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# **NORMAL PRESENTATIONS**

**JULY 26, 1990**



**8:00 AM - 12:00 PM**

## Specific Nursery Cultural Practices that Affect Field Performance of Longleaf Pine Seedlings

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Abstract.--We report results of two studies. In the first, longleaf pine (Pinus palustris) seedlings were grown at the Soup Carolina Coastal Nursery in 1983 at a density of 9 seedlings/ft with vegetative inoculum of Pisolithus tinctorius (Pt) or no inoculation, and with or without vertical root pruning in mid-August and again in late September. Evaluation of seedlings at lifting showed that vertical root pruning decreased shoot/root ratio and increased Pt index of inoculated seedlings. Average root collar diameters (RCD) ranged from 10.1 to 11.2 mm and were not affected by the treatments.

Representative seedlings (>8 mm RCD) were outplanted by machine (February 1984) on three sites at the Savannah River Forest Station (SRFS), Aiken, SC. Site 1 is an Americus loamy sand (site index 65), site 2 is a Lakeland sand (site index 60), and site 3 is a Troop sand (site index 65). All sites were prepared by shearing, raking, and bedding in late 1982. After 3 years, Pt ectomycorrhizae and vertical root pruning significantly increased survival, total height, percentage of seedlings in active height growth, and RCD, as well as seedling and plot volumes. Site 1 supported the best growth of seedlings.

In study two, seedlings were grown at the Experimental Nursery, Athens, GA, in 1984 at four bed densities (6, 9, 12, and 15 seedlings/ft) with and without vertical root pruning, and with and without vegetative inoculum of Pt. Evaluation of seedlings at lifting showed that cull percent (<8 mm RCD) increased with increasing bed densities (9.5 to 55.7%) and that seedling sizes (RCD and weight) and Pt ectomycorrhizal development decreased with increasing densities. Root pruning significantly increased Pt ectomycorrhizal development at all densities. Representative seedlings (>8 mm RCD) from each nursery treatment were outplanted by machine in February 1985 on site 1 described above. After 4 years, low bed density, lateral root pruning, and Pt ectomycorrhizae significantly improved seedling survival, total height, percent seedlings in active height growth, RCD, and seedling and plot volumes. The best treatment combination (6 seedlings/ft, Pt ectomycorrhizae, and vertical root pruning) increased plot volumes by over 2.6 times compared to the worst treatment combination (15 seedlings/ft, natural ectomycorrhizae, and no vertical root pruning).

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Longleaf pine (*Pinus palustris* Mill.) once dominated the Sandhills of the Southern United States. Its deep root system makes it the most desirable pine species to plant on fast-draining soils. Unfortunately, after many decades of poor plantation establishment and delayed initiation of height growth, most land managers have converted most of the nearly 60 million acres of these soils to other pine species (Mann 1969). Only a small portion of the approximately 7.3 million acres of forest land in the Sandhills of Georgia, the Carolinas, and northern Florida is stocked in longleaf pine today.

In order to correct this problem, much nursery research in recent years has concentrated on improving the field performance of bare-root longleaf pine seedlings. It was necessary to modify nursery cultural practices developed for other southern pines. Low bed density, vertical root pruning, inoculation to form *Pisolithus tinctorius* ectomycorrhizae, different fertility regimes, and a change in sowing time dates from spring to fall were combined to produce seedlings with RCD >12 mm or 0.5 inch, a morphological characteristic considered necessary for suitable field performance (May 1985, Hatchell 1985, Hatchell 1986, Hatchell and Marx 1987, South 1987). Recently, Hatchell and Muse (1990) provided evidence that 6 or more first-order lateral roots combined with high root fibrosity resulting from vertical root pruning contributed more to improved field performance than did large root-collar diameters.

In 1987, nursery production and outplanting protocols were developed by the USDA Forest Service for the artificial regeneration of longleaf pine at the Savannah River Site, Aiken, SC. This protocol incorporated many of the nursery cultural practices mentioned above as well as some that have not been published. Results of the two studies described here contributed to the development of this protocol.

## MATERIALS AND METHODS

**Study 1.**--This study was designed to determine the influence of vertical root pruning (side cutting) and inoculation<sub>2</sub> to form *P. tinctorius* ectomycorrhizae at a low bed density (9 seedlings/ft ) on seedling morphology and field performance of longleaf pine.

The study was installed in the South Carolina Coastal Nursery, St. George, SC. Soil was fumigated with a mixture of methyl bromide (98%) and chloropicrin (2%) in March 1983. Assay of pre- and post-fumigation samples for nematodes and pythiaceous fungi indicated that fumigation was effective (Marx et al. 1984). Commercial 10-10-10 fertilizer at 500 lbs/acre was disked into the soil. Soil analysis (A & L Laboratories, Inc., Memphis, TN) of samples collected after fumigation and fertilization revealed a pH of 4.8, 2.1% organic matter, 0.06% total nitrogen, and 42, 75, 66, and 219 ppm of P, K, Mg, and Ca, respectively.

Four 10-foot-long plots were laid out in each of five nursery beds. Vegetative inoculum of *P. tinctorius* (Pt) was broadcast on the soil surface at a rate of 1.3 liter/yd in one plot in each replicate bed (block) and was

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<sup>1</sup> Cordell, C.E., D.H. Marx and D.W. Omdal. (in press). Operational application of ectomycorrhizal fungi in forest tree nurseries - 1990. Proceedings of the Southern Forest Nursery Association, Biloxi, MS. July 1990.

mixed with hand tools into the upper 4 inches of soil. Inoculum was a commercial batch produced by Sylvan Spawn Laboratory (Marx et al. 1989); it had a bulk density of 370 g/l, pH 5.0, and a moisture content of 51%. The remaining three plots in each of the test beds were not inoculated (NI plots) to provide control seedlings with only naturally occurring ectomycorrhizae. All nursery test plots were sown in rows by hand in late April 1983 with seed collected from a seed collecting area in the Georgia Coastal Plain. The remaining nursery bed lengths were row-sown by conventional means. Nursery beds were mulched with pine straw and irrigated as needed. In mid-June, seedlings in test plots were thinned to 9 seedlings/ft with a minimum of 1-inch spacing between seedlings within rows. During the growing season,  $\text{NH}_4\text{NO}_3$  at 50 lbs N/acre and  $\text{K}_2\text{SO}_4$  at 18 lbs K/acre were applied in June, July, August, and September. Captan 50-WP (2 lbs/acre) was applied after sowing, Manzate 200 (1.5 lbs/acre) was applied every 7 days from mid-May to early October, Benlate 50-WP (2 lbs/acre) was applied monthly from June through August, and Oxyfluorfen (Goal 2C) was applied at sowing and in June and July. In mid-August and late September, one Pt and one NI plot in each of the five replicate blocks were vertically root pruned to an 8-inch depth.

In mid-February 1984, seedlings were undercut to 8 inches, vertically root pruned and hand lifted in February 1985. Seedlings were graded and culls discarded. Plantable seedlings had RCD >8 mm (0.3 inch) and were free of rust galls and foliar blight caused by Rhizoctonia. Twenty-five seedlings were randomly selected from each plot and measured for RCD and top and root fresh weights. Ectomycorrhizae were visually estimated at 5X magnification. Six-hundred seedlings of each treatment were randomly selected and 24 bundles of 25 seedlings each were packed in wet peat moss in seedling bags and stored at 40° F.

The three outplanting sites were located on the Savannah River Forest Station, Savannah River Site, Aiken, SC. Site 1 is an Americus loamy sand with an estimated site index of 65. Site 2 is a Lakeland sand with a site index of 60. Site 3 is a Troup sand with a site index of 65. All sites, located on upland ridges, were site prepared by shearing, raking, and bedding in the summer 1983. Prior to stand harvest in 1980, each site supported either loblolly or longleaf pines and various hardwoods.

Seedlings from the four nursery treatments were machine planted on each site in mid-February 1984. A single row of 25 seedlings/treatment was planted (6 X 10 ft spacing) in each of 8 blocks per site. A border row of nursery-run seedlings was planted around each block. Survival, RCD, and height were obtained at the end of each of the first three growing seasons. Results from the nursery and field tests were subjected to analyses of variance, and means were separated by Duncans Multiple Range Test at P = 0.05.

Study 2.--The purpose of this study was to determine the effects of vertical root pruning, gt ectomycorrhizae, and different bed densities (6, 9, 12, and 15 seedlings/ft ) on seedling morphology and field performance of longleaf pine.

The study was installed at the USDA Forest Service's Experimental Nursery, Athens, GA. Eight wood-frame nursery beds (20 ft long, 4 ft wide, and 2 ft deep) were filled with a 2:1:1 volume mixture of forest loamy sand, sand, and milled pine bark (92% sand, 4% clay, and 4% silt) and fumigated in mid-April

1984. Commercial 10-10-10 fertilizer at 1000 lbs/acre was mixed into the upper 6 inches of soil. Four beds were inoculated with Pt as in Study 1 and the other four beds were left for natural inoculation (NI). Each bed was separated into four 5-ft long plots. In mid-April 1984 plots were randomly selected and longleaf pine seeds (same source as Study 1) were sown by hand in 8 rows approximately 5.5 inches apart. In early June, seedlings were thinned in assigned plots to obtain bed densities of 6, 9, 12, or 15 evenly-spaced seedlings/ft<sup>2</sup>. Seedlings in half of each plot were vertically root pruned between rows with a flat shovel to a depth of 8 inches in mid-August and again in late September. During the growing season, fertilizer was applied as in Study 1. The only pesticide used on these seedlings was Benlate 50-WP (4 lbs/acre) applied monthly from April through October. The experimental design was two ectomycorrhizal conditions (Pt and NI), four bed densities (6, 9, 12, and 15 seedlings/ft<sup>2</sup>) and with or without vertical root pruning. Each of the 16 treatments was replicated 4 times.

Seedlings were undercut to 8 inches, vertically root pruned and hand lifted in February 1985. Seedlings were graded and culls discarded. Cull standards were the same as Study 1. Ten seedlings were randomly selected from each of the 64 subplots and assessed for RCD, top and root fresh weights, and ectomycorrhizal development. Two-hundred seedlings from each of the 16 treatments were randomly selected, grouped into 8 bundles of 25 seedlings each, packed in moist peat moss in seedling bags and stored at 40°F until outplanting.

Seedlings were outplanted in late February 1985 on Site 1 described in Study 1. The experimental design, seedling measurements, and statistical analyses were the same as Study 1.

## RESULTS

Study 1.--Pt ectomycorrhizae increased root weights and decreased shoot/root ratios of nursery seedlings (Table 1). Root pruning did not affect RCD or seedling weights, but significantly increased Pt ectomycorrhizal development. After 3 years in the field, seedlings with Pt ectomycorrhizae had improved survival and/or growth on all sites (Table 2). Seedlings with Pt ectomycorrhizae and root pruning treatments were significantly larger in most measurements than seedlings with only natural ectomycorrhizae and no pruning. Seedlings with Pt ectomycorrhizae and root pruning had 96, 92, and 163 percent larger plot volumes than seedlings without pruning and with only natural ectomycorrhizae on Sites 1, 2, and 3, respectively. There was no apparent relationship between initial shoot/root ratio and field performance. Survival and growth of seedlings, regardless of treatment, was significantly better on Site 1 than on the other two sites. However, differences between nursery treatments were more pronounced on Site 3, the least productive site.

Study 2.--Nursery treatments, especially bed density, had very significant effects on seedling morphology (Table 3). At 15 seedling/ft<sup>2</sup>, over 50 percent of seedlings were culls, regardless of ectomycorrhizal or root pruning treatment. As expected, fewer culls (8 to 10%) were associated with a density of 6 seedlings/ft<sup>2</sup>. In most instances, the cull percentage was the same at 9 and 12 seedlings/ft<sup>2</sup> densities. Generally, seedlings with Pt ectomycorrhizae and/or root pruning were larger in RCD and shoot and root fresh weights and had lower shoot/root ratios than seedlings from other treatments within a bed

density treatment. More Pt ectomycorrhizae were found in the lower density/root pruned treatments than other treatments. Overall, the effects of Pt ectomycorrhizae on seedling morphology were no different from those of seedlings with only naturally occurring ectomycorrhizae. Root pruning had only nominal effects on seedling measurements within a bed density treatment.

Results after 4 years in the field showed significant effects of the nursery treatments (Table 4). Generally, seedlings with the greatest RCD and weight from the best nursery treatment combinations had better survival and growth than smaller seedlings from less effective nursery treatments. However, there were some exceptions. Seedlings from the natural ectomycorrhizae/root pruned/lower densities nursery treatments did not exhibit improved survival and growth over initially smaller seedlings from higher densities but identical other treatments.

Shoot/root ratios were not related to field performance. Seedlings from the Pt ectomycorrhizae/pruned/6 seedlings/ft treatment had a shoot/root ratio of 2.25 and those from the natural ectomycorrhizae/pruned/12 seedlings/ft treatment had a shoot/root ratio of 2.28. Seedlings from these treatments with nearly identical shoot/root ratios from the nursery had differences in survival of nearly 20% and differences in plot volumes of over 85%. Survival was more than doubled and plot volumes were nearly tripled with seedlings from the bet nursery treatment combination (Pt ectomycorrhizae/pruned/6 seedlings/ft ) as compared to those from the worst treatment combination (natural ectomycorrhizae/not root pruned/15 seedlings/ft ).

## DISCUSSION

0 These two studies served as a prelude to a more comprehensive study (Hatchell and Muse 1990) which showed that vertical root pruning increases root fibrosity (number of secondary lateral roots) and ectomycorrhizae. Seedlings with fibrous root systems (root pruned) and as few as 6 strong first-order lateral roots (FOLR) performed as well in the field as seedlings with low root fibrosity (not pruned) but 14 or more FOLR. As fibrosity increases, the number of ectomycorrhizae will also increase.

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Table 1. Morphological characteristics of longleaf pine seedlings produced in the South Carolina Coastal Nursery, St. George, SC, as affected by nursery treatments

Nursery treatments <sup>2</sup>	Root-collar diameter	Fresh weight		Shoot/root ratio	% of short roots with ectomycorrhizae formed by:		Pt index <sup>3</sup>
		Shoot (g)	Root (g)		Pt (%)	All fungi (%)	
Pt-pruned	10.8a	27.5a	17.5a	1.57b	48a	59a	81a
NI-pruned	10.1a	22.9a	12.9b	1.78ab	0	37b	0
Pt-not pruned	11.1a	31.0a	15.9ab	1.95a	36b	51a	68b
NI-not pruned	11.1a	27.2a	13.7b	1.99a	0	33b	0

<sup>1</sup> Plantable seedlings had root-collar diameters >8 mm (0.3 inches) and did not show visual symptoms of Rhizoctonia blight or fusiform rust.

<sup>2</sup> Pt = Pisolithus tinctorius ectomycorrhizae ; NI = naturally occurring ectomycorrhizae formed mainly by Thelephora terrestris; Pruned = vertically root pruned to 8-inch depth between seedling rows in mid-August and in late September.

<sup>3</sup>Pt index = a x (b/c) where a = percent of seedlings with Pt ectomycorrhizae, b = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and c = percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.



Table 2. Field performance of longleaf pine after 3 years on three sandhills sites following specific nursery treatments at the South Carolina Coastal Nursery.

Outplanting sites and nursery cultural treatments <sup>2</sup>	Survival	Active height growth <sup>3</sup>	Height	Root-collar diameter	Seedling volume (x 10 <sup>2</sup> ) <sup>4</sup>	Plot volume index <sub>2</sub> <sup>4</sup> (x 10 <sup>2</sup> )
	(%)	(%)	(cm)	(cm)	(cm <sup>3</sup> )	(cm <sup>3</sup> )
<b>Site 1</b>						
Pt-pruned	82.0a	93a	54.1a	3.70a	9.3a	19.4a
NI-pruned	73.5ab	87b	49.2ab	3.61a	8.3a	15.5ab
Pt-not pruned	77.0ab	90ab	53.1a	3.71a	9.2a	17.3a
NI-not pruned	69.5b	87b	38.1b	3.39a	5.6b	9.9b
Means - site 1	75.5A	89A	48.7A	3.60A	8.1A	15.5A
<b>Site 2</b>						
Pt-pruned	63.0a	91a	47.4a	3.46a	7.6a	12.7a
NI-pruned	67.5a	81b	38.8ab	3.12b	5.3b	9.0b
Pt-not pruned	58.5a	83b	39.9ab	3.19b	5.9b	9.3b
NI-not pruned	59.0a	81b	31.4b	3.02b	4.2b	6.6b
Means - site 2	62.0B	84AB	39.3B	3.20B	5.7B	9.4B
<b>Site 3</b>						
Pt-pruned	72.0a	83a	32.8a	3.29a	5.0a	9.2a
NI-pruned	59.5b	66b	22.5ab	2.79ab	2.7b	4.0c
Pt-not pruned	63.0b	83a	28.5ab	3.11ab	3.9ab	6.7b
NI-not pruned	53.0b	68b	18.9b	2.66b	2.2b	3.5c
Means - site 3	61.9B	75B	25.7C	2.96C	3.4C	5.9C

<sup>1</sup> Means for treatments on each site followed by a common lowercase letter are not significantly different at P = 0.05; Site means followed by a common uppercase letter are not significantly different at P = 0.05.

<sup>2</sup> Pt = Pisolithus tinctorius ectomycorrhizae; NI = naturally occurring ectomycorrhizae formed mainly by Thelephora terrestris; Pruned = vertically root pruned to 8 inch depth between seedling rows in mid-August and in late September.

<sup>3</sup> Percent of surviving trees with > 10 cm height growth.

<sup>4</sup> Seedling volume = height x (root collar diameter)<sup>2</sup>; Plot volume index = total of seedling volumes per plot.

Table 3. Morphological characteristics and cull percentages of longleaf pine seedlings following various nursery treatments at the Experimental Nursery, Athens, GA.

Nursery treatments <sup>2</sup>	Cull (%)	Root-collar diameter (mm)	Fresh weight (g)		Shoot/root ratio	Pt index <sup>3</sup>
			Shoot	Root		
<b>Pt ectomycorrhizae pruned</b>						
6 seedlings/ft <sup>2</sup>	10.1c	15.1a	72.9a	32.4b	2.25b	65a
9 seedlings/ft <sup>2</sup>	32.9b	13.1ab	58.0ab	21.8ab	2.66ab	65a
12 seedlings/ft <sup>2</sup>	36.3b	12.7b	48.0b	16.3a	2.94ab	60a
15 seedlings/ft <sup>2</sup>	53.8a	12.5b	50.0b	16.0a	3.13ab	45bc
<b>not pruned</b>						
6 seedlings/ft <sup>2</sup>	9.4c	14.6a	74.3a	21.8ab	3.41a	25c
9 seedlings/ft <sup>2</sup>	30.2b	13.9ab	57.9ab	17.7a	3.27a	30c
12 seedlings/ft <sup>2</sup>	37.4b	13.2ab	55.0ab	17.0a	3.24a	40bc
15 seedlings/ft <sup>2</sup>	56.8a	12.2b	47.0b	15.5a	3.03ab	35c
Mean Pt ectomycorrhizae	33.4A	13.4A	57.9A	19.8A	2.99A	
<b>Natural ectomycorrhizae pruned</b>						
6 seedlings/ft <sup>2</sup>	10.5d	14.0a	75.1a	24.9a	3.02ab	0
9 seedlings/ft <sup>2</sup>	28.9b	12.8b	59.0ab	18.1ab	3.26a	0
12 seedlings/ft <sup>2</sup>	39.4c	12.4b	46.5b	20.4ab	2.28b	0
15 seedlings/ft <sup>2</sup>	56.7a	12.6b	47.5b	18.8ab	2.53b	0
<b>not pruned</b>						
6 seedlings/ft <sup>2</sup>	7.9d	14.0a	63.1ab	19.4ab	3.25a	0
9 seedlings/ft <sup>2</sup>	34.3bc	13.1ab	59.6ab	18.0ab	3.31a	0
12 seedlings/ft <sup>2</sup>	35.5bc	12.5b	42.1b	17.6b	2.39b	0
15 seedlings/ft <sup>2</sup>	55.4a	12.1b	41.8b	17.5b	2.39b	0
Mean natural ectomycorrhizae	33.6A	12.9A	54.3A	19.3A	2.80A	

<sup>1</sup> Means in a common ectomycorrhizal treatment sharing a common lowercase letter are not significantly different at P = 0.05; overall means of ectomycorrhizal treatments sharing a common uppercase letter are not different.

<sup>2</sup> Pt = Pisolithus tinctorius ectomycorrhizae; NI = naturally occurring ectomycorrhizae formed mainly by Thelephora terrestris; Pruned = vertically root pruned to 8 inch depth between seedling rows in mid-August and in late September.

<sup>3</sup> Pt index = a x (b/c) where a = percent of seedlings with Pt ectomycorrhizae, b = average percent of feeder roots with Pt ectomycorrhizae (including 0 percent for those without Pt), and c = percent of feeder roots with ectomycorrhizae formed by Pt and other fungi.

Table 4. Field performance of longleaf pine after 4 years on a sandhills site 1 following specific nursery treatments at the Experimental Nursery, Athens, GA.

Nursery treatments <sup>2</sup>	Survival	Height <sup>3</sup>	Root-collar diameter	Seedling volume (x 10 <sup>2</sup> ) <sup>4</sup>	Plot volume index <sub>2</sub> <sup>4</sup> (x 10 <sup>3</sup> )
	(%)	(cm)	(cm)	(cm <sup>3</sup> )	(cm <sup>3</sup> )
Pt ectomycorrhizae					
pruned					
6 seedlings/ft <sup>2</sup>	82.4a	98.8a	4.9a	2.5a	52.6a
9 seedlings/ft <sup>2</sup>	85.6a	87.6abc	4.6ab	2.1abc	43.9ab
12 seedlings/ft <sup>2</sup>	83.2a	84.4abc	4.5bc	1.8bc	38.0b
15 seedlings/ft <sup>2</sup>	80.8a	75.6bc	4.5bc	1.7bc	33.8bc
not pruned					
6 seedlings/ft <sup>2</sup>	63.2bc	91.6ab	4.6ab	2.2ab	35.0bc
9 seedlings/ft <sup>2</sup>	52.0c	74.4c	4.4bc	1.6c	21.6d
12 seedlings/ft <sup>2</sup>	73.6ab	82.4abc	4.6abc	1.9bc	35.6bc
15 seedlings/ft <sup>2</sup>	64.0bc	73.0c	4.3c	1.6c	25.0cd
Mean Pt ectomycorrhizae	73.1A	83.5A	4.6A	1.9A	35.7A
NI ectomycorrhizae					
pruned					
6 seedlings/ft <sup>2</sup>	82.4a	94.6a	4.7a	2.4a	49.1a
9 seedlings/ft <sup>2</sup>	69.6ab	82.0ab	4.6ab	1.9ab	32.7b
12 seedlings/ft <sup>2</sup>	68.8ab	76.4b	4.5b	1.6b	28.4bc
15 seedlings/ft <sup>2</sup>	69.6ab	74.0b	4.4b	1.6b	28.0bc
not pruned					
6 seedlings/ft <sup>2</sup>	46.4cd	75.0b	4.4b	1.6b	19.5cd
9 seedlings/ft <sup>2</sup>	59.2bc	76.0b	4.4b	1.6b	23.2bcd
12 seedlings/ft <sup>2</sup>	52.8cd	72.0b	4.4b	1.5b	20.0cd
15 seedlings/ft <sup>2</sup>	40.0d	71.6b	4.3b	1.5b	14.6d
Mean NI ectomycorrhizae	61.1B	77.7B	4.5A	1.7A	26.9B

<sup>1</sup> Means in a common ectomycorrhizal treatment sharing a common lowercase letter are not significantly different at P = 0.05; means between ectomycorrhizal treatments sharing a common uppercase letter are not different.

<sup>2</sup> Pt = Pisolithus tinctorius ectomycorrhizae; NI = naturally occurring ectomycorrhizae formed mainly by Thelephora terrestris; Pruned = vertically root pruned to 8-inch depth between seedling rows in mid-August and in late September.

<sup>3</sup> From 98 to 100 percent of seedlings in all treatments were in active height growth (>10 cm).

<sup>4</sup> Seedling volume = height x (root collar diameter)<sup>2</sup>; Plot volume index = total of seedling volumes per plot.