MYCORRHIZAE IN FOREST NURSERIES

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At the Wilmington, N. C., conference in 1972, Dr. Donald Marx from the Forestry Sciences Laboratory at Athens, Georgia, presented an excellent introduction to the mycorrhizal fungi along with the benefits and desirability of developing and utilizing this most interesting group of soil fungi in forest nurseries. As was pointed out, this group of fungi are symbiotic parasites rather than parasitic pathogens on seedling roots. The two common classes of mycorrhizae are ectomycorrhizae and endomycorrhizae. Ectomycorrhizae are formed on the root surface of such species as pines, spruce, fir, beech, hickory, and most oaks. Endomycorrhizae are also formed on the root surface but their hyphae are much more loose, sparse, and inconspicuous as compared to the tight thick fungus mantle formed by ectomycorrhizae. Endomycorrhizae enter the cortical tissues of the feeder roots of such species as sycamore, sweetgum, ash, yellow-poplar, boxelder, locust, maples, and many other hardwoods. Most research and field studies have been conducted on ectomycorrhizae. Therefore, the remainder of this paper will be devoted to this class of mycorrhizae.

Dr. Marx also pointed out the following experimentally proven benefits of ectomycorrhizae to forest trees:

1. Increased physiologically active absorbing surface of the feeder root system.
2. Increased absorption and accumulation of essential elements--especially N, P, K, and Ca.
3. Increased functional age (longevity) of feeder roots.
4. Conversion of normally unavailable soil nutrients to available form for absorption by tree roots.
5. Increased heat and drought tolerance of tree hosts.
6. Biological deterrents to feeder root infections by root pathogens such as _Phytophthora_ and _Pythium_ spp.

The following factors were listed as stimulatory to mycorrhizal development of forest trees:

1. High light intensity
2. Low or moderate soil fertility
3. Good soil aeration and moisture relations
4. High soil organic matter
5. Soil reaction of pH 4.5 to 5.5
6. Soil temperatures between 65 and 85°F.

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Extremes in any of these factors, as well as certain pesticides such as methyl bromide commonly used in forest tree nurseries, usually cause a decrease in mycorrhizal development and/or function on seedling feeder roots. However, ectomycorrhizae produce abundant air-borne spores throughout the year and, consequently, are capable of rapid recolonization of fumigated soil. In addition, certain species of mycorrhizal fungi have been observed to be more ecologically adaptable than others and, consequently, are more capable of synthesizing mycorrhizae under soil environmental extremes as long as susceptible seedling feeder roots are available.

Nurserymen must be concerned with growing healthy vigorous seedlings with high field survival capabilities as well as good seedbed and packing shed appearances. These factors represent a good durable product which is essential for good business. The seedling product in this case must often withstand severe adverse climatic and soil factors at the planting site--not to mention the planting shock sometimes encounted. The development and utilization of mycorrhizal feeder roots on seedlings to satisfy particular requirements will be most beneficial in the development of a more durable and higher quality product.

During the spring of 1973, pathologists at the Forestry Sciences Laboratory, Athens, Georgia, and Forest Pest Management, SEA-S&PF, Asheville, North Carolina, joined forces to establish a cooperative mycorrhizal field study in selected southeastern forest nurseries. Cooperative nursery plot studies were established with the States of Florida (Andrews Nursery), Georgia (Morgan Nursery), and North Carolina (Edwards Nursery). Mycelium and basidiospores of the mycorrhizal fungus _Pisolithus tinctorius_ were prepared in the Forestry Sciences Laboratory at Athens, Georgia and used as the mycorrhizal inoculum source for the field plots. Replicated and check plots were established to facilitate treatment comparisons and statistical analysis. All plot sites were fumigated with methyl bromide (summer or fall-1973 or spring-1973) prior to plot establishment. Selected pine species hosts were planted on the plots at each nursery. Slash, loblolly, and sand (Ocala variety) pines were planted at Andrews (Florida). Loblolly, sand (Choctowhatchee variety), and Virginia pines were planted at Morgan (Georgia). Loblolly, Virginia, and white pines were planted at Edwards (North Carolina).

Successful P. _tinctorius_ basidiospore as well as mycelium inoculations were obtained at all three nurseries. However, growth stimulation was quite variable at each nursery. This was due primarily to such factors as high soil fertility and optimum seedling growth conditions (Andrews Nursery), extremely high populations of competitive soil microorganisms (Morgan Nursery), and adverse climatic (spring seedbed flooding) and poor growth conditions (Edwards Nursery).

At the Morgan Nursery _Pythium_ spp. propagule counts of over 60/gm. of soil and parasitic nematode assays of over 1,000 per pint of soil were recorded in some of the plots. These competitive microorganisms almost completely nullified the mycorrhizal inoculations at this nursery resulting in only a small insignificant amount of P. _tinctorius_ mycorrhizal development and corresponding seedling growth response.

The story was reversed at the Andrews Nursery. Good P. tinctorius mycorrhizal development was obtained on all three pine species--loblolly, slash, and sand. However, the high soil fertility and optimum growing conditions at this nursery produced comparable seedlings on both the inoculated and uninoculated plots. Consequently, there was no significant difference in fresh root and top biomass weight between treatments for either of the three pine seedling species.

The best results in terms of both P. tinctorius mycorrhizal development and pine seedling growth (increased biomass production) were obtained at the Edwards Nursery (Table 1). The percent of mycorrhizal development from P. tinctorius mycolium inoculations was 62%, 68%, and 37% on the feeder roots of loblolly, Virginia, and white pines, respectively. Mycorrhizal development from basidiospore inoculations was 43%, 6%, and 17%, respectively, on the same three species. Growth response (increased biomass production) on P. tinctorius mycelium inoculated plots was highly significant (1% sig. level) for all three pine species. There was an 140% increase in biomass on loblolly pine plots and a 100% or more increase on the Virginia and white pine plots. In addition, the P. tinctorius basidiospore inoculated loblolly pine plots showed a biomass increase of 85% which was also significant at the 1% sig. level. However, Virginia and white pine basidiospore inoculation results were insignificant both in terms of P. tinctorius mycorrhizal development and increased biomass production.

During the winter of 1973-74, field mycorrhizae outplanting studies were established in western North Carolina (near Edwards Nursery) and central Florida (Withlacoochee State Forest near Brooksville, Florida). Seedlings were selected from the respective species and mycorrhizal seedbed treatments at the Edwards and Andrews Nurseries, respectively. Good and poor sites were selected at each outplanting location by respective state forest personnel. A randomized block design was established at each of the four sites in a manner to isolate the various treatments tested and facilitate statistical analysis of data collected. Five blocks, with complete randomization and isolation of treatments per block, were established at each site. The study is scheduled for five years duration and initial data will be collected during the fall of 1974. The relative performance of the seedling treatments on these outplanted plots will represent the real "proof of the pudding" concerning the benefits of P. tinctorius mycorrhizal feeder-root development on the survival and growth of these five pine species in the field.

SUMMARY AND CONCLUSIONS

1. The mycorrhizal fungus Pisolithus tinctorius was successfully inoculated into the natural seedbeds at three southeastern United States nurseries.

2. The fungus formed mycorrhizae on the feeder roots of five southeastern United States pine species--loblolly, slash, Virginia, sand, and white.

3. Excellent and highly significant growth responses (increased seedling biomass production) were obtained on P. tinctorius mycelium inoculated loblolly, Virginia, and white pine plots at the Edwards Nursery in North Carolina.

Table 1. Mycorrhizal Field Plot Study Results - Edwards Nursery – Morgantown, N. C. - 1973

Pine Species	Pisolithus tinctorius Mycorrhizal treatment											
	Veg. Mycelium				Basidiospores				Noninoc. Controls			
	Biomass		Mycorrhizae		Biomass		Mycorrhizae		Biomass		Mycorrhizae	
	Amt.1/	Inc.2/	Total3/	P.t.4/	Amt.	Inc.	Total	P.t.	Amt.	Inc.	Total	P.t.
		%	%	%		%	%	%			%	%
Loblolly	12.5	140	64	62	9.6	85	68	43	5.2	0	50	0
Virginia	18.2	110	72	68	10.5	20	58	6	8.8	0	47	0
White	3.2	100	47	37	1.9	19	27	17	1.6	0	17	0

1/ Root and shoot fresh wt. - gms.

2/ Percent biomass increase over noninoculated controls.

3/ Percent of feeder roots with any ectomycorrhizal development.

4/ Percent of feeder roots with *Pisolithus tinctorius* development.

4. All P. tinctorius inoculated plots produced better seedlings (more biomass) than uninoculated plots.

5. Both mycorrhizal inoculation techniques (mycelium and spores) are feasible for use in southern nurseries. However, the basidiospore inoculum is much more economical and practical to use. Dry viable basidiospores of P. tinctorius have been successfully stored under refrigeration at the Forestry Sciences Laboratory for three years. Mycelium should be beneficial for restricted production of highly customized seedlings for specific reforestation requirements. This might include seedlings for adverse and difficult to establish sites along with high-value needs such as seed orchard root stock, container plantings, recreation areas, Christmas trees, and ornamentals.

6. The requirement of effective soil fumigation to reduce populations of such competitive root-damaging soil microorganisms as Pythium spp. and nematodes was clearly demonstrated in the data obtained from the Morgan Nursery. All basic research and field study results collected to date indicate that effective soil fumigation, synchronized as closely as possible with the mycorrhizal fungus inoculation date, is mandatory to achieve effective mycorrhizal seedling root development. The present data also shows the rapid recolonization potential of methyl bromide fumigated soils by the common indigenous mycorrhizal fungus Thelephora terrestris. This occurred even at the Andrews Nursery where over 70% T. terrestris mycorrhizal roots were present on uninoculated slash pine plots that were fumigated with 600 lbs. methyl bromide in April, 1973.

7. As previously stated, the real "proof of the pudding" concerning the benefits achieved from a mycorrhizal fungus such as P. tinctorius will be expressed in the field survival and growth data obtained from the outplanting plots. However, it is not difficult to ascertain the probable survival and growth benefits furnished to transplanted seedlings by a mycorrhizal fungus such as P. tinctorius with its apparent increased survival capabilities under adverse conditions. For example, results obtained from a 5-month-old loblolly pine outplanting study established on a coal spoil site in Kentucky showed 95% survival of seedlings with 85% P. tinctorius mycorrhizal roots as compared to only 5% survival of seedlings with 85% T. terrestris and 0% P. tinctorius mycorrhizal roots. The common T. terrestris ecotomycorrhizal fungus probably accounts for 90% or more of the mycorrhizal feeder root development on southern pine seedling species.

FUTURE WORK

1. Studies to determine more practical seedbed inoculation methods.

2. Expanded field studies to determine additional benefits (qualitative and quantitative) to selected seedling host species.

3. Studies (laboratory and field) designed to obtain similar information concerning endo- and ectomycorrhizae on hardwood seedling species.

4. Additional studies to determine the host ranges of selected mycorrhizal fungi. *Pisolithus tinctorius* now has a known host range of 22 pine species along with eucalyptus, Norway spruce, Douglas fir, and European birch.

As indicated, this work has been and will continue to be oriented towards practical field application. The continued cooperation of all agencies concerned (Research, State and Private Forestry, State agencies, and Industry) will help to assure the most rapid accumulation of experimentally proven and field-tested results along with assimilation of these results for practical field application.