Propagation of Mycorrhizal Plants for Restoration

Ted St. John

Tree of Life Nursery, P. O. Box 736, San Juan Capistrano, California 92693

There is increasing interest in the propagation of native plants for use in habitat restoration. Mycorrhizae are essential to the survival of most native plant species and a defining feature of functional ecosystems. Native plant nurseries are now being called upon to provide mycorrhizal plants for habitat restoration (St. John, 1993). Production of mycorrhizal plants requires a source of inoculum, care in providing growing conditions favorable for both plant and symbiosis, and an ability to verify successful colonization.

INTRODUCTION

Rampant and often uncontrolled development in southern California has brought about a belated realization that our unique biological communities are almost gone. Habitat restoration—the rebuilding of native ecosystems—is supported by environmental regulations and has now reached the status of an industry. In recent years, Tree of Life Nursery has been supplying mycorrhizal plants for habitat restoration.

Most plant species form the beneficial fungal association known as mycorrhiza. The fungus colonizes both root tissue and soil and acts as a bridge for transport of phosphorus and other nutrients. Mycorrhizal fungi are usually destroyed by grading and other severe disturbances; their propagules are typically slow to reinvade disturbed sites. Thus, uninoculated mycorrhiza-dependent plants may be placed in the field and expected to become self-sufficient without their symbiotic partners.

Mycorrhizal types are defined by structure and kind of host plant. The most common involve aseptate fungi of the order Glomales and are usually called vesicular-arbuscular mycorrhizae (VAM). The structure of VAM is not evident without special staining procedures. Typical VAM host species include most crop and ornamental plant species.

Ectomycorrhizae (ECM) involve fungi of the basidiomycetes and ascomycetes. ECM host plants include pines, firs, and spruce, along with most of the other commercial timber species. Other ECM hosts are oaks, beeches, willows, and the tropical family Dipterocarpaceae.

The Ericaceae and other families have mycorrhizae that are different in structure from either VAM or ECM. A fuller description of all of these types can be found in Harley and Smith (1983).

Mycorrhizae have a number of beneficial effects on host plants, the most prominent of which is improved phosphorus nutrition. Due to the slow diffusion of phosphate ions, much of the supply in the soil is out of reach of unaided roots. Mycorrhizal fungi absorb this more distant phosphate, which is then passed to the root (Tinker 1978).

Mycorrhizae are able to reduce stresses of various kinds (Sylvia and Williams, 1992). In some cases reduced stress is a side-effect of improved phosphorus nutrition. VAM can improve drought tolerance through a combination of improved nutrition and other mechanisms (Hardie, 1985; Nelson, 1987; Safir et al., 1972).

There has been considerable experimental work on the possibility that mycorrhizae confer disease resistance. Linderman (1994) concluded that a large share of the protective effect is due to improved phosphorus nutrition and increased vigor of mycorrhizal plants. Most of the remaining effect, at least with VAM, is probably due to antagonistic microorganisms that often accompany mycorrhizal inoculum. Such protective effects are clearly most effective when both VAM and associated pathogen antagonists are introduced before any pathogens become established.

In many cases the only practical way to introduce mycorrhizal inoculum to a restoration site is mycorrhizal carrier plants: plugs or other inexpensive plants, normally part of the intended flora, that have been made mycorrhizal in the nursery.

INOCULATION IN THE NURSERY

Unless the growing medium contains field soil, it is unlikely to contain propagules of VAM fungi. If the plants are to become mycorrhizal, they must be intentionally inoculated and given growing conditions that favor development of the symbiosis.

Mycorrhizal inoculum, the mixture of roots, hyphae, and spores that give rise to new colonization (Ferguson and Woodhead, 1982; Menge, 1984), can be purchased from commercial sources, collected from the field, or produced in-house. Commercial sources are available for both VAM and ECM. Commercial inoculum is usually of much higher quality than field soil or in-house inoculum. Field-collected soil is a reliable source of pathogens and insects.

Mycorrhizal fungi tend to be non-specific for hosts. That is, most species of fungus can form mycorrhizae with most suitable hosts. However, there is considerable specificity for soil pH and other characteristics (Cordell and Marx, 1994; Mosse, 1975). It is important that the fungi used in the nursery be suitable for both the growing medium and the field soil for which the plants are destined. In some applications, the best choice may be a mixture of fungal species from the site for which the plants are intended.

Some conditions commonly found in nurseries can prevent mycorrhiza formation. The most important of these are low light intensity, excess fertilization, improper watering, extreme temperatures in the root zone, and the presence of root pathogens.

VAM inoculation essentially consists of mixing inoculum uniformly through the growing medium or of placing a small amount of inoculum under a transplant. ECM inoculum may be spores or vegetative mycelium, applied in any of several ways (Castellano and Molina, 1990). Inoculation rate has a significant effect on mycorrhizal colonization and plant performance (Cordell and Marx, 1994).

It is very important that nursery staff realize that inoculum is perishable. The inoculum must not be allowed to heat up in the sun or freeze. If the material was delivered as an air-dried preparation, it should not be stored damp.

The best response to mycorrhizae is usually realized with early inoculation (Abbott and Robson, 1981). For practical reasons, nursery managers may decide to await the first transplant before inoculating.

It is important to verify the success of inoculation, a task that is more difficult with VAM than with ECM. Determination of VAM status requires chemical treatment of roots to remove cytoplasm and selectively stain the fungi (Phillips and Hayman, 1970). The fungal structures in the roots must then be distinguished from any other fungi in

the roots. By contrast, ECM are often visible with the unaided eye or a hand lens.

The nursery that intends to undertake a mycorrhizal plant program must be prepared to make some procedural changes. The first decision is the kinds of plants and mycorrhizae required. Most host species form only a single type of mycorrhiza (i.e. VAM or ECM), but cottonwoods and a few other species form both VAM and ECM.

Nursery growing media may have to be changed to accommodate the symbiosis (Cordell and Marx, 1994). It may become necessary to substitute biocontrol for certain pesticides and to choose other pesticides for compatibility with mycorrhizae (Trappe et al., 1984). The customers must be made aware of the need to handle the plants carefully, not over-fertilize, and assure that the roots of bare-root plants are not damaged (Cordell and Marx, 1994).

A very important question is how to verify the success of inoculation. If no one on staff is able to stain and interpret roots, an outside laboratory or consultant may be able to do so. Soil and Plant Lab, Orange, CA, has recently begun to offer mycorrhizal determinations.

LITERATURE CITED

- **Abbott, L. K.** and **A. D. Robson.** 1981. Infectivity and effectiveness of vesicular arbuscular mycorrhizal fungi: Effect of inoculum type. Aust. J. Agric. Res. 32 (4):631-639.
- Castellano, M. A. and R. Molina. 1990. Mycorrhizae. p. 101-167. In: T.D. Landis, R.W. Tinus, S.E. McDonald, and J.P. Barnett (eds.) The container tree nursery manual. Volume five. The biological component: nursery pests and mycorrhizae.
- Cordell, C.E. and D.H. Marx. 1994. Effects of nursery cultural practices on management of specific ectomycorrhizae on bareroot tree seedlings. p. 133-151. In: F.L Pfleger and R.G. Linderman (eds.). Mycorrhizae and plant health. APS Press, St. Paul, Minnesota.
- Ferguson, J.J. and S.H. Woodhead. 1982. Production of endomycorrhizal inoculum. A. Increase and maintenance of vesicular-arbuscular mycorrhizal fungi. p. 47-54. In: N.C. Schenck (ed.). Methods and principles of mycorrhizal research. The American Phytopathological Society, St. Paul.
- **Hardie, K.** 1985. The effect of removal of extraradical hyphae on water uptake by vesicular-arbuscular mycorrhizal plants. New Phytologist 101(4):677-684
- Harley, J.L., and S.E. Smith. 1983. Mycorrhizal symbiosis. Academic Press, London.
- Linderman, R.G. 1994. Role of VAM fungi in biocontrol. p. 1-25. In: F.L. Pfleger and R.G. Linderman (eds.). Mycorrhizae and plant health. APS Press, St. Paul, Minnesota.
- Menge, J.A. 1984. Inoculum production. In: C.L. Powell and , D. J. Bagyaraj. (eds.). Va Mycorrhiza. CRC Press, Boca Raton, Florida.
- Miller, R.M. and J.D. Jastrow. 1992. The role of mycorrhizal fungi in soil conservation. p. 29-45. In: Bethlenfalvay, G.J., and R.G. Linderman (eds.). Mycorrhizae in sustainable agriculture. ASA Special Publication Number 54. American Society of Agronomy, Inc., Madison, Wisconsin.
- Mosse, B. 1975. Specificity in VA mycorrhizas. p. 469-484. In: F. E. Sanders, B. Mosse, and P. B. Tinker (eds.). Endomycorrhizas. Academic Press, London.
- Nelsen, C. E. 1987. The water relations of vesicular-arbuscular mycorrhizal systems. p. 71-91. In: G. E. Safir (ed). Ecophysiology of VA mycorrhizal plants. CRC Press, Boca Raton.
- **Phillips, J.M.** and **D.S., Hayman.** 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55:158-161.

- Safir, G.R., J.S. Boyer, and J.W. Gerdemann. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. Plant Physiol. 49:700-703.
- **St. John, T.V.** 1993. Benefits of mycorrhizae in revegetation and restoration. In: J. E. Keeley (ed.). Interface between ecology and land development in California. Southern California Academy of Sciences, Los Angeles, California.
- Sylvia, D.M. and S.E. Williams. 1992. Vesicular-arbuscular mycorrhizae and environmental stresses. P. 101-124. In: G. J. Bethlenfalvay and R. G. Linderman (eds.). Mycorrhizae in sustainable agriculture. American Society of Agronomy, Madison, Wisconsin.
- **Tinker, P.B.H.** 1978. Effects of vesicular-arbuscular mycorrhizas on plant nutrition and plant growth. Physiol. Veg. 16:743-751.
- **Trappe, J.M., R. Molina,** and **M. Castellano.** 1984. Reactions of mycorrhizal fungi and mycorrhiza formation to pesticides. Ann. R. Phyto. 22:331-359.

QUESTION-ANSWER FRIDAY MORNING

Barbara Selemon: Will mycorrhizae work with exotic plants? Can mycorrhizae be introduced after the plant has been grown for 1 year?

Ted St. John: It is not too late. You can certainly inoculate after the fact. It's not the best thing to do when plants are in large containers. With special plants or for research purposes, it has been done. For the first question, whether the fungus is suitable, the only real question here is whether your plants are the vesicular-arbuscular type. If they are, then these general fungi are suitable. In fact, the same species are probably found in their home countries. They tend to be globally distributed and there are about 200 species. You do have to make sure it's the right kind of fungus and the fungi tend to be quite specialized for soil although they are very unspecialized with regard to host. The fungus that comes from an acid soil will not work in a neutral or basic soil, for instance.

Christy Alterman: How can you tell the difference between fungus gnat larvae and shore fly larvae?

Karen Robb: If they have a dark brown head capsule it is diagnostic for fungus gnats?

Christy Alterman: Would your potato idea attract shore flies as well or is it specific for fungus gnats?

Karen Robb: The potato slices or cores attracts only fungus gnat larvae.

Andrew Davis: Are mycorrhizae affected by herbicides (e.g., RoundUp) that are applied to the soil around landscape plants?

Ted St. John: No, herbicides are not usually directly damaging to the fungi, although if you were to eliminate the host plant over a long period of time the fungi would gradually die out.

Andrew Davis: How long is long?

Ted St. John: The only answer is, it depends on the host plant, how it propagates itself and the soil. Some fungi have spores that are quite resistant. One to two years there will probably be a significant amount of inoculum, but 3 to 5 years is too long.