MYCORRHIZAL INOCULATION OF CONTAINER PLANTS

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INTRODUCTION

The majority of the work reported here took place at Tree of Life Nursery, which produces about 300 species of California native plants for both horticultural and revegetation purposes.

With the revegetation plants, especially, we are concerned that they leave the nursery in a condition that will allow them to survive the rigorous conditions of a land restoration job. Those conditions include no irrigation except natural rainfall, little or no fertilization, and minimal help against weeds and herbivorous animals. For that reason we have established a program of inoculation with mycorrhizal fungi.

WHAT ARE MYCORRHIZAE?

Roots of most field-grown plants are found to contain fungal tissue. The fungi may form a cloak around the root, or may exist as individual strands (hyphae) among the root cells, but in each case the fungus extends from the root into the soil. These fungi are known to be beneficial for the plant. The association that they form with roots is called mycorrhiza. The word "mycorrhiza" refers to the association of both organisms, not to the fungus by itself. The plant is called the "host plant," and the fungus is referred to as the "mycorrhizal fungus."

Mycorrhizae of several types differ from each other in the kinds of fungi that enter the root and the details of the fungus-root-interface. Two of the several kinds of mycorrhiza are most likely to be of concern in the nursery. These are ectomycorrhizae (ECM) and vesicular-arbuscular mycorrhizae (VAM).

ECM mycorrhizae are found primarily in the dominant trees of temperate forests and in a few genera in the tropics. Among California natives, examples of ECM host plants include chamise, oaks, pines, willows, and cottonwoods. The fungi form a layer around short side roots, and ectomycorrhizae can often be seen with the unaided eye. Many of the fungi of ECM produce mushrooms or other large fruiting structures.

The most widespread type of mycorrhiza is vesicular-arbuscular mycorrhizae (VAM). This kind of mycorrhiza was named for two kinds of characteristic structures: vesicles, which are globular oilstorage organs, and arbuscules, which are finely-branched fungal networks within the cells. In the arbuscules, the fungus passes phosphorus to the host plant, and receives carbohydrates from the plant. This mutually beneficial exchange is the fundamental feature of the mycorrhizal symbiosis.

VAM are found in most crop, ornamental, and native plants, and their fungi are the most abundant microorganisms in many soils (8). The fungi can infect a very wide range of host plants. The same species of VAM fungus may be associated with liverworts, ferns, and maple trees; thus one or a few species of fungus may serve an entire plant community. Host species among California native plants include grasses, *Ceanothus* spp., most members of the legume and rose families, and many others. VAM are not normally visible without special staining and magnification.

Other kinds of mycorrhiza are found in the Ericaceae, Orchidaceae, and several small tropical families. California native plants with their own kinds of mycorrhizae include *Arctostaphylos* spp., Madrone, and *Rhododendron* spp. Harley and Smith (7) gave detailed descriptions of the known kinds of mycorrhiza.

It is clear that most kinds of plants, when growing in their natural habitats, are host to one or more of these kinds of mycorrhiza. Although numerous accounts have been published of the mycorrhizal status of various plant species, the list is far from complete.

The most important kind of mycorrhiza for the nursery trade is VAM, because it is by far the most widespread type, and because the natural dispersal of VAM fungi is slow. Ectomycorrhizal fungi often are carried by the wind, but the very large VAM fungal spores must move by animal vectors or movement of soil. These fungi must be intentionally introduced if the plants are to benefit from the mycorrhizal symbiosis.

WHAT ARE THE BENEFITS OF MYCORRHIZAE?

The growth response that can be brought about by mycorrhizae is legendary. Inoculated plants commonly show growth rates double or more those of comparable uninoculated controls. The increased growth rate is the result of enhanced mineral nutrition.

Mycorrhizae greatly improve uptake of phosphorus (and probably zinc and copper) because of spatial distribution of the hyphae; the fine, branched fungal filaments. The root removes phosphorus quickly from a zone near the root surface, but because phosphorus moves through the soil very slowly, a zone of depletion forms around the root. The mycorrhizal hyphae cross the depletion zone and take up phosphorus that is beyond the reach of the unaided root (20).

The special ability of mycorrhizae to take up phosphorus is of interest in the nursery. Not every nurseryman is aware that soilless mixes, unlike natural soils, allow phosphorus to be leached out. Not many modern nurserymen would consider rock phosphate for serious nursery use, but Graham and Timmer (6) showed that within the first five weeks of a 16 week experiment, rock phosphate was a better source of phosphorus than superphosphate.

To be efficiently used, rock phosphate has to be added in large amounts. Rock phosphate is inexpensive when purchased in bulk, and can be safely applied in large quantities. However, it must be used in combination with mycorrhizae. The mycorrhizal fungi make the otherwise unavailable rock phosphate useful to the plant (6). In our experience, osmotically-coated slow-release fertilizer has provided a steady supply of phosphorus, and has been compatible with mycorrhizae. A low-P, slow-release formulation with a long release time, combined with vesicular-arbuscular mycorrhizae inoculation has solved some phosphorus-related problems that have plagued us seasonally for several years.

It is difficult to avoid a boom-and-bust cycle when highly soluble phosphorus is used, because the superphosphate is leached out with irrigation. While a mycorrhizal program is necessary when rock phosphate is used, a mycorrhizal program is very difficult with superphosphate, since the high initial phosphorus concentration inhibits mycorrhiza formation. Later in the cycle, soilless media cannot retain even the relatively low concentration of phosphorus needed by mycorrhizal plants.

There have been reports of mycorrhizal benefits other than improved phosphorus nutrition, including better tolerance of transplanting (2, 13) and higher drought resistance (17, 18). Mycorrhizal plants perform better in saline conditions (14), and may be more resistant to pathogens (19). Some of these "non-phosphorus" effects of mycorrhizae may actually be side effects of improved phosphorus nutrition.

Mycorrhizae do not enhance the uptake of nitrogen (4), but do aid nitrogen fixation by improving the phosphorus supply. Both leguminous (9) and actinorrhizal (16) nitrogen fixers have been shown to perform markedly better when mycorrhizal.

Mycorrhizae have important effects in the soil, beyond their effects on individual host plants. Mycorrhizal fungi are important agents of soil structure (21) because their hyphae bind soil particles into aggregates.

Mycorrhizae have brought about important advantages in the nursery, such as relief from phosphorus-induced stunting (10). However, the main focus of a nursery's mycorrhizal program should be the performance of the plant after it goes into the ground.

Inoculating in the nursery improves the chances of good transplant recovery and early growth. It also insures against a complete lack of native mycorrhizal fungi at the site, as occurs on recently graded or severely eroded ground.

WHAT IS MYCORRHIZAL INOCULUM?

Inoculum is material that carries viable propagules of mycorrhizal fungi. This usually means a combination of fungal spores, root fragments, and mycelium. Inoculum may be purchased from a commercial supplier, produced in-house by a nursery (5, 12), or collected from the wild. Commercial sources of mycorrhizal inoculum are generally the most sensible option. NPI of Salt Lake City, Utah produces a VAM product called "Nutri-Link." Mycorr Tech, Inc., of Pittsburgh, Pennsylvania, produces several ECM fungi in liquid culture. Other suppliers of each kind of inoculum have been in production at various times. Tree of Life Nursery is producing inoculum for in-house use, and is considering offering several isolates for use by other nurseries.

VAM inoculum can be produced in-house, although the process brings with it several unexpected difficulties. The fungus must be grown with a living host plant, usually in greenhouse cultures. Host plants for this purpose are most commonly fast-growing tropical grasses, which produce abundant spore crops in summer conditions. Lower quality inoculum can be produced in winter greenhouse conditions, but only by shifting to a host (such as celery) that tolerates short photoperiods. The container medium must be relatively low in nutrients to encourage the spread of the fungus rather than rapid top growth of the plant. It is very important that the amount of phosphorus be low in relation to the supply of cations and micronutrients. The balance between phosphorus and nitrogen, which can also be inhibitory to mycorrhizae, is particularly sensitive.

The culture medium should be steamed since the cultures have to be free of unwanted soil organisms. The mix must be formulated with components that are free of "autoclave toxicity," toxic forms of manganese and other factors that can greatly reduce plant growth.

Container-grown inoculum is harvested after two to six months, depending on conditions, and may be chopped up for storage and handling. Inoculum is usually stored in a refrigerator, where it may be expected to retain most of its viability for several months. If the inoculum has been mixed with nursery potting medium, the bulk mix must be sheltered from harmful conditions. Deleterious influences include extremes of temperature, such as may occur in a mix made with "green" organic matter. The inoculum should not be allowed to dry to extremely low moisture content, or to experience excess moisture, which will encourage the growth of pathogens.

INOCULATION IN THE NURSERY

To initiate a mycorrhizal inoculation program, the nurseryman must make several important decisions:

- Which plant species are to be inoculated?
- What kind of mycorrhiza do they normally support (ECM, VAM)?
- What species of mycorrhizal fungi are appropriate for the intended uses for the plants?
- At what stage will the inoculation be carried out?
- How will the inoculation be carried out?
- How much inoculum will be required for each plant?
- What special care should be exercised during growth of the plants?
- Will the grower "certify" mycorrhizal plants at the time of sale?

CHOICE OF PLANTS AND FUNGI

Pines, oaks, willows, and a few other groups of plants are ECM hosts, and members of the family Ericaceae have several specific kinds of mycorrhizae. Most plants have the VA type of mycorrhiza; thus a plant of unknown mycorrhizal status should be assumed to be a VAM host.

The fungi should also be chosen with care. Every species of mycorrhizal fungus has a range of tolerance for each environmental variable, and it follows that some fungi will be preferable to others in any given set of conditions. The most important considerations determining suitability of the fungi are pH and other soil properties. Since the primary objective is performance after outplanting, mycorrhizal fungi should be chosen for conditions in the field rather than the nursery.

In the final analysis, most nurseries will have to choose fungi primarily on the basis of availability. A commercially available VAM fungus known to occur naturally in slightly acid soils is *Glomus etunicatum*. For neutral to slightly basic soils, *Glomus intraradices* is available commercially. As demand for mycorrhizal plants grows, other fungi may become available for unusual or difficult conditions. Our own objective is to pre-inoculate container plants with a mixture of fungi native to the vicinity of the intended planting site.

MECHANICS OF MYCORRHIZAL INOCULATION

Inoculation may be carried out at the germination or rooting stage, at an early transplant, in the final container, or at the time of outplanting. The earlier stages are preferable because maximum benefit is realized from inoculation of plants early in their lives. Most host plant seedlings can support mycorrhizae as soon as lateral roots have appeared. Inoculation in the seed or cutting flat would give the best early boost to plant growth, and can hasten development of roots (1, 11, 23). However, inoculation at that stage may be impractical if fungicides must be used to control damping-off. After performing several tests on timing of inoculation, we have found that the first transplanting is the most practical time to inoculate.

Highly concentrated inoculum, such as that available from commercial sources, may be mixed throughout the potting medium, or a small amount of inoculum may be introduced separately to each pot. The amount required per pot is a function of the quality of the inoculum and should be based upon the supplier's recommendation. For VAM spore inoculum about 300 spores per plant has been effective (5), but hyphal and root fragments are often more important propagules than spores. The inoculum is placed in the center of the pot, just below the roots. The roots should be in contact with the inoculum as the soil is pressed in place.

Larger plants already in their final containers may be inoculated in place by cutting into the root zone, where future growth will carry the roots through the inoculum.

Inoculation in an outdoor nursery bed can be carried out by banding (introducing inoculum into a slit cut in the soil beside a row of growing plants). Riffle and Maronek (15) discussed various means of inoculating ECM fungi with basidiospores, which can be applied in hydromulch, mixed into soil, dusted onto seedlings, or pelleted with seed in a clay carrier.

CARE OF MYCORRHIZAL PLANTS

In order to assure that mycorrhiza formation proceeds smoothly, certain key steps in plant growing are modified to accommodate the symbiosis.

The conditions of light, temperature, and moisture that promote photosynthesis and root growth in host plants are generally the conditions that promote colonization by mycorrhizal fungi. However, mycorrhizal plants are sensitive to fertilization. Close control has to be maintained on concentrations of nitrogen and phosphorus during the establishment phase. One form of fertilization that has worked well is resin-coated slow release fertilizer, such as Osmocote 18-6-12 (Sierra Chemical Company, Milpitas, CA). Comparable products rated for eight or more months are likely to work as well. The fertilizer should be incorporated at the manufacturer's lowest recommended rate. Supplemental feeding may be carried out as needed by top-dressing with the same slow-release material, or with soluble nutrients if the immediate needs of the plants are not exceeded. Nitrogen and phosphorus are both potentially inhibitory. No sacrifice of growth rate need be accepted, but it will be necessary to apply fertilizers with precision.

Sometimes the nursery's potting mix will have to be modified for compatibility with mycorrhizae. Media consisting only of organic materials, or solely of soil conditioners such as sand, perlite, or vermiculite, have often been unsatisfactory (3), but any of these can be useful as components of the medium. At the liner stage, we have successfully used a medium consisting of about 40% organic materials (bark, shavings, and sawdust of redwood, white fir, or pine) and 60% inorganic amendments (#2 and #3 perlite, vermiculite, and sand). Peat moss is a common source of trouble, with some peats giving good results and others inhibitory to the symbiosis. Soil or clay components in the potting mix appear to improve colonization, but are not required. Any soil components should be tested for toxicity that may develop as a result of steam pasteurization. The pH of the mix must be adjusted to match the optimum pH of the mycorrhizal fungus. Most commonly used VAM fungi will colonize roots if the pH of the medium is near pH 6.5.

Many pesticides can be inhibitory to the symbiosis (22); care should be taken in selecting and applying any pesticides that must be used. Fungicides are generally the most damaging. A few examples of fungicides that are thought to be compatible with ectomycorrhizal (ECM) fungi are benomyl, captan, and subdue. Examples of fungicides that appear to be compatible with vesicular-arbuscular mycorrhizal (VAM) fungi are copper sulfate and subdue. Because conditions and dosages vary considerably, tests of each pesticide should be carried out in-house before large-scale pesticide applications are undertaken.

Another potential problem is soil temperature, especially when plants are grown outdoors without shade. Mycorrhiza formation may be reduced or inhibited by high temperatures in the growing medium.

CERTIFICATION OF MYCORRHIZAL PLANTS

Certification of mycorrhizal plants is a valid concern when mycorrhizal container plants have been specified for a restoration or revegetation job. Certification requires an independent laboratory that is prepared to evaluate the mycorrhizal status of sample material. We have approached a commercial soil laboratory about a certification program, but at this writing the program has not yet been in place.

CONCLUSIONS

The result of our mycorrhizal work has been routine production of mycorrhizal plants, focusing on a few key species. The best approach for each nursery, and the means for solving practical problems will depend on the grower's objectives and on the specific circumstances. The important generalities about a mycorrhizal program at a commercial nursery can be summarized:

- Mycorrhizae are natural part of the life of most plant species.
- Mycorrhizae aid uptake of nutrients and enhance drought resistance, thus helping to make plants less dependent on irrigation and fertilization.
- Mycorrhizae may provide immediate benefits in the nursery, but the primary objective of the program should be performance of the plants after outplanting.
- Mycorrhizal inoculation can be incorporated into a nursery's routine, but may require some accommodation in cultural procedures.

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VOICE: I have a question for Dr. St. John. Do you know what mycorrhizal organisms are in ''Nutri-Link'', and could you elaborate on the circumstances where it could be used successfully in a nursery production situation?

T.V. ST. JOHN: Right now in ''Nutri-Link'' they are marketing two fungal species, *Glomus intraradicas* and *Glomus etunicatum.* They are using these two species after test screenings showed that these give a good response with a number of hosts under a variety of conditions and a range of climates.

Plants that grow very poorly until they are planted out in the ground indicate a mycorrhizal problem. There are some plants where it is nearly impossible to give them enough phosphorus without a symbiot—without mycorrhizae; *Liquidambar* is an example of this. Certain forest tree seedling nurseries show spotty growth. This may be due to uneven distribution of mycorrhizae. More even growth may be obtained by intentionally introducing mycorrhizae.

VOICE: How specific are these mycorrhizal fungi?

V.T. ST.JOHN: The ones people work with are quite non-specific but for some ectomycorrhizal fungi there are cases where they are quite host specific.

BRUCE BRIGGS: In order to prevent the loss of phosphate, if you use aluminum in a non-soil medium what would be the effect on mycorrhizae?

V T. ST.JOHN: Aluminum is quite toxic, but it is not soluble until the pH is very low, too low to be used. The main effect is that it would bind up the phosphorus—the phosphorus is there but the plants can't get it.

ANN KYTE. Carolyn, I am wondering if you have published on your work with molecular plant breeding

CAROLYN NAPOLI. Yes, it has been published in the April, 1990, issue of the journal, *Plant Cell*, which can be found in most university libraries.

VOICE: Dr. MacDonald, you mentioned ''Agro-Diagnostics'' kits for the detection of certain fungus species. Are these easy to use and how expensive are they?

JAMES MACDONALD: They are really very simple to use. Anyone could do it. In regard to expense, they are still doing marketing studies—whatever the market will bear. They want to go commercial with a *Phytophthora* test kit in the winter of 1990-91. I am sure you will hear more about the costs in the future.

VOICE: To Dr. MacDonald—I am wondering if you have done any work on preventing *Phytophthora* and a cure for it.

JAMES MACDONALD. In terms of chemical control, there are many pesticides that can be used. One of the problems with these is that you must detect the pathogen at the very earliest possible stage. By the time you can see the problem in the crop the pathogen is very well established, which limits the effectiveness of these materials. Given the current political climate against the use of pesticides, it is very fortuitous that we have these early diagnostic aids coming along for very early detection of the fungi.