Mycorrhizae Nursery Management for Improved Seedling Quality and Field Performance[1]

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Abstract.--Nursery and field outplanting studies have repeatedly demonstrated that selected ecto- and endomycorrhizae on nursery seedlings reduce culls and improve field survival and growth. Mycorrhizae are significantly affected by nursery soil factors such as pH, drainage and moisture, fertility, and organic matter, and by cultural practices such as soil fumigation, cover crops, and pesticide applications. Seedling lifting, storage, and planting practices should be designed to retain the maximum number of feeder roots and associated mycorrhizae as possible. Inoculum of several species of ectomycorrhizae is commercially available, along with the necessary technology and machinery to be incorporated into standard bare-root and container nursery operations. Nurserymen and foresters are challenged to utilize mycorrhizae technology as an integral component of seedling production and forest regeneration.

INTRODUCTION

Seedling quality and field performance are largely governed by processes occurring under the soil surface in the root zone of seedlings. Absorption of water and nutrients is a function of the amount and quality of growing root tips or feeder roots. The feeder roots of most tree species are infected by specialized fungi that form beneficial associations called mycorrhizae (fungus-roots). These symbiotic structures greatly increase root absorption efficiency and are vital to the survival and growth of both the host tree and the fungus. Compared to nonmycorrhizal roots, those infected by mycorrhizal fungi have increased absorptive capacity, nutrient fixation, resistance to soil pathogens, and longevity. As the main interface between seedling and soil, mycorrhizae are a key measure of root system quality and are a vital component of integrated nursery management.

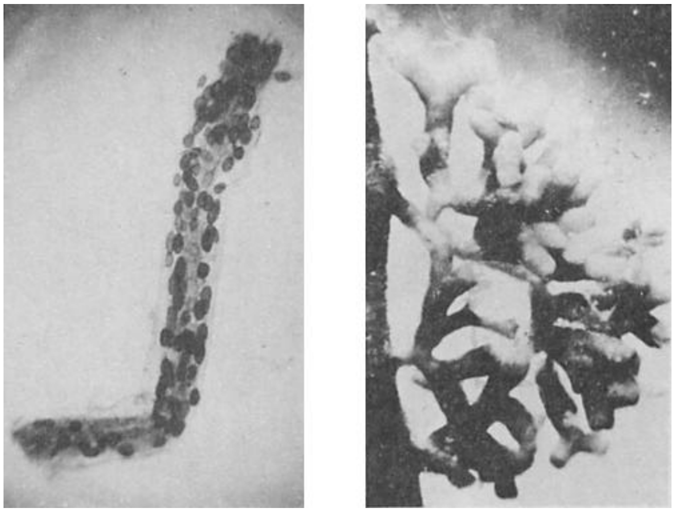

Figure 1.--Hardwood seedling feeder root infected with the endomycorrhizal fungus, Glomus sp. (left) and a mass of Pisolithus tinctorius (Pt) ectomycorrhizae on a southern pine seedling root (right).

Mycorrhizae are of two biological types: endomycorrhizae, which actually penetrate host cells; and ectomycorrhizae, which grow between the root cells and cover the root surface with a mantle of fungus hyphae (Fig. 1). Most hardwood

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tree species, including maple, sweetgum, sycamore, ash, walnut, and poplar, along with some conifers, including cypress, redwood, and arborvitae, form endomycorrhizae and depend on them for normal growth. This mycorrhizal type occurs on all agronomic crops, including nursery cover crops such as sorghum, corn, and the grasses. Ectomycorrhizal fungi are associated with tree species which include pine, spruce, fir, alder, beech, oak, and hickory. Both ecto- and endomycorrhizal fungi have very broad host ranges.

Endomycorrhizal fungi penetrate cortical cells of infected roots and form nutrient-exchanging structures (arbuscles) inside them. A loose network of fungal hyphae grows from the feeder root surface, extending the effective area of the root system. Endomycorrhizal roots absorb and utilize nutrients, particularly phosphorous, better than nonmycorrhizal roots. Thick-walled spores (vesicles) may develop in feeder root tissue, on the root surface, or in the root zone. These microscopic "vesicular-arbuscular" (VA) mycorrhizal fungi do not modify root morphology or produce conspicuous above-ground fruiting bodies, as do the ectomycorrhizal fungi.

Ectomycorrhizal feeder roots are visibly different from nonsymbiotic roots. They usually appear swollen, forked, more prolific, and differently colored. Fungal hyphae cover the feeder root in a dense mantle. Strands of fungal hyphae radiate into the soil and to the bases of fruiting bodies produced by these fungi. Ectomycorrhizal fungi depend on their hosts for simple carbohydrates, amino acids, and vitamins to complete their life cycles and produce their spore-disseminating fruiting bodies. They benefit their hosts by increasing water absorption and accumulation of nitrogen, phosphorous, potassium, calcium, and other nutrients (Marx 1977).

Extensive mycorrhizae research conducted by the USDA Forest Service and a number of cooperating forestry agencies has identified the primary functions of mycorrhizae in tree seedling physiology and the nursery management factors that limit mycorrhizal establishment. Technology has been developed recently for the artificial inoculation of bare-root and container nurseries with selected ectomycorrhizal fungi. Several types of commercial inoculum are currently available for selected ectomycorrhizal fungi and can be operationally utilized in forest tree nurseries. Techniques have been developed to identify and quantify ectomycorrhizae occurring on seedling root systems utilizing ectomycorrhizae as a measure of seedling quality. In numerous container and bare-root nursery studies, along with forest and reclaimed mineland outplanting studies, selected ectomycorrhizae have significantly increased seedling quality and field performance. Provided with this unique technology, nurserymen, foresters, and mineland reclamation specialists are challenged to understand and utilize mycorrhizae as an integral component of nursery seedling production and forest regeneration.

BENEFITS

Ectomycorrhizae

Most conifer tree species, including all pines, cannot grow without ectomycorrhizae. This obligate dependency of trees on their fungal symbionts has been thoroughly substantiated through extensive laboratory and field research, and through unsuccessful attempts to introduce tree species into areas where their symbiotic fungi were not present. After the ectomycorrhizal fungi were introduced, trees were successfully established (Marx 1980). In forest tree nurseries in the United States, there is seldom a total absence of ectomycorrhizal fungi. Seedlings form ectomycorrhizal associations with naturally occurring fungi that originate from windblown spores produced by fruiting bodies in adjacent windbreaks, seedling beds, or forest stands. In nurseries where cultural practices or new field conditions have reduced ectomycorrhizal fungus populations, seedlings grow poorly and do not respond to increased fertilization. Pockets of seedlings that do have ectomycorrhizae or even had ectomycorrhizae established earlier in the season, have increased stem caliper and height, improved foliage color, and a more balanced shoot:root ratio than adjacent stunted seedlings which are deficient in ectomycorrhizae.

The ectomycorrhizal fungi that occur most commonly in bare-root nurseries, such as _Thelephora terrestris_ (Tt), are ecologically adapted to the favorable growing conditions in nursery soils. However, these fungi are poorly adapted to the adverse conditions of many reforestation and reclamation sites. Research by the USDA Forest Service has focused on one particular ectomycorrhizal fungus, _Pisolithus tinctorius_ (Pt), which is especially tolerant of extreme soil conditions, including low pH, high temperature, drought, and toxicity. The conditions, which occur on many forest sites, inhibit other naturally occurring ectomycorrhizal fungi and their host trees (Marx, Cordell, and others 1984). Pt was selected because of its adaptability, ease of manipulation, wide geographic and host range, and demonstrated benefits to trees, both in the nursery and on reforestation and reclamation sites.

Many conifer and some hardwood species on a variety of nursery sites have been artificially inoculated with Pt by treating seedling containers and prefumigated nursery seedbeds (Fig. 2). Effective Pt vegetative inoculum has consistently improved the quality of nursery seedlings. National container and bare-root nursery evaluations have demonstrated the effectiveness of several formulations of Pt inoculum on selected conifer seedling species (Marx, Ruehle, and others 1981; Marx, Cordell, and others 1984). During the past 10 years, over 125 bare-root nursery tests have been conducted in 38 states. A companion evaluation of container seedlings also demonstrated the effectiveness of commercial Pt vegetative inoculum in 18 nurseries in 9 states and Canada. Inoculated seedlings have significantly outperformed uninoculated checks (Fig. 3) that contained only naturally occurring ectomycorrhizae (predominantly Tt). Results obtained from 34 nursery tests conducted during 3 years showed that Pt inoculation of southern pine seedlings increased fresh weight by 17 percent, increased ectomycorrhizal development by 21 percent, and decreased the number of cull seedlings at lifting time by 27 percent (Fig. 4). The nursery failures that have occurred have been correlated with such factors as ineffective Pt inoculum, excessively high soil pH (above 6.5), improper nursery cultural practices, pesticide toxicity, or severe climate (Cordell 1985).

Inoculated seedlings have been planted on routine forestation sites, strip-mined areas, kaolin wastes, and Christmas tree farms scattered over the United States. Currently, over 100 Pt ectomycorrhizal outplantings involving 12 species of conifers are being monitored in 20 states. Over 75 of these outplantings contain southern pine species (primarily loblolly [<u>Pinus</u> <u>taeda</u> L.] and slash pine [P. <u>elliottii</u> Engelm. var. <u>elliottii</u>]) in the Southern United States. Most of these outplantings have been established since 1979; consequently, benefits to mature forest stands cannot be estimated. At widespread locations, however, tree survival and early growth of several conifer species have been significantly improved by Pt inoculations in the nursery. A significant increase (25+x) in tree volume is still being observed on Pt-inoculated eastern white (P. <u>strobus</u> L.), loblolly, and Virginia (P. <u>virginiana</u> Mill.) pines over check trees after 10 years in western North Carolina. Loblolly pine volume was 31 percent higher, and white pine volume was 151 percent higher than in uninoculated checks. Outplantings established by the Ohio Division of Mineland Reclamation on mineland reclamation sites in southern Ohio during 1982 and 1983 showed an average survival increase of 23 percent and 24 percent, respectively, for Virginia and eastern white pine seedlings over routine nursery seedlings after 2 years in the field. Treating longleaf pine (<u>Pinus</u> <u>palustris</u> Mill.) seedlings with Pt inoculum in the nursery increased their survival over uninoculated checks by 1'7 percent after 3 years in the field in four Southern States. Inoculation of longleaf pine with Pt, in combination with selected cultural practices in the nursery and a benomyl root treatment prior to field planting, has significantly increased the field survival and early growth of bare-root seedlings (Kais, Snow, and Marx 1981; Hatchell 1985).

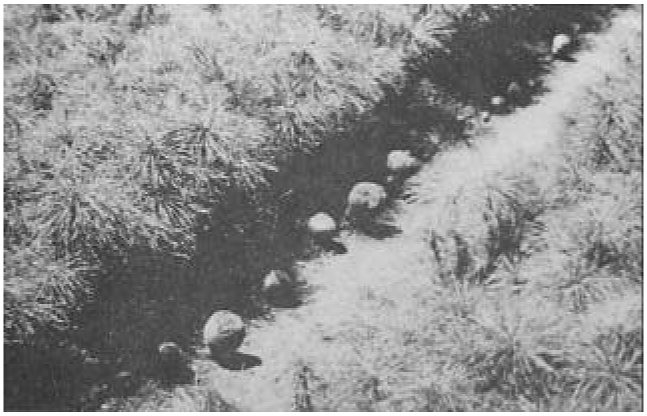

Figure 2.--Abundant Pt fruiting body production between 2-0 eastern white pine seedbeds prefumigated and inoculated with commercial Pt vegetative inoculum.

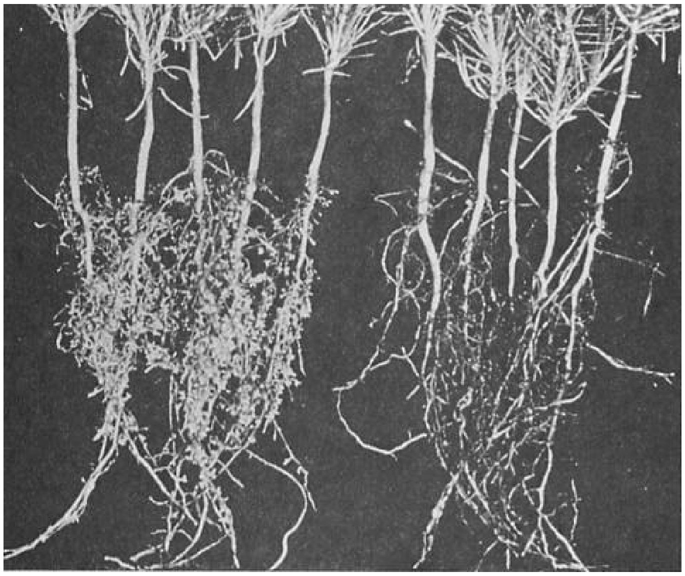

Figure 3.--1-0 loblolly pine seedlings with Pt ectomycorrhizae (left) and with only naturally occurring ectomycorrhizae (right).

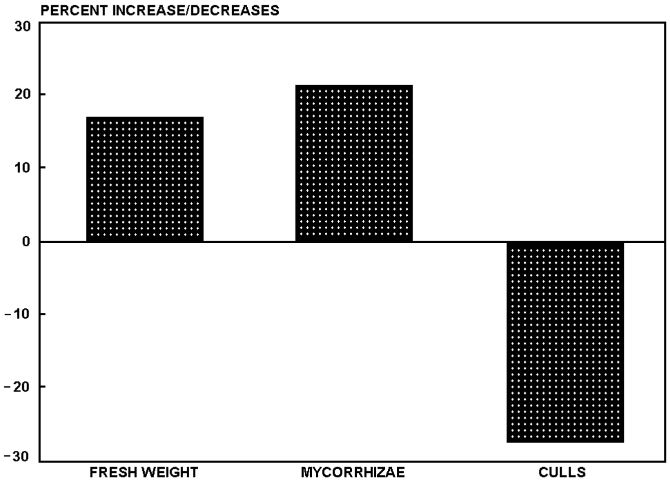

Figure 4.--Increases in seedling fresh weights and ectomycorrhizal development and decreases in the number of culls are obtained by inoculating seedlings with Pt.

After 8 years on a good-quality, routine forestation site in southern Georgia, a 50 percent increase was observed in volume/acre growth of Pt-inoculated loblolly pine over controls. The improvement was correlated with continued Pt-inoculated tree growth during seasonal periods of severe water deficit.[5] Similar relationships have been found in other field studies. Root systems with abundant Pt ectomycorrhizae are apparently more capable of extracting water and essential nutrients from soil during periods of extreme water stress than are root systems with fewer ectomycorrhizae or with other species of ectomycorrhizal fungi. These reported benefits do not even show the full potential of Pt, because as the fungus thrived on inoculated treatment plots and spread to uninoculated plots, treatment integrity was lost after 3 years.

Endomycorrhizae

Any nurseryman who has encountered stunted, chlorotic hardwood seedlings in a prefumigated bed, despite proper fertilization, irrigation, and disease control, is fully aware of the benefits provided by endomycorrhizal fungi. Nursery studies have repeatedly shown increases in the quality of seedlings with endomycorrhizae, compared to those without endomycorrhizae (Fig. 5). Root and stem weight of black cherry, boxelder, green ash, red maple, sweetgum, sycamore, and black walnut seedlings were significantly increased following treatment with VA mycorrhizal fungi (Kormanik, Schultz, and Bryan 1982). Black walnut seedlings grown in nursery soils infested with VA fungi retained their leaves longer, extending the effective growing season by 6 to 8 weeks and resulting in greater root and shoot biomass production (Kormanik 1985). Benefits from endomycorrhizae were greatest at phosphorous levels below 'j5 ppm (150 lb/acre). At higher soil phosphorous concentrations, nonmycorrhizal seedlings grew as well as endomycorrhizal seedlings (Kormanik et al. 1982; Kormanik 1985). In field studies where available phosphorous was low (10-15 ppm), hardwood seedlings that had abundant lateral roots and endomycorrhizae did not die back as much after outplanting as those with few lateral roots and poor endomycorrhizal development. In most forest soils, long-term benefits from endomycorrhizal treatments in the nursery are difficult to determine because nonmycorrhizal root systems are quickly colonized by naturally occurring VA fungi (Kormanik 1985).

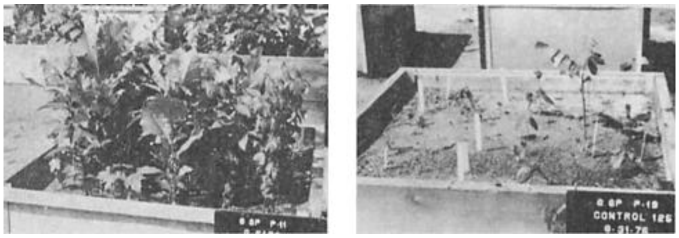

Figure 5.--Inoculation with a VA endomycorrhizal fungus increased seedling biomass of eight hardwood species (left) compared to noninoculated seedlings (right).

Figure 6.--Observed correlation between increased number of sweetgum seedling primary lateral roots (= or > 1 mm diameter) and improved seedling quality.

In the extended process of evaluating root system development in relation to VA fungi, a high correlation was found between the number of primary lateral roots (1 mm or more in diameter) and seedling performance after outplanting. In a 1-year-old sweetgum plantation, height, root-collar diameter, and survival increased and top dieback decreased (Fig. 6) as the number of lateral roots increased (Kormanik 1986). The previously observed correlation between the number of lateral roots and seedling quality remained consistent as additional tree species were examined. Findings may be applicable to conifers as well as hardwoods and ecto- as well as endomycorrhizal host trees. While the effects of lateral root morphology appear to be independent of mycorrhizal condition, they demonstrate the importance of assessing root systems as a component of seedling quality.

Identification and Quantification

A nurseryman who hopes to maximize seedling quality should learn to recognize and perhaps quantify the dominant mycorrhizal types occurring on seedlings. Ectomycorrhizal fungi are most easily identified by their fruiting bodies--the numerous puffballs or mushrooms that develop some time after seedlings have been colonized. The fungi can also be recognized on the basis of distinct morphology of ectomycorrhizal feeder roots. Although over 2,000 ectomycorrhizal fungi are known, only a few (1 to 3) species usually are found in a nursery. On western fir, spruce, and pine seedlings, gilled mushrooms of <u>Laccaria</u>

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[5] Marx, D.H., C.E. Cordell, and A. Clark. 1987. Eight-year performance of loblolly pine with <u>Pisolithus</u> ectomycorrhizae on a good quality forest site. Manuscript in press. USDA Forest Service, Southeastern Forest Experiment Station, Institute for Mycorrhizal Research and Development, Athens, Ga. [Submitted to Southern Journal of Applied Forestry.]

(Fig. 7a) and *Hebeloma* (Fig. 7b) species, pored mushrooms of *Suillus* species (Fig. 7c), and puffballs of *Rhizopogon* species (Fig. 7d) are common. On or near pine seedlings in the South, puffballs of *Pisolithus tinctorius* (Fig. 7e) and the papery thin, funnel-shaped mushrooms of *Thelephora terrestris* (Fig. 7f) frequently occur. Puffballs of *Rhizopogon* species, which have white, homogeneous centers, can easily be distinguished from those of *Pisolithus tinctorius* by their lack of peridioles or small sacs of spores within the context. Recognizing and separating ectomycorrhizal species on the basis of root morphology requires a trained eye, but the different colors and shapes of ectomycorrhizae can be distinguished with practice. Whereas nonmycorrhizal feeder roots are generally thin, with texture and color similar to the larger roots, ectomycorrhizae usually are swollen, forked or many-branched, and differently textured and colored from the rest of the root system.

During quantitative and qualitative seedling evaluations, a relative measure of the amount of mycorrhizal occurrence is more useful than identification of the ectomycorrhizal fungi on a sample of seedlings. Sampling techniques have been developed to estimate the proportion of a seedling's feeder roots that are ectomycorrhizal. In measured lengths of lateral roots, numbers of feeder roots with and without ectomycorrhizae are counted (Anderson and Cordell 1979). Such laborious examinations may be required for research studies, but they are impractical for estimates of large quantities of operational seedlings. A reliable estimate can be determined by visual examination of seedling root systems that have been rinsed clean in water. An estimated percentage of ectomycorrhizal feeder roots is assayed to each seedling and averaged for the whole seedling sample. With experience, a seedling can be evaluated in a matter of seconds. These estimates provide values that can be compared among samples, inventory dates, or even different crop years. As nursery management practices are refined, it becomes possible to monitor the mycorrhizal component of seedling quality.

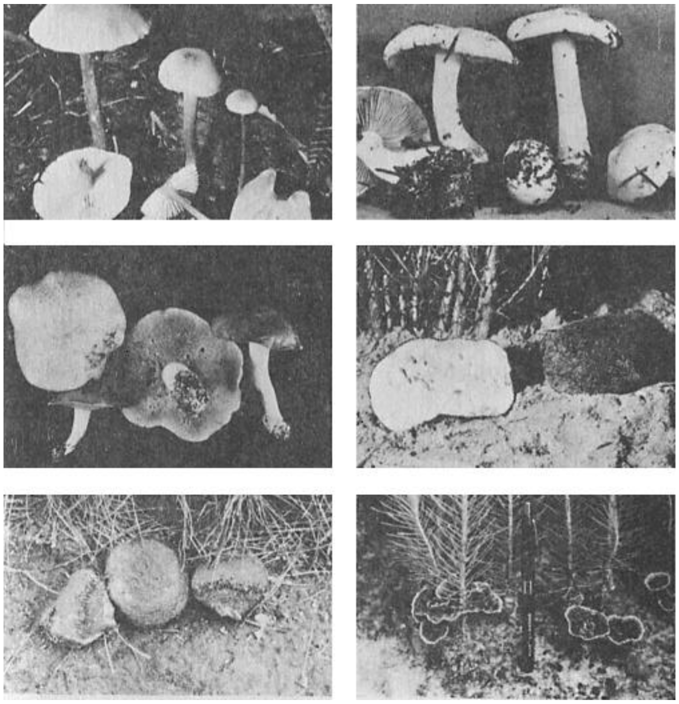

Figure 7.--Characteristic ectomycorrhizal fungus fruiting bodies of (a) *Laccaria* sp., (b) *Hebeloma* sp., (c) *Suillus* sp., (d) *Rhizopogon* sp., (e) *Pisolithus tinctorius*, and (f) *Thelephora terrestris*.

Unlike ectomycorrhizae, the VA endomycorrhizal fungi produce no morphological changes or structures visible to the unaided eye. Endomycorrhizae can only be identified by their microscopic hypha and vesicle morphology, and by the host association in which they occurred. In bare-root nurseries, seedling stunting, chlorosis, and top dieback are often indicators of poor endomycorrhizal development. Endomycorrhizal deficiencies may result from soil fumigation or from fungicide applications that eliminate or drastically reduce soil populations of the fungi. Endomycorrhizal deficiencies also occur in new seedling production areas with insufficient populations of appropriate endomycorrhizae. Although endomycorrhizae can be identified. and quantified, monitoring for possible deficiency symptoms appearing among endomycorrhizal seedings is more practical.

MYCORRHIZAE NURSERY MANAGEMENT

Endomycorrhizae or ectomycorrhizae in nurseries can be increased by modifying nursery management practices, as well as by artificial mycorrhizal inoculation. Guidelines for mycorrhizal nursery management pertain more to maintaining healthy seedling root systems than to the requirements of a particular species of mycorrhizal fungus. Enhancement of mycorrhizal fungi is inseparable from increased seedling quality. Management for increased mycorrhizal development is not limited solely to establishing the symbiotic structures on roots. One must consider development and retention of seedling feeder roots and mycorrhizae from seed sowing to seedling lifting in the nursery and to planting the trees in the field. Nurserymen, field foresters, and tree planters must be made aware of the two symbiotic living organisms they are handling--the tree seedling and its complement of mycorrhizal fungi.

Soil and Cultural Factors

Nurserymen strive to maintain optimal soil conditions for seedling growth. Having evolved with their host trees, mycorrhizae generally require the same moisture, fertility, and pH as the tree seedlings, but tolerance for extreme or adverse conditions does vary. Mycorrhizae are adapted to the full range of forest soils, from heavy clays to coarse sands, but their responses to nursery practices vary with the soil type. For example, ectomycorrhizae on southern pine. seedlings in deep sands may have much reduced tolerance of the systemic fungicide triadimefon (Bayleton) as compared to ectomycorrhizae occurring in clayey nursery soils. Soil fumigation with methyl bromide formulations is generally more effective in lighter, sandy soils than in heavy clays, which bind the chemical and prevent complete penetration. Similar interactions between soil texture and composition and mycorrhizae may occur for other cultural practices, including irrigation, fertilization, and application of other pesticides.

Soil pH

The pH of nursery soils has a profound effect on mycorrhizal establishment and growth. As a measure of the balance of acid and basic chemical activity in a soil, pH indicates limitations to the availability of nutrients, the pattern of nutrient absorption and exchange in the root zone, and even the composition of micro-organisms (mycorrhizal fungi, saprophytes, and soil pathogens) in the root zone. Although mycorrhizal synthesis occurs on trees in soils with wide pH ranges throughout the world, pH of nursery soils should approximate the optimum for the tree species and the forest soil type. For endomycorrhizae on hardwoods, Kormanik (1980) recommended maintaining soil pH between 5 and 6. He cited a study in which satisfactory endomycorrhizal synthesis and sweetgum seedling growth occurred at pH 4.5 and 5.5, but not at pH 6.5 or 7.5. Ectomycorrhizae also are usually favored by slightly acidic soils, and some, such as Pt, are severely inhibited by soil pH over 6.5. Most ectomycorrhizal fungi have a pH optimum between pH 4 and 6 when grown in pure culture, but by manipulating the amount and chemical formulation of nutrients, this range can be extended or shifted to more acidic or alkaline pH optimums.

The indirect effect of soil pH on nutrient availability in soils may be more important in mycorrhizae formation than the direct effects of pH on the fungus (Slankis 1974). All the macronutrients are more available above pH 6. Pt thrives in nursery soils under standard fertilization regimes, at pH 4.5 to 5.5, and on acid mine spoils with soil pH as low as 3. Vegetative inoculum formulations of Pt produced at pH greater than 6.0 were not as effective as inoculum produced at pH below 6.0 (Marx et al. 1984). An additional hazard of high soil pH in the production of both conifer and hardwood seedlings is the increased activity of soil pathogenic fungi, such as Fusarium and Pythium, which cause damping off and root rot.

Soil Drainage and Moisture

For satisfactory mycorrhizal development and seedling growth, nursery soils must have adequate soil drainage but sufficient soil moisture. In dry soils, free water is unavailable to roots, and nutrient absorption and exchange stop. However, irrigation generally maintains adequate soil moisture for seedling growth. In soils with excess water, oxygen deficiency inhibits the growth of both symbiotic fungi and tree roots. Respiration is greater in mycorrhizal roots than in noninfected roots. Prolonged flooding profoundly changes root physiology, decreasing phosphorous fixation, decreasing permeability to water and nutrients, arresting growth, and eventually killing roots (Slankis 1974). Seedlings grown in poorly drained soils are subject to damping off and root rot diseases caused by fungi with spores motile in water, such as Pythium and Phytophthora. Where drainage is poor, soil conditions must be improved by leveling, subsoiling, or adding amendments.

Soil Fertility

As with other soil factors influencing mycorrhizal development, fertility should be maintained at levels required for ample host seedling growth. Excessively high levels of certain nutrients, particularly nitrogen and phosphorous, may change chemical balances within seedling root systems, limiting mycorrhizal infection. As pH rises above 6, high phosphorous and nitrogen levels may be especially discouraging to mycorrhizal fungi. With soil pH at or below 6, however, seedlings grown under high fertility (especially nitrogen).have produced abundant Pt ectomycorrhizae. Hardwood seedlings grown under. high phosphorous fertility (greater than 200 ppm) have reduced endomycorrhizal synthesis (10-35% down from 40-75%) without reducing seedling growth. Kormanik (1980) recommends maintenance of 75 to 100 ppm phosphorous for good hardwood seedling and VA mycorrhizal development. Kormanik also recommends up to 10 applications of nitrogen, totaling 500 lb/acre, scheduled to capture late season height growth of hardwood seedlings following root development. Increasing total nitrogen from 250 to 500 lb/acre was accompanied by a 50-percent increase in height growth and approximately a 40-percent increase in root collar diameter of endomycorrhizal sweetgum seedlings, justifying the added nitrogen cost.

Soil Fumigation

Effective soil fumigation is necessary to control against weeds, nematodes, insects, and injurious soil fungi. Unfortunately, fumigation also kills existing populations of mycorrhizal fungi. Ectomycorrhizal fungi are quickly replenished by high numbers of windblown spores from mushrooms and puffballs. Replenishment occurs so readily in most nurseries, that spring rather than fall fumigation is required before artificial ectomycorrhizae inoculations to minimize competition from these naturally occurring fungi.

Spread only by physical movement of soil and water, endomycorrhizal fungi are slow to return to prefumigation levels. VA fungi populations are highly variable in fumigated areas and build up in the soil only after one or more crops are grown. By growing cover crops between soil fumigation and sowing of tree seedlings, endomycorrhizal populations are at effective levels for seedling production. If certain soil pathogens, such as Cylindrocladium sp., were not of greater danger than having insufficient endomycorrhizae, soil fumigation should be avoided all together.

Cover Crops

In addition to building up endomycorrhizal populations, cover crops between seedling crops rest the soil, increase organic matter content, and improve soil structure. Crops of corn, sudex, sorghum, millet, or grasses are effecttive in building up VA fungi in the plant roots and in soil. Winter as well as summer cover crops will increase endomycorrhizae. Although sorghum induced highest densities of VA fungal spores, sweetgum seedlings grown in compartments planted with corn, millet, sudex, and sorghum were of comparable quality and size (Kormanik, Bryan, and Schultz 1980). Crops with longer growing seasons have greater potential for root growth and spore production. Use of any cover crop after fumigation must be accompanied by careful monitoring of any chronic soil-borne disease problems that may occur in particular nursery soils.

Pesticides

Many pesticides of various types are used in nurseries, and the effects of individual chemicals on seedling growth or mycorrhizal synthesis are seldom known. The effects of herbicides and insecticides on mycorrhizae are particularly unexplored. However, many effects of commonly used fungicides have been documented. The fungicides captan and benomyl are recommended for use in conjunction with operational Pt inoculation of bare-root nurseries. Metalaxyl (Ridomil or Subdue), an effective fungicide against Phytophthora root rot, has no deleterious effect on ectomycorrhizae on Fraser fir when used at recommended dosages. Perhaps the most widely used fungicide in southern pine nurseries is the systemic fungicide, triadimefon (Bayleton), used to control fusiform rust. Triadimefon seed treatments which provide rust control through southern pine seedling emergence, have no negative impact on naturally occurring or artificially-introduced ectomycorrhizal fungi. However, foliar applications applied three to four times during the rust season (May-June) suppress ectomycorrhizal development until late in the growing season. Pt ectomycorrhizae are particularly susceptible to this fungicide. Normally, by lifting time, naturally occurring ectomycorrhizae, mostly Thelephora terrestris, have recolonized the root system. Negative impact on seedling quality is hotly debated, but the effects on mycorrhizae are well substantiated. Any and all pesticides, prior to operational use in nurseries, should be evaluated for their effects on mycorrhizal development as well as seedling growth.

Shading

Shade-tolerant conifer seedlings require some degree of physical shading. Too much shading reduces photosynthesis and soil temperatures to the degree that mycorrhizae cannot form. The optimum level of shade must be found that protects seedlings from scorching but does not inhibit mycorrhizae.

Root Pruning

At the proper depth and distance from seedlings, root pruning stimulates formation of compact root systems and increased mycorrhizal development. Injury of the root tips initiates greater carbon allocation to the root system, which causes the increased root growth. This practice increases the amount of mycorrhizal feeder roots proximal to the seedling stem, effectively increasing the amount of mycorrhizae that will be retained with the seedling during lifting and handling.

Seedling Lifting, Storage, and Planting

Special care must be taken during all stages of seedling handling to maintain sufficient root systems and mycorrhizae. Mycorrhizae are delicate structures. They can be ripped off and left behind in seedling beds during lifting, desiccated in storag4, or cut off prior to field planting. For sustained seedling quality, lifting and handling techniques must be modified to minimize damage to feeder roots and mycorrhizae. Stripping of roots adds severe negative impacts on seedling field performance (Marx and Hatchell 1986). Full bed seedling harvesters are less destructive than single- or double-row lifters. Condition of the root systems should be checked during the entire lifting process; even slight reductions in tractor speed can greatly reduce damage to the roots as seedlings are lifted.

During transfer of seedlings from the field to the packing room and at all other times when seedlings are handled, special care is required to avoid drying of the roots by exposure to wind and sun. The procedure by which seedlings are packed influences their ability to endure storage and survive field planting. If extended storage is required, Kraft paper bags with a polyethylene seal will maintain seedling moisture better than seedling bales. Cold storage is vital to slow seedling respiration. Studies comparing packing material have determined that seedling survival is better when peat moss, clay, or inert superabsorbents are used rather than hydromulch (Cordell, Kais, Barnett, and Affeltranger 1984). The material should be distributed through the bag, not simply dumped at the bottom or top. Better results are obtained when all root systems are coated or at least in contact with the packing material. Numerous studies have documented the effects of long-term storage on seedling quality. For most tree species and their mycorrhizae, storage for 2 to 6 weeks is not harmful. Beyond the threshold for each species, however, significant negative effects can occur.

Seedling quality is vulnerable to any one or more limiting factor. Even if quality is maintained through seedling growth, lifting, and storage, it could still be severely reduced by improper transportation to the planting site or rough handling during planting. Tree planters should understand proper planting methods and the reasons for them. Where possible, seedlings should be transported under refrigeration. If that is not possible, they should be covered and stacked with spacers to avoid high temperature buildup inside the seedling containers. For machine or hand planting, root pruning at the planting site should be avoided because it eliminates carefully nurtured feeder roots and mycorrhizae. High temperature, high winds, and low humidity kill feeder roots and mycorrhizae very rapidly. The first priority in planting should always be to maintain seedling viability and vigor. The rate at which acres are planted is of no consequence if the seedlings do not survive.

Ectomycorrhizal Fungus Inoculations

Ectomycorrhizal Fungus Inoculum

Until recently, artificial inoculation of Pt or any other ectomycorrhizal fungus species was limited because procedures, commercial fungus inoculum, and necessary equipment were not readily available to nurserymen. The USDA Forest Service has been cooperating with several private companies to develop different types of commercial ectomycorrhizal inoculum, along with equipment and procedures needed for inoculating bare-root and container-grown seedlings. In addition to Pt ectomycorrhizal inoculum, strains of Hebeloma sp., Laccaria sp., and Scleroderma sp. are currently available. The types of Pt inoculum that are available are vegetative inoculum from Mycorr Tech, Worthington, Pennsylvania, spore pellets, spore-encapsulated seeds, and bulk spores from either International Forest Tree Seed Co., Odenville, Alabama, or SouthPine, Inc., Birmingham, Alabama. A nursery seedbed applicator (Fig. 8) has been developed to accurately place Pt vegetative inoculum in seedbeds prior to sowing in bare-root nurseries. Inoculum is applied in bands under seed rows at desired depths (Fig. 9). Use of the applicator has reduced the amount of vegetative inoculum needed by 75 percent and reduced time and labor requirements as compared to broadcast application.

Inoculum Costs

There is a wide range in the cost of commercial Pt inoculum (Table 1). Cost of the each inoculum type also varies with such factors as

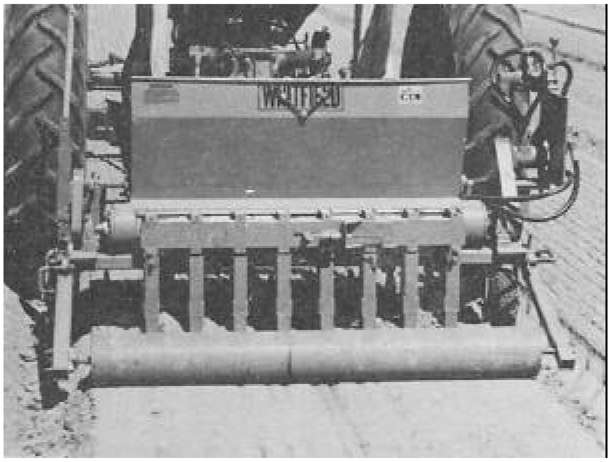

Figure 8.--A commercially available machine applies bands of commercial Pt vegetative inoculum to a bare-root nursery seedbed.

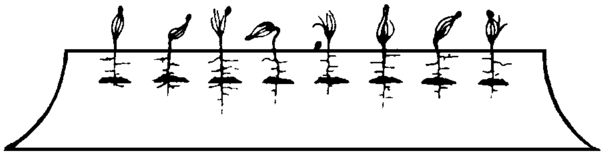

Figure 9.--Diagram of a bare-root nursery seedbed shows bands of Pt vegetative inoculum under seedling rows in root zones.

Table 1.--Commercial Pt inoculum costs.[1]

Pt inoculum type	Inoculum cost per 1,000 seedlings	planted hectare	planted acre
Vegetative mycelium	$10.00	$17.94	$7.26
Spore-encapsulated seeds	$ 2.22	$ 3.98	$1.61
Spore pellets	$ 2.75	$ 4.93	$2.00
Double-screened[2] bulk spores	$ 0.43	$ 0.77	$0.31

[1] Cost estimates are for loblolly and slash pine bare-root nurseries (269 seedlings/m^2 or 25 seedlings/ft^2) and forest plantings (1.8 x 3.0 m or 6 x 10 ft. spacing; 1,794 trees/ha. or 726 trees/ac.) in the Southern United States.

[2] Double screening is required for even flow through spray nozzles. Standard bulk spores are only screened once.

nursery seedling density, seed size for spore-encapsulated seeds, and field planting spacing. In 1987, the Pt vegetative inoculum costs for bare-root nurseries per unit of forest product were reduced 25 percent by increasing nursery seedbed inoculation efficiency, improving effectiveness of inoculum, and decreasing application rates. The vegetative mycelium is sold on a volume (liter) basis, while the spore inocula are all sold on a weight (pound) basis.

Inoculation Procedures

Operational procedures vary among the different commercial Pt inoculum types, but with any inoculum, the biological requirements of a second living organism are added to those of the seedling. Special precautions are necessary for shipping, storing, and handling the Pt inoculum, as well as for lifting, handling, and field planting of seedlings. For successful Pt inoculation in bare-root seedbeds, populations of pathogenic and saprophytic fungi and native ectomycorrhizal fungi that may already be established in the soil must be reduced by spring soil fumigation. Prior to spring sowing, vegetative inoculum can be broadcast on the soil surface and incorporated into the fumigated seedbeds or it can be machine-applied with greater effectiveness and efficiency. For container-grown seedlings, vegetative inoculum can be incorporated into the growing medium before filling the containers or placed at selected depths in the growing medium in the container. Bulk spores can be sprayed, drenched, or dusted onto growing medium for containerized seedlings and onto seedbeds in bare-root nurseries. Spore pellets can either be incorporated into the growing medium or seedbed soil, or they can be broadcast on the soil surface, lightly covered, and irrigated. Spore pellets have been applied at several nurseries with a standard fertilizer spreader (Fig. 10). Spore-encapsulated seeds can be sown by conventional methods. A major disadvantage of the Pt spore inoculum is the absence of a reliable means of determining or controlling spore viability. Consequently, Pt ectomycorrhizal development has been considerably less consistent and effective with spore inoculum than with vegetative inoculum.

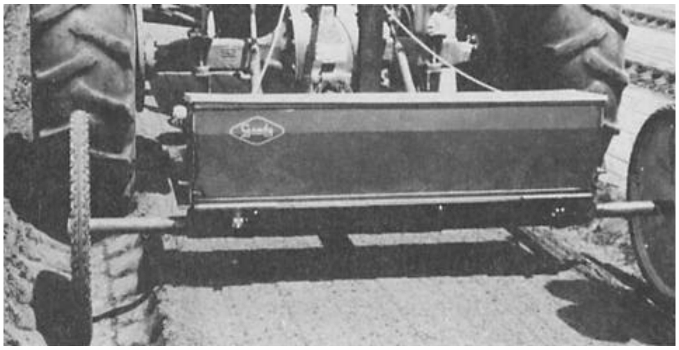

Figure 10.--Commercially available Pt spore pellets are applied to a nursery seedbed with a standard fertilizer applicator.

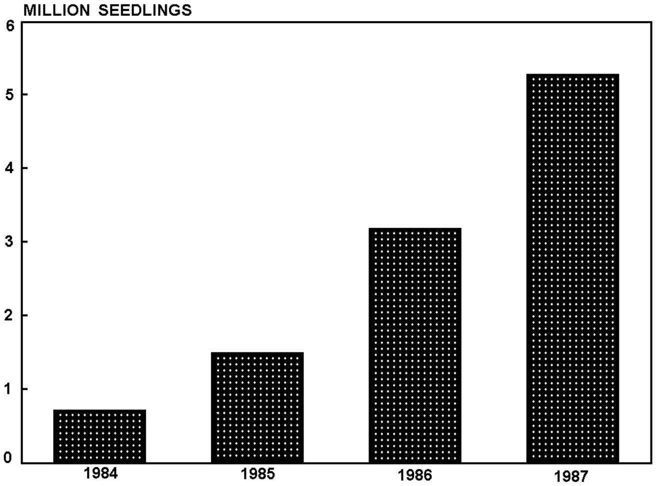

Figure 11.--Increased Pt-inoculated custom seedling production in bare-root and container seedling nurseries, 1984-87.

Operational Applications

The demand for Pt-tailored nursery seedlings has significantly increased during the past 4 years, despite the added costs and financial difficulties that most forestry agencies are currently experiencing. Since 1984, annual demand for tailored seedlings has increased 10-fold from 0.5 million to 5 million seedlings (Fig. 11). During the spring of 1986, Pt vegetative inoculum was operationally applied at 10 bare-root nurseries in the Southern and Central United States. Approximately 2_million seedlings of 9 conifer and 1 hardwood species were produced. In addition, over 1 million pine seedlings were inoculated with spore pellets. During the spring of 1987, Pt vegetative inoculum was applied at five bare-root nurseries in the Southern and Central United States. More than 3 million seedlings of five conifer and one hardwood species were inoculated. More than 2 million seedlings are being produced at a South Carolina State nursery for the USDA Forest Service, Savannah River Forest Station, and the United States Department of Energy. This represents the largest single application of an ectomycorrhizal fungus in a forest tree nursery to date. Over 2 million additional pine seedlings were inoculated with spore pellets at two bare-root nurseries in North Carolina and South Carolina and a container seedling nursery in Alabama.

Endomycorrhizal Fungus Inoculations

Although the technology required to produce VA mycorrhizal inoculum and to inoculate soils and plants is available and in use on certain agricultural and orchard crops that are highly dependent on endomycorrhizae, artificial inoculation of forest tree seedlings is not generally feasible. For most tree species, the phosphorous threshold is low enough that increased fertilization can remedy the effects of endomycorrhizal deficiencies. In addition, within several months, indigenous VA fungi on most reforestation sites colonize root systems of seedlings that were deficient in endomycorrhizae at the nursery. However, artificial inoculation may be beneficial if continued endomycorrhizal deficiencies and subsequent reductions in seedling quality occur at a nursery despite modifications in fertilization, fumigation, and crop rotation.

Different methods of artificial inoculation with variable potential benefits may be utilized. Nurserymen can add endomycorrhizal forest soils to the nursery soil, add soil from an area previously used to produce endomycorrhizal seedlings, or build up VA fungi populations through cover cropping. Soil or roots from the cover crop area can be spread over a deficient area and tilled into the soil. A potential problem with any of these methods is that soil pathogens can be introduced or increased by the same processes that introduce or increase VA fungi. Commercially available pot cultures of endomycorrhizal hosts grown under aseptic conditions can provide potentially cleaner and more effective inoculum consisting of soil and roots. Various types of VA fungal inocula are currently produced by NPI (Native Plants, Inc.), Salt Lake City, Utah 84108. This endomycorrhizal "starter" inoculum can be used to introduce appropriate VA fungi into fumigated or naturally deficient soils. Cover cropping can then be used to build up the VA fungal populations to effective levels for the production of endomycorrhizal seedlings.

CONCLUSION

Symbiotic relationships between tree seedlings and mycorrhizal fungi are the rule in nature. Conifer and hardwood nursery seedlings require adequate quantities and quality of either ecto- or endomycorrhizae to meet seedling quality standards. Minimum quantities or amounts of mycorrhizae are required to provide adequate field survival and growth. For southern pines produced in bare-root nurseries, this minimum ectomycorrhizae quantity has been established at 35 percent of the total seedling feeder roots on 90 percent or more of the seedlings. It should be emphasized that this 35 percent must be pres-ent when the pine seedlings are planted in the field. The quality of ectomycorrhizae for a planting site depends on the host tree-fungus species combination; optimum combinations can be produced by inoculating seedlings for specific applications, such as mineland reclamation. Custom production of mycorrhizal seedlings has been incorporated into bare-root and container nursery operations. The quality of mycorrhizae and of seedlings can also be improved through careful management of existing ecto- or endomycorrhizae.

Regardless of the selected alternatives, nurserymen, field foresters, and tree planters must be aware that they are dealing with two symbiotic living organisms--the tree seedling and the mycorrhizal fungus. Both must be nurtured to provide seedlings of the highest quality for field forestation. The tree seedling-mycorrhizal fungus symbiotic relationship is an integral component of nursery seedling production. Any estimates of seedling quality that exclude quantitative and qualitative mycorrhizal assessments are incomplete and unrealistic.

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