MYCORRHIZAE AND CONTAINERIZED FOREST TREE SEEDLINGS 1/

Donald H. Marx 2/ and James P. Barnett 3/

Abstract.--A review of the ecological and physiological effects of mycorrhizae on tree growth suggests that mycorrhizae should be encouraged on containerized seedlings. In a test of 3-month-old loblolly pine seedlings grown in Japanese paperpots, artificial introduction of inocula of the ectomycorrhizal fungus Pisolithus tinctorius increased seedling growth at low soil fertility and improved field performance. Seedlings mycorrhizal from natural inocula and grown in soil of much higher fertility performed even better.

INTRODUCTION

The feeder roots of forest trees are associated with highly specific, root-inhabiting fungi. The infected feeder roots are called mycorrhizae. The relationship between roots and these fungi is symbiotic since both partners benefit from the association. The value of mycorrhizae to tree growth has been known by forest scientists for several decades. Certain trees, such as Pinus, will not grow and develop normally without mycorrhizae. This paper reviews some well-established information on mycorrhizae and shows how it may be applicable to containerized seedlings.

Anatomically, mycorrhizal feeder roots can be separated into three classes--ectomycorrhizae, endomycorrhizae, and ectendomycorrhizae.

ECTOMYCORRHIZAE

This class occurs naturally as feeder roots on pine, fir, spruce, larch, eucalyptus, beech, birch, oak, hickory, and other trees in North America. Ectomycorrhizae can be distinguished macroscopically from nonmycorrhizal roots because they are swollen and usually are forked. Forking of feeder roots can also be stimulated by many factors other than ectomycorrhizal fungus infection. Ectomycorrhizae may be simple, nonforked (monopodial); "Y" shaped or forked (bifurcate); multiforked (coralloid); or in other configurations. A monopodial ectomycorrhiza of pine may be as small as 1 X 2 mm (diameter and length), and a complex coralloid ectomycorrhiza may be as large as 10 X 15 mm. Most nonmycorrhizal feeder roots of pine are approximately 1 X 2-4 mm.

Under a microscope, hyphae of ectomycorrhizal fungi can be observed growing internally around the primary cortical cells of the root forming the Hartig net; thus the prefix ecto. The Hartig net formed by the hyphae of the fungus appears to replace the middle lamella, a layer normally composed of pectins which cements the cortical cells. These fungi do not infect meristematic or vascular tissues. Hyphae of the fungal symbionts normally surround the feeder root in a tightly interwoven pattern that is called the fungus mantle. Mantles of ectomycorrhizae range from one or two hyphal to several dozen hyphal diameters in thickness. Ectomycorrhizae may be white, brown, yellow, black, blue, or blends of these colors. Overall color is apparently determined by the color of the hyphae that form the fungus mantle. Thick, individual hyphae or strands of hyphae that are generally the same color as the mycorrhizae frequently radiate from fungus mantles into the soil.

1/Paper presented at North American Containerized Forest Tree Seedling Symposium, Denver, Colorado, August 26-29, 1974.

2/Principal Plant Pathologist, USDA Forest Service, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Carlton Street, Athens, Georgia 30602.

3/Principal Silviculturist, USDA Forest Service, Southern Forest Experiment Station, Alexandria Forestry Center, Pineville, Louisiana 71360.

The major fungi which form ectomycorrhizae are Basidiomycetes, which produce mushrooms and puffballs. Certain species of Ascomycetes, such as truffles, are also ectomycorrhizal with trees. Spores of these fungi, produced in their fruit bodies in great numbers, are easily carried by wind and water and are the chief means of geographic spread of the fungi. In soil the fungi spread primarily by hyphae growing from one root to another when roots grow adjacent to or contact one another.

Many species of fungi may be ectomycorrhizal with a single tree species, an individual tree, or even a small segment of lateral root. As many as three different fungi have been isolated from an individual ectomycorrhiza of pine. Mycologists have estimated that over 2200 species of ectomycorrhizal fungi exist on roots of trees in North America. Many of these fungi have broad host ranges and form ectomycorrhizae on trees in diverse genera, while others form ectomycorrhizae only with a limited number of tree species. Many other species of fungi which form mushrooms and puffballs are not symbiotic with tree roots, but function simply as saprophytes which decompose organic matter in forest soil.

ENDOMYCORRHIZAE

Most economically important agronomic crops, forage crops, and ornamentals, as well as fruit and nut trees, form endomycorrhizae. Important forest tree species such as maples, gums, sycamore, cottonwood, locust, poplars, elms, and other trees not forming ectomycorrhizae normally form endomycorrhizae. Certain species of forest trees may have both ecto- and endomycorrhizal associations.

Endomycorrhizal fungi form a loose network of hyphae on feeder root surfaces instead of a dense fungus mantle characteristic of lost ectomycorrhizae. Most often, these fungi form large, conspicuous, thick-walled spores on the root surfaces, in the rhizosphere,and sometimes within the cortical tissues of feeder roots. Hyphae of the endomycorrhizal fungi penetrate the cell walls of the epidermis and grow into the cortical cells of the root; thus he prefix endo. The hyphae which infect cortical cells may develop either branched absorbing structures (haustoria), called arbuscules, or thin-walled, spherical to ovate vesicles. Sometimes both structures are formed within the same tissue. The term "vesicular-arbuscular" (VA) mycorrhizae has been coined to denote this type of endomycorrhizae. Certain symbionts form structures which are anatomically different from the common VA mycorrhizae. As in ectomycorrhizae, endomycorrhizal infection does not progress into meristematic or vascular tissues. Unlike ectomycorrhizae, endomycorrhizal infection does not significantly change the appearance of feeder roots. Microscopic examinations, therefore, are necessary to distinguish endomycorrhizal from nonmycorrhizal roots.

The fungi which form endomycorrhizae with trees are mainly Phycomycetes. They do not produce large, above-ground fruit bodies or windblown spores. These fungi spread within the soil by hyphae growing from root to root, and are disseminated from one area to another by runoff water and by man or animals causing the movement of infested soil or plant material. Many endomycorrhizal fungi of forest trees belong to the genus Endogone. Numerous other genera have recently been erected and many more undoubtedly are yet to be described. These fungi are so widespread that it is extremely difficult to find natural soils anywhere in the world that do not contain them. In the absence of a host, the spores of the fungi are able to survive in a dormant state for many years in the soil. Based on the limited amount of work done on endomycorrhizae of forest trees, most fungus species tested have a very broad host range. For example, Endogone mosseae will form endomycorrhizae with sycamore, soft maple, cottonwood, yellow poplar, sweetgum, and black locust. This fungus will also form endomycorrhizae with agronomic crops such as cotton, corn, soybeans, sorghum, and pepper, and horticultural crops such as citrus and peach.

ECTENDOMYCORRHIZAE

This class of mycorrhizae has been found on roots of certain conifers and has the features of both ecto- and endomycorrhizae. The taxonomic classification of the fungi involved is still in doubt. These symbionts may belong to a distinct group of fungi, or they may actually be ectomycorrhizal fungi which form a different morphological type of mycorrhizae. Anatomically, ectendomycorrhizae may or may not have a thin fungus mantle, but do have the Hartig net between cortical cells. Hyphae, usually of a smaller diameter, penetrate into the primary cortex cells in a manner that resembles certain types of endomycorrhizal infection. Ectendomycorrhizae are rarely found on trees in forest soils, but are almost exclusively confined to seedlings of pines in nurseries, in formerly treeless areas, or in soils with adverse conditions. Pine seedlings that form ectendomycorrhizae in nurseries will eventually form ectomycorrhizae after transplanting in the field.

BENEFITS OF MYCORRHIZAE TO FOREST TREES

Important benefits of ectomycorrhizae to forest tree growth have been demonstrated. Certain of these benefits are thought to apply to endomycorrhizae as well, but only limited information is available on their value to forest trees. Even less is known about the value, if any, of ectendomycorrhizae to tree growth.

Trees with abundant ectomycorrhizae have a much larger, physiologically active root-fungus surface area for nutrient and water absorption than trees with few ectomycorrhizae. This increase in surface area comes not only from the multibranching habit of most ecto-mycorrhizae, but also from the growth of hyphae of the fungus symbionts emanating from the ectomycorrhizae out into large soil volumes. These hyphae, which may be extensively developed, function as additional nutrient and water-absorbing entities. Ectomycorrhizae also in-crease longevity of feeder root function because they persist longer on root systems than nonmycorrhizal roots. Ectomycorrhizae absorb and accumulate in the fungus mantle numerous elements, such as nitrogen, phosphorus, potassium, and calcium, more rapidly than non-mycorrhizal roots. The fungi of ectomycorrhizae are able to biologically decompose certain complex mineral and organic substances in soil and render certain nutrients from these materials available to the tree. The presence of abundant ectomycorrhizae appears to increase tolerance of trees to drought, high soil temperature, soil toxins (organic and inorganic), and extremes of soil pH caused by high levels of sulfur or aluminum. Ectomycorrhizae also deter infection of feeder roots by root patho-gens which are common in many tree nurseries and forest soils. The fungus mantle is a mechanical barrier to cortical cell infection by these pathogens, and mechanisms provided by symbionts other than mechanical ones provide additional protection.

FACTORS AFFECTING MYCORRHIZAL DEVELOPMENT

Generally, any condition which regulates feeder root development affects mycorrhizae. The main factors influencing the susceptibility of tree roots to mycorrhizal infection are photosynthetic potential, soil fertility, and soil aeration. Extremes of other factors, such as soil temperature, pH, organic matter, etc., also affect either the fungal symbionts or the capacity of the host to produce feeder roots. Low light intensity on foliage (below 20% of full sunlight) reduces, or may completely inhibit, mycorrhizal development. Excess soil fertility, especially high levels of nitrogen and phosphorus, have similar effects. These two factors apparently influence the biochemi-cal status of roots, such as levels of root sugars or the synthesis of feeder roots, which are prerequisites to symbiotic infection. Roots growing rapidly in soils having high fertility may actually outgrow their fungal symbionts. Certain soil factors may directly affect the viability of infective propagules (hyphae or spores) of the fungal symbionts or their growth on roots. Extremes of soil temperature, pH, and moisture, as well as antagonistic soil organ-isms, can influence the activity of fungi and, thereby, suppress mycorrhizal development.

Fungicides and fumigants used for control of soil-borne diseases may inhibit or stimulate mycorrhizal development. Selective chemical control of feeder root pathogens, such as nematodes and pythiaceous fungi, facilitate additional growth of healthy new roots which, subsequently, may permit increased mycorrhizal development. Treatment of tree seed with various fungicides to control damping-off and with repellents to minimize loss of seed to birds and mammals has no apparent effect on early mycorrhizal development on seedlings. Apparently, most mycorrhizal fungi are not as susceptible to pesticides as once thought. The fact that hundreds of millions of tree seedlings are grown annually throughout North America with abundant mycorrhizae on their roots supports this view. All of these seedlings are either sprayed or dusted, or the soil is drenched with a wide variety of insecticides, fungicides, herbicides, and nematicides periodically during their growth in the nursery, and when these seedlings are lifted, their roots normally have abundant mycorrhizae. This does not mean that pesticides (or other soil factors) do not have a selective and detrimental effect on species of fungal symbionts which would normally pre-dominate on the root systems, but it does show that not all symbionts are eradicated. However broad spectrum fumigants applied to soil to eliminate weeds and pathogens are nonselective and either eliminate or severely reduce the amount of mycorrhizal fungi in the soil. Eradication of endomycorrhizal fungi from soil is more of a problem than eradication of ecto-mycorrhizal fungi. The latter fungi usually quickly recolonize fumigated soil because they produce wind-disseminated spores throughout the year. Deficiencies of ectomycorrhizal fungi in fumigated soil have been reported, however. In these instances, the soil was not colonized by spores of appropriate fungi. The weather may have been unfavorable for mushroom production in nearby forests, causing an absence of spore inoculum, or the source of spores may have been too far from the fumigated soil for adequate colonization.

The consequence of soil fumigation to endomycorrhizal fungi is far more serious. Once these fungi have been eradicated from soil, natural reinfestation is very slow or nonexistent because their spores are not readily disseminated by wind. Endomycorrhizae Develop after soil fumigation from inoculum of the symbionts still viable in soil depths be yond effective penetration of fumigants. Inoculum is sometimes carried in runoff water or heavy rain splash from nonfumigated areas of soil. It may be brought in on contaminated cultivation equipment or on windblown non -fumigated soil. All of these processes are both slow and uncertain.

The above information was intentionally presented in a general manner so that a broad overview could be condensed into a limited space. Two recently published texts (Hacskaylo, 1971; Marks and Kozlowski, 1973) thoroughly describe the ecological and physiological aspects of mycorrhizae.

PRACTICAL CONSIDERATIONS

Most of the information on the value of mycorrhizae to forest trees has been obtained by comparing the responses of mycorrhizal to nonmycorrhizal tree seedlings. These comparisons have been essential for defining the type and degree of benefits derived by plants from different mycorrhizal associations. As a result, we know that any mycorrhizae on roots of trees are far better than no mycorrhizae at ill. However, another valid aspect of mycorrhizae has often been overlooked; i.e., certain species of mycorrhizal fungi are significantly more important to tree growth under certain conditions than other species. One such beneficial fungus is Pisolithus tinctorius, a basidiomycete which forms ectomycorrhizae with numerous species of pine, spruce, and Eucalyptus.

Schramm (1966), reporting plant colonization of coal refuse wastes in Pennsylvania, observed that trees such as pine, poplar, oak, and alder only became established after ectomvcorrhizae had formed. Of the hundreds of species of ectomycorrhizal fungi potentially available from adjacent forests for spore colonization of these sites, only a few species, such as Pisolithus tinctorius and Thelephora terrestris, were capable of surviving to any extent on these adverse sites. This result strongly suggests an ecological adaptation of these fungal symbionts to mined sites. Since Schramm's report, we, and others (Hacskaylo, 1972; Hile and Hennen, 1969), have observed this apparent adaptation. We have found Pisolithus tinctorius to be the most prevalent ectomycorrhizal fungus on roots of pine and spruce in strip-mined coal spoils in Kentucky, Virginia, Alabama, Pennsylvania, Indiana, Missouri, and Ohio, as well as on pine in strip-mined kaolin clay spoils in Georgia. This fungus is also very prevalent on pines growing on sheet-eroded clays of low fertility in various parts of the United States, such as the Piedmont of the Southeast and the Copper Basin of Tennessee. Many of these sites,, especially the spoils, have limiting factors such as low fertility, periodic high soil temperatures, and phytotoxic levels of sulfur, manganese, and aluminum, which severely hinder routine reforestation and revegetation. In controlled tests, P. tinctorius increased the tolerance of pines to high soil temperatures (Marx, et al., 1970; Marx and Bryan, 1971) and instead of being suppressed, actually grew better with high sulfur levels (Hile and Hennen, 1969). Because P. tinctorius tolerates these adverse conditions, it is better adapted to function as a mycorrhizal symbiont on disturbed sites than the ubiquitous T. terrestris. T. terrestris, an ectomycorrhizal fungus naturally occurring on pine and other tree seedlings in nurseries, is present on roots of most outplanted seedlings. However, unlike P. tinctorius, it fails to survive severe soil conditions. Generally, the more adverse the site the more P. tinctorius and the less T. terrestris is present.

At the Southeastern Station, we are developing methods for growing thousands of seedlings of various pine species that are ectomycorrhizal with P. tinctorius. Our working premise is simple--since P. tinctorius can colonize roots of seedlings on disturbed sites from naturally occurring spores, and, since these seedlings appear to grow vigorously following this colonization, P. tinctorius can be artificially established in the nursery on roots of the initial transplant stock. Tests will show whether seedlings with ecologically adapted, physiologically active feeder root systems survive and grow better than other seedlings on adverse sites.

Recently, we artificially infested fumigated nursery soil in small experimental plots with vegetative mycelial inocula of P. tinctorius. 4/ The fungus was grown in a medium of vermiculite, peat moss, and nutrients in 2-liter jars. After 3 to 4 months, the fungus had completely permeated the vermi-

4/ Marx, D. H., and W. C. Bryan. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with PisoZithus tinctorius. Ms. in review for Forest Sci.

culite particles. The inoculum was removed from the jars, passed through a 0.7 cm mesh screen, leached with tap water to remove the nonassimilated nutrients, broadcast over recently fumigated soil, and hand-chopped into the soil to a depth of 4 inches. After 7 months, P. tinctorius had stimulated more than a 100% increase in biomass production of loblolly pine seedlings. The root systems of these seedlings were completely colonized by P. tinctorius and the proliferation of feeder roots was outstanding. Seedlings growing in soil without P. tinctorius were ectomycorrhizal from naturally occurring spore inoculum of T. terrestris and other fungal symbionts. Growth of these latter seedlings and morphology of their ectomycorrhizae were comparable to that normally observed on seedlings grown in pine seedling nurseries. Later, we infested soil with P. tinctorius in state nurseries in Georgia, North Carolina, and Florida and grew several species of southern pines. The results were very rewarding. For example, loblolly pines in the North Carolina nursery symbiotic with P. tinctorius approached almost double the size of seedlings mycorrhizal from the naturally occurring fungi on control seedlings. Thousands of these tailored pine seedlings have been outplanted on a variety of sites, including routine reforestation sites. These results showed that P. tinctorius can be artificially introduced into fumigated nursery soils--that it can compete with other fungal symbionts and form abundant ectomycorrhizae on pine seedlings.

We recently also found that basidiospores of P. tinctorius, which are available in nature in great quantities, are effective inocula for mycorrhizal synthesis.5/ Bulk lots of spores were successfully collected dry, held for long periods in cold storage, and applied to soil in a variety of ways to synthesize mycorrhizae on seedlings. The use of basidiospores to inoculate nursery soils is more economical than vegetative inoculum which must be prepared under laboratory conditions.

LOBLOLLY PINE CONTAINER STOCK AND ISOLITHUS TINCTORIUS ECTOMYCORRHIZAE

The principles underlying the ecology and physiology of mycorrhizal fungi discussed earlier have specific application to containerized forest tree seedlings. Certain practices used in growing container stock, such as high soil fertility and sterile soil media, will discourage significant ectomycorrhizal development and, thereby, could be responsible for increasing transplanting shock, especially under adverse field conditions. Unfortunately, only limited published information is available concerning the value of ectomycorrhizae to containerized seedlings. Hartigan (1969) found that inoculation of Monterey pine seedlings grown in polyurethane foam greatly increased seedling growth in the greenhouse. The preset of mycorrhizae on seedlings was essential to his objectives; his seedlings were to be outplanted in Australian soils void of significant quant ities of indigenous fungal symbionts.

We recently began a joint study to investigate the value of ectomycorrhizae to loblolly pine containerized seedlings, and felt that the techniques recently developed with P. tinotorius could have immediate practical application to this method of seedling production. We artificially infested soil in containers with P. tinctorius in an attempt to improve growth and survival of loblolly pine seedlings. This study was done in a greenhouse at the Alexandria Forestry Center in Louisiana. Three different growing media in 3 X 15 cm Japanese Paperpots were evaluated: 1) Kys-mix, a commercial potting medium consisting of peat, vermiculite, and perlite (5:3:2 by volume) and a low level of complete nutrients; 2) fumigated (MC-2 methyl bromide) topsoil, peat, and sand (1:1:1 by volume) fertilized weekly with 20-20-20 fertilizer at about 100 ppm NPK; and 3) same media as in 2 but fertilizer applied only after 5 and 10 weeks. The soil media were tested in combination with four mycorrhizal treatments: 1) 5 liters of vegetative mycelial inoculum of P. tinctorius per cubic foot of soil mixture prepared as previously described; 2) 1 g of basidiospores of P. tinctorius per cubic foot soil medium; 3) 3 g of basidiospores of P. tinctorius per cubic foot soil medium; and 4) control soil mixture without P. tinctorius. There were approximately 1.1 billion basidiospores of P. tinctorius per gram. Inoculum was mixed with soil in a concrete mixer. Each medium-treatment combination was replicated 4 times, and each replication included 100 pine seedlings. Groups of 10 loblolly pine seedlings per replication were harvested 4, 6, 8, 10, and 12 weeks following germination. Ectomycorrhizal development, height, and root and foliar-stem dry weights were recorded at each harvest date. After 12 weeks, 25 seedlings per replication were also outplanted in September 1973 on a previously disked site of a Beauregard silt loam. Only 3-month field data is available at this time.

Both the soil media and inoculation with P. tinctorius treatments affected seedling growth (Table 1). Seedlings grown in the

5/ Marx, D. H. Synthesis of ectomycorrhizae on loblolly pine seedlings by basidiospores of Pisolithus tinctorius. Ms. in review.

TABLE 1.--Growth and ectomycorrhizal development of containerized loblolly pine seedlings grown in different soil mixtures-fertilizers and inocula of *Pisolithus tinctorius*.

Mycorrhizal Treatments[1/]	After 12 Weeks in Greenhouse: Root Dry Wt. (mg)	Shoot Dry Wt. (mg)	Height (cm)	Ectomycorrhizae[2/] %	Ectomycorrhizae[2/] Ratio	After 3 Months in Field: % Survival	Height (cm)
-- Soil Mixture At Low Fertility --							
Control	96	170	6.1	46	0:1	81	6.4
Mycelium	86	211	8.0	81	4:1	88	7.9
Spores - Low	69	114	5.2	38	1:2	85	5.2
Spores - High	87	168	6.4	76	6:1	84	6.7
-- Soil Mixture At High Fertility --							
Control	109	307	8.3	26	0:1	88	7.6
Mycelium	61	266	8.1	36	1:20	94	8.5
Spores - Low	47	167	5.9	28	0:1	82	5.5
Spores - High	72	240	7.6	14	0:1	92	7.6
-- Commercial Soil Mix With Nutrients --							
Control	90	428	14.4	29	0:1	95	13.7
Mycelium	87	373	12.9	63	6:1	98	13.4
Spores - Low	97	305	9.5	34	1:6	94	11.4
Spores - High	112	323	10.3	40	1:2	92	11.0

1/ Control = No fungal symbiont added; Mycelium = 5 liter vegetative mycelial inoculum of P. tinctorius per cubic foot soil mixture; Spores - Low = 1 g basidiospores P. tinctorius per cubic foot soil mixture; and Spores - High = 3 g basidiospores P. tinctorius per cubic foot soil mixture.

2/ Percent mycorrhizae is based on number of ectomycorrhizae in proportion to total number of feeder roots. Ratio column represents approximate ratio of mycorrhizae formed by P. tinctorius to those formed by naturally occurring fungi not artificially introduced.

commercial mix were largest. Those grown in soil receiving weekly fertilizer applications were significantly larger in above-ground parts than those receiving only 1/6 that amount. Seedlings grown in soil with low fertility had better root systems than those grown at higher fertility levels. Soil mixture and fertility strongly affected ectomycorrhizal development of P. tinctorius. Control seedlings grown in the commercial soil mix and those in soil mixture with high fertility were larger than those with P. tinctorius; however, in the low fertility soil mixture the vegetative mycelial inoculum treatment induced the greatest foliar-stem growth. It is apparent that the capacity of P. tinctorius to form ectomycorrhizae was strongly inhibited by high soil fertility. High fertility also strongly inhibited mycorrhizal development from naturally occurring fungi, since nearly twice as many mycorrhizae were found on control seedlings grown at low fertility than on controls grown at higher soil fertility. Mycorrhizal development was first detected on seedlings growing in soil of low fertility with mycelial inoculum of P. tinctorius 6 weeks after germination. Appreciable mycorrhizae were not detected until after 8 weeks in the basidiospore treatments. In all cases, other fungi formed mycorrhizae in mixture on the roots with P. tinctorius. Based on past experiences with P. tinctorius, this result suggests either that the mycelial inoculum lost some viability during the nearly 96 hours of storage prior to use or that the soil mixtures contained indigenous inocula of other competing fungal symbionts. Either or both conditions could be responsible for the presence of other fungal symbionts on the root systems in addition to P. tinctorius.

Field survival of the test seedlings from all treatments was good. Seedlings grown in the commercial medium survived significantly better than those grown in other soil media. There was no statistical differences in survival due to the mycorrhizal treatments. The lack of major differences due to treatments probably reflects the late summer planting date and the excellent growing conditions for seedlings during their 3 months in the field. Additional field measurements will be made over the next 2-3 years to further evaluate the treatments.

It is too soon to draw any major conclusions from this study. However, it does show that high soil fertility inhibited development of ectomycorrhizae--those formed by P. tinctorius and those formed by naturally occurring fungi. Also, the techniques for infesting soil with P. tinctorius appear to be practical for container stock. In the absence of conclusive data on effects of mycorrhizae on performance of containerized tree seedlings, it still seems logical to assume that they are highly important. Any procedure that encourages mycorrhizal development on containerized stock should therefore be explored.

LITERATURE CITATIONS

Hacskaylo, E.
1972. Mycorrhizae: The ultimate in reciprocal parasitism. Bioscience 22: 577-583.

Hacskaylo, E.
1971. Mycorrhizae. Proc. First North American Conf. on Mycorrhizae, April 1969, Urbana, Illinois. Mis. Publ. 1189, USDA Forest Service.

Hartigan, D.
1969. A new look at pine propagation. Austral. Timber J. 35(1): 59-60, 63.

Mile, Nancy, and Hennen, J. F.
1969. In vitro culture of PisoZithus tinctorius mycelium. Mycologia 61: 195-198.

Marks, G. C., and T. T. Kozlowski (Eds.)
1973. Ectomycorrhizae--Their ecology and physiology. Academic Press, New York and Canada. 444 pp.

Marx, D. H., and W. C. Bryan.
1971. Influence of ectomycorrhizae on survival and growth of aseptic seedlings of loblolly pine at high temperatures. For. Sci. 19: 37-41.

Marx, D. H., W. C. Bryan, and C. B. Davey.
1970. Influence of temperature on aseptic synthesis of ectomycorrhizae by Thelephora terrestris and Pisolithus tinctorius on loblolly pine. For. Sci. 16: 424-431.

Schramm, J. R.
1966. Plant colonization studies on black wastes from anthracite mining in Pennsylvania. Trans. Amer. Phil. Soc. 56(1): 1°4.

Question: If you don't have mycorrhizae on your container seedling until you plant it in the field, do the mycorrhizal fungi in the soil have any adverse effect on the seedling or will they stimulate its growth?

Marx: We have never experienced grow inhibition due to mycorrhizal infection of species under any set of situations that I'm familiar with. It has always been a tremendous stimulation to growth especially with pine.

Question: Will fungicide use in the greenhouse have any effect on mycorrhizae that would be on seedling roots?

Marx: Nearly a billion seedlings are grown in North America each year, and great amounts of fungicide, insecticide, and herbicide are drenched on the nursery soil every growing season. The fact that these seedlings are heavily mycorrhizal shows, I think, that fungicides will not have a limiting effect, although some fungi will be inhibited. We have found no detrimental effect from any pesticide that has normally been used in nursery practices in the South. This is not to say, however, that certain indigenous species of fungi may not be inhibited by fungicide.

Question: How do we determine whether a particular plant species normally requires mycorrhizal fungi for vigorous growth?

Marx: The only way is to test it. We know that almost all of our tree species, in fact all of them to my knowledge, are mycorrhizal under field conditions. We cannot say that mycorrhizae are absolutely necessary for all tree species, but growth is stimulated by the presence of mycorrhizal fungi on the root systems. We know that certain species of fungi are far more beneficial than others.

Question: What effect does high concentrations of chlorine and sodium have on the development of mycorrhizae?

Marx: We know that plants grown on the Atlantic coastline, both natural and planted, are heavily endomycorrhizal, so here we have another example of ecological adaptation to site. Generally speaking, most mycorrhizal fungi are probably inhibited by high levels of sodium and chlorine that give you a neutral or alkaline pH reaction, but I'm sure there are species of fungi that are adapted to these adverse chemical conditions.

Question: What medium is used to produce your vegetative inoculum of Pisolithus tinctorius?

Marx: We use vermiculite with enough peat moss in it to give a pH around 5.5. This is moistened with a nutrient medium we use to grow the fungus in pure culture. We grow it for approximately 3 months, then leach with tap water to remove the nonassimilated nutrients, because if you add this enriched medium to the soil, other microorganisms will build up tremendously. We are able to store the reached inoculum for various periods of time and it remains effective.

Question: How widespread is Pisolithus tinctorius on the North American Continent?

Marx: Two weeks ago it was found in Mexico throughout the high elevation areas where pines are quite common. We have found it in Quebec, and we've found it throughout the United States wherever we've looked for it. We have found it wherever pines grow naturally, whether the site is highly productive or adverse.