POSSIBLE REFINEMENTS IN CONTROLLED POLLINATION OF SOUTHERN PINES

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At what stage of female strobilus ("flower") development is controlled pollination most effective, in terms of seed yield?

When weather or workload makes it impossible to pollinate at this optimum flower stage, what are the earliest and latest stages at which pollination can be justified?

What is the latest stage at which pollination bags may be installed or the earliest they may be removed without danger of contamination by wind-borne pollen?

Does high laboratory germination of a compatible pollen insure good seed set if the pollen is applied to highly receptive flowers? At what level of laboratory germination percent must pollen be considered too poor to be working taking up the tree?

A definite answer to any of these questions should increase either the effectiveness or the flexibility of controlled-pollination programs. It might increase both.

In 1958 and 1959 we tried to answer these questions by control-pollinating, in various ways, 11 different prolific longleaf pine trees (Pinus palustris Mill.) near Many, Louisiana.: We enclosed 1 ,551 flowers in 707 synthetic sausage-casing bags , as described in Southern Forest Experiment Station Occasional Paper 136, 1954. We control -pollinated with a number of different lots of both longleaf and slash pine (Pinus elliottii Engelm) pollen, both fresh and stored, as needs of individual studies dictated; in some instances we utilized wind-borne longleaf pollen also. We applied treatments in units of 5 to 10 bags per tree, and always replicated on at least 2 trees, usually on 3.

We recorded results in terms of: (1) Bags from which cones matured; (2) Percentages of flowers maturing into cones; (3) Full seeds per flower originally pollinated; and (4) Full seeds per cone matured. When only longleaf pollen had been used, we determined full seeds by cutting. When slash pollen has been applied, we expressed results in terms of viable seeds as determined by nursery sowing.

The yields of full or viable seeds per flower pollinated and per cone matured proved to be the most effective criteria for evaluating the experimental treatments, and are therefore presented in the attached tables.

Adverse weather, some individual-tree incompatibilities, insect damage to flowers and cones, depredations by squirrels, and the loss of one tree to lightning left us with fewer data than we had hoped to get. Analyses of variance although they had been planned, were applicable in none of the studies. Nevertheless some points were definitely proved and others strongly indicated, and are reported and commented on here.

Flower Stages

The 5 series of controlled pollinations described here involved recognition of from 2 to 6 of the 7 flower stages defined by Cumming and Righter in U. S. Department of Agriculture Circular 792, 1948. These are: (1) (Flower) buds small; (2) (Flower) buds large; (3) (Flower) buds opening; (4) Flowers partly open; (5) Flowers (at) maximum (receptivity); (6) Flowers closed; and (7) Cones enlarging.

We necessarily adapted and applied the classification of stages in the light of our experience with the flowers and flowering habits of the southern pines, which differ in detail from those of the western pines on which the classification was originally based.

Stage or Stages at Which to Pollinate

In 1958 we bagged at early stage 1, and pollinated separate lots of bags at stages 2, 3, 4, 5, and 6. That year, at each stage on each tree, we pollinated half the bags with longleaf pollen and half with slash. In 1959 we bagged at very early state 1, and pollinated, separately, at all stages from 1 to 6 inclusive, with slash pollen only.

The results showed definitely that pollination of bagged longleaf flowers at stage 2, with either longleaf or slash pollen, can yield a few viable seeds, and that pollination at stage 3 is quite likely to produce some seeds. The yield even from stage 3, however,, is likely to be less than that from stages 4 or 5, Pollination at stage 3 only or at stage 2 only may sometimes be justifiable, but we do not recomment it for quantity production of control-pollinated seed, or (except as a last resort) for important crosses.

Flowers pollinated at what we classified as stage 4 yielded erratically. Some gave little or no seed, but in general they produced considerably more than the flowers pollinated at

stage 3, and in one instance each year they excelled the best yield of flowers pollinated at stage 5. As a rule, when flowers can more conveniently be pollinated at stage 4, deferment till stage 5 seems hardly justifiable, and when flowers have been pollinated at stage 4, only extraordinary circumstances would seem to justify repollinating them at stage 5.

By and large, best results were obtained by pollinating at stage 5.

In 1958, to our surprise, our pollinations yielded almost as much viable seed from flowers we had classified as stage 6 as from those we had classified as stage 5, and more than from those we had classified as stage 4. We got some viable seed from stage-6 pollinations on one tree in 1959. As stage 6 is, by definition, past receptivity, it is evident that in practice we had misclassified as stage 6 a considerable number of flowers still in late stage 5. This misclassification raises questions as to just how dependably stages 5 and 6 can be differentiated by the unaided eye, especially when the flowers are viewed through the sausage casing. Moreover, it immediately calls into question the safety of removing bags while flowers are still in what we have been accustomed to calling stage 6.

Stage at Which to Bag

On each of 3 longleaf trees in 1959 we bagged flowers at stages 1, 2, 3, and 4, at the rate of 10 bags per stage per tree.

The flowers bagged at stage 1 were exposed to wind pollination until the bags were installed, None of them received any supplemental artificial pollination.

At this point we realized that the flowers might be escaping pollination because there was as yet little or no longleaf pollen in the air, rather than because they were non-receptive. Therefore, at stages 2, 3, and 4, we dusted half of the flowers with longleaf pollen before we put the bags on.

Two trees in this study set seed from flowers bagged at stage 4, one of them from both wind-pollinated and artificially pollinated flowers, and the other only from the flowers pollinated artificially before bagging. Neither tree set seed from flowers bagged at stage 3 or earlier. The third tree set no seed from flowers bagged at any stage.

These results strongly confirm the receptivity of stage-4 flowers that was shown by the stage-of-pollination studies. In the stage-of-pollination studies there was much loose pollen in the bogs. Some of this presumably could have reached the flowers, through agitation of

the bags, and most or all of the viable seed must have developed from pollen grains lodging on or between the flower scales while the flowers were still in stage 4.

The results further suggest that, though receptive, stage-4 flowers (at least on the trees flowering earliest in the season) may escape pollination because of the scantiness of air-borne pollen.

The results also strongly suggest that bagging at stage 2 is amply early for controlled pollinations in which a small percentage of contamination by wind-borne pollen can be tolerated. In commercial hybridization by baggin, for example, it might be far cheaper to accept 2 percent or even 5 percent of seedlings of the female parent species than to exclude stage 2 flowers from bagging.

POLLEN GERMINATION TESTS

In 1958 we tested individual lots of 2-year-old longleaf pollen with current laboratory germinations of 92, 87, 76, *74, 65,* and 58 percent against fres longleaf pollen germinating 92 percent. We applied each of the 7 pollens to flowers in stages 4 and 5 on each of 3 longleaf trees.

One tree produced a few full seeds from the stored pollen germinating 65 percent and a second tree developed a few from the stored pollen germinating 87 percent. Neither yielded full seeds from any other pollen lots, even from the fresh check.

The third tree produced full seeds from 6 of the 7 lots, but not in proportion to pollen germination per cent. In general, the fresh pollen gave the best results; in particular, it far excelled all stored lots in full seeds per flower pollinated and in full seeds per cone that matured. Among the stored lots, that germinating 65 percent yielded the greatest number of full seeds per flower pollinated. The lots germinating 74 and 87 percent were next best, in that order.

In 1959 we similarly pollinated 3 longleaf trees with 1-year-old slash pollens germinating 99, 92, and 80 percent, with fresh slash pollen germinating 99 percent. On 2 of the trees, the stored pollen germinating 80 percent equalled or excelled the stored pollens germinating 92 and 99 percent, both in viable seeds per flower pollinated and in viable seeds per cone matured. On 1 of the 2, the 80-percent stored pollen out-yielded even the fresh check. Squirrels got so many of the cones from the third tree that results can not be assessed.

It appears that pollen germinations of 90 to 99 percent in the laboratory do not insure success in crossing. At the same time, pollen germinating as little as 65 percent may be well worth using in attempts to make greatly desired crosses.

Summary and Conclusions

While the studies just described were not advanced in design or technique, they have proved or strongly indicated the following:

Bagged flowers can mature into cones and produce viable seeds if pollinated at any stage from 2 to the very end of 5. Yields from pollination at stages 4 and 5 are most certain and abundant, but pollination at stage 3 or even at stage 2 may repay the effort under some circumstances.

It is very difficult to distinguish, in practice, between flowers at the end of stage 5 (and hence still receptive) and those that have passes into stage 6 and become non-receptive When avoidance of contamination by wind-borne pollen is imperative, flowers must not be debagged until far into or past stage 6.

When avoidance of contamination is imperative, flowers must be bagged at stage 1, but when a small per centage of contamination is preferable to excessive cost, flowers may be bagged at stage 2.

The laboratory germination indicates only roughly the capacity of the pollen to effect fertilization, and pollen germinating as little as 65 percent may be well worth using in attempts to make certain greatly desired crosses.

Table 1 .--Seeds per flower pollinated and per cone matured in stage-of-pollination studies.

1958 STUDY

Pollen used and flower stage at which applied	Full[1] or viable[2] seeds, by longleaf female parents			
	Per flower pollinated		Per cone matured	
	Tree 11-4	Tree 1-16	Tree 11-4	Tree 1-16
Longleaf pollen at stage:				
2	0.2	0.0	2	--
3	9.7	1.8	33	44
4	22.8	1.2	60	32
5	22.0	9.4	66	51
6	17.1	17.9	48	78
Slash pollen at stage:				
2	.8	.0	8	--
3	1.2	.0	7	--
4	.9	.0	4	--
5	2.4	16.4	9	49
6	1.2	5.4	7	41
	1959 STUDY			
	7-24	6-6	7-24	6-6
Slash pollen at stage:				
1	0.0	--	--	--
2	.0	.0	--	--
3	1.6	.0	4	--
4	6.8	1.5	10	3
5	8.1	2.2	9	3
6	1.2	.0	2	0

1. By cutting test in studies in which only longleaf pollen was used.
2. By seedling counts in nursery in each study in which part or all of pollinations were made with slash pollen.

Table 2.-- Seeds per flower pollinated and per cone matured in 1959 stage-of-bagging study

Pollination before installation of bags.	Flowers bagged at stage:	Full[1] of viable[2] seeds, by longleaf female parents					
		Per Flower pollinated			Per Cone matured		
		Tree 7-21	Tree 7-22	Tree 7-23	Tree 7-21	Tree 7-22	Tree 7-23
By wind only	1	0.0	0.0	0.0	--	--	--
	2	.0	.0	.0	--	--	--
	3	.0	.0	.0	26	--	--
	4	7.6	.0	.0	--	--	--
Wind plus artificial	2	.0	.0	.0	--	--	--
	3	.0	.0	.0	--	--	--
	4	33.5	6.1	.0	38	9	--

I. 2. See footnotes, table 1.

Table 3,--Seeds per flower pollinated and per cone matured in studies of laboratory germination of pollen

1958 STUDY OF LONGLEAF POLLEN

Period stored	Notes on storage	Laboratory germination	Full[1] or viable[2] seeds, by longleaf female parents: Per Flower pollinated: Tree 1-23	Tree 1-16	Tree 1-11	Per cone matured: Tree 1-23	Tree 1-16	Tree 1-11
Years		Percent						
0	Fresh check	92	25.5	0.0	0.0	91	0	0
2	Sealed	92	1.7	.0	.0	13	--	--
2	Sealed	87	2.4	.0	.1	5	--	3
2	Sealed	76	.5	.0	.0	6	--	--
2	Unsealed	74	3.1	.0	.0	14	--	--
2	Unsealed	65	3.4	.8	.0	11	21	--
2	Sealed	58	.0	.0	.0	--	--	--
		1959 STUDY OF SLASH POLLEN						
			7-3	7-16		7-3	7-16	
0	Fresh check	99	5.8	0.9		20	2	
1	Unsealed	99	3.3	1.1		7	1	
1	Unsealed	92	2.5	2.2		6	3	
1	Unsealed	80	5.8	2.8		13	3	

1.2. See footnotes, table 1.