The Use of Mycorrhizae in Native Plant Production

Native Plant Production
University of Washington
June 2006

Figure 1. Fungal hyphae interacting with root tip
(From http://www.jasons-indoor-guide-to-organic-and-hydroponics-gardening.com/beneficial-mycorrhizae.html)

Background on Mycorrhizae

What is it? A mycorrhiza (singular) is a symbiotic *relationship* between a plant and a mycorrhizal fungus.

What does it do? The hyphae of the fungi spread through the soil and infect the roots of plants creating specialized structures for the exchange of nutrients. This relationship generally benefits both organisms by providing carbon to the fungi and increased nutrient uptake (primarily phosphorus) for the plant. Each mycorrhizal plant and fungi is typically involved in multiple simultaneous relationships. It has also been demonstrated that this network of underground connections can facilitate a plant-to-plant exchange of nutrients.

Figure 2. Fungal hyphae penetrating a root cell and forming a highly branched arbuscule where nutrient exchange takes place.

How widespread is it? Estimates range from 70-90% of all plant species are involved in mycorrhizal associations. Notably, two large and diverse plant families are non-mycorrhizal, the Brassicaceae and Proteaceae.

How important is it? Some mycorrhizal relationships are obligatory, others facultative and in certain situations the fungus can even have a parasitic relationship with the plant. The importance of this relationship for plant health varies depending on the plant and fungal species involved. These relationships are complex and not easily understood. For example Douglas-fir, an obligatory mycorrhizal tree, is estimated to associate with 2000 species of mycorrhizal fungi. Not all of these fungi confer the same benefits to the

Douglas-fir and their relative abundance and competitive ability will influence the level of benefit received by the tree from its mycorrhizal partners. Additionally, mycorrhizae are believed to play a role in maintaining plant community structure by conferring a greater benefit to some plant species than to others.

Types of Mycorrhizae: At least six types of mycorrhizae have been classified, but for simplicity's sake we can generally break them down into two groups: the ectomycorrhizae and the endomycorrhizae. Ectomycorrhizae coat the outside of roots and infect the cell walls of root tissue. They associate with woody plants and produce fruiting bodies. Many of our edible forest mushrooms are ectomycorrhizal fungi. This is a vastly more diverse group of fungi, but associates with only a minority of the plant species.

Endomycorrhizal fungi actually penetrate into root cells and form tree like structures called arbuscules, which are thought to aid in nutrient exchange. They are also called arbuscular mycorrhizal fungi (or, AM fungi). These are a completely different division, or phyla, of fungi and do not produce mushrooms. Instead they have tiny underground fruiting bodies that are rarely seen by people. Endomycorrhizal fungi are far less diverse than ectomycorrhizae and comprise only six genera. They however associate with the vast majority of plants—both woody and herbaceous.

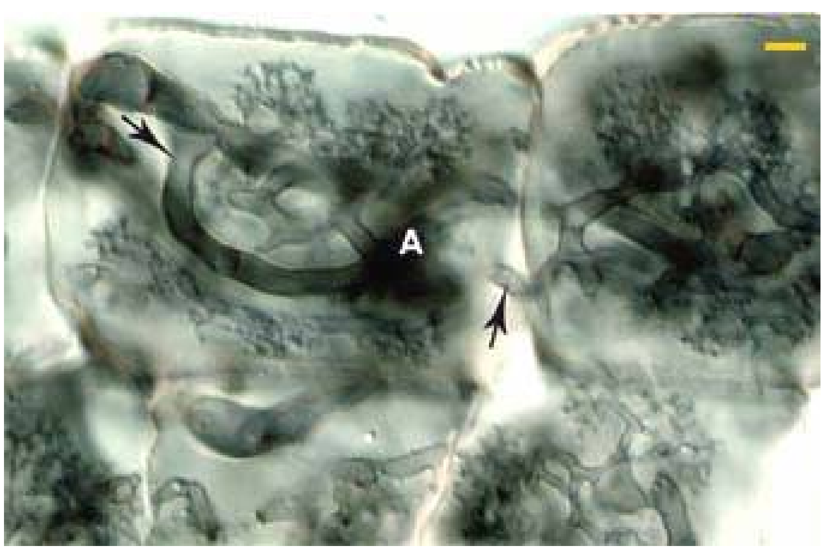

Figure 5. Endomycorrhizal fungi inside of root cells. Hyphae are evident inside

and passing between individual cells (arrows). Dark masses of arbuscular structures are present (see A).

How to Use Commercially Available Mycorrhizae in Nursery Production

Applying mycorrhizal inoculants is becoming an increasingly common practice in the nursery industry. Whether nurseries are producing native, ornamental, or agricultural plants, adding mycorrhizae can lower mortality rates and reduce water and fertilizer consumption. However, there is much uncertainty concerning where to obtain the fungi, what types to use, the quality of commercially available products and the most effective application methods.

Over thirty companies sell mycorrhizal inoculants. These may contain varying amounts of different species of fungi, different percentages of viable spores, as well as additives such as fertilizers, and hydrogels. Some inoculants contain spores specific to particular species, while others contain a broad mixture. When choosing a commercially produced inoculant, the first step is to determine a specific plant's needs and the present soil conditions. For instance conifers such as spruce and fir use ectomycorrhizae, while maples use endomycorrhizae. A comprehensive list of forest trees and the specific fungi that colonize them can be found in "The Container Tree Nursery Manual" available at http://www.rngr.net/Publications/ctnm. [6]

Companies and services: There are currently many companies that sell mycorrhizal inoculants. Some are geared towards nurseries while others market more to the landscaping industry. Many of the products contain additives such as "biostimulants" and hydrogels which might be undesirable in a restoration site. [16] A company called Mycorrhizal Applications is based in the Pacific Northwest and offers customized mixes based on plant type and soil conditions (personal communication). The best way to learn about the products is to contact the suppliers directly. A listing of commercial producers of mycorrhizal inoculum is available at: http://fungus.ucdavis.edu/outvend.html.

Commercially produced inoculants: Buying commercially produced mycorrhizal inoculants can be expensive, but it is much less labor-intensive than collecting forest soil. In large-scale production, it is by far the better option. Commercial products are more uniform, and may have higher concentrations of spores. Because they are a combination of endo- and ectomycorrhizae, one product can be used on several different types of plants. Also, forest soil might come laden with weeds and pathogens. [6] The cost of mycorrhizal inoculation varies from two hundred to thousands of dollars per acre depending on what products and application methods are used. [22]

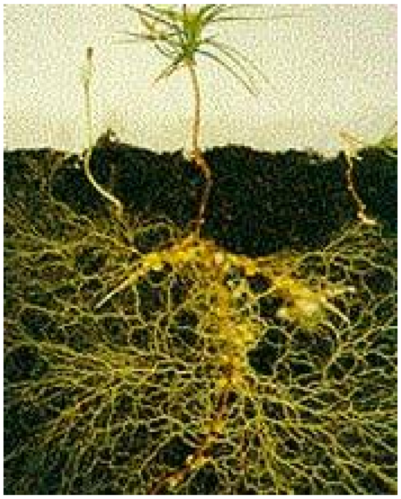

Figure 3. Pine seedling mycorrhizal network.
(Plant Health Care, Inc)

Quality: One supplier provides information on its website showing that its inoculum has the highest percent of spores. However these numbers can be deceiving as they are total counts, but only a fraction of those spores may be effective for a particular plant or in specific soil conditions. It is important to contact the supplier to find out specifically what types of fungi are in their inoculum and in what percentages. Because nursery conditions vary widely, a diligent nursery manager would keep detailed records on what suppliers were used, field conditions and success rates. Because root/soil/mycorrhizal relations are only poorly understood, it is worthwhile for nurseries to conduct their own experiments and determine what works best for them. [6]

Benefits: As discussed above, there are many ways that mycorrhizae facilitate healthy plant growth. Mycorrhizae increase the surface area of a plant's root system and, as a result, the plant is able to absorb more water. This increase in water uptake increases the survival of transplants and facilitates drought resistance. In some cases, mycorrhizae increase a plant's access to nutrients such as phosphorus and zinc, which can be important in tropical soils where phosphorus availability is low. [7] In addition, mycorrhizae can increase nitrogen fixation for nodule-forming plants.

Plants that receive proper nutrition are more able to resist disease, and this is one way that mycorrhizae help plants resist soil borne diseases. The most obvious mechanism for protection against diseases is the barrier that ectomycorrhizae create when they coat the outside of the root. Mycorrhizae compete with pathogenic microorganisms and may actually exude toxins and antibiotics to protect the plant. [2] Other benefits include improved soil structure due to the mycorrhizae binding soil particles into aggregates—allowing water to infiltrate and increasing oxygen in the rhizosphere. [5] Finally, root-feeding nematodes are less likely to attack mycorrhizal plants. [1]

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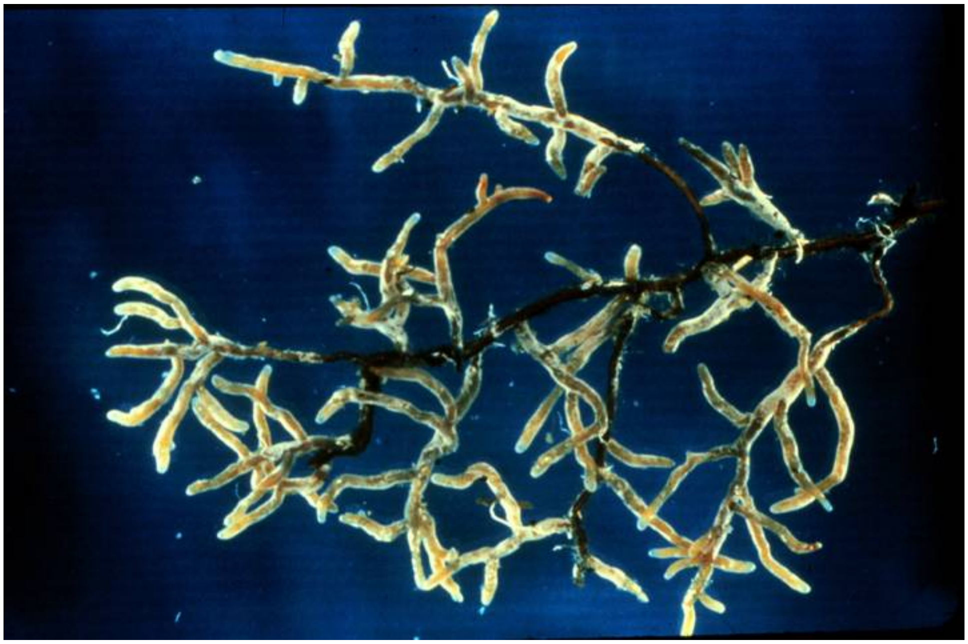

Figure 4. White sheath of ectomycorrhizal fungi on grape roots.

Because nursery soils are often highly disturbed, fumigated, or sterile planting mixes, mycorrhizal inoculation may be necessary for plant growth. Monterey pine plantations in New Zealand initially failed due to lack of suitable mycorrhizae in the soil. In general, if a nursery is adjacent to native forest, mycorrhizae will establish from spores on their own. [12] However if a nursery is not in close proximity to a forest, if its practices are interfering with mycorrhizal establishment, or if plant health is sub par, buying commercially produced mycorrhizal inoculant can be a good option.

Container nurseries have limited plant-to-plant mycorrhizal spread, as the fungus can not spread vegetatively through the soil and contact another plant. Therefore mycorrhizal colonization depends on wind-blown spores landing on each plant. Only certain types of mycorrhizae have spores which are spread by wind, so inoculating container plants is the best way to ensure establishment (see below). [6]

Application methods/Products (see page 9 for further details)**:**

- Soil from forest sites can be added to nursery beds or containers.
- Spores can be added to irrigation or applied to seeds before sowing.

- Mycelial fragments can be added to the soil.
- In the case of a non-container nursery, nurse plants are plants spread throughout the nursery with established mycorrhizae. These mycorrhizae can then spread through the soil to seedlings.
- Seedling root systems can be dipped directly into a gel containing spores.
- The most important function of the application is to place the spores as close to the root zone as possible.

Assessment for mycorrhizae: Seedlings should be assessed to determine levels of ectomycorrhizal colonization. The Container Tree Nursery Manual contains detailed assessment instructions. [(5)] Note that assessment of endomycorrhizal colonization requires laboratory techniques beyond the scope of most nurseries.

Mycorrhizae in the nursery and field: It is important to consider that the mycorrhizae that aid in the production of healthy nursery plants may not function the same way in the field. Certain mycorrhizae can only grow in the controlled, favorable soil in the nursery but are ineffective in droughty or poor-quality soils common in restoration sites. [(11)] There are several solutions to this problem. One is to inoculate seedlings with soil from the intended planting site. If seedlings are not grown for a particular site, commercially produced inoculum containing hardy species may prove effective. Alternatively, inoculum can be added in the field at the time of planting. However, it is preferable to have a plant that already has suitable mycorrhizal colonization at the time of planting or there may be a lag time while the new mycorrhizae establishes.

Nursery Practices: Soil fumigation, pesticides, and fungicides can kill mycorrhizae. Over-fertilizing and over watering can also reduce mycorrhizal populations. In general, practices that build healthy soil are beneficial to mycorrhizae. [(12)]

Use of Native Mycorrhizae

Who uses it? Often in the specifications for contract native plant production, there is a requirement to produce plant material using mycorrhizal inoculation. The inoculum can come from native topsoil or may be commercially available. It may be more cost effective to use native topsoil as the source of inoculum. (13) Plant material grown with inoculum is especially useful when used in post-fire restoration, mining reclamation, and contaminated sites. (4) Site soil should be tested to determine if there are already mycorrhizae present, whether the soil is toxic or has the proper soil properties to enhance colonization. (13)

Drawbacks: Inoculum obtained from a small amount of native soil can be mass-produced when growing mycorrhizal plants; however, this may be costly and time-consuming. (13) Removing "donor" soil from natural areas may cause existing plants to suffer from root disturbance as well as open up areas to invasion of weed species. (21) Because donor soil may also contain non-native pathogens such as Sudden Oak Death (SOD), there is a danger of infecting nursery plants. Native mycorrhizae may not be able to provide protection from these alien pathogens. In addition, donor soil may contain a seedbank and/or rhizomes of weedy species.

When phosphorus is readily available in soil, mycorrhizae may either be "inhibited" or actually take energy from the host plant. (13)

TECHNIQUES

Supplies you need to produce your own AM fungal inoculum:

The following guidelines are an excerpt from *Use of Mycorrhizae in Restoration of Hawaiian Habitats* by J. N. Gemma and R. E. Koske. (7)

- **Containers**: One gallon or larger pots can be used to produce your "pot culture." Allow for drainage so that water does not accumulate in the bottom of the pot.

- **Growth Medium**: A good growth medium for inoculum production consists of quartz or basaltic sand (construction blue sand) or a pasteurized sandy soil with good drainage. The basaltic sand should be well rinsed to remove the finer dust particles. Coral sand should not be used.

- **AM Fungi**: Collect fine roots or soil from the root zone of native vegetation from the habitat you are restoring or from specific plants that are likely to be mycorrhizal [see Appendix I for a list of plant species that form AM associations].

- **Seeds of a "Host" Plant:** [A fast-growing native plant species should be used as a host plant. Researchers in Hawaii have used corn as a host plant.]

- **Low-Phosphorus Fertilizer**: For best results, use fertilizer low in phosphorus or slow release fertilizer pellets (e.g. 17-6-10 with micronutrients). You can make your own low-phosphorus fertilizer by mixing the following ingredients into 2 gallons of water: 1 teaspoon of Peters 20-0-20 fertilizer, a fifth of a teaspoon (0.9 g) of Epsom salts ($MgSO_4$), and 1_ milliliters of a concentrated PO_4 fertilizer solution called Quick Start (4-12-4) made by Miracle-Gro. You can measure milliliters in a child's measuring spoon for liquid medicine that is available at most drug stores.

Step-by-step procedure for producing inoculum*:*

1. Add 10 to 30% of your collected soil that contains AM fungi or 1 cup of fine roots per gallon of medium and mix thoroughly with your sand growth medium. If you are using a slow release fertilizer, add 1 tsp of slow release fertilizer pellets (17-6-10 with micronutrients) for every gallon of potting soil. Set up several pots so that you will produce sufficient inoculum for later use.

2. Sow "host" plant seeds in your container. Grow 4-6 plants.

3. After seedlings emerge, fertilize weekly with about 1 cup of the low-P liquid fertilizer solution (see above) per gallon of potting if you are not using the slow release pellets in step 1. Water in between fertilizer applications.

4. Let plants grow for about four months and then stop watering, allowing the plants and potting mixture to dry slowly and completely in the greenhouse over a period of 2-3 weeks.

5. Remove the above-ground portion of the "host" plant and discard.

6. Chop the roots and mix them in with the sand. A clean pair of clipping shears is useful. This root and sand mixture is your concentrated AM fungal inoculum. The inoculum will remain effective for at least one year if stored in plastic containers and kept in a cool, dry area. You can use this inoculum as your AM fungal source to produce more "pot cultures," to add to potting mix to raise mycorrhizal plants in the greenhouse, as well as to inoculate non-mycorrhizal plants that are being transplanted to the field.

Field soil incorporation

Inoculum may be broadcast or tilled into field soil, though this requires large quantities of inoculum in order to facilitate rapid colonization. Side dressing of inoculum near seeds and seedlings can be effective with less product. A very successful method is to layer inoculum under the seeds. [12]

For more information about how to produce mycorrhizal plants in the greenhouse, see Gemma and Koske's easy to follow guidelines at: http://www.hawaii.edu/scb/docs/science/scinativ_mycor.html.

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