POLLEN QUANTITY AFFECTS CONE AND SEED YIELDS

IN CONTROLLED SLASH PINE POLLINATIONS

David L. Bramlett[1/]

Abstract.--Controlled pollination treatments ranging from 0-32 puffs of pollen per bag were tested on slash pine in the Georgia Forestry Commission's Arrowhead Seed Orchard, Pulaski County, Georgia. Cone survival, cone size, seed yield, and seed efficiency increased with increased quantities of pollen. Filled seed yields ranged from 0 in the unpollinated cones to 100 seeds per cone in the 32-puff treatment. The efficiency of production of viable seeds was five times greater for the 32-puff treatment than the 2-puff treatment. Applying large quantities of pollen would enable the seed orchard manager to pollinate fewer bags, yet still provide adequate amounts of seed for progeny testing or advanced breeding.

KEYWORDS: Seed orchard, seed efficiency, seed production, *Pinus elliottii*.

Controlled pollination is required in progeny tests, advanced breeding programs, and interspecific hybridization of southern pines. This work is time-consuming and expensive and should be designed to produce seeds as efficiently as possible. Seed yields from controlled pollinations, however, have been much lower than the seed potential of the cones (DeBarr and others 1975; Snyder and Squillace 1966). One possible cause of low seed yields is application of too little pollen. This study evaluated the effect of the pollen quantity on the seed and cone yields from controlled pollinations of slash pine.

METHODS

Five slash pine trees, each representing a different clone, in the Georgia Forestry Commission's Arrowhead Seed Orchard, Pulaski County, Georgia, were selected as study trees. On each tree, 48 flower bearing branch tips were tagged and 6 branches were randomly assigned to each of 8 pollination treatments.

Treatments 1 through 6 were controlled pollinations with 0, 2, 4, 8, 16, and 32 puffs- of pollen applied to each bag with a standard syringe and bulb pollinator (Mergen and others 1955). One puff of the syringe was equal to roughly 0.06 ml of pollen or approximately 6 x 10' individual pollen grains.--/ The pollen

1/ Plant Physiologist, Southeastern Forest Experiment Station, USDA Forest Service, Macon, Georgia.
2/ A puff of the syringe pollinator was generated by expelling the air from the bulb.
3/ Personal corn nication, Fred Matthews, USFS, Athens, Georgia.

supply was a fresh, five-tree polymix of unrelated clones from the Arrowhead Seed Orchard. Pollen was applied at only one time to each bag. Treatments 7 and 8 were wind pollinations and did not include any supplemental pollen. All study trees were sprayed with Guthion insecticide at monthly intervals from April-October of 1975 and 1976. Furadan 10G was applied to each study tree in February 1976, at a rate of eight ounces per inch of tree diameter. In addition, fiberglass screen cages (Bramlett and others 1977b) were installed on the conelets in treatments 1 through 7 in 1975. The new vegetative growth was released from the distal end of screens in spring of 1976 and the screens maintained until cone maturity.

All mature cones were collected in September 1976. From each study tree, five cones in each treatment were randomly selected for analysis. Sample cones were air dryed until the scales began to separate and then dried in a forced-draft oven at 40°C for 24 hours. All loose seeds were extracted from the cone. Then all cone scales were removed and examined to determine the numbers of total scales, fertile scales (seed potential), aborted ovules, and unextracted seeds. All developed seeds from the cone were radiographed and classified as filled, empty, malformed, fungus damaged, or incomplete gametophyte by the method of Bramlett and others (1977a).

Seed germination was measured after 30 days in sand flats on greenhouse benches. Analyses of variance were used to evaluate the effects of pollen treatments on the cone, seed, and germination performance of the study trees.

RESULTS AND DISCUSSION

Cone Survival

Unpollinated flowers frequently aborted before cone maturity and had an average of 28 percent survival on the five study trees. Flowers pollinated with 2 puffs of pollen averaged 55 percent survival. Treatments with 4, 8, 16, and 32 puffs had an average of 75, 77, 75, and 70 percent survival respectively, but the mean survival values for all pollination treatments (2-32 puffs) were not significantly different. Wind-pollinated flowers averaged 71 percent survival with screens and 70 percent without screens.

Some cone losses were caused by mechanical (broken branches, etc.), insect, and fungus damage. Dead cones with no external symptoms of damage were classed as aborted. Cones not present at the periodic counts were recorded as missing. When the survival percentage of cones was adjusted to consider only mortality in the aborted and missing classes, cone survival averaged 87 percent in the controlled pollinated cones; 88 percent in wind-pollinated cones, and 36 percent in unpollinated cones.

Cone Morphology

The few unpollinated cones that survived were much smaller than the pollinated cones. Both cone length and width increased with increasing quantities of pollen applied (table 1). From these data it appears that the developing seed influence, to some degree, the scale development and size of the mature cone.

Table 1.--Average cone size, number of scales, and seed potential from pollination treatments in five slash pine study trees

Pollination treatment (puffs)	Cone length 1/	Cone width 1/	Fertile scales 2/	Total scales 2/	Seed potential 2/
	----mm ---	---	---------	number	--------
0	98	33	86	158	171
2	111 c	40 c	86	159	171
4	114 bc	41 c	91	166	183
8	113 bc	42 c	86	162	172
16	120 bc	46 b	89	164	177
32	123 h	47 b	90	167	179
Wind	115 bc	46 b	90	164	180
Wind 3/	133 a	51 a	89	161	179

1/ Values not followed by the same letter are significantly different at the 0.05 level of probability by Duncan's New Multiple Range Test. Treatment I was not included in analysis of variance because of missing values due to conelet abortion.
2/ No significant differences between treatments.
3/Cone and corselets not protected with screen cages.

Caging significantly reduced the size of wind-pollinated cones (treatment 7 vs treatment 8). Apparently the cage reduced light intensity and thus reduced the quantity of photosynthate supplied to the developing cone. No other measured morphological trait (including the number of fertile scales, total scales, and the seed potential) was influenced by the pollination treatment (table 1).

Seed Yield

The total number of seed as well as the filled seed yield from mature cones varied directly with the quantity of pollen applied to the flowers (table 2). As expected, unpollinated cones yielded no seeds. Total seeds/cone increased from 57 with 2 puffs of pollen to 138 with 32 puffs. Wind-pollinated cones averaged 157 seeds per cone with screens and 129 seeds per cone without screens.

As pollen quantity increased from 2 to 32 puffs, the yield increased from 29 to 100 filled seeds per cone. Wind-pollinated cones averaged 120 filled seeds per cone with screen cages and 114 filled seeds per cone without screen cages.

Table 2.--Average seed yields and seed losses from pollination treatments in slash pine

Pollination treatment (puffs)	Aborted ovules		Developed seed					
			Seed losses					
	First year 1/	Second year 2/	Incomp. 2/	Malform. 2/	Fungus 1/	Empty 1/	Filled 1/	Total 1/
	number							
0	171	0	0	0	0	0	0	0
2	111 ab	3	6	0	3 abc	21 abc	29 c	57 c
4	117 a	1	4	0	2 bc	20 bc	38 c	64 c
8	90 b	3	3	0	6 ab	24 abc	45 c	79 c
16	60 c	5	4	0	8 a	24 abc	77 b	113 b
32	39 cd	3	4	0	7 ab	27 ab	100 a	138 ab
Wind	19 d	4	4	0	2 bc	32 a	120 a	157 a
Wind 3/	46 c	4	1	0	0 c	14 c	114 a	129 b

1/ Values not followed by the same letter are significantly different at the 0.05 level of probability by Duncan's New Multiple Range Test. Treatment 1 was not included in analysis of variance because of missing values due to conelet abortion.
2/ No significant differences between treatments.
3/ Cones and conelets not protected with screen cages.

Seed Losses

Since the only product of value to the tree improvement program is filled seeds, all other classes of ovules and seeds are considered as seed losses. These losses include ovules that aborted before seedcoat development.

In contrast to the seed yields, the seed lost as first-year aborted ovules, i.e., ovules aborting during conelet stage (Bramlett 1974), decreased as the quantity of applied pollen increased (table 2). All ovules in unpollinated cones (171 per cone) were classified as first-year aborted. In the 32-puff treatment an average of 39 ovules per cone were in this category. These results confirm a previous report that lack of viable pollen causes the ovule to abort during the first year of development (McWilliam 1959). The larger number of aborted ovules in the unscreened, wind-pollinated cones indicates that some ovules were lost to seedbugs. These observations substantiate the report of DeBarr and others (1975) that both insects and lack of pollen cause abortion of ovules in slash pine seed orchards.

Very few second-year aborted ovules were observed in the study and pollination treatments were not significantly different. None of the second-year ovules appeared to abort from insect damage.

Developed seed losses were classed as incomplete gametophytes, malformed, fungus, or empty seed. No insect damage was observed on the seed radiographs.

Seeds with incomplete gametophytes were infrequent in all treatments and although some malformed seeds were observed in all pollination treatments, all treatments averaged less than one malformed seed per cone.

Some seeds were lost to fungi in all treatments except the wind pollination treatments with no screen cage. These seed losses could be related to the nutritional status of the cone or to the increased fungal damage in the constrictive cage. Caged branches frequently contained dead or damaged needles and fungal mycelium on the cone scales.

The empty seed class included the typical "pops" in which only a remanent of the embryo and gametophyte are present. These seed losses increased slightly in number as the pollen quantity increased, but the percentage of empty seeds decreased because fewer total seeds were produced in cones that received less pollen. The number of empty seeds, however, was greater in the wind-pollinated cones with screen cages than in cones without screens.

Seed Production Efficiency

The efficiency of seed production was rated for each pollination treatment by comparing actual with potential yields of cones, seeds, and seedlings. Thus, cone efficiency (CE) is the ratio of healthy mature cones to pollinated flowers. Only unpollinated flowers had noticeably reduced cone efficiency, although the 2-puff pollination did have a lower mean cone efficiency than other pollination treatments (table 3).

Seed efficiency (SE), the ratio of filled seed to the seed potential for each cone, is the most important measure of pollination effectiveness. It compares the number of seeds the normal reproductive system produces with the number of functional ovules in the cone.

Extraction efficiency (EE) measures the success of seed extraction from mature cones. The degree of cone opening was the most important factor in seed extraction. The slash pine cones evaluated in this study generally opened well and extraction efficiency averaged 90 percent. Some cones with fungal mycelium on the scales did not open as well as the cones with no fungi. In general, there was little or no effect of the pollen treatments on seed extraction.

Germination efficiency (GE) is the ratio of the number of germinated seed to number of filled seed produced by the cone. The germination percentage was generally less for caged cones than for wind-pollinated, uncaged cones. Apparently, the reduced germination was related to the higher frequency of fungus damage and incomplete gametophyte development in the caged cones.

Seed Orchard to Nursery Efficiency

The seed orchard to nursery efficiency (SO-NE) was calculated as a product of cone efficiency, seed efficiency, extraction efficiency, and germination efficiency:

$$SO\text{-}NE = CE \times SE \times EE \times GE.$$

The SO-NE considers the loss ratios of flowers to cones, seed potential to filled seed, extraction of seed, and the germination process. This value was then a ratio of the number of seedlings produced from the cones collected from a given number of pollinated flowers. For example, in the 2-puff treatment, the overall cone efficiency was 55 percent (CE = 0.55) and the seed efficiency was 16 percent (SE = 0.16). The extraction efficiency was 82 percent (EE = 0.82) and the percent germination of filled seeds was 70 percent (GE = 0.70). Thus, the SO-NE would equal 0.55 x 0.16 x 0.82 x 0.70 = 0.05. In comparison, in the 32-puff treatment, the following values were observed: CE = 0.70; SE = 0.56; EE = 0.91; and GE = 0.73. Thus, SO-NE = 0.70 x 0.56 x 0.91 x 0.73 = 0.26 or 5 times as great as the 2-puff treatment. The SO-NE for wind pollinations was 0.32 caged cones and 0.36 for uncaged cones.

Table 3.--Cone, seed and seedling efficiency from slash pine cones pollinated with varying quantities of pollen

Pollination treatment (puffs)	Cone efficiency 1/6/	Seed efficiency 2/6/7/	Extraction efficiency 3/6/7/	Germination efficiency 4/8/	Seed orchard nursery efficiency 5/
	----------	----------	Percent	----------	----------
0	28 a	0	0	0	0
2	55 b	16 c	82 bc	70	5
4	75 b	21 c	84 abc	62	8
8	77 b	27 c	77 c	64	10
16	75 b	44 b	93 ab	65	20
32	70 b	56 a	91 ab	73	26
Wind	71 b	67 a	95 a	70	32
Wind 9/	70 b	63 a	96 a	84	36

1/ Cone efficiency (CE) = Pollinated flowers/mature cones.
2/Seed efficiency (SE) = Filled seed/seed potential.
3/Extraction efficiency (EE) = Extracted developed seed/total developed seed.
4/ Germination efficiency (GE) = Germinated seed/filled seed.
5/Seed orchard-nursery efficiency (SO-NE) = CE x SE x EE x GE.
6/ Values not followed by the same letter are significantly different at the 0.05 level of probability by Duncan's New Multiple Range Test.
7/ Treatment 1 was not included in analysis of variance because of missing values due to conelet abortion.
8/ No significant differences between treatments.
9/Cones and conelets not protected with screen cages.

CONCLUSIONS

Seed and seedling yields were greatly increased in controlled pollinations of slash pine by applying large volumes of pollen (32 puffs) into each pollination bag. The overall effect of the higher density of pollen was an increased efficiency of the seed production process. On this basis, it appears that a given number of seedlings can be produced from fewer bags by increasing the amount of pollen applied to each bag. These procedures, however, would require larger quantities of pollen to be collected, processed, and stored.

The efficiency statements indicated the key stages in which seed losses were occurring. In this study, treatment effects were most important in influencing the seed yield per cone. The seed efficiency of the highest level of controlled pollination, however, did not equal the wind-pollinated control cones. It is possible that even higher yields of seed could be obtained by pollinating each bag two or more times.

Insect protection is a necessity for the success of controlled pollinations in most seed orchards. In this study, the screen cages reduced the number of first-year aborted ovules but also appeared to cause increased fungus damage, poorer gametophyte development, and poorer seed germination in the mature cones. Systemic insecticides are preferable for insect control after controlled pollinations.

LITERATURE CITED

Bramlett, D. L., E. W. Belcher, Jr., G. L. DeBarr, G. D. Hertel, R. Karrfalt, C. W. Lantz, T. Miller, K. D. Ware, and H. 0. Yates, III. 1977a. Cone analysis of southern pines. Gen. Tech. Rep., USDA, For. Serv. (In press).

Bramlett, David L., William G. Lewis, and Gary L. DeBarr. 1977b. Screen cages to protect pine cones from seedbugs. Tree Planters' Notes (In press).

Bramlett, David L. 1974. Seasonal development and loss of Virginia pine ovules and seed. Seed Sci. and Technol. 2: 285-292.

DeBarr, G. L., D. L. Bramlett, and A. E. Squillace. 1975. Impact of seed insects on control-pollinated slash pine cones. Thirteenth South. For. Tree Improv. Conf. Proc. 1975: 177-181.

McWilliam, J. R. 1959. Interspecific incompatibility in Pinus. Am. J. Bot. 46: 425-433.

Mergen, Francois, Harry Rossoll, and Kenneth B. Pomeroy. 1955. How to control the pollination of slash and longleaf pine. USDA, For. Serv. Res. Pap. SE-58, 14 p. Southeast. For. Exp. Stn., Asheville, N. C.

Snyder, E. B., and A. E. Squillace. 1966. Cone and seed yields from controlled breeding of southern pines. USDA, For. Serv. Res. Pap. SO-22, 7 p. South. For. Exp. Stn New Orleans, La.