STEM CUTTING PRODUCTION AND ROOTING IN A SLASH PINE DIALLEL MANAGED FOR RAPID CUTTING PRODUCTION[1]

M.E. Mason[2] and C.D. Nelson[3]

Abstract: Seventeen crosses from a five-parent diallel mating design of slash pine were tested for stem cutting production and rooting ability. Seedling progeny were grown in pots, fertilized weekly, and hedged as soon as suitable length (> 7 cm) cuttings were available. A total of seven hedging and rooting cycles were completed in the first 14 months. Each seedling stock plant was scored in each cycle for the total number of usable cuttings produced and the percentage of cuttings set that rooted. Overall mean cutting production per seedling increased from 5.6 in cycle 2 (1.0 in cycle 1) to 15.8 in cycle 4, then fell to 10.4 in cycle 6. Overall mean percent rooting decreased from 67% in cycle 2 (95% in cycle 1) to 28% in cycle 5. The decrease in percent rooting was much more rapid than anticipated, and may reflect seasonal effects or a negative impact of the rapid-cycling management system. Cycles 1-4 (age=early) were characterized by increasing cutting production and good rooting (>55%), while cycles 5-6 (age=late) showed level to decreasing production and poor rooting (<35%). Family and age effects and family x age interaction were significant for both cutting production and percent rooting. Cumulative numbers of cuttings rooted per stock plant ranged from 4 to 95. Subsequent rooting trials have shown a reversal in the decreasing trend for cutting production and percent rooting, suggesting an opportunity for successful clonal multiplication using a rapid-cycling technique.

Keywords: *Pinus elliottii*, genetic variation, vegetative propagation, rooted cuttings

INTRODUCTION

Vegetative propagation has played an important role in the genetic improvement and analysis of southern pines. The routine ability to propagate selected trees by grafting has greatly accelerated the rate and level of improvement in operational tree breeding programs. Stem cutting propagation has been a useful research tool for analyzing the genetic control of important traits (Nelson et al. 1993a, Frampton and Huber 1995), but has largely failed to move beyond the research phase (McRae et al. 1993). Recent advances in macro- and micro propagation, as well as the increasing potential of gene transformation and genetic engineering, have increased the interest in vegetative propagation research and clonal forestry applications.

---

[1]Presented at the 24th Southern Forest Tree Improvement Conference, June 9-12, 1997, Orlando, FL.

[2]Biologist, USDA Forest Service, Southern Institute of Forest Genetics, Harrison Experimental Forest, 23332 Hwy 67, Saucier, MS 39574, mason@datasync.com.

[3]Genetics Project Leader, International Paper Company, Southlands Experiment Forest, 719 Southlands Road, Bainbridge, GA 31717, dana.nelson@ipaper.com. Formerly, Research Geneticist, USDA Forest Service, Southern Institute of Forest Genetics.

Successful use of stem cutting propagation for research or production purposes depends upon the ability to efficiently multiply selected genotypes. Vegetative multiplication of pine and many other forest trees is limited by the reduction and near loss of rooting ability as clones age (Libby et al. 1974). Because of this limitation, early selection and clonal multiplication are critically important (Wülisch 1984). The greater the number of cuttings that can be rooted in the first year and developed into productive stock plants, the larger the final multiplication potential of the clone.

The Southern Institute of Forest Genetics (SIFG) has employed stem cutting propagation as a research tool for many years (Hare 1974, Nance and Nelson 1989). As part of ongoing research into the genetic interaction of fusiform rust disease (Jewell 1961, Jewell and Mallett 1967, Dinus and Griggs 1975, Griggs and Walkinshaw 1982, Nelson et al. 1993b), we have been vegetatively propagating seedling progeny from a five-parent diallel of slash pinee The initial propagation goal was to produce adequate numbers of rooted cuttings from each seedling to allow time and space replicated inoculations with a series of fungal isolates (Nance et al. 1992). A rapid-cycle hedge management system (Nelson et al. 1993a) was employed in an attempt to maximize cutting production in the first year from seede This paper discusses the results of the first year's propagation efforts.

MATERIALS AND METHODS

Population

Parent trees were originally selected as superior wild trees for inclusion in early (1950s) breeding programs at the SIFG. Open-pollinated progeny were included in field tests and fusiform rust resistance screening experiments. Based upon results from these studies (Jewell 1961, Jewell and Mallett 1967), the trees were selected for inclusion in a diallel mating design for further disease resistance work (Griggs and Walkinshaw 1982). Control pollinations for the current work were conducted in 1989 and 1990. The mating design and number of seedlings tested per family are shown in Table 1. Seeds were collected, processed, and stored frozen until use in November 1991.

Stock Plant Initiation and Maintenance

Seeds were germinated on wet vermiculite in a growth chamber (25°C, 16 hour photoperiod) and transplanted to a peat:vermiculite mix in Ray Leach stubby cells (7 in3)e Seedlings were grown in a greenhouse (16 hour photoperiod) for 16 weeks before hedging was initiated. At 28 weeks, the seedlings were transplanted to one gallon pots containing a pine bark:peat:vermiculite mix and grown outside under ambient temperature and light conditions for the remainder of the experiment. Seedling stock plants received weekly fertilizer treatments of Peters 20-20-20 applied at 200 ppm nitrogen. Stock plants also received periodic insecticide treatment to control aphids and mites.

Table 1. Mating design, pedigree codes, and number of progeny tested per familye

♀/♂	18-27[1] (SIFG 1001)	8-7 (SIFG 1002)	9-2 (SIFG 1003)	18-26 (SIFG 1004)	18-61 (SIFG 1006)
18-27	11[2]/30[3]	12/10	13/30	14/20	15/35
8-7	21/35	22/30	23/15	24/20	25/35
9-2				34/20	35/15
18-26	41/20	42/20	43/20	44/10	45/30

'Tree identification, SIFG xxxx is the database clone number (Mason et al. 1993).
[2]family code, parents are coded 1-5 and families are coded as female x male (ie. 12 is 1 x 2).
[3]number of seedlings used in current experiments.

Cutting Collection and Rooting Conditions

Stock plants were hedged and cuttings were collected at approximately 6 to 8 week intervals beginning at 16 weeks from germination. On each cutting collection date, all potential stem cuttings were removed. A random subset (from 80 to 100%) of the usable (>7 cm) cuttings was set for propagation. Cuttings were treated with commercially available rooting powder containing 0.3% IBA, and set into a peat:perlite mix in Ray Leach fir cells (3 in ^{3}). The propagation greenhouse was maintained at moderate temperatures (<90°F) and high humidity (>85%) using a combination of evaporative cooling, air conditioning, intermittent mist, and fog. After 8 to 10 weeks, the stem cuttings were scored for rooting and transferred out of the propagation environment.

Data Collection and Analysis

During each hedging cycle the number of usable cuttings (NrCut) per stock plant was countede All shoots longer than about 7 cm were considered usable. In cycles 1, 3, and 5-7, all usable cuttings were set, while 5 and 10 cuttings per stock plant were set in cycles 2 and 4, respectivelye After 8 to 10 weeks in the rooting environment, the cuttings were removed from the containers and inspected for rooting. For each stock plant, the number of cuttings developing one or more primary roots was recorded. Proportion rooted (pRoot) was calculated as the number of cuttings rooted divided by the number of cuttings set. The number of propagules (NrProp) was calculated as the product of the number of usable cuttings and the proportion rooted (NrCut*pRoot). Since either all or a random subset of the cuttings were set in each cycle, we assumed that the discarded cuttings would root at the same rate as those actually set. The number of usable cuttings produced were not recorded for cycle 5, so cycle 5 production values (NrCut) were estimated to be the same as cycle 4. Rooting data for cycle 7 were inadvertently lost, so proportion rooted was estimated to be the average of the proportion rooted for cycles 5 and 6. Statistical analysis were conducted using SAS version 6.11 (SAS Institute Inc. 1996). Diallel analysis were completed using the program DIALL (Schaffer and Usanis 1969).

RESULTS AND DISCUSSION

Overall rooting percentages varied from 95% in cycle 1 to 28% in cycle 5 (Table 2). Production values ranged from 5.6 in cycle 2 to 15.8 in cycle 4. A definite trend in proportion rooted and cutting production was observed (Figure 1). Two distinct periods can be identified based on rooting and production. The early period (cycles 1-4) was characterized by increasing cutting production and *rooting above 55%, while the late (cycles 5-7) exhibited* level to declining production and rooting below *35%.* This distinct change in propagation performance was noted previously (Nelson et al. 1993a) in a study examining rapid-cycle propagation in loblolly pinee We are uncertain as to the cause of this decline. Possible explanations include biological and/or cultural effects. In both experiments, seasonal effects (photoperiod and temperature) were confounded with seedling development. The rapid-cycle technique may have induced the decline due to imbalanced nutritional status and/or a negative effect from recurrent wounding and tissue loss. In any case the effect appears transient as these stock plants have performed well in later years of rooted cutting propagation (H.E. Stelzer personal communication).

Table 2. Overall Summary of rooting experiments by cycle. Bracketed values are estimates as described in the text.

Cycle	Estimated Age (weeks)	Average NrCut	pRoot	pSet	Number of clones set	Average cumulative NrProp
1	16	1.0	0.95	1e0	395	0.95
2	24	5e57	0.67	0.86	394	4.81
3	30	7.39	0.56	1e0	383	9.10
4	36	15.8	0.60	0.62	382	18.6
5	42	[15.2]	0.28	0e57	372	23.1
6	48	15.3	0.35	1e0	335	28e3
7	54	10.4	[0.33]	1.0	250	31e 8

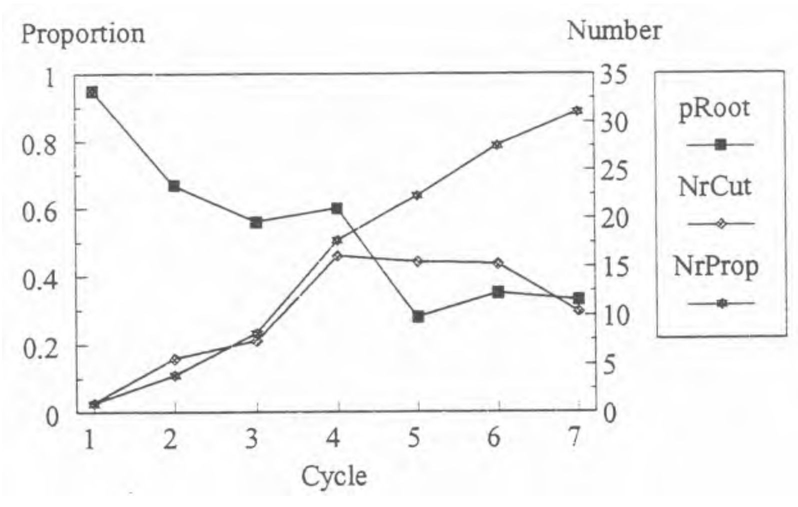

Figure 1. Plot of proportion rooted, number of cuttings produced, and cumulative number of propagules per stock plant for 17 families (395 clones) of slash pine.

The age by family interaction was significant ($p<.05$) for each trait (Table 3). For number of cuttings and number of propagules the interaction was primarily due to scale effects, as the error variance was much greater late than early (Table 4). Changes in rank were much more important for proportion rooted, however the higher and lower ranked families early tended to remain so late, or move to the middle. No families moved from one extreme to the other. This was not the case, however, for clones-within-families (data not shown). Clones tended to change ranks to a much higher degree. In fact on a per cycle basis, many clones tended to alternate between higher and lower rooting percentages.

Table 3. Analysis of age and family effects on proportion rooted and number of cuttings and number of propagules.

Source	df	pRoot ms	pRoot p>F	NrCut ms	NrCut p>F	NrProp ms	NrProp p>F
Age	1	15.1	***	23364	***	2621	***
Family	16	.130	***	2372	***	811.6	***
Age X Family	16	.051	**	768.2	***	121.0	*
Error	735:756:735	.024		208.1		64.3	

significance levels: *** <.001, **<.01, *<.05

Table 4. Analysis of family effects by age on proportion rooted and number of cuttings and number of propagules.

Source	df	pRoot ms	pRoot p>F	NrCut ms	NrCut p>F	NrProp ms	NrProp p>F
Age=Early (cycles 1-4)							
Family	16	.053	***	307.6	***	239.4	***
reciprocal	1	.111	*	282.3	*	396.5	**
maternal-1, 18-27	1	.052		273.0	*	343.1	**
maternal-2, 8-7	1	.007		18.4		66.4	
maternal-4, 18-26	1	.023		171.1		123.0	
self vs outcross	1	.329	***	584.3	***	857.5	***
self-1, 18-27	1	.061		32.8		147.3	
self-2, 8-7	1	.404	***	915.9	***	1228	***
self-4, 18-26	1	.127	*	120.9		223.3	*
Error	378:378:378	.020		49.8		38.8	
Age=Late (cycles 5-7)							
Family	16	.126	***	2832	***	680.2	***
reciprocal	1	.013		176.0		27.9	
maternal-1, 18-27	1	.005		178.4		47.6	
maternal-2, 8-7	1	.001		178.4		20.5	
maternal-4, 18-26	1	.001		0.0		6.44	
self vs outcross	1	.416	***	1799	*	1887	***
self-1, 18-27	1	.382	***	118.9		697.3	**
self-2, 8-7	1	.105		6520	***	1416	***
self-4, 18-26	1	.079		112.0		91.4	
Error	357:378:357	.029		366.4		91.2	

significance levels: *** <.001, **<.01, *<.05

Within early and late ages, family effects were highly significant (p<.01) for all traits (Table 4). Over the early period an average of 18 propagules were produced per stock plant, and only 13 in the late period. The ranges in family means for number of propagules per stock plant were 13 to 27 early, and 8 to 25 late. Reciprocal effects were generally significant in the early cycles and non-significant late (Table 4). Maternal effects were significant only for parent 1 in the early cycles for number of cuttings and number of propagules. Self effects were significant early and late, but variable by parent. Relative to outcross performance, parent 4 selves were similar, however parent 1 selves produced the most cuttings and parent 2 selves the most propagules.

Combining data over ages shows similar trends (Table 5)-- family effects are highly significant, self effects are significant and variable, while reciprocal and maternal effects are not significante Overall, an average of 70 usable cuttings per stock plant were produced and 44% rooted. Figure 2 presents the ranges in family and clone-within-family means for all traits. The ranges in family means were 47 to 91 cuttings produced, 32% to 57% rooted, and 19 to 50 propagules produced. Clone-within-family variance was analyzed using binary rooting data (0¯not rooted, 1=rooted). Clone variance was highly significant during the early and late periods and over all cycles (data not shown). The *ranges* in clone *means over* all families were *12 to 159* cuttings produced, *10%* to 82% rooted, and 4 to *95* propagules produced.

Table 5. Analysis of family effects over cycles 2 through 7 on proportion rooted and number of cuttings and number of propagules.

Source	df	pRoot ms	pRoot p>F	NrCut ms	NrCut p>F	NrProp ms	NrProp p>F
Family	16	.069	***	4744	***	1449.0	***
reciprocal	1	.03		904.2		549.2	
maternal-1, 18-27	1	.009		892.9		616.1	
maternal-2, 8-7	1	.000		311.3		158.7	
maternal-4, 18-26	1	.011		171.1		170.8	
self vs outcross	1	.346	***	4433	**	4839	***
self-1, 18-27	1	.215	***	26.8		1939	**
self-2, 8-7	1	.129	**	12323	***	5472	***
self-4, 18-26	1	.104	*	465.6		546.2	
Error	378	.018		585.7		178.6	

significance levels: *** <.001, **<.01, *<.05

Diallel analyses were completed using the fixed model, with reciprocals pooled and without selves. Table 6 gives the results for each trait over all cycles. Both general combining ability (GCA) and specific combining ability (SCA) were non-significant for proportion rooted. For number of cuttings and number of propagules, GCA was significant at p< .01 and SCA was significant at p<.05. GCA and SCA effects for each parent and cross are given in Table 7. Clearly parents 1 and 5 were superior in GCA for numbers of cuttings and propagules, while crosses 1 x 3 and 4 x 5 were highest in SCA and cross 1 x 5 was lowest.

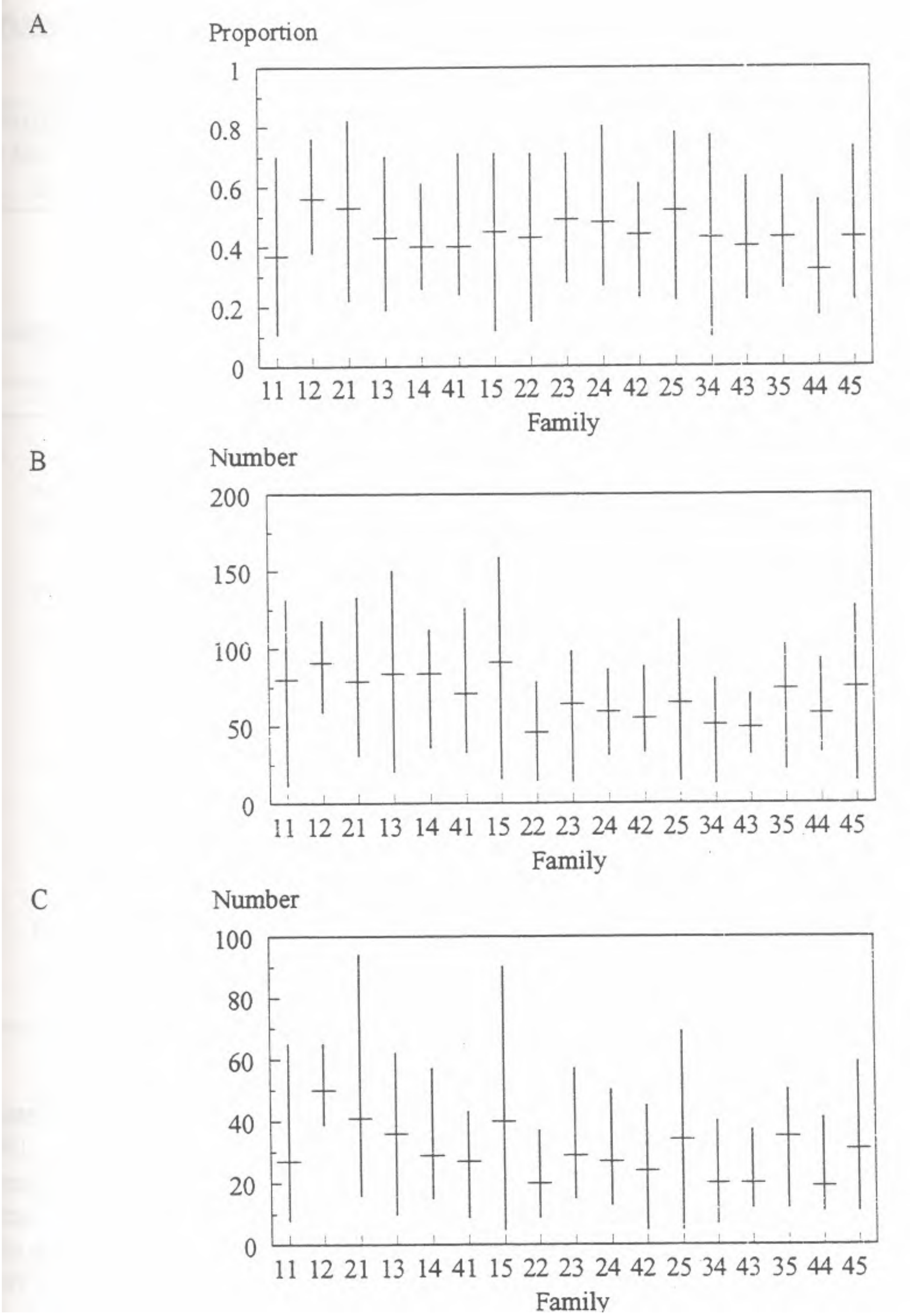

Figure 2. Clone-within-family range for proportion rooted (A), number of cuttings produced (B), and number of propagules produced (C). The bar is plotted from the minimum clone-within-family mean to the maximum clone-within-family mean. The tick mark is plotted at the family mean.

Table 6. Diallel analysis of proportion rooted, number of cuttings and number of propagules.

Source	df	pRoot		NrCut		NrProp	
		ms		ms	F	ms	F
GCA	4	0.1194	0.6905	6777	14.48**	2450	12.25**
SCA	5	0.1039	0.6009	1304	2.78*	613	3.76*
Error	315	0.1729		468		200	

significance levels: *<.05, **s .01

Table 7. Estimates of general (GCA) and specific (SCA) combining abilities for proportion rooted, number of cuttings and number of propagules.

	pRoot	NrCut	NrProp
General Means	0.480	61.655	25.273
GCA			
parent 1, 18-27	0.005	8.331	3.317
parent 2, 8-7	0.041	-2.174	2.728
parent 3, 9-2	-0.025	-4.715	-3.241
parent 4, 18-26	-0.025	-6.129	-5.152
parent 5, 18-61	0.000	3.779	2.009
SCA			
1 x 2	0.031	1.365	4.884
1 x 3	-0.013	9.329	3.387
1 x 4	-0.013	1.042	-4.209
1 x 5	-0.006	-11.488	-5.099
2 x 3	0.011	2.167	0.474
2 x 4	-0.014	-3.503	-2.506
2 x 5	0.004	-4.147	-2.616
3 x 4	0.009	-7.861	-1.288
3 x 5	0.006	-1.786	-1.448
4 x 5	-0.005	8.061	4.923

The rapid-cycle hedge management system appears to offer promise as a means to efficiently multiply slash pine genotypes. In comparison to loblolly pine (Nelson et al. 1993), slash pine appears to respond better to the rapid-cycle technique. However, improvements in the technique aimed at lessening the mid-season decline are necessary to improve production, especially for the poorer performing families and clones. In addition, serial propagation of the rooted cuttings could be used to further increase productivity (Libby et al. 1974, Wülisch 1984). First season production for the best families and clones were in excess of 40 and 60 rooted cuttings per seedling stock plant, respectively, with an overall average of 32. This level appears sufficient to produce about 500 stock plants per clone in a two year period, which is more than adequate for producing research materials and approaching a level necessary for larger scale applications (Foster et al. 1981).

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Tom Caldwell, Jim Hamaker, Gay Flurry, Hershal Loper, and Larry Lott. A copy of the computer software DIALL compiled for use on the PC was kindly provided by Dr. Floyd Bridgewater. Please note that the use of trade names does not imply endorsement by the USDA Forest Service.

LITERATURE CITED

Dinus, RJ, and MM Griggs. 1975. Rust-free slash pines surviving artificial inoculation potentially useful for resistance breeding. Forest Science 21: 275-277.

Foster, GS, FE Bridgewater, and SE McKeand. 1981. Mass vegetative propagation of loblolly pine--a revaluation of direction. In: Proceedings of the 16th Southern Forest Tree Improvement Conference, Blacksburg VA, pp. 311-319.

Frampton, LA Jr., and DH Huber. 1995. Clonal variation in four-year-old loblolly pine in coastal North Carolina. In: Proceedings of the 23rd Southern Forest Tree Improvement Conference, Asheville NC, pp. 254-264.

Griggs, MM, and CH Walkinshaw. 1982. Diallel analysis of genetic resistance to *Cronartium quercuum* f. sp. *fusiforme* in slash pine. Phytopathology 72: 816-818.

Hare, RC. 1974. Chemical and environmental treatments promoting rooting of pine cuttings. Canadian Journal of Forest Research 4: 101-106.

Jewell, FF. 1961. Artificial Testing of intra- and interspecies southern pine hybrids for rust resistance. In: Proceedings of the 6th Southern Forest Tree Improvement Conference, Gainesville FL, pp. 105-109.

Jewell, FF, and SL Mallett. 1967. Testing slash pine for rust resistance. Forest Science 13: 413-418.

Libby, WJ, AG Brown, and JM Fielding. 1974. Effects of hedging radiata pine on production, rooting, and early growth of cuttings. New Zealand Journal of Forestry Science. 2: 263-283.

Mason, ME, CD Nelson, TD Caldwell, and WL Nance. 1993. Plant materials database development at the Southern Institute of Forest Genetics. In: Proceedings of the 22nd Southern Forest Tree Improvement Conference, Atlanta GA, pp. 298-305.

McRae, JB, HE Stelzer, GS Foster, and TD Caldwell. 1993. Genetic test results from a tree improvement program to develop clones of loblolly pine for reforestation. In: Proceedings of the 22nd Southern Forest Tree Improvement Conference, Atlanta GA, pp. 424-433.

Nance, WL, GA Tuskan, CD Nelson, and RL Doudrick. 1992. Potential applications of molecular markers for genetic analysis of host-pathogen systems in forest trees. Canadian Journal of Forest Research 22: 1036-1043.

Nance, WL, and CD Nelson. 1989. Restriction fragment length polymorphisms and their potential use in marker assisted selection in southern pine. In: Proceedings of the 20th Southern Forest Tree Improvement Conference, Charleston SC, pp. 50-59.

Nelson, CD, TD Caldwell, and JM Hamaker. 1993a. Stem cutting production and rooting performance in an S2 population of loblolly pine. In: Proceedings of the 22nd Southern Forest Tree Improvement Conference, Atlanta GA, pp. 154-161.

Nelson, CD, RL Doudrick, WL Nance, JM Hamaker, and B Capo. 1993b. Specificity of host:pathogen interaction for fusiforme rust disease on slash pine. In: Proceedings of the 22nd Southern Forest Tree Improvement Conference, Atlanta GA, pp. 403-410.

Schaffer, HE, and RA Usanis. 1969. General least squares analysis of diallel experiments: A computer program - DIALL. Genetics Department Research Report Number 1. North Carolina State University, Raleigh NC, 61 p.

Wülisch, GV. 1984. Propagation of Norway spruce cuttings free of topophysis and cyclophysis effects. Silvae Genetica 33: 215-219.