# Growing Mycorrhizal Native Plant Species

**Shengjun Lu**
Bitterroot Restoration, Inc., 55 Sierra College Boulevard, Lincoln, California 95648

## INTRODUCTION

Since its establishment in 1986, Bitterroot Restoration, Inc. (BRI) headquartered in Corvallis, Montana has been doing ecological restoration on severely disturbed lands. The focus of much of our work has been on arid, semi-arid, and sensitive, high-elevation areas, as well as sites severely disturbed by mining and pollution. Many mining companies, the National Park Service, the Environmental Protection Agencies, other federal and state agencies, and the private sector in the western United States of America work with us because we offer comprehensive restoration services and provide site specific native plants for our restoration projects. During the past several years, we have been trying to incorporate root-associated microorganisms into our restoration processes. Today, I will share with you our philosophy and approach to the restoration process, and our successful mycorrhizal program.

## OUR PHILOSOPHY AND APPROACH

We at BRI believe that site specific native plants and their associated rhizosphere microorganisms must be utilized for successful restoration on environmentally sensitive and severely disturbed sites. Native plant species, through millions of years of natural selection, have adapted to their indigenous environments. Rhizosphere microorganisms are not only part of the ecosystems, but also critical to both establishment and long-term survival of plants in a sustainable plant community. Planting a site with shrubs and trees is not always enough; an ecological approach must be used.

A general approach to building a restoration plan follows these steps:

1) Gather baseline information. This would include comprehensive information obtained from an undisturbed reference plant community. On the disturbed site, information would be collected including: species composition, soil data, microorganisms, succession stage, habitat, and conditions limiting vegetation survival and growth. This initial site visit and analysis is very important.
2) Establish a site-specific restoration plan by carefully selecting the major components of vegetation and closely associated microorganisms, soil amendments, and a time line leading to the installation.
3) Collect plant propagules and microorganism inocula.
4) Propagate plants, isolate and culture microorganisms, and inoculate plants with microorganisms.
5) Harden-off plants and induce dormancy.
6) Amend soil.
7) Install plants on the sites and ameliorate micro-site conditions.
8) Modify and update original restoration plan with monitoring.

## WHY MYCORRHIZAE ARE IMPORTANT?

First, mycorrhizal fungi are naturally occurring components of soil ecosystems. Seventy percent or more of the species of angiosperms, and most or all species of gymnosperms are mycorrhizal (Harley, 1991). Because mycorrhizal fungi may not exist on severely disturbed sites, we need to restore both vegetation cover and rhizosphere microorganisms in the soil.

Second, mycorrhizal fungi increase the uptake and translocation of water and nutrients for plants by significantly increasing the soil volume that the roots can effectively explore. The range of maximum values of mycorrhizal fungal mycelium length to root length are <1 to 592 m $cm^{-1}$ of root (Sylvia, 1986). This means that the mycelia length increases effective root length by up to 59,200 fold. I am sure some of you are very familiar with Read's incredible picture of a small seedling with mycorrhizal hyphae (Read, 1991). One can imagine, from this picture, how the mycorrhizal fungi increase the root absorption area in the soil.

Third, mycorrhizal fungi reduce plant transplanting shock by increasing plant drought tolerance (Pigott, 1982; and Walker et al., 1989). This is extremely important for initial plant survival on arid and severely disturbed sites. The number one issue facing restorationists with these sites is plant survival. Incorporating mycorrhizal fungi into plant production process can dramatically increase plant survival after installation. One should realize that while mycorrhizae may not significantly increase plant growth in the nurseries, they are critical to plant survival and successful revegetation on harsh and severely disturbed sites (Evans, 1997).

Last, mycorrhizal fungi alleviate the toxicity of heavy metals to plants (Denny and Wilkins, 1987; and Jones and Hutchinson, 1986). Heavy metals in soil at mining sites, and Super-Fund sites are toxic, even to native plants. However, mycorrhizal fungi can help plants resist heavy metal toxicity through sequestering these metals in the fungal hyphae. Inoculation of these plants greatly increases a plant's initial survival and long-term growth on these sites. Additional benefits include disease suppression and improvement in soil structure.

## OUR MYCORRHIZAL PROGRAM

Mycorrhizal fungi are classified according to the type of relationship they have with root cells and by their culturing requirements. The major groups of mycorrhizae are ectomycorrhizae, endomycorrhizae, and ectendomycorrhizae.

The plants we grow at BRI that form ectomycorrhizae include the genera: *Abies, Alnus, Betula, Picea, Pinus, Populus, Quercus,* and *Salix.* The hyphae of these ectomycorrhizal fungi do not penetrate root cells. They grow between and outside the root cells and form a layer called the Hartig net. Ectomycorrhizae are usually very distinct and easily recognized by their visible structures (Castellano and Molina, 1989). This type of mycorrhizal fungi can be cultured with artificial semisolid or liquid media. At BRI, we use either fruiting bodies collected from specific sites, or commercially available spores to inoculate all our ectomycorrhizal plants in greenhouse 6 to 10 weeks after sowing of seeds. Before planting, mycorrhizal colonization rates are assessed. We have been very successful in colonizing all our ectomycorrhizal plants.

However, most of the native plant species we grow at BRI are endomycorrhizal species. Endomycorrhizal fungal hyphae penetrate into root cells and occasionally form structures called vesicles and arbuscles. They are also referred to as vesicular-

arbuscular mycorrhizae (VAM). This type of mycorrhizal structure is not easily visible. One has to rely on chemical staining and de-staining methods to examine them microscopically (Rajapakse and Miller 1992). Culturing this type of fungi in semisolid or liquid media is not possible. They must be cultured directly with host plants. Characteristically, mycorrhizal fungi spread very slowly in the soil as they can only be carried by root growth or distributed by soil disturbances. Consistent with the restoration plan, we collect native plant roots and soils from restoration sites, isolate the target mycorrhizal fungal spores in the laboratory, increase the mass with host plants in our research greenhouse, and inoculate target plants with these fungi. After monitoring the plants to ensure that we achieve at least 30% success rate of colonization to total root mass for each seed lot, we inform our clients of the status of the plants. If the colonization percentage of a seed lot is less than 30%, we reinoculate. We have grown most endomycorrhizal native plants successfully including the genera: *Amelanchier, Chrysothamnus, Juniperus, Prunus, Purshia, Rhus, Ribes, Rubus,* and *Rosa*.

Ectendomycorrhizae have characteristics of both ectomycorrhizae and endomycorrhizae. These mycorrhizal fungi penetrate into root cells as well as grow outside of roots as ectomycorrhizal fungi. Native plant species we have grown with this type of mycorrhizal association are in the genera *Arctostaphylos* and *Vaccinium*. *Arctostaphylos* is sometimes referred to as *arbutoid*, and *Vaccinium* as *Ericoid* (Smith and Read, 1997). For the genus *Arctostaphylos*, we have grown native plants with site-specific inoculant cultured in our research greenhouse, as well as commercial ectomycorrhizal sources. We have been particularly successful colonizing *Arctostaphylos uva-ursi*. For *Vaccinium* species, our approach and method are consistent. However, it has been very difficult to stain and observe the mycorrhizal fungi in the laboratory. Future work needs to be done to improve and verify our results.

In summary, we have successfully grown many mycorrhizal plant species native to the western United States. We continue to research and develop techniques for mycorrhizal fungal collection, isolation, culturing, inoculation, and colonization analysis. We have incorporated our mycorrhizal program into our normal propagation routine and have seen positive results of mycorrhizal inoculation in the field. Our future goal is to incorporate not only mycorrhizal fungi, but also other beneficial microorganisms into our plant propagation and restoration work.

## LITERATURE CITED

**Castellano, M.A.,** and **R. Molina.** 1989. Mycorrhizae, pp. 101-167. In: T.D. Landis, R.W. Tinus, S.E. McDonald, and J.P. Barnett (eds.). The container tree nursery manual. Dept Agric., Forest Service. Vol. 5. Agriculture Handbook, 674. Washington D.C.

**Denny, H.J.,** and **D.A. Wilkins.** 1987. Zinc tolerance in *Betula spp*. IV. The mechanism of ectomycorrhizal amelioration of zinc toxicity. New Phytol. 106:545-553.

**Evans, M.** 1997. Mycorrhizal inoculation of California native plants in containers. Comb. Proc. Intl Plant Prop. Soc. 47:262-263.

**Harley, J.L.** 1991. Introduction: The state of the art, pp.1-23. In: J.R. Norris, D. Read, and K. Varma (eds.) Techniques for mycorrhizal research. Academic Press, San Diego.

**Jones, M.D.** and **T.C. Hutchinson.** 1986. The effect of mycorrhizal infection on the response of *Betula papyrifera* to nickel and copper. New Phytol. 102:429-442.

**Pajapakse, S.** and **J.C. Miller, Jr.** 1992. Methods for studying vesicular-arbuscular mycorrhizal root colonization and related root physical properties. pp.761-782. In: J.R. Norris, D. Read, and K. Varma (eds.). Techniques for mycorrhizal research. Academic Press, San Diego.

**Piggot, C. D.** 1982. Survival of mycorrhiza formed by *Cenococcum geophilum* Fr. in dry soils. New Phytol. 92:513-517.

**Read, D.J.** 1991. Mycorrhizal fungi in nature and semi-natural plant communities, pp. 27-53. In: Ecophysiology of ectomycorrhizae of forest trees. M. Wallenberg Foundation Symposia Proceedings. Stockholm, Sweden.

**Smith, S.E.** and **D.J. Read.** 1997. Mycorrhizal symbiosis. 2nd ed., Academic Press, San Diego.

**Sylvia, D.M.** 1992. Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi, pp. 513-525. In: J.R. Norris, D. Read, and K. Varma (eds.) Techniques for mycorrhizal research. Academic Press, San Diego.

**Walker, R.F., C.D. West, S.B. McLaughlin,** and **C.C. Amundsen.** 1989. Growth, xylem pressure potential, and nutrient absorption of loblolly pine on a reclaimed surface mine as affected by an induced *Pisolithum tinctorius* infection. Forest Sci. 35:569-581.