United States
Department of
Agriculture

Forest Service

Southeastern Forest
Experiment Station

Research Note
SE-358

May 1990

Inoculation of Fall- and Spring-Sown Longleaf Pine Seedlings With *Pisolithus tinctorius*

Donald H. Marx and Charles E. Cordell

ABSTRACT

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 in a forest industry nursery in Alabama.

Keywords: Specific ectomycorrhizae, vegetative inoculum, basidiospore inoculum, seedling quality.

Nursery and outplanting studies of longleaf pine (*Pinus palustris* Mill.) show that inoculation of fumigated nursery soil to form *Pisolithus tinctorius* (Pers.) Coker & Couch (Pt) ectomycorrhizae, together with other nursery cultural practices, increases seedling quality and field performance (Hatchell 1985a, 1985b, 1987; Hatchell and Marx 1987; Kais and others 1981). The other required practices include low seedbed density, even spacing of seedlings in rows, lateral-root pruning, and optimal nutrient and water supplies. That treatment combination produces more seedlings with root-collar diameters greater than 1 cm and increases root fibrosity and ectomycorrhizal development.

Inoculation with Pt is routinely done in the spring, as seeds are sown in recently fumigated soil. With longleaf pine, however, soil fumigation and seed sowing can also be done in the fall. Fumigation, sowing, and inoculation in the spring yields more Pt ectomycorrhizae than fumigation, sowing, and inoculation in the fall. The difference has been attributed to increased competition from naturally occurring ectomycorrhizal fungi, such as *Thelephora terrestris* Ehrh. ex Fr., for seedling roots during the fall, winter, and early spring (Hatchell 1985b). It may also reflect poor survival of Pt inoculum in soil during these periods when seedling root growth and development of ectomycorrhizae are at their lowest. Results from spring inoculation of fall-sown seedlings have not been reported.

The purpose of the nursery study and operational test reported here was to compare the results of fall sowing and fall inoculation, fall sowing and spring inoculation, and spring sowing and spring inoculation with vegetative and spore inoculum of Pt. The factors of interest were ectomycorrhizal development and growth of the longleaf pine seedlings.

Materials and Methods

The study was installed at the Taylor Forest Tree Nursery, Trenton, SC. Plots for fall sowing were fumigated by standard procedures in early October 1986, and plots for spring-sown treatments were fumigated in mid-March 1987. At both dates, a formulation of 98 percent methyl bromide and 2 percent chloropicrin was applied at the recommended rate. After soil fertilization with 1,150 kg/ha of 10-10-10 commercial fertilizer and bed shaping, 4.56-m-long plots, each separated by a 1.52-m-long buffer strip, were laid out in blocks of five plots for fall sowing treatments and three plots for spring-sowing treatments. All treatments were replicated five times (blocks). Plots randomly selected for fall sowing and

---

Donald H. Marx is Institute Director and Chief Plant Pathologist, Institute of Tree Root Biology, Athens, GA; **Charles E. Cordell** is Plant Pathologist, Forest Pest Management, Region 8, Asheville, NC.

fall inoculation (November 19) and those selected for spring sowing and spring inoculation (March 24) with vegetative inoculum treatment were inoculated by machine at a rate of 0.33 L inoculum/m^2 of soil surface (Cordell and others 1988). Seeds were sown in all plots in seven drills by machine immediately after the above treatment was applied and mulched 1 cm deep with fumigated pine straw. Seeds were a 1985 wild unimproved collection from the coastal plain of South Carolina. Plots for fall sowing and spring inoculation (March 24) with vegetative inoculum were trenched 10 cm deep by 3 cm wide with a flat shovel in the six spaces separating the seven rows of seedlings. Vegetative inoculum of Pt at 0.33 L/m^2 of soil surface was placed in the trench by hand, and the trench was closed. The characteristics of the Pt vegetative inoculum (isolate 301) were similar for both inoculation dates. Bulk density was 397 g/L, moisture content was 41 percent, and pH was 5.4. Vegetative inoculum was produced by standard procedures (Marx and others 1984). Seedlings in all plots were thinned on March 24 to approximately 130 seedlings/m^2 with equal spacing between seedlings.

Basidiospores of Pt were collected in September 1986 from fruit bodies produced in operationally inoculated (isolate 301) beds of loblolly pine (*Pinus taeda* L.) seedlings in the Taylor Forest Tree Nursery. Spores were filtered through a 50-μ screen, dried at room temperature with 35 to 45 percent relative humidity to approximately 18-percent moisture content, and stored at 5 °C until used. Spores were applied after sowing by spraying a water suspension at 0.65 g spores/m^2 of soil surface on the same day vegetative inoculum was applied. Spore application was done by adding 30 mL of water plus two to three drops of Tween 20 to a 50-mL glass vial containing 3.6 g of basidiospores. The vial was vigorously shaken for 1 to 2 minutes and decanted into a 4-L plastic bottle containing 3 L of water. After mixing the contents vigorously, the suspension was decanted into a 12-L capacity backpack sprayer modified with an F80-06 fan-tip nozzle. Water was added to 12 L. With frequent agitation, the 12-L water suspension was sprayed on a test plot. The sprayer was refilled with another 12 L of water and shaken. This volume also was sprayed onto the plot to clean the sprayer of remaining spores and to leach spores into the soil. Within 2 hours of spraying, all plots were irrigated with approximately 5 mm of water to further leach spores into the soil.

In early April 1987, 4.48 kg a.i./ha of the fungicide Captan was sprayed on all plots. In early August and October 1987, seedlings were vertically root pruned 15 cm deep and undercut to 15 and 20 cm, respectively. The systemic fungicide benomyl (Benlate) at 2.2 kg a.i./ha was sprayed on all plots immediately after each root pruning. Applications of 168 kg/ha of ammonium nitrate (33% N) were broadcast on all plots four times at monthly intervals from mid-May to mid-August. Plots were irrigated with approximately 1.25 cm of water immediately after application of fungicides and fertilizer.

In early December 1987, all plots were undercut to 20 cm. Three 0.3- by 1.2-m subplots in each test plot were randomly selected, and seedlings were removed by hand. Subplot seedlings were combined, counted, graded to determine cull ($\leq$8 mm root-collar diameter, RCD) percentage, and 25 plantable-size seedlings (>8 mm RCD) were randomly selected as sample seedlings. These were measured for RCD and root and shoot fresh weights. Ectomycorrhizal development was visually assessed at 5 X magnification, and data on Pt ectomycorrhizae were transformed to a Pt index (Marx and others 1984). Analysis of variance and Duncan's new multiple range test were used to determine the statistical significance of differences between treatments.

Results and Discussion

Ectomycorrhizal development by Pt from either inoculum applied at any time did not affect seedling size (table 1). Sowing date significantly affected seedling size. Bed density averaged 116 seedlings/m^2 and cull rate averaged 14.5 percent; neither was significantly affected by the treatments. Type of inoculum and when it was applied had a significant effect on Pt ectomycorrhizal development. As reported earlier (Hatchell 1985a), fall sowing and fall inoculation with vegetative inoculum did not produce Pt indices >50. Spores applied at this time produced few Pt ectomycorrhizae and a much lower Pt index. Vegeta-

Table 1 – Growth and ectomycorrhizal development of longleaf pine seedlings at different times of seed sowing and inoculation treatments with vegetative or spore inocula of *Pisolithus tinctorius* (Pt) at the Taylor Forest Tree Nursery in South Carolina

Inoculum treatment	Root-collar diameter	Fresh weight		Percent of feeder roots ectomycorrhizal with–		Pt index[1]
		Tops	Roots	Pt	All fungi	
	mm	*g*				
		Fall sown - spring inoculated				
Vegetative	12.6a	21.6	12.7a	27ab	41a	63a
Spore	11.9ab	20.8a	11.9a	8c	43a	14c
Control	11.7ab	20.3a	11.8a	0	36a	0
		Fall sown - fall inoculated				
Vegetative	12.3a	21.8a	13.5a	19b	38a	37b
Spore	11.8ab	20.7a	12.8a	4c	41a	6d
		Spring sown - spring inoculated				
Vegetative	10.4b	17.2ab	10.4ab	34a	46a	68a
Spore	10.3b	16.8ab	10.6ab	9c	34a	17c
Control	10.6b	16.4b	9.8b	0	29a	0

Within columns, means followed by a common letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

[1] Pt index = A x (B/C) where A = percent of seedlings with any ectomycorrhizae formed by *P. tinctorius*, B = average percent of feeder roots with ectomycorrhizae formed by *P. tinctorius* (including 0 percent for those without *P. tinctorius*), and C = average percent of feeder roots with ectomycorrhizae formed by *P. tinctorius* and other fungi.

tive inoculum applied at spring sowing produced numerous Pt ectomycorrhizae (Pt index 68), but these seedlings were somewhat smaller than those sown in the fall. These results also agree with those reported earlier on seedlings sown and inoculated in the spring (Hatchell 1987). Spore inoculum produced over twice as many Pt ectomycorrhizae when it was applied at spring sowing as when it was applied in the fall, but the highest Pt index was only 17.

The most interesting result was from the fall sowing and spring vegetative inoculum treatment. These seedlings formed as many Pt ectomycorrhizae (Pt index 63) as those in the spring sowing and spring vegetative inoculum treatment. Because of fall sowing, these seedlings were also larger in RCD than those sown in the spring. Pt formed inadequate amounts of ectomycorrhizae from spore inoculum applied at this time.

Operational Test

During April 1988, an operational test with Pt vegetative inoculum was made on longleaf pine seedlings sown in the fall of 1987 and the spring of 1988 at the International Paper Company, Super Tree Nursery, Selma, AL. The nursery inoculations were conducted in a similar manner (spring fumigation, fertilizer, pesticides, and sampling and assessment procedures) as that at the Taylor Forest Tree Nursery except fall sown – fall inoculation treatments were not applied. At this nursery, the between-row inoculations were done with a machine built by R.A. Whitfield Manufacturing Company,[1] Mableton (Atlanta), GA. This prototype consisted of a modified lateral root pruner with a double-disk and inoculum metering device to permit controlled inoculum rate, depth, and width in each between-row trench (fig. 1). The inoculum rate, depth, and width were the same as at the Taylor Forest Tree Nursery. In general, the prototype between-row applicator performed effectively and efficiently. Approximately 100,000 seedlings were inoculated with the machine. Ectomycorrhizal root assessments at lifting showed that the applicator was equally effective in both treatments in the formation of Pt ectomycorrhizae on the longleaf pine seedlings (table 2). However, Pt ectomycorrhizal development and subsequent Pt indices were relatively low (16 and 22) in both treatments. A primary factor responsible for these relatively poor results could be the late April inoculation date, which allowed additional time for competition for feeder roots by naturally occurring ectomycorrhizal fungi on these seedlings. At lifting, seedlings sown in the fall had larger root-collar diameters than those sown in the spring.

Spring placement of vegetative inoculum near roots of seedlings sown the previous fall apparently reduces the opportunity for competition by naturally occurring ectomycorrhizal fungi and increases the inoculum potential and early root colonization by Pt. This procedure should also reduce the time required for inoculum survival since roots should grow into the inoculum faster than roots developing from spring-sown seeds. A primary requirement for consistent and effective results with fall-sown longleaf pines is early spring inoculation, which reduces the time between root growth and inoculation. Early spring inoculation increases the opportunity for Pt inoculum to capture feeder roots and reduces the competitiveness of other fungi for the same feeder roots.

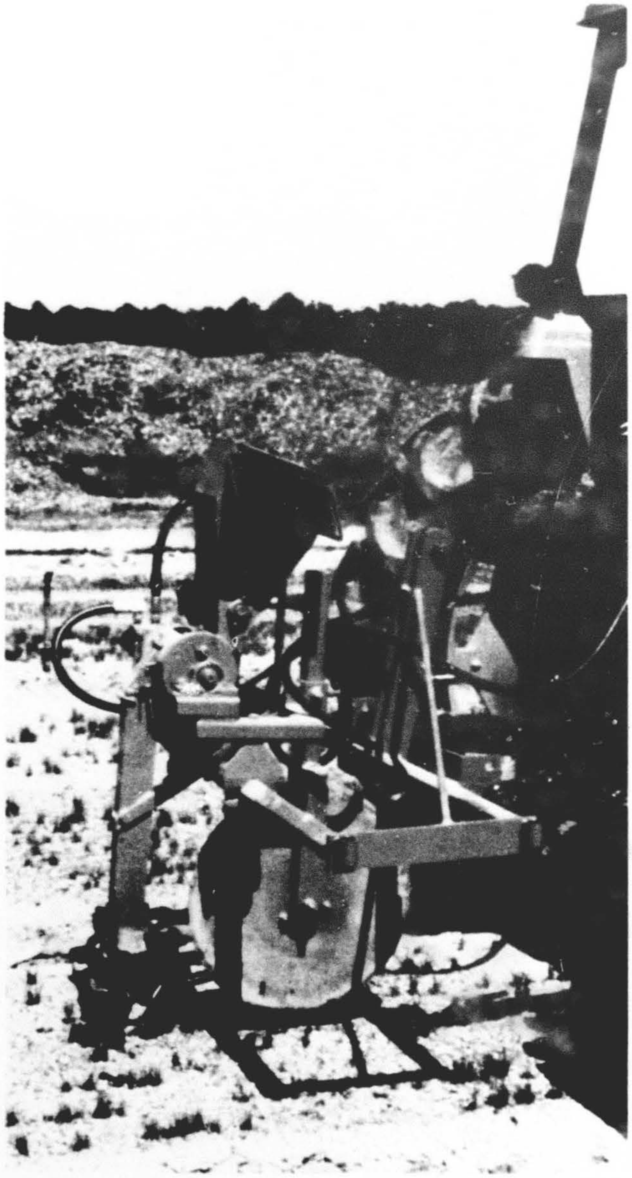

Figure 1 – Prototype of machine for applying Pt vegetative inoculum between rows of longleaf pine seedlings sown the previous fall.

---

[1] The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture of any product or service to the exclusion of others that may be suitable.

Table 2—Growth and ectomycorrhizal development of longleaf pine seedlings at different times of seed sowing and inoculation treatments with machine-applied vegetative inoculum of *Pisolithus tinctorius* (Pt) at the International Paper Company nursery in Alabama

Inoculum treatment	Root-collar diameter	Percent of feeder roots ectomycorrhizal with–		Pt index[1]
		Pt	All fungi	
	mm			
Fall sown– spring inoculated	10.4a	6a	14a	22a
Spring sown– spring inoculated	8.2b	4a	15a	16a

Within columns, means followed by a common letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

[1] Pt index = A x (B/C) where A = percent of seedlings with any ectomycorrhizae formed by *P. tinctorius*, B = average percent of feeder roots with ectomycorrhizae formed by *P. tinctorius* (including 0 percent for those without *P. tinctorius*), and C = average percent of feeder roots with ectomycorrhizae formed by *P. tinctorius* and other fungi.

Literature Cited

Cordell, C.E.; Marx, D.H.; McFee, David. 1988. Pt ectomycorrhizal fungus operational inoculations and management in southern forest tree nurseries, 1988. In: Proceedings: 1988 Southern Forest Nursery Association; 1988 July 25-28; Charleston, SC: 187-199.

Hatchell, Glyndon E. 1985a. Nursery cultural practices affect field performance of longleaf pine. In: South, David B., ed. Proceedings of the international symposium on nursery management practices for the southern pines; 1985 August 4-9; Montgomery, AL. Auburn AL: Auburn University: 148-156.

Hatchell, Glyndon E. 1985b. Seedling quality and field performance of longleaf pine seedlings affected by ectomycorrhizae and nursery cultural practices. In: Shoulders, Eugene, ed. Proceedings of the 3d biennial southern silvicultural research conference; 1984 November 7-8; Atlanta, GA. Gen. Tech. Rep. SO-54. New Orleans, LA: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station: 395-402.

Hatchell, Glyndon E. 1987. Nursery cultural practices, seedling morphology, and field performance of longleaf pine. In: Phillips, Douglas R., comp. Proceedings of the 4th biennial southern silvicultural research conference; 1986 November 4-6; Atlanta, GA. Gen. Tech. Rep. SE-42. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station: 61-66.

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

Kais, Albert G.; Snow, Glenn A.; Marx, Donald H. 1981. The effects of benomyl and *Pisolithus tinctorius* ectomycorrhizae on survival and growth of longleaf pine seedlings. Southern Journal of Applied Forestry 5:189-195.

Marx, D.H.; Cordell, C.E.; Kenney, D.S. [and others]. 1984. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on bare-root tree seedlings. Forest Science Monograph 25. 101 pp.

Southeastern Forest Experiment Station, P.O. Box 2680, Asheville, North Carolina 28802

The Forest Service, U.S. Department of Agriculture, is dedicated to the principle of multiple use management of the Nation's forest resources for sustained yields of wood, water, forage, wildlife, and recreation. Through forestry research, cooperation with the States and private forest owners, and management of the National Forests and National Grasslands, it strives—as directed by Congress—to provide increasingly greater service to a growing Nation.