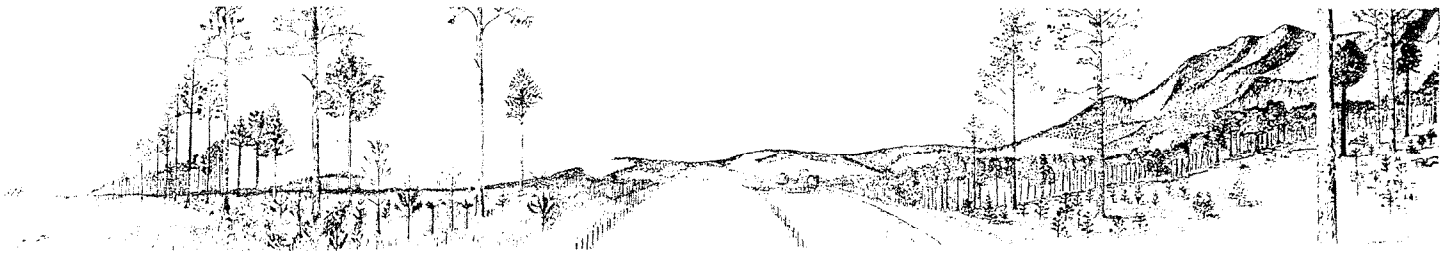

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FRESH AND STORED POLLEN FROM SLASH AND LOBLOLLY PINES COMPARED FOR SEED YIELDS

Abstract. --Seed yields showed no consistent differences between fresh and stored pollen from 8 years of controlled pollination on slash pine and 4 years on loblolly pine. Collection of male strobili at the proper stage of pollen maturity was an important factor in obtaining good seed yields from stored pollen. Criteria are described which were useful in determining when to collect male strobili.

One of the major problems in tree breeding is obtaining sufficient sound seed from controlled pollinations to conduct well-designed progeny tests. Many factors influence the yield of seed obtained from a given number of female strobili some 18 months after pollination. Excellent progress has been made in decreasing losses from some of these factors, such as insects and disease. Some factors which may not be controllable are severe weather, premature conelet drop, damage or loss of first-year cones during mature cone collection, and collection of immature cones. The use of pollen which has been stored from one breeding season until the next has also been associated with poor yields of control-pollinated seed.[1] [2] Our data, however, show that seed yields are similar using either fresh or stored pollen.

The information reported here was accumulated as part of a study of breeding methods in slash pine (Pinus elliottii Engelm.) and loblolly pine (P. taeda L.). The study is being carried out by the Southeastern Forest Experiment Station, USDA Forest Service, in cooperation with the Georgia Forestry Commission at the Commission's Arrowhead and Horse-shoe Bend seed orchards.

METHODS

Since the methods used in pollen handling may play a large part in successful controlled breeding, they are described in more than normal detail.

[1] Campbell, T. E., and Wakeley, Philip C. Possible refinements in controlled pollination of southern pines. Sixth South. Conf, Forest Tree Impr. Proc. 1961: 121-128. 1961.

[2] Snyder, E. Bayne, and Squillace, A. E. Cone and seed yields from controlled breeding of southern pines. South. Forest Exp. Sta., U. S. Forest Serv. Res. Pap. SO-22, 7 pp. 1966.

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Pollen collection. --The techniques used in collecting the male strobili (microsporangiate strobili) varied with age of the orchards.

From 1958 through 1961 the male strobili were collected from the ortets because they were not being produced in sufficient quantity by ramets in the orchards. Branches having male strobili which had not yet shed pollen were cut off about 15 to 25 cm. below the strobili cluster and the needles trimmed to a length of about 5.5 cm. The branches were placed in a polyethylene bag with a handful of moist sphagnum moss for transportation. At the laboratory the branch ends were reclipped, placed in a bottle of water, and kept in a greenhouse until just before pollen shed.

Determination of male strobili maturity is essential to the collection of well-developed pollen which will keep well in storage. Although there is no substitute for field experience, the criteria for strobilus maturity which we have found most helpful are:

(1) A rapid and noticeable elongation of the strobilus axis starting at the base 1 to 3 days before pollen is released. This elongation causes lighter-colored tissue to be exposed between the scale tips.

(2) The basal scales loosen, causing the lower third of the strobilus to appear rough.

(3) The basal portion of the strobilus becomes limber enough to bend when slight pressure is put on the tip.

(4) The mature, but unshed, pollen becomes so dry that squeezing of the strobilus no longer yields a yellow exudate.

As ramets in the orchard came into production, pollen collection was simplified. Clusters of male strobili were enclosed in sausage-casing bags during December or early January; depending on genotype and climate, this can speed male strobili maturity from 3 to 7 days. The bagged clusters were collected when the strobili were judged to be mature. In some cases small amounts of pollen had shed in the bag before collection.

Pollen extraction and storage. --Mature clusters of strobili were put in clean sausage-casing bags or kraft paper bags and hung to finish drying at room temperature maintained at 30" to 32" C. ; air movement was provided by a small fan.

Dry pollen which had shed from the strobili was extracted from the bag through a funnel attached to the bag base. Pollen was judged sufficiently dry if it flowed freely, without clumping and without sticking to the sides of the storage bottle. Square glass bottles of about 80-ml. capacity, not more than half full of pollen and plugged with cotton, were stored in a household refrigerator at 2" **C.**

Polymix preparation. --In preparing a batch of polymix pollen, equal amounts of pollen from 7 to 30 trees were used. When prepared from stored pollen, selection of the pollen for the polymix was based on results of a germination test (3 days at 26° C. in distilled water). Pollens used in the polymixes tested 60 percent or higher.

Cone collection and seed extraction. --Cones which resulted from controlled-pollination were collected as near full maturity as possible. Cones were considered ripe when the cone scales had begun to separate (cracking sound when cone is squeezed). Conditions for cone opening varied considerably, but usually took place indoors in warm rooms with supplemental air circulation.

Extracted seed were dewinged by hand-rubbing and cleaned at the Eastern Tree Seed Laboratory, Macon, Georgia. Seed yields were based on the number of seed per sound cone collected.

RESULTS

The results are based on several years of data from controlled-crosses in which different polymixes of fresh or stored pollen were used (table 1). The following comparisons were analyzed:

Slash pine

(1) The pooled seed yields from all mixes made with fresh pollen averaged 44.3 seed per cone and were compared with those from stored pollen, which averaged 37.3 seed per cone. There was no significant difference between seed yields from fresh and stored pollen.

(2) Polymix I (fresh pollen) was compared with polymix II (stored pollen). Different male parents were used in preparing these mixes. Seed yields from 26 female parents on which both mixes were used were compared statistically. Seed yields from polymix I were conclusively greater ($P < 0.001$)[3] than those from polymix II.

(3) Polymix III, made from fresh pollen, was compared with polymix IV, prepared from stored pollen of the same trees. Controlled pollinations were made on 13 female parents common to both mixes. Seed yields of these trees from polymix IV were conclusively greater than those from polymix III.

(4) Polymix IV was also compared with polymix V, made from fresh pollen a little later in the same season. The male parents in the mixes differed, but both mixes were used on eight common female parents and the yield from those parents compared. Seed yields from polymix IV were conclusively greater than those from polymix V.

[3] Miller, David A. 'Significant' and 'highly significant.' **Nature** (London) 210: 1190. 1966.

(5) Polymix IX, composed of fresh pollen from 15 trees, was compared with polymix X, made from stored pollen. Ten of the pollen parents used were common to both mixes. Based on the seed yields from all trees pollinated with these mixes, fresh pollen yielded conclusively more seed than the stored pollen. Five female parents were pollinated with both mixes. Seed yields from these trees were significantly higher when polymix IX had been used.

Loblolly pine

(1) The pooled seed yields from all polymixes of fresh pollen averaged 32.9 seed per cone and were compared with those from stored pollen, which averaged 27.6 seed per cone. There was no significant difference in seed yields from the use of fresh and stored pollen

(2) Polymix III, composed of stored pollen, was compared with polyrnix IV, made of fresh pollen. Pollinations had been made on 20 female parents with both mixes. There were no significant differences in seed yield between the pollen mixes.

Table 1. --Results of controlled pollination using fresh and stored pollen

Polymix designations	Pollen condition	Pollination year	Male parents in polymix	Female parents pollinated	Cones collected	Average seed yield per cone[1]	
				Number			
SLASH PINE							
II	fresh	1958	30	66	839	54.4	CON.[2]
	stored	1959	28	27	6	34.8	
III	fresh	1959	12	13	240	24.8	CON.
IV	stored	1960	12	73	907	56.8	CON.
V	fresh	1960	20			47.3	
VI	stored	1961	12	35	353	50.4	
VII	fresh	1961	10	42	483	36.9	
IX	fresh	1962	15	11	65	58.3	CON.
X	stored	1963	14	40	730	35.7	
XI	stored	1964		23	310	19.1	
XII	stored	1965	15	12	175	27.0	
LOBLOLLY PINE							
I	stored	1961	21	69	1,053	33.6	
II	fresh	1961	26	27	217	29.5	
III	stored	1962	7	34	407	24.7	NS
IV	fresh	1962	10	42	475	29.4	
V	fresh	1963	13	12	423	39.7	
VI	stored	1964	15	39	710	24.4	

[1] Seed yields are weighted by the number of sound cones collected.
[2] Conclusively greater difference, $P < 0.001$.

DISCUSSION

Analyses of the data from 8 years of controlled-pollination of slash pine and 4 years of loblolly pine show that seed yields from stored pollen can be comparable to those from fresh pollen. Because this conclusion is based on records obtained in the course of work on an applied breeding program, it is difficult to pinpoint exactly what produced such a favorable result. The extraction and storage facilities available are less than those considered ideal," so the explanation we consider most logical lies in the collection and handling of the male strobili.

Unless climatic factors have already caused damage, male strobili collected immediately prior to normal pollen release and handled with reasonable care will yield a high proportion of viable pollen. If fully mature, stored pollen requires little additional drying and is probably able to endure the rigors of cleaning, storage, and use following storage somewhat better than pollen obtained from earlier collection and possibly too harsh extraction and prestorage preparation.

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*Snyder, E. Bayne. Extracting, processing, and storing southern pine pollen. USDA Forest Serv. South. Forest Exp. Sta. Occas. Pap. 191, 14 pp. 1961.

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