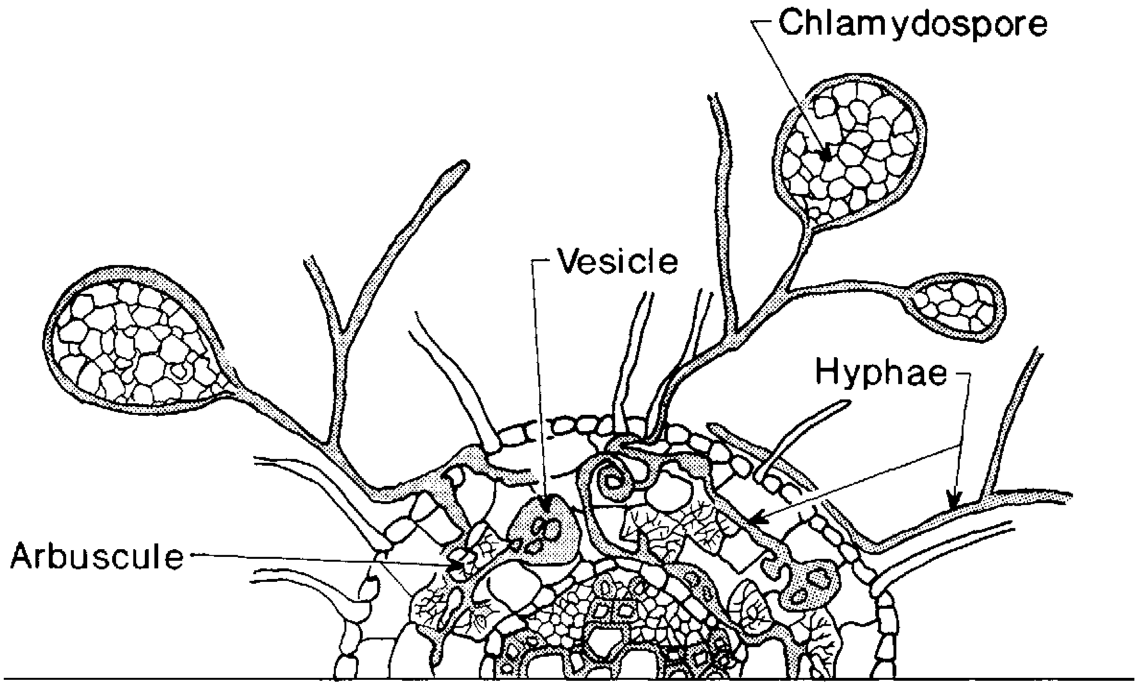

Figure 2.—Representative cross section through a vesicular-arbuscular mycorrhizal root. Reproduced from Zak, 1985 (*127*); used with permission of J. C. Zak.

production of thick-walled intercellular and intracellular structures known as vesicles are characteristics of VAM infections. The arbuscule is the primary site of nutrient transfer between the fungus and plant, whereas vesicles are believed to be storage organs and may function as inoculum.

The beneficial aspects of mycorrhizal associations include the following: (1) mycorrhizae increase nutrient and water absorption by increasing the absorbing surface area of the root system, and the fungal hyphae serve as extensions of the root system; (2) mycorrhizae increase nutrient mobilization through biological weathering by breaking down complex minerals and organic substances; (3) mycorrhizae serve as a biological deterrent and physical barrier to root infection by soil pathogens; (4) mycorrhizae may provide tolerance to heavy-metal accumulation by restricting the translocation of metals from roots to shoots, as the ions are absorbed on the cell wall of the hyphae in the root; and (5) evidence exists that mycorrhizae may provide the host plant with growth hormones such as auxin, cytokinin, and gibberellin. The mycorrhizal fungi benefit by utilizing photosynthates and derivatives from the host plant.

The purpose of this U.S. Bureau of Mines information circular is to review the literature relating to some of the difficulties of establishing plants on mining-related wastes because of adverse chemical, physical, and biological characteristics; to describe ecological and soil factors that affect mycorrhizae-plant associations; and to provide general information on current inoculation technologies. This work is in support of the Bureau's program to help minimize the environmental impacts of mining.

MINE SOIL CHEMICAL, PHYSICAL, AND BIOLOGICAL LIMITATIONS

Mining-related waste materials are characterized to determine if the replaced soils (coal) or tailing materials (hard rock) are suitable for plant growth and development. Spatial variability in the properties of mine soil, tailing, or other mining-related waste must be assessed to determine chemical, physical, and biological limitations to mine waste stabilization through revegetation. Variability in mining-related wastes is most likely due to the mining process itself, whether it be open pit hard-rock mining or coal surface mining. The physical disturbance caused by the removal and replacement of soil and overburden over mineral or coal deposits, together with the waste material from ore processing, affects the chemical, physical, and biological processes within the disturbed soil or waste. Additionally, variability in soil properties results from differences in climate, topography, vegetation, and parent material prior to disturbance. Successful stabilization of mining-related disturbances through revegetation depends on the knowledge of the chemical, physical, and biological limitations of a site prior to or at the time of stabilization.

CHEMICAL LIMITATIONS

Chemical characterization of mine soils and waste materials is necessary to determine whether a particular parameter or element exceeds an optimum value, suggesting a potential chemical problem in using the mine soil or waste materials as a medium for plant growth. The potential for stabilization through revegetation is greatly dependent upon the rooting medium pH, organic matter content, concentration of plant-essential nutrient elements, and presence of trace elements.

Soil Reaction

Soil reaction is the degree of soil acidity or alkalinity as measured by pH. Soil pH is the negative logarithm of hydrogen ion (H^+) activity of a soil. By specifying activity rather than concentration, it is recognized that there are other hydrogen ions in the soil system, such as in organic matter and the mineral structure. Soil pH is a measure of the hydrogen ion activity in the soil solution (*19*).

The degree of acidity or alkalinity is expressed in terms of the pH scale, 0 (extremely acid) to 14 (extremely basic or alkaline), while pH of 7.0 is neutral. Lower pH values are an indication of various degrees or intensities of acidity, while higher pH values indicate varying intensities of alkalinity. Because the pH scale is logarithmic, the intensity of acidity or alkalinity changes tenfold for each unit change in pH.

The determination of soil pH is one of the most common and important chemical measurements that can be made in mine soil, overburden, tailing, or other types of mining-related waste materials. It is useful for predicting and defining many of the problems associated with vegetative stabilization of lands disturbed by mining, as plant species vary in their tolerance of acid or alkaline conditions (*120*). Additionally, based on soil reaction, the concentration of plant-essential mineral elements and other trace mineral elements (such as aluminum, cadmium, chromium, nickel, lead, selenium) may be toxic, or sufficient or deficient (limiting) for the mineral nutrients. A relationship exists between the pH of the soil solution and nutrient availability. The soil pH may influence nutrient absorption and plant growth in two ways: (1) through the direct effects of the hydrogen ion or (2) indirectly, through its influence on nutrient availability and the presence of toxic ions (*18*). The indirect effect is of greater significance; for example, as the pH is raised from 5.0 to 8.0, iron, manganese, and zinc become less available, while molybdenum availability increases. At pH values below 5.0, aluminum, iron, and manganese are soluble in sufficient quantities to be phytotoxic. In general, most plants grow well within a pH range of 5.5 to 7.5.

Low pH values may also be an indication of weathered sulfide minerals such as pyrite and marcasite. When sulfide minerals such as iron disulfides in mine soils, overburden, tailings, and other mining-related wastes are exposed to water and oxygen, sulfide is oxidized to produce iron oxyhydroxide minerals and acidity, resulting in low pH values. The rate of pH change and sulfide oxidation is related to the crystal structure of the iron-disulfide; temperature, moisture, surface area of the materials, and oxygen concentration; and the presence of iron-oxidizing bacteria (*Thiobacillus ferrooxidans*) (*20*).

Essential Elements

Plant growth is largely regulated by the supply of available nutrients to the root system and by the efficiency of the active absorption of nutrients arriving at the root surface. The magnitude of nutrient supply to root surfaces depends on (1) the concentration of ions in the soil solution, (2) the sorption-desorption capacity of the soil, which allows ions in the exchangeable pools to replenish the soil solution as ions are taken up by plants, and (3) the efficiency of nutrient transport through the soil solution to absorbing sites, either by mass flow or diffusion (*10, 22, 116*). The bulk of available forms of plant nutrients are in the soil solution at low concentrations and move to root surfaces by diffusion. The rate-limiting step in the absorption of low-mobility ions is the diffusion of ions through the soil solution. Because the rate of diffusion is lower than the rate of absorption, regions of nutrient depletion develop around roots. There are two ways that roots modify nutrient inflow: by size and distribution and by their capacity for nutrient uptake (kinetics). VAM modify the nutrient uptake properties of a root through the development of an extramatrical hyphae network that is capable of absorbing nutrients and transporting them to root cortical cells (*10*).

Sixteen mineral elements have been recognized as essential nutrients for plant growth. Three criteria of essentiality (*8*) must be met for an element to be considered essential: (1) a given plant must be unable to complete its life cycle in the absence of the mineral element, (2) the function of the element must not be replaceable by another mineral element, and (3) the element must be directly involved in plant metabolism as a component of an essential plant constituent, such as an enzyme, or it must be required for a distinct metabolic step, such as an enzyme reaction. Of the 16 essential nutrients, 9 are used in relatively high concentrations and are referred to as "macronutrients." These are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur. The sources of carbon, hydrogen, and oxygen are mainly air and water, while the source of the other six macronutrients is the soil solids.

The remaining essential mineral nutrients are referred to as "micronutrients" because they are essential only in very low concentrations in plants; however, they are not any less essential than macronutrients. The micronutrients are iron, manganese, zinc, copper, boron, molybdenum, and chloride. The source of all micronutrients is the soil solids.

Macronutrients are generally constituents of organic compounds found in plants, such as proteins and nucleic acids, while micronutrients are predominantly constituents of enzyme molecules.

Nutrients may be classified into groups according to their physical and chemical behavior: (1) soluble: ions dissolved in soil solution; (2) exchangeable: elements adsorbed in exchangeable form in mineral particles and organic matter; (3) iron-manganese oxide bound: elements bound in nonexchangeable form in mineral particles and organic matter; (4) organically bound: elements present in soluble and insoluble forms in organic matter; and (5) residual: residual from organic fraction (*96, 114*). Nutrient availability to plants is strongly influenced by the physical and chemical behavior of the nutrient, whether bonded and bound or ionic and free to move (*96*).

When mineral elements, including the essential elements, are present in either deficient or toxic concentrations, plant growth and productivity can be adversely affected. In general, the common growth-production versus nutrient-supply curve has three well-defined regions: deficient, sufficient, and toxic. In the first region, growth rate increases with increasing nutrient supply. In the second, the growth rate or productivity reaches a maximum and remains unaffected by nutrient supply because there is a sufficient supply of nutrients for normal plant growth and development. In the third region, the growth rate falls with increasing nutrient supply. Plant toxicity responses involve both essential and nonessential mineral elements.

Essential nutrients, particularly nitrogen, phosphorus, and potassium, are often deficient in reconstructed mine soils and tailing materials (*81-83*). In situations in which topsoil and its associated organic matter are not replaced, nitrogen is almost always deficient for plant growth (*120*). Deficiencies of other macronutrients and micronutrients do exist on mine soils, but in most instances these deficiencies are site specific and have not been well defined (*120*). Toxicity occurs when mineral elements, including the essential nutrient elements, are present in concentrations that are abnormally high. Elements that have been found in mine soils and tailing materials at concentrations considered toxic to plants include aluminum, arsenic, barium, boron, cadmium, copper, iron, lead, manganese, nickel, selenium, and zinc (*81-83*, *120*).

Organic Matter

Soil organic matter can be broadly defined as all organic materials contained in soil and can be viewed as a complex heterogenous mixture of living, dead, and decomposing plant, animal, and microbial tissues and detritus (*9*). In general, the higher the organic matter content of soils, the more productive they are. The Soil Science Society of America (*107*) has defined soil organic matter as the organic fraction of soil exclusive of undecayed plant and animal residues. "Soil organic matter" is often used synonymously with "humus." Typically, soil organic matter represents the organic constituents in soil, excluding undecayed plant and animal tissues, their partial decomposition products, and the soil biomass (*110*). Soil organic matter contains most of the organic compounds synthesized by living organisms and includes (1) identifiable high-molecular-weight organic materials such as polysaccharides and proteins, (2) simpler substances such as sugars, amino acids, and other small molecules, and (3) humic substances (*63*).

Soil organic matter is frequently divided into two fractions, humic and nonhumic substances. Humic substances are the dominant components of soil organic matter (*62*). Humic substances consist of a complex mixture of organic compounds that are relatively inert (*62-63*). Despite attempts, humic substances have not yet been separated into discrete components. Humic substances are defined as a series of relatively high-molecular-weight, brown- to black-colored substances formed by secondary synthesis reactions (*107*). Humic substances have been vaguely placed into three operational fractions: humic acid, fulvic acid, and humin.

Humic acid is the dark-colored organic material that can be extracted from soil by various reactions. Humic acid is the fraction of humic substances that is not soluble in water under acidic conditions (pH < 2), but is soluble at higher pH values (*3*). Fulvic acid is the mixture of organic substances remaining in solution upon acidification of a dilute alkali extract from soil (fulvic acid fraction) and the colored material remaining in solution after removal of humic acid by acidification (*107*). Fulvic acid is the fraction of humic substances that is soluble in water under all pH conditions (*3*). Humin is the fraction of soil organic matter that is not dissolved upon extraction of a soil with a dilute alkali (*107*). It is the fraction of humic substances that is not soluble in water at any pH value (*3*).

Nonhumic substances are all those materials that can be placed in one of the categories of discrete compounds (*63*). These compounds include polysaccharides and sugars, proteins and amino acids, fats, and simple organic acids.

The addition of organic residues to coal mine soils and hard-rock tailing materials, along with the development of organic matter, directly and indirectly affects plant growth and development on mine waste sites. Organic matter may affect mine soil or tailing fertility *indirectly* as a source of nitrogen, phosphorus, sulfur, potassium, and micronutrients for plant nutrition through the soil biological processes of decomposition, oxidation, and reduction; by buffering against changes in soil or tailing pH; by increasing the cation-exchange capacity of the mine soil or tailing; and by binding metal ions that otherwise might be leached out of the mine soil or tailing surface (*23*). Mine soil fertility may be *directly* affected by organic matter through the supply of defined biochemical compounds to plant roots or the supply of humic substances that serve as carriers of micronutrients or growth factors, resulting in various biochemical effects at the cell wall, membrane level, or in the cytoplasm (*23*).

Soil organic matter may also affect the physical properties of mine soils and tailings by improving soil structure, thereby improving water-to-air ratios in the rhizosphere; improving the water-holding capacity of the soil, helping to maintain the water regime of the soil; improving the absorption of energy from the sun and heating of the soil; and acting as a binding agent to hold soil particles together in the development of soil aggregates, contributing to the development of a crumb soil structure, which is more resistant to soil erosion. The binding of soil particles by soil organic matter is a major contributor to the development of soil structure.

The presence of organic matter also helps to increase the population of beneficial soil microorganisms through increases in nutrient availability, a source of energy for microbial growth. Recovery of soil microbial activity in mine soils has been found to be directly related to organic matter and nitrogen accumulation (*111*).

Trace Elements

The distribution of trace elements in undisturbed soils is influenced by several factors, including parent material, mineralogy, organic matter content, particle size distribution, soil horizonation, soil age, drainage, vegetation, and atmospheric input (*37*). In mining-disturbed soils or wastes, high concentrations of trace elements influence the success of vegetative stabilization of these sites. Byproducts of mined ore bodies (including sulfides of cadmium, copper, lead, nickel, and zinc) and smelting (including sulfur dioxide and metal oxides of cadmium, lead, and zinc) significantly contribute to high trace element concentrations in these wastes.

The term "trace element" (heavy metal) is used to describe transition metals that may or may not be essential for plant growth. All of the micronutrient ions (iron, manganese, zinc, copper, and molybdenum) and most of the trace elements (for example, aluminum, cadmium, chromium, lead, mercury, and nickel) are toxic at concentrations above ranges typically found in soils (*17*). Bohn (*17*) states that naturally occurring high concentrations of trace elements are rare in soils, except for widespread aluminum phytotoxicity in acid soils. Soil contamination by toxic elements is generally the result of human activities such as mining and smelting, disposal of municipal and industrial wastes, agricultural practices such as pesticide use, atmospheric precipitation of industrial pollutants, and automobile emissions.

The *total* soil concentration is a poor indicator of a trace element's availability to plants, since it is the soil solution concentration of an element that governs plant availability (*17*). Use of total soil concentration as a criterion to assess the potential effects of trace element contamination implies that all forms of a given trace element have an equal impact on the environment, an assumption that cannot be defended (*114*). The mining waste material can be partitioned into specific fractions that can be extracted selectively using appropriate reagents. The use of sequential extractions furnishes detailed information about the origin, mode of occurrence, biological and physicochemical availability, mobilization, and transport of trace metals (*114*). One generality can be made concerning trace elements: availability of trace element cations decreases with increasing pH and clay content, while the availability of trace element anions increases with pH.

Researchers have identified various mechanisms by which trace elements are retained in soil: (1) adsorption reactions, including complex ion and ion-exchange reactions, (2) precipitation reactions, (3) complexation with organic matter, and (4) incorporation into plant tissues and decay products.

Evans (*38*) identified two mechanisms involved in the retention of trace elements by soil. First, metals may be retained by adsorption reactions involving complex ions and ion pairs (inner- and outer-sphere complexes) with the surfaces of mineral and organic constituents. Second, metals may be retained by precipitation reactions leading to the formation of new secondary mineral phases.

For nonspecifically adsorbed ions, the nature of the ion adsorption process is dependent on the relative charges of the metal species in solution and the charge of the charged surface. For specifically adsorbed ions, the extent of adsorption depends on the intrinsic formation constant of the metal-surface complex and on the pH of the soil solution (*38*). The alkali and alkaline earth metals, such as sodium, calcium, and magnesium, are held in soil by cation-exchange reactions, while large monovalent cations, such as potassium, rubidium, and cesium, can be adsorbed specifically by clay minerals (*38*).

Most anions and many metallic cations can form complex ions with charged mineral surfaces through the process of ligand exchange. These complexes occur most readily on oxide and hydroxide surfaces. This specific adsorption of metallic ions through ligand exchange occurs most readily for metals that hydrolyze in water, such as the rare earths. The adsorption reaction involves the formation of complex ions between the hydroxo-metal complex and the negatively charged, deprotonated surface of oxides, hydroxides, and oxyhydroxides of aluminum, manganese, and iron (*38*). This pH-dependent reaction increases to a maximum as the pH is raised.

Complexation with organic matter is a third mechanism by which mine soils and tailings retain trace elements. The extent of metals retention by humic and fulvic acid fractions of organic matter has been shown by Schnitzer (*102*). Typical results of these experiments show decreasing retention by organic matter from left to right:

At pH 4.7,

mercury = iron = lead = aluminum = chromium
> cadmium > nickel = zinc > cobalt
> manganese.

At pH 5.8,

mercury = iron = lead = aluminum = chromium
= copper > cadmium > zinc > nickel
> cobalt > manganese.

The retention mechanisms involved in complexation with organic matter include complex ion, ion-exchange, and precipitation reactions. Complex ion associations can be formed between metallic cations and coordinating functional groups found in humic substances (*38*). The functional groups behave like complexant organic ligands. Because most organic ligands contain more than one donor atom, additional linkages may be formed with the metal ion to give chelated complexes (*38*). For this to occur, the ligand must contain at least two donor atoms capable of bonding to the same metal ion and positioned within the ligand so that the formation of a ring is sterically possible (*38*). Donor atoms include oxygen, nitrogen, and sulfur.

Humic substances contain a highly complex mixture of functional groups whose metal-complexing abilities suggest that many types of sites may be involved in metal binding (*38*).

A fourth mechanism by which trace elements are retained is through incorporation into plant tissues and decay products. Some plants have evolved a high degree of metal tolerance. Some plant species are able to withstand higher concentrations of trace elements than others through morphological and physiological adaptations. Plant roots can exclude trace elements, or plants can limit trace element translocation from root tissues to aboveground tissues, thus retaining trace elements in the soil.

The concentrations of trace elements in mining wastes may be several orders of magnitude higher than the concentrations in undisturbed soil. It is possible that the precipitation of trace elements as secondary minerals occurs in mining wastes. Among the most important precipitates are oxides, oxyhydroxides, hydroxides, carbonates, and sulfides; with phosphates and silicates being less important (*38*).

One of the most important precipitation reactions occurring in soils is the hydrolysis reaction involving the hydroxide ligand. Hydrolysis reactions can precipitate hydroxide and oxyhydroxides if the concentration of the metal ion and the ion activity product exceeds the value of the solubility product (*38*). Metals that might occur as hydroxides under some soil conditions are aluminum, cadmium, copper, iron, and zinc. These metals hydrolyze in water, with the extent of hydrolysis increasing with higher pH values. The hydroxide complexes of some metals, especially aluminum hydroxide and iron hydroxide anions at neutral or slightly alkali pH, control the behavior of these metals in soils (*38*).

The extent of precipitation of metallic carbonates and sulfides in soil is controlled not only by the solubility products of the individual carbonates and sulfides, but also by the partial pressure of gaseous carbon dioxide for carbonates and gaseous hydrogen sulfide for sulfides (*38*). This precipitation occurs because the content of carbonate and sulfide anions is controlled by the amount of carbon dioxide and sulfide dissolved in the soil water (*38*). Metals that might be expected to precipitate as carbonates include calcium, strontium, barium, iron, zinc, cadmium, and lead.

Hydrogen sulfide forms from the biological reduction of sulfate ions under anaerobic conditions. When the redox potential in soil is low, generally because of anaerobic conditions due to excess water, hydrogen sulfide ions will form; however, when the redox potential is higher, the soil is more oxidizing and the sulfide minerals will be oxidized. The stability of sulfides depends on the state of oxidation or reduction of the soil. Because there is an associated production of protons during oxidation of sulfide minerals to sulfate, the minerals will generally dissolve (*38*). Metals expected to occur as sulfides under reducing conditions include silver, nickel, zinc, cadmium, lead, mercury, and iron.

PHYSICAL LIMITATIONS

Plant establishment and growth on mine soils and wastes are often limited by a physical effect rather than a chemical imbalance (*35*). Soil physical properties that are known to affect plant growth on mine soils and wastes include soil texture and structure, bulk density, pore space, soil water, and soil color.

Soil Texture and Structure

Soil texture is a measurement of the size of the mineral particles and refers to the relative proportion of particles of various sizes in a given soil (*18*). Particle size analysis is used to measure the percentages of the primary separates: sand, silt, and clay. The diameter limits of these classes are sand, 2 to 0.05 mm; silt, 50 to 2 µm; and clay, less than 2 µm.

Soil textural classes (relative percentages of sand, silt, and clay) are used to identify the textural makeup of soils and give an indication of some of their physical properties (*18*). Soil texture is determined using particle size data and a textural triangle (*108*). Twelve textural classes are recognized; they are clay, sandy clay, silty clay, clay loam, sandy clay loam, silty clay loam, loam, sandy loam, silt loam, silt, loamy sand, and sand. Physical features of soil affected by soil texture include water movement and retention, water availability to plants, bulk density, soil erodibility, and soil consistence. Soil texture also affects the soil cation-exchange capacity, a chemical parameter. Medium-textured materials, such as loams, clay loams, silt loams, and silty clays, are suitable for plant growth, while coarse-textured sands and fine-textured materials (silty clays and clays) are less suitable for plant growth and development.

Soil structure refers to the overall aggregation or arrangement of the primary separates (*18*). A soil profile may be dominated by a single structural pattern, or a number of types of aggregates can be found within a soil profile from horizon to horizon. Individual aggregates are known as peds. Soils that do not have aggregates with naturally preserved boundaries (peds) are considered structureless (*19*) and are characterized by one of two forms, either single grain or massive. There are four primary types of structure that refer to the shape of the peds: platelike, prismlike, blocklike, and spheroidal. Ped shape, together with size, is used to define soil structure. Soil structure can influence water movement, heat transfer, aeration, bulk density, and pore space (*18*).

Mine soils are different from undisturbed soils in the kind and distinctness of peds (*35*). Undisturbed soils generally exhibit well-developed structure in their upper horizons, while mine soils show little or no soil structure throughout their profile. Older mine soils may exhibit weak granular structure in the surface horizons because of root growth, wetting and drying, freezing and thawing, and organic matter enrichment (*35*). Mine soils should become more like undisturbed soils with time since the same processes that have occurred in the development of undisturbed soils are active in mine soils (*35*).

Soil Bulk Density and Pore Space

Bulk density is defined as the mass of dry soil per unit bulk volume, which includes both soil solids and pore space (*107*). Vogel (*120*) states that high bulk density is the most troublesome soil feature of reconstructed soils on surface-mined land. Soils that are loose and porous will have lower bulk density values, while those that are more compact will have higher bulk density values and a resulting loss of soil structure. Surface bulk densities of mine soils are usually greater than those of undisturbed soils because of their compacted state, lack of structure, and high coarse fragment content (*24*).

Compaction is a result of soil-handling operations (topsoil removal, topsoil stockpiling, overburden regrading, and topsoil replacement) by heavy equipment under the wrong conditions (high soil moisture values, for example). Compaction causes a reduction in pore space, which inhibits or restricts the movement of air into the soil and the movement and storage of water within the soil. High bulk density values may also restrict plant root expansion and penetration into the soil.

The pore space of a soil is that portion of the soil bulk volume that is occupied by pores, air, and water (*107*). The amount of pore space is determined by the arrangement of solid soil particles. If the particles lie close together, as in sands or compacted soils, the total porosity is low; however, if they are arranged in porous aggregates (such as soils high in organic matter) the porosity is high (*18*). In general, one-half the volume of soil is thought of as pore space, and the size, shape, and continuity of these voids will affect the movement of air and water in the soil.

Soil structure has a great influence over soil pore space. Soil horizons with structure defined as granular, blocky, prismatic, or columnar will have much less resistance to soil air and water movement and root extension than will soil horizons with structure that is massive or platy.

Two types of soil pores are generally recognized, macropores and micropores. Although pore types are not clearly separated, macropores characteristically allow the ready movement of air and percolating water, while in micropores air movement is greatly impeded and water movement is restricted to capillary movement. Undisturbed soils tend to be more porous than mine soils, with a developed system of cracks and fissures; however, pores in mine soils are typically larger (*85*).

Soil Water

The measurement of soil moisture parameters provides information on the amount of water present in the mine soil or waste, the amount of water available for plant use, and the movement of water within the mine soil or waste profile. Available moisture in the root medium is a major factor in successful stabilization of mine soils and wastes. Plant-available water is generally considered to be the difference between the water content at field capacity (the amount of water held after excess gravitational water has drained away) and the water content at the permanent wilting point (water content when plants undergo irreversible wilting).

Available plant water is directly influenced by soil texture. In coarser textured mine soils or wastes, such as materials with high sand, gravel, or rock fragments, the water-holding capacity is significantly reduced because of larger pore spaces in the material, but most of the water held will be available to plants. In general, coarse-textured materials have low plant-available water and are droughty.

The finer textured mine soils and wastes (clays) have a greater capacity to hold water, but they hold water in a closer association, limiting its availability to plants. Soils that are high in silt are capable of holding the most plant-available water, with silt loams, loams, and clay loams having the greatest water-holding capacity. The textural differences and organic matter deficiencies of mine soils and wastes compared with undisturbed soils account for their lower water-holding capacities and plant-available water.

The water content of mine soils and mining-related wastes is controlled by the infiltration rates. Infiltration rates also control the amount of surface runoff from mine soils and wastes. Low infiltration rates indicate a potential for high runoff, erosion, and inadequate recharge of mine soil water (*35*). These consequences of low infiltration may prevent plant growth and development. Infiltration is influenced by many different factors, including soil texture, structure, porosity, pore size distribution, existing water content, and organic matter content (*35*).

Soil Color

Soil color is probably the most easily recognizable physical feature of a soil. It may also be related to specific chemical and biological properties of a soil. Soil color affects the temperature of the surface layers of a soil. Black or dark-colored soil absorbs more heat from the sun, resulting in elevated soil surface temperatures that can be lethal to plant seedlings. These higher temperatures can also lead to soil drying. A black soil color is also an indication of the presence of organic matter. Lighter colored soils are not as warm, nor do they dry as quickly. Red-colored soil may contain elevated levels of free iron oxides common in well-oxidized soils. Under reducing conditions, the removal of free iron generally results in a gray or bluish-gray color.

BIOLOGICAL LIMITATIONS

The biological components of soil contribute to soil development and to the development and maintenance of a vegetative cover. Although the chemical and physical properties of mine soils and wastes are important in determining initial plant establishment and growth, biological factors are essential for the successful maintenance of soil fertility and evolution of a stable plant system (*119*). The presence of soil flora and fauna is essential for the long-term survival and growth of most plant species and the re-establishment of natural ecosystems (*120*). The development of a stable ecosystem depends upon the processes of primary production, decomposition, and nutrient cycling. There is no doubt that rapid establishment of primary producers on disturbed land should be the goal of stabilization programs. However, once vegetation is established, the rate at which essential elements are released from dead plant residues and returned to the plant system, along with the rate at which stable organic matter accumulates on disturbed sites, will be largely determined by the biological characteristics of mine wastes. The development of a self-sustaining vegetative cover on mine soils and wastes is ultimately dependent on the establishment of decomposition and mineralization processes (*119*).

The biological component of soil consists of diverse groups of organisms that range in size from the larger rodents, worms, and insects to microscopic bacteria and fungi. These organisms, either individually or in complex interactions, are an integral part of numerous physiological activities associated with plants and in soil processes (*120*). Mining processes generally disrupt these organisms, their interactions, and physiological activities.

The activities of soil organisms range from physical disintegration of plant residues by insects and earthworms to the complete decomposition of these residues by microorganisms, including bacteria, fungi, and actinomycetes. Organic matter alteration and decomposition of plant residues release nutrients that are available for plant uptake, including but not limited to nitrogen, phosphorus, and sulfur. Microorganisms are also obligatory for nitrogen fixation (bacteria), nitrogen and sulfur cycling (bacteria), fermentation of manure and compost piles (actinomycetes), root nodulation of nonleguminous plants (actinomycetes), and development of mycorrhizal associations (fungi).

The establishment, rate of development, density, and community composition of microorganisms in reconstructed mine soils and other mining-related wastes will be influenced by the physical and chemical characteristics of the material and the availability of organic matter. The environmental factors that influence the density and composition of bacteria, actinomycetes, and fungi are discussed below.

Bacteria

Moisture, aeration, temperature, organic matter, acidity, and inorganic nutrient supply are the primary environmental factors that affect soil bacteria. Moisture affects microbial activity in two ways: (1) it must be available for microbial development, and (2) where it is excessive, the bacterial flora decreases because of waterlogging, which limits gaseous exchange and lowers the available oxygen supply, creating anaerobic conditions (*4*). Alexander (*4*) states that maximum bacterial density is found in regions of fairly high moisture content with the optimum level for aerobic bacteria activity occurring where soil moisture is between 50 and 75 pct of the soil's water-holding capacity. Under anaerobic conditions only those bacteria tolerant of low oxygen levels or complete anaerobiosis are capable of surviving.

All biological processes are governed by temperature, including microbial activity. Most bacteria are mesophilic; their optimum temperature for growth and proliferation ranges from 25° to 35° C (*4*).

The largest bacterial numbers are found in soils with high organic matter content (*4*). Additions of carbonaceous materials, green manures, and crop residues stimulate bacterial numbers. This effect, however, is most pronounced during the first months of decomposition, disappearing after a period of 1 year (*4*).

Many soil bacteria are inhibited by highly acidic or alkaline conditions, with optimum pH for most species near neutrality. As the pH is reduced, the number of bacteria within the soil microbial community is also reduced (*4*).

Inorganic nutrients are required by bacteria even though organic carbon is the major constituent of their energy supply. Applications of inorganic nutrients serve a dual function since they are utilized by both the plant and bacteria (*4*).

Actinomycetes

For actinomycetes, the stage of the life cycle that predominates, the size of the community, its biochemical transformations, and the genera and species found are determined by the ecological factors acting within the ecosystem (*4*). The primary edaphic factors that influence actinomycetes are the status of organic matter, pH, moisture, and temperature.

The availability of carbon directly affects the community size of actinomycetes; thus, the concentration of actinomycetes increases with organic matter content. When organic amendments are added to the soil, bacterial and fungal flora usually proliferate initially, but actinomycetes populations generally do not respond until later stages of decomposition (*4*). This suggested to Alexander (*4*) that greater growth rates and biochemical versatility of bacteria and fungi make them initial organisms of decomposition, while actinomycetes appear only after more readily available compounds have been metabolized and competition has been reduced.

Actinomycetes are not tolerant of low soil pH values, with the limiting pH for most strains at approximately 5.0; pH values ranging from 6.5 to 8.0 are most favorable for their development (*4*).

Actinomycetes are severely limited under conditions of waterlogging or where soil moisture is 85 to 100 pct of water-holding capacity. All common soil actinomycetes require aerobic conditions for metabolism; under conditions of anaerobiosis, they do not develop or spread. Additionally, actinomycetes are not as greatly influenced by dry soil conditions as are bacteria. The number of actinomycetes remains high as soils dry out; however, their relative incidence decreases because of their lack of tolerance to arid conditions (*4*). The optimum temperature range for the growth and development of actinomycetes is from 28° to 37° C.

Fungi

Fungi (mushrooms, molds, mildews, and yeasts) vary in abundance, physiological activity, and biochemical activities with time on any single site (*4*). Organic matter status of the soil, pH, organic and inorganic fertilizers, moisture, aeration, temperature, position in the soil profile, season of the year, and vegetative composition are all environmental factors that influence the fungal community.

The number of filamentous fungi in the soil varies directly with the content of utilizable organic matter (*4*). Incorporation of crop residues, green manures, and other carbonaceous materials into the soil not only improves the nutrient content of the soil, but also increases the size of the fungal community. There is a succession of fungal species following the additions of a carbon source, through its decomposition.

Many fungal species are adapted to a wide range of soil pH values, from highly acidic to highly alkaline conditions. Since both bacteria and actinomycetes are not commonly found in acidic conditions, fungi dominate the microbial communities in areas of low pH (*4*). The presence of fungi in acid soils is not the result of these areas' providing optimum conditions for their existence, but a consequence of the lack of microbial competition for food reserves. Fungi are the dominant microorganism in acid soils because of their tolerance to low soil pH values and the narrow range of pH values in which most bacteria and actinomycetes can function (*4*). Fungi are responsible for a large portion of the biochemical transformations that occur in acid soil habitats.

Soil water has a direct effect on the abundance and functions of fungi (*4*). When the water supply or water-holding capacity of the soil is low, the capacity of fungi for catalyzing chemical transformations may be lacking. When moisture is excessive, the diffusion of oxygen that is necessary for aerobic metabolism is inadequate to meet microbiological demands, and fungal populations decrease.

In general, filamentous fungi are strict aerobes. This dependency on oxygen helps to explain the high concentration of fungi in the upper few centimeters of soil and the absence of fungi in the lower levels of undrained peat and from swamps and bogs (*4*). Like bacteria and actinomycetes, most species of fungi are mesophilic in their temperature relationships. The effects of moisture and temperature on fungal composition and density can be shown through seasonal changes. Spring warmth and moisture are usually beneficial to fungal populations, but periods of summer drought or cold winters significantly reduce the activity of fungal populations in soil (*4*).

ECOLOGICAL AND SOIL EFFECTS ON MYCORRHIZAE

INTRODUCTION TO LITERATURE REVIEW

The establishment of mycorrhizae-plant associations on mine wastes is influenced by the effects of soil chemical and physical properties on mycorrhizal fungi. Soil pH, essential elements, organic matter, trace elements, moisture, aeration, temperature, and light affect mycorrhizal colonization of plant roots on mine waste sites. Mosse (*77*) suggests that specificity may be determined more by the interaction of a fungal strain with the soil than of the fungus with its host. The ways in which soils influence the ecology and symbiotic behavior of mycorrhizae are poorly understood (*36*). This section reviews some of the soil factors that may affect mycorrhizal establishment on mining wastes. Mycorrhizal colonization may be directly related to the successful establishment of plants on mine waste sites. Of the 6,507 species of angiosperms that have been studied, 70 pct are consistently found to be mycorrhizal and 12 pct are facultatively mycorrhizal (*117*). Those species not able to form mycorrhizae belong mainly to the Cruciferae, Chenopodiaceae, Cyperaceae, and Juncaceae families.

ECTOMYCORRHIZAE AND VESICULAR-ARBUSCULAR MYCORRHIZAE

Ectomycorrhizae are more limited in distribution among plant species and have a greater morphological uniformity than do VAM. About 3 pct of higher plants form ectomycorrhizae (*10*). Most ectomycorrhizae are restricted entirely to tree species (*91*). Ectomycorrhizae have their greatest host range among temperate forest trees, and boreal and high-elevation forest are exclusively ectomycorrhizal (*78*). Most conifer species and some hardwood species cannot grow in the absence of ectomycorrhizal fungi. It is estimated that more than 2,000 species of ectomycorrhizae exist; most are basidiomycetes, but there are some ascomycetes (*91*). Ectomycorrhizal species that have been studied in detail or identified on mine land and mine wastes include *Pisolithus tinctorius* (Pers.) Coker & Couch, *Thelephora terrestris* Ehrh. ex Fr., *Cenococcum geophilum* Fr., *Suillus* spp., *Laccaria* spp., and *Inocybe* spp. For a list of mycorrhizal fungi reported to occur on mine spoils, tailings, or wastes (both ectomycorrhizae and VAM) see Danielson (*31*), and for a listing of ectomycorrhizal fungi genera see Mosse (*78*).

Vesicular-arbuscular mycorrhizal fungi are the most widespread and important root symbionts of all mycorrhizal associations (*78, 91*). About 80 pct of all land plants form this type of mycorrhizae. These fungi are found in both agricultural and forest soils throughout the world. Hosts include most families of angiosperms and gymnosperms, including Rosaceae, Gramineae, and Leguminosae. Approximately 150 species of VAM are recognized (*100*); all are zygomycetes (*75*). *Glomus* spp., *Scutellispora gregaria* (Schenck & Nicol.) Waller & Sanders, *Scutellispora pellucida* (Nicol. & Schenck) Waller & Sanders, and *Acaulospora laevis* Gerd & Trappe are among the VAM species studied or identified on mine land or mine wastes.

VAM species occur over a broad spectrum of plant habitats and soils, exhibiting little if any host specificity (*125*). They do, however, vary in their sensitivity and response to environmental factors. These fungi are obligate symbiots and, as such, require carbohydrates from their plant host. The carbohydrate drain from the host may have negative consequences for biomass production of the host, especially under environmental stress conditions (*103*).

VAM fungi are not, in general, limited in their host range, as isolates can infect a variety of plant species (*51*). Except for the sporophores of basidiomycetes and ascomycetes, all mycorrhizal fungi are strictly soil or root inhabiting and have no aerial stage (*78*). Dissemination vectors of mycorrhizal fungi include (1) spread from one living root to another, (2) spread by spores and other propagules in drainage water and wind-blown particles, and (3) spread by animal vectors (*78*). A noninfected plant may become more rapidly infected with VAM through spreading or connecting of hyphae from one living root to another than if the source of infection is spores or dead roots. Additionally, plants may become infected by different species of mycorrhizae and become connected with a mycelial system much larger than the system a noninfected plant would develop on its own (*80*). Mycorrhizal links may assist plant establishment by allowing seedlings to become infected with mycorrhizae more rapidly and become connected to a larger mycorrhizal mycelium network supported by carbon from other plants. If carbon passes from well-illuminated plants to shade plants and mineral nutrients flow from higher nutrient to lower nutrient plants, this could reduce competitive dominance by some plants and promote coexistence and species diversity. Mycorrhizal links may promote nutrient cycling by passing mineral nutrients from one plant to another or by releasing nutrients from dying roots to living roots, thus increasing ecosystem productivity by keeping the nutrients within the plant biomass rather than releasing nutrients into the soil solution (*80*). Ectomycorrhizae are dispersed as spores through the air, while VAM depend mainly on root contact, moving water, or soil fauna for dissemination.

SOIL FACTORS AND FUNGAL ACTIVITY

The rate of mycorrhizal development is directly related to soil chemical and physical properties. Information is presented on the soil factors that influence the formation of mycorrhizae-plant associations.

Chemical

Soil Reaction

Soil pH has a direct effect on fungal populations; extreme acidity can reduce or completely retard the growth of some fungal species. Ectomycorrhizal fungi are assumed to be acidophilic (*42, 54, 104*). McAfee and Fortin (*67*) identified pH as one of the soil variables influencing ectomycorrhizal colonization of *Pinus banksiana* Lamb. and *Picea mariana* (Mill.) BSP seedlings. Soil pH was positively correlated with mycorrhizal formation on *Picea mariana*, since mycorrhizal formation increased with increasing soil pH values; on *Pinus banksiana* mycorrhizal formation increased with decreasing soil pH. Fungal growth occurs at pH values between 3.2 and 6.5, with optimal mycelial growth for most isolates at a soil pH range of 4.5 to 5.5. The pH in the soil solution is important through its influence on oxidation-reduction equilibrium, the solubility of nutrients, and the ionic form of the mineral nutrients (*94*).

Ectomycorrhizal fungi vary in their pH optimum, which suggests that they may adapt to the pH of the soil. McAfee and Fortin (*67*) suggest, however, that the association of pH with mycorrhizae formation on *Pinus banksiana*, an acidophilic species, may be the result of a plant-mediated process. Hendrix (*52*) found that when *Pinus taeda* L. seedlings were transplanted onto pyritic coal spoil, soil pH of the root zone was lower in seedlings naturally infected with *Pisolithus tinctorius* (average pH value of 3.6) than in those not infected (average pH value of 4.1). Trees infected with *Pisolithus tinctorius* were green and robust in appearance, while noninfected trees were chlorotic with dieback symptoms. Although *Pisolithus tinctorius* will not control acid production in mine spoils, it may enable its hosts to thrive on acid spoils by controlling acid accumulation and enabling the plant to withstand or tolerate acid by excluding the uptake of aluminum, iron, and manganese (*55*).

Hung and Trappe (*54*) evaluated the response of 10 species of ectomycorrhizae over a range of pH from 2 to 7. The species tested fell into five growth patterns: (1) growth of *Amanita muscaria* (L. ex Fr.) Pers. ex Hook. was significantly higher at pH 6 than at any other value; (2) growth of *Hebeloma crustulimifrome* (Bull. ex St. Am.) Quel. increased significantly as pH was increased from 2 to 7; (3) growth of *Laccaria laccata* (Scop. ex Fr.) Berk. & Br., *Piloderma bicolor* Erikss. & Hjortst, and *Suillus lakei* (Murr.) Smith & Thiers was best at pH values ranging from 4 to 6, while growth of *Pisolithus tinctorius* (isolate S-471) was best at pH values ranging from 3 to 5; (4) growth of *Rhizopogon vinicolor* Smith and *Thelephora americana* Lloyd was significantly better at pH values ranging from 3 to 6; and (5) growth of *Cenococcum geophilum* and *Pisolithus tinctorius* (isolate S-431) was best at pH values ranging from 3 to 7. Hung and Trappe (*54*) recommend that isolates that grow well over a large range of pH would be preferred for field inoculations over species that grow well over a more restricted range.

VAM resemble ectomycorrhizae in their wide range of tolerance to soil pH (*78*). Root infection by VAM fungi has been shown to occur over a pH range of 4.2 to 7.0. *Glomus diaphanum* Morton & Walker has been found in abandoned or partially reclaimed surface mine land characterized as acidic and high in aluminum (*36*). VAM have also been reported in alkaline soil conditions.

Studies conducted to evaluate pH effects on spore germination and growth indicate that optimal pH conditions are slightly acidic (5.5 to 6.5). Spore numbers and viability of VAM decline with increasing acidity, which may be related to the lack of suitable hosts on highly acidic soils or to the effects of hydrogen ions. Porter (*88*) found that decreasing soil pH to 4.3 decreased spore germination and hyphal growth of *Glomus* species. Additionally, pH acidification to 5.3 decreased spore germination by one-third and hyphal growth by one-eighth of that obtained in untreated solutions.

The optimum pH for spore germination differs with each VAM species and the environment in which it is found; however, germination occurs within a pH range that is acceptable for plant growth. Porter (*89*) found that spores of *Glomus* species were found only in soil with a pH of 6.8; however, large numbers of *Acaulospora laevis* spores were found in soils with a pH range of 4.5 to 4.9. The relationship between soil pH and the distribution of spores in the field is correlated with the relationship between soil pH and the germination of spores and growth of hyphae (*88*). Porter (*89*) concludes that spoil pH is a major determinant of the distribution of VAM.

In a study on the effectiveness of several VAM fungi with the legume *Macroptilium atropurpureum* (DC.) Urb., Medina (*68*) hypothesized that the native VAM used may be sensitive to changes in soil pH and that they may have been rendered ineffective by lime (calcitic limestone) applications. Since the initial pH of the soil used in the study was 4.5, it was necessary to lime the soil for satisfactory establishment and growth of *Macroptilium atropurpureum*. Although the indigenous population of VAM fungi in the soil was high (two propagules per gram of soil), the native population was ineffective in stimulating the growth of *Macroptilium atropurpureum* in lime-amended soil.

Essential Elements

The formation of ectomycorrhizae- and VAM-plant associations is affected by soil inorganic nutrient levels. Low to moderate soil fertility enhances mycorrhizal development, while high soil fertility may reduce or in certain instances prevent mycorrhizal development. The benefits of mycorrhizae-plant associations are greatest when a rapidly growing plant with high internal phosphorus requirements is grown on phosphorus-deficient or phosphorus-fixing soil (*46*). Plants are not expected to benefit from a mycorrhizae-plant association if a readily available source of nutrients is present. Soil nutrients of primary importance and concern in the formation of mycorrhizae-plant associations are nitrogen and phosphorus.

Available phosphorus (phosphate) in the soil solution under normal conditions is usually very low, 10^{-6} to 10^{-7} *M* (0.01 to 0.1 mg L^{-1}) (*17*). Plant-available phosphate accounts for only about 1 to 5 pct of the total phosphorus content (*10*). The remaining forms of phosphorus, both organic and inorganic, are not directly available for absorption by plants. Vesicular-arbuscular mycorrhizal plants draw most of their phosphate from the soluble pool more efficiently than nonmycorrhizal plants; however, increasing the soluble phosphate levels in soil reduces the overall percentages of VAM colonization (*10*). Increasing soluble phosphate levels decreases both the extent of extramatrical mycelium and the number of arbuscules formed (*2, 106*).

Kothari (*59*) found that hyphal length was significantly greater at low phosphorus than at high phosphorus supply. Louis and Lim (*61*) found that the isolate *Glomus clarum* Nicol. & Schenck from a soil with low phosphate availability was more efficient in increasing growth, nodulation, and nitrogenase activity of *Glycine max* (L.) Merr., with more mycorrhizal infection, than was the isolate from soils with high phosphate availability. In soils with high phosphate availability, there was no difference in the behavior of the two isolates. These results suggested to Louis and Lim (*61*) the need of evaluating VAM isolates in relation to host efficiency for field studies.

In kaolin spoil, Gruhn (*45*) found that the growth of *Liquidambar styraciflua* L. was increased to a greater degree by VAM inoculation than by phosphorus addition. Nonmycorrhizal plants failed to grow and develop normally regardless of the level of phosphorus addition.

High concentrations of soil phosphorus have been shown to inhibit mycorrhizae formation. Phosphorus fertilization was found to decrease VAM root colonization of *Allium cepa* L. by 20 pct (*41*). Spore production was higher on plants that were not fertilized with phosphorus than on those that were; the average difference in the number of spores produced was 108 (*41*). Gruhn (*45*) found that phosphorus additions inhibited both mycorrhizal colonization of *Liquidambar styraciflua* and VAM sporulation. For a given phosphorus requirement, variability among plant species in response to mycorrhizae infection is thought to be related to either variability in the inherent capacity to acquire phosphorus or variability in phosphorus utilization (*58*). The choice of the phosphorus level to supply mycorrhizal transplants involves balancing the apparently contradicting goals of vigorous growth and adequate infection (*121-122*). The effect of soil phosphorus on symbiosis varies with the specific host and endophyte (*69*).

Hatch (*50*) suggested that the intensity of infection by ectomycorrhizae was greater under conditions of low or unbalanced nutrient supply. It has also been determined that the formation and continued development of VAM are inversely related to phosphorus supply (*78*). Actual soil phosphorus and nitrogen levels supporting increased or decreased infection levels are variable and depend on host utilization rates, VAM species, and the availability of phosphorus and nitrogen in any given soil (*97*).

Additionally, it has been postulated that mycorrhizae may contribute to phosphorus nutrition of host plants through nonspecific surface acid phosphatases that are capable of hydrolyzing organic phosphorus compounds. The production of inorganic phosphorus from organic sources by mycorrhizae using enzyme-catalyzed hydrolysis is another means by which plants may obtain phosphorus (*15*). Kroehler (*60*) found that inorganic phosphate is an important regulator of surface phosphatase activity. There was a consistent inverse relationship between phosphorus concentration and phosphatase activity, with production induced before growth limitations began. Available soil phosphorus plays a role in the regulation of mycorrhizal phosphatase activity and production (*60*).

VAM may increase nitrogen concentration in plant shoots. A number of mechanisms for improved nitrogen nutrition are based on ^{15}N and include (1) the improvement of symbiotic biological nitrogen fixation, (2) direct uptake of nitrogen by VAM, (3) facilitated nitrogen transfer, a process by which the nitrogen fixed by nodulated plants benefits non-nitrogen-fixing plants growing nearby, and (4) enzymatic activities involved in nitrogen metabolism (*10*).

Greenhouse and field studies have shown that VAM improve growth, nodulation, and nitrogen fixation in legume-*Rhizobium* symbiosis (*11, 13, 51*). This effect arises from the dependency of nitrogen fixation on an adequate supply of phosphate to the root and nodules. Barea (*12, 14*) has shown that VAM inoculation enhanced biological nitrogen fixation of legumes in a way that is similar to phosphate fertilization.

VAM hyphae can take up and translocate both nitrate and ammonium, thereby increasing the amount of nitrogen taken up by the plant (*105*). Intercropping with nitrogen-fixing plants enriches the soil with nitrogen, providing nitrogen directly to companion plants. It has been hypothesized that VAM can improve nitrogen transfer through a network of linked VAM mycelia (*80*). The nitrogen released by legume roots through exudation or decay can be used by non-nitrogen-fixing plants.

Mycorrhizae also increase the uptake of nutrients besides nitrogen and phosphorus, although relatively few studies have shown a direct role of VAM in plant uptake of nutrients other than nitrogen and phosphorus (*10*). Studies have shown that calcium, potassium, and zinc uptake is increased when plants have mycorrhizal associations. Gruhn (*45*) found that inoculation of *Liquidambar* *styraciflua* with *Gigaspora margarita* Becker & Hall resulted in increased foliar concentrations of phosphorus by 90 pct, calcium by 53 pct, and magnesium by 87 pct over the concentrations found in nonmycorrhizal plants. In most studies, a lack of appropriate controls makes it difficult to distinguish whether a VAM effect is the result of an improvement in nutrient uptake by the extramatrical mycelium or an indirect consequence of VAM balancing the phosphate status of the plant (*10*).

Organic Matter

The amount of organic matter present affects the physical and chemical properties of a soil. Typically, the number and types of microorganisms increase with applications of organic matter; this can be attributed to the addition of an available energy source, oxidizable carbon. Mining typically results in the loss of soil organic matter and reductions in microbial populations. In mine wastes that are low in organic matter, some form of organic material will have to be added to establish vegetative cover on the site. The development of a self-sustaining vegetative cover on mine wastes is dependent on establishment of decomposition and mineralization processes.

Ectomycorrhizae- and VAM-plant associations may be stimulated by substances produced by organic matter and the properties of organic matter. McAfee and Fortin (*67*) found that the amount of soil organic matter significantly affected mycorrhizal formation on *Pinus banksiana* seedlings. Allen and MacMahon (*6*) found that stored, respread topsoil had significantly lower phosphorus, nitrogen, and organic matter than soil from an undisturbed site. These changes were correlated with changes in soil fungal characteristics. Fungal hyphal length was low at the disturbed site, and the hyphae were randomly dispersed; no VAM hyphae or spores were observed. The undisturbed site had distinct, consistent patterns in terms of the soil and fungal characteristics measured. Organic matter, total fungal, VAM spore counts, and hyphae length were significantly higher under shrub vegetation than in the areas between shrubs (*6*). Additionally, the presence of mycorrhizae in the organic or litter layers may aid in the cycling of nutrients in the ecosystem, as well as play a role in nutrient conservation.

Trace Elements

The presence of trace elements (heavy metals) in the soil may also affect mycorrhizae-plant associations. Mycorrhizal fungi are known to enhance the uptake of essential metal micronutrients such as copper, zinc, manganese, and iron, whose mobility and concentration in soils are low. Kothari (*59*) found that the concentrations of zinc and copper in *Zea mays* L. were increased 1.7 to 3.8 times for zinc and 2 to 3 times for copper because of mycorrhizal inoculation. This feature of enhanced uptake of metals by mycorrhizae would be a disadvantage for both the fungi and host when metals are present in the soil at toxic concentrations.

Dixon and Buschena (*34*) found that the addition of heavy metals to potting media significantly decreased infection by the ectomycorrhizae fungi *Suillus luteus* (L. ex Fr.) S.F. Gray with *Pinus banksiana* and *Picea glauca* (Moench) Voss. High levels of cadmium, copper, lead, and nickel eliminated ectomycorrhizal formation of the roots of *Picea glauca*. Additions of cadmium and nickel above 10 mg kg^{-1} significantly reduced ectomycorrhizal formation on *Pinus banksiana* roots. The ability to form mycorrhizal structures, such as the fungal mantle, was suppressed by cadmium, copper, lead, and nickel; however, this occurred only at higher concentrations. At low and intermediate soil metal concentrations, the fungal mantle formed by ectomycorrhizae ameliorated the toxicity effects of heavy metals (*34*). The inability of mycorrhizae to ameliorate heavy-metal toxicity at higher concentrations suggests that the protective mechanism of binding metals to root or fungal structures or the precipitation of metal oxalates in intercellular space of the fungi or plant had reached a saturation point.

The degree of metal tolerance of mycorrhizal fungi may be an important factor to the host plant (*28*). Not all fungi are equally effective. Colpaert and Van Assche (*28*) suggest that in soils with high concentrations of heavy metals, mycorrhizal fungi are submitted to a strong selection for metal tolerance. As an example, five of six ectomycorrhizal isolates collected at a zinc-contaminated site (average zinc concentration of 3,166 $\mu g\ g^{-1}$) were strongly zinc tolerant, while those same isolates collected at a noncontaminated site were not tolerant. These researchers also found that the mechanisms for specific metal tolerance were different; there is an implication of a specific biochemical mechanism for metal tolerance, since a strong zinc tolerance does not result in a copper tolerance and vice versa. Since the external mantle of mycelium of ectomycorrhizal fungi is involved in the uptake of minerals, the retention of heavy metals by these fungal tissues may be important in excluding metals from the plant. Colpaert and Van Assche (*28*) suggest that fungi with an extensive external mantle of mycelium may be more suitable to accomplish a filter function than fungi without this feature.

Harris and Jurgensen (*49*) found that no mycorrhizal development occurred on willow (*Salix* spp.) or hybrid poplar (*Populus* spp.) when grown on copper tailings, indicating that elevated copper levels were inhibitory to the formation of mycorrhizae. In the same experiment, roots of willow and poplar formed abundant mycorrhizae in iron tailings, indicating that iron tailings were not inhibitory to the formation of mycorrhizae-plant associations. Substrate pH was not a factor since the pH of the copper tailings ranged from 7.7 to 8.1 and the pH of the iron tailings ranged form 7.4 to 7.5 (*49*).

In a study to evaluate and characterize the role of mycorrhizae in uptake and accumulation of heavy metals by *Pinus taeda* under different concentrations and pH levels, Wong (*124*) found that the metal concentration in the roots decreased with increasing solution acidity. The concentration of aluminum, lead, and zinc in the roots was not significantly different between mycorrhizal and nonmycorrhizal roots exposed to different metal concentrations and pH levels. There were, however, morphological changes in the mycorrhizal structure due to pH and metal treatments. The fungal mantle of mycorrhizal roots exposed to 1 mg kg^{-1} lead at pH 3 had deteriorated more than after exposure at the same lead concentration, but at pH of 4 or 5. Additionally, cell modifications occurred in some of the root cortical cells adjacent to the Hartig net hyphae. Exposure to 50 mg kg^{-1} lead at pH 3 resulted in failure of the fungal mantle and Hartig net development and loss of mycorrhizal symbiosis (*124*). When exposed to different aluminum concentrations and pH levels, roots became mycorrhizal at all treatment levels; however, there was a decrease in the percentage of mycorrhizal infection at pH 3 (*124*).

Aluminum in solution reduces foliar phosphorus concentration by chemically binding phosphorus in the root-free space, reducing its availability for plant uptake and translocation (*25-26*). In a study on how ectomycorrhizal association between *Pisolithus tinctorius* and *Pinus rigida* Mill. altered seedling response to aluminum in the rhizosphere, Cumming and Weinstein (*29*) found that inoculation enhanced seedling growth and prevented development of aluminum toxicity symptoms. At a concentration of 200 μM aluminum, shoot and root growths of *Pinus rigida* seedlings were reduced by 47 and 45 pct, respectively, compared with control values. Additionally, nonmycorrhizal seedlings exposed to 200 μM aluminum exhibited a 44-pct increase in water loss, but aluminum exposure did not affect transpiration in seedlings inoculated with mycorrhizae. *Pinus rigida* seedlings inoculated with *Pisolithus tinctorius* exhibited unaltered growth, physiological function, and ionic relations when exposed to aluminum (*29*). The reduction in foliar aluminum in mycorrhizal seedlings suggests to Cumming and Weinstein (*29*) that the presence of a vigorous mycorrhizal symbiont inhibits the passage of aluminum into the stele. Further, these researchers suggest several possible mechanisms for this inhibition, including the following: (1) fungal tissue surrounding the pine roots may act as a sink for aluminum, binding it to cell wall sites and extrahyphal slime; (2) the fungus may segregate aluminum internally; (3) infected roots may exclude aluminum; or (4) the fungus may produce and excrete organic substances that chelate, detoxify, or alter the chemical activity or availability of aluminum in solution.

Physical

Soil Water

Soil moisture is very important for the formation of mycorrhizae through a complex of direct and indirect effects that soil water has on mycorrhizal infection and growth promotion (*78, 94, 96, 104*). Miller (*74*) found that soil moisture characteristics were significantly correlated to propagule levels, while soil chemical characteristics were not. Soil moisture is a part of the soil-plant-atmosphere water continuum; moisture is important in terms of its availability for uptake by the plant and the maintenance of the plant water status. Indirectly, soil moisture is important through its effects on nutrient availability, aeration of the soil, root development, and microorganism activity (*97*).

Mycorrhizal fungi vary in their response to soil moisture conditions. The abundance of ectomycorrhizal fungi has been correlated with soil moisture levels; in very dry or very wet soils ectomycorrhizae may be absent. Excessive soil moisture has been shown to limit the formation of ectomycorrhizae because of oxygen deficiencies that limit the development of the fungus and root system. Under anaerobiosis, because of excessive soil moisture, reducing conditions exist that may result in the production of toxic substances such as hydrogen sulfide, manganese, and other organic acids, which limit mycorrhizal development. The tolerance of mycorrhizal fungi to excess soil moisture is dependent on the physiological state of the roots and their ability to supply oxygen to the fungus (*104*).

Different groups of fungi vary in how they are affected by excessive soil moisture (*109*). Several fungal groups have hydrophobic mycelium, which create air pockets in the soil; under saturated conditions these fungi should be able to survive without a decrease in activity or colonization (*109*). Soils that are poorly drained and remain saturated for long periods of time will decrease mycorrhizal infection efficiency (*97*). McAfee and Fortin (*67*) found that drainage was an important factor in colonization of indigenous ectomycorrhizae on *Picea mariana*. Mycorrhizal infection increased in the well-drained sites.

Dry soil moisture conditions can also limit ectomycorrhizae. Redmond (*92*) found that excessively dry soil conditions resulted in death of ectomycorrhizae. Restoration of normal soil moisture levels following periods of drought may result in reinfection of regenerated roots. Studies have shown that soil moisture deficits decreased the abundance of mycorrhizae formed by all fungi tested except *Cenococcum geophilum* (*73, 126*). This species is tolerant to lower water potentials and exhibits maximum growth at a water potential of −1.5 MPa when grown in pure culture. Hacskaylo (*47*) attributed the more vigorous growth under drought conditions to reduced vigor of competing fungi. In general, ectomycorrhizal abundance and infection is greatest between the extremes of excessive and deficient soil moisture contents, between field capacity (−0.033 MPa) and the permanent wilting percentage (−1.5 MPa).

VAM occur over a wider range of soil water contents than do ectomycorrhizae (*78*). Infection of plants by VAM has been found in the arid regions of the United States and in the marsh soils. Mycorrhizal phosphorus supplies are likely to be more advantageous for plant growth under arid conditions than under wet conditions since the diffusion coefficient of phosphate in soil is linearly related to soil moisture content (*40*). Most aquatic plants and plants growing in very wet areas are generally nonmycorrhizal. The lack of VAM formation under saturated conditions has been attributed to low availability of oxygen (*57, 98*).

Spore germination of VAM fungi is best at soil moisture contents between field capacity (−0.033 MPa) and soil saturation (*53*). Spore germination decreases with decreasing water potentials below in situ field capacity. Root infection by VAM fungi is usually most rapid when soil water content is between field capacity and the permanent wilting point. Miller (*74*) demonstrated that survival of VAM propagules in stockpiled topsoil is related to the soil-water potential under which the soils were stored. Bioassays of stored soils taken from stockpiles with a high moisture content produced less infection then did soils taken from dryer stockpiles. Data indicate that if soils are to be stored, stockpiling should be limited to time periods when soil water potentials are below −2 MPa to ensure propagule survival (*74*).

As was the case for ectomycorrhizae, the optimum water content for plant growth is also the optimum for root infection by VAM. Cerligione (*21*) reported an overall decrease in percent VAM colonization of *Schizachyrium scoparium* (Michx.) Nash as soil water availability decreased.

Aeration

The oxygen content of surface layers is relatively high and usually stable, being sufficient for mycorrhizae-root associations (*78*). An exception occurs when anaerobiosis is induced by waterlogging, as discussed previously, in the section on fungi. Low oxygen availability in flooded soils has been shown to be partially responsible for the inhibition of ectomycorrhizal formation (*48*). Miller (*73*) reported that edaphic factors associated with high VAM potential were related to soil and plant moisture availability and soil aeration. Positive associations were found for sand and porosity and negative associations for water-retention ability and silt. The carbon dioxide concentration in soil air varies widely, and the possible effects of carbon dioxide concentration on mycorrhizae are not known (*78*). The results of several studies have implied that the lack of oxygen reduces mycorrhizae infection through its effects on spores and their germination since oxygen is transported from plant shoots to roots.

Temperature

Mycorrhizal development is thought to be strongly temperature dependent, particularly where soil temperature varies considerably with the season (*78*). Mosse (*78*) states that in temperate zones soil temperatures may be the major limiting factor in infection and spread of mycorrhizae. Soil temperature is important for several reasons. Excessive soil temperature can reduce seedling establishment by direct injury to tissues in germinating seedlings, by its regulatory effect on root metabolism, which influences water and nutrient uptake by root systems, and by its influence on the growth and infection processes of mycorrhizal fungi (*94*). Plant species differ in their response to temperature in growth, translocation, root extension, and photosynthesis, and these differences impact mycorrhizal establishment and function (*97*). Extreme temperatures not only restrict the growth of tree seedlings, but also limit the ability of mycorrhizal fungi to function (*42*). Temperature requirements of mycorrhizal isolates must be determined to culture inoculum effectively, to attain good mycorrhizae development following inoculation, and to maintain satisfactory survival and growth (*118*).

In general, optimum temperature for ectomycorrhizal growth is greater than the temperatures the fungi would encounter in the field (*78*). Although mycorrhizae infection may occur at lower temperatures, higher soil temperatures generally accelerate the infection process. Dixon (*33*) imposed four soil temperature treatments on *Quercus veluntina* Lam. seedlings inoculated with the ectomycorrhizae fungus *Pisolithus tinctorius* to assess the influence of soil temperature on ectomycorrhizal development and root and shoot growth. Temperature treatments were 18°, 23°, 28°, and 33° C. As soil temperature increased, seedling root and shoot growth decreased, but ectomycorrhizal development increased. No *Pisolithus tinctorius* was found on the seedlings at 18° and 23° C, small amounts were identified at 28° C, and significant amounts were present at 33° C. In a second experiment, Dixon (*33*) found that ectomycorrhizal development at 31° C was 2.5 times greater than that observed at 26° C. *Pisolithus tinctorius* has been shown to be tolerant of high temperatures (*65*).

Cline (*27*) studied isolates of four ectomycorrhizal fungi in pure cultures to determine their response to temperature. Isolates of *Pisolithus tinctorius*, *Cenococcum geophilum*, *Suillus granulatus* (L. ex Fr.) O. Kuntze, and *Thelephora terrestris* Ehrh. ex Fr. were used. Liquid culture plates of geographically distinct isolates of each mycorrhizal fungus were placed in incubators without light at 16°, 21°, 27°, 32°, and 38° C. Each isolate exhibited one of six general patterns of mycelial growth in relation to temperature: (1) normal distribution, (2) skewed to the left (higher temperature optimum), (3) skewed to the right (lower temperature optimum), (4) gradual decrease in growth with increased temperature, (5) rapid decrease to zero growth with increased temperature, and (6) constant low level of growth over all temperatures.

Pure culture growth of *Pisolithus tinctorius* was more tolerant of high temperatures than any of the other fungi tested (*27*). Optimal growth occurred between 21° and 32° C, depending on the isolate considered. Northern latitude isolates generally had lower optimal temperatures for mycelial growth than did southern latitude isolates. Changes in *Pisolithus tinctorius* growth habit in response to temperature included a change in color of mycelium from golden brown at low temperatures (16° to 27° C) to bright yellow at high temperatures (32° and 38° C) and earlier cessation of growth at high temperatures than at low temperatures.

The temperature optimum for *Cenococcum geophilum* mycelial growth occurred at 27° C; however, growth occurred at temperatures between 16° and 27° C, depending on the isolate considered. Optimal growth for *Suillus granulatus* also varied with the isolate used; however, mycelial growth occurred primarily at 21° through 27° C, with some growth occurring at 32° C. Isolates of *Thelephora terrestris* exhibited mycelial growth at all temperature treatments except 38° C. The high degree of variability in mycelial growth in pure culture between isolates of the same species in response to temperature suggests to Cline (*27*) that fungal genotype can significantly influence growth study results and that particular isolates and species may be better suited for mycorrhizal inoculation programs.

In a study on the influence of temperature on the mycorrhizal associations of *Pinus radiata* D. Don, Theodorou and Bowen (*115*) found that there were significant mycorrhizal growth differences at temperatures between 16° and 33° C, with optimal growth occurring between 23° to 25° C, and with significant declines between 20° and 15° C and at temperatures greater than 25° C. Poor colonization by mycorrhizal fungi at 16° C compared with 20° C indicates the necessity to select fungi for field inoculations based on root colonization at soil temperatures appropriate to the area and season as well as on the ability of the mycorrhizae to stimulate plant growth (*115*).

It has also been demonstrated that certain mycorrhizal fungi have evolved that are adapted to low temperatures. Grossnickle and Reid (*44*) inoculated three conifer species with three ectomycorrhizal species and established these seedlings on a high-elevation site (3,200 m) in Colorado. Seedling height and diameter growth, bud activity, and survival were monitored. After four growing seasons, seedlings inoculated with *Suillus granulatus* had greater growth rates than seedlings inoculated with *Pisolithus tinctorius* or *Cenococcum geophilum*. Examination of the root systems of *Pinus contorta* Dougl. and *Pinus flexilis* James showed extensive growth of *Suillus granulatus* and a native fungus on the root systems, while *Pisolithus tinctorius* and *Cenococcum geophilum* were not found on the roots (*44*). Root systems of *Picea engelmannii* Parry had low levels of root infection for all mycorrhizal treatments. Grossnickle and Reid (*44*) state that both *Pisolithus tinctorius* and *Cenococcum geophilum* were not adapted to the environmental conditions of this high-elevation mine site. Lack of development by these mycorrhizal fungi was attributed to high soil pH, short growing season, and cooler soil temperatures (*43*).

Parke (*84*) studied the effects of root zone temperature on native ectomycorrhizal and VAM-forming fungi in southwestern Oregon using soil from disturbed (clearcut) and undisturbed sites. *Pseudotsuga meniessii* (Mirb.) Franco, *Pinus ponderosa* Dougl. ex P. Laws & C. Laws, and *Trifolium subterraneum* L. were grown at 7.5°, 13°, 18.5°, 24°, 29.5°, and 35° C. After 14 weeks (*Pseudotsuga meniessii* and *Pinus ponderosa*) or 10 weeks (*Trifolium subterraneum*), mycorrhizal formation was determined along with root growth characteristics. Optimal temperature for the formation of both ectomycorrhizae and VAM occurred when soil temperatures ranged from 18.5° to 24° C. This was true for both disturbed and undisturbed soils. In the soils studied, mycorrhizal formation was moderate at 7.5° and 13° C, but was reduced or prevented at or above 29.5° C (*84*). Temperature optima for mycorrhizal development reflected the adaptation to climate and coincided with the temperature optima for active root growth (*84*). The native fungi identified in the study were *Cenococcum geophilum* (ectomycorrhizae) and *Glomus tenue* (Green.) Hall (VAM). In areas that are to be reforested or afforested, soils should be inoculated with the mycorrhizal fungi having the proper temperature tolerances. Additional research is needed to evaluate the success of ectomycorrhizal fungi on plants used in cold-climate reclamation.

Variations in soil temperature affect the development of VAM; however, comparisons between endophytes from different climates are lacking, indicating that it is possible that other strains and species may be temperature adapted (*78*). Mosse (*78*) states that since most species of VAM fungi are worldwide in their distribution, it is likely that temperature adaptation is common.

In general, temperatures higher than ambient are preferred for the enhanced development of VAM fungi in plant roots (*79*). The temperature range at which VAM develop depends on the fungal species and the environments to which they are ecologically adapted (*99*). Schenck and Smith (*101*) showed that colonization of *Glycine max* roots, sporulation, and plant growth enhancement by six species of VAM fungi were temperature dependent. The degree to which temperature affected colonization, sporulation, and plant growth varied with the mycorrhizae species tested.

Allen (*5*) surveyed the occurrence of VAM spores and root infection in several successional alpine areas of the Beartooth Mountains, MT. To determine the yearly and seasonal variation in mycorrhizae, Allen examined roots from undisturbed and mined areas that either were abandoned (approximately 25 years old) or had been revegetated 3 and 7 years previously. Mycorrhizal spore counts and percent root infection varied among species, areas, and collection dates (*5*). Plant colonization was slow in the alpine area, but once plants were established mycorrhizal fungi colonized quickly. The 3-year-old revegetated area had infection equaling that in some undisturbed areas. The revegetated areas had only 1 mycorrhizal species, while the undisturbed and older areas had more than 11 species (*5*). As with ectomycorrhizae, it is important to inoculate areas with the VAM fungi having the proper temperature tolerances. Owing to slow host growth in mountainous or cool-temperature climates, prolonged persistence of introduced symbionts is essential; this must be monitored to determine the real effects of inoculation (*31*).

Light

Light or solar radiation provides two basic needs of plants: (1) establishment of a satisfactory thermal environment and (2) energy required for photosynthesis (*94*). Several experiments have shown that mycorrhizal development is affected by light received by the shoot of the host plant (*78*). Research has shown that high light intensities favor the establishment of both ectomycorrhizal and VAM fungi. Photoperiod or day length has also been shown to have positive effects on mycorrhizal colonization. It is not clear whether the effects of light intensity are determined by the total light energy received or whether photoperiod and intensity have separate effects (*78*). Additional research is needed on the effects of light on mycorrhizae development in plants.

FIELD INOCULATION AND INOCULUM TECHNOLOGY

Deliberate inoculation of soil and plants with specific mycorrhizal fungi may have one of the following objectives: (1) to increase total inoculum potential or (2) to establish more efficient fungal symbionts than those present (*78*). Mosse (*78*) states that two general principles apply to field inoculations: first, for strongly mycorrhizae-dependent plant species, any mycorrhizal fungus is better than none, and second, indigenous mycorrhizal fungi are not necessarily the most efficient, particularly when soil conditions are changed or new plant species are introduced. Mycorrhizal inoculum will probably lead to successful mycorrhizal infection in the field when viable inoculum is placed in the root zone of actively growing plants that are not heavily fertilized (*71*).

FIELD INOCULATION

There are four basic types of inocula available: (1) colonized soil, (2) infected roots, (3) pure cultures of fungi, and (4) spores (*78*). Additionally, microbial populations have been introduced into soil through various organic soil amendments. Sundberg (*112*) found increased fungal populations in sludge-amended coal mine spoil and attributed some of this increase to the fungi present in the sewage sludge prior to its application. Danielson (*32*) determined that peat used as an organic soil amendment contained viable VAM propagules, indicating the importance of specific amendments in the rapid reestablishment of VAM-plant associations. There are various techniques available for introducing mycorrhizae inoculum into the field. Ectomycorrhizal and VAM inoculation procedures include broadcast inoculation, banding inoculum below the seeds, slurry dips, basidiospore inoculations (ectomycorrhizae only), pelletizing seed, and mycorrhizal seedlings and roots (*95*).

Broadcast inoculation involves spreading a known quantity of inoculum over a given area of soil surface and mixing the inoculum into the soil to a depth of 10 to 20 cm before seeding (*95*). Pine duff, sporocarps and spores, and pure culture vegetative mycelium have been applied in this manner.

Inoculum placement below the seed in a layer or band facilitates the concentration of inoculum near developing roots (*95*). Most types of mycorrhizal inoculum can be applied using this technique. The banding inoculation technique requires one-third as much inoculum as the broadcast method (*95*). Banding or side-dressing with mycorrhizal inoculum next to seedlings or seeds is also effective (*71*). Band placement must be in the zone of root proliferation to be effective.

Slurries of mycorrhizal inoculum are prepared by mixing the inoculum with water and a carrier (*95*). Bare-root or container-grown seedlings are inoculated by dipping them into the slurry prior to planting.

Basidiospore inoculation (ectomycorrhizae only) and VAM spore inoculation involve suspending spores in water and leaching them into soil, mixing dry spores directly into the soil, coating seeds with spores prior to sowing, dusting spores on roots of nonmycorrhizal seedlings, or mixing spores in a carrier and applying them after sowing (*95*). Spores applied to the soil should be incorporated. This method is advantageous, but more information is needed on spore transport and storage, viability, inoculum concentration, and application methods.

Coating seeds with spores involves incorporation of basidiospores in an external matrix of encapsulated seed (*95*). Adhesives such as methyl cellulose have been used to coat seeds with mycorrhizae (*71*). VAM inoculum has also been formulated into a nonseed granule or a multiseeded pellet (*90*). Pelleting forces the seedling radicle to pass through the inoculum, becoming infected with the fungi contained in the pellet before coming in contact with indigenous mycorrhizal fungi in soil. Pelleting is accomplished with either fungal spores or soil inoculum. Multiseeded pellets may impair seed germination through competition for soil moisture. Additionally, pelleted seeds are bulky and restrict the position of mycorrhizal inoculum to the seed surface (*90*). Nonseeded granulated inoculum has the advantage that it is commercially available, can be stored for several months with minimal loss of viability, and is suitable for machine drilling (*90*). Granulation, however, can depress inoculum viability.

Transplanting mycorrhizal seedlings has been a successful inoculation method. Mycelia spread to adjacent noninfected seedlings. Mycorrhizal roots have also been used successfully for inoculating seedlings. Roots with abundant mycorrhizae should be selected as inoculum and should be incorporated while fresh and prior to sowing seeds (*95*).

One of the greatest impediments to VAM inoculation in the field is the amount and weight of inoculum required (*90*). Pure cultured inoculum of VAM fungi is not available, and field plots have been inoculated by placing VAM-infested soil below each seed. Rates have ranged from 2 to 50 g soil per seed, with very high inoculum rates (100 to 167 st ha^{-1}) used to establish mycorrhizal plants on coal spoils. Average soil depth used was 10 to 16 mm (*90*).

INOCULUM TECHNOLOGY

Successful production of ectomycorrhizal seedlings is dependent on the type and age of inoculum used, timing of inoculation, inoculum density, inoculum placement in the growing medium, and host and fungus interactions (*95*). Riffle and Maronek (*95*) list the times when mycorrhizal inoculum can be applied: (1) before seeds are sown, (2) when seeds are sown, and (3) after seedlings emerge. The most efficient times are before or when seeds are sown, or when cuttings are propagated.

No ectomycorrhizal fungus can complete its life cycle in the absence of mycorrhizal association. Additionally, these fungi cannot grow saprophytically in natural soils. As a result, inoculation programs must place the inoculum in the rooting zone of seedlings where roots can grow into the inoculum (*66*). Several types of natural and laboratory-produced inocula have been used, including soil, spore, and vegetative inoculum (*66*).

Soil containing natural inoculum is the most widely used medium of inoculation. The best results are obtained when freshly collected soil is used rather than soil that has been stored for several months. A major problem with soil inoculum is that the species of ectomycorrhizal fungi in the inoculum cannot be controlled, so it is possible that the most desirable fungi for the association may not be present. Additionally, soil inoculum may contain harmful microorganisms and noxious weeds along with the ectomycorrhizae fungi (*66*).

Successful inoculations with specific ectomycorrhizae on tree seedlings have resulted when fungal sporophores and spores have been used as inoculum. Inoculum composed of spores is mixed with a carrier and used with various field inoculation methods (broadcast, seed coating, pelletizing, etc.). Marx and Kenney (*66*) list several advantages and disadvantages of using spore inoculum. Major advantages of spore inoculum include the following: (1) they do not require an extended growth phase; (2) they are light weight; and (3) they have long survival. Disadvantages are as follows: (1) there is no standard laboratory test to determine spore viability; (2) sufficient sporophores may not be available every year; (3) it takes time for spores to germinate and infect a root—it may take 3 to 4 weeks longer than vegetative inoculum for some fungi; and (4) there is a lack of genetic definition.

The most biologically sound method of inoculation is the use of pure mycelial or vegetative inoculum (*66*). Ectomycorrhizal fungi, however, are difficult to grow in the laboratory. Selection and production of vegetative inoculum involve several steps, which are outlined below.

The most important step in an inoculation program is selection of the fungi (*66*). Among ectomycorrhizae there are physiological differences that can be used as a criterion for their selection. Host specificity is a physiological trait that should be considered in the selection process, and the fungus selected should have the physiological capacity to form extensive ectomycorrhizal development on the desired host (*66*). A second criterion that should be considered is isolate variability within any candidate symbiont. Several isolates from different tree hosts and geographic regions should be used to determine the amount of variation that exists between isolates (*66*). The selected fungus should also be able to grow in pure culture and withstand manipulation. Once growth characteristics of a fungus are confirmed, its capacity to withstand physical, chemical, and biological manipulations should be determined. Production of large quantities of vegetative inoculum of a fungus is of little value if the inoculum cannot survive manipulation.

Adaptation of the selected fungus to the site on which it is to be used is another important selection criterion. The selected fungus must be adapted to a wide range of environmental and edaphic factors present at the planting site. The fungus must be able to form ectomycorrhizae on seedlings as soon as the roots are produced. Marx and Kenney (*66*) present several examples of cultural procedures developed for producing vegetative inoculum for a variety of fungi.

Inoculum of VAM fungi may consist of spores, mycelia, and infected root pieces containing vesicles or chlamydospores. The most common method for producing VAM inoculum, however, is the soil culture method (*39, 70*). Inoculum is supplied from infected soil, roots, or soil sievings (*51*). Spores of the various species are removed from soil or roots and used to establish pot cultures (*39*). Spores produced from these cultures are removed by wet sieving, and species are identified and isolated. Identified spores are then used to establish new pot cultures. Establishment of axenically grown plants inoculated with single spores is the most desirable way to establish pure cultures of VAM fungi. Results of several experiments, however, have shown that synthesis of VAM occurs more rapidly when mycorrhizal root pieces rather than spores are used as inoculum (*1, 16*). Duvert (*36*) states that, from a physiological point of view, standardization of inoculum is easier with infected root pieces than with spores.

CONCLUDING STATEMENT

Mining wastes initially lack viable mycorrhizal fungal populations, and thus, the establishment of a vegetative community will be delayed. Mycorrhizae inoculations can enhance the productivity of mine waste sites if mycorrhizae are absent, available soil phosphorus is low, and the plant species used in revegetation respond to mycorrhizal infection. Mycorrhizal inoculation of mine waste may be critical for the establishment of a viable, diverse, and self-sustaining plant community.

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