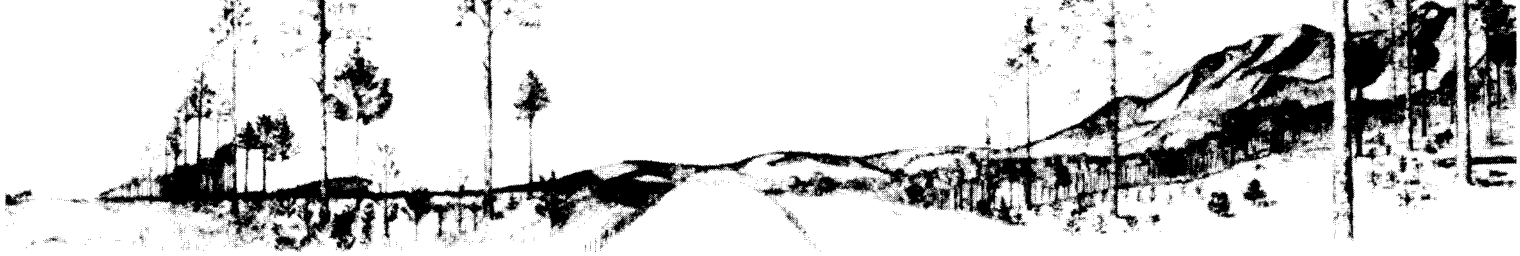

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PERFORMANCE OF CHOCTAWHATCHEE SAND PINE SEEDLINGS INOCULATED WITH ECTOMYCORRHIZAL FUNGI AND OUTPLANTED IN THE SANDHILLS OF NORTH FLORIDA

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ABSTRACT.-Studies were conducted on the effect of the ectomycorrhizal symbiont *Pisolithus tinctorius* (Pt) on nursery production and field performance of both container-grown and bare-root Choctawhatchee sand pine (CSP). In two separate experiments, seedlings inoculated with Pt were planted on prepared sites on the sandhills of north Florida. Inoculation with Pt improved the quality of bare-root CSP in the nursery. Field survival and early growth were not affected by mycorrhizal treatment. The reasons for the failure of Pt inoculation to improve outplanted seedling performance during these experiments are discussed.

Keywords: ***Pinus clausa*** var. ***immuginata, Pisolithus tinctorius, Thelephora terrestris,*** container stock. reforestation, seedling quality.

It is generally recognized that if forest tree species such as ***Pinus*** are to grow normally, it is essential that ectomycorrhizae develop on their roots. However, not all mycorrhizal fungi are equally beneficial or adapted to the wide range of soil and climatic conditions supporting tree growth. In his studies of coal spoil wastes in Pennsylvania, Schramm (1966) noted that of the numerous species of mycorrhizal fungi potentially available from adjacent forested sites, only ***Pisolithus tinctorius*** (Pt), ***Thelephora terrestris*** (Tt), and a few other species were able to survive on these adverse sites and form ectomycorrhizae. Marx and Bryan (1971) have also noted this adaptation of Pt to such adverse sites and reported that this symbiont increases the tolerance of pines to high soil temperatures.

The sandhills of north Florida without question can be described as adverse sites. These soils are droughty, infertile, and surface soil temperatures become as high as 7 1°C when sites are exposed by site preparation. Pine seedlings planted in these severe conditions are definitely subjected to periods of stress. Of the pine taxa that have been tested, the Choctawhatchee variety of sand pine (***Pinus clausa*** var. ***immuginata*** D.B. Ward), a species endemic to this area, shows the greatest promise for sandhill reforestation (Brendemuehl;[3] Burns 1973).

Despite the fact that Choctawhatchee sand pine (CSP) is obviously well suited for sandhill reforestation, two deficiencies that may be related to mycorrhizal development have been noted in the early growth of this species. Seedling growth in the nursery is often uneven; the larger seedlings have excellent mycorrhizal development, while the smaller ones usually have few mycorrhizal roots. In addition, height growth of CSP seedlings during the first 2 or 3 years in the field is often less than that of Ocala (***P. clausa*** var. ***clausa*** (Chapm.) Vasey) or slash (***P. elliottii*** Engelm.) pine, species that frequently fail to attain merchantable size when planted in the sandhills.

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[3]Brendemuehl, R. H. Options for management of sandhill forest land. Southeastern Forest Experiment Station, Marianna, Florida.[In process.]

Reported here are the results of two experiments which were intended to improve the development of CSP planting stock and to increase early growth of these seedlings when transplanted to **sandhill** sites. One study was of bare-root nursery stock, the other was of container-grown stock.

METHODS AND MATERIALS

Nursery Inoculation-Seedling Production

CSP seedlings intended for bare-root planting were produced during 1975 in a nursery located on the Chipola Experimental Forest, Calhoun County, Florida. Soils in this nursery are infertile, excessively drained sands of the **Lakeland** series. A portion of the nursery that had never been in production was used for this study. The area was cleared of all vegetation, triple superphosphate was broadcast at the rate of 268 kg/ ha, and the site was tilled with a rototiller.

Early in April when soil temperatures were above **16°C,** the nursery site was fumigated with methyl bromide (MC-2) released under polyethylene at the rate of 1 kg/ $10 m^2$ of nursery area. Four days later the polyethylene was removed, the site was tilled, and the nursery beds were formed. Four days following this, the vegetative Pt inoculum, prepared according to procedures described by Marx and Bryan **(1975),** was applied by hand to three nursery beds at the rate of 10.8 liter/ $10 m^2$. The inoculum was mixed into the soil with a rotovator to a depth of 10 to 15 cm. On three additional beds, vermiculite without Pt inoculum was applied and mixed with the soil to allow the effects of the inoculum upon the seedlings to be separated from any effects of vermiculite alone. A third set of nursery beds used to produce the control seedlings received no inoculum.

Seed obtained from natural CSP stands in west Florida were sowed in rows 15 cm apart and at a rate calculated to produce approximately 325 seedlings per square meter of nursery bed. The nursery fertilization schedule developed by Brendemuehl and Mizell(1978) for production of CSP seedlings was followed.

Pt basidiocarps were first detected in the inoculated beds in early September and were removed at weekly inspection periods during the balance of the year to prevent their spread to adjacent control beds.

In January 1976, the seedlings were lifted and 90 seedlings per treatment were weighed (top weight and root weight) and the roots were evaluated for mycorrhizal development. Seedlings used for field planting were graded for size and mycorrhizal development. The seedlings lifted from Pt beds had a very low percentage of their roots colonized with Pt. Many of the Pt ectomycorrhizae were developed on roots deeper in the soil and were lost during lifting. Therefore, plantable seedlings used to evaluate this treatment in the field plots had only 5 to 10 percent Pt ectomycorrhizae and approximately 25 percent other ectomycorrhizae (mostly Tt). Also, seedlings with less than 10 cm of stem supporting live needles or seedlings damaged in lifting were considered culls and were not planted. The seedlings were machine-planted the same day at an approximate spacing of 1.8 by 3 meters. About 125 seedlings were planted on the individual treatment plots, each measuring 21 by 30 meters. All plots were separated by a 3-meter isolation strip.

The five sites on the Chipola Experimental Forest selected for the field plantings were typical of the sandhills and had supported scrub oak and wire grass before being double chopped in preparation for planting. All five sites had excessively drained sand soil profiles to a depth of at least 3 meters. Laboratory analyses of samples taken from the surface horizon (0 to 20 cm) of each site showed that there was little variation among them. Average values for the soil characteristics analyzed were: **pH**, 5.3; organic matter, very low; **CaO,** 84 kg/ ha; **MgO**, 15 kg/ha; P_2O_5, 6 kg/ ha; and K_2O, 17 kg/ha.

One and two growing seasons after planting (November 1976 and **1977),** survival counts were made and surviving trees in all plots were measured for root-collar diameter and height. After determining the average height and root-collar diameter of all seedlings in a plot, the smallest and largest seedlings were harvested. A third seedling was also chosen so that the average height and root-collar diameter of the three seedlings approximated (within 10 percent) the averages for that plot. The top fresh weight of these seedlings was determined **onsite** immediately after cutting the stems at ground level. Another three seedlings were randomly selected from each plot for mycorrhizal assessment and dug up with a shovel. After carefully removing most of the soil, the roots were visually assessed for mycorrhizal development. All growth data were subjected to an analysis of variance.

Container Inoculation-Seedling Production

Container-grown CSP seedlings were produced in a greenhouse in Athens, Georgia. The seed were from the same seed lot used the previous year in the nursery inoculation study.

Inocula of Pt, isolate 138, and Tt, isolate 201, were produced in peat moss-vermiculite nutrient medium and processed as reported by Marx and Bryan (1975). Mycorrhizal inoculum was mixed in a 1:6 v/v ratio with milled pine bark previously fumigated with methyl bromide. Autoclaved Pt inoculum was mixed with fumigated pine bark at the same rate for the control seedlings. Spencier LeMaire Rootrainers® (Hillson model, 150 cm^3/cavity) were filled with the growing medium mixed with inoculum.

In May 1976, all containers were placed on greenhouse benches and sowed with several seed per cavity. After germination, seedlings were thinned to one per cavity. Seedlings were watered as needed, and fertilized until August with a commercially available water-soluble NPK fertilizer (23: 19: 17) and chelated iron as previously described (Ruehle and Marx 1977). In August, all seedlings were transported to a greenhouse at the Chipola Experimental Forest.

Before outplanting, 10 seedlings were randomly selected from each of the three groups of seedlings. Their roots were carefully separated from the potting medium and visually assessed for mycorrhizal development. Pt seedlings averaged 64 percent Pt and 8 percent naturally occurring Tt, Tt seedlings averaged 68 percent Tt, and controls averaged 40 percent naturally occurring Tt. All seedlings were of uniform size regardless of treatment; they averaged 19 cm in height and 2 mm in root-collar diameter.

Three planting sites-one on the Chipola Experimental Forest and two on private land in Washington County, Florida-were selected and double shopped with a duplex brush cutter 3 to 4 months before planting. The sites were similar, with infertile, excessively drained sandy soils (Lakeland or Kershaw soil series) which supported scrub oak and wire grass before being prepared for planting to pines. The soil profile at each site was sand to a minimum depth of 3 meters.

Laboratory analyses of soil samples collected from the 0- to 20-cm surface of the soil profile at each site indicated that the study areas were quite similar in terms of chemical characteristics, although site 1 was somewhat more fertile (table 1).

In December, seedlings were handplanted at a spacing of 2.4 by 2.7 meters into 25-tree plots, each comprised of five rows of five seedlings. To minimize contamination, all plots were separated by 3-meter unplanted strips. Treatments were assigned to plots in a randomized block design of five replications.

In November 1978, 2 years after planting, the survival, height, rootcollar diameter, selection and harvesting, top fresh weight, and mycorrhizal assessment of outplanted container-grown seedlings were determined by the same procedures as for the bare-root outplantings.

Table 1 .-Chemical properties of soils at outplanting sites of the container-grown seedling study

Site	Organic matter	Soil property				
		pH	P_2O_5	MgO	K_2O	CaO
				kg/ha		
1 (Forests & Lakes)	Very low	5.3	3.4	30.2	32.5	313.6
2 (Hunt)	Low	5.1	3.4	14.6	21.3	100.8
3 (Chipola)	Very low	5.2	3.4	14.6	16.8	89.6

RESULTS AND DISCUSSION

Field survival of both bare-root stock (nursery inoculation) and container-grown stock (container inoculation) was greater than 90 percent for all plantings and did not vary by mycorrhizal treatment (table 2.) Treatments had no effect on survival. These results were not unexpected since similar results had been obtained in experimental and operational plantings of CSP seedlings which were correctly handled and planted.

Average height, diameter, and aboveground biomass of seedlings did not vary by mycorrhizal treatment (tables 3 and 4). In the bare-root outplanting, this may have been due to the low percentage of Pt ectomycorrhizal development (5 to 10 percent).

In a container-stock outplanting test with shortleaf pine (Ruehle and others, In press), seedlings colonized with Pt performed no better than control seedlings. Excavation of samples of the container stock after 2 years showed that Pt failed to spread to the new roots which had emerged from the root plugs. The root configuration was markedly different on container-grown seedlings than bare-root seedlings and probably accounted, to some degree, for the lack of spread of Pt and the consequent lack of growth response with the container stock. Similarly, after excavating sample seedlings of inoculated CSP container stock 2 years after planting, we found that Pt again failed in most cases to spread to the new roots that had emerged from the root plug:

Table 2.-Effect of mycorrhizal treatment on second-year survival of CSP seedlings

Seedling type and planting site	Treatment'			
	Control	Pt	Tt	Vermiculite
	Percent			
Nursery Chipola	97.2	97.6	--	99.4
Container Site 1 (Forests & Lakes)	99.2	97.6	99.2	--
Container Site 2 (Hunt)	96.8	96.8	95.2	--
Container Site 3 (Chipola)	95.2	92.0	92.0	--

"Control-no inoculum, Pt—*Pisolithus tinctorius* inoculum, Tt—*Thelephora terrestris* inoculum. vermiculite-vermiculite carrier applied with no inoculum.

Table 3.—Effect of mycorrhizal treatment on second-year growth of CSP seedlings

Planting site	Treatment[a]							
	Control		Pt		Tt		Vermiculite	
	Height	Diameter	Height	Diameter	Height	Diameter	Height	Diameter
	cm	*mm*	*cm*	*mm*	*cm*	*mm*	*cm*	*mm*
Nursery Chipola	64.0	--	61.6	_	--	_	64.6	--
Container Site 1 (Forests & Lakes)	14.3	14.5	18.9	15.9	19.1	16.3	--	--
Container Site 2 (Hunt)	49.7	8.4	50.3	8.5	44.8	7.2	--	--
Container Site 3 (Chipola)	66.1	13.0	69.8	13.5	68.6	13.0	--	--

[a]Control-no inoculum, Pt—*Pisolithus tinctorius* inoculum, Tt—*Thelephora terrestris* inoculum. vermiculite-vermiculite carrier applied with no inoculum.

Table 4.-Aboveground biomass of CSP seedlings. Means of five replicates

Treatment	Number of seedlings per plot	Seedling stem volume[a] (cm^3)	PVI[b] ($\div 10^3$)	Top fresh weight	PWI[c] ($\div 10^3$)
		SITE 1			
Pt	24.4	205.9	5.00	432.7	10.56
Tt	24.8	219.4	5.42	494.0	12.20
X	24.8	161.0	4.00	409.4	10.17
		SITE 2			
Pt	24.2	39.6	.96	117.1	4.30
Tt	23.8	23.5	.58	143.8	3.44
X	24.2	39.7	.93	175.3	4.21
		SITE 3			
Pt	23.0	133.3	3.07	304.8	7.05
Tt	23.0	124.6	2.87	331.7	7.35
X	23.8	120.4	2.83	328.7	7.46

[a]Seedling volume (cm^3) = (rootcollar diameter)^2 × height.
[b]PVI is plot volume index (cm^3) = mean seedling volume × number surviving seedlings.
[c]PWI is plot weight index (g) = mean seedling top fresh weight × number surviving seedlings.

Planting site	Proportion of inoculated plugs still colonized with Pt	Average percent of Ft colonization on roots which had emerged from the plugs
1	13 of 15	23
2	11 of 15	10
3	13 of 15	20

In the CSP container-stock outplanting, as in the shortleaf container-stock study (Ruehle and others, In press), we found extensive colonization by indigenous symbionts of roots that had emerged from the plugs. We concluded that this natural inoculation partly accounted for the low percentages of Pt colonization listed in the tabulation above. Seedlings sampled from the other treatments also had abundant ectomycorrhizae formed by indigenous symbionts both in plugs or roots that emerged from the plugs. Such early colonization has been previously reported by Tainter and Walsted (1977) for container-grown loblolly pine on Coastal Plain sites.

One factor that could partly account for lack of response to Pt inoculation in our bare-root seedling study was the low percentage colonization by Pt (5 to 10 percent) on seedlings after lifting. To obtain significant growth response of bare-root pine seedlings on reforestation sites, a threshold level of at least half of all ectomycorrhizae on seedlings at planting must be formed by Pt (Marx and others 1977; Ruehle and others, In press; Kais and others[4]).

In the nursery phase of this study, the seedlings grown in soil with Pt were much more uniform in size than those grown in the uninoculated beds. The high degree of seedling uniformity as a result of Pt inoculation should help to make CSP seedling production more efficient. Unfortunately, too many Pt ectomycorrhizae were lost during the lifting and grading of these seedlings. This study, then, indicates that inoculation with Pt can improve nursery grade of bare-root stock and suggests that more research into the inoculation, increased colonization, and handling of CSP seedlings with Pt ectomycorrhizae is warranted.

[4] Kais, A. G., G. A. Snow, and D. H. Marx. The effects of benomyl and *Pisolithus tinctorius* ectomycorrhizae on survival and growth of longleaf pine seedlings. Southern Forest Experiment Station, Gulfport, Mississippi. [In process.]

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