Low Level Inbreeding Effects on Germination, Survival, and Early Height Growth of Slash Pine

P.A Layton and R.E Goddard

ABSTRACT

Four progeny tests containing back, half-sib, and out crosses of slash pine were established near Lake Butler, Florida; two each in 1980 and in 1981. Height and survival were measured in 1982 and 1983. Crossing types were not significantly different for these traits in the four tests. For the three tests in which all cross types were planted, average survivals for back, half-sib, and out crosses were 86%, 83%, and 99%, respectively. Average heights for back, half-sib, and out crosses were 57, 59, and 63 cm in 1982 and 132, 137, and 150 cm in 1983, respectively. Height and survival for the crossing types are inversely related to the inbreeding coefficient. Seed germination of the same families was tested in a greenhouse. Percent germination at nineteen days was 94, 78, and 94 percent for back, half-sib, and out crosses, respectively. Means were not significantly different.

Additional Keywords: *Pinus elliottii* var. *elliottii*, inbreeding depression.

Previous inbreeding studies of forest trees have considered mainly self-pollination. Results show inbreeding adversely affected seed yield, germination, seedling morphology, and tree vigor and growth (Table 1) and concluded that inbreeding should be avoided if at all possible. However, small effective population size precludes avoidance of inbreeding. Van Buijtenen (1975) suggests separation of breeding and production populations with the breeding population subsetted into small groups where inbreeding is allowable. The production population would contain one selection from each group. Inbreeding and inbreeding depression would only affect the breeding population; the production population being totally out crossed except for an estimated 2.5% selfing (Squillace and Goddard, 1982). Within breeding groups the average co-ancestry after 10 generations is predicted to be less than 0.425 for two mating designs (Layton 1978).

What then are the effects of low levels of inbreeding? Squillace and Kraus (1962) estimated that with an increase of 0.1 in the inbreeding co-efficient, slash pine (*Pinus elliottii* var. *elliottii* Englem.) had five fewer sound seeds per cone, an 8% decrease in germination, a 7% decrease in rate of germination, and a 4% decrease in seedling height. Their study provided some insight of the effect of low levels of inbreeding; however, as slash pine

[1/] Graduate Assistant and Professor, respectively, School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611. The authors thank James Hickman and Owens-Illinois, Inc., Southern Woodlands for their assistance in this study.

Table 1.--Studies of various conifer species presenting evidence of inbreeding depression.

Species	Common Name	Study	Inbreed Cross Used
Pinus elliottii var. elliottii Engelm.	Slash pine	Squillace and Kraus 1962	Self Back Full-sib
		Snyder 1968, 1972	Self
		Gansel 1971	Self Back Half-sib Full-sib
Pinus taeda L.	Loblolly pine	Franklin 1969	Self
Pinus monticola Dougl.	Western white pine	Barnes 1964	Self
		Bingham 1973	Self
Picea abies	Norway Spruce	Andersson et al. 1974	Self
Pinus banksiana Lamb.	Jack Pine	Fowler 1964	Self
Pinus strobus L.	Eastern white pine	Fowler 1964	Self
Pseudotsuga menziesii (Mirb.) Franco	Douglas-fir	Orr-Ewing 1954	Self
		Sorensen and Miles 1982	Self
Pinus ponderosa Dougl. ex. Laws.	Ponderosa pine	Sorensen and Miles 1982	Self
Sequoia sempervirens (D. Don) Endl.	Redwood	Libby et al. 1981	Self

breeding has progressed, interest in the effects of inbreeding has increased. Further study is needed to increase the knowledge of these effects over time, and over a wide range of genotypes.

MATERIALS AND METHODS

In 1975, the University of Florida Cooperative Forest Genetics Research Program initiated a study to examine effects of low level inbreeding on slash pine growth. The study was designed to include back crosses, half-sib crosses, out crosses, and selfs, but the self-pollinations were not completed. The inbreeding coefficients for the progeny of these crosses, assuming no prior inbreeding, are 0.0, 0.125, and 0.25 for out, half-sib, and back crosses, respectively (Squillace and Kraus 1962). Control pollinations were made at the Owens-Illinois, Inc. seed orchard in White Springs, Florida. The orchard contained mostly second generation selections of slash pine from open-pollinated progeny tests with two to five different half-sib clones of 11 families available for crossing. In 1980 two tests (3A and 3B) were planted near Lake Palestine in Baker County, Florida on a cleared and bedded site. Ten replications of six families of each of the three cross types (back, half-sib, and out) were planted in four-tree row plots in each test.

In 1981, two tests were established nearby in Union County, Florida on a bedded but much wetter site. Test 4A contained only half-sib and out crosses, while the main test, 4B, had all three cross types. Five-tree row plots were replicated ten times in each test.

Comparisons among tests were not possible because only one cross was common to all four tests while two crosses were common to three of the tests. Sixty-seven different crosses were represented in all tests (Table 2).

Plot means were obtained for survival and height from measurements in 1982 and 1983. Variances for each test in each year were analyzed using the Statistical Analysis System (Helwig and Council 1979). Factors considered in the model were cross type (back, half-sib, and out), families within cross type, replications, replication by cross type interaction, and experimental error (Table 3).

In 1982 seed from 47 of the crosses (8 out, 36 half-sib, and 3 back crosses) were evaluated for germination. From 50 to 100 seeds per family were germinated in flats of vermiculite in a greenhouse. Seeds were soaked in water 24 hours and placed in flats. The number of seed available and the number of germinated seed at approximately two-day intervals from day eight to day nineteen was recorded.

Percent germination after 19 days was computed. Results were analyzed using analysis of variance for a completely randomized design. Germination rates were calculated by the formula:

$$\text{Germination Rate} = \frac{\text{Germination count at 10 days}}{\text{Germination count at 19 days}} \times 100.$$

These data were analyzed in the same manner as percent germination.

Table 2. Families present in four slash pine progeny tests by crossing type.

Back	Test 3A	3B	4A	4B
1901 x 249-55				X
1902 x 249-55	X	X		X
1903 x 249-55				X
0301 x 17-57	X			
0601 x 84-57	X			
0804 x 106-56				
0805 x 106-56	X	X		
3701 x 11-57	X	X		
0201 x 262-55	X	X		
0202 x 262-55	X			
3601 x 248-56				X
0307 x 17-57	X			
3602 x 248-56				X
Out				
0603 x 0402				
0703 x 0201	X	X		
0703 x 0301	X	X		
0703 x 0402	X			
0703 x 0603	X	X		
0703 x 0804	X	X		
0703 x 1902	X	X		
0703 x 3701	X			
0802 x 0301	X			
0804 x 0305	X			
0401 x 0608			X	X
0402 x 0608			X	X
0403 x 0608				X
0702 x 0306			X	X
0802 x 1301			X	X
0802 x 3602			X	X
0804 x 3602			X	X
0807 x 0702			X	X
0807 x 1301			X	X
0807 x 3602			X	X
0809 x 1301			X	X
0809 x 3602			X	X
3701 x 3607			X	X
3702 x 3602			X	X

Half sib	Test 3A	3B	4A	4B
0602 x 0603	X		X	X
0607 x 0603	X			
0610 x 0603	X	X		
0802 x 0804	X	X		
0802 x 0805	X	X	X	X
0804 x 0805	X	X		
0807 x 0804	X			
0302 x 0307			X	
0303 x 0301				X
0303 x 0305				X
0304 x 0301				X
0304 x 0305				X
0401 x 0402				X
0402 x 0401			X	X
0403 x 0401			X	X
0602 x 0608				X
0603 x 0604				X
0603 x 0608			X	X
0606 x 0603				X
0610 x 0604				X
0611 x 0608				X
0703 x 0702				X
0802 x 0801			X	X
0804 x 0801			X	X
1903 x 1901			X	X
1903 x 1902			X	X
2001 x 2003				X
2004 x 2003				X
3601 x 3602			X	X
0303 x 0307				X

Table 3.--Expected mean squares and degrees of freedom for the model used to analyze height and survival.

Source	df	Expected Mean Squares
Total	179	
Cross	2	$\alpha_e^2 + 6\alpha_{r \times c}^2 + 10\alpha_{f(c)}^2 + 60\alpha_c^2$
Family (Cross)	12	$\alpha_e^2 + 6\alpha_{r \times c}^2 + 10\alpha_{f(c)}^2$
Replication	9	$\alpha_e^2 + 18\alpha_r^2$
Replication x Cross	18	$\alpha_e^2 + 6\alpha_{r \times c}^2$
Error	138	α_e^2

RESULTS AND DISCUSSION

Survival

Survival in each of the four tests was over 80%. In tests 3A and 3B initial survival values were 88.6% and 88%, respectively. Tests 4A and 4B, planted one year later, averaged 81.6% and 81.2%, respectively. There were no significant differences in survival between back, half-sib, and out crosses. Outcrosses had better survival than the inbreed crosses in three of the tests (Table 4). The differences in survival between out crosses and inbred crosses

Table 4.--Survival by crossing type for four slash pine progeny tests planted in Florida, 1980 and 1981.

Test	Age (yrs)	Type of Cross: Back	Half-sib	Out
3A	2	86.4	86.7	92.3
	3	86.0	85.4	91.7
3B	2	83.0	90.0	91.0
	3	83.0	90.0	91.0
4A	1	-	82.0	80.4
	2	-	81.1	80.4
4B	1	82.2	79.3	82.8
	2	81.8	78.8	82.3

was below eight percentage points. There were significant differences for families within cross types in Test 4A and 4B for both first and second year survival measurements. Site conditions, especially the presence of standing water, may have influenced survival of tests 4A and 4B.

Height

Heights were not significantly different among cross types. Test 4B had significant differences for families within crosses at age one, but there were no significant differences in this factor at age two. Height in tests 4A and 4B was greater at ages one and two than height at age two and three in tests 3A and 3B (Table 5). Differences reflect site quality. On the average out crosses were taller than half-sib and back crosses for both ages in tests 3A and 3B. Height differences between out crosses and inbred crosses increased from age two to three in both of these tests (Table 5). Test 4A followed the same pattern. Half-sib crosses averaged 0.3 cm taller than back crosses at age one in test 4B, but at age two were 9.4 cm shorter, a 5.5% decrease in height when compared to the out-cross mean height.

Table 5.--Mean height by crossing type for four slash pine progeny tests planted in Florida, 1980 and 1981.

Test	Age (yrs)	Control (cm)	Back Cross (cm)	Deviation from Out Cross (%)	Half-sib Cross (cm)	Deviation from Out Cross (%)	Out Cross (cm)
3A	2	54.2	51.6	-10.8	52.5	-9.7	57.8
	3	110.7	101.3	-16.8	107.8	-11.5	121.8
3B	2	61.9	59.1	-11.2	60.2	-9.4	66.5
	3	146.4	137.3	-13.6	143.3	-9.8	158.9
4A	1	62.6	-	-	61.7	-3.1	63.6
	2	166.6	-	-	162.5	-4.9	171.0
4B	1	64.0	61.3	-4.7	64.7	+0.005	64.3
	2	165.3	157.5	-7.5	160.8	-5.5	170.2

The general trend for heights was for out crosses to be tallest, followed by half-sib and back crosses (Figure 1). Although more variable, means by individual parent have similar height trends (Table 6). In a few individual cases, however, no inbreeding depression was apparent. This may be due to experimental error, but suggests that some genotypes contain few recessive deleterious genes.

Germination

Percent germination and germination rates were not significantly different between cross types. Average percent germination was 93.9%, 77.9%, and 91.3% for back, half-sib, and out crosses, respectively. Mean germination rate for back crosses was 98.5%, followed by out crosses, 83.8%, and half-sib crosses, 78.7%, however, estimates of back cross germination are based on only three crosses. Out crosses had better percent germination and germination rates than half-sib crosses.

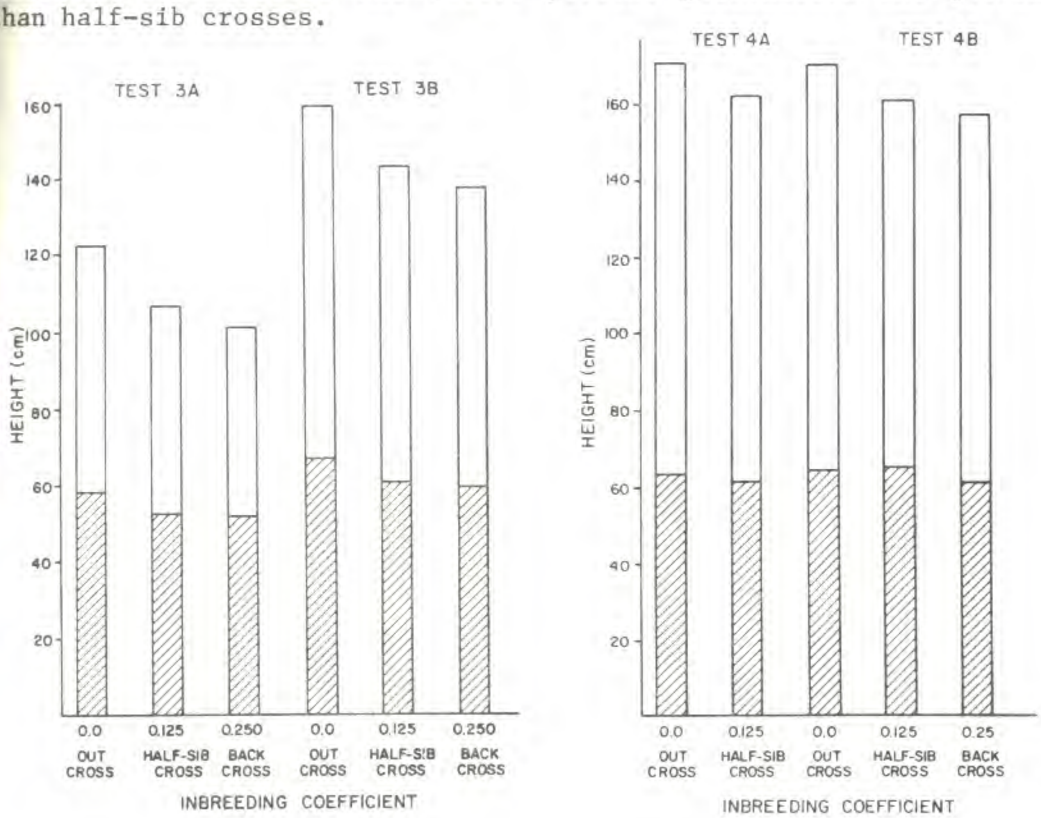

Figure 1. Mean heights by crossing type for four slash pine progeny tests planted in Florida, 1980 and 1981. Shaded areas are heights at ages 2 and 1 and clear areas are heights at ages 3 and 2 for the 1980 and 1981 plantings, respectively.

SUMMARY AND CONCLUSIONS

No significant differences occurred in early height, survival, or germination of out, half-sib, and back crosses of slash pine. Height and survival were inversely related to the inbreeding coefficient. These data agree with other studies of inbreeding depression.

The material available for the several plantings was insufficient for comparison among tests, therefore, data from each planting must be interpreted independently. The trend of lower height growth with increase in inbreeding coefficient was similar in all plantings, and to date, the suspected inbreeding depression seems to be increasing with age of the material planted.

Table 6.--Mean progeny heights when parents are crossed as back, half-sib, or out crosses for two slash pine tests planted in Florida, in 1980 and 1981.

Test	Age	Parent	Mean Height		
	(yrs)		Back Cross	Half-sib Cross	Out Cross
			(cm)		
3A	3	0201	89	-	127
		0301	135	-	103
		0601	138	118	-
		0603		121	114
		0802		114	103
		0804	88	102	111
		0805	90	123	-
		1902	94	-	137
		3701	101	-	83
		Test Average	101	108	122
4B	2	0301	-	159	181
		0401	-	178	200
		0402	-	164	181
		0403	-	165	204
		0608	-	155	187
		0702	-	97	172
		0802	-	156	176
		0804	-	79	174
		1901	173	162	-
		1903	132	162	-
		3601	170	209	-
		3602	167	209	164
		Test Average	157	161	170

LITERATURE CITED

Andersson, E., R. Jansson, and D. Lindgren. 1974. Some results from second generation crossings involving inbreeding in Norway spruce (*Picea abies*). Silvae Genet. 23:34-43.

Barnes, B. V. 1964. Self-and cross-pollination of western white pine: A comparison of height growth of progeny. USDA For. Serv. Res. Note INT-22. 3 pp.

Bingham, R. T. 1973. Possibilities for improvement of western white pine by inbreeding. USDA For. Serv. Res. Paper INT-144. 18 pp.

Fowler, D. P. 1964. Effects of inbreeding in red pine, *Pinus resinosa* Ait. IV. Comparison with other northeastern *Pinus* species. Silvae Genet. 14:76-81.

Franklin, E. C. 1969. Inbreeding depression in metrical traits of loblolly pine (*Pinus taeda* L.) as a result of self-pollination. Technical Report 40. N.C. State Univ. Coop. Prog., Raleigh. 19 pp.

Helwig, J. T. and K. A. Council. 1979. SAS user's guide. 1979 edition. SAS Institute, Inc., Cary, N.C. 494 pp.

Gansel, C. R. 1971. Effects of several levels of inbreeding on growth and oleoresin yield in slash pine. Eleventh South. For. Tree Tmprov. Conf. Proc. pp. 174-176.

Layton, P. A. 1978. Comparison of two advanced generation mating designs for loblolly pine (*Pinus taeda* L.). Master's Thesis, Texas A & M Univ. College Station. 47 pp.

Libby, W. J., B. G. McCutchan, and C. Millar. 1980. Inbreeding depression in selfs of redwood. Silvae Genet. 30:15-25.

Orr-Ewing, A. L. 1954. Inbreeding experiments with the Douglas-fir. For. Chron. 30:7-20.

Snyder, E. B. 1968. Seed yield and nursery performance of self-pollinated slash pines. For. Sci. 14:68-74.

Snyder, E. B. 1972. Five year performance of self-pollinated slash pines. For. Sci. 18:246.

Sorensen, F. C. and R. S. Miles. 1982. Inbreeding depression in height, height growth, and survival of Douglas-fir, ponderosa pine, and noble fir to 10 years of age. For. Sci. 28:283-292.

Squillace, A. E. and J. F. Kraus. 1962. Effects on inbreeding on seed yield, germination, rate of germination, and seedling growth in slash pine. For. Genet. Wkshp. Proc., Macon, Georgia. pp. 59-63.

Squillace, A. E. and R. E. Goddard. 1982. Selfing in clonal seed orchards of slash pine. For. Sci. 28:71-78.

van Buijtenen, J. P. 1975. Advanced generation breeding. Thirteenth South, For. Tree Improv. Conf. Proc. pp. 63-72.