ECTOMYCORRHIZAL INOCULATION OF NURSERY SEEDBEDS AND CONTAINER GROWING MEDIA

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I will answer four questions during this presentation: What are mycorrhizae? Are mycorrhizae necessary for growth and survival of nursery and container seedlings? How are nursery beds and container growing media inoculated with mycorrhizal fungi? What can the nurseryman do to promote development of mycorrhizae on tree seedlings?

What are mycorrhizae?

A mycorrhiza, which means literally "fungus root", is an association composed of root tissues and fungal mycelium. They exist in a symbiotic relationship; the fungus and plant root cells directly transmit substances to each other for use in their metabolic activities. Mycorrhizae occur naturally on nearly all flowering plants and are essential for optimum growth of most tree species. Without them many plants, including our most important timber species, could not survive in the competitive biological communities that occur in natural soil habitats.

Three types of mycorrhizae are formed on tree species based on the interrelation of fungal hyphae and host root cells:

1. Ectomycorrhizae

   Ectomycorrhizae occur normally on roots of such species as pine, fir, larch, spruce, beech, oak, birch, and hickory. With few exceptions most fungal symbionts of ectomycorrhizae produce mushrooms as fruiting bodies.

   The sequence of events in ectomycorrhizal formation follows a specific pattern. Feeder roots of seedlings initially come in contact with hyphae from germinating spores or other propagules in the soil. These hyphae, stimulated into rapid growth by root exudates, envelop the entire root tip with a dense sheath of mycelium called the hyphal mantle. As root cells divide and elongate, the fungus, by secreting pectolytic enzymes, penetrates the root, and grows between cells of the cortex. The living cortical cells are thus isolated by fungal hyphae, and the pattern formed by hyphae in the cortex is referred to as the Hartig net. Fungal invasion of the root is restricted to the intercellular region of the cortex. As infected root cells divide and elongate, growth substances secreted by the fungi cause the roots to be shorter than noninfected roots, and sometimes to branch. In pine, the branching is usually dichotomous. Root hairs do not develop. Thus, a very organized and morphologically distinct "organ" develops.

2. Endomycorrhizae

Endomycorrhizae occur on more plant species than any other type. They are found on the roots of many shrubs and tree species, including maple, redwood, sweetgum, yellow-poplar, ash, Russian-olive, buffaloberry, and apple.

Endomycorrhizal fungi produce subterranean, nearly microscopic spores. These fungi grow on surfaces of rootlets as individual threads or loose hyphal strands during infection. They secrete substances that dissolve a small portion of the cell wall, thus allowing fungal hyphae to penetrate root hairs and other epidermal cells behind the meristematic region, and grow into the cortical cells. Inside the cortex cells, the hyphae form minute branches (arbuscules) or swellings (vesicles). The term vesicular-arbuscular (VA) mycorrhizae has been used to describe this type of infection. In contrast to ectomycorrhizae, most endomycorrhizal infections produce very little or no change in the physical appearance of roots.

3. Ectendomycorrhizae

Ectendomycorrhizae have been reported only on tree species that are usually ectomycorrhizal. Ectendomycorrhizae have the typical organization of ectomycorrhizae plus intracellular penetration by hyphae. They were originally thought to represent a transitional stage between the ectomycorrhizal and endomycorrhizal types. Now, however, the formation of at least some ectendomycorrhizae has been ascribed to distinct, but yet unidentified fungi.

Ectendomycorrhizae are most common on pines in tree nurseries. They are rarely found in forest soils. If transplanted on roots of seedlings to a forest soil, the fungal symbiont does not develop and newly formed roots are invaded by ectomycorrhizal fungi. However, ectendomycorrhizae have been found to persist in mature trees in prairie soils in Nebraska. In these soils, the pH usually remains nearly neutral, and is not representative of conditions found in most forests.

A considerable amount of research has provided several experimentally proven benefits of ectomycorrhizae to forest trees. There is a large physical increase in the physiologically active absorbing surfaces of seedling root systems because of mycorrhizal formation. This increase includes mycorrhizae per se as well as hyphae that grow out of the ectomycorrhizae into large volumes of adjacent soil. As a result, ectomycorrhizae can more easily absorb and accumulate water and various nutrients, especially nitrogen, phosphorus, potassium and calcium, than nonmycorrhizal roots. Ectomycorrhizal fungi increase the functional longevity of feeder roots, and also decompose certain complex organic substances and minerals in the soil and make essential nutrients from these materials available to the tree. Ectomycorrhizae also increase the tolerance of pine seedlings to drought, high soil temperatures, and extremes of soil acidity. Ectomycorrhizae also act as biological deterrents to feeder root infections by root pathogens such as Phytophthora and Pythium spp.

Research on the benefits of endomycorrhizae to forest trees has not been as extensive as research on ectomycorrhizae. Endomycorrhizae are known to be more efficient in absorption of water and nutrients, especially phosphorus, than nonmycorrhizal roots. Endomycorrhizae probably do not have a significant role in protecting hosts from feeder root diseases since they do not form a fungal mantle which protects susceptible root tissues.

Are mycorrhizae necessary for growth and survival of nursery and container seedlings?

Most woody plants depend on mycorrhizal fungi for absorption of nutrients from soil. Although seedlings have been produced in nurseries when adequate nutrients have been supplied and pathogens are controlled, addition of mycorrhizal fungi has dramatically improved growth and survival of seedlings. In fact, survival of tree seedlings planted on sites lacking mycorrhizal fungi, such as prairie soils devoid of trees for long periods of time, non-forested areas, and mining spoils, is dependent to a great extent upon seedlings being infected or inoculated with a suitable mycorrhizal fungus. Nonmycorrhizal seedlings planted on sites devoid of mycorrhizal fungi may die unless infection occurs from wind-dispersed spores. If these seedlings survive, it is likely that they will remain severely stunted until a mycorrhizal root system develops. Nonmycorrhizal seedlings planted in forested areas normally will develop mycorrhizae as their new roots emerge, but depending on environmental conditions, mycorrhizal infection may require a long period of time.

The following examples of mycorrhizal inoculation in nurseries illustrate that mycorrhizae are indeed essential for normal tree growth.

For approximately 20 years, seeds of many species were imported into Puerto Rico in attempts to establish pine on the island. The seedlings, planted in nursery beds, grew a few inches in height, became chlorotic, then showed symptoms of extreme phosphorus deficiency, stagnated and died. In 1955, soil inoculum from a 5-year old pine stand growing in southeastern United States was added to an experimental plot of slash pine seedlings in the Puerto Rican mountains. Within 3 years the effects were dramatic. Uninoculated plants were not more than 1 foot tall and had just a small tuft of needles at their branch tips. In contrast, inoculated plants were 8 feet tall, vigorous, and fully needled.

During the spring of 1937 the first crop of conifers were seeded at the Iowa State Forest Nursery, Ames, Iowa. Four pine species were seeded in beds mulched with pine needles collected from a vigorous 14-year-old pine plantation; four other conifer species were seeded but not mulched with pine needles. By early August, seedlings not mulched were stunted, whereas seedlings in mulched beds had a spotted appearance. In certain parts of these beds the seedlings were making vigorous growth, while in others they were stunted. At the termination of the growth period the vigorous seedlings were about twice the size of the stunted ones. Examination revealed that vigorous seedlings had abundant ectomycorrhizae, and the stunted seedlings had few or none. This relationship held for all seedlings from mulched beds.

During the winter many nonmycorrhizal seedlings in the mulched beds died. During the second growing season, however, the size of areas supporting vigorous seedlings increased, and many of the previously unhealthy seedlings regained their normal foliage color and made good growth. Winter mortality was very conspicuous in beds not originally mulched with pine needles. In late spring of the second season, however, a few small areas of vigorous seedlings appeared, and these seedlings were mycorrhizal. These areas enlarged slowly, but by the close of the second season about 85% of the seedlings were dead.

Inoculation of new coniferous seedbeds was attempted to determine if the pine needles used to cover the seedbeds were a source of mycorrhizal fungi. In the spring of 1938, duff and humus-rich soil were obtained from the plantation which had furnished the pine needles used for inoculation of seedbeds in 1937. This inoculum was applied at the rate of 1 bushel per 250 ft of seedbed, and the bed then was sown to Scots pine. At the end of the second growing season (1939) many of the uninoculated seedlings were dead, while those still alive averaged under 2 inches in height. The inoculated seedlings averaged 7 inches in height.

A second study at Iowa State Nursery showed that mycorrhizal development in a prairie nursery soil could be promoted with phosphorus fertilization. White pine seedlings, which without inoculation and fertilization were chlorotic and nonmycorrhizal, started vigorous growth and developed numerous mycorrhizae as the result of moderate phosphorus fertilization. However, better results and mycorrhizal development were achieved by duff inoculation in which newly introduced fungal species established a symbiotic relationship with pine without phosphorus fertilization. Such results demonstrate that (1) mycorrhizal fungi are not completely lacking in prairie soil but exist in a dormant condition, probably as spores or resistant hyphae, (2) existing mycorrhizal fungi may be activated by phosphorus fertilization, and (3) existing species belong to less effective species and duff inoculum contained different mycorrhizal fungi that greatly improved growth of white pines.

Extensive mycorrhizal inoculation and supplemental treatments were made in nursery seedbeds at Oakes, Towner, Denbigh, and Bottineau, North Dakota in the 1940's. In 1938, a Prairie States Forestry Project nursery was established at Oakes, North Dakota on land that had produced successive crops of wheat and corn from 1918 to 1936 (Wright, 1940). No mycorrhizal inoculum was introduced in the seedbeds. In the second growing season, Wright observed a patchy, uneven appearance of pondeorsa pine seedlings, and found a wide variation in the number of mycorrhizae on the root systems.

In the spring of 1941, soil acidification and fertilization, mycorrhizal inoculation, and combinations of these treatments were applied in experimental plots at Oakes nursery in an attempt to correct the patchy character of vigorous seedlings. Mycorrhizal inoculum consisted of pure cultures of Amanita muscaria and Boletus granulatus, and soil from a bed of 6-year-old white spruce transplants at Denbigh Nursery near Towner, North Dakota. The experiment revealed that the best method of treating seedbeds for production of ponderosa pine, on a square foot basis, was addition of 1/8 oz sulphuric

acid, 1/8 oz 20% super-phosphate, and 1 lb of nursery soil. The beneficial effects of the treatments to seedling growth were attributed to the rapid spread of mycorrhizal fungi in the acidified beds, and to improvement in conditions for phosphate and iron assimilation in the same beds. The treatment that included pure cultures of mycorrhizal fungi was not effective in stimulating seedling growth since the fungi did not thrive in the alkaline soil (pH 7.5).

In general, results of conifer seedbed treatments at Oakes nursery were similar to those obtained from experiments conducted at Denbigh, Bottineau, and Towner Nursery, Towner, North Dakota. At Towner, the most successful mycorrhizal inoculation method was application of nursery soil to seedbeds at the rate of 10 to 20 tons per acre. The soil, collected from an established nursery, was plowed or disked into the top 2 inches of seedbed soil while moist. The procedure was effective only if there was a high soil phosphate level (1/4 oz of 15-30-15 nitrophoska/ft^2) and heavy acid treatment (1/4 oz sulphuric acid/ft^2) of the soil.

More recently Goss (1960) studied ponderosa pine mycorrhizae in a wide variety of soils in field shelterbelts, in several Nebraska nursery soils, and in sandy soils of the Nebraska National Forest. He found that occurrence of mycorrhizae in virgin grassland soils was relatively rare compared with their occurrence in natural forested areas of the eastern United States. He was able to establish mycorrhizae in virgin nonmvcorrhizal grassland soils by amending these soils with a duff-soil mixture collected from a 50-year-old ponderosa pine stand in the Nebraska National Forest. In addition, he found that severe chlorosis of ponderosa pine seedlings in some virgin grassland soils could be prevented or diminished by inoculations with a duff-soil mixture which induced mycorrhizal formation.

Several researchers have studies in progress on production of container stock with mycorrhizal inoculation, but at present there are few published reports concerning the value of ectomycorrhizae to container seedlings. Sphagnum peat moss and vermiculite are ingredients now used as growing medium in the production of container seedlings. Such growing medium generally lack mycorrhizal fungi, and mycorrhizal inoculum must be added to produce ectomycorrhizal seedlings.

How are nursery beds and container growing medium inoculated with mycorrhizal fungi?

Ideally, to gain maximum benefit from mycorrhizal inoculations, seedlings must be associated with fungal symbionts which are best suited to specific tree species, and are most tolerant to environmental conditions in the outplanting site. Because of the known physiological variability between mycorrhizal fungi, hosts can be expected to respond differently to different fungi. Some mycorrhizal fungi are more beneficial to tree survival and growth than others. Other species are more ecologically adapted to certain sites.

Types of mycorrhizal inoculum available for inoculation of nursery seedbeds or container growing media include (1) soil duff from forest stands or old nurseries, (2) mycorrhizal seedlings and roots, (3) spores and fungal fruiting bodies, and (4) pure cultures of mycorrhizal fungi.

Soil duff inoculum

Incorporation of duff from natural stands, plantations, or old nurseries has been a successful method of mycorrhizal inoculation of nursery seedbeds and container growing media. Typical examples include inoculation of nurseries in Iowa and North Dakota as previously discussed. For initial application, a thin layer of freshly collected duff, obtained from the upper few inches of the soil from an established forest stand, is spread on the surface of a nursery bed and mixed thoroughly with the soil beneath. For container seedlings, a satisfactory method of inoculation is to mix duff at a ratio of 1 part to 9 parts growing medium, by volume.

There are several advantages and disadvantages of using duff as a source of mycorrhizal fungi. Application of duff is a reliable, easy, and simple method of inoculation. Duff not only provides a mixed population of mycorrhizal fungi, but it is also a source of added organic matter. In addition, duff can supply microflora and microfauna beneficial to litter decomposition and nutrient cycling. However, this method of inoculation can be expensive due to the large amount of duff required for nursery inoculation (transportation costs are high if inoculum has to be shipped long distances). Also, the time of transportation should be as short as possible, and the soil must be kept moist and protected from excessive temperatures because mycorrhizal fungi, in general, are not very heat-tolerant.

The greatest disadvantage of using duff inoculum is the risk of introducing new pests into a nursery. Duff from healthy plantations and natural stands contains an indiscriminate mixture of fungi and other organisms, including perhaps root pathogens and parasites such as plant-parasitic nematodes. For this reason, duff should not be collected from stands where known root pathogens are present. Similarly, soil from old nurseries should not be used as an inoculum source because it may contain damping-off fungi, plant-parasitE nematodes, insects, weed seeds, and other nursery pests.

Mycorrhizal seedlings and roots

Use of living mycorrhizal seedlings as inoculum in large scale nursery practice is uncommon. However, transplanting mycorrhizal seedlings in nursery beds has been a successful inoculation method where application of soil duff has not been effective. This type of inoculum was first used in Indonesia and was a reliable method for production of Merkus pine (Pinus merkusii).Mycorrhizal seedlings are planted in seedbeds at 1 to 2 meter intervals, and from there mycelia spreads to adjacent seedlings. At time of lifting, some seedlings are left in beds to infect the next crop of seedlings. Disadvantages of this method are slow and uneven spread of fungal mycelia from mycorrhizal mother trees, and the possibility of introducing pests and diseases.

Incorporation of mycorrhizal root systems into soil has also been successfully used for inoculation of nursery seedlings.

Spores and fruiting bodies

Spores and fungal fruiting bodies (certain mushrooms and puffballs) also have been successfully used as mycorrhizal inoculum. Fruiting bodies of ectomycorrhizal fungi are normally chopped into small pieces before incorporation into nursery soil or container growing medium, and function primarily as spore inoculum (Trappe, 1977).

Basidiospores of mycorrhizal fungi have been suspended in water and leached into nursery soil, mixed mechanically without water directly into soil, or coated on tree seeds. Dry basidiospores of Pisolithus tinctorius mixed with moist vermiculite, broadcast on fumigated soil, and mixed into soil mechanically has been an effective method of soil infestation (Marx, 1976). However, the quantity of spores needed for a specific volume of soil or seed has not been clearly defined. In pot experiments, a concentration of 55 million P. tinctorius spores per 800 cm^3 of soil synthesized more ectomycorrhizae on loblolly pine than did greater or lesser concentrations (Marx, 1976). Maximum ectomycorrhizal development on Monetery pine has been obtained by coating each seed with 3000 Rhizopogon luteolus spores (Theodorou and Bowen, 1973).

Basidiospores of some ectomycorrhizal fungi can be readily collected from fruiting bodies, and spore incorporation in nursery soil and container growing media is a simple and inexpensive method of inoculation. However, there are still several problems to resolve before spores of mycorrhizal fungi can be used extensively as inoculum for production of seedlings. The availability of viable inoculum in sufficient quantities to infest nursery soils is one problem. Spores can be collected only during periods of the growing season when weather conditions are favorable for production of fruiting bodies, and the occurrence of fruiting bodies is erratic from year to year. An effective method for storage of spores is needed. Dry storage of P. tinctorius spores in darkness at 5 C for 1 week to 34 months has not significantly affected mycorrhizal development on loblolly pine (Marx, 1976). Viability of spores has been difficult to determine in the laboratory. The best way to determine spore viability at present is in mycorrhizal synthesis tests with seedlings.

Pure cultures of mycorrhizal fungi

Inoculations with pure cultures of mycorrhizal fungi are necessary when a desirable mycorrhizal species is lacking or when existing mycorrhizal species are insufficiently virulent. This method of inoculation eliminates the risk of introducing pathogens, but only those fungi that grow on culture media can be used. Several mycorrhizal fungi grow slowly or not at all in pure culture, and it is time consuming and expensive to produce sufficient quantities of vegetative mycelium for inoculation purposes.

In the laboratory, vegetative mycelium is produced by aseptically growing mycorrhizal fungi for three to four months in 1 to 1.5 liter volumes of vermiculite-peatmoss substrate moistened with a nutrient solution. After fungal mycelia have thoroughly permeated this substrate, the inoculum is screened to 5 mm dimension and thoroughly leached with cold tap water to remove nonused nutrients so that build-up of saprophytic organisms in the

soil is avoided when inoculum is added. After leaching, inoculum can be dried at a low temperature (30 C) in a forced air oven. Dried inoculum (12% moisture) mixes more homogenously with nursery soil or dry container growing media than wet inoculum, and also can be stored for several weeks at 5 C without any appreciable loss of viability. Researchers have found that dried P. tinctorius vegetative inoculum can be stored for 12 weeks at 5 C or 8 to 10 weeks at 22 or 30 C without loss of viability.

Commerical production of vegetative mycelium of P. tinctorius is now being investigated. Dr. Richard Tinus, plant physiologist, U. S. Forest Service, Rocky Mountain Forest and Range Experiment Station, and I are currently participating in a nationwide test designed to evaluate P. tinctorius inoculum produced by a commercial laboratory with P. tinctorius at the U. S. Forest Service Laboratory in Athens, Georgia, for ability to synthesize mycorrhizae and improve growth of nursery and container seedlings. Tests with container ponderosa and Scots pine at Bottineau, N. D., and with ponderosa pine at Bessey Nursery, Halsey, Nebraska, were installed in the spring of 1977. Evaluation of these tests will be made after the 1977 growing season. Additional testing of inoculum is planned for 1978. Although more extensive field testing is needed, there is a possibility that commercially produced vegetative mycorrhizal inoculum will be available for use in practical forestry within the next few years.

Several investigators have successfully used vegetative mycelium of mycorrhizal fungi as inoculum for synthesis of ectomycorrhizae on pine seedlings in forest nurseries and in container greenhouses. In nearly all nursery tests conducted by Dr. Donald Marx and his colleagues at Athens, Georgia, southern pine seedlings with Pisolithus ectomycorrhizae were significantly taller and of better quality after one growing season in the nursery than routine, non-inoculated seedlings. Pines with Pisolithus ectomycorrhizae also exhibited better survival and growth after two years on routine reforestation sites in Florida and North Carolina and on a variety of drastically disturbed sites in the southern region (Marx, Bryan and Cordell 1977). In a cooperative study with Dr. Richard Tinus, vegetative mycelium of six ectomycorrhizal fungi and pine duff were mixed separately with container growing medium (1:1 vermiculit peat moss) at ratios of 10 and 20% by volume, immediately seeded with Pinus ponderosa or P. sylvestris, and seedlings grown in the U. S. Forest Service greenhouse at Bottineau, North Dakota for one year. We have found that good ectomycorrhizal development occurred on seedlings with P. tinctorius or pine duff. For Scots pine, treatment with duff resulted in heavier fresh stem weights and larger root-collar diameters than treatment with pure cultures of mycorrhizal fungi. For ponderosa pine, inoculation with mycorrhizal inoculum has resulted in greater seedling weights and root collar diameters than with no inoculation. Mixing mycorrhizal inoculum in growing medium at 20% was no better than using a 10% rate. First season survival of seedlings in an out-planting at Denbigh, N. D. was very good for both tree species from the pine duff treatment.

In the current nationwide testing of Pt vegetative inoculum, the following procedures for inoculation of experimental nursery beds are being used. Prior to inoculation, seedbeds were fumigated to eliminate feeder root pathogens (plant-parasitic nematodes and fungi) that destroy sites for mycorrhizal development. After aeration of fumigated soil, inoculum was evenly broadcast by hand over the soil surface of seedbeds at rates of 50 to 150 ml per ft2

and thoroughly mixed into the upper 6 inches of soil with a hoe. Each seedbed then was seeded according to normal nursery procedures. The application of inoculum prior to seeding will allow sufficient time for mycorrhizae to develop and benefit seedling growth during the first growing season in the nursery. Inoculated nursery beds are maintained using normal procedures for irrigation and application of fertilizers, pesticides and herbicides. For inoculation of container growing medium, vegetative mycelium of Pt was mixed at a ratio of 1 part inoculum to 7.5, 15, and 30 parts of growing medium, by volume. Following seeding, normal growing procedures are followed except that a moderate nutrient level is maintained in the growing medium.

What can the nurseryman do to promote development of mycorrhizae on tree seedlings?

Many procedures carried out in nurseries affect soil factors, such as fertility, aeration, temperature, and pH which influence the development of seedling feeder roots, growth of mycorrhizal fungi, and formation of mycorrhizae. The effect of certain nursery procedures on formation of mycorrhizae of tree seedlings will be discussed under the following headings: (1) application of biocides, (2) application of fertilizers, (3) adjustment of soil reaction, (4) shading, (5) watering, and (6) application of organic matter.

Application of biocides

Partial soil disinfection with chemicals to control pathogens and weeds is a common practice in modern nursery management. One question arises from this practice: What effect do these chemicals have on mycorrhizal fungi? Fumigation with broad spectrum fumigants not only controls pathogenic organisms but also reduces populations of mycorrhizal fungi in seedbeds during the first growing season. Retardation of mycorrhizal formation after use of methyl bromide has been reported by several investigators.

Although spores and hyphae of mycorrhizal fungi in the uppermost soil layers penetrated by fumigants may be destroyed, hyphae in the lower soil depth may remain alive and infect roots as they grow into these depths. In addition, soil may be reinfested by wind-disseminated spores of ectomycorrhizal fungi from mushrooms from nearby forested areas. However, a considerable delay in mycorrhizal development may occur if weather conditions are unfavorable (dry or cold) for mushroom production. If nurseries are located in remote areas away from forested areas, distances of spore dissemination may be too far for effective soil reinfestation. Relative uniform reinoculation of fumigated soil may require one or more growing seasons. If reinvasion is not uniform. erratic seedling growth with stunted nonmycorrhizal seedlings may result. Therefore soil infestation with mycorrhizal inoculum may be advisable.

Selective chemicals that control feeder root pathogens promote growth of healthy new feeder roots which subsequently may provide increased mycorrhizal development. Certain nematicides, such as ethylene dibromide and Nemagon, do not appear to suppress formation of ectomycorrhizae on pine seedlings (Hacskaylo and Palmer, 1957).

Many hardwood trees and shrubs have mycorrhizae that are synthesized by endomycorrhizal fungi. When endomycorrhizal fungi have been killed by soil fumigation, soil reinfestation usually is very slow because these fungi do not produce spores which can be wind disseminated. Reinfestation of fumigated

soil by endomycorrhizal fungi may originate from inoculum still viable in the soil at depths beyond penetration of fumigants, from windblown or washing from adjacent non-fumigated soil, or from non-fumigated soil brought in on cultivation equipment.

The following recent reports illustrate the impact of fumigants on formation of endomycorrhizae in nursery soils. Citrus seedlings grown in a nursery fumigated with 400 lb per acre of a 3:1 mixture of methyl bromide and chloropicrin, grew unevenly (Kleinschmidt and Gerdemann, 1972). Healthy plants were mycorrhizal, whereas stunted chlorotic plants were not mycorrhizal When stunted seedlings were transplanted into sterilized soil that had been inoculated with the endomycorrhizal fungus Glomus mosseae (Syn. Endogone mss. they grew normally, but noninoculated seedlings remained stunted. Fumigation with methyl bromide (Dowfume MC-2 and MC-33) at rates of 250 and 350 lbs per acre, followed by seeding 2 to 6 weeks later, has resulted in very erratic seedling growth of hardwoodsduring the first growing season at Lincoln-Oakes Nursery, Bismarck, N. D. A test was conducted at this nursery with three fumigants and one fungicide to determine their effects on development of endo-mycorrhizae (EDM) on honeysuckle, Russian-olive, buffaloberry, and green ash seedlings. Methyl bromide reduced, but did not inhibit, endomycorrhizal formation on these seedlings during the first growing season. Tree seedlings from nonfumigated soil had 98% occurrence and 91% density EDM compared to W, occurrence and 79% density EDM for seedlings from fumigated soil (Riffle, 1911

In nursery practice, it is sometimes necessary to use a wide variety of O° fungicides, nematicides, insecticides and herbicides for production of nurser! stock. Although certain pesticides may depress the activity of some mycorrhi fungi, most mycorrhizal fungi apparently are not as vulnerable to pesticides as once believed. Treatment of seeds with fungicides and bird or animal repellents has no apparent effect on early mycorrhizal development of seedling (Marx and Barnett, 1974). Also, the majority of reports on translocation of systemic fungicides indicate that the amount transported from foliage within the plant downward into the roots is small (Erwin, 1973), and should have little significance on development of mycorrhizal fungi.

Fungicide seed treatment raises the question:Do basidiospores of ectomycorrhi fungi added to pine seeds remain viable in the presence of fungicides on the seedcoat? Recent nursery and greenhouse tests in Australia have revealed that seed coat dressings of captan and zineb inhibited ectomycorrhizal development on Monterey pine seedlings grown from seeds inoculated with Rhizopogon luteolus basidiospores (Theodorou and Skinner, 1976). However, these fungi-cides did not inhibit mycorrhizal development by naturally occurring fungi distributed throughout the soil.

Application of fertilizers

Intelligent regulation of nutrient condition is one way by which maximum benefit from ectomycorrhizae may be obtained. McComb and Griffith (1946) have shown that some indigenous species present in a prairie soil were able, when activated by moderate phosphorus fertilization, to synthesize mycorrhizae with white pine seedlings. The development of mycorrhizae can also be promoted by the addition of other nutrients. In general, a moderate and balanced supply of nitrogen, phosphorus, and potassium stimulates the development of

mycorrhizae on tree seedlings. Excess soil fertility,especially high levels of nitrogen and phosphorus, reduces the degree of mycorrhizal infection. Extremely low nutrient levels limit growth of both fungi and seedling roots.

Soil reaction

It may be necessary to improve soil conditions in forest nurseries by acidification. Acidification alone may be effective to promote mycorrhizal development, or it can be used in conjunction with inoculation. Most ectomycorrhizal species are acidophilic. Species grown in pure cultures have maximum mycelial growth at pH 4 to 6. Extremes in pH, however, affect viability of fungal hyphae and spores or their growth on roots.

Variation in pH optimum among different strains of the same mycorrhizal species suggests that these fungi adapt to soil pH. Nurseries in Nebraska with soil pH 7.8 have produced mycorrhizal ponderosa pine (Goss, 1960). The sparse development of ectomycorrhizae in neutral or alkaline soil may not be due to the acidophilic nature of mycorrhizal fungi. The indirect effect of soil pH on available soil nitrogen may be more important to mycorrhizal formation than direct effects on the fungus per se. Some investigators ascribe the inhibitory effect of alkaline soils on mycorrhizal formation to increased availability of nitrogen resulting from enhanced nitrification, rather than from high pH (Richards and Wilson, 1963).

Shading

Shading of nursery beds and greenhouses is sometimes used in the production of nursery stock. Care should be exercised so that light received by foliage is not significantly reduced for extended periods of time. There is a direct correlation between light intensity and mycorrhizal infection. Abundant mycorrhizal development of pine seedlings occurs under condtions of full sunlight and with sufficient nutrient levels for adequate but not lush root growth. When light intensity on foliage is low (below 25% full sunlight), the reduced amount of photosynthetic substances translocated to the roots may completely inhibit mycorrhizal development.

Soil moisture and aeration

Moisture content in soil is important for the survival, development, and activity of various mycorrhizal fungi. In fact, available moisture, aeration, and temperature directly affect the growth of roots and mycorrhizal fungi in nursery soil. These soil factors also can influence mycorrhizal development indirectly since feeder roots must be formed before mycorrhizal development can take place. Deep tillage of nursery beds to eliminate compacted layers and to provide better water drainage directly affect such soil factors. Whereas abundant development of mycorrhizae may be found in moist locations, fewer mycorrhizae are usually found on dry sites and flooded areas.

Application of organic matter

Addition of organic matter usual ly promotes the formation of mycorrhizae. However, the factor actually promoting the growth of seedling roots and development of mycorrhizae in materials used as organic amendments in nurseries is not well known. For example, favorable effects of forest duff on seedling growth may be due to a combination of many factors, such as addition of mycorrhizal fungi, nutrients, or the effect of duff on physical (aeration) properties of the nursery soil.

LITERATURE CITED

Erwin, D. D. 1973. Systemic fungicides: disease control, translocation, and mode of action. Ann. Rev. Phytopathol . 11:389-422.

Goss, R. W. 1960. Mycorrhizae of ponderosa pine in Nebraska grassland soils. Nebr. Agr. Exp. Stn. Res. Bull. 192, 47 p.

Hacskaylo, E., and J. G. Palmer. 1957. Effects of several biocides on growth of seedling pines and incidence of mycorrhizae in field plots. Plant Dis. Reptr. 41:354-358.

Kleinschmidt, G. D., and J. W. Gerdemann. 1972. Stunting of citrus seedlings in fumigated nursery soils related to the absence of endornycorrhizae. Phytopathology 62:1447-1453.

Marx, D. H. 1976. Synthesis of ectomycorrhizae on loblolly pine seedlings with basidiospores of Pisolithus tinctorius. For. Sci. 22:13-20.

Marx, D. H., and J. P. Barnett. 1974. Mycorrhizae and containerized forest tree seedlings. North Am. Containerized For. Tree Seed. Symp. Proc. 1974:85-92.

Marx, D. H., W. C. Bryan, and C. E. Cordell. 1977. Survival and growth of pine seedlings with Pisolithus ectomycorrhizae after two years on reforest-ation sites in North Carolina and Florida. For. Sci. 23:(in press) .

McComb, A. L., and J. E. Griffith. 1946. Growth stimulation and phosphorus absorption of mycorrhizal and non-mycorrhizal northern white pine and Douglas-fir seedlings in relation to fertilizer treatment. Plant Physiol.21:

Mikola, P. 1973. Application of mycorrhizal symbiosis in forestry practice. p. 383-411. In Marks and T. . Kozlowski. (ed) Ectomycorrhizae Their Ecology and Physiology. Academic Press, New York, 444 p.

Richards, B. N., and G. L. Wilson. 1963. Nutrient supply and mycorrhiza development in Caribbean pine. For. Sci. 9:405-412.

Riffle, J. W. 1977. Soil fumigation suppresses endomycorrhizal development on broadleaf seedlings in a northern plains nursery. Proc. Amer. Phytopathol. Soc. 4:(in press).

Theodorou, C., and G. D. Bowen. 1973. Inoculation of seeds and soil with basidiospores of mycorrhizal fungi. Soil Biology and Biochemistry 5:765-771.

Theodorou, D., and M. F. Skinner. 1976. Effects of fungicides on seed inocula of basidiospores of mycorrhizai fungi. Aust. For. Res. 7:53-58.

Trappe, J. M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Ann. Rev. Phytopathol. 15:203-222.

Wright, E. 1940. Annual report of activities of the Lincoln Branch, Division of Forestry Pathology covering period from March 8, 1939 to February 21, 1940. Typed office report. 54 p.