STEM-CUTTING PRODUCTION AND ROOTING PERFORMANCE IN AN S2 POPULATION OF LOBLOLLY PINE[1]

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Abstract.-- An inbred population of loblolly pine (*Pinus taeda* L.) was formed by self-pollinating for two generations. The first generation (SI) was grown in field plots on the Harrison Experimental Forest (HEF) in south Mississippi. All flowering (female and male) SI trees, 16 years after planting, were self-pollinated to produce the second generation (S2). Seedlings of the S2 families and a related outcross control family were repeatedly hedged to promote the production of multiple shoots. On each of nine hedging dates, the shoots were collected and set for rooting under intermittent mist. Each seedling hedge was scored for the number of useable cuttings produced and for the number of cuttings that later developed supporting root systems. The average number of useable cuttings per seedling increased from 1.0 to 22.2, while the proportion of cuttings that rooted decreased from 0.41 (in cycle 2) to 0.01. All families responded similarly over the nine cycles for both traits. The differences between the outcross control and the S2 families were significant for cutting production in six cycles and non-significant for proportion rooted in all cycles. The poor rooting response to this rapid hedging and propagation system was unexpected and was not observed in a comparable study of SI and outcrossed families of slash pine (P. *elliottii* Engelm. var. *elliottii*). Additional studies will be necessary to determine the cause of this response and whether the response applies to a wider range of loblolly pine genotypes.

Keywords: vegetative propagation, inbreeding, *Pinus taeda* L.

INTRODUCTION

Inbred line development followed by hybrid breeding has several drawbacks as an improvement method for the southern pines (Franklin 1969a, 1969b; Snyder 1972; Sniezko and Zobel 1986). Some of these drawbacks are similar to those encountered in corn improvement where the inbreeding-hybridization method is preferred. Both species are highly heterozygous and, as a result, suffer from severe inbreeding depression, making line development and testing difficult. Additionally, both species are extremely difficult to propagate clonally, reducing the opportunity for within-line selection and presenting problems with the commercial increase of promising hybrids. The most obvious drawback to the inbreeding-hybridization method for southern pine improvement is the generation length, which is currently 5 to 10 years under optimal conditions.

Beyond inbred line development for hybrid breeding purposes, inbreeding can produce useful stocks for genetic research. Inbred materials are useful for calibrating genetic diversity measures (Kuhnlein et al. 1990), revealing novel mutants available for genetic mapping or gene cloning (Franklin 1969c), developing near isogenic lines for gene function and transformation studies, and for producing homogeneous seed-propagated populations. Obviously, most of the

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same drawbacks that apply to the inbreeding-hybridization improvement method also apply to inbred line development for genetic research purposes. However, the potential benefits seem to outweigh the drawbacks, especially considering the recent technological advances in molecular genetics, and accelerated breeding and clonal propagation of the southern pines (Nance and Nelson 1989).

In light of these advances, we have initiated an inbreeding program within a loblolly pine population of east Texas origin. The objective of this program is to produce as many multi-generation, self-pollinated lines as possible in as short a time as possible. Tests of inbreeding depression will be made during this process in an effort to develop performance versus homozygosity (inbreeding coefficient) curves for several traits. Toward this end, we are currently producing SO, SI, S2, and S3 seed within the study population. Here we describe results from a vegetative propagation study in which we repeatedly removed all shoots (stem-cuttings) from six S2 families and an outcrossed control family and evaluated the families and clones for stem-cutting production and rooting performance.

MATERIALS AND METHODS

Population

Founding parent trees were originally selected (randomly) as parents in a 10-tree half-diallel mating design involving 6 seed sources (Synder 1967). Trees in two sources— Conroe and Nacogdoches, Texas— were self- and cross-pollinated according to a half-diallel design with selfs. The self-pollinated families (S1) were sown in the Harrison Experimental Forest (HEF) nursery (20 miles north of Gulfport, MS) and outplanted to a field site on the HEF. After 16 years in the field, all flowering SI trees were self-pollinated (February 1988) to produce the S2 generation (F, inbreeding coefficient, =0.75). Fourteen trees were flowering, thirteen with female and male flowers and one with female only. The female-flower-only tree was pollinated with pollen from eight unrelated SI trees. At cone-collection time, the cones from the different crosses on this tree were mixed, effectively resulting in a pollen-mix (half-sib) family (F=0). Table 1 gives the pollination and germination data for the 13 S2 crosses and the 1 outcross.

All S2 families and the outcross control family were germinated after cold stratification and sown in Ray Leach pine (4 in^3) cells (April 1990). For families with 22 or less sound seeds (Table 1), all seeds were used. Twenty seeds were used for the other S2 families, and 50 seeds for the outcross control. The seedlings were hedged initially 9 weeks after germination and later transplanted to the HEF Cutting Orchard. Several of these genotypes in each family will be clonally propagated as rooted cuttings and moved into the accelerated breeding program to serve as parents for the S3 generation.

Experiment and Propagation Methods

Remnant seeds from 6 of the 13 S2 families and the outcross control family were stratified, germinated, and sown in Ray Leach stubby (7 in^3) cells (June 1991). Following germination, the 6 S2 families were represented by 12 to 29 seedlings and the outcross control by 38 seedlings. These seedlings were arranged into a randomized complete block (RCB) design with four replications, placing one-quarter of the seedlings from each family in each replication. Over a 19-month period (October 1991 to April 1993), the seedlings were hedged 9 times (Table 2). The length of time between hedgings varied depending on the population's rate of response to the hedging. When most (approximately 90% based on visual examination) of the shoots had elongated to at least 7 cm, the seedlings were hedged. In March 1992, after the third hedging, the seedling hedges w ere transplanted to 3.78-L pots containing pine bark, peat, and vermiculite (2:1:1) and moved outdoors.

Table I. Identification, seed, and germination data for the 14 SI loblolly pine parent trees.

SI family ID[a]	SIFG clone[b]	seed extracted	sound seed[c]	sound seed (%)	germinated / sown
2-5	1238	4500	120	2.7	13/20
2-5	1239	415	22	5.3	16/20
3-7	1240	2685	108	4.0	14/20
3-5	1241	100	13	13.0	9/13
3-4	1242d	350	13	3.7	8/13
2-8b	1243	1290	21	1.6	18/21
2-8b	1244	2500	61	2.4	18/20
3-8	1245	3350	90	2.7	13/20
3-8	1246	1835	96	5.2	18/20
2-5	1247	715	41	5.7	17/20
2-5	1248d	23	2	8.7	2/2
3-7	1249e	835	398	47.7	47/50
2-8a	1250	435	19	4.4	17/19
2-8b	1251	180	10	5.6	8/10
S2 family	totalsd	18378	616	3.35	171/220

[a] Source ID - Parent tree (within-source) ID from the original half-diallel mating design (Synder 1967), where source 2 is Conroe, TX, and source 3 is Nacogdoches, TX.

[b] SIFG (Southern Institute of Forest Genetics) clone ID number (Mason et al. 1993), clones with the same SI family ID are full-siblings.

Number of seeds remaining after floating in distilled water (estimate of the number of filled seeds).

[d] Clone 1242 produced very little pollen and most cones on clone 1248 were damaged by pollen isolation bags.

[e] Clone 1249 was cross-pollinated with 8 unrelated SI pollens and is excluded from the totals.

On each hedging date, the number of useable cuttings (> 7 cm) for stem-cutting propagation were counted (#Cutt) and removed, and a randomly selected subset of these cuttings was immediately set for propagation. The subset included all cuttings for the first 4 hedgings (propagation cycles) and 10 or 15 cuttings per seedling for the last 5 cycles (Table 2). The cuttings were dipped in Hormodin 2 (a commercially available rooting powder containing 0.3% IBA), set in Ray Leach fir (3 in^3) cells containing a 2:3 ratio of peat and perlite, and placed in a propagation greenhouse. Moderate temperature (s 85 °F) and high humidity (> 85%) were maintained in the greenhouse with evaporative cooling, air conditioning, intermittent mist, and fog. Over the course of the study, both the seedling hedges and their cuttings were maintained in the RCB arrangement. When rooting was nearly complete (Table 2), the cuttings were moved to a standard greenhouse and scored for rooting— number of cuttings with a supporting root system (#Root). From this number, and the number of cuttings set (#Set), the proportion of rooted cuttings (pRoot) was calculated.

Data Analysis

Proportion of cuttings with supporting root systems (pRoot) and number of useable cuttings (#Cutt) were subjected to the analysis of variance procedure using the following models:

Family analysis within cycles--

$$Y = R + F + R*F + E,$$

Family analysis among cycles--

$$Y = P + R(P) + F + P*F + R*F(P) + E,$$

Clone analysis within reps among cycles--

$$Y = P + F + P*F + C(F) + E,$$

where Y is either pRoot or #Cutt, R is replication, F is family, P is propagation cycle, C is clone, and E is error. Analyses were restricted to cycles 2 through 9, since only 1 cutting was available per seedling in cycle 1. In the experimental design, replications were nested in propagation cycles (R(P)), and clones were nested in families (C(F)) and in replications. Thus, the clone analyses were restricted to within replications, resulting in four independent experiments. Prior to analysis, both independent variables were transformed- arcsin(pRoot) and $(\#Cutt+0.5)^{-0.5}$ -to stabilize the error variance (Steel and Torrie 1980). Proc GLM (SAS 1990), with Type III sums of squares, was used to compute the analyses of variance. A weighted analysis was utilized for pRoot, with the number of cuttings set (#Set) used as the weight. Terms involving replication and clone were considered random, while all others were considered fixed. F-tests were computed with the "random" statement and "test" option in proc GLM.

Table 2. Timing and magnitude of the propagation cycles.

Propagation cycle	Date hedged	Elapsed time(days)	Avg. #Set[a]	Avg. #Cutt[b]	Time(wks) in rooting	Avg. pRoot[c]
1	2 Oct 91	65	1.0	1.0	13	0.14
2	15 Nov 91	43	3.9	4.0	14	0.41
3	2 Mar 92	106	3.8	3.8	12	0.12
4	1 May 92	59	8.6	8.6	9	0.03
5	19 Jun 92	49	14.7	16.0	12	0.03
6	30 Jul 92	41	14.0	15.6	8	0.01
7	13 Sep 92	45	9.7	15.9	8	0.01
8	23 Nov 92	66	10.3	15.3	9	0.01
9	13 Apr 93	141	16.1	22.2	11	0.01

[a] Average number of cuttings set per seedling per cycle.
[b] Average number of useable cuttings per seedling per cycle.
Average proportion of cuttings that rooted per cycle.

RESULTS AND DISCUSSION

The percentage of sound seeds among the S2 families was very low, ranging from 1.6 to 8.7 (Table 1). The highest yielding clone, 1238, produced 120 sound seeds out of 4,500 seeds extracted. The outcross seed was 47.7% sound, a value 5.5 to 30.0 times greater than the S2 families. Germination values among the S2 families were moderate to high, ranging from 62% to 100%. The outcross seed attained 94% germination.

The mean number of useable cuttings per seedling hedge for each family is plotted over propagation cycles in Figure 1. The number of useable cuttings increased significantly between cycles 3 and 4, 4 and 5, and 8 and 9. The pattern of increasing number of useable cuttings was consistent for all families. Figure 1 also gives the mean number of useable cuttings per hedge in a comparable study of slash pine. The slash pine seedlings (3 SI and I 1 outcross families) were started 3 months later than the loblolly, but were given essentially the same treatment.

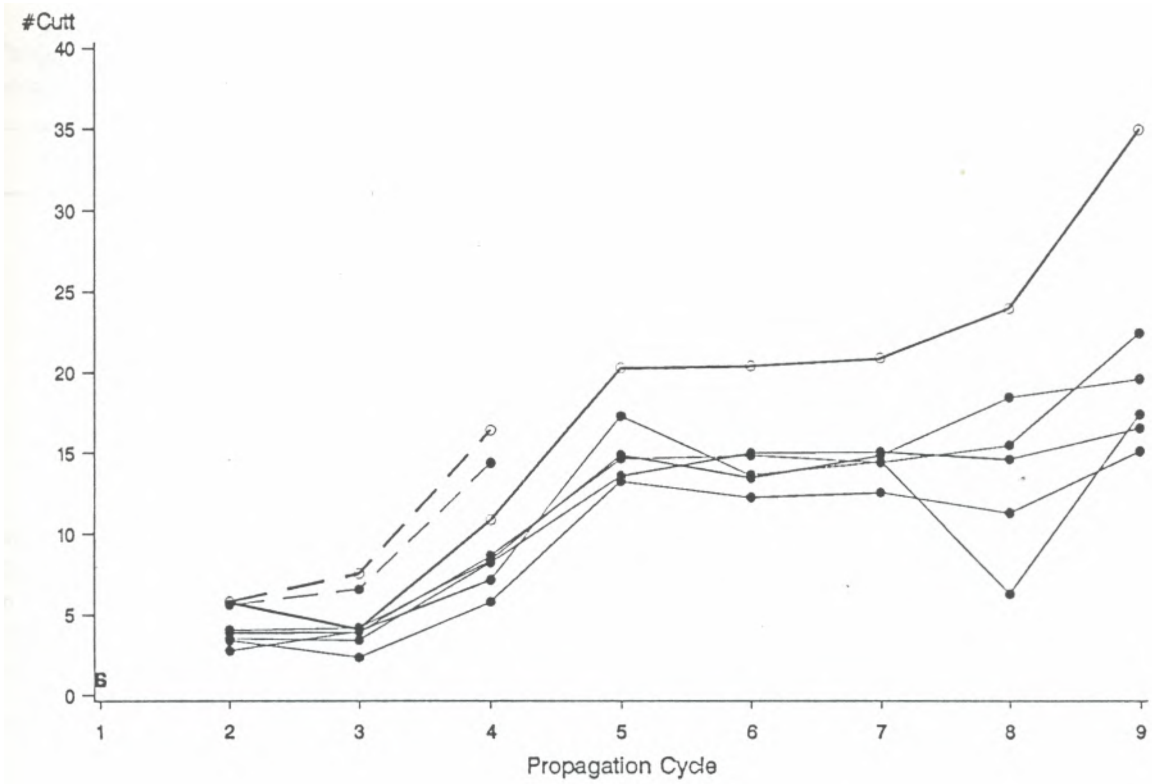

Figure 1. Plot of stem-cutting production (#Cutt) over propagation cycles for 5 S2 and 1 outcrossed family of loblolly pine and SI s (mean of 3 families) and outcrosses (mean of 11 families) of slash pine. Solid lines and 'L' represent loblolly pine and dashed lines and 'S' represent slash pine. Thin lines with filled circles represent the S2 and SI families and thick lines with empty circles represent the outcross families. The slash pine families were treated similarly and are included as a treatment control.

The mean proportion of cuttings rooted for each family is plotted over propagation cycles in Figure 2. The proportion rooted decreased dramatically between cycles 2 and 3 and 3 and 4. After cycle 4, the rooting proportion remained very low. The same pattern was observed for all families, which was unanticipated, since the rapid method of hedging and propagating was expected to maintain the seedling hedges in a juvenile stage for an extended period of time (5 or more years in a standard hedging system) (T.D. Caldwell, unpublished data).

Figure 2 also gives the mean proportion of cuttings rooted in the slash pine study. The slash pine cuttings received the same treatments and the rooting was completed at the same time in the same propagation greenhouse. However, since the slash pine seedlings were started later, cycles 2 to 5 of the loblolly correspond in time-of-year to cycles 1 to 4 of the slash. The slash pine data for both SI and outcrossed families showed no decline in proportion rooted through cycle 4 (Figure 2). From both a stem-cutting production and rooting performance perspective, it appears that slash pine will be more responsive to this propagation method.

Results of the family analyses of variance are presented in Table 3. As expected, propagation cycle was the dominant factor. Family was highly significant for both traits; however, the cycle*family and rep*family(cycle) interactions were also significant. Within cycles, family was always non-significant for proportion rooting and nearly always significant for number of useable cuttings (Table 3). The significant family variation was primarily due to the better performance of the outcross control, especially for number of useable cuttings. The outcross control produced the most cuttings in all cycles and attained the highest rooting proportion in four cycles (Figures 1 and 2). Results of the clone analyses of variance are presented in Table 4. Clone(family) variation was significant only in replication 2 for proportion rooted. In contrast, clone(family) variation was significant in all four replications for number of useable cuttings.

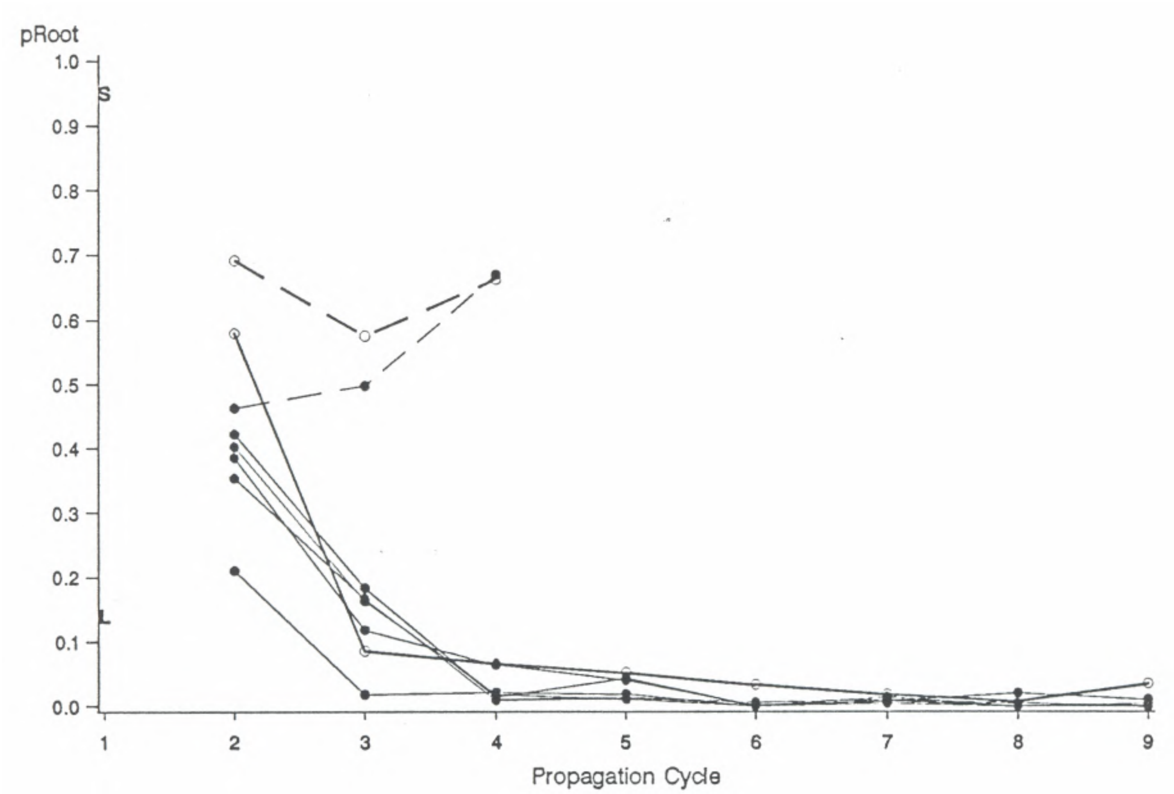

Figure 2. Plot of proportion of cuttings rooted (pRoot) over propagation cycles for 5 S2 and 1 outcrossed family of loblolly pine and S I s (mean of 3 families) and outcrosses (mean of 11 families) of slash pine. Solid lines and 'L' represent loblolly pine and dashed lines and 'S' represent slash pine. Thin lines with filled circles represent the S2 and SI families and thick lines with empty circles represent the outcross families. The slash pine families were treated similarly and are included as a treatment control.

The results of this study suggest a problem with the application of a rapid hedging and propagating system to loblolly pine. The frequent hedgings appear to have detrimentally affected the seedling hedges' potential to produce rootable cuttings. The number of useable cuttings increased, as expected, through cycle 5, then leveled off through cycle 8, and then increased again during cycle 9. The large increase in cycle 9 suggests that the hedges' potential to produce increasing numbers of cuttings was left intact. In the study, cycle 8 preceded and cycle 9 succeeded the first dormant season for the hedges. The increase in number of useable cuttings from cycle 8 to 9 was probably due to the occurrence of favorable physiological changes in the hedges induced by the intervening dormant season. For the same reason, we surmised that rooting performance would recover considerably in cycle 9, but it did not. Thus, the rootability loss does not appear to be closely related to dormancy or season. The seedling hedges will be outplanted to the HEF Cutting Orchard and periodically tested to further investigate these time trends in stem-cutting production and rooting performance.

Table 3. Results of the family analyses of variance of number of useable cuttings (#Cutt) and proportion of cuttings rooted (pRoot).

Family analysis among cycles--

Source	df	#Cutt MS	#Cutt F	pRoot MS	pRoot F
Cycle	7[a]	117	98.1***	21.4	55.9***
Rep(Cycle)	24	1.20	1.80*	0.40	1.68*
Family	5[b]	20.1	29.3***	0.97	4.31***
Cycle*Family	35	1.27	1.85**	0.41	1.66*
Rep*Family(Cycle)	120	0.69	1.48**	0.25	1.52***
Error	962	0.47		0.16	

Notes: Based on the family analysis within cycles model (see Materials and Methods)- Rep*Family was significant (p=0.05) in Cycles 3, 4, and 8 and Family was significant (p=0.05) in Cycles 2, 4, 5, 6, 7, and 9 for #Cutt; and Rep*Family was significant (p=0.05) in Cycles 2, 4, and 6 and Family was non-significant (p=0.05) in all Cycles for pRoot. The outcross control was highest in #Cutt in all eight cycles and hi g hest in pRoot in cycles 2, 6, 7, and 9.
* significant at 0.05 level
** significant at 0.01 level
*** significant at 0.001 level
[a] Cycles 2 through 9.
[b] One S2 family was excluded from the analyses due to low number of seedling hedges.

Table 4. Results of the clone analyses of variance of number of useable cuttings (#Cutt) and proportion of cuttings rooted (pRoot).

Clone analysis within reps among cycles--

Rep	Source	df	#Cutt MS	#Cutt F-test	pRoot MS	pRoot F-test
1	Clone(Family)	32	1.92	***	0.10	ns
	Error	204	0.28		0.13	
2	Clone(Family)	30	1.57	***	0.30	**
	Error	193	0.28		0.15	
3	Clone(Family)	29	1.40	***	0.17	ns
	Error	195	0.28		0.22	
4	Clone(Family)	40	1.23	***	0.11	ns
	Error	239	0.37		0.15	

Notes: Statistical model included Cycle, Family, and Cycle*Family (see Materials and Methods). F-tests were computed for Clone(Family), only.
ns Non-significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

Additional experiments will be required to determine whether the fast decline in rooting performance is applicable only to the studied inbred loblolly pine population or to a wider range of inbred and outcrossed loblolly genotypes. The similar performance pattern of the outcrossed control family to the S2 families suggests that inbreeding depression is not the primary cause of the reduction in rooting performance. However, it will be necessary to test several inbred lines, each represented by inbred and outcrossed seedlings of each generation, to adequately estimate the effect of inbreeding. To accomplish this, we are currently breeding this loblolly pine population to produce SO, S1, S2, and S3 seed. Several of the S2 genotypes cloned as rooted cuttings in the present study will serve as parents for the S3 generation.

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LITERATURE CITED

Franklin, E.C. 1969a. Mutant forms found by self-pollination of loblolly pine. Journal of Heredity 60:315 -320.

Franklin, E.C. 1969b. Inbreeding depression effects and their influences on selection programs. Silvae Genetica 18:194.

Franklin, E.C. 1969c. Inbreeding as a means of genetic improvement of loblolly pine. In: Proc. 10th South. For. Tree Imp. Conf., Houston, TX, pp. 107-115.

Kuhnlein, U., D. Zadworny, Y. Dawe, R.W. Fairfull, and J.S. Gavora. 1990. Assessment of inbreeding by DNA fingerprinting: Development of a calibration curve using defined strains of chickens. Genetics 125:161-165.

Mason, M.E., C.D. Nelson, T.D. Caldwell, and W.L. Nance. 1993. Plant materials database development at the Southern Institute of Forest Genetics. In: Proc. 22nd South. For. Tree Imp. Conf., July 1993, Atlanta, GA, (in press).

Nance, W.L. and C.D. Nelson. 1989. Restriction fragment length polymorphisms and their potential use in marker assisted selection in southern pine improvement. In: Proc. 20th South. For. Tree Imp. Conf., Charleston, SC, pp. 50-59.

SAS Institute Inc. 1989. SAS/STAT User's Guide. Version 6, Fourth Edition, Volume 2, Cary, NC. 846 p.

Sniezko, R.A. and B.J. Zobel. 1986. Seedling height and diameter variation of various degrees of inbred and outcross progenies of loblolly pine. Silvae Genetica 37:50-60.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. Second Edition, McGraw-Hill Book Co., New York. 633 p.

Synder, E.B. 1967. Cooperative diallel crosses of loblolly pine: Study plan. FS-SO-1401.11.23, Gulfport, MS. 15 p.

Synder, E.B. 1972. Five-year performance of self-pollinated slash pines. Forest Science 18:246.