NATIONAL *PISOLITHUS TINCTORIUS* ECTOMYCORRHIZAE NURSERY EVALUATION

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ABSTRACT

During the past several years, the U. S. Forest Service has been conducting extensive research and field application studies concerning the practical application of the ectomycorrhizal fungus, *Pisolithus tinctorius,* to bare-root and container seedling nurseries. Positive results, involving increased ectomycorrhizal development on seedling feeder roots, increased seedling biomass, and decreased cull percentages have been obtained on a variety of conifer and some hardwood seedling species over a wide geographic range in the United States. In 1978, the National P. *tinctorius* mycorrhizae evaluation was expanded to include 33 bare-root nurseries in 28 states along with container seedling studies in 8 states (including Hawaii) and Canada. The objective of this evaluation was to compare the effectiveness of P. *tinctorius* inoculum produced by the Mycorrhizal Institute - Athens, GA and Abbott Laboratories - Chicago, IL for ectomycorrhizal seedling feeder root formation, seedling growth and quality, and tree survival and growth in subsequent field outplantings. Preliminary evaluation results show the Abbott P. *tinctorius* inoculum to be nearly comparable to the Georgia inoculum in producing ectomycorrhizae on a variety of conifer and some hardwood (oaks) species in both bare-root and container seedling nurseries. Consequently, these results could represent a significant potential increase in nursery seedling quality along with timber volumes. Barring unforeseen problems, Abbott Laboratories anticipates the commercial availability of P. *tinctorius* inoculum in 2-3 years.

Additional keywords: Forestation, bare-root nurseries, container seedlings, adverse sites, windbreaks, shelter belts, Christmas trees, hardwoods, conifers, *Abies concolor, A. fraseri, Pinus banksiana, P. clausa, P. echinata, P. elliottii, P. nigra, P. palustris, P. ponderosa, P. strobus, P. sylvestris, P. taeda, P. virginiana, Quercus macrocarpa, Picea mariana, Pseudotsuga menziesii.*

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INTRODUCTION

The mycorrhizal fungi are the beneficial fungi in forest tree nurseries, field forestation, and natural stands. Two primary types of mycorrhizae are the ectomycorrhizae and the endomycorrhizae. The ectomycorrhizae form a rather conspicuous fungus mantle on the surface of the feeder roots primarily on conifers (pine, fir, spruce, hemlock, larch, Douglas-fir) along with some hardwoods (oaks, birch, eucalyptus). The endomycorrhizae develop within the root cortical cells primarily on hardwoods such as walnut, poplar, maple, sweetgum, sycamore, and ash. The ectomycorrhizae also produce a variety of fruiting bodies such as puffballs and mushrooms above ground; their spores are wind-disseminated. However, the endomycorrhizae produce their spores on infected root surfaces below ground and, consequently, are limited to localized distribution in the soil by water, soil tillage, etc. Both types of mycorrhizae occur on a wide variety of plant hosts throughout the temperate zones of the world; their occurrence constitutes the rule rather than the exception in nature.

PAST RESEARCH AND FIELD EVALUATIONS

During the past several years, the Mycorrhizal Institute of U. S. Forest Service at Athens, Georgia, has been conducting extensive research on mycorrhizae (1). This research has centered around one particular ectomycorrhizal fungus, *Pisolithus tinctorius (P.T.),* and its application to forest tree nurseries and field forestation. Based on the successful results obtained from these pioneer laboratory and greenhouse studies, field evaluations were initiated in selected southern nurseries to evaluate the effectiveness of *P.t.* artificial inoculations in producing tailored ectomycorrhizae on a variety of seedling hosts and geographic locations in the Southeast (2). The effectiveness of *P.t.* on seedling growth and quality along with tree survival and growth responses in followup outplantings were also evaluated. Artificial seedbed inoculations with *P.t.* spores and a mycelium-vermiculite-peat inoculum were evaluated in three southern nurseries--one each in North Carolina, Georgia, and Florida (2).

Results obtained from these studies were highly successful. Significant increases in ectomycorrhizal production along with seedling growth and quality were obtained on the majority of the P.t. inoculated plots as compared with uninoculated check-plots. However, the P.t. mycelium-vermiculite-peat inoculum was more effective than the spores. Seedling fresh weights on loblolly pine *(Pinus taeda),* slash pine *(P. elliottii),* sand pine (P. *clausa),* Virginia pine *(P. virginiana),* and eastern white pine *(P. strobus),* were increased over 100 percent in one growing season - the normal seedling rotation for these southern hard pines except for eastern white pine which is 2 years. The common indigenous ectomycorrhizal fungus, *Thelephora terrestris,* produced abundant mycorrhizae (50+ percent) on the uninoculated plots. This mycorrhizal fungus accounts for 75+ percent of the ectomycorrhizae on pine seedling roots in southern nurseries. Followup field outplantings were established in North Carolina and Florida to determine the effects of different *P.t.* ectomycorrhizae levels on tree survival and growth. Significant increases in plot volume (tree survival X ht. X basal diameter^2) have been realized in two

sites each in North Carolina and Florida after 4 years in the field. An average increase in plot volume of 46 percent has been obtained on plots planted with seedlings inoculated with *P.t.* mycelium in the nursery as compared with the check plots planted with uninoculated (natural *T. terrestris* inoculated) seedlings (3).

NATIONAL *P.t.* ECTOMYCORRHIZAE NURSERY EVALUATION - 1977

Following these results, Abbott Laboratories in Chicago, Illinois, became very interested in the commercial production and practical application of *P.t.* for forest tree nurseries and field forestation. Subsequently, a National *P.t.* ectomycorrhizae nursery evaluation was initiated in the spring of 1977. This is a cooperative evaluation by the Mycorrhizal Institute, the Southeastern Area, Forest Insect and Disease Management Staff, Abbott Laboratories, and various state and industry agencies. The 1977 evaluation was conducted in 17 nurseries in 15 states from Virginia to California and involved seven species of pines along with white fir *(Abies concolor)* and Fraser fir (A. *fraseri).* The objectives of this evaluation were to compare the effectiveness of P.t. mycelium-vermiculite-peat inoculum produced by the Mycorrhizal Institute, with that commercially produced by Abbott Laboratories for mycorrhizae production, seedling growth and quality, and tree survival and growth in tree nurseries and field plantings. Results obtained with the Georgia P.t. inoculum were highly successful on all host species in all nurseries (table 1). Artificial seedbed inoculations with this inoculum source produced an average of 25 percent P.t. ectomycorrhizae, increased seedling fresh weights 13 percent, and decreased seedling culls 32 percent on the seven species of pines in the 17 nurseries. Positive P.t. ectomycorrhizae development was also obtained on the two species of fir but these results are not included in this summary. Results obtained with the Abbott *P.t.* inoculum (high rate: 150 ml/ft^2 in seedbeds) were considerably less effective than the Georgia *P.t.* inoculum (table 1). Seedbed inoculation with this inoculum source produced an average of 4 percent P.t. ectomycorrhizae on the same 7 species of pine in the 17 nurseries. However, there was no difference in seedling fresh weights and very little difference in seedling culls using this inoculum source as compared with the uninoculated check plots. The Abbott P.t. inoculum did produce some degree of ectomycorrhizae on all the seven pines and two fir species inoculated in the 17 nurseries. Similar comparative results between the Georgia and Abbott *P.t.* inoculum sources were also obtained in a companion container seedling evaluation conducted in five locations from North Carolina to Oregon. Subsequent discussions with scientists at Abbott Laboratories revealed probable production problems with the initial P.t. inoculum batches in 1977. These problems have since been identified and, hopefully, corrected.

EXPANDED NATIONAL P.t. ECTOMYCORRHIZAE NURSERY EVALUATION - 1978

In 1978, the National *P.t.* mycorrhizae nursery evaluation was expanded to include 33 bare-root forest tree nurseries in 28 states from Delaware to Florida and Oregon. Seedling species involved include 11 species of pines along with two varieties of Douglas-fir *(Pseudotsuga menziesii)* and Fraser fir. The evaluation objectives are the same as

TABLE 1.--Overall summary of 1977 test in 17 nurseries (1-0) using laboratory (Ga) and Abbott (Abb) produced vegetative inoculum of *Pisolithus tinctorius* (*Pt*) to form ectomycorrhizae on seven species of pine.

Treatment	Seedlings fresh wts. (gm) Top	Roots	Total	% Ectomycorrhizae by: *Pt*	Other	Total	Percent of seedlings with *Pt*	Percent cull seedlings	Total No. *Pt* fruit bodies
Ga 100	12.2	4.8	17.0	25	12	37	93	16.1	43
Abb 150	10.8	4.2	15.0	4	26	30	26	23.3	12
Abb 100	10.8	4.2	15.0	3	27	30	23	22.0	3
Abb 50	10.5	4.1	14.6	3	29	32	19	23.7	8
Control	10.8	4.2	15.0	<1	30	30	3	23.7	1
% increase of Ga 100 over control	13.0	14.3	13.3	N.A.	N.A.	23	N.A.	32.1	N.A.

for the 1977 evaluation - comparison of Georgia and Abbott P.t. inoculum effectiveness for mycorrhizae feeder root formation, seedling growth and quality, and tree survival and growth in nursery seedbeds and field plantings. As in previous nursery seedbed evaluations, randomly selected prefumigated seedbed plots were inoculated with either the Georgia or Abbott *P.t.* mycelium-vermiculite-peat inoculum immediately prior to planting. Prefumigation with soil fumigants equivalent to methyl bromide - 98% + chloropicrin - 2% and methyl bromide - 67% + chloropicrin - 33% has been mandatory in obtaining successful P.t. nursery seedbed inoculations The dried inoculum mixture (approximately 12 percent moisture content) was sprinkled evenly on 4 X 4 ft (16 ft^2) plots at four dosage rates - Georgia - 100 ml/ft, Abbott - 200, 100, 50 ml/ft^2. The inoculum was then chopped into the upper 3-4 inch soil surface with a garden tool. Each of the five treatments - four P.t. treatments and one check -were replicated five times in a randomized 5-block design. Following the inoculation, conventional seeding, mulching and all other nursery cultural practices were maintained as usual. The nursery phase of this evaluation is scheduled for 1 to 3 years, depending on the rotation length of the seedling host species. Followup field outplantings are also scheduled for a 5-year duration to compare the relative effectiveness of various degrees of Abbott and Georgia *P.t.* mycorrhizae feeder root formation on tree survival and growth. A companion container seedling study, also using the Abbott and Georgia *P.t.* inoculum, with similar objectives and followup outplantings is also being conducted in eight states (including Hawaii) and Canada during 1978.

SUMMARY AND CONCLUSIONS

1. The ectomycorrhizal fungus *Pisolithus tinctorius* can be successfully artificially inoculated into prefumigated nursery seedbeds and greenhouse containers on a wide variety of conifer (pines, firs, Douglas-fir) and some hardwood (oaks) species and in a wide geographic range within the United States. The Georgia *P.t.* inoculum has produced 20 percent or more P.t. ectomycorrhizae on the feeder roots of bare-root Fraser fir in western North Carolina, several species of pine throughout the south, and ponderosa pine (P. *ponderosa)* at Placerville, California, along with container-grown jack pine (P. *banksiana)* in Maine and Canada, several species of pine in Nebraska and longleaf pine (P. *palustris)* in Louisiana and Georgia.

2. Artificial inoculations with the Georgia *P.t.* mycelium-vermiculite-peat inoculum significantly increased seedling growth and quality (fresh weights) in nursery seedbeds along with significant increases in tree survival and growth in field plantings. These results suggest significant potential economic benefits to forest tree nurseries and field forestation. For example, the 32 percent reduction in cull seedlings obtained in the 17 nurseries during 1977 would result in the production of 85 million more plantable seedlings annually in the present 55 southern nurseries. Based on present seedling values ($12/M), this represents an annual seedling monetary benefit of $1,020,000. The 46 percent increase in plot volume realized on the Georgia *P.t.* treatment plots on the four outplanting sites in North

Carolina and Florida after 4 years in the field represents a considerably larger potential benefit to field forestation. This increase in plot volume represents a potental annual increase of 17.25 million cords (1.55 billion ft) in pine wood volume in 13 southern states from Virginia to Texas. Based on present round wood values, this represents an annual potential increase in southern pine wood volume values of $345 million

3. Although the results obtained with the Abbott *P.t.* inoculum were considerably less successful than those with the Georgia P.t. inoculum in 1977, the production of some *P.t.* mycorrhizae in practically all the nurseries inoculated with this commercially-produced inoculum is highly significant and gratifying. Preliminary mid-season examinations of the 1978 nursery studies show the Abbott P.t. inoculum to be nearly comparable to the Georgia *P.t.* inoculum in producing ectomycorrhizae on a variety of conifer species in several bare-root nurseries. Results obtained from the 1978 container seedling evaluation also show the Abbott P.t. inoculum as comparable to the Georgia *P.t.* inoculum in the production of P.t. mycorrhizae on a variety of seedling hosts from such diverse locations as Canada, Maine, Nebraska, and Louisiana. Over 15 percent P.t. ectomycorrhizae have been produced by the Abbott inoculum on shortleaf pine (P. *echinata)* in Louisiana, bur oak *(Quercus macrocarpa)* in North Dakota, Austrian (P. *nigra),* Ponderosa and Scotch (P. *sylvestris)* pine in Nebraska, and on jack pine in Canada and Maine.

4 Consequently, those of us who have been working on this project for the past several years are now optimistic concerning the practical application of the P.t. ectomycorrhizal fungus to nursery and container-grown conifer seedlings for the production of "tailored" seedling products. The "tailored" seedling product may have a variety of forestry-related uses such as adverse site forestation, windbreaks or shelter belts, seed production areas, and Christmas trees along with increased timber volumes. Abbott Laboratories shares this optimism with us and has allocated a considerable effort in technology development and financial expenditure for the commercial production of this mycorrhizal fungus product. Barring unforeseen problems, Abbott anticipates the commercial availability of the P.t. inoculum in 2-3 years.

5. Future followup evaluations are being planned by the Southeastern Area concerning the practical inoculation of bare-root nursery seedbeds utilizing a modification of conventional seeding or mulching equipment.

6. Meanwhile, the Mycorrhizal Institute, along with Abbott Laboratories, are continuing and expanding their cooperative efforts with various Federal, State, industry, and university agencies in widespread locations throughout the United States and several foreign countries concerning the research, development, and application of other suitable ecto- and endomycorrhizal fungi in both bare-root and

container nurseries. The Mycorrhizal Institute has recently expanded its research and development effort to include the endomycorrhizae of specific species of hardwoods.

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