MYCORRHIZAE IN RESTORATION

Reforestation Technologies

BACKGROUND

The "Symbiotic Relationship" between plants and microbes started about 450 million years ago when vegetation began to transition from an aquatic environment to terrestrial locations. Without water bathing their roots and nutrients washing by in the tides and streams, their dry land conditions required the plants to form a pact with microbes that could supply moisture and plant nutrients in exchange for food, especially carbohydrates. This symbiotic relationship between plants and beneficial microbes remains a critical component of natural ecosystem diversity.

BENEFITS OF MYCORRHIZAL INOCULANTS

Mycorrhizal fungi play a very critical role in the plant's soil / microbial matrix by both weight and biomass, it comprises about 80% or more of all the living biology in the soil. It also functions as a vector that connects plants to other soil microbes. When soils are disturbed and the natural biology disrupted, many organisms have the ability to regenerate quickly, however arbuscular mycorrhizal fungi re-colonize sites very slowly. Amending soils with a mycorrhizal inoculant at time of seeding or transplanting can expedite the process of restoring a full consortia of microbes to the site more rapidly.

The benefits of a mycorrhizal colonization include: better establishment of preferred native species, suppression of undesirable non-mycorrhizal species such as mustard, pigweed and other weeds that rapidly develop a monoculture presence. More importantly, mycorrhizal hyphae release Glomalin throughout the soil, known as Super Soil Glue. Glomalin, a soil glue that restructures dirt into living soil. It helps to aggregate soil particles so that space is created for the flow of moisture and air in heavy soils. In sandy soils, the glue binds particles together and creates areas that retain moisture, attract root development a create a knitted web of root, glue and aggregates that more effectively resist the pressures of wind and water erosion of the soil. http://www.ars.usda.gov/is/ar/archive/sep02/soil0902.htm

WHEN ARE MYCORRHIZAL INOCULANTS RECOMMENDED IN RESTORATION PROJECTS?

If soil is stockpiled for over two years, in tall piles without any host plants to maintain a mycorrhizal presence, then an inoculant should be used in any revegetation applications. When seeding or planting is conducted directly into un-amended parent material, mycorrhizal inoculants will also improve regeneration results.

HOW TO TELL IF SOILS HAVE A NATIVE MYCORRHIZAL POPULATION

If the soil has an aggregated structure, or is crumbly with root fragments present that are attached to aggregate soil particles there is a good possibility that there is a mycorrhizal presence. If the soil has no structure, slakes or has an unpleasant smell, then it is likely deprived of mycorrhizal fungi. To determine the presence and amount of mycorrhizal present, a lab test can be done to assess the amount of spores and hyphae in the soil or the amount of mycorrhizal spores in the roots.

WHAT SPECIES OF MYCORRHIZAL FUNGI WORK BEST ON RESTORATION PROJECTS?

Mycorrhizal fungi form associations with 90% of plants. There are four different families of mycorrhizae. Ecto (meaning outside the roots) form associations with many conifer trees including pines, spruce, hemlock, fir, etc as well as some hardwood trees such as oaks and birch. Ericoids, including azaleas, rhododenruns, cranberries and blueberries form an association with ericoid mycorrhizae. Orchids have their own specific mycorrhizae. Almost every other plant with few exceptions form associations with arbuscular mycorrhizae. As most restoration projects use grasses, forbs, and woody plants in the revegetation mix, arbuscular mycorrhizal fungi such as AM 120 (Arbuscular Mycorrhizal inoculant containing 120 propagules per gram or more) works best.

ESTABLISHMENT AND SURVIVAL

Sugarite Mine Restoration Project, Raton NM

Mine Site Photo, 2000

Mine site Photo, 2002

The performance of the restoration strategy which included the use of AM 120 is evident in the photo of the Sugarite Mine. The re-vegetation project was so successful that it received an award from the EPA.

CARBON DIOXIDE SEQUESTRATION

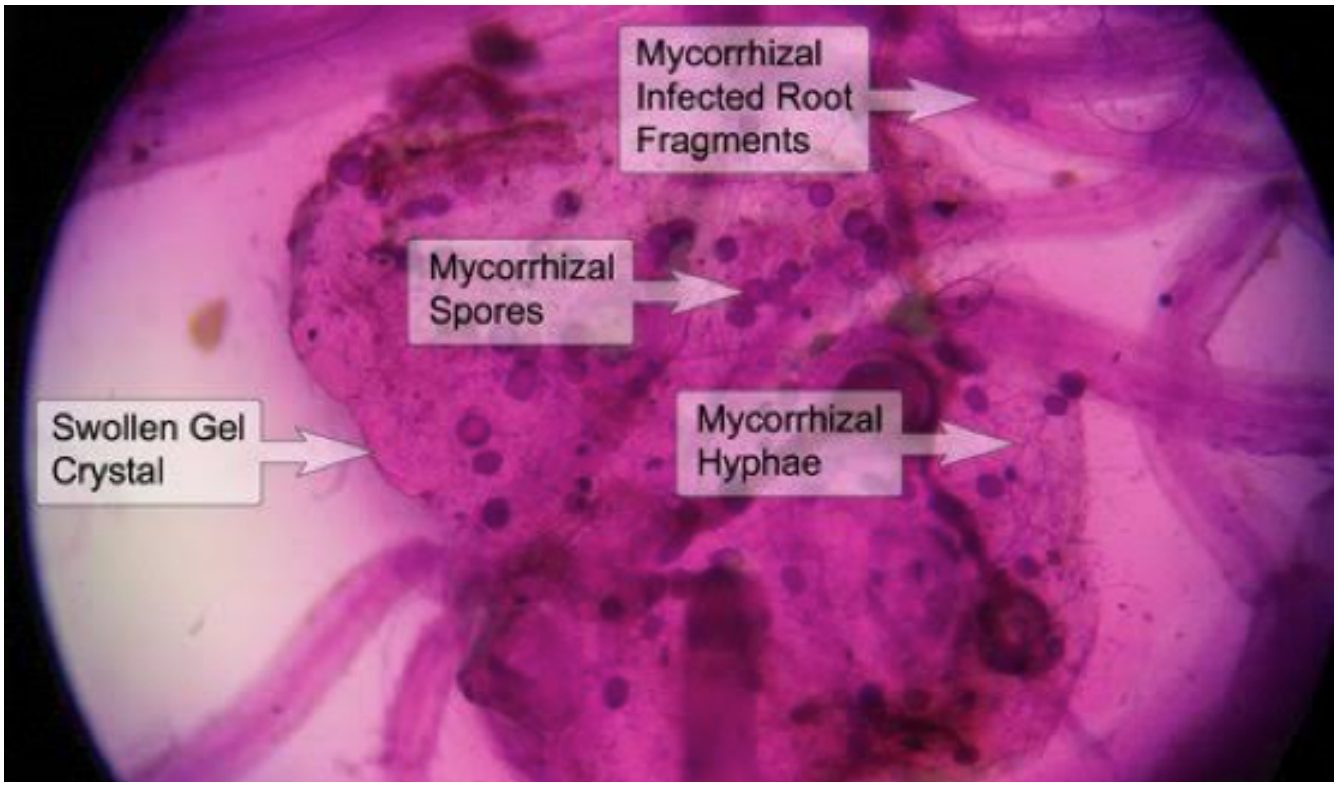
According to research conducted by the USDA/ARS, approximately 30% more CO2 is removed from the atmosphere by plants with a mycorrhizal association. But of equal or more importance, is how the carbon is used by fungi. Much of it is converted Glomalin.

NUCLEAR HAZARDOUS MATERIALS AND HEAVY METAL SEQUESTRATION

The decommissioning of the Nuclear weapons facility in Harrison, OH posed a major environmental challenge. The 300 acre wetland project in the complex required an unusual approach for plant establishment. The site was contaminated with large quantities of Uranium that can enter into solution in the presence of phosphates. The use of conventional fertilizers containing phosphorous could potentially exacerbate the problem. The project management team in charge of the mitigation project chose a program that replaced the phosphate fertilizer with Am 120 mycorrhizal inoculant to help stabilize the Uranium and reduce the potential for future contamination. The use of Mycorrhizal inoculum to manage and sequester several heavy metal contaminants is well doc mented in several research papers.

WHAT IS A PROPAGULE?

Mycorrhizae fungi are present as either spores, the reproductive form of the organism or hyphae that are microscopic threadlike filaments that are the vegetative form of the fungus. Spores and hyphae are also present inside the roots of the host crop that was used to culture the microbe. Both spores and hyphae are capable of colonizing a host plant once they have made contact with



the plant's roots. Therefore fragments that contain spores or hyphae are considered propagules or inoculating agents.

HOW ARE PROPAGULES MEASURE?

It is a procedure where a sample of the inoculum is placed in a blender with water to break up the medium, then runned through a series of screens to draw off the majority of the spores, hyphae and root fragments. The sample containing the inoculum is then stained with a dye so that it stands out under a microscope. There is an independent laboratory that can provide a propagule count report to clients, see attached example.

WHAT IS THE DIFFERENCE BETWEEN A SINGLE SPECIES AND A MULTIPLE SPECIES BLEND?

AM fungi is described as "Adaptive" and "Cosmopolitan", this refers to certain species of mycorrhizae that can adjust to a range of biological, geological and climatic conditions. For instance Glomus Intraradices has been found in abundance from the Arctic Tundra to the Amazonian Rainforest, the key with Glomus Intraradices, however is that it requires a nutrient rich soil. Certain species of mycorrhiza fungi perform better in nutrient poor sites, including sites with an unusually low pH or with an odd chemical composition. RTI recommends, that if the site to be restored has a reasonable amount of organic matter and nutrients, the single species Glomus Intraradices is a good choice. But it the soil on the site has low nutrient ranges, low organic ranges and are comprised of mostly parent material, a blend of 3 diverse species may be a better selection.

WHAT SPECIES OF MYCORRHIZAL FUNGI ARE AVAILABLE AT RTI FOR RESTORATION APPLICATIONS?

RTI cultures a total of nine different species, but because of the proven performance, we highly recommend that our Glomus Intraradices "Single Species" be used on nutrient and organic rich sites and a regional "Multi Species" be used on more complex and poorer soils. Please see the attached panel with species and descriptions list.

HOW TO APPLY MYCORRHIZAL INOCULANTS

While a range of species are available in granular, powder and liquid forms, restoration projects are best treated with a granular product. The mycorrhizal inoculant must be positioned with the seed in the soil, applications of inoculum to the surface of a site are not effective. The fungus must make contact with the roots to achieve colonization. The three most common methods of applying an inoculant are:

1. Through a hydro seeder. The granular inoculant can simply be incorporated into the tank along with the seed and fertilizer, and be applied in the first pass. A second pass of a mulch or fiber cover is recommended.

2. A drill box application. If a conventional seed drill is being used, the mycorrhizal inoculant can either be mixed in with the seed or if the seeder has a second legume box, the inoculant can be applied by through that.

3. Broadcasting seed, fertilizer and inoculant: If seed is surface broadcast, mycorrhizal inoculant can be mixed with the seed or applied in a separate pass after seeding or fertilizing. An additional pass with a chain type drag harrow is recommended to both cover the seed and inoculant.

WHAT ARE THE RECOMMENDED RATES FOR THE APPLICATION OF A MYCORRHIZAL INOCULANT?

The standard rate developed by the California Department of Transportation (CALTRANS) is 3.6 million propagules per acre or 8.895 million propagules per hectare. This equates to 82.6 propagule per square foot or 1 propagule for every 1.74 square inches. This is an appropriate

rate to promote early and rapid colonization of the germinating seed. Lower rates of 2 million propagules are acceptable on flat, nutrient rich sites, however it will take longer for the entire site to become fully colonized. AM 120 is packaged in 20 lb. bags which contain a minimum of 1.2 million propagules. Therefore an application of 3 / 20 lb. bags will meet the specification number.

WRITING SPECIFICATIONS FOR MYCORRHIZAL INOCULANTS

This is a typical specification that is based on the original California Department of Transportation (CALTRANS) specification of 3.6 million live propagules per acre:

3.5.4 Mycorrhizal Inoculation

The planting areas will receive granular mycorrhizal inoculum. The inoculum shall be comprised of a single species, Glomus Intraradices or a blend of multiple species with Glomus Intraradices comprising at least 50% of the mix. Endomycorrhizal inoculum is a live material, it will be stored, transported, and applied at temperatures of less than 90 degrees Fahrenheit (32°C). If temperatures exceed 90 degrees Fahrenheit, the inoculum must be covered or incorporated within three hours of its application.

Application during Seeding

Endomycorrhizal inoculum will be applied in the same application as the seeds, in no case will endomycorrhizal inoculum be applied after the seeds. Inoculum must be applied with hydro-seeding equipment within one hour of addition to the mixing tank. Inoculum should be applied at the rate of 3,600,000 live propagules per acre (8.895 million live propagules per hectare), based on the guarantee of the supplier or the analysis returned by an independent laboratory. This is usually approximately 60 pounds of inoculum per acre, but brands may vary.

This specification is more recent and includes measures for field testing and confirmation of propagule minimum levels. It also includes a clause requiring the supplier to supply any additional inoculum required to bring the propagule count up to the specified amount per acre if the test indicates that minimum levels have not been met.

333.07 Soil Inoculants

Mycorrhizal Inoculants should consist of spores, mycelium and mycorrhizal root fragments in a solid carrier suitable for handling in dry applications. The carrier shall be the material in which the inoculum was originally produced and may include organic materials, vermiculite, perlite, calcined clay or other approved materials consistent with proper application and good plant growth.

Each endomycorrhizal inoculum shall carry a supplier's guarantee of number of propagules per unit weight or volume of bulk material. Inoculant should contain a minimum of 50% Glomus Intraradices with a propagule count of 120 per gram of which a minimum of 20 spores per gram shall be present in random tested sampling. A representative 100 gram sample (from a

re-mixed bag in order to obtain a homogeneous sample) from each bag of material supplied to the project shall be obtained 30 days prior to application for verification of spore count (a rounded ½ cup kitchen measuring scoop will hold roughly 100 grams of material). Contractor shall provide independent testing results of actual counts of viable spores using standard spore extraction methods as described by Schenck et al in "Methods and Principles of Mycorrhizal Research, University of Florida. These methods and testing services are provided by the following independent laboratories:

Western Laboratories, Inc.
211 Highway 95
Parma, ID 83660
Tel: 800-658-3858
Contact: Harry Kreeft

U of Florida, Soil & Water
2169 McCarty Hall,
PO Box 110290
Gainesville, FL 32611-0290
Tel: 352-392-1951, ext 220
Contact: Abid Al Agely

MycoRoots
1970 NW Lance Way
Corvallis, OR 97330-2209
Tel: 541-752-0339
Contact: Efren Cazeres

Inoculant shall be transported and stored in areas with a temperature of less than 90o F. Use a dust mask when handling the material. Product shall be AM – 120 -3 or equal

HOW TO DETERMINE THE SUCCESS OF THE MYCORRHIZAL INOCULATION PROCESS.

Vegetative Cover:

The primary purpose of inoculation is to restore soil biology to the revegetated site. If most of the plants are natives, an early indicator of success is the percentage of cover developing that is comprised of these natives. Ruderals (non mycorrhizal plants such as members of the brassica family, e.g. mustard, the amaranthae family, e.g. pigweed and other weedy invasive species) may appear early but over one to two seasons, they should disappear and be replaced by native plants and more desirable introduced species that are normally included in restoration or erosion control seed mixes.

Plant Diversity:

Plant communities with a strong mycorrhizal association tend to be moderately to very diverse. Because the growth patterns of different plants vary, it is believed that diversity allows the fungi to spread its symbiotic association between a range of plants that may be more active at different times during the year. This provides benefits to not only the fungal symbiont but to the plant community as a whole, assuring a strong mycorrhizal network that can provide a nutrient and moisture supply throughout the growing seasons.

Lab Tests for Root Colonization:

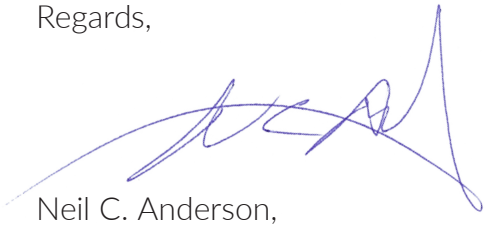
The ultimate process by which the success of inoculation can be determined is by a lab test that looks at the presence of mycorrhizal fungi within the roots of the plants. The three labs noted in this document provide a root staining process that should provide information about the level of

colonization. While mycorrhizal hyphae (the vegetative form) that provides nutrients and moisture to plants becomes active immediately after the plants become colonized, it takes several weeks to months before the reproductive form of the organism begins to show up in roots. It is therefore important that a planting only be tested after at least 14 weeks of growth.

FURTHER INFORMATION

Soil Biology is a critical part of virtually all sustainable ecosystems. RTI has over twenty years of experience in the culturing and production of premium mycorrhizal inoculants. The company is committed to providing the best technical support possible to all clients and associates in the habitat and erosion control industries. Please do not hesitate to contact us by e-mail or telephone for technical assistance. We will do our absolute best to answer any of your questions.

Regards,



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