Arbuscular Mycorrhizal Inoculation in Nursery Practice

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Abstract -The beneficial plant-fungus association known as arbuscular mycorrhiza (AM) or vesiculararbuscular mycorrhiza (VAM) is known to improve phosphorus uptake, drought tolerance, and resistance to pathogens, among other benefits. The symbiosis is to a large extent a buffer against unfavorable soil conditions, and the benefits are generally more readily apparent in the field than in the nursery. Even so, plants intended for revegetation, habitat restoration, or forestry should be inoculated in the nursery with appropriate mycorrhizal fungi.

Fungal propagules must be placed in the root zone rather than on the surface of the medium. In the nursery inoculation is carried out by incorporating inoculum in the medium, by placing inoculum below the seedling at a transplant stage, or by dipping bare-root stock in adhesive-treated inoculum. Since the spores and other propagules of AM fungi are large and quickly settle out of suspension, it is unlikely that a successful method will be developed to apply inoculum through an irrigation system. The best procedures for each nursery will depend on properties of the inoculum, the species of plants, and site-specific factors that involve integration of the mycorrhizal program into existing nursery practices.

Fertilization in excess of the plant's current needs often reduces mycorrhizal colonization; thus fertilization procedures must almost always be modified to accommodate a mycorrhizal program. Similarly, fungicide and pesticide applications must be planned for compatibility with the symbiosis. Increasing demand for quality habitat restoration, and the mycorrhizal plants that it requires, will likely make a serious mycorrhizal program look attractive to increasing numbers of nursery managers.

INTRODUCTION

The endomycorrhizal symbiosis, also called arbuscular (AM) or vesicular-arbuscular mycorrhiza (VAM) is found in about 70% of the plant species examined to date, and is found somewhere in 80 to 90% of the world's plant families (Trappe 1987). Mycorrhiza is best known for dramatic growth responses, sometimes as much as thirty or more times the growth rate of otherwise comparable plants. These growth responses are usually related to phosphorus nutrition, and are most pronounced in soil of low fertility (Tinker 1978).

Other effects of mycorrhiza make less dramatic photographs, but may be more meaningful when nursery plants go out to a restoration or reforestation site. Other effects include drought tolerance, plant diversity, soil structure, resistance to pathogens, and ecosystem functionality.

Drought tolerance is commonly higher in mycorrhizal than non-mycorrhizal plants. Whether this is a direct effect of mycorrhizal fungi, or simply a side benefit of improved phosphorus nutrition is still in debate (Hardie 1986; Nelsen 1987).

Plant diversity is commonly higher after mycorrhizal inoculation (Gange et al. 1990; Fitter and Read 1994; St. John 1996). The effect is exerted mainly through improved survival of less common species (Grime et al. 1987).

The filaments of mycorrhizal fungi (the hyphae) are key players in the formation of soil structure (Bethlenfalvay and Schüepp 1994). Soil structure is a primary concern in restoration and reforestation. Some degraded forest soils are said to no longer support tree growth because they lack structure (Perry et al. 1987).

Wild plants in healthy ecosystems suffer very little from root disease, because soil pathogens are in balance with other beneficial soil organisms (St. John 1993). The mycorrhizal symbiosis is a key player in this balance, since it selectively favors beneficial soil organisms (Linderman 1994). The selective effect includes plant growth-promoting rhizobacteria (PGPR), organisms that improve plant growth by mechanisms that are still under discussion (Kloepper et al. 1991).

The beneficial effects of the mycorrhizal symbiosis are less evident in the favorable conditions of a nursery than in the harsh conditions of the final field site. Mycorrhizal inoculation is less likely to accelerate the nursery crop than it is to improve the customer's planting success.

The objective in habitat restoration is not simply to make plants grow, but to form a functional ecosystem. The characteristics of functional ecosystems include productivity, sustainability, retention of nutrients, resistance to invasive species, and biotic interactions (Ewel 1987), properties that all depend heavily upon rnycorrhiza.

Tree of Life Nursery produces plants primarily for use in habitat restoration, where the conditions are difficult at best. The mycorrhizal program at this nursery has become a major selling point for the product and a major source of recognition for the nursery.

LACK OF NATIVE FUNGI

No one doubts the importance of mycorrhizal fungi in restoration and forestry. The only question is whether the fungi are already present on site, and will thus take care of themselves. Conditions in which native mycorrhizal fungi are typically lacking, and must be intentionally added, include land that has been graded, that is badly eroded, is overgrazed, is frequently disked, or that previously supported a nearly pure stand of strictly ectomycorrhizal trees. On graded land the top soil has been carried away, or the profile has been inverted. Eroded land usually lacks top soil and thus mycorrhizal fungi (Hall 1980; Powell 1980). Overgrazed land lacks microorganisms due to the poor condition of the vegetation (Bethlenfalvay et al. 1985). Mechanical disturbance, like disking, breaks up the mycelium in the soil and reduces its viability (McGonigle et al. 1993). Forests dominated by pines may lack propagules of endomycorrhizal fungi (Kovacic et al. 1984), as may other ectomycorrhizal forests (Gerdemann 1968).

In general, it is an advantage for the plants to already be mycorrhizal at the difficult time of transplanting. For this reason, restoration and reforestation container and bare root plants are best inoculated before outplanting.

It is of particular importance to note that mycorrhizal fungi are lacking or in low concentration in soilless nursery media (Graham and Timmer 1984) and in fumigated nursery beds (Hattingh and Gerdemann 1975). If the plants are to leave the nursery in the mycorrhizal condition, they will have to be intentionally inoculated early in production.

MYCORRHIZAL INOCULUM

The material that carries propagules of mycorrhizal fungi is called inoculum. Several kinds of fungal structures can serve as propagules, and all are of value in mycorrhizal inoculum. Propagules Include spores, mycorrhizal root fragments, and pieces of mycelium. Spores are generally considered the most resistant to adverse environmental conditions, but are slower than other propagules to colonize new roots. Mycelial fragments are usually the fastest to colonize new roots.

A significant limitation in practical use of mycorrhizal fungi is the size of the propagules. The spores are in the range of 1/10 millimeter in diameter, the largest of all fungal spores. Hyphal fragments that are large enough to constitute good inoculum may be that size or larger. A result is that they quickly settle out of suspension and do not readily pass through apertures of small diameter. Thus endomycorrhizal inoculum does not suit itself to distribution through a liquid handling system. Material applied to the surface of soil or even a very open container mix is likely to remain on the surface, and out of reach of the roots. This is in contrast to ectomycorrhizal inoculum, which works well when applied to the surface of a container mix.

In addition to fungal structures, the inoculum usually includes a carrier. The carrier may be sand, soil, peat, clay, or other solid substrate. Suspensions of fungus plus carrier in a viscous liquid, such as certain polymer formulations, work well as root dips. The polymers are likely to remain too expensive to serve as carriers for direct field application.

CHOICE OF FUNGI

Trappe (1977) suggested that ectomycorrhizal fungi should be chosen for the final project site, not for convenience in the nursery. The same could be said for endomycorrhizal fungi, which vary in their responses to soil properties, especially pH. While there is no specificity between fungus and host plant, there are preferences that can be expressed in field or greenhouse experiments (Brundrett 1991; Johnson et al. 1992). That is, some fungal species work better with particular host plants.

The best way to assure a good fit between plant, soil, and fungus may be to isolate a mixture of native species from undisturbed vegetation on the same soil (Daft 1983; Perry et al. 1987). Unfortunately, native fungi are often more difficult to culture than the proven "generic" strains, and in any case require more time and expense to produce. Further, there is no assurance that the native fungi of the undisturbed soil will still be appropriate for the altered conditions after disturbance (Stahl et al. 1988).

Most often, the nursery manager must select from a very short list of commonly available commercial strains. At the very minimum, the selected fungi must be suitable for the soil pH at both the nursery and the final planting site. Two fungi now being offered in commercial preparations are Glornus intraradices and G. etunicatum. Both are strains originally tested and made available by NPI, a company no longer in the inoculum business. G. intraradices has provided good growth responses in a wide range of host plants, at soil pH from about 6 to 8.5 or higher. and G. etunicaturm has been most effective in moderately acid soils.

INOCULUM PLACEMENT

A guiding principle in mycorrhizal work is that the moculum must go into the root zone (Hayman et al. 1975; Ferguson and Menge 1986). The roots of new seedlings must be able to grow through the inoculum, unlikely if the inoculum is placed on the soil surface. The propagules of mycorrhizal fungi are large and will not readily wash into the soil, so even an open, loose container mix is difficult to penetrate.

For plants grown from seed in their final containers, the most cost-effective option may be to mix the inoculum into the container medium. This is likely to use more inoculum than might be necessary with other inoculation methods, but requires less labor. Nurseries that do not prepare their own container mixes may be able to persuade their medium suppliers to incorporate the inoculum.

Inoculation may be of greatest benefit to the plant when done at the earliest possible stage. However, germination and rooting stages can be difficult times to inoculate because the facilities commonly have low light intensity, heavy use of chemicals , and very wet medium, making mycorrhizal colonization difficult. For plants that are moved one or more times during the production cycle, the first transplant may be a more practical time to inoculate. Most plants at Tree of Life Nursery are inoculated by placing two mL of granular moculum, containing well over 100 propagules, beneath the transplant. The cost of such inoculation is about a penny per container.

An additional possibility is a root dip. This has been used for ectomycorrhizal (ECM) species, and would be most appropriate at the end of the container phase of production. The nursery might dip bare-root plants before delivering them to the customer, or might dip container plants that were not made mycorrhizal during production. The slurry must contain ingredients that make the suspension viscous to keep the propagules from settling out rapidly, and must act as an adhesive. The root dip would also protect the root systems from desiccation.

The combined benefits of mycorrhizal inoculation may help the nursery meet environmental standards. The improved nutrient uptake of mycorrhizal plants means that nutrient solutions can be less concentrated, and that less will leach through the medium and into the ground water. Pathogen antagonists that are often associated or favored by mycorrhizal fungi may allow a reduction in other chemicals. There is a delicate balance between enough fertilizer and pesticide to maximize production, and too much for the tolerance of the beneficial organisms. The correct balance will depend on plant species and many factors that are specific to each nursery, and can only be fine-tuned by experimentation on site. The need for procedural changes is least with fumigated bare-root beds and greatest with high-tech indoor systems in small containers.

Perhaps the easiest place to begin a mycorrhizal program is in bare root beds. Fumigation has killed all native inoculum, and many plant species perform very poorly after fumigation. ECM fungi often disperse to the site quickly by wind-blown spores, but AM fungi may require months or years to arrive if not introduced by the nursery manager. Some growers of coast redwood, western red cedar, pacific yew, and other endomycorrhizal species have introduced native forest soil into the fumigated beds, and yew, and have realized substantial benefits from doing so. Unfortunately, forest soil contains both desirable and undesirable organisms, and its use can be a very risky practice. Good quality commercial inoculum bypasses these risks, and can be introduced by banding or disking it into the soil. If the plants go into the bed as seedlings rather than seeds, they can be made mycorrhizal in the container or can be treated with a root dip at the time of outplanting.

A final nursery option is inoculation as the plants leave the nursery. This allows the nursery to use chemicals and methods that may prevent mycorrhizal colonization, but still make the plants mycorrhizal soon after outplanting. A root dip, as described above, is probably the most cost-effective option for this kind of application.

If the nursery has not made the plants mycorrhizal, the customer may wish to inoculate the plants at the field site. This may be done with a root dip or by dropping a pre-packaged "tea bag" of inoculum in each planting hole. Endomycorrhizal inoculum, packaged in tea bags with or without compatible fertilizer formulations, is now available.

Finally, inoculum in a solid carrier can be incorporated into the soil by broadcasting followed by disking, or by broadcasting onto freshly ripped ground, followed by dragging. A method rapidly gaining favor in habitat restoration is a specially-modified land imprinter. The imprinter places inoculum one to four inches in the soil, then shapes the soil to provide spatial heterogeneity and facilitate water infiltration. It applies seed in firm capillary contact with the soil. This single-pass operation has proven very cost effective in land restoration. The method has provided dramatic improvements in plant survival and species diversity (St. John 1996). The cost of inoculum in field application has ranged from $300 to $500 per acre.

DOSAGE

The amount of inoculum required for a particular application is an important question, because it directly influences both the cost of the operation and the chances for success. For container use, the recommended number of propagules per plant has drifted downward, from several hundred a few years ago to much lower numbers now.

The recommendations come from inoculum suppliers and researchers, and the basis for each recommendation is not always clear. Obvious motivations for recommending high doses are to be sure it works and to sell more product. This last motivation can be self-defeating, since the cost of the product becomes noncompetitive at high doses. Our own recommendations are based on spatial dispersion of propagules in the medium, and on empirical tests of dosage rates. Tests are continuing, and as lower rates are tested our recommendations may go down.

It is more difficult to understand why a supplier would recommend a very low dose. One supplier with a particularly low propagule count actually recommends a container plant rate that can provides fewer propagules than the number of containers! The supplier may not realize the error, but a clear advantage of such a recommendation is that the product appears inexpensive.

VERIFICATION OF RESULTS

No mycorrhizal program can be successful without a means of verifying results, since inoculation can and will fail for numerous reasons. Low light intensity, short photoperiod, low carbon dioxide concentration, and the presence of excess ethylene are common problems in the greenhouse. Cold temperatures, excess fertility, incompatible pesticides, incompatible medium ingredients, contaminated water, and wet medium may prevent colonization either indoors or outdoors. It Is critical that environmental conditions and procedural changes be checked at every step.

Another problem is that mycorrhizal roots look just like ordinary roots to most observers. You may be faced with competitors who will claim their product is mycorrhizal, while saving themselves the expense of a serious mycorrhizal effort. Customers are now beginning to request proof of this claim.

Setting up an in-house mycorrhizal lab requires training, microscope equipment, and the use of toxic chemicals. There is at least one commercial laboratory, in Corvallis, Oregon, that will stain and interpret roots for about $30 per sample. Your plant pathologist may be able to provide this service, or train you to do it yourself.

SUMMARY

Mycorrhiza is a normal and necessary part of plant roots if the plants are to be used in habitat restoration or forestry. Mycorrhizal plants are more independent and better prepared for existence at the restoration or reforestation site. There is now considerable demand for mycorrhizal plants in commercial projects. Most plant species used in restoration, and several used in forestry, are endomycorrhizal hosts. The considerable benefits of mycorrhiza promote not only health and survival of the individual plant, but of the ecosystem as well. A functional ecosystem is not possible without mycorrhizal fungi and a range of associated beneficial organisms.

Inoculation in the nursery is entirely feasible, but may require procedural changes to accommodate the symbiosis. Media, fertilizer, chemicals, and environmental conditions may all have to be adjusted. Successful colonization is probably most practical in fumigated bare root beds, and most difficult in greenhouse crops with automated watering and fertilization.

Direct inoculation in the field is now practical, and may be the best alternative when nursery inoculation is unsuccessful or impractical. Plants may be treated at planting time with a root dip, with inoculum in the planting hole, or by incorporation of inoculum into the field soil.

By adopting a mycorrhizal program, the nurseryman may expect more efficient use of fertilizer and less need for pesticides. These will provide immediate savings in materials and better environmental compliance, which may well offset any added costs of inoculation. The procedures are demanding, however, and the program will require time to establish.

The difficulties involved in initiating a mycorrhiza program, and the lack of evident responses in fertile nursery soils, have made it difficult to persuade nursery managers to undertake routine inoculation. It is easy to conclude that anything as invisible as mycorrhiza takes care of itself, a mistaken impression in the case of habitat restoration. Demands for success in restoration is likely to increase the pressure for mycorrhizal inoculation in the nursery.

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LITERATURE CITED

Bethlenfalvay, G. J.; R. A. Evans, and A. L. Lesperance. 1985. Mycorrhizal colonization of crested wheatgrass as influenced by grazing. Agronomy Journal 77:233-236.

Bethlenfalvay, G. J., and H. Schüepp. 1994. Arbuscular mycorrhizae and agrosystern stability. In: Gianinazzi, S., and H. Schüepp (Eds.). Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhäuser-Verlag, Basel.

Brundrett, M. C. 1991. Mycorrhizas in natural ecosystems. Pages 171-313 in: A. Macfaydn, M. Begon, and A. H. Fitter, Eds. Advances in Ecological Research. Vol. 21. Academic Press, London.

Daft,-M. J. 1983. The influence of mixed inocula on endomycorrhizal development. Plant and Soil 71:33 1337. The Hague, Netherlands; Nijhoff/Junk.

Ewel, J. J. 1987. Restoration is the ultimate test of ecological theory. pp. 31-33 in: W. R. Jordan, M. E. Gilpin, and J. D. Aber (eds.). Restoration Ecology: a synthetic approach to ecological research. Cambridge University Press, Cambridge.

Ferguson, J. J., and J. A. Menge. 1986. Response of citrus seedlings to various field inoculation methods with Glomus deserticola in fumigated nursery soils. Journal of the American Society of Horticultural Science 111:288-292.

Francis, R., and D. J. Read. 1994. The contributions of mycorrhizal fungi to the determination of plant community structure. Pp. 11-25 in: A. D. Robson, L. K. Abbott, and N. Malajczuk (eds.). Management of mycorrhizas in agriculture, horticulture, and forestry. Kluwer Academic Publishers, The Netherlands.

Gange, A. C., Brown, V. K., and L. M. Farmer. 1990. A test of mycorrhizal benefit in an early successional plant community. New-Phytologist 115:85-91.

Gerdemann, J. W. 1968. Vesicular-arbuscular mycorrhizae and plant growth. Annual Review of Phytopathology 6:397-418.

Graham. J. H.. and L. W. Timmer. 1984. Vesiculararbuscular mycorrhizal development and growth response of rough lemon in soil and soilless media: Effect of phosphorus source. Journal of the American Society Horticultural Science 109:118-121.

Grime, J. P., J. L. M. Mackey, S. H. Hillier, and D. J. Read. 1987. Floristic diversity in a model system using experimental microcosms. Nature 328:420-42 1.

Hall, I. R. 1980. Growth of Lotus pedunculatus Cav. in an eroded soil containing soil pellets infested with endomycorrhizal fungi. New Zealand Journal of Agricultural Research 23:103-105.

Hardie, K. 1986. The role of extraradical hyphae in water uptake by vesicular-arbuscular mycorrhizal plants. P. 651-655 in: V. Gianinazi-Pearson and S. Gianinazi (eds.). Physiological and genetical aspects of mycorrhizae. Proceedings of the 1st European symposium on mycorrhizae, Dijon, 1-5 July, 1985.

Hattingh, M. J. and J. W. Gerdemann. 1975. Inoculation of Brazilian sour orange seed with an endomycorrhizal fungus. Phytopathology 65:1013-1016.

Hayman, D. S., A.M. Johnson, and 1. Ruddlesdin. 1975. The influence of phosphate and crop species on Endogone spores and vesicular-arbuscular mycorrhiza under field conditions. Plant and Soil 43:489-495.

Johnson, N. C., D. Tilman, and D. Wedin. 1992. Plant and soil controls on mycorrhizal fungal communities. Ecology 73:2034-2042.

Kloepper, J. W., R. M. Zablotowicz, E. M. Tipping, and R. Lifshitz. 199 1. Plant growth promotion mediated by bacterial rhizosphere colonizers. Beltsville-Symposia-in-Agricultural-Research. 1991, No. 14, 315-326. Presented at a symposium held May 8-11, 1989 at Beltsville, Maryland, USA.

Kovacic, D. A., St. John, T. V., & Dyer, M. 1. (1984). Lack of vesicular-arbuscular mycorrhizal inoculum in a Ponderosa Pine forest. Ecology 65:1755-1759.

Linderman,-R. G. 1994. Role of VAM fungi in biocontrol. P. 1-26 in: F. L. Pfleger and R. G. Linderman (eds.). Mycorrhizae and plant health. APS Press, St. Paul.

McGonigle, T. P., and M. H. Miller. 1993. Responses of mycorrhizae and shoot phosphorus of maize to the frequency and timing of soil disturbance. Mycorrhiza 4:63-68.

Nelsen, C. E. 1987 Chapter 5. The water relations of vesicular-arbuscular mycorrhizal systems. Pp. 71-91 in G. E. Safir. Ecophysiology of VA mycorrhizal plants. CRC Press, Boca Raton.

Perry, D. A., R. Molina, and M. P. Amaranthus. 1987. Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. Canadian Journal of Forest Research 17:929-940.

Powell, C. L. 1980. Mycorrhizal infectivity of eroded soils. Soil Biology and Biochemistry 12:247-250.

St. John, T. 1996. Specially-modified land imprinter inoculates soil with mycorrhizal fungi (California). Restoration and Management Notes 14:84-85.

St. John, T. V. 1993. The importance of mycorrhizal fungi and other beneficial organisms in biodiversity projects. P. 99-105 in: T. D. Landis. Proceedings, Western Forest Nursery Association. USDA Forest Service General Technical Report RM-221.

Stahl, P. D., S. E. Williams, and M. Christensen. 1988. Efficacy of native vesicular-arbuscular mycorrhizal fungi after severe soil disturbance. New-Phytologist 110:347-354.

Tinker, P. B. H. 1978. Effects of vesicular-arbuscular mycorrhizas on plant nutrition and plant growth. Physiologia Vegetal 16:743-751.

Trappe, J. M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annual Review of Phytopathology 15:203-222.