Opportunities to Improve Ectomycorrhizal Colonization within a Nursery Inoculation Program

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Abstract

In order to propagate site-adapted containerized plants inoculated with the appropriate mycorrhizal fungi it is essential to understand mycorrhizal associations vary widely in structure and function. A nursery inoculation program should consider the following factors that will affect the success of ectomycorrhizae colonization: selection, timing and setting of inocula application, growing media characteristics, fertility levels, and the use of fungicides.

Introduction

It is possible to state broad generalizations about the structure and function of the different mycorrhizal types that colonize the dominant vegetation in a gradient of climatic zones (Read 1984). Ericaceous plants, which dominate the acidic, high-organic heathland soils of the subarctic and subalpine regions are colonized by a group of ascomycetous fungi, giving rise to the ericoid type of mycorrhiza. This mycorrhizal type is characterized by extensive growth within (intracellular) the cortical cells, but little extension into the soil. Moving along the environmental gradient, coniferous trees replace ericaceous shrubs as the dominant vegetation. These trees are colonized by a wide range of mostly basidiomycetous fungi that grow between (intercellular) the root cortical cells forming the ectomycorrhizal type of fungi. Ectomycorrhizal fungi may produce large quantities of hyphae on the root and in the soil. At the warmer and drier end of the environmental gradient, grasslands often form the dominant vegetation. The fungi form arbuscles or highly branched structures within the root cortical cells, giving rise to the arbuscular type of mycorrhizae (Sylvia 1986).

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Ectomycorrhizae (ECM) are found on most tree species in temperate forests such as pines, spruces, firs, larch, birch, aspen, oak, and hickory. Mycorrhizal fungi usually proliferate both in the root and in the soil, and by this mechanism, mycorrhizae increase the effective absorptive surface area of the plant. In nutrient poor or moisture deficient soils, the increase of the absorptive area by the hyphae can lead to improved plant growth and reproduction. As a result, mycorrhizal plants are often more competitive and better able to tolerate environmental stresses than are nonmycorrhizal plants. Mycorrhizal fungi also interact with root pathogens. One major role of mycorrhizal fungi is the protection of the root system from endemic pathogens such as *Phytophtora* and *Pythium*. Lastly, ecological restoration often occurs on old mined land sites known to have high concentrations of heavy metals. Contingent upon the plant species and type of contaminant, mycorrhizae are able to filter some heavy metals to tolerable amounts for the plants (Norland 1993).

Ectomycorrhizal Structures

Most plants with ECM have roots with a modified lateral root branching pattern. This pattern, heterorhizy, consists of short mycorrhizal lateral roots supported by a network of long roots. The long and short roots in heterorhizic root systems are fundamentally similar in structure, but short roots normally grow much more slowly than long roots (Kubikova 1967). Many ECM also have a sheath, or mantle, of fungal tissue that may completely cover the absorbing root. The mantle increases the surface area of the absorbing root and often affects fine-root morphology, resulting in root bifurcation and clustering. Pine roots with ECM are easily recognized by their bifurcated roots.

Mycorrhizal fungi also produce a hyphal network within the soil. This network consists of individual strands of hyphae and/or relatively undifferentiated bundles of hyphae called mycelial strands (Agerer 1991). The absorbing surface area of the root can be greatly increased by the presence of aggregated mycelia. Roussea *et al.* (1994) found that while the extramatrical mycelia accounted for less than 20% of the total nutrient absorbing surface mass in pine seedlings, they contributed nearly 80% of the absorbing surface. Some fungi also produce aggregated hyphal strands (rhizomorphs), which contain specialized conducting hyphae (sclerotia) which are resistant storage structures.

Developmental Stages

Early colonization begins when the fungi adhers to the root epidermal cells near the apex of young actively growing feeder root. Attached hyphae have been observed 1-2 days after first contact with the root. After ECM associations have been established mycorrhizal short roots continue to grow by elongation and branching.

Later colonization is characterized by hyphae that have penetrated between the epidermal and cortical cells and formed a labyrinthine structure called the Hartig net that is able to form 2-4 days after root contact by the fungus. This extensive network is the site of nutrient and water exchange to the seedling by the fungi in return for photosynthates produced by the seedling.

The hyphal network that interconnects the structures produced by mycorrhizal fungi in the soil can also produce fungal fruit bodies used for reproduction. The reproductive structures of ECM fungi include epigeous fungi (mushrooms, puffballs, coral fungi, etc.) and hypogeal fungi, subterranean structures (truffles or truffle-like fungi).

Factors Affecting Colonization

There are several factors that may affect whether or not inoculated seedlings are successfully colonized by ectomycorrhizal fungi. A primary consideration is the selection of inoculum. According to Marx and Kenny (1982) the most biologically sound inoculum is vegetative. Although spores can be collected and stored for years, they may take 3 to 4 weeks longer than vegetative inoculum to germinate and infect a root. However, Castellano and Molina (1989) reported the successful inoculation of six million container-grown Douglas-fir seedlings through the incorporation of a spore suspension of *R. vinicolor* into a fertilizer injector system and misting the spores onto the seedlings. At Bitterroot Restoration, Inc., many ectomycorrhizal species were successfully colonized by applying a commercial spore inoculum "Plant Success Soluble" by Mycorrhizal Applications as a soil drench. Prior to purchasing commercial inoculum, careful consideration should be given to the selection of the fungus species. Fungi for nursery applications should be early-stage with the physiological capacity to form abundant mycorrhizae on the desired hosts (Cordell and Marx 1994). Preference should be given to fungal species that fall into the multistage classification that occur in young and old forests alike in order to enhance the growth or stress tolerance of the host once outplanted. For example, several *Rhizopogon* species have been found from the nursery stage through forest rotation age in Chile (Garrido 1986).

Another consideration is the timing of the inocula application. Attention should be given to the seedling stage of growth upon inoculation. The seedling should have a fairly extensive feeder root system. Following inoculation ECM feeder roots grow much more slowly than the longer lateral roots Kubikova (1967) thus suppressing the growth of the seedling. The restricted growth of short roots may be necessary to allow ECM fungi time to form an association, since these fungi have difficulty colonizing more rapidly growing roots (Chilvers and Gust 1982).

The seedling should also have enough leaf surface area to produce enough photosynthates for continued growth and support of the fungus, which may assimilate as much as 20% of the carbohydrates produced by the host. Otherwise, suppressed growth of the seedling may result from the allocation of host photosynthates to the fungus. Gagnon and Langlois (1991) observed later colonization was more favorable to the growth of *rubra* L.

The setting in which the inoculation occurs can also affect the success of ectomycorrhizae colonization. The ability of mycorrhizal fungi to readily convert host-derived carbohydrates into forms specific for the fungi is influenced by many of the same factors affecting seedling metabolism such as temperature and photosynthetic active radiation (PAR). Ideally, inoculation should take place within a greenhouse where the temperate range is moderate. The inoculated seedlings should be under grow lights or moved out into direct sunlight. Intensities below 20% PAR have been shown to significantly reduced ectomycorrhizal development (Marx 1991).

The characteristics of the growing media such as texture can significantly affect the development of ectomycorrhizae on the host seedlings. Most ectomycorrhizal fungi favor good drainage and aeration (Cordell and Marx 1994). Growing media containing coarse-textured particles such as vermiculite promote ectomycorrhizal development.

The pH of the medium and irrigation water can also be important. Most fungi have pH requirements similar to their hosts, but some can tolerate unusual deviations such as *Rhizopogon*. Hung and Trappe (1983) found that an isolate of *Rhizopogon vinicolor* would grow well over a span of 4 pH units. On the other hand, research conducted by Marx and Kenney (1981) demonstrated a commercial formulation of mycelial inoculum of *Pisolithus tinctorius* had higher colonization rates in a much narrower pH range of 4.5 to 6.0. Preference should be given to fungal species having a greater tolerance to a broad pH range.

The effects of soluble N and P on ectomycorrhizae colonization may be an important factor depending upon the ectomycorrhizal species. spp. are able to form associations with many genera including; *Pinus, Picea, Abies, Pseudotsuga, etc.* (Smith and Read 1997). *Rhizopogon* spp. are little affected by high levels of soluble fertilizer. Inoculation with these fungi in commercial nurseries has been successful without altering the routine fertilization regime (Tyminska *et al.* 1986). In contrast, several researchers have documented increased levels of soluble N and P lowered ectomycorrhizal colonization of *Pisolithus tiuctorius (Crowly et* a1.1986, Rupp and Mudge 1985). Ideally, fertilization formulations can be manipulated for optimum seedling growth without detrimentally affecting mycorrhizal colonization.

Over the past 45 years many synthetic sulfur and other organic fungicides have been developed to replace the harsh, less selective inorganic materials. Thiazoles (etridiazol, ethaboxam, truban, terrazole, etc.) would be the fungicides of choice for nurseries growing ectomycorrhizal hosts (Landis *et al.1989).* The 5-membered ring of the thiazoles is cleaved rather quickly under soil conditions to form either the fungicidal isothiocyanate (-N=C=S) or a dithiocarbamate, depending on the structure of the parent molecule (Ware 1991). Dithiocarbamates (ferbam, polycarbamate, thiram, etc.) tend to inhibit mycorrhizal colonization, their use should be avoided. The dicarboximides (iprodione, captan, procymidone, etc.) are usually not inhibitory at low application rates; they can even be stimulatory. However, avoid drenching with these compounds, as they can be detrimental to ectomycorrhizae (Pawuk *et al.* 1980) .

There are non-destructive and destructive sampling methods for detecting mycorrhizae. A non-destructive method entails pulling the seedling plug out of its'container and determining the absence or presence of ECM through the visual detection of aggregates of hyphae. In many cases there may not be a proliferation of hyphae due to the optimal growing environment. A preferred method that destroys the integrity of the plug without causing any mechanical damage to the root system is to place the plug in a tray of water overnight to gently remove the majority of the growing media. When the detection rates of the two sampling methods were compared at Bitterroot Restoration, a 15% increase in the detection of mycorrhizae for *Pinus ponderosa* and a 40% increase for *Pinus edulis* were observed when using the destructive method of removing the growing media. Once the growing medium was removed, the bifurcation caused by the ectomycorrhizal colonization was easily identified using a hand lens.

Conclusions

The establishment of an optimal growing environment for the seedling will greatly facilitate mycorrhizae colonization. The fungal symbiont should be able to tolerate a broad pH range and proliferate without adjusting the routine fertilization regime using soluble nitrogen and phosphorus. Familiarity with the different fungicidal compounds and their effects on ectomycorrhizae will mitigate accidental kills. Preference should be given to ECM that fall into the multistage classification. In most cases, the objective of a nursery inoculation program is not to achieve a physiological response of the host while in the nursery, but rather to establish symbiosis so that it can be effectively transferred to the field.

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