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FINAL REPORT
1975-1989

RESEARCH ON:

A. RECLAMATION OF BORROW PITS AND DENUDED LANDS

B. BIOCHEMICAL ASPECTS OF MYCORRHIZAE OF FOREST TREES

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Project Summary

This final research report covers the period 1975 through 1989. Funding to the Institute for Mycorrhizal Research and Development (now the Institute of Tree Root Biology) for these 15 years totaled $459,347. This report furnishes a budget breakdown by year, a list of completed studies and ongoing studies, and a list of publications which resulted from the research accomplished by Institute scientists and their cooperators. Some 15 field studies are still ongoing and will not be completed for several years. The research accomplished can conveniently be placed into four categories; I. Research on borrow pit rehabilitation with 12 publications; II. Research on artificial regeneration of southern pines with 34 publications; III. Research on artificial regeneration of eastern hardwoods with 16 publications; and IV. Cooperative research with the University of Georgia on biochemical aspects of mycorrhizae with 5 publications. A total of 67 publications resulted from this research. Thirty-four were research publications and 33 were publications involved with technology transfer of the results to various potential user groups. DOE funding defrayed about 20 percent of the total cost of the research. The remainder was funded by the Southeastern Forest Experiment Station.

Three major accomplishments from this research are:

1. Procedures to successfully reclaim borrow pits with sludge, subsoiling and seedlings with specific mycorrhizae.

2. Protocols to successfully artificially regenerate southern pines, particularly longleaf pine, and certain eastern hardwoods.

3. Basic understanding of the biochemistry of mycorrhizae and the discovery of a new pathway for sucrose utilization in plants.

Completed Nursery and Field Studies[1]

Effect of high levels of phosphorus on development of sweetgum seedlings inoculated with *Glomus fasciculatus*. FS-SE-4551-51. P.P. Kormanik and W.C. Bryan. Planted February 1977.

Survival and growth of container grown loblolly pine seedlings with and without ectomycorrhizae formed by specific fungi. FS-SE-4551-48. J.L. Ruehle and D.H. Marx. Planted December 1977.

Response of longleaf, sand, and loblolly pines to nursery inoculation with *Pisolithus tinctorius* ectomycorrhizae and to soil amendments applied during outplanting on a deep sandy site in the Carolina sandhills. FS-SE-4551-56. G.E. Hatchell. Planted February 1979.

Effects of root dips of benomyl and captan on seedling response and mycorrhizal development of outplanted longleaf, sand, and loblolly pines. FS-SE-4551-62. G.E. Hatchell. Planted February 1980.

Growth and ectomycorrhizal development by *Pisolithus tinctorius* and *Thelephora terrestris* on different clones of *Pinus taeda* in fumigated nursery soil. FS-SE-4551-60. D.H. Marx. Planted February 1980.

The effect of ectomycorrhizae removal on survival and growth of loblolly pine seedlings with *Pisolithus* or natural ectomycorrhizae on an upland site in South Carolina. FS-SE-4551-65. D.H. Marx. Planted January 1981.

Field performance of loblolly pine seedlings with and without *Pisolithus* ectomycorrhizae on a reforestation site at the SRP, Aiken, SC. FS-SE-4551-83. D.H. Marx and C.E. Cordell. Planted December 1982.

Field performance of ectomycorrhizal container-grown pine seedlings that have been chemically root pruned. FS-SE-4551-87. J.L. Ruehle. Planted July 1983.

Field performance of loblolly pine seedlings with various amounts of *Pisolithus* and natural ectomycorrhizae resulting from nursery inoculation and application of ferbam and bayleton fungicides. FS-SE-4551-86. D.H. Marx and C.E. Cordell. Planted January 1984.

Field performance of bare-root longleaf pine seedlings with Pisolithus ectomycorrhizae and root pruning versus direct seeding with spore inoculation. FS-SE-4551-75. G.E. Hatchell and D. H. Marx. Planted February 1984.

Containerized longleaf pine with Pisolithus tinctorius ectomycorrhizae machine planted at the Savannah River Forest Station. J.L. Ruehle and D.H. Marx. Planted November 1984.

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[1] Forest Service policy dictates that all research studies have a written comprehensive study plan reviewed both inside and outside the Forest Service prior to initiation of research. This involves not only the evaluation of the merits of the science but also the validity of the experimental design and statistical evaluation. The study plan document with its supporting research data, maps, and other information is a permanent record of the specific research study until the study is closed.

Ongoing Field Studies[1]

Reforestation of borrow pits by use of specific mycorrhizal fungi, soil amendments, and site preparation. FS-SE-4551-31. D.H. Marx, C.R. Berry, J.L. Ruehle, P.P. Kormanik and W.C. Bryan. Planted December 1975. (Three studies involved.)

Comparison of sludge and fertilizer applications on establishment and growth of seedlings of two sweetgum ecotypes endomycorrhizal with Glomus mosseae and Glomus etunicatus. FS-SE-4551-54. P.P. Kormanik. Planted February 1978.

Interactions of subsoiling and solid sludge on soil physical and chemical factors and growth of Pinus taeda L. and a Festuca sp. FS-SE-4551-55. C.R. Berry and D.H. Marx. Planted December 1978.

Fiber yield and affect of Botryosphaeria spp. on recurrent coppicing of 11-year-old sweetgum. FS-SE-4551-107. P.P. Kormanik and J.L. Ruehle. Planted December 1978.

Interaction of VAM, sweetgum family, and root grade on plantation development. FS-SE-4551-70. P.P. Kormanik. Planted December 1978. (Three studies involved.)

The feasibility of growing hardwood species on a borrow pit amended with either fertilizer and lime or sewage sludge. FS-SE-4551-69. C.R. Berry. Planted March 1982.

Effects of subsoiling, sewage sludge, and VA mycorrhizae on growth of sweetgum on a high quality site. FS-SE-4551-88. C.R. Berry and P.P. Kormanik. Planted February 1984.

Interaction of endomycorrhizae and soil phosphorus from the nursery on field response of black walnut seedlings. FS-SE-4551-78. P.P. Kormanik. Planted December 1984.

Effects of morphological attributes of longleaf pine seedlings on field performance. FS-SE-4551-97. G.E. Hatchell, J.L. Ruehle and D.H. Marx. Planted December 1985. (Three studies involved.)

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PUBLICATIONS

I. BORROW PIT REHABILITATION

Research

Berry, Charles R. and Donald H. Marx. 1976. Sewage sludge and *Pisolithus tinctorius* ectomycorrhizae: Their effect on growth of pine seedlings. For. Sci. 22:351-358.

Four levels of dried sludge (0, 69, 138, and 275 metric tons/ha) and infestation of soil with the fungal symbiont *Pisolithus tinctorius* or control inoculum were combined factorially in heavy clay soil from a severely eroded site to determine their effects on growth of *Pinus taeda* and *P. echinata* seedlings. Addition of sludge to soil caused highly significant increases in growth of both pine species. Seedlings of both pine species with *Pisolithus* ectomycorrhizae were significantly taller than those with ectomycorrhizae formed by naturally occurring fungi. Loblolly pine seedlings with *Pisolithus* ectomycorrhizae also had significantly greater stem diameters and foliar and root fresh weights than control seedlings. Foliar zinc increased and foliar aluminum decreased with increased sludge levels. Loblolly seedlings with *Pisolithus* ectomycorrhizae also had significantly less foliar zinc than control seedlings. There were no correlations between sludge levels or *Pisolithus* ectomycorrhizae and amounts of 13 other elements in the foliage. The effects of *Pisolithus* ectomycorrhizae were greatest at the intermediate sludge level (138 tons/ha) on both loblolly and shortleaf pine seedlings. Based on these results, the interaction of dried sewage sludge with *Pisolithus* ectomycorrhizae appears to have valuable potential in the improvement of pine growth on eroded forest sites.

Berry, Charles R. and Donald H. Marx. 1980. Significance of various soil amendments to borrow pit reclamation with loblolly pine and fescue. Reclam. Rev. 3:87-94.

Loblolly pine seedlings with ectomycorrhizae formed by *Pisolithus tinctorius* (Pt) or naturally occurring *Thelephora terrestris* (Tt) were planted on a borrow pit in South Carolina in plots with no amendment; with fertilizer plus dolomitic limestone alone and with pine bark or bottom ash or bark and ash together; or with dried sewage sludge alone and with bark or ash or bark and ash together. All plots were subsoiled, disked and seeded to fescue grass before planting pine seedlings. Naturally occurring Pt formed abundant ectomycorrhizae on all Tt seedlings by the end of the first season, precluding any specific ectomycorrhizal fungus effect for the duration of the study.

Sewage sludge alone or with bark or ash amendments dramatically improved pine seedling growth and grass biomass in comparison with other soil treatments. Mean seedling volume (D^2H) was 28 times greater and grass biomass was five times greater in the sludge plots than on nonsludge plots. Generally, soil amended with sludge contained more N, P, organic matter, and had a higher cation exchange capacity than soil of other treatments. Foliage of pine seedlings in sludge-amended soil also contained more N and less Ca than other seedlings. The significance of these results to reclamation of borrow pits is discussed.

Berry, C.R. 1985. Subsoiling and sewage sludge aid loblolly pine establishment on adverse sites. Reclam. Reveg. Res. 3:301-311.

Eight intensities of subsoiling (in which depth, direction and spacing of furrows were varied) were compared to disking on borrow pit reclamation plots amended with 17 mt ha^{-1} sewage sludge or inorganic fertilizer (1121 kg ha^{-1}) and lime (2242 kg ha^{-1}). After 1 year, soil N and P levels in sludge plots were significantly higher than in fertilizer plots. K was low in all plots. Foliar N was significantly increased in trees on sludge plots, but foliar K was significantly higher in trees on fertilizer plots.

Loblolly pines grown on sludge plots grew an average of 37% more in height and 76% more in DBH than trees grown on fertilizer plots. On fertilzer plots, trees grew faster on plots subsoiled 46 cm deep than on plots subsoiled 92 cm deep from which, apparently, there was more leaching of nutrients. On sludge plots, trees grew faster when subsoiling was 92 cm deep than when it was 46 cm deep. Other variations in subsoiling, i.e., distance between furrows and whether in one direction or two directions, had little effect on growth of trees. On sludge plots, all subsoiling treatments produced better growth than disking. On fertilizer plots, only half of the eight subsoiling treatments produced better growth than disking. Production of herbaceous biomass was significantly greater on sludge plots or on subsoiled fertilizer plots than on fertilizer plots that had been disked.

Berry, C.R. 1985. Growth and heavy metal accumulation in pine seedlings grown with sewage sludge. J. Environ. Qual. 14:415-419.

Loblolly pine seedlings were grown in microplots amended with 34 or 68 Mg ha^{-1} of one of five sewage sludges; (i) old sludge from Athens, GA, (ii) fresh sludge from Athens, GA, and sludges of undetermined age, (iii) from Aiken, SC, (iv) from Newberry, FL, and (v) from Chicago, IL. Newberry sludge and fresh Athens sludge supported growth comparable to the inorganic fertilizer control. Seedlings grown with Chicago sludge grew well at the 68 Mg ha^{-1} rate (23.6 cm in height, 4.2 cm in root collar diameter and weighing 8.3 g) but were not as large as those grown with fertilizer (22.8 cm in height, 5.3 cm in root collar diameter and weighing 12.2 g). Seedlings grown with old Athens or Aiken sludge did not grow well and most of them were not of plantable size (i.e. 15 cm in height and 3.5 mm in root collar diameter). *Pisolithus tinctorius* did not form ectomycorrhizae on seedlings grown with the sludge as well as on seedlings grown with fertilizer (in most cases <23% of short roots compared to 63% for controls). *Thelephora terrestris* formed ectomycorrhizae as readily on seedlings grown with the sludge as those grown with fertilizer when inoculation with the fungus was artificial; natural ectomycorrhizae, however, did not form as readily with some sludges as with fertilizer. When grown with Chicago sludge, roots, stems, and needles of seedlings had significantly more cadmium (two- to fourfold) than seedlings grown with other sludges. Poor growth of seedlings grown in old Athens sludge is assumed to be due to deficiencies of macronutrients, since fresh Athens sludge supported good growth. With Aiken sludge and to a lesser degree, Chicago sludge, toxicity of heavy metals is considered to be a possible factor contributing to poor seedling growth.

McNab, W. Henry and Charles R. Berry. 1985. Distribution of aboveground biomass in three pine species planted on a devastated site amended with sewage sludge or inorganic fertilizer. For. Sci. 31:373-382.

Five-year-old loblolly, shortleaf, and Virginia pines growing on sites initially planted with 11,960 seedlings/ha on plots amended with 34,000 kg/ha of dried sewage sludge or with 896 kg/ha of 10-10-10 inorganic fertilizer and 1,417 kg/ha of CaO were felled, separated into stem and branches plus foliage, and sampled, to determine relative amounts of wood, bark, and foliage. On plots amended with sewage sludge, Virginia pine produced significantly more total tree biomass than the other species, and shortleaf produced significantly less. Regardless of fertility treatment, less than half of the total tree biomass for shortleaf and loblolly consisted of branches, but dry weight of branches for Virginia pine was twice that of the main stem. In terms of biomass composition, trees growing on plots amended with sewage averaged about 8 percent more wood as a percentage of total tree weight and proportionately less foliage than trees on plots amended with inorganic fertilizer. Regression equations based on independent variables of stem diameter at ground line and total height were developed to estimate total tree dry biomass. Estimated stand biomass was more than three times greater on the sludge plots than on fertilizer plots.

Ruehle, John L. 1980. Growth of containerized loblolly pine with specific ectomycorrhizae after 2 years on an amended borrow pit. Reclam. Rev. 3:95-101.

A borrow pit with exposed subsoil in South Carolina was graded level and deep subsoiled. Plots were amended with processed sewage sludge or commercial fertilizer followed by a seeding with fescue. Container-grown loblolly pines colonized with *Pisolithus tinctorius*, *Thelephora terrestris*, or no ectomycorrhizae were planted by hand on the plots one year after site preparation.

Two years after planting on sludge-amended plots, seedlings initially colonized with *Pisolithus* had greater height, root-collar diameter, and seedling volume (D^2H) than *Thelephora* or control seedlings. The means for these three growth parameters on seedlings planted on fertilized plots were no different between *Pisolithus* and *Thelephora* seedlings, but *Pisolithus* seedlings were greater than controls. There was no difference in survival among mycorrhizal treatments on the sludge plots. Survival and seedling volume were integrated into plot volume indices (PVI). Seedlings in sludge plots had greater PVI than seedlings on fertilized plots. *Pisolithus* seedlings on sludge plots had 265 and 528% greater PVI after 2 years than *Thelephora* or control seedlings.

Containerized loblolly seedlings tailored with *Pisolithus* can be successfully established and rapid growth obtained on a subsoiled borrow pit amended with sewage sludge. This procedure may be applicable to thousands of acres of similar borrow pits left by highway and construction work.

Kormanik, Paul P. and Richard C. Schultz. 1985. Significance of sewage sludge amendments to borrow pit reclamation with sweetgum and fescue. USDA For. Serv. Res. Note SE-329, 7 pp.

Sweetgum (*Liquidambar styraciflua* L.) and fescue (*Festuca arundinocea* Schreb.) were planted after subsoiling and sludge applications to depths of 0, 0.64, 1.27, and 2.54 cm to reclaim a borrow pit in the Lower Piedmont of South

Carolina. Sludge treatments in combination with subsoiling significantly improved establishment of fescue and growth of sweetgum, but all combinations of fescue with sludge significantly reduced first-year survival of sweetgum. Because survival was stabilized after the first growing season, allelopathy of fescue to sweetgum had little effect in this study. Fifth-year total height growth (0.74 m) of sweetgum on the nonamended plots was rated not acceptable, but on plots amended at even the lowest rate of sludge, height growth (2.75 m) was acceptable. Total heights (ca 3.6 m) after 5 years on plots amended at the two highest sludge rates were equal to or greater than 5-year heights reported for sweetgum on reforestation sites. The increase in total nitrogen available on the site after 5 years exceeded the amount initially present in the sludge, which suggests either a significant atmospheric contribution or that organic matter enhanced nitrogen fixation.

Technology Transfer

Berry, Charles R. 1981. Sewage sludge aids reclamation of disturbed forest land in the southeast. pp 307-316 in Proceedings Symposium of Utilization and Municipal Wastewater and Sludge for Land Reclamation and Biomass Production. Pittsburgh, PA.

Berry, Charles R. 1986. Reclamation of severely devastated sites with dried sewage sludge in the Southeast. pp 497-507 in The Forest Alternative for Treatment and Utilization of Municipal and Industrial Wastes. D.W. Cole, C.L. Henry and W.L. Nutter (eds.), University of Washington, Seattle.

Berry, Charles R. 1987. Subsoiling improves growth of trees on a variety of sites. pp 360-367 in Proceedings 4th Biennial Southern Silvicultural Research Conference, Atlanta, GA. USDA For. Serv. Gen. Tech. Report SE-42.

Berry, Charles R. 1987. Use of municipal sewage sludge for improvement of forest sites in the southeast. USDA For. Serv. Res. Paper SE-266, 33 pp.

Ruehle, John L. 1982. Mycorrhizal inoculation improves performance of container-grown pines planted on adverse sites. pp 133-135 in Proceedings Southern Containerized Forest Tree Seedling Conference, Savannah, GA. USDA For. Serv. Gen. Tech. Report SO-37.

II. ARTIFICIAL REGENERATION OF SOUTHERN PINES

Research

Hatchell, G.E., C.R. Berry and H.D. Muse. 1985. Nondestructive indices related to aboveground biomass of young loblolly and sand pines on ectomycorrhizal and fertilizer plots. For. Sci. 31:419-427.

Evidence is presented to support the use of root-collar diameter squared x total height (D^2H) as a surrogate measure for aboveground biomass of young pine seedlings. Statistical analyses were based on data from ectomycorrhizal and fertilizer plots of four outplanting sites of loblolly pine (Pinus taeda L.) and one outplanting site of Choctawhatchee sand pine (P. clausa var. immuginata D.B. Ward) in the southeastern United States. D^2H performed well compared with other surrogate measures of interest: H, D, D^2, and DH. Also, analyses of D^2H, log (D^2H), and the associated weight and log (weight) data showed that claims of significance based on these surrogate measures agree closely with claims based on the corresponding weight analyses. The concept of plot volume index (PVI), which is defined as the total of D^2 values for the surviving trees in the plot, is discussed. Appropriate statistical analysis procedures are presented for PVI as a plot biomass surrogate measure based on total D^2H.

Hatchell, Glyndon E. and Donald H. Marx. 1987. Response of longleaf, sand, and loblolly pines to *Pisolithus* ectomycorrhizae and fertilizer on a sandhills site in South Carolina. For. Sci. 33:301-315.

Bareroot seedlings were established on a deep sandy soil to test the responses of three pine species with ectomycorrhizae formed by *Pisolithus tinctorius* (Pt) or naturally occurring fungi in the nursery and with one of three fertilizer treatments applied during planting. Longleaf pine (*Pinus palustris* Mill.) had better survival and growth with Pt through seven growing seasons, but the effect of fertilizer was not significant for either ectomycorrhizal condition. *Pisolithus tinctorius* significantly increased the percentage of longleaf seedlings in active height growth after 3 years. *Pisolithus tinctorius* significantly improved growth of Choctawhatchee sand pine (*P. clausa* var. *immuginata* D.B.Ward) through four growing seasons, but survival of this species exhibited a significant interaction of ectomycorrhizal and fertilizer treatments. *Pisolithus tinctorius* significantly improved survival and growth of loblolly pine (*P. taeda* L.) only in the first growing season, and this species responded significantly to fertilizer in terms of increased survival and early growth. Ranking of 3- to 17-year growth of the three species was sand pine>loblolly pine>longleaf pine. Longleaf and Choctawhatchee sand pines, which are well adapted to sandy sites and are resistant to fusiform rust infection, are good choices for planting on deep sandy soils in the South Carolina sandhills.

Hatchell, Glyndon E. and H. David Muse. 1990. Nursery cultural practices and morphological attributes of longleaf pine bare-root stock as indicators of early field performance. USDA For. Serv. Res. Paper SE-277, 34 pp.

Seven morphological characteristics of 1-0 longleaf pine (*Pinus palustris* Mill.) stock were evaluated on a total of 1,600 seedlilngs representing eight combinations of nursery cultural treatments. Seedlings were outplanted completely at random, with respect to rows and planting positions, on a deep sandy site in South Carolina. Field performance of individual seedlings was evaluated after two growing seasons that were extremely dry. Statistical tests were derived for determining differences in field performance of groups of seedlings, both within and between treatments. Groups of seedlings that received vertical root-pruning treatment (sidecutting) in the nursery and possessed key attributes -- either 14 or more strong, first-order lateral roots for a low-fibrosity root system or a minimum of 6 strong laterals for medium- or high-fibrosity root system -- met the preset goal of 80 percent survival and 60 percent of planting stock in active height growth after two growing seasons. Within a pruning treatment, all seedlings possessing these key morphological attributes, regardless of root-collar diameters, performed equally well in the field. For each of the three fibrous-root ratings, seedlings that received vertical root pruning in nursery beds had significantly higher survival than unpruned seedlings. Vertical root pruning also significantly increased the proportion of planting stock in active height growth after the second growing season.

Kais, Albert G., Glenn A. Snow and Donald H. Marx. 1981. The effects of benomyl and Pisolithus tinctorius ectomycorrhizae on survival and growth of longleaf pine seedlings. South. J. Appl. For. 5:189-194.

Benomyl applied to roots of longleaf pine (Pinus plaustris Mill.) seedlings at planting significantly reduced brown-spot disease and increased survival, root collar diameter, and early height growth on two sites in Mississippi. Seedlings with half or more of all ectomycorrhizae formed by Pisolithus tinctorius (Pers.) Coker and Couch in the nursery had significantly better survival and growth; Pisolithus ectomycorrhizae did not appreciably affect brown-spot disease. The benefits of benomyl and Pisolithus ectomycorrhizae were most obvious when combined. More than 75 percent of seedlings treated with benomyl and with more than half of all ectomycorrhizae formed by Pisolithus initiated height growth after 3 years. Forty-seven percent of seedlings with only Thelephora terrestris ectomycorrhizae and without benomyl exhibited height growth. The combined use of benomyl to control brown-spot disease and Pisolithus ectomycorrhizae to stimulate early height growth may overcome the major handicaps that have limited artificial regeneration of longleaf pine in the South.

Marx, Donald H. and Glyndon E. Hatchell. 1986. Root stripping of ectomycorrhizae decreases field performance of loblolly and longleaf pine seedlings. South. J. Appl. For. 10:173-179.

Survival and growth of loblolly and longleaf pine seedlings were significantly reduced after 2 years in the field by physical removal (stripping) of specific ectomycorrhizae before planting. The tests were done in South Carolina during the drought years of 1981 and 1982. Loblolly pine seedlings were all the same size at planting. Two sizes of longleaf pine seedlings were produced by varying nursery bed density. After 2 years, mean survival for loblolly pine was 71, 60, 56, and 39% for seedlings with Pisolithus tinctorius (Pt) ectomycorrhizae that had 0, 33, 66, and 100%, respectively, of the ectomycorrhizae stripped. Pt seedlings with 0 or 33% stripping of ectomycorrhizae were significantly larger than seedlings with more Pt ectomycorrhizae removed. Mean survival of loblolly pine with ectomycorrhizae from natural inoculation (NI) was 61, 52, 39, and 38% with 0, 33, 66, and 100%, respectively, of the ectomycorrhizae stripped. Growth of NI seedlings was significantly less than that of Pt seedlings; NI seedlings also produced fewer new lateral roots than Pt seedlings in the first growing season. Survival and early growth (stem caliper increment) of longleaf pine seedlings were affected more by stripping ectomycorrhizae than by different initial root-collar diameters caused by different bed densities (7.5 and 15 seedlings per ft^2). Survival of seedlings from the low bed density averaged (Pt and NI) 59, 48, and 37%, and that of seedlings from the higher bed density averaged 63, 40, and 27% after stripping 0, 50, and 100% of the ectomycorrhizae, respectively. Seedlings from the higher bed density with Pt ectomycorrhizae survived better (48%) than those with NI ectomycorrhizae (38%). Thus, the quality and quantity of the ectomycorrhizal condition of loblolly and longleaf pine seedlings from the nursery are highly significant in determining outplanting success, especially during years of low rainfall.

Marx, Donald H. 1987. Triadimefon and *Pisolithus* ectomycorrhizae affect second-year field performance of loblolly pine. USDA For. Serv. Res. Note SE-349, 6 pp.

Survival and growth of loblolly pine 2 years after planting on a productive site (site index 80) in South Carolina were not affected by suppression of naturally occurring ectomycorrhizae caused by triadimefon spraying in the nursery to control fusiform rust. However, seedlings with abundant *Pisolithus tinctorius* ectomycorrhizae (Pt index 84) sprayed with fermate in the nursery were significantly larger after 2 years than seedlings with lesser amounts of *Pisolithus* ectomycorrhizae sprayed with triadimefon and seedlings with naturally occurring ectomycorrhizae sprayed with either fungicide. Seedlings of the fermate-*Pisolithus* ectomycorrhizae treatment had significantly more starch in roots than other seedlings at planting. Adequate monthly rainfall prevented severe soil moisture stress. Nearly 23 percent of seedlings sprayed with triadimefon in the nursery developed fusiform rust galls of field origin, while only 16 percent of seedlings sprayed in the nursery with fermate developed such galls.

Marx, Donald H. and Charles E. Cordell. 1988. Bed density and *Pisolithus* ectomycorrhizae affect morphology of loblolly pine seedlings. pp 70-79 in Proceedings Southern Forest Nursery Association, Charleston, SC.

Loblolly pine seedlings were grown at 25, 30, 35 and 40 seedlings/ft^2 with either *Pisolithus tinctorius* (Pt) or naturally occurring (NI) ectomycorrhizae at the Taylor State Nursery, Trenton, SC, in 1987. Uniform spacing of seedlings was maintained in each bed density. Average root collar diameter (RCD) and weight decreased as bed density increased, but the averages were within acceptable size limits at all bed densities. Fifty-four percent of the NI seedlings grown at 40/ft^2 had <3.6 mm (9/64 in.) RCD while only 23% were this small at 25 seedlings/ft^2. Pt ectomycorrhizae decreased the percentage of seedlings with <3.6 mm RCD at all densities. Less than half of the NI seedlings had >30 percent ectomycorrhizal development and more than half of the Pt seedlings had >45 percent development at all bed densities.

Using an average RCD of >3.5 mm for the desired seedling population, about 38 seedlings/ft^2 with natural ectomycorrhizae and 42 seedlings/ft^2 with Pt ectomycorrhizae were produced. However, when culls (<3.0 mm RCD) were removed, 30 and 32 plantable seedlings/ft^2, respectively, were produced. At 30 seedlings/ft^2, average RCD was 3.7 and 3.9 mm for NI and Pt seedlings with cull percentages of 10 and 7.5, respectively. Larger seedlings with RCD >4.0 mm of both mycorrhizal treatments were produced at the 25 seedlings/ft^2 density with an average of <3 percent culls.

Marx, Donald H. and Charles E. Cordell. 1990. Development of *Pisolithus tinctorius* ectomycorrhizae on loblolly pine seedlings from spores sprayed at different times and rates. USDA For. Serv. Res. Note SE-356, 7 pp.

Ectomycorrhizae of *Pisolithus tinctorius* developed on loblolly pine (*Pinus taeda* L.) seedlings in a bare-root nursery after basidiospores were sprayed at different rates and different times. *Pisolithus tinctorius* ectomycorrhizae dominated root systems throughout the growing season from vegetative inoculum yielding a final Pt index of 75. Spores applied at 0.6 g/m^2, 1.2 g/m^2, or 1.8 g/m^2 of soil surface at sowing produced similar amounts of ectomycorrhizae through the growing season and at lifting each produced the desired Pt index >50. Except for the highest spore rate applied 1 month after sowing, spores applied at any rate 1 or 2 months after sowing did not dominate the root systems or produce final Pt indices >50. Procedures needed to effectively apply spores by spraying are discussed.

Marx, Donald H. and Charles E. Cordell. 1990. Inoculation of fall- and spring-sown longleaf pine seedlings with Pisolithus tinctorius. USDA For. Serv. Res. Note SE-358, 5 pp.

Vegetative inoculum of Pisolithus tinctorius (Pt) placed in the spring in trenches between rows of longleaf pine seedlings sown the previous fall formed as many Pt ectomycorrhizae by lifting as did vegetative inoculum applied by machine just prior to spring sowing. Fall-sown seedlings had consistently larger root-collar diameters than the spring-sown seedlings. Spores applied by spraying in the fall or spring on seedlings sown in the fall or spring formed inadequate amounts of Pt ectomycorrhizae. A prototype machine for applying Pt vegetative inoculum between rows provided favorable results in an operational-level inoculation on a forest industry nursery in Alabama.

Ruehle, John L. 1982. Field performance of container-grown loblolly pine seedlings with specific ectomycorrhizae on a reforestation site in South Carolina. South. J. Appl. For. 6:30-33.

Container-grown loblolly pine seedlings (Pinus taeda L.) with Pisolithus tinctorius, Thelephora terrestris, or no ectomycorrhizae (control) were outplanted on a South Carolina reforestation site. Two years after planting, seedlings initially colonized with Pisolithus or Thelephora had greater survival, height, and root collar diameter than control seedlings. Growth data were integrated into seedling volume indices (D^2H). Seedlings with ectomycorrhizae at planting had nearly a three-fold greater D^2H than seedlings initially without ectomycorrhizae. These results provided additional field validation for the hypothesis regarding benefits of ectomycorrhizae on this type of planting stock.

Ruehle, John L. 1983. The relationship between lateral-root development and spread of Pisolithus tinctorius ectomycorrhizae after planting of container-grown loblolly pine seedlings. For. Sci. 29:519-526.

Container-grown loblolly pine (Pinus taeda L.) seedlings with Pisolithus ectomycorrhizae were planted in microplots containing fumigated or nonfumigated forest soil. At 4-week intervals during a 20-week period after planting, seedlings were excavated to determine the pattern of lateral root egress and spread of Pisolithus ectomycorrhizae on laterals. Between 75 and 80 percent of the laterals egressed from the bottom of the plugs in both fumigated and nonfumigated soil. Hyphal strands of Pisolithus spread from the plugs and developed ectomycorrhizae at greater distances from the plugs on lateral roots of seedlings in fumigated soil than on seedlings in nonfumigated soil The results indicated that root configuration of container-grown loblolly seedlings initially influences lateral root egress (first 20 weeks) after planting. Soil microbial associates, i.e., native symbionts and pathogenic fungi, of the more vertically egressed roots in nonfumigated soil apparently do not favor development of Pisolithus ectomycorrhizae.

Ruehle, J.L., D.H. Marx and H.D. Muse. 1984. Calculated nondestructive indices of growth response for young pine seedlings. For. Sci. 30:469-474.

The relationship between aboveground weight (W) and root-collar diameter squared times total height (D^2H) was modeled using data on 2- and 3-year-old pine seedlings growing in mycorrhizal plots at several locations in the southeastern United States. The linear and log-log models fit the individual data sets very well, as shown by correlation coefficients ranging from 0.87 to 0.98 for the linear model and from 0.90 to 0.98 for the log-log model. Included is a discussion of the criteria needed to establish D^2H, or a function of D^2H, as a suitable surrogate measure of biomass. The evidence presented shows that D^2H and log (D^2H) are interval scaled measures of W and log (W), respectively, thus validating these nondestructive indices for use in comparing treatment effects on mycorrhizal research plots.

Ruehle, John L. 1985. Lateral-root development and spread of Pisolithus tinctorius ectomycorrhizae on bare-root and container-grown loblolly pine seedlings after planting. For. Sci. 31:220-225.

Bare-root and container-grown loblolly pine (Pinus taeda) seedlings with Pisolithus tinctorius (Pt) ectomycorrhizae were planted in microplots containing forest soil. During a 22-week period after planting, seedlings were excavated at 4-week intervals to determine the pattern of lateral root egress and rate of development of Pt ectomycorrhizae on egressed laterals. The pattern for lateral root growth from original root systems differed between bare-root and container-grown seedlings. Bare-root seedlings produced 60 percent more laterals and had significantly more horizontal lateral root egress from the B (middle) horizontal zone of the original root system than container-grown seedlings. Bare-root seedlings also had significantly more Pt ectomycorrhizae on egressed laterals and these mycorrhizae were found at significantly greater distances from the original root system than those on container-grown seedlings after 22 weeks. The implications of these results on field performance of seedlings are discussed.

Ruehle, John L. 1985. The effect of cupric carbonate on root morphology of containerized mycorrhizal pine seedlings. Can J. For. Res. 15:586-592.

Loblolly (Pinus taeda L.), longleaf (Pinus palustris Mill.), shortleaf (Pinus echinata Mill.), and eastern white (Pinus strobus L.) pine seedlings inoculated with Pisolithus tinctorius (Pers.) Coker & Couch (Pt) were raised in a greenhouse in containers painted with latex paint containing 50 g/L cupric carbonate ($CuCO_3$). To determine the effects of $CuCO_3$ on root development and morphology, seedlings were sampled in the middle and at the end of the test. The treatment was effective in preventing most first-order laterals of all species from growing down the container wall, yet it had no significant effect on seedling height, root-collar diameter, or top and root fresh weight. Cupric carbonate had little effect on ectomycorrhizal formation on loblolly and shortleaf seedlings, but significantly increased it on longleaf seedlings and decreased it on eastern white seedlings. Using $CuCO_3$ to alter root morphology of containerized southern pine seedlings inoculated with Pt has the potential for developing stock that will grow long laterals near the soil surface for anchorage and increase the rate of spread of Pt ectomycorrhizae from the plug to new roots after planting.

Technology Transfer

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Hatchell, Glyndon E. 1985. Nursery cultural practices affect field performance of longleaf pine. pp 148-156 in Proceedings International Symposium on Nursery Management Practices for the Southern Pines, D.B. South (ed.), Montgomery, AL.

Hatchell, Glyndon E. 1987. Nursery cultural practices, seedling morphology, and field performance of longleaf pine. pp 61-66 in Proceedings 4th Biennial Southern Silviculture Research Conference, Atlanta, GA. USDA For. Serv. Gen. Tech. Report SE-42.

Cordell, C.E., G.E. Hatchell and D.H. Marx. 1990. Nursery culture of bare-root longleaf pine seedlings. pp 38-51 in Proceedings Symposium on the Management of Longleaf Pine, R.M. Farrar, Jr. (ed.), Long Beach, MS. USDA For. Serv. Gen. Tech. Report SO-75.

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Cordell, Charles E. and Donald H. Marx. 1981. Ectomycorrhizae: Present status and practical application in forest tree nurseries and field plantings. pp 34-39 in Proceedings Western and Intermountain Nurserymen's Conference, Boise, ID. USDA For. Serv. Gen. Tech. Report INT-109.

Cordell, Charles E., Donald H. Marx, James R. Lott, and Donald S. Kenney. 1981. The practical application of Pisolithus tinctorius ectomycorrhizal inoculum in forest tree nurseries. pp 38-42 in Forest Regeneration, Proceedings Symposium on Engineering Systems for Forest Regeneration, American Soc. Agric. Engineers, St. Joseph, MI.

Marx, D.H. 1982. Mycorrhizae in interactions with other microorganisms. B. Ectomycorrhizae. pp 225-228 in Methods and Principals of Mycorrhizal Research, N.C. Schenck (ed.), American Phytopathol. Soc., St. Paul, MN.

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Marx, D.H. and N.C. Schenck. 1983. Potential of mycorrhizal symbiosis in agricultural and forest productivity. pp 334-347 in Challenging Problems in Plant Health, T. Kommedahl and P.H. Williams (eds.), American Phytopathol. Soc., St. Paul, MN.

Cordell, Charles E. and Donald H. Marx. 1982. The operational application of Pisolithus tinctorius ectomycorrhizae in forest tree nurseries for custom

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Cordell, Charles E. and Donald H. Marx. 1985. Benefits and application of ectomycorrhizae in southern forest tree nurseries. pp 244-250 in Proceedings International Symposium on Nursery Management Practices for the Southern Pine, D.B. South (ed.), Montgomery, AL.

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Cordell, C.E., D.W. Omdal and D.H. Marx. 1989. Operational ectomycorrhizal fungus inoculations in forest tree nurseries: 1989. pp 86-92 in Proceedings Intermountain Forest Nursery Association, Bismarck, ND. USDA For. Serv. Gen. Tech. Report RM-184.

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Ruehle, John L. 1987. Field performance of ectomycorrhizal container-grown pine seedlings that were chemically root pruned. p 103 in Mycorrhizae in the Next Decade, Practical Applications and Research Priorities, D.M. Sylvia, L.L. Hung, and J.H. Graham (eds.), 7th North American Conference on Mycorrhizae, Gainesville, FL.

III. ARTIFICIAL REGENERATION OF EASTERN HARDWOODS

Research

Kormanik, Paul P., William C. Bryan and Richard C. Schultz. 1977. Influence of endomycorrhizae on growth of sweetgum seedlings from eight mother trees. For. Sci. 23:500-506.

Sweetgum seedlings from eight mother trees were grown in fumigated soil with or without the endomycorrhizal fungus Glomus mosseae at four levels of soil fertility for one growing season in nursery microplots. Nonmycorrhizal seedlings of all families died or failed to exceed 5 cm in height regardless of soil fertility. Endomycorrhizal seedlings suffered little mortality, averaged about 36 cm in height, and fertility level did not significantly influence their biomass. These results demonstrate that to increase the percentage of plantable seedlings in nurseries sweetgum seedlings must be endomycorrhizal. The data further suggests that adequate endomycorrhizal inoculum in nursery beds can allow the use of less fertilizer than has been customary for production of sweetgum seedlings.

Schultz, R.C., P.P. Kormanik, W.C. Bryan and G.H. Brister. 1979. Vesicular-arbuscular mycorrhiza influence growth but not mineral concentrations in seedlings of eight sweetgum families. Can. J. For. Res. 9:218-223.

Seedlings of eight half-sib sweetgum (Liquidambar styraciflua L.) families were grown for 6 months in a fumigated soil mixture, with or without inoculum from a mixture of Glomus mosseae and Glomus etunicatus fungi at levels of 140, 280, 560, and 1120 kg/ha of 10-10-10 fertilizer. All seedlings received three additions of 187 kg/ha of N during the growing season. Inoculated seedlings had significantly greater biomass, height, and stem diameters at each fertilizer level than nonmycorrhizal control seedlings. Significant differences in growth occurred between families in mycorrhizal plants. However, fertlilizer did not significantly affect growth or nutrient uptake of the seedlings. Inoculation with VA mycorrhizal fungi did not increase N, P, K, or Mg concentrations in the leaves, stems, or roots. Leaves of VA mycorrhizal seedlings had higher concentrations of calcium but stems and roots had lower concentrations of this element than the nonmycorrhizal seedlings. Seedlings with endomycorrhizae contained higher absolute quantities of each nutrient simply because of their greater biomass. The results suggest that the role of VA mycorrhizal fungi in the initial growth of sweetgum seedlings may be the result of physiological stimuli other than increased nutrient uptake.

Kormanik, Paul P., W. Craig Bryan and Richard C. Schultz. 1980. Increasing endomycorrhizal fungus inoculum in forest nursery soil with cover crops. South. J. Appl. For. 4:151-153.

Corn, millet, sudex, and sorghum were all effective cover crops for increasing inoculum density of vesicular-arbuscular fungi (Glomus spp.) in nursery soils. Spore production was increased approximately 7 to 12 times, depending on the cover crop used. Sweetgum seedlings did not differ significantly in size on plots previously planted with any of the four cover crops. Eighty-nine percent of the sweetgum seedlings grown after cover cropping had root-collar diameters exceeding the minimum (0.25 inch) recommended for outplanting of this species.

Kormanik, Paul P., William C. Bryan and Richard C. Schultz. 1981. Effects of three vesicular-arbuscular mycorrhizal fungi on sweetgum seedlings from nine mother trees. For. Sci. 27:327-335.

Soil in microplots was infested with three vesicular-arbuscular (VA) symbionts then sown with seed of half-sib progeny from nine sweetgum mother trees. The VA treatments were Glomus fasciculatus, Glomus spp. (a mixture containing both Glomus mosseae and Glomus etunicatus), or a VA mixture of several fungi from the genera Glomus and Gigaspora. Before the seed was sown, all plots had calcium standardized and received an application of commercial fertilizer. During the growing season, NH_4NO_3 was applied to all plots in equal portions. Mycorrhizal seedlings with G. fasciculatus were slightly but not significantly larger in both height and root-collar diameter than were seedlings with the Glomus spp. or the VA mixture. Seedlings from all VA treatments were approximately 32 cm tall with root-collar diameters of approximately 0.70 cm. Nonmycorrhizal seedlings averaged 4.5 cm in height and 0.19 cm in root-collar diameter. Progeny from four of the mother trees were consistently larger than those from the other five, but progeny from three of these five were consistently smaller regardless of the VA mycorrhizal treatment. From a given mother tree, no significant difference in progeny ranking was observed among treatments. Significant differences in percentage of roots infected and in intensity of infection within root segments were found among the three treatments, but these differences were not correlated with seedling growth. There were some differences in mineral analyses of tissue from seedlings among the different mycorrhizal treatments and the control but no consistent trends were observed in any of the parameters in the tests.

Schultz, Richard C., Paul P. Kormanik and William C. Bryan. 1981. Effects of fertilization and vesicular-arbuscular mycorrhizal inoculation on growth of hardwood seedlings. Soil Sci. Soc. Am. J. 45:961-965.

Eight hardwood species were grown in fumigated soil without vesicular-arbuscular mycorrhizae (VAM) or in soil infested with a mixture of Glomus mosseae and Glomus etunicatus. Three fertilizer treatments of 140, 560, and 1,120 kg/ha of 10-10-10 fertilizer were established in combination with two mycorrhizal treatments. Ten equal applications of NH_4NO_3, totaling 1,680 kg/ha, were added to all the treatment plots during the growing season. For six of the eight species, the VAM seedlings showed greater height and diameter growth and dry weight production than nonmycorrhizal seedlings. Sugar maple (Acer saccharum Marsh.) and walnut (Juglans nigra L.) displayed no height growth differences. Only boxelder (Acer negundo L.), of the inoculated seedlings, consistently responded to increases in fertilizer level. Nonmycorrhizal seedlings generally showed increased growth with increased fertilizer applications. The growth of the nonmycorrhizal seedlings at the higher fertilizer levels was not sufficient to produce plantable seedlings for artificial regeneration. A difference in host preference for the Glomus spp. symbionts is suggested by the large difference in infection between species. Infection values varied from a high of about 80% for sycamore (Platanus occidentalis L.), green ash (Fraxinus pennsylvanica Marsh.), and boxelder to a low of 40% for sugar maple and sweetgum. The growth data suggest that high quality seedling stock of most of these hardwood tree species can be obtained in nurseries as long as cultural practices in the nursery encourage VAM development.

Kormanik, Paul P. 1982. The influence of vesicular-arbuscular mycorrhizae on the growth and development of eight hardwood tree species. For. Sci. 28:531-539.

Eight hardwood forest species were grown in fumigated soil without vesicular-arbuscular mycorrhizal (VAM) fungi or in soil infested with either *Glomus fasciculatus* (GF), a mixture of *Glomus mosseae* and *G. etunicatus* (GM), or a mixture of several fungal species in the genera *Glomus* and *Gigaspora* (GG). With the exception of sugar maple, VAM development increased stem weight of seedlings by 2- to 80-fold over nonmycorrhizal controls. Root weight of all seedlings was increased by 4- to 70-fold by VAM. Generally, GF stimulated more seedling growth than other fungi. Laboratory assays of the root samples indicated that feeder root infection by the fungi varied from 55 to 85 percent, but generally there were no significant differences among the VAM treatments within tree species. Differences among hosts were observed in the amount of hyphae, arbuscules, and vesicles produced by the fungi, which could be attributed to growth and development characteristics among hosts and VAM fungi. The data suggest that high-quality seedling stock of these hardwood tree species can be obtained in nurseries where cultural practices in the nursery encourage VAM development.

Schultz, Richard C. and Paul P. Kormanik. 1982. Vesicular-arbuscular mycorrhiza and soil fertility influence mineral concentrations in seedlings of eight hardwood species. Can. J. For. Res. 12:829-834.

Eight hardwood species were grown under two sets of fertilizer and vesicular-arbuscular mycorrhizae (VAM) treatments. In the first study three treatments of 140, 560, and 1120 kg/ha of 10-10-10 (% N, P_2O_5, and K_2O, respectively) fertilizer were added to fumigated soil with or without a mixture of *Glomus mosseae* Nicol. and Gerd. and *Glomus etunicatus* Becker and Gerd. (GM). In the second study, seedlings were grown with VAM treatments of (i) the same *Glomus* (GM) mixture as in study 1, (ii) *Glomus fasciculatus* (Thaxter) Gerd. and Trappe (GF), or (iii) mixed cultures of several *Glomus* and *Gigaspora* species (GG). A fertilizer treatment of 280 kg/ha of 10-10-10 was added to all seedlings. All treatments, in both studies, also received 10 equal applications of NH_4NO_3, totaling 1680 kg/ha, during the growing season. No single nutrient was consistently higher in nonmycorrhizal or VAM seedlings in either study and no symbiont produced consistently high concentrations of all nutrients in all species. Uninoculated seedlings frequently had higher N, K, Ca, and Mg concentrations than VAM seedlings. Inoculated seedlings generally had higher total P concentrations than uninoculated seedlings. For uninoculated seedlings of five of the species, P concentrations increased with higher fertility levels. Seedlings inoculated with GM and GG had higher P concentrations than those inoculated with GF. In numerous instances, uninoculated seedlings had higher mineral concentrations than VAM seedlings even though the uninoculated seedlings were always the smallest. This suggests that VAM provide stimulation other than or in addition to the enhanced nutrient uptake.

Kormanik, Paul P. 1983. Third-year seed production in outplanted sweetgum related to nursery root colonization by endomycorrhizal fungi. pp 49-54 in Proceedings 17th Southern Forest Tree Improvement Conference, Athens, GA.

A sweetgum plantation was established from nursery seedlings grown in soil infested with vesicular-arbuscular mycorrhizal (VAM) fungi and amended with phosphorus at 25 ppm (Bray II); control seedlings were grown without VAM fungi and with soil P adjusted to 800 ppm. At the end of the third year, seed production was observed on 78 young trees, 75 from the VAM treatment and 3 from the control. In another plantation established from seedlings grown in VAM fungus-infested nursery soil and outplanted on a site that received different fertilization regimens with sewage sludge, heavy seed production was observed in both the fourth and fifth years. Observations in both plantations indicate that early seed production can be attributed to both VAM fungal root colonization and high levels of available soil P on the planting site.

Kormanik, Paul P. 1985. Effects of phosphorus and vesicular-arbuscular mycorrhizae on growth and leaf retention of black walnut seedlings. Can. J. For. Res. 15:688-693.

Black walnut seedlings were grown in fumigated soil without vesicular-arbuscular (VA) mycorrhizal fungi or in soil infested with *Gigaspora margarita*, *Glomus fasciculatus*, or *Glomus macrocarpum*. For each mycorrhizal treatment, three levels of available phosphorus (P), 25, 50, and 75 ppm, were provided. With 25 and 50 ppm P, the presence of VA mycorrhizae significantly improved leaf retention and root weight of all seedlings. At 75 ppm P, seedling development was not affected by mycorrhizal treatment. Within a given mycorrhizal condition, there were only minor differences in growth parameters across P levels. In the nonmycorrhizal treatments, all growth parameters significantly improved at the 75 ppm P treatment, while little difference could be detected between 25 and 50 ppm P. The number of lateral roots with a diameter of 1.0 mm or larger and root weight of seedlings were not affected by soil P level within a mycorrhizal treatment, but each increment of soil P increased root weight but not the number of lateral roots of seedlings in the nonmycorrhizal treatment. Seedlings that were mycorrhizal with *G. margarita* had more dense root colonization and were characteristically larger than seedlings that were mycorrhizal with either *G. fasciculatus* or *G. macrocarpum*.

Kormanik, Paul P. 1985. Development of vesicular-arbuscular mycorrhizae in a young sweetgum plantation. Can. J. For. Res. 15:1061-1064.

Sweetgum seedlings with vesicular-arbuscular mycorrhizae formed by *Glomus etunicatum* or *Glomus deserticola* in nursery soil with 30 ppm available phosphorus (P) and nonmycorrhizal seedlings grown in nursery soil with 800 ppm available P were outplanted and whole trees were excavated periodically over the next 5 years in the plantation to follow mycorrhizal development. Four months after outplanting, roots of all initially nonmycorrhizal seedlings had formed vesicular-arbuscular mycorrhizae and the degree of root colonization was comparable to that of initially vesicular-arbuscular mycorrhizal seedlings. New feeder roots did not develop on seedlings of any treatment until almost 5 months after planting. By the end of the first growing season and for the remainder of the study, vesicular-arbuscular mycorrhizae development was approximately the same on all seedlings. The proportion of feeder roots colonized by vesicular-arbuscular mycorrhizal fungi stabilized at 65 to 70%; approximately 56% of the cortical tissues of all feeder roots were colonized with arbuscles, vesicles, and hyphae. Periodic assays of the soil in the plantation showed that vesicular-arbuscular mycorrhizal fungal spores gradually declined from an initial high of 3600 spores to 620 spores per 100-cm^3 soil sample after 5 years. This decline was probably caused by crown closure of the sweetgum trees which gradually suppressed understory vegetation.

Technology Transfer

Kormanik, Paul P., William C. Bryan and Richard C. Schultz. 1977. Quality hardwood seedlings require early mycorrhizal development in nursery beds. pp 289-293 in Proceedings 14th Southern Forest Tree Improvement Conference, Gainesville, FL.

Kormanik, Paul P. 1979. Biological means of improving nutrient uptake in trees. pp 293-311 in The Ecology of Even-aged Forest Plantations, E.D. Ford, D.C. Malcolm and J. Atterson (eds.), Proceedings Division 1, IUFRO, Edinburgh.

Kormanik, Paul P., W. Craig Bryan and Richard C. Schultz. 1980. Procedures and equipment for staining large numbers of plant root samples for endomycorrhizal assay. Can. J. Microbiol. 26:536-538.

Kormanik, Paul P. 1981. Effects of nursery practices on vesicular-arbuscular mycorrhizal development and hardwood seedling production. pp 63-67 in Proceedings 1980 Southern Nursery Conference, Lake Barkley, KY. USDA For. Serv. Tech. Publ. SA TP-17.

Kormanik, Paul P. 1981. A forester's view of vesicular-arbuscular mycorrhizae and their role in plant development and productivity. pp 33-37 in Mycorrhizal Associations and Crop Production. New Jersey Agric. Exp. Station Res. Report No. R04400-01-81. Rutgers University.

Kormanik, Paul P. 1984. Application of vesicular-arbuscular mycorrhizal fungi in forestry. pp 48-54 in Application of Mycorrhizal Fungi in Crop Production, J.J. Ferguson (ed.), Gainesville, FL.

IV. BIOCHEMICAL ASPECTS OF MYCORRHIZAE - Cooperative Research Agreement. Dr. Clanton C. Black, Biochemistry Department, University of Georgia and Southeastern Forest Experiment Station. No. 102, 12-11-008-876. FY86 to FY89; total funds $75.5K.

Research

Black, Clanton C., Laszlo Mustardy, S.S. Sung, P.P. Kormanik, D.-P. Xu, and Nachman Paz. 1987. Regulation and roles for alternative pathways of hexose metabolism in plants. Physiol. Plantarum 69:387-394.

Plant cells have two cytoplasmic pathways of glycolysis and gluconeogenesis for the reversible interconversion of fructose 6-phosphate (F-6-P) and fructose 1,6-bisphosphate (F-1,6-P_2). One pathway is described as a maintenance pathway that is catalyzed by a nucleotide triphosphate-dependent phosphofructokinase (EC 2.7.1.11:ATP-PFK) glycolytically and a F-1,6 bisphosphatase (EC 3.1.3.11) gluconeogenically. These are non-equilibrium reactions that are energy consuming. The second pathway, described as an adaptive pathway, is catalyzed by a readily reversible pyrophosphate-dependent phosphofructokinase (EC 2.7.1.90;PPi-PFK) in an equilibrium reaction that conserves energy through the utilization and the synthesis of pyrophosphate. A constitutive regulator cycle is also present for the synthesis and hydrolysis of fructose 2,6-bisphosphate (F-2,6-P_2) via a 2-kinase and a 2-phosphatase, respectively. The pathway catalyzed by the ATP-PFK and F-1,6-bisphosphatase, the maintenance pathway, is fairly constant in maximum activity in various plant tissues and shows less regulation by F-2,6-P_2. Plants use F-2,6-P_2 initially to regulate the adaptive pathway at the reversible PPi-PFK step. The adaptive pathway, catalyzed by PPi-PFK, varies in maximum activity with a variety of phenomena such as plant development or changing biological and physical environments. Plants can change F-2,6-P_2 levels rapidly, in less than 1 min when subjected to rapid environment change, or change levels slowly over periods of hours and days as tissues develop. Both types of change enable plants to cope with the environmental and developmental changes that occur during their lifetimes. The two pathways of sugar metabolism can be efficiently linked by the cycling of uridylates and pyrophosphate required for sucrose breakdown via a proposed sucrose synthase pathway. The breakdown of sucrose via the sucrose synthase pathway requires half the net energy of breakdown via the invertase pathway. Pyrophosphate occurs in plant tissues as a substrate pool for biosynthetic reactions such as the PPi-PFK or uridine diphosphate glucose pyrophosphorylase (EC 2.7.7.9;UDPG pyrophosphorylase) that function in the breakdown of imported sucrose. Also, pyrophosphate links the two glycolytic/glyconeogenic pathways; and in a reciprocal manner pyrophosphate is produced as an energy source during gluconeogenic carbon flow from F-1,6-P_2 toward sucrose synthesis.

Sung, S.S., P.P. Kormanik, D.P. Xu and C.C. Black. 1989. Sucrose metabolic pathways in sweetgum and pecan seedlings. Tree Physiol. 5:39-52.

Sucrose metabolism and glycolysis were studied in one- to two-year-old seedlings of sweetgum (Liquidambar styraciflua L.) and pecan (Carya illinoinensis (Wangenh.) C. Koch). The sucrose synthase pathway was identified as the dominant sucrose metabolic activity in sucrose sink tissues such as terminal buds and the root cambial zone. The sucrose synthase pathway was completely dependent on uridine diphosphate and pyrophosphate and it was activated by fructose 2,6-bisphosphate. Both acid and neutral invertases were less active than sucrose synthase in sucrose sink tissues. According to the magnitude of seasonal changes in activity, sucrose synthase, the pyrophosphate-dependent phosphofructokinase, and fructokinase were identified as adaptive enzymes, whereas neutral invertase, uridine diphosphate-glucopyrophosphorylase, phosphoglucomutase, and the nonspecifc, nucleotide triphosphate-dependent phosphofructokinase were identified as maintenance enzymes. The periodically high activities of pyrophosphate-dependent phosphofructokinase indicate that pyrophosphate can serve as an energy source in trees. The observations support the hypothesis that sucrose glycolysis and gluconeougenesis in plants proceed by a network of alternative enzymes and substrates.

Xu, Dian-Peng, Shi-Jean S. Sung, Tadeusz Loboda, Paul P. Kormanik and Clanton C. Black. 1989. Characterization of sucrolysis via the uridine diphosphate and pyrophosphate-dependent sucrose synthase pathway. Plant Physiol. 90:635-642.

The breakdown of sucrose to feed both hexoses into glycolytic carbon flow can occur by the sucrose synthase pathway. This uridine diphosphate (UDP) and pyrophosphate (PPi)-dependent pathway was biochemically characterized using soluble extracts from several plants. The sucrolysis process required the simultaneous presence of sucrose, UDP, and PPi with their respective K_m values being about 40 millimolar, 23 micromolar, and 29 micromolar. UDP was the only active nucleotide diphosphate. Slightly alkaline pH optima were observed for sucrose breakdown either to glucose 1-phosphate or to triose phosphate. Sucrolysis increased with increasing temperature to near 50°C and then a sharp drop occurred between 55 and 60°C. The breakdown of sucrose to triose-P was activated by fructose 2,6-P_2 which had a K_m value near 0.2 micromolar. The cytoplasmic phosphofructokinase and fructokinase in plants were fairly nonselective for nucleotide triphosphates (NTP) but glucokinase definitely favored ATP. A predicted stoichiometric relationship of unity of UDP and PPi was measured when one also measured competing UDPase and pyrophosphatase activity. The cycling of uridylates, UDP to UTP to UDP, was demonstrated both with phosphofructokinase and with fructokinase. Enzyme activity measurements indicated that the sucrose synthase pathway has a major role in plant sucrose sink tissues. In the cytoplasmic sucrose synthase breakdown pathway, a role for the PPi-phosphofructokinase was to produce PPi while a role for the NTP-phosphofructokinase and for the fructokinase was to produce UDP.

Technology Transfer

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