POLLEN HANDLING

by

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I have divided the subject into hastening of pollen shedding, pollen extraction, and pollen storage. All experiments mentioned, other than by cited authors, were conducted at the Southern Institute of Forest Genetics during 1956.

Hastening Pollen Shedding

First, how can pollen be obtained days or weeks ahead of natural shedding? Mergen (3) grafted longleaf catkin-bearing scions on slash stock in late December and obtained pollen in time for use on slash flowers. Placing severed branches in jars of nutrient solutions is a complementary method which may be used later in the season and requires less labor and space. The following data were obtained chiefly from severed cuttings. The technique was to:

1. Collect catkin-bearing stems about 12 inches in length.

2. Make a slanting cut on the base of the stems with a very sharp grafting knife.

3. Plunge the basal end into a surface-sterilized container filled with water.

4. Place in greenhouse and keep containers filled with water,

Results from collecting catkin-bearing stems of slash pine up to five weeks before natural shedding are shown in Table 1. Nuclear stages were determined by the simple acetocarmine smear technique (4,5). When catkin-bearing branches were taken before the tetrad stage, no pollen was recovered. At the tetrad stage partially viable pollen was recovered. The next slide illustrates the earliest stage of microsporogenesis which matured any viable pollen. The next slide shows the four immature pollen grains escaping from their microspore cases. Viable pollen matured when catkins were collected at this stage.

Treatments, in addition to those just outlined, might permit earlier collections. Unfortunately, it was not possible to test various treatments at most of the stages, but a battery of treatments was applied 4 to 6 weeks before natural shedding. This was done to avoid conflicting with the pollination program. Although no viable pollen was produced, it is thought that treatments were effectively screened as measured by catkin elongation. Catkin elongation is an essential process

Table 1.-Catkin lengths, date of shedding, germination percentage at 10 months, and stage of pollen from slash pine catkins collected at intervals beginning 5 weeks before natural shedding.

Date of Cutting	Catkin length		Shed before natural shedding	Pollen	
	Initial	Final		Germination	Stage at cutting
	mm.	mm.	days	%	
12/22	11	11	No pollen	-	premeiotic
12/26	12	12	do	-	do
12/29	14	14	do	-	do
12/31	14	14	do	-	do
1/2	20	20	do	-	1st division /
1/4	18	18	do	-	do meiosis
1/6	18	18	do	-	do
1/9	17	17	do	-	2nd division /
1/13	17	27	11	-	do meiosis
1/16	19	40	11	50	tetrads
1/18	18	20	8	-	do
1/20	20	25	8	-	do
1/24	22	36	6	-	immature pollen
1/30	23	38	2	-	do
2/1	25	40	3	-	do visible
2/5	27	45	2	-	prothallial / (?)
2/9	46	46	0	-	do

in pollen shedding. Therefore catkin elongation is the basis for the following recommendations from four factorial experiments. Part of the experiments are shown in the next slide. The recommendations are:

1. Boil cut bases of stems one minute before placing them in final solutions. We made the transfer from the boiling water by tongs and a vial of water so that the cut ends were not exposed to air after boiling.

2. Use a cutflower preservative, or a sugar plus fungicide, especially if high temperatures are to be encountered.

3. Strip needles from stems.

4. Incubate at 25°C.

5. Supplement daylight with continuous artificial light. The beneficial effect of this is attributed to extra heat rather than to a light effect. The next slide shows the apparatus used for testing interrupted darkness and supplemental light.

In the next slide only the stems in the first jar were boiled; no treatment was applied to the second; and the third is a bottle graft. Boiling was the best treatment out of the many tried and in most cases resulted in the shedding of non-viable pollen. The benefit of this treatment is attributed to improved water absorbtion. It had no effect when applied four days before natural shedding probably because water absorption was not a limiting factor within so short a time. Effectiveness of special treatments such as boiling is limited to applications at intermediate nuclear stages. They can't be applied too early or too late.

As an illustration of the possibilities of forcing, longleaf catkin-bearing branches were cut four days before natural shedding. They were placed in water and received no special treatment. Outside conditions were warm and windy, yet we beat mother nature at her best by getting catkins to shed pollen a day or two sooner than those on the outside.

Pollen Storage

Assume that we have extracted pollen and now want to store it for a year. What are some of the conditions affecting its viability? What makes pollen so hard to keep? Table 2 gives some of the answers. Here, pollens with three different initial moisture contents were stored in different amounts per same sized bottle for 10 months. If pollen had a low initial moisture content, viability was maintained, if it had a high one its germination percentage deteriorated. However, if a small dmount of wet pollen was stored, the moisture escaped before fungi or other deletarious agents could ruin the pollen. Other tests have shown that

Table 2.--Germination percentages of different amounts of pollen with different initial moisture contents stored unsealed at 5° - 10°C

Amount per 24 ml. Shell vial	Initial moisture content percentage		
	12	29	51
ml.	%	%	%
4	94	78	73
8	95	76	58
12	90	77	54
16	89	80	49
20	91	79	47
Mean germination Percentage	92	78	56

pollens exposed to a dry atmosphere will attain the same equilibrium moisture content regardless of their initial moisture contents. The speed of drying to a safe moisture content is dependent on the mass of the pollen stored.

What is the correct moisture content and relative humidity for storage? Duffield (2) suggested storage at 25% relative humidity (R.H.). We stored pollen over a saturated solution of potassium acetate to give this R. H. The viability of this pollen was identical to that stored unsealed in a refrigerator and its moisture content after 10 months of storage was 14%. By coincidence, this moisture content is the approximate threshold for fungal activity in organic substrates. Thus there is considerable evidence that a pollen moisture content near 14% is desirable.

What makes pollen spoil when sealed in stored vials? Our results after 8 to 10 months show that pollen with an initial moisture content of 44% was a total loss whereas pollen at a 14% moisture content remained fully viable.

Pollen Extraction

The last column of Table 2 shows that wet pollen may be saved if it is dried. This can be done in many ways. For instance, vacuum desiccation has been used by us with limited success. However, an alternative is to extract under conditions such taht the pollen is already at its correct moisture content. Extraction of dry pollen resulted in better viability after storage, double the yield, freedom from sawfly larvae, and elimination of the extra desiccation operation. The lower humidity probably inhibited the hatching of the insects' eggs.

How can dry pollen be produced? The next slide shows how dry pollen was obtained experimentally. This set up is too elaborate to be used extensively but does illustrate factors in extraction. Here we have a sealed cabinet containing five sealed compartments. The next slide is a close up showing that each compartment has a Placerville-type extractor, chemical solution for controlling humidity, and a fan for circulating air. The cloth is a thin cotton batiste easily penetrated by the circulating air. Minute quantities of pollen escaping through it are not a contamination problem because of the sealed compartment. However, when pollen-proof canvas was used, as at Placerville, wet pollen was produced because the dry air could not penetrate it sufficiently. I think the best procedure is to force air at controlled humidity through the funnel and canvas bag as is done at Placerville.

Pollen with a 147, moisture content was produced in 36 hours at 25°C. and R.H.'s of 37 and 537,, respectively, for longleaf and slash pine. Duffield (1) suggested extraction at 15 to 307. R.H. These figures will vary according to the sample size, treatment time, temperature, velocity of circulating air, and the size and the moisture content of individual catkins.

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