PRODUCING AND TESTING LARGE NUMBERS OF SELF-FERTILIZED LOBLOLLY PINE SEEDLINGS

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Abstract.--Self-fertilization combined with selection is the fastest way to fix favorable alleles in breeding populations. However, inbreeding depression dramatically reduces the average numbers of self-fertilized seeds . In fact, the numbers of viable seeds from self-fertilizations are often so few that it may not be practical to maintain a parental line in the breeding population. If self-fertilization is to be a part of a breeding strategy it is important to know how much effort will be required to produce adequate numbers of self-fertilized seedlings for subsequent selection and breeding. Forty, first-generation loblolly pine *(Pinus taeda* L.) parents were self pollinated in a seed orchard in an operational trial. The results of that breeding and testing trial through the first two years in field plantings are reported and the implications in planning a breeding strategy are discussed

Keywords: Pinus taeda, breeding strategy, self-fertilization, inbreeding depression

INTRODUCTION

Inbreeding offers a means of rapidly increasing homozygosity in breeding populations. Coupled with selection, the frequency of favorable alleles will theoretically increase as will the additive genetic variance. Self-fertilization (hereafter, selfing) is the most rapid form of inbreeding and has the advantage of a greater among-family selection intensity than for bi-parental crosses. With selfing, the parents with the best general combining abilities will be mated to themselves, and thus can be advanced to the next generation without carrying genes from poorer parents. Since selfing is perfect assortative mating,

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the additive genetic variance among lines will increase rapidly. Although selfing has the greatest potential to produce genetic gains rapidly it also has some critical disadvantages. Inbreeding depression in metrical traits and in reproductive capacity is well documented for many conifers (See Williams and Sovolainen , 1995 for a thorough review). Both of the largest loblolly pine breeding programs in the southern United States have adopted the use of sublines to avoid inbreeding depression in progenies used to establish plantations (Lowe and van Buijtenen 1989, McKeand and Bridgwater 1992). Thus, the critical question for breeders is how to maintain breeding populations composed of parents with reduced vigor and reproductive capacity. One of the major hurdles to implementing a breeding strategy incorporating self-pollinations is in the early generations. Models of embryonic lethal allele systems in conifers suggest that these can be purged in a few generations of selfing (Bishir and Namkoong 1987). However, the difficulty of producing sufficient numbers of S_1 progenies for testing and selection should not be minimized. Herein, we report an operational trial of self-pollination on 40, first-generation loblolly pine *(Pinus taeda* L.) parents. The results of breeding and testing through the first two years in field plantings are reported and the implications in planning a breeding strategy are discussed

METHODS

Fresh pollen, pollen that had been processed and stored in a freezer since 1985 and 1986 at 4 C°, and pollen that had been dried in a vacuum in a freeze desiccator and stored since 1982 and 1983 at 4 C° were used to accomplish the self-pollinations. Polymix crosses were made using one of two 5-parent mixes of pollen frozen in 1986.

Pollinations were made in the Weyerhaeuser Company's Lyons, Georgia Seed Orchard in the spring of 1987. Counts of surviving conelets were made in the fall of 1988 and cones were harvested in October of 1989. Seeds were extracted and numbers of total seeds were counted. The numbers of filled seeds were determined from radiographs. Seeds were sown in a greenhouse in Raleigh, North Carolina in April and May of 1992. Surviving self- and cross-pollinated sibs from 25 families were planted in a split plot experimental design near Lyons, Georgia on January 14-15, 1993. Main plots were pollination types. Heights to each whorl of branches and total heights were measured at the end of the first growing season in the greenhouse and again in June, 1993. Total heights were measured in January, 1993 and 1994.

RESULTS AND DISCUSSION

There were no significant differences among the pollen types in the total numbers of seeds per cone produced nor in the percentages of filled seeds per cone. In fact, fresh pollen produced approximately average numbers of total and filled seeds per cone. There three pollen types ranked as expected for total seeds, filled seeds and percentage filled seeds (Table 1). Lower numbers of total seeds were produced in both self and polymix crosses. The percentage of filled seeds was less for polymix than wind pollinations, probably because the polymix males were related as half-sibs to the female parents in 11 of the matings.

Table 1. Average cone and seed statistics for selfed and outcrossed parents and three pollen types.

Pollen Parent	Numbers of Cones	Total Seeds	Filled Seeds	Empty Seeds	% Filled Seeds
WIND	198	132	99	33	72
POLYMIX	152	88	58	30	61
SELF	365	94	9	85	9

Conelet abortion during the first growing season after pollination was not great nor significantly different for wind and self pollinations (Table 2). Conelet abortion during the second year was important and significantly more self-pollinated conelets were lost for both polymix and selfed cones. Only about 1 in 5 wind-pollinated strobili and 1 in 7 self- or polymix-pollinated strobili survived until harvest. Conelets and cones which had been damaged by insects or disease were excluded from the counts.

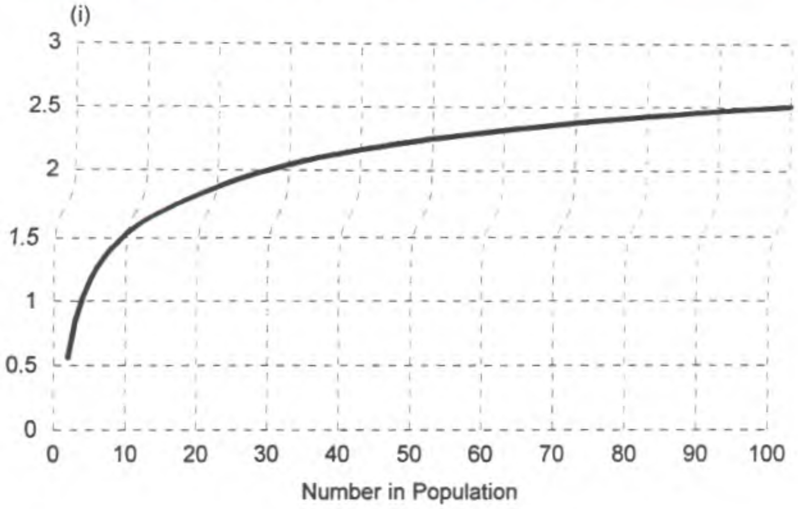
Table 2. Strobilus survival for self and outcrossed parents and three pollen types.

Pollen Parent	Numbers of Pollination Bags	Mean Number of Strobili Pollinated	Conelet per Strobilus Pollinated Year 1	Cone per Strobilus Pollinated Year 2
WIND		3.1	0.92	0.63
POLYMIX	99	3.6	0.98	0.55
SELF	252	3.6	0.97	0.50

Thus, producing large numbers of self-pollinated seeds requires pollinating large numbers of female strobili. Furthermore, the effort required is increased if there is no prior knowledge about which parents produce very low percentages of filled seeds when selfed.

When a breeding and testing plan are developed the question of how many S1 progenies to plant arises. Examination of Figure 1 suggests that at least 20 individuals should be planted for efficiency. That is, the marginal increase in expected selection differential per individual added is greatest from two to twenty and decreases thereafter. It may in fact be desirable to plant more than twenty individuals since mortality, selection based on multiple traits and the need for insurance, all suggest planting more individuals.

Figure 1. Standardized selection intensity (i) for selecting one individual from varying population sizes less than 400. (After Becker, 1984).



The effort required to produce a desired number of S, seedlings is a direct function of : (1) the number of strobili pollinated, (2) the germination percentage for filled S, seeds, and (3) the numbers of filled seeds produced per strobilus pollinated. The number of strobili that can be pollinated is determined by the availability of pollen and female strobili and the investment that can be made in making controlled pollinations. The percentage of filled seeds that can be expected to germinate is, on average, 75% to 85% (Franklin, 1969 and McKeand, N.C. State University, Pers. Comm., respectively). The numbers of filled seeds expected per strobilus pollinated is more problematical. The assumptions made to produce the data in Table 3 were that 75% of filled, S, seeds would produce a seedling, and that there would be an average of 3.6 strobili per pollination bag (Table 2).

Thus, if 50 seedlings were desired for outplanting, and one was willing to accept that only half of the parents would reach that goal, the number of pollination bags that would have had to be used per parent could be calculated as ((1/1.27 filled seeds per strobilus pollinated)/3.6 strobili per bag) x (50 seedlings / 0.75 seedling per seed) = 15 bags. If no prior knowledge is available, the number of pollinations to be made on each parent must assume that each parent will produce the number of filled seeds per strobilus pollinated for which the plan is developed. Thus, all 40 parents in the seed orchard represented in Table 3 would have had to have 15 pollination bags installed and pollinated for a total of 600 bags. As the percentage of filled seeds per strobilus pollinated decreases, the numbers of strobili that must be bagged increases exponentially (Table 3).

Table 3. Estimated numbers of pollination bags to produce desired numbers of S, seedlings for outplanting. Assumptions were 75% germination of filled, S, seeds and 3.6 strobili per pollination bag.

% of 40 Parents Producing Desired # of Seeds	Filled Seeds per Strobilus Pollinated	# of Bags Per Parent to Produce n seeds			Total # of Bags to Produce n seeds		
		n=20	n=30	n=50	n=20	n=30	n=50
2.5	22.62	1	1	1	40	40	40
5	15.43	1	1	1	40	40	40
10	8.25	1	1	2	40	40	80
15	7.1	1	2	3	40	80	120
20	6.32	1	2	3	40	80	120
25	4.47	2	2	4	80	80	160
30	4.15	2	3	4	80	120	160
35	3.67	2	3	5	80	120	200
40	2.22	3	5	8	120	200	320
45	1.87	4	6	10	160	240	400
50	1.27	6	9	15	240	360	600
55	1.15	6	10	16	240	400	640
60	0.94	8	12	20	320	480	800
65	0.63	12	18	30	480	720	1200
70	0.52	14	21	36	560	840	1440
75	0.48	15	23	38	600	920	1520
80	0.44	17	25	42	680	1000	1680
85	0.33	22	33	56	880	1320	2240
90	0.24	31	46	77	1240	1840	3080
95	0.19	39	59	98	1560	2360	3920
100	0.11	67	100	167	2680	4000	6680

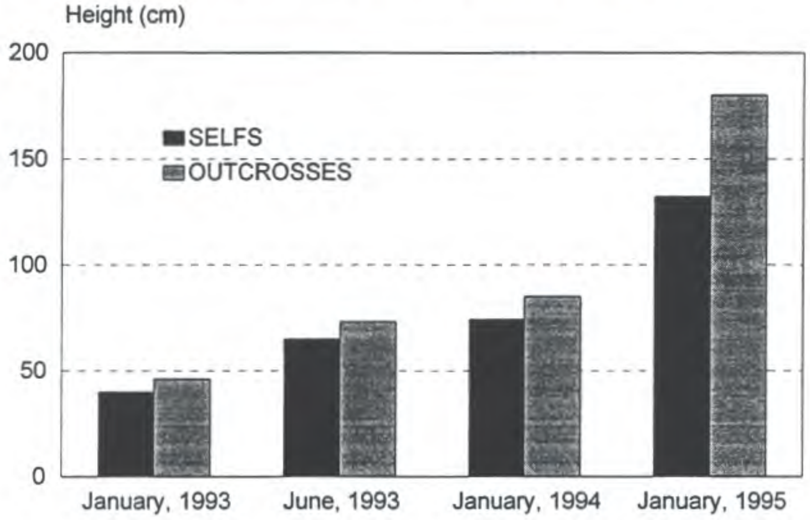
Once the population of S, progenies has been planted, survival until selection age becomes a concern. After one year in the field there was significantly more mortality in S, progenies than in their outcrossed siblings (Table 4). Most S, mortality occurred during the first year after planting (21% mortality) but continued through year 2 (7% additional mortality). Survival was determined for all trees including damaged trees and trees that had been used as border row trees.

Table 4. Survival of outcross and self progenies for 2 years after planting.

	Percentage Surviving			
Pollen Type	January 1993 (Planted)	June 1993 (5 months)	January 1994 (1 year)	January 1995 (2 years)
Outcross	100	100	97	97
Seff	100	99	79	72

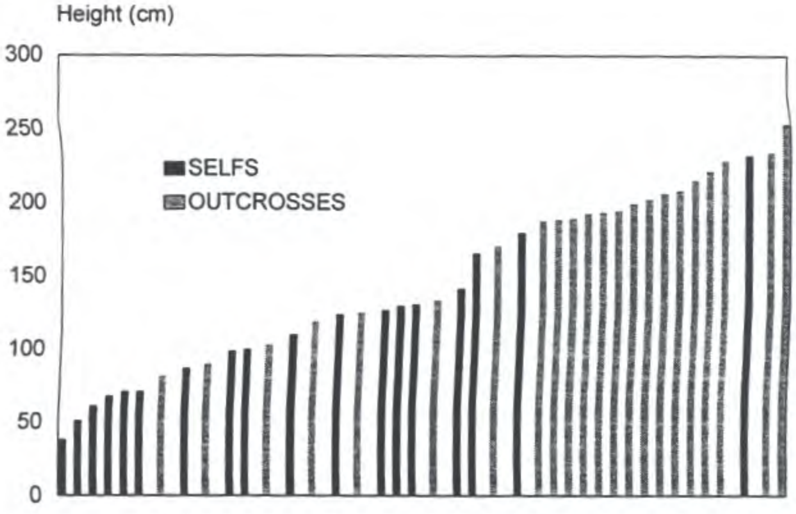
the field outcross siblings were 36 % taller than their S
S
siblings (Figure 3, for example).

Figure 2. Average height growth of self and outcross progenies.



Thre are two important issues to consider when contemplating selection in trials of self-pollinated progenies. First, there is a real probability that the fastest growing S, progenies are the result of pollen contamination and are not selfs. Secondly, selecting the fastest growing S, progenies may not be the best way to increase general combining ability. If there is a substantial amount of non-additive variance contributing to the genetic variance, the correlation between self- and out-cross performance may not be high (Williams and Sovolainen , 1995).

Figure 3. Heights of self and outcross progenies from a single parent after 2 years in the field.



If Phenotypic selection is to be done in tests such as those reported here, it may be possible to make selections at fairly young ages. After two years in the field, there were 127 S, progenies representing 17 parental lines that survived and were undamaged. Six of these had trivial numbers of individuals upon which to compare height ranks over time (four lines had two individuals and two had only one). Of the remaining 11 lines, the tallest individual after two years was also tallest after one year for five lines. If the tallest individual from each of the 11 lines had been selected after five months in the field their average rank would be 5 after two years in the field. If the tallest individual from each of the 11 lines had been selected after one year in the field their average rank would be 2 after two years in the field. Further assortment among individuals within lines may occur as they grow older. However, examination of the heights of all individuals from a typical line over the period of the study suggests that much of the assortment may have occurred during the first two years in the field.

SUMMARY AND CONCLUSIONS

The basis for estimating the effort required to produce desired numbers of S, loblolly pine seedlings for testing is provided. This should be a useful tool for breeders who plan to make large numbers of self-pollinations of non-inbred loblolly pines. If phenotypic selections for height growth are to be made among S, progenies in field trials, it may be possible to do so after two years in field tests.

The large amount of effort required to produce large numbers of S, progenies, their subsequent lower germination and survival rates, and uncertainty about the efficacy of selecting good general combiners from selfed lines suggest that embarking upon such a program be done with caution.

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