ENDOMYCORRHIZAE: THEIR IMPORTANCE IN NURSERY PRODUCTION OF HARDWOOD SEEDLINGS

P. P. Kormanik, W. C. Bryan and R. Schultz[1/]

Abstract.--Early spring infection by endomycorrhizal fungi may be the solution to producing quality 1-0 hardwood seedlings in tree nurseries. Initial results obtained from experiments at Athens, Georgia, indicate all hardwoods tested benefit significantly from endomycorrhizal fungus infection and that one species, sweetgum, exhibited an obligate physiological requirement for these root symbionts. Applications of 141, 280, 560, and 1120 kg/ha of 10-10-10 fertilizer combined with a top dressing of NH4NO3 of up to 1680 kg/ha did not result in appreciable growth of nonmycorrhizal sweetgum seedlings. Fertilized endomycorrhizal sweetgum seedlings averaged between 35 and 40 cm in height, while the fertilized nonmycorrhizal controls averaged 5 to 7 cm tall. Significant differences among the fertilizer treatments were not found.

Additional keywords: Fumigation, Liquidambar styraciflua L., mass inoculation.

High-quality seedlings are a prerequisite to the successful establishment of hardwood plantations. Consistent nursery production of such seedlings is now the major obstacle to implementation of a hardwood regeneration program throughout the South. Techniques for plantation establishment have already been developed for use with suitable seedlings. Slow initial growth and failure of hardwood plantations now appear to be the result of using seedlings of a substandard morphological quality. Endomycorrhizae provide a way to overcome this problem.

Four years ago at this Conference, Dr. Donald Marx (1973) of the Forestry Sciences Laboratory in Athens, Georgia, presented a paper "The Importance of Mycorrhizae in Forest Nurseries." Although there is little need to read his data here, his predictions have materialized in only four short years. Mass inoculation of nursery beds with ecologically adapted, ectomycorrhizal fungi has become the newest tool available for nurserymen growing pine (Marx and Bryan 1975; Marx and others 1976). The response of field foresters to outplanting trials has been enthusiastic. Similar techniques are now being perfected for inoculating hardwood nursery beds with endomycorrhizae. That research and its implications for managing hardwood nurseries are reported here.

= Principal Silviculturist and Plant Pathologist, respectively, Southeastern Forest Experiment Station, USDA Forest Service, Athens, Georgia 30602 and Assistant Professor, School of Forest Resources, University of Georgia, Athens.

Today, our knowledge of endomycorrhizae is comparable to what Marx described for ectomycorrhizae in 1972. We have been able to demonstrate in small nursery plots that seedlings of many hardwood species have either an obligate requirement for, or exhibit pronounced growth stimulation from, the symbiotic relationship with endomycorrhizal fungi. By "obligate requirement" we mean that some hardwood seedlings will not develop normally until their roots become infected with appropriate endomycorrhizal fungi. As in 1972, data on performance of outplanted seedling are lacking. However, we do know large, healthy hardwood seedlings always grow better in the field than smaller ones. Although more research is needed, results to date show that hardwood seedlings of the required morphological quality for regeneration can be produced. It will be the nurseryman's responsibility to supply those seedlings for tomorrow's hardwood plantations.

OCCURRENCE AND CHARACTERISTICS OF ENDOMYCORRHIZAE

There are hundreds of species of endomycorrhizal fungi in the United States. Most natural soils harbor many different species simultaneously. Because endomycorrhizal fungi, like most fungi, readily undergo mutation, the possibilities for developing strains adapted to selected sites or tree species are almost limitless. Spores from these fungi, unlike those of ectomycorrhizae, are disseminated through soil, water movement, and root contact rather than by wind. Thus, their recolonization of fumigated nursery soils is often slow.

Roots become infected when they contact spores of endomycorrhizal fungi in the soil. These spores are stimulated to grow by exudates from the root. Thus, the endomycorrhizal fungi are dependent upon the host for their existence. This dependence may account for the evolutionary development of endomycorrhizae that has resulted in their broad host range. Endomycorrhizal roots are not readily distinguishable from nonmycorrhizal roots by the naked eye. They usually have an extensive, loose network of hyphae dotted with large, thick-walled, yellowish-brown spores on the feeder root surface. These spores are easily visible under a microscope at a relatively low magnification. The hyphae penetrate the root epidermis and grow into the deeper cortical tissue. Neither meristematic nor vascular tissue are infected by mycorrhizal fungi. The hyphae or strands of hyphae also radiate from the root surface into the soil and greatly increase the effective absorption area of the root. According to accepted theories, these external hyphal strands transport water and mineral elements that might otherwise be unavailable to the host plant. Furthermore, some tree species, i.e., sweetgum (*Liquidambar styraciflua* L.), apparently cannot develop normally unless the roots become endomycorrhizal.

Researchers do not yet know all the physiological ramifications of this symbiotic relationship. However, this lack need not be of concern to nurserymen at this time. The important point is that the successful growth of many species of hardwoods in the nursery may be dependent upon the presence of endomycorrhizae.

Unfortunately, although endomycorrhizae are ubiquitous in most soils, the fumigation essential in many nurseries drastically reduces or eliminates the indigenous populations. Recolonization can be well along by late summer, but there is usually insufficient time during the remainder of the growing season for infected seedlings to reach adequate size for successful outplanting.

WHAT RESEARCH IS DOING

At the Forestry Sciences Laboratory in Athens, Georgia, we are developing methods to shorten or eliminate the long period required for recolonization of endomycorrhizal fungi. We hope to do this with ecologically adapted fungi tailored for specific tree species and outplanting sites.

For several years before and after Marx's presentation to this group in 1972, the major emphasis in our program was directed at the fungi themselves. We had to learn how to handle pure cultures of endomycorrhizal fungi for inoculum. These techniques are even more difficult than those for endomycorrhizal fungi, since endomycorrhizal fungi cannot yet be grown on artificial media in the laboratory. The fungi need the roots of plants in order to grow. We must still work within this limitation, but we have been relatively successful in growing and maintaining our populations.

In the last two or three years, we have learned how to put endomycorrhizal fungi on seedling roots to determine their role in seedling development in beds. The early preliminary results, even without the best technology, have been encouraging.

Although we now know that sweetgum has an obligate requirement for endomycorrhizae, we have found no difference in growth between infected seedlings fertilized with 141, 280, 560, or 1120 kg/ha of 10-10-10 plus side dressings of NH_4NO_3 of up to 1680 kg/ha. The nonmycorrhizal controls at the various fertilizer levels averaged about 5 cm in height at the end of the growing season, while the infected seedlings averaged over 35 to 40 cm. For two successive years we have observed and measured such growth differences and are confident that, if sweetgum seedlings are growing, they are infected with some endomycorrhizal fungi.

Early spring infection of the roots will be the key to producing larger sweetgum seedlings in the nursery. We are sure that with modified inoculation techniques we can produce sweetgum seedlings averaging 60 to 75 cm in height with root collar diameters of 1 to 1.5 cm in beds of 60 to 80 seedlings/m . We have already produced many individual seedlings that approach these dimensions. Machines are available to plant such seedlings, and the forest-based industries in the South are demanding seedlings of this size for their hardwood regeneration programs.

We have not extensively studied the influence of endomycorrhizae on species other than sweetgum. But in studies designed to test our inoculation procedures, we found that white (*Fraxinus americana* L.) and green ash (F. *pennsylvanica* Marsh), boxelder (*Acer negundo* L.1 black cherry (*Prunus serotina* Ehrh.), red maple (*Acer rubrum* L.), and black locust (*Robinia pseudoacacia* L.) may also possess an obligate requirement for endomycorrhizae. However, because of weakness in our early technology, most of the control seedlings of these species became infected late in the summer. Table 1 contains a compilation of data

Table 1.--Mean height and stem growth of four species of 5-month-old seedlings in fumigated soil with and without addition of endomycorrhizal inoculum, 1975

	Noninoculated		Natural soil inoculum		*Glomus mosseae*		*Glomus fasciculatus*	
Species	Height	Stem diam.	Height	Stem diam.	Height	Stem diam.	Height	Stem diam.
	cm							
Green ash	38.2	0.91	79.4	1.5	60.6	1.2	83.2	1.6
White ash	9.4	0.23	37.8	0.82	21.9	0.52	37.2	0.77
Boxelder	30.6	0.81	44.4	0.99	40.4	0.95	57.9	1.2
Black cherry	6.9	0.21	34.1	0.51	28.5	0.49	36.9	0.63
	Percent growth increase							
Green ash			107	65	59	32	118	76
White ash			302	257	132	126	296	235
Boxelder			45	22	32	17	89	48
Black cherry			394	143	313	133	434	200

from a preliminary test completed in 1975. If the seedlings had been harvested in early August when infection became obvious, the obligate nature of this symbiotic relationship would have been readily apparent. Larger and more comprehensive studies are now installed on larger and deeper microplots in our experimental nursery, where our test conditions are more easily maintained.

FUTURE RESEARCH AND RECOMMENDATIONS

Several areas need more research before mass inoculation of nursery beds with endomycorrhizal fungi can be considered for routine application. We have not tested ecologically adapted strains of endomycorrhizae as we have with ectomycorrhizae. It appears, however, that any endomycorrhizal association in sufficiently large numbers is much better than none. Thus, today, the nurserymen may be able to reap benefits from indigenous endomycorrhizal fungi occurring in their nursery soils. Tomorrow, they will be able to inoculate beds with strains or species of endomycorrhizal fungi ecologically adapted for the desired hardwood species and a range of outplanting sites.

One practice that nurserymen might start today is growing a good endomycorrhizal host plant such as sorghum (*Sorghum vulgare* Pers.) the year they fumigate their nursery beds. By late summer or early fall the soil could be examined to determine whether endomycorrhizal fungi have recolonized the beds in sufficient numbers to assure adequate infection of the next hardwood seedling crop. If recolonization has been successful, the sorghum could then be plowed under during the fall and the beds reshaped and planted to hardwoods the following spring. We have confidence in this method and hope to do research on it during the next year.

Another management practice that still needs additional research before definite recommendations are possible is the level of NH_4NO_3 needed during the growing season. Seedlings infected in early spring can grow 300 to 800 percent taller than noninfected ones or those which become infected in late summer. To sustain this growth, it may be necessary to apply ammonium nitrate five or six times during the growing season at cumulative dosages of 560 to 1150 kg/ha. Until we have more precise information about inoculation techniques, we cannot confound our experiments with this important variable.

CONCLUSION

For over a decade the importance of hardwood species to forest-based industries in the South has been increasing. This new emphasis on hardwood management can have as great an impact on southern forestry as that experienced with pine after World War II. Foresters have been aware for some time of the great productive potential of hardwood species, and farsighted industrial leaders now share this enthusiasm. Although the commitment to hardwoods has been made, it will be up to nurserymen to grow the quality seedlings needed in this new management program. We in research must first obtain information that can make endomycorrhizal mass inoculation practical; nurserymen must then put this information into use.

LITERATURE CITED

Marx, D. H. 1973. The importance of mycorrhizae in forest nurseries. Proc. Southeast. Area Forest Tree Nurserymen's Conf., East. Sess. Wilmington, NC, Aug. 1972, p. 188-192.

Marx, D. H. and W. C. Bryan. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with *Pisolithus tinctorius*. Forest Sci. 21: 245-254.

Marx, D. H., W. C. Bryan and C. E. Cordell. 1976. Growth and

fungal symbiont *Pisolithus tinctorius.* Forest Sci. 22: 91-100.