A Practical Look at Mycorrhizal Fungi in Nurseries: Part Two

In the last issue of FNN we discussed what mycorrhizae are, the differences between ectomycorrhizae (ECM) and vesicular-arbuscular mycorrhizae (VAM), benefits of encouraging mycorrhizae in nurseries, and how to determine if you have a mycorrhizal problem. This time we will finish up with a discussion of the development of mycorrhizae in nurseries, the various types of mycorrhizal inocula, how and when to inoculate, and the effect of nursery cultural practices on mycorrhizal development.

Development of mycorrhizae in nurseries

Although wild plants become inoculated with mycorrhizal fungi soon after they germinate, inoculation in nurseries is slowed or even completely eliminated by cultural practices. However, considerable variation occurs between ECM and VAM and between bareroot and container nurseries.

Soil fumigation in bareroot nurseries eliminates all species of fungi and so mycorrhizal colonization is delayed. Reinoculation occurs more quickly with ECM fungi because they can spread either by airborne spores or by mycelial growth through the soil. Most fumigated soil becomes recolonized by the end of the first growing season. However, because VAM fungi lack an airborne spore stage, they can only slowly reinvade sterilized soil by mycelial growth or inadvertent transfer of colonized soil. It may take months or even years for VAM fungi to completely recolonize fumigated seedbeds.

Mycorrhizal colonization is much different in container nurseries because artificial growing media are essentially sterile and containers are sterilized between crops. And, as we discussed in Part 1, container seedlings grow perfectly normal without mycorrhizae because all the various growth-limiting factors are being provided culturally. In nurseries close to vegetation supporting the proper species of mycorrhizae, spores of ECM fungi do blow into container nurseries and colonize the seedlings. Some ECM fungi, such as *Thelephora terrestris,* thrive in the nursery environment, so this type of mycorrhizae is very common in container seedlings. The chocolate brown fungi can be seen growing out the bottom of containers, and sometimes forms a sheath around the stem of conifer seedlings (Figure A). Reinvasion of VAM fungi is completely inhibited when artificial growing media are used. So, considering the problems with natural reinvasion of mycorrhizal fungi in nurseries, some growers inoculate their nursery stock.

Inoculating nursery stock

When considering an nursery inoculation program, several things need to be considered:

1. *The proper species of fungi for each crop.*
2. *The most appropriate type of inoculum for your nursery system.*
3. *The proper timing and technique.*
4. *Cost effectiveness.*

Species selection

There are two possible routes to take when selecting a mycorrhizal fungi for inoculation: selection of a species adapted to a broad range of hosts or site conditions, or selection of a species adapted to a specific host or particular type of outplanting site. Obviously, the first step is to select a fungus that can colonize the plant species you wish to inoculate. Most VAM fungi and ECM species such as *Cenococcum geophilum, Pisolithus tinctorius,* and *T. terrestris* have broad host ranges. Broadly-adapted mycorrhizal fungi are advantageous because many nursery crops can be inoculated at the same time. Also, the inoculated seedlings would be adapted to a wide variety of outplanting site conditions. On the other hand, host-specific or site-specific mycorrhizal fungi will produce maximum seedling performance in a given application.

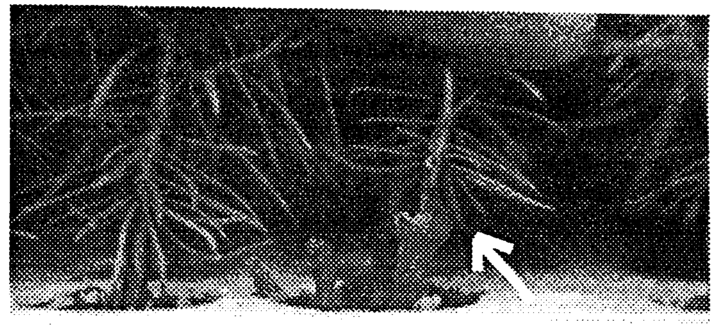

Figure A. Brown felt-like fruiting bodies of Telephora terrestris often fruit on the stem of seedlings , or on bottom of containers.

Types o f mycorrhizal inocula

There are two basic categories of inocula currently being used in forest and conservation nurseries: spores, or vegetative inoculum (Figure B). Fungal spores of ECM are obtained from fruiting bodies collected from wild stands, or VAM spores can be sieved from soil collected in the root zone of host plants. Vegetative inoculum is produced from fungal mycelia grown in pure culture on an artificial medium. Although several different ECM fungi have been successfully produced, VAM fungi are very difficult to grow in pure culture.

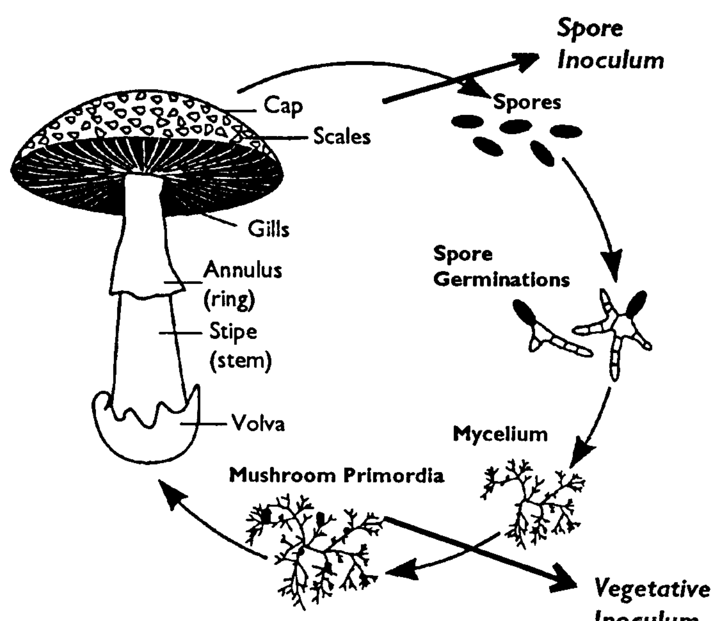

Figure B. Two types of Mycorrhizal Inocula (Modified from Molina and others, 1993)

ECM inoculum can also be obtained by collecting duff or soil from forest stands, or from around seedlings known to be colonized with the desired fungus. While simple and proven effective, this method has several drawbacks. Collecting soil is time-consuming, and the inoculum is bulky and expensive to transport. Besides, you never really know which fungi you are collecting, and this technique also carries the risk of introducing unwanted fungi, weeds or other pests into the nursery. Another option is to collect the mushrooms, puffballs, or truffles of the desired fungus in the wild and harvest the spores for inoculum. This method is relatively simply and inexpensive, and has been effectively used in several countries. Its main limitation is that identifying and collecting fruiting bodies of ECM fungi requires time and training, and it is restricted in some areas due to overharvest for commercial food markets.

Because VAM fungi have an extremely wide host range, some nurseries raise their own inoculum. Host plants are "pot cultured" or raised in special seedbeds, where the fungus is first inoculated on a host-like sorghum or clover, allowed to proliferate on that host, and then the mycelium, spores and host roots are used as inocula for the seedling crop. Some growers collect the relatively large VAM spores by wet sieving of soil. Other nurseries collect root trimmings of VAM hosts during seedling harvesting, and use them to inoculate the next crop. While having many of the same drawbacks as the collecting native soil or duff, there is less risk of pest introduction when using soil or seedling roots from pot cultures or from nursery beds.

Both spore inoculum and vegetative inoculum are available commercially. Several firms have developed sophisticated techniques for culturing both ECM and VAM fungi on artificial media. Other companies collect fruiting bodies of ECM fungi, harvest the spores, and sell them to nurseries (see list of suppliers at the end of this section).

How and when to inoculate

The method of inoculum application, and the required amount of inoculum will vary between container and bareroot nurseries. For either type of nursery, there are three possible times to inoculate: at the time of sowing, during the crop cycle, or during outplanting.

Bareroot seedlings can be inoculated with either VAM or ECM fungi by incorporating soil or duff containing native mycorrhizae into the top layer of the seedbed. Spore inoculum can also be applied to seed prior to sowing. Vegetative inoculum or spore inoculum can be mixed with a carrier and banded in the seed drill rows during sowing. Fluid drilling of a suspension of inoculum has also been tested and found to be fairly successful.

In container nurseries, ECM fungal spores can be applied to seed before sowing. Vegetative inoculum of ECM or VAM fungi can be incorporated into the growing media prior to filling the containers. ECM spores can also be applied in a water suspension either by hand, or through the existing irrigation system 6 to 12 weeks after sowing.

Seedlings could also be inoculated at the time of outplanting when vegetative inoculum could be added to the planting hole as the seedling is planted. However, inoculating during outplanting has many drawbacks and inoculation success is greater in the nursery.

Cost effectiveness

Mycorrhizal inoculation must make sense from an economic as well as biological point of view. If you feel that inoculation might improve the performance of your seedlings, the next step is to do a cost: benefit analysis. Compute the total cost of inoculation including inoculum price and application costs and compare that to the benefits either in the nursery or on the outplanting site. Document the savings associated with less fertilizer, disease reduction, higher seedling survival, and increased growth. Be sure to consider the improved marketability of seedlings which have been inoculated with a specific mycorrhizal fungus. A nursery could advertise that their seedlings were mycorrhizal and the associated benefits, just like they do for seedling size, seed origin, and vigor.

Cultural practices affecting mycorrhizae

Mycorrhizal fungi are sensitive to several factors in the nursery environment, and so growers must modify some cultural practices following inoculation.

Fertilization

This is probably the most critical cultural factor because mycorrhizae develop most readily in soils or media of relatively low fertility. Many fungi are inhibited by the high fertilization rates commonly used in nurseries, especially nitrogen and phosphorus. Some fungal species, such as Laccaria laccata and *Rhizopogon vinicolor,* are less affected by high fertility and so are easier to manage in a nursery environment. The type and amount of fertilizer can also influence mycorrhizae formation (Figure C) and so slow-release formulations are recommended for some species.

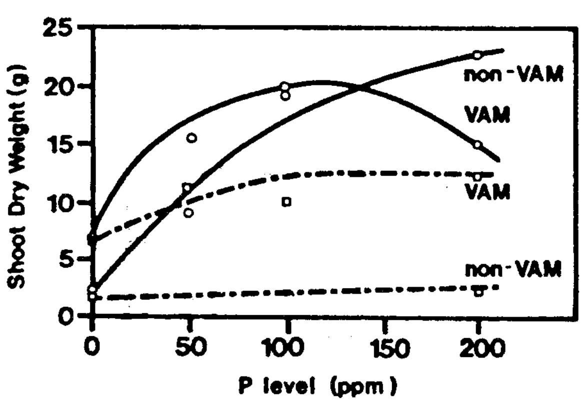

Figure C. Amount and type of fertilizer (soluble P-- rock P---) affect both mycorrhizae and seedling growth. (Modified from Walker, 1992).

Root culture

Cultural practices that encourage proliferation of roots in the upper soil layers will result in seedlings whose root systems become mycorrhizal more rapidly and completely. Practices such as root wrenching and lateral pruning in bareroot nurseries will produce more roots that are suitable for colonization by mycorrhizal fungi. Container nurseries can encourage fibrous root development by using more porous growing media and containers treated to promote chemical root pruning.

Soil management

Physical and chemical characteristics of the soil or growing medium are important for the formation of mycorrhizae. Many mycorrhizal fungi prefer a mildly acidic environment with a high percentage of organic matter. Porosity affects mycorrhizae both directly and indirectly: porous, well-drained soils and growing media promote good fibrous root growth and provide more oxygen for both roots and fungi.

Pesticides

Some pesticides, especially soil sterilants and fumigants, inhibit or kill mycorrhizal fungi. In fact, soil fumigation with methyl bromide/ chloropicrin is used to eliminate competitors before inoculating with a desired mycorrhizal fungus. Most fungicides are designed to control specific fungi and so growers should consult the literature or mycorrhizae experts before use. Although recommended rates of most other pesticides are not damaging, high rates and frequent application are discouraged.

Conclusions and Recommendations

Well, have I confused you completely? The decision on whether to inoculate your seedlings is complicated and so if you are thinking about mycorrhizal inoculation, please consider the following:

1. Consult a specialist - Unless you have a good understanding of the various fungi/ host combinations and the inoculation process, please contact a mycorrhizal consultant before you get started.

2. Start with a small trial - The tendency is to immediately apply a new cultural treatment on an operational scale, but don't. Ralph Shugert relays a horror story of using a commercial inoculum on some cuttings to stimulate rooting. Luckily, he installed a small field trial because the treated cuttings became diseased with Rhizoctonia root rot and he ended up loosing all of them.

3. Be sure to include a control - As with any new cultural practice, remember to establish a control area so that you can make a valid comparison. The control must be of the same species, seed source, and receive all the same cultural treatments as the trial. I know this sounds obvious, but I have visited nurseries and seen trials without controls or the control seedlings were of a different seed source.

4. Monitor the trial frequently and document results - The tendency is to get excited about a new practice, establish some sort of trial, and then get busy with normal day-to-day operations and forget to check back on a regular basis. Without frequent observations, you may miss some critical event that affects the outcome of the trial. Even if everything goes smoothly and you get good results, remember to take some measurements and photographs to document the trial (Figure D).

Figure D. Comparative photographs of seedlings from mycorrhizal inoculation trials provide effective documentation (Walker 1992).

Sources of mycorrhizal inocula:

Forest Mycorrhizal Application
P.O. Box 1181
Grants Pass, OR 97526 USA

PHONE/FAX: 503-476-3985

They offer specific strains of ECM spore inocula, including *Rhizopogon* spp., for commercial conifers at a cost of around $1.50 per M seedlings. Biogrow[R], a new VAM inoculum, is also being offered on a trial basis. They have worked with nurseries across the US and around the world, and offer consulting services.

Mikko-tek Labs
P. O. Box 2120
Timmins, ON P4N 7X8
CANADA

PHONE: 705-268-3536
FAX: 705-268- 7411

This firm produces commercial quantities of vegetative ECM mycorrhizal inoculum such as *Laccaria* spp. and *Paxillus* spp. for both conifer and hardwood stock, and also has lab facilities for evaluating the performance of inoculated seedlings.

MycorrTech InG
440 William Pitt Way
Pittsburg, PA 15231330 USA

PHONE: 412-826-5488
FAX.• 412-826-523

They sell vegetative inoculum of *Pisolithus tinctorius* and other ECM fungi, including *Hebeloma crustuliniforme* and *Laccaria laccata*, for conifers and hardwoods. They also provide consulting for anyone considering a mycorrhizal inoculation project.

Tree of Life Nursery
P.O. Box 736
San Juan Capistrano, CA 92693
PHONE: 714-728-0628
FAX: 714-728-0509

They can provide generic and site-specific VAM inoculum made from calcined clay, which can be incorporated into seedbeds or growing medium. Applications include forestry, restoration, and agriculture—contact Ted St. John for details.

Sources of inoculated growing media:

Premier Enterprises Ltd
326 Main St Red Hill, PA
18076 USA PHONE: 800
424-2554 FAX: 21S
679-4119

They sell a growing medium called "Mycori-Mix", which is a mixture of sphagnum peat moss, vermiculite, and perlite that has been inoculated with the VAM fungi *Glomus intraradix.*

Sources of inoculated seedlings:

Carino Nurseries
P.O. Bax 538
Indiana PA 15701
PHONE: 412-463-3350
FAX.• 412-463-3050

They have been working with vegetative ECM inoculum from Mycorr-Tech for the past several years, and will be offering a limited supply of Mycorr-ized[R] conifer seedlings for sale starting this fall.

Western Forest Systems, Inc.
1509 Ripon
Leiviston, ID 83501
PHONElFAX.• 20&743-0147

They have been treating their conifer stock with spore inoculum from Forest Mycorrhizal Application for a couple of years, and are

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