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.

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.