SUPPLEMENTAL MASS POLLINATION OF SINGLE CLONE ORCHARDS FOR THE PRODUCTION OF SOUTHERN PINE HYBRIDS

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Abstract.--Supplemental mass pollination, "mistblowing", of a multiclone pitch pine orchard with loblolly pine pollen produced an average of 11% hybrid seed. Results of a controlled pollination study indicate that mistblowing isolated single clone pitch pine orchards would produce much higher percentages of pitch x loblolly hybrids. This technique could also be used to mass produce other commercially valuable southern pine hybrids.

Additional keywords: Pinus rigida, P. taeda, inbreeding depression, seed orchard, supplemental mass pollination.

The northern range of loblolly pine, Pinus taeda L., extends into Maryland, Delaware, and even southern New Jersey (Little, 1971) . Unfortunately when loblolly is planted in colder areas to the north and west, it is susceptible to snow and ice damage and winter desiccation. Pitch pine, P. rigida Mill., although cold hardy, is noted for slow growth and poor form. Having observed fast growing hybrids between the two species growing around loblolly plantations in Maryland and New Jersey, Dr. Silas Little of the U.S. Forest Service saw the potential of using the hybrid for reforestation in the northeastern United States.

In the early 1960's, the U.S. Forest Service and Westvaco signed a cooperative agreement to breed and field test pitch x loblolly pine hybrids. Under the direction of Dr. Little and Fred Trew of Westvaco, the cooperative intensively selected 33 loblolly pines in Maryland and Delaware, and 32 pitch pines from Virginia, West Virginia, Maryland, New Jersey, Pennsylvania, New York, Massachusetts, New Hampshire, and Maine. In 1964, a clonal breeding orchard was established at the Northeastern Forest Experiment Station field office in New Lisbon, NJ. By 1968, enough female strobili were present to initiate controlled pollinations. The first test plantations were established in 1971 (Little and Trew, 1979). To date, there are over 50 hybrid plantations in several northeastern and midwestern states.

Although there currently is a strong demand for hybrid seedlings, the controlled pollination technique used to produce the hybrid seed is far too costly for mass production. Reforestation with hybrids on a large scale is dependent upon development of economical mass production techniques. Supplemental mass pollination is one of several techniques under consideration.

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Mistblowing experiments have been conducted at the multiclone New Lisbon orchard for several years. Allozyme analysis of seed collected from the 1976 mistblowing indicate that an average of 11% of the seed was hybrid (Joly and Adams, 1983). The remaining seed was either self pollinated or was outcrossed to other clones of pitch pine in the orchard or wild pitch pines from the surrounding vicinity. Much higher percentages of hybrids must be produced in order for mistblowing to become operational.

A single clone pitch pine orchard, isolated from all outside sources of compatible pollen, mistblown with loblolly pollen can yield only two types of seed: self pollinated and hybrid. If the pitch clone selected exhibits a significant degree of inbreeding depression, most of the seed will be hybrid.

MATERIALS AND METHODS

All breeding work was conducted at the U.S. Forest Service field office in New Lisbon, NJ. Controlled crosses were made in loblolly pine and pitch pine seed orchards established in 1963-64 by the Northeastern Forest Experiment Station and Westvaco. 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 controlled 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 I and II (Bramlett and O'Gwynn, 1980). When they reached stage V, 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 VI.

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 400 - 45o 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 Na0Cl 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 300 C and 16 hours of darkness at 200 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).

In February of 1984, 28 self pollinated, 28 loblolly pollinated, and 28 self + loblolly pollinated seedlings from clone 76 were planted in Ray Leach super cells. (Only clone 76 was used because there were not enough seedlings from the other clones). The seedlings were grown in a heated greenhouse under supplemental lighting. In September of 1984 the seedlings were transplanted into one gallon containers.

RESULTS

Clones 71, 76, and 79 did not produce any cones when unpollinated (table 1), but clone 62 developed eight cones from the initial 13 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 counted 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).

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 > loblolly > 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.

As of June 1985, seedling survival is 93% for the hybrids, 54% for the selfs and 61% for the self + loblolly pollinated seedlings. The hybrid seedlings are an average of three times taller than the selfs. There is no overlap between the two groups as the shortest hybrid is still taller than the tallest self. The self + loblolly pollinated seedlings have segregated into two populations, fast growing and slow growing. The average height of the fast growing seedlings is equivalent to that of the hybrids and the average height of the slower growing seedlings is equivalent to the of the selfs.

DISCUSSION

Use of inbreeding depression to facilitate production of hybrids is certainly not a new idea. It is widely used in crop breeding and its application to forest genetics was discussed by Wright in 1976. The effects of inbreeding depression can be used to increase the percentage of pitch x loblolly hybrids in various ways. In the four clones of pitch pine tested, self pollinated cones always had fewer filled seeds that loblolly pollinated cones. Although southern pine pollen is capable of self pollination and fertilization (Bramlett, 1981), embryo collapse is more likely to occur when a pitch pine ovule is self pollinated than when loblolly pollinated. Thus there

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

should be more hybrids than selfs in mistblown seedlots (assuming equal pollen volumes).

All four clones of pitch tested had lower seed germination upon selfing than with loblolly pollination. Reduced germination of selfed seeds in the nursery seedbed will further increase the percentage of hybrids.

Inbreeding depression in survival and growth of selfed seedlings, as seen in clone 76, indicates yet another means of increasing the percentage of hybrids. As the seedlings grow in the seedbed, there is competition for light, nutrients, water, and space. Many of the smaller self pollinated seedlings will suffer and die as a result. In addition, many of the surviving selfs can be rogued out of harvested mistblown seedlings during standard nursery grading operations. In our greenhouse experiment, it appears that all of the selfs could be rogued out of the self + loblolly seedling group.

It is impossible to accurately estimate percentages of hybrids which could be produced by mistblowing single clone orchards based solely on data from this experiment. The actual percentage of hybrids produced will depend upon the degree of inbreeding depression expressed by the orchard clone, compatibility of the orchard clone to the pollens used, the amount of pollen mistblown, and the timing of the application. Even so, it seems safe to predict that in clone 62, where 53.0 seedlings per cone developed when loblolly pollinated compared to only 2.8 per cone when self pollinated, the percentage of hybrids in mistblown seedlots would be very high.

CONCLUSION

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

Establishing several single clone orchards and mistblowing each with a variety of compatible loblolly pollens would 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.

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 variability justifying the additional expense (Kellison, 1971) (Bridgewater and Trew, 1981). Mistblowing single clone orchards 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 the production of other commercially valuable southern pine hybrids.

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