

# PROCEEDINGS

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IMPACT OF NURSERY MANAGEMENT PRACTICES ON HERITABILITY  
ESTIMATES AND FREQUENCY DISTRIBUTIONS OF FIRST-ORDER  
LATERAL ROOTS OF LOBLOLLY PINE

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Abstract.--Frequency distribution and heritability of first-order lateral root (FOLR) numbers in 1-0 seedlings were followed for 5 years for 115 different half-sib seedlots from the Georgia Forestry Commission's Arrowhead and Baldwin Seed Orchards. In 1986 and 1987, seedlings were permitted unrestricted growth under management conditions similar to those practiced in most forest tree nurseries in the Southern United States. Seedlings were placed in four different classes based on the number of FOLR with proximal diameters above a threshold value. These classes were 0-3, 4-5, 6-7, and >8 FOLR. In 1988, 1989, and 1990, management practices were altered to restrict height growth by 30 to 40 percent for each of four FOLR groupings. The associated reduction in seedling sizes had very little affect on either FOLR family mean heritability estimates or the assumption of truncated normality for FOLR data. Restricting growth did reduce FOLR proximal diameters, but in each data set the seedlings in the FOLR group with the fewest FOLR (0-3 in 1986 and 87, 0-2 in subsequent years) were always significantly smaller than those in the other groups. Not only were seedlings in this first FOLR group of smaller size, they also had unfavorable traits: succulent stems, preponderance of primary needles, and poorly developed or absent terminal buds.

Key Words: restricted and unrestricted growth, artificial regeneration

INTRODUCTION

Protocols for predicting the growth potential of progeny from specific mother-trees have been under development since tree improvement programs were initiated in the late 1940s. Heritability trials for tracking specific traits are standard procedures in all tree improvement programs. However, heritability estimates for given morphological and physiological traits vary significantly among different tests, and between fertility levels in the same tests, and estimates for juvenile and mature trees are often poorly correlated (Zobel and Talbert 1984, Bailian et al 1989, Gerhold and Stanton 1987, Bridgwater and Williams 1987). It is difficult to identify the causes of variation in heritability estimates for a specific trait because heritability estimates carry

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large associated errors, even when large numbers of families and progeny are used (Zobel and Talbert 1984). Silviculturists and forest managers generally agree that early testing for traits that influence progeny performance and subsequently improved yields are necessary if we want meaningful gains in the near future.

Before inorganic fertilizers, pesticides, and modern irrigation systems were heavily used in forest tree nurseries, seedling grading systems based primarily upon stem morphological characteristics proved effective (Wakeley 1954). Growing conditions were not ideal, seedling growth was unrestricted, and the grading systems identified small noncompetitive seedlings. Modern management practices, however, significantly increased the sizes of all the southern pine seedlings in seedbeds. As a result, morphological grading no longer worked well, and it rapidly fell from favor among nursery managers.

In both tree improvement programs and artificial regeneration systems in the Southern United States, the production of seedlings of uniform size became highly desirable. Seedling production methods, therefore, were altered to restrict the growth of the fastest growing seedlings (USDA 1985). Greater uniformity in seedling sizes was generally achieved by restricting growth with mechanical procedures such as top clipping, root wrenching and undercutting. Uniformity had a considerable biological cost. The more vigorous and competitive seedlings were adversely affected by repeated top clipping while the slower growing noncompetitive seedlings benefited. These mechanical procedures produced uniform seedlings in which differences in growth potential after outplanting could not be identified.

In recent studies of the heritability and frequency distribution of numbers of first-order lateral roots (FOLR) in half-sib loblolly pine progeny, the seedlings were grown using currently accepted fertility and water regimes in southern forest tree nurseries but growth of seedlings was unrestricted (Kormanik and Muse 1989, Kormanik et al. 1990). In these early trials, even the smallest seedlings in practically all of the half-sib seedlots were from 12 to 20 cm taller than Wakeley's grade 2 seedlings and had comparable or larger root collar diameters (RCD) (3.2 mm). Heritability estimates of FOLR numbers, based on plot means, were found to be ca  $0.76 \pm .09$  (Kormanik et al. 1990). Over 13,000 seedlings were established over a 2-year period in field research studies. Only diseased and mechanically damaged seedlings were excluded from these outplanting trials. Even though severe droughts occurred throughout the Southeast during this period, survival of these seedlings averaged between 80 and 85 percent.

Identification of FOLR characteristics was based upon unrestricted growth of seedlings that permitted nursery bed competition ( $280/m^2$ ) but resulted in favorable root/top ratios. Such large seedlings were undesirable for outplanting in a commercial operation. Even the progeny of the best mother trees were not uniform in size. Unrestricted growth, under these conditions, allowed the competitive ability of seedlings in the seedbeds to be expressed. Non-competitive individuals all appeared to share several undesirable stem traits such as succulent stems, little secondary needle development, and lack of terminal buds. These undesirable traits were also noted by Wakeley (1954), but have received little emphasis since the late 1950s because mechanical procedures have been used to obtain seedling uniformity since that time.

Since changes in management practices significantly altered the value of stem morphology as an indicator of quality (Wakeley 1954), we became concerned that management practices that restricted seedling development might also affect the lateral root morphology. If so, heritability estimates and our ability to assess seedling competitiveness based upon root morphological development might also be affected. These concerns led to the studies we now describe.

## METHODS

All study seedlings were grown at the Institute of Tree Root Biology (ITRB), Whitehall Experimental Nursery, Athens, Georgia. All mother-tree seedlots were obtained from the Georgia Forestry Commission, which collected them in 1986 through 1990.

Procedures for growing seedlings with unrestricted growth in 1986 and 1987 are reported in detail elsewhere (Kormanik et al. 1990). Progeny from 12 mother trees in 1986 and 25 mother trees in 1987 were included in the test. The two criteria of FOLR were: (1) regardless of length, diameter at the proximal end  $\geq 1$  mm; and (2) maturity, rigidity, and suberization of the periderm.

In 1988, 34 half-sib seedlots were used. Total nitrogen application was reduced from approximately 70 ppm to 25 ppm (rates equivalent to 140 and 50 lbs N/acre) for the entire growing season. Nitrogen ( $\text{NH}_4\text{NO}_3$ ) was applied at 2.5 ppm (5 lbs N/acre) in mid-May and again in the last week of May; and at 5 ppm (10 lbs N/acre) on June 5, June 23, July 2, and July 20. Fall N application was avoided to enhance fall foliar discoloration common to loblolly pine seedlings. The proximal diameter of roots to be counted was reduced to 0.5 mm to reflect the reduction in seedling sizes resulting from reduced soil fertility, and the FOLR count classes were altered to 0-2, 3-5, 6-7 and  $\geq 8$  to reflect a reduction in overall seedling development. The FOLR classes used in 1986-1987 were 0-3, 4-5, 6-7, and  $\geq 8$ .

Water was applied as needed until the final nitrogen top dressing. After that, water was applied weekly at 3.0 cm per week when rainfall did not occur or was limiting. Watering was continued until first frost in mid-November. The seedlings were lifted and outplanted in late January. A total of 200 seedlings from each mother tree (100 from each replication) were included in the out-planting, and only mechanically damaged or rust-infected seedlings were excluded from the data set. No more than 1 percent of the seedlings in any family were infected with rust. Approximately 6,800 graded seedlings grown in the 1988 nursery trial were outplanted.

In 1988, we began to assay seedlings for the enzymes involved in sucrose utilization to assess sink strengths of roots and tops throughout the year. The three enzymes that catalyze sucrose breakdown (sucrose synthase, acid invertase, and neutral invertase) were assayed according to procedures reported by Sung and others (1989a and b). The purpose of these assays was to determine if seasonal patterns in the activity of these three enzymes could help in clarifying the causes for the periodicity in growth between roots and tops of loblolly pine seedlings. Beginning in mid-July and continuing for 12 months, the activities of sucrose breakdown enzymes in the taproot and stem cambial tissue extracts were measured biweekly with a Beckman DU-7 spectrophotometer. Composite samples

of 3 g of cambial tissue were obtained by scraping the exposed cambial surface of 15 to 50 debarked stems or roots. More seedlings were needed earlier in the season and fewer later as the seedling sizes increased. Activities of all three sucrose breakdown enzymes were assayed from a single root or stem extract.

In 1989, 34 loblolly pine half-sib seedlots were included in the test. Essentially the same management protocol as in 1988 was followed throughout the growing season. In mid-September, after budset was completed in over 90 percent of the seedlings in all seedlots, nitrogen was applied at the rate of 10 ppm (rate equivalent to 20 lbs N/acre) to enhance carbon production. Based on 1988 enzyme assays, we believed that this was when carbon would be shunted to the roots. The total amount of N applied was 35 ppm (rate equivalent to 70 lbs N/acre). Again, 100 seedlings from each replication from each mother tree were lifted in mid-December. None of the seedlings in this trial were outplanted. However, over 2,000 seedlings were transplanted to other nursery beds and during the next 12 months sucrose metabolism was monitored in these transplants by periodically assaying the three enzymes followed in 1988.

During the 1990 growing season, 24 additional half-sib seedlots were included in the trials. The quantity of nitrogen applied by mid-July was the same as in the previous 2 years, but at bud set in September only 7.5 ppm (rate equivalent to 15 lbs N/acre) were applied. The total for the year was thus 30 ppm (rate equivalent to 60 lbs N/acre). Watering was altered from previous trials. In 1990, three model R irrometers (Irrometer Company, Riverside, CA) were placed in each bed; all were placed in the approximate bed center to a depth of 15 to 20 cm (6 to 8 inches). One was placed in the middle of the bed and the other two were placed approximately 3.5 (ca 10 feet) from either end. After the last nitrogen treatment in mid-July, supplemental watering was used only after the irrometers within a bed all registered 70 centibars for 3 consecutive days. At that time, 3 cm of water were applied. The seedlings were harvested in late February. No field testing was done, but more than 1,000 seedlings were transplanted within the nursery to follow the seedlings for one additional year. The same number of seedlings from each mother tree were used in the heritability data set as in previous trials.

#### ANALYSIS

Data from each experiment were examined by ANOVA. For each year's data, the FOLR sample cumulative distributions for individual families were compared using the Smirnov nonparametric procedure (Conover 1980). Underlying theoretical distributions for FOLR were then examined using Kolmogorov's goodness of fit procedures. For each year's data and for the combined data, hypotheses of approximate normality and truncated normality were tested using nontransformed and square-root transformed FOLR values.

Family mean heritability was estimated for each year's data based on the analysis of variance of plot means. Calculations were based on  $h^2 = (MSF - MSF_{xR}) / MSF$  where MSF and MSF<sub>xR</sub> denote the respective mean squares for family and family by replication interaction. Since the data for many families did not fit a normal distribution, the within-plot variance estimate was not utilized in estimating heritability. It is recognized that failing to correct for within-plot variance tends to inflate the heritability estimate; however,

since individual family sample sizes were large, the resulting inflation was considered negligible.

## RESULTS AND DISCUSSION

The ANOVAs used to calculate heritability estimates for the data set for each year are shown in Table 1, and the heritability estimates are shown in Table 2. The percentage of seedlings in each FOLR group and average heights and root collar diameters of the seedlings for all combined mother trees are shown in Table 3.

Table 1. ANOVA of FOLR plot means for loblolly pine for years 1986-1990.

Source	df	Sum of squares	Mean squares	Variance component est.
1986				
reps	3	7.282634	2.437545	
families	11	30.149740	2.740885	0.527047
fam x reps	33	20.879008	0.632697	0.632697
1987				
reps	1	0.083988	0.083988	
families	24	22.516832	0.938201	0.359221
fam x reps	24	5.274240	0.219760	0.219760
1988				
reps	1	0.334448	0.334448	
families	33	20.402580	0.618260	0.197262
fam x reps	33	7.350273	0.222736	0.222736
1989				
reps	1	0.441934	0.441934	
families	19	8.005344	0.421334	0.158946
fam x reps	19	1.965398	0.103442	0.103442
1990				
reps	1	2.618002	2.618002	
families	23	13.903398	0.604496	0.209351
fam x reps	23	4.273248	0.185793	0.185793

In 1986 and 1987, seedlings were permitted unrestricted growth under soil fertility and water regimes comparable to those in most southern pine nurseries (Kormanik et al. 1990, USDA 1985). The one exception was that we used less nitrogen top dressing and eliminated fall applications of both nitrogen and potash. Seedling sizes, heritability estimates, and FOLR groupings were comparable for these 2 years (Table 2 and 3). The initial field survival (>80%) and first year development of these large seedlings, both established during the

Table 2. Family mean heritability estimates for number of FOLR of loblolly pine seedlings.

Year	No. families	$h^2$ est	S.E.
1986	12	0.769	0.170
1987	25	0.766	0.292
1988	34	0.640	0.140
1989	20	0.754	0.138
1990	23	0.693	0.158
Pooled*		0.712	0.088

\* Pooled data is presented only to illustrate the uniformity of the data sets in spite of the altered nursery practices and were not used in statistical analyses reported here.

extreme drought years of 1987 and 1988, were satisfactory. It was obvious, however, that the seedlings were large enough to cause problems for commercial machine planting and too large for typical hand planting.

Stem heights and RCD were smaller in all FOLR groups in 1988 than in 1986-87, when growth was restricted (Table 3). Normal fall needle discoloration occurred but no attempt was made to eliminate it through fall application of nitrogen. First-year survival of these seedlings was ca 85 percent at the Savannah River Forest Station, Aiken, South Carolina.

In 1989, the nitrogen applied in September prevented normal needle discoloration during the winter but did not result in any obvious elongation in stems and little differences in FOLR proximal root diameters. The heritability values were numerically comparable to those in the 1986-87 tests and not significantly different among the five data sets (all differences are less than 1 standard deviation). A beneficial response to the September nitrogen application was more abundant feeder root and mycorrhizal development that seemed to accompany the increase in fall root metabolism. We did not have a nitrogen control to verify this observation, but increased mycorrhizal development after mid-August has been reported by others (Marx et al. 1986, Marx and Cordell 1987).

In 1990, we again topdressed seedlings with nitrogen in the fall but we also began controlling moisture after the final nitrogen application in mid-July. Monitoring moisture allowed us to more closely control height growth. This further reduction in top growth did not affect the heritability estimates of FOLR development and yet resulted in desirable RCDs. The reduction in percentage of seedlings in the lowest FOLR group here was due directly to six families. In each of the previous 5 years, different half-sib seedlots were used and normally only one to three lots produced seedlings with significantly lower percentages of seedlings in the lowest FOLR group. In 1990, 6 of the 24 seedlots had 11 percent or fewer seedlings in the lowest FOLR group. This difference, however, did not significantly affect the heritability estimates or

Table 3. Average heights and root collar diameters by FOLR groups for 1986-1990 studies for combined families<sup>1</sup>

FOLR group	Total seedlings (%)	Height (cm)	RCD (mm)
1986			
0-3	36	40.4	4.0
4-5	25	46.4	5.1
6-7	21	48.2	5.8
>8	18	49.8	6.5
1987			
0-3	38	38.4	3.8
4-5	20	46.5	5.4
6-7	18	49.1	6.0
>8	24	50.0	7.1
1988			
0-2	33	28.9	3.2
3-5	47	32.8	4.0
6-7	15	34.4	4.6
>8	5	35.0	5.0
1989			
0-2	35	27.4	2.8
3-5	36	32.9	4.0
6-7	16	34.7	4.7
>8	13	36.4	5.5
1990			
0-2	18	22.7	3.2
3-5	49	26.5	4.2
6-7	20	29.1	4.8
>8	13	29.5	5.4

<sup>1</sup> Compiled data sets on file for each mother tree by Georgia Forestry Seed Orchard designation at the Institute of Tree Root Biology, Athens, Georgia.

the truncated normality of this data set compared to those for other years. As in 1989, fall application of nitrogen appeared to enhance root and mycorrhizal development in comparison with development in years when no nitrogen was applied in the fall.

When it became apparent in the mid-1980s that FOLR numbers were associated with competitive ability of open-pollinated seedlings in the nursery (Kormanik 1986), studies were initiated at the Institute of Tree Root Biology to determine the approximate heritability of FOLR numbers for loblolly pine. During the past

5 years, it has become clear that half-sib progeny are not equal in their competitive potential even against their own siblings in nursery beds. We also determined that nitrogen and supplemental watering could be used to regulate seedling sizes. These cultural treatments to control seedling growth had only a minor influence on root morphology. Silviculturally, the FOLR relationship is of considerable interest because it provides a basis for grading loblolly pine seedlings according to their early competitive potential.

When assessing root development of loblolly pine we had noted that regardless of seedling development in different State nurseries, low numbers of FOLR were associated with specific undesirable stem characteristics. These traits were similar to those reported by Wakeley (1954) for his small cull or grade 3 seedlings. While these stem characteristics were useful as indicators when we set up the FOLR groupings used in the initial heritability studies in 1986-87, we had not expected them to be expressed when we permitted unrestricted growth of seedlings (Kormanik and Muse 1986, Kormanik et al. 1990).

In 1988, seedlings were sampled throughout the year to biochemically assess stem and root growth and development. As in sweetgum, pecan, potato, and lima bean (Sung et al. 1989a and b), sucrose synthase activity was the dominant sucrose breakdown enzyme activity in stems and roots of loblolly pine. The seasonal patterns of sucrose synthase activities in stems and in roots coincided with stem and root morphological development reported by Wakeley (1954). Sucrose synthase activity in seedling roots lagged behind that in stems until early fall. Throughout the winter, roots continued to utilize sucrose, but at a slower rate than in the fall. Stem sucrose synthase activity was lowest during the winter and did not return to a high level until mid-March. During active shoot elongation, the previous year's stems and roots were both competing for sucrose with newly formed shoots. In April and May, stem sucrose synthase activity decreased 40% and root activity decreased 70% from levels measured in March. Neutral invertase activities in stems and roots were lower than those of the other enzymes throughout the year, and no seasonal patterns were detected. Acid invertase was more active than neutral invertase at all times. Acid invertase activity sometimes was higher than sucrose synthase activity. We are in the process of correlating acid invertase and sucrose synthase activities in seedlings under various environmental stresses.

High enzyme activity in stems reflects high use of sucrose for stem elongation. Our approach to restricting top growth therefore was to limit the nitrogen supply when stems were elongating. We did not know how these changes would affect FOLR development, but we expected significant changes in heritability estimates based on other published papers. The expected changes did not occur (Table 2). FOLR diameter at the proximal end declined when top growth was reduced, but other morphological characteristics of FOLR did not change. The reduction in FOLR diameter would have resulted in an increase in percentage of seedlings in the smallest FOLR category in 1988 if the 0-3 category had been maintained. This reduction in class ranges had little effect on heritability estimates (Table 2), which are based on total number of FOLR and not on FOLR classes. On the basis of stem characteristics--stem succulence, high proportion of primary needles, and lack of or poor development of terminal buds associated with individual FOLR numbers--the critical FOLR diameter was reduced from 1.0 mm to 0.5 mm.

## CONCLUSION

Restricting loblolly pine seedling height growth by taking advantage of carbon allocation distribution patterns does not affect the FOLR heritability estimates or the truncated normality observed in loblolly pine seedlings with unrestricted growth. Morphological stem characteristics common to seedlings in the 0-3 FOLR group with unrestricted top growth were found in the 0-2 FOLR group when growth was restricted. Thus it appears that restricting seedling development in the nursery by reducing soil fertility when stems are elongating will permit identification of noncompetitive seedlings. Such identification has important practical implications. FOLR contributions to later competitiveness are currently being tested in field plantings.

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