



The Use of Mycorrhizae in Mined Land Reclamation

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Mined land sites are generally known to be nutrient poor and contain soils that are in dire need of stabilization to prevent erosion. Marked by the beginning works of J. R. Schramm, mine reclamation practices have included the use of mycorrhizal inocula to establish successful plant communities on mined sites (Danielson 1985). Mycorrhizae benefit the vegetation by increasing a plant's ability to survive in a nutrient poor and water deficient environment (Norland 1993). In undisturbed ecosystems, mycorrhizal relationships occur naturally. Mined sites, however, are chemically, physically, and biologically altered and often lack the necessary quantity of mycorrhizal fungi to sustain a tolerant plant community (Norland 1993). Several types of mycorrhizal inocula are currently in use and will be examined according to their practicality and economy to site specificity.

Benefits of mycorrhizae on mined land sites

Five types of mycorrhizae are recognized – ectomycorrhizae (ECM), vesicular-arbuscular mycorrhizae (VAM), and three others that are species restricted (Ericoid, Orchid, and ectendomycorrhizae) (Norland 1993). ECM are common to woody plants and characterized by a sheath of hyphae that surround the plant root. VAM penetrates the cell walls of the plant and forms arbuscules (used for nutrient transfer) and vesicles (recognized as sites of lipid storage) within the roots. VAM is host obligate, while ectomycorrhizae can exist without a plant host, but needs one to complete its life cycle. Both ECM and VAM can be found on mined sites, but VAM species have been found to colonize mine wastes more than ECM (Danielson 1985).

Dissemination can occur in several ways. Spores can remain ungerminated for an extensive period of time during unfavorable conditions. They can be transferred via water drainage, wind, and redeposited by animals after consumption. The spores of mycorrhizal fungi have been shown to survive the digestive processes of small animals and insects and still germinate (Ponder 1980). Also, infection does seem to occur more rapidly through the spread of an infected root to an uninfected root than by spore germination (Rives 1980). Mined lands are usually deficient in natural plant cover and lack the necessary mycorrhizae to establish a root system to stabilize the soil, maintain moisture, and obtain nutrients (Helm and Carling 1990). The top soil is usually removed during mining which eliminates natural inoculation on the site (Rives et al. 1980).

Mining also affects many soil factors, such as pH, fertility, toxicity, bulk density, and soil moisture, which, in turn, reduce the VAM propagules in the soil that would be necessary for mycorrhizal dependent plants to thrive (Fuge 1986).

Mycorrhizal fungi act as providers and protectors for plants. For example, nitrogen, phosphorus, and potassium are deficient in mine soils and tailings and can be increased in plant intake by mycorrhizae (Norland 1993). Other essential nutrients such as calcium, magnesium, sulfur, iron, zinc, aluminum, and sodium have shown increases in plant intake with mycorrhizal fungi (Daft and Hacskeylo 1976). Higher concentrations of metals which can be harmful to plants (such as

aluminum, arsenic, barium, boron, cadmium, copper, iron, lead, manganese, nickel, selenium, and zinc), can exist on mined sites and, at the same time, be filtered to tolerable amounts by the fungi for the plant (Norland 1993). Increasing plant hormones and acting as a barrier to plant pathogens are other benefits to the plant provided by mycorrhizae (Fuge 1986). Mycorrhizae can also alleviate the stress of higher surface temperatures and acidity that mined sites may have (Danielson 1985). Not only can mycorrhizae improve plant growth, but provide some resistance to drought and salinity as well (Duvert et al. 1990).

Mycorrhizal inoculation techniques

Several factors should be considered before applying any type of inoculum. First, effectiveness of inoculation form is usually dependent on fungi species, climate, and ecosystem (Abbott and Robson 1981). Second, a particular fungus may not necessarily do as well with a host in different environmental conditions or with the same host under varied environmental conditions (Abbott and Robson 1981). Also, inocula, in general, will be most successful when applied in the root zone of actively growing plants without heavy fertilization (Norland 1993). Inoculum sources should be given strong considerations. Inocula from old mine spoils with established vegetation and from neighboring undisturbed sites are valuable sources (Danielson 1985, Helm and Carling 1990). Finally, with all types of inoculum, there are practical difficulties of storage, reactivation and applications on a large scale (Mosse et al. 1981).

Mycorrhizal inoculum comes in four forms for application – infested soil, infected plant roots, pure cultures of fungi, and spores (Mosse et al. 1981). The earliest attempts to introduce mycorrhizal fungi was with infested soil (Mosse et al. 1981). Soil inoculum is the most widely used natural inoculum (Marx and Kenny 1982). Peat, sludge, stockpiled soil, and soil from neighboring sites are all sources for soil inoculum (Norland 1993). An advantage of using soil inoculum is that it could contain nitrogen fixing bacteria. Also, soil inoculum consists of spores, roots and hyphae that all may be able to inoculate seedlings (Helm and Carling 1990). Application and collection of soil inoculum is easier than other types (Helm and Carling 1990). Disadvantages are that the species within the soil cannot be controlled, there is no guarantee the desired fungi are contained in the soil, and the actual transportation of large loads of soil is difficult and costly (Marx and Kenny 1982).

The first successful use of root pieces to inoculate seedlings was in 1958 by Peuss, Winter and Meloh (Biermann 1983). Root pieces tend to be lighter than blended pot culture material, colonize more rapidly than spores, and contain intraradical vesicles that have been found to be viable after two years of cold storage in pot cultures (Biermann 1983). Disadvantages of root pieces include an increased potential of introduced pathogens and varied infectivity (Biermann 1983). Pathogens can be reduced if proper sanitation is practiced and a surface sterilant is applied to destroy some of the organisms. Care should be taken in the application of the sterilant since large amounts may kill the fungus (Biermann 1983).

Spores as inoculum date back to the eighteenth century (Marx and Kenny 1982). Spores do not require an extended growth phase and are lightweight (Norland 1993). Also, spores can be collected and stored for years (Marx and Kenny 1982). Although spores can be viable for an extended amount of time, there is no standard laboratory test to determine spore viability

(Norland 1993). It may also take 3 to 4 weeks longer than vegetative inoculum to germinate and infect a root. Other disadvantages include a possible insufficient availability of sporophores every year and lack of genetic definition of the spores(Norland 1993).

The most biologically sound inoculum is vegetative (Marx and Kenny 1982). The fungus should be able to grow in pure culture and withstand manipulation (Norland 1993). A commercial formulation of mycelial inoculum has been produced called MycoRhiz (*Pisolithus tinctorius*) (Marx and Kenny 1982). The most successful results of MycoRhiz showed when an abundance of hyphae, a pH between 4.5 and 6.0, low levels of bacterial and fungal contaminants, and low amounts of residual glucose (Marx and Kenny 1982). ECM are, unfortunately, difficult to grow in the laboratory and large amounts of inoculum for large scale application are scarce (Marx and Kenny 1982).

Several techniques are available to apply inocula. Two common techniques are broadcasting inoculation at the soil surface and banding inoculum at the root zone (Riffle and Maronek 1982). Broadcasting has been used for soil, spore, and vegetative inoculum (Norland 1993). Broadcasting consists of spreading a known amount of inoculum over an area of soil surface and then mixing the inoculum into the soil to a depth of 10 to 20 cm. before seeding (Riffle and Maronek 1982). Large amounts of inoculum are also needed for broadcasting to ensure complete coverage of seedbeds (Riffle and Maronek 1982). Banding inoculation, on the other hand, requires only a third of the amount of inoculum as broadcasting since the inoculum is placed in a band in a concentrated area near developing roots (Norland 1993). Side-dressing of the inoculum is also effective with seedlings or seeds (Norland 1993). When using container seedlings, banding inoculum is more effective than the broadcasting technique (Riffle and Maronek 1982). Banding inoculation has been shown more effective than seed pelleting (Menge and Timmer 1982). Most types of inoculum can be applied with banding inoculation. Banding is time and labor saving compare to other techniques, but does require the use of a machine applicator (Riffle and Maronek 1982).

Slurries and seed pelleting are additional techniques that are successful with the use of spore inoculum (Marx and Kenny 1982). Slurries are a mixture of water, a carrier and the inoculum which roots are dipped into prior to planting (Norland 1993). Seed pelleting consists of coating seeds with spores or soil inoculum (Norland 1993). Nonseed granules are also available for VAM and are advantageous since they can be stored for several months, and can be used in conjunction with machine drilling (Norland 1993). Inoculum viability, however, is affected by granulation (Norland 1993).

Two other techniques are basidiospore inoculation and mycorrhizal seedlings (Riffle and Maronek 1982). Basidiospore inoculation and VAM spore inoculation can be accomplished by mixing spores in water and leaching them into soil or mixing dry spores into the soil (Norland 1993). More information is needed on application methods, concentrations of spores, and transport and storage of spores, but the techniques have been found to be advantageous (Riffle and Maronek 1982). Mycorrhizal seedlings have been used to infect other plant roots . The mycelia from these transplanted seedlings spread to desired plant roots (Riffle and Maronek 1982). Roots with abundant mycorrhizae should be selected and incorporated while fresh (Norland 1993).

Discussion

The use of mycorrhizal propagules have been proposed as a cost efficient long term technique to establish vegetation on mined land (Fuge 1986). Past revegetation efforts have used seed, typically grass and legumes, or transplants of woody species plus fertilization and irrigation (Helm and Carling 1990). Repeated application of fertilizer is expensive and may not reestablish plant communities that are self-sustaining (Helm and Carling 1990). Sludge is an inexpensive source of nitrogen and phosphorus used in mine reclamation, but has been shown to often add heavy concentrations of metals that reduces plant growth and affects VAM productivity (Fuge 1986). Use of local topsoil as a mycorrhizal inoculum improved the growth of balsam poplar and alder on an abandoned coal mine site in Palmer, Alaska (Helm and Carling 1990). Mycorrhizal inoculum used in conjunction with other techniques could improve reclamation success. Dual infections of bacterium and a mycorrhizal fungus resulted in increased vegetative success in coal wastes sites in Pennsylvania (Daft and Hacskeylo 1976).

Mycorrhizae are an essential piece to reclamation success on mined lands. Resources, site and host specificity, environmental conditions and time commitment need to be evaluated to determine which inoculum and technique would be best suited for possible applications.

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