SINGLE CLONE ORCHARD PRODUCTION OF PITCH X LOBLOLLY HYBRIDS

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ABSTRACT--Inbreeding depression tests with four clones of pitch pine indicate that select clones may be sufficiently self sterile to make supplemental mass pollination of isolated single-clone seed orchards with loblolly pollen an effective and economical means of producing superior pitch x loblolly hybrids.

INTRODUCTION

Studies conducted by the United States Forest Service indicate that interspecific hybrids between pitch pine, Pinus rigida Mill., and loblolly pine, Pinus taeda L., may be valuable timber trees for forestry use in the northeastern United States. Many hybrid families combine the fast growth and excellent form of loblolly with the cold hardiness and drought resistance of pitch (Little and Trew, 1979). Certain hybrid families also seem to be more resistant to fusiform rust, Cronartium fusiforme, than their corresponding loblolly parents (Trew, 1982).

To date, 29 first generation hybrid plantations have been established in nine states (Kentucky, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Virginia, and West Virginia). Planting sites differ in elevation, climate, soils, and past land use (Little and Trew, 1979). While results vary among plantations, certain families of fast growing, well formed hybrids have been identified. Results from the plantations have led to a strong demand for hybrid seed and seedlings.

Hybrids used in the test plantations have been produced by standard control pollination techniques (Bramlett and O'Gwynn, 1981), which are thought to be too costly for mass production (Bridgewater and Trew, 1981). Various techniques are now under consideration by the Pitch x Loblolly Cooperative including tissue culture, rooting, induced male sterility, flower stimulation, and supplemental mass pollination (mistblowing). So far, none of these methods have proven to be both effective and economical.

Supplemental mass pollinations have been conducted at the Forest Service clonal orchards in New Lisbon, N.J. since 1975. These operations are performed by using a backpack airblast sprayer to blow pollen onto receptive female conelets. Allozyme analysis of seed harvested from a multiclone pitch pine orchard mistblown with loblolly pollen in 1976 indicated that an average of only 11% were hybrids (range 1% -32%) (Joly and Adams, 1983). A shortleaf pine, Pinus echinata Mill., seed orchard mistblown with slash pine, Pinus

receptive female conelets. Allozyme analysis of seed harvested from a multiclone pitch pine orchard mistblown with loblolly pollen in 1976 indicated that an average of only 11% were hybrids (range 1% -32%) (Joly and Adams, 1983). A shortleaf pine, Pinus echinata Mill., seed orchard mistblown with slash pine, Pinus elliotti Engelm., pollen produced an estimated 10.7% hybrids (Bridgewater and Trew, 1981).

To increase the percentage of pitch x loblolly hybrids in mistblown seedlots the percentage of selfed and outcrossed seed must be reduced. Outcrossing can be minimized by establishing a single-clone orchard isolated from other sources of pitch pollen. While complete pollen isolation may be impossible, orchards in the mid-Atlantic and New England states could be situated at least three to five kilometers from the nearest source of pitch pollen. Mistblowing such orchards with loblolly pollen will yield two types of seed: self pollinated and hybrid. Predominantly hybrid seed will be produced if there is a significant reduction in the number of filled seeds upon selfing. Inbreeding depression effects on germination, survival, and growth provide additional means of reducing the number of selfs in nursery beds of mistblown seedlings.

This study was designed to determine if any of four pitch pine clones known to produce good pitch x loblolly progeny meet inbreeding depression criteria for single-clone orchard use.

MATERIALS AND METHODS

All breeding work was conducted at the U.S. Forest Service field office in New Lisbon, N.J. Controlled crosses were made in loblolly and pitch pine seed orchards established in 1963-64 by the Northeastern Forest Experiment Station and the Westvaco Corporation. Each clonal orchard consists of phenotypically superior selections planted in rows of 8-16 ramets to facilitate controlled pollination.

Four clones of pitch pine were selected as female parents for the study. The clones were chosen on the basis of precocious flowering and their demonstrated ability to produce good pitch x loblolly hybrids. Two clones each of loblolly pine and pitch pine were chosen as pollen parents. All pollen used was fresh and tested for viability. Ortet data on the female and male clones used are listed below.

clone #	county	state	height	age
		Pitch Pine Females		
62	Tompkins	New York	95'	160
71	Plymouth	Massachusetts	85'	114
76	Carroll	New Hampshire	68'	63
79	Oxford	Maine	75'	110
		Pitch Pine Males		
15-54	Rabun	Georgia	90'	51

16-269	Burke	North Carolina	64'	40
		Loblolly Pine Males		
4-32	Worcester	Virginia	92'	42
7-56	Williamsburg	South Carolina	90'	36

Pollination bags were mounted on twenty branch tips per ramet, each with a minimum of two female strobili (from hereon referred to as conelets). Four branch tips per ramet were marked as open pollinated controls. Only one ramet per female clone was used in order to avoid possible variation between ramets. Six pollen treatments were applied to each clone. Each treatment was replicated in four pollination bags. The pollen treatments were:

1) No Pollen: unpollinated to test for complete conelet isolation.
2) Self: pollen from the same clone to test for self compatibility versus inbreeding depression.
3) Self + Loblolly: pollen from the same clone plus loblolly pollen in a 1:1 mix to simulate the conditions in a mistblown single clone orchard.
4) Loblolly: a mixture of two loblolly pollens to test the ability of the pitch clone to hybridize with loblolly.
5) Outcross Pitch: a mixture of two pitch pollens to test the effectiveness of the control pollination technique with presumably highly compatible pollen.
6) Open Pollinated: unbagged wind-pollinated control.

All crosses were made using standard control pollination techniques. Beginning in early May 1982, sausage casing style pollination bags were mounted over branch tips with the aid of aluminum rings for added support. Conelets were bagged while in stages one and two (Figure 1), not yet receptive for pollination. When they reached stage five, 0.50cc - 0.75cc of fresh pollen was injected by hypodermic syringe into each bag. The conelets were treated twice at two day intervals to bracket the period of maximum receptivity. The bags were removed when the conelets reached stage six and were no longer receptive.

Two growing seasons are required for pitch pine cones to mature. In September 1983, the cones were harvested. They were kept separate according to treatment and bag number. Conelet abortion was determined by subtracting the number of cones harvested from the number of conelets pollinated. Cones were placed in used paper pollination bags with clear plastic on the upper side in an unheated greenhouse for drying. Cones from clones 71, 76, and 79 opened by January 3, 1984. Cones from clone 62 were serotinous and were opened by heating in an oven at 40° - 45° C. Seeds were extracted from each cone by hand and tallied for each bag separately. The number of seeds per cone was determined by dividing the number of seeds per bag by the number of cones per bag.

As fresh pitch pine seed does not require stratification (USDA, 1974), the seed was ready for germination. All seed was surface

sterilized with 0.7 molar NaOC1 for 15 seconds and placed directly on moist blotter paper in germination trays. Two trays of approximately 60 seeds each were prepared for the four replicates of each treatment. Seed was germinated under eight hours of light at 30° C and sixteen hours of darkness at 20° C for 14 days.

At the end of the germination period, the numbers of normal and abnormal germinants were counted. The remaining seeds were opened to determine whether or not they were filled. The percentage of filled seed was determined by dividing the total number of germinants plus the number of ungerminated filled seed by the initial number of seeds placed in each germination tray. Numbers of filled seeds per cone were determined by multiplying the number of seeds per cone by the percentage of filled seed. Numbers of germinated seedlings per cone were determined by multiplying the number of seeds per cone by the percent germination of all seed (filled and empty).

RESULTS

Clones 71, 76, and 79 did not produce any cones when unpollinated (Table 1), but clone 62 developed eight cones from the initial thirteen conelets bagged. The cones were significantly smaller than those resulting from other pollen treatments. Although normal wings developed, the seeds were small and rudimentary, and are not included as seeds in Table 1. Abortion of self pollinated conelets varied by clone. While all self pollinated conelets of clones 71 and 76 developed into mature cones, in clone 79 only one of fifteen developed. In clone 62, self pollinated conelets aborted less often than those from the outcross pitch treatment.

The total number of seeds per cone was not affected by inbreeding depression. In clones 62 and 71, the number of seeds per cone in the self pollination treatment was actually greater than the number of seeds per cone in outcross pitch pollination. (Data from clone 79 are difficult to assess due to low sample size in the outcross pitch treatment because of damage to three of the four pollination bags during the breeding season. Despite indications of severe inbreeding depression in several characteristics, data from clone 79 will not be analyzed further).

The number of filled seeds per cone resulting from outcross pitch and loblolly pollination of clones 62, 71, and 76 was far greater than those in the self pollination treatment. Clones 71 and 76 produced more filled seed from outcross pitch pollination than from loblolly pollination, but clone 62 produced more with loblolly pollination. In clones 62, 71, and 76 the self + loblolly mix treatment is intermediate between self pollination and loblolly pollination.

Inbreeding depression effect on germination was determined by comparing percent germination of filled seed. In all three clones, percent germination follows the same pattern: outcross pitch > labially >

self + loblolly > self. In all cases, self pollination yielded fewer seedlings per cone than the other pollen treatments. The self + loblolly pollen treatment always produced more seedlings per cone than self pollination but fewer than loblolly pollination.

DISCUSSION

All four clones tested show evidence of inbreeding depression. This supports the findings of others (Ledig and Fryer, 1974) (Wright, 1976).

There is clonal variation in the ability of pitch pine to develop mature cones without pollination. This supports the findings of McLemore (1977) with loblolly, slash, longleaf, and shortleaf pines. Based on incomplete results from clone 79, there appears to be clonal variation in abortion of self pollinated pitch pine conelets, however additional studies are required to confirm this behavior.

The total number of seeds per cone was unaffected by selfing, but the number of filled seeds per cone was sharply lower. Viable pollen must be present in the pollen chamber for a seed to develop. Since the presence of a seedcoat indicates that pollination has taken place (Bramlett, 1981), empty seeds are probably the result of embryo collapse and not lack of pollination (McCall and Kellison, 1981). Embryo collapse in self pollinated seed is thought to be the result of expression of deleterious recessive alleles in the embryo (Orr—Ewing, 1957). Embryo collapse in hybrid seed may also be due to differential physiology between species.

Germination of filled seed was lower for self pollination than for other pollen treatments, probably because of of the expression of lethal and sub—lethal genes during germination. Self pollinated embryos appear to carry the largest genetic load and outcross pitch embryos the least.

The self + loblolly pollen treatment was designed to simulate the conditions in an isolated single—clone pitch pine orchard mistblown with loblolly pollen. In clones 62, 71, and 76 mixing self and loblolly pollen together always produced more germinants than when only self pollen was applied. In clone 62 the difference was very significant, 45.1 seedlings per cone versus 2.8 seedlings per cone. The increase in number of germinants was due to the loblolly pollen and the additional seedlings are presumably hybrids.

Studies by Franklin (1974) indicate that the first pollen reaching an ovule is most likely to fertilize that ovule. Early application of mistblown pollen, in conjunction with attempts to retard development and shedding of orchard pollen, will result in loblolly pollen reaching ovules before self pollen, thus further increasing the percentage of hybrids in mistblown seedlots.

CONCLUSION

Results of this experiment indicate that mistblowing isolated single—clone pitch pine seed orchards with loblolly pollen may be an effective and economical technique for mass producing pitch x loblolly hybrids.

The percentage of hybrids in mistblown seedlots will depend upon the degree of inbreeding depression of the pitch clone and its compatibility with the loblolly pollens used. Lower survival and slower growth of selfed seedlings provide additional means of increasing the percenta [g]e of hybrids at
the nursery.

Establishing several single—clone orchards and mistblowing each with a variety of compatible loblolly pollens could reduce problems associated with low genetic variability. Ease of access must be weighed against adequate pollen isolation when deciding upon the number of orchards to be located in any particular area. The optimum number of such orchards and pollens required to provide good genetic variation is open to debate.

In order for this technique to become accepted, it must be cost effective. Currently, mistblowing is being carried out operationally in several southern pine seed orchards. Mistblowing in seed orchards may become commonplace as a means of alleviating pollen shortages and to circumvent inbreeding, with increases in seed yield and variablity justifying the additional expense (Kellison, 1971) (Bridgewater and Trew, 1981). Mistblowing single—clone pitch pine orchards with loblolly pollen should be equally cost effective.

While this study was designed to test the feasibility of single—clone orchard production of pitch x loblolly hybrids, the technique appears to be readily adaptable for production of other commercially valuable southern pine hybrids.

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Figure 1. Developmental Stages of Southern Pine Conelets

STAGE 1

The female flower bud (♀) is small and tightly enclosed within the bud scales. One to several flower buds may occur in a lateral position on a vegetative shoot bud (v). This is a good time to identify potential branches for pollination, but it is still too early to bag the flowers. Bagging at this stage causes female flowers to be receptive before fresh pollen is available.

STAGE 2

Flower buds have enlarged, but the flower primordia are still enclosed within the bud scales. Light-colored scales are noticeable at the tip of the bud. Isolation bags should be installed now.

STAGE 3

The flower has begun to emerge through the top of the scales. Flowers are normally red, pink, or light green. Since flowers may have received pollen of unknown origin, it is too late to bag.

STAGE 4

The flower has elongated and extends beyond the bud scales, but the lower one-third to one-half of the flower is still enclosed by the bud scales. Delay pollination.

STAGE 5

The flower has completely emerged from the bud scales. Typically, the bud scales are rolled back, and the flower scales approach a right angle with the axis of the conelet. The flower is at its maximum receptivity because the opening between the scales and the bracts offers the greatest access to pollen. Stage 5 normally lasts from one to several days. Pollinate at this stage.

STAGE 6

The flower is no longer receptive because the growth of the scales completely closes the openings. Pollinations in stage 6 produce no seeds.

(Bramlett and O'Gwynn, 1980)

Table 1. SUMMARY OF CONTROLLED POLLINATIONS

Clone #	Pollen Treatment	Conelets Pollinated	Cones Matured	Seeds per Cone	Filled Seeds per Cone	% Germination of Filled Seed	Germinants per Cone
62	No Pollen	13	8	0.0	_	-	-
	Self	16	11	94.8	3.4	82.4	2.8
	Self + Loblolly	13	6	110.3	53.7	84.0	45.1
	Loblolly	12	4	120.0	60.4	87.8	53.0
	Outcross Pitch	12	5	89.2	30.6	92.4	28.3
	Open Pollinated	18	17	116.0	45.5	82.3	37.7
71	No Pollen	15	0	-	-	-	
	Self	14	14	92.5	13.6	69.1	9.4
	Self + Loblolly	20	20	78.7	17.6	82.4	14.5
	Loblolly	16	16	87.5	27.5	94.9	26.1
	Outcross Pitch	17	13	83.2	67.1	96.9	65.0
	Open Pollinated	17	15	82.7	46.9	95.1	44.6
76	No Pollen	18	0		-	-	
	Self	16	18	58.3	25.7	72.0	18.5
	Self + Loblolly	15	14	62.1	27.0	82.6	22.3
	Loblolly	12	12	78.2	31.7	87.8	27.8
	Outcross Pitch	12	12	71.3	59.9	95.3	57.1
	Open Pollinated	12	12	81.3	73.0	97.0	70.8
79	No Pollen	14	0				
	Self	15	1	100.0	11.5	55.7	6.4
	Self + Loblolly	12	0				
	Loblolly	11	7	118.0	57.3	97.0	55.6
	Outcross Pitch	4	1	90.1	79.2	96.5	76.4
	Open Pollinated	17	16	125.3	102.1	97.5	99.5