THEORETICAL IMPACT OF POLLEN VIABILITY AND DISTRIBUTION ON THE NUMBER OF STROBILI TO USE FOR CONTROLLED POLLINATIONS IN LOBLOLLY PINE

D. L. Bramlett, F. E. Bridgwater, J. B. Jett, and F. R. Matthews[1/]

Abstract.--The number of female strobili to pollinate per controlled cross in loblolly pine can be estimated from three factors: (1) in vitro pollen viability, (2) pollen distribution frequency within the ovules, and (3) the expected cone survival of pollinated flowers. In this study empirical data on the number of pollen grains per ovule were obtained from sampling loblolly pine conelets 2 weeks after pollination. The frequency distribution for the number of pollen grains per ovule was then used to develop a nonlinear model to estimate pollination effectiveness. Adjustments for less than the maximum number of seeds per cone and for empty seed losses resulted in estimates of filled seed per cone for varying levels of pollen viability. From these data, the numbers of flowers required to produce 300 filled seed per cross are presented for four levels of cone survival. These guidelines can improve breeding effectiveness and efficiency for loblolly pine.

Additional Keywords: *Pinus taeda,* tree breeding, tree improvement

INTRODUCTION

Controlled pollinations are a vital component of the recurrent selection and breeding program of southern pines. Production of adequate seeds for genetic testing requires both an effective and efficient controlled pollination procedure. An effective program would have a high success rate for completion of the attempted prescribed crosses. For example, it is important to have all the cells of the mating design completed before outplanting. However, an effective program could have a high completion rate but would not necessarily be efficient in terms of the required resources such as the number of pollination bags installed, the amount of pollen required, or the labor employed.

[1/] The authors are respectively: Plant Physiologist, Southeastern Forest Experiment Station, Macon, GA; Plant Geneticist, Southeastern Forest Experiment Station, Raleigh, NC; Associate Director, Cooperative Tree Improvement Program, School of Forest Resources, N.C. State University, Raleigh, N.C.; and Plant Pathologist, Southeastern Forest Experiment Station, Athens, GA.

We wish to acknowledge the assistance of Dr. M. Buford, Virginia Polytechnic Institute and State University and Mr. W. D. Smith, North Carolina State University in developing the theoretical model used in this study.

An efficient program could provide a high completion rate for attempted crosses yet should not utilize more than the necessary amount of resources. Some safety margin is prudent, but consistent overproduction of cones or seeds is certainly not cost effective. It would be better to refine the breeding program so that more crosses were completed in a given year rather than to overproduce seeds from a smaller number of crosses.

In research studies to evaluate the factors affecting the success of controlled pollinations, pollen viability appears to be the single most important factor relating to high seed yield per cone (Matthews and Bramlett 1985). Correct timing of female receptivity and the delivery of the pollen to the ovules are important factors but if the pollen is not of high quality, the seed yield and seed quality may be seriously reduced.

Large-scale breeding programs must routinely deal with the subject of pollen viability because of the impact this variable has on how aggressively breeding efforts can be pursued. The North Carolina State Cooperative Tree Improvement Program maintains a large, centralized pollen storage bank to expedite the Cooperative's second-generation breeding program. The Cooperative's program involves the sharing of the control-pollination workload and the exchange of pollens. In order to meet the biological time constraints imposed by the pollination season, it is necessary to use stored pollen.

The Cooperative annually receives and processes for storage some 600 to 800 pollen lots. In the course of processing, several decisions are made that take into account pollen viability. Moisture content and percentage of germination in vitro are determined upon receipt of the pollen. When moisture content exceeds 15 percent, the pollen is dried in an extractory as described by Sprague and Snyder (1981) to a moisture content of 8 to 10 percent. This initial drying is done to stabilize pollen quality until final preparations for long-term storage can be completed. Subsequent drying to approximately 4 percent moisture is done by freeze-drying and the pollen is vacuum sealed in 10 ml vials for long-term storage. Germination tests (0.5 percent agar) are only done before pollen storage. Both research and experience indicate that properly stored pollen suffers little loss in viability for at least several years (Goddard and Matthews 1981).

Pollen lots with viabilities as low as 10 percent are stored when no other pollen of higher viabilitiy is available. This approach assumes that even at viabilities as low as 10 percent, some seed set will be achieved when appropriate amounts of pollen and proper timing are utilized in making controlled pollinations. The low-viability pollen will be employed where required rather than delay breeding for a year. When sufficient pollen is available with viability greater than 50 percent, 80 ml of each pollen lot are put into storage (eight vials of 10 ml each). This amount is considered sufficient to complete the anticipated crosses involving any given pollen even when pollen viabilities approach 10 percent. When the viability for a pollen lot is less than 50 percent, additional pollen is requested the following spring to upgrade the inventory. As long as a cross has not been completed, the plan is to replace or upgrade pollen inventories when the prestorage viability is less than 50 percent.

The complexities of managing a large pollen bank with constantly shifting inventories are eased with a computer-based record system. Matching pollen availability to the availability of female strobili also requires good records to avoid inefficiencies in breeding efforts and lost time.

This paper presents the impact of pollen viability on the effectiveness and efficiency of controlled pollinations in loblolly pine. Empirical data were used to construct a generalized model to generate the pollination effectiveness at varying viability levels. The pollination effectiveness was then transformed to the expected seed yields per cone and the number of strobili estimated to produce 300 filled seed for progeny testing.

EFFECTIVE CONTROLLED POLLINATIONS

The pine reproductive system consists of the megasporangiate strobilus (female flower), and the microsporangiate strobilus (male catkin). The female flowers are in an upright position on the tips of the new vegetative growth and are phenologically synchronized in development to be at maximum receptivity during the peak of pollen release from the male catkins. As the wind transports pollen to the female flower, individual pollen grains adhere to micropyle arms and are transported to the pollen chamber via a pollination droplet. In controlled pollination, breeders try to simulate the natural wind pollination process. Wind pollen is excluded with an isolation bag and the selected pollen is injected into the bag by the breeder. Key elements of successful breeding are (1) correct timing of pollen application, (2) providing adequate quantities and distribution of the pollen to the flowers, and (3) maintaining a high viability and vigor of the pollen.

Up to 10 million pollen grains may be injected into a single pollination bag, and the female flower may have a large amount of pollen between the cone scales, yet only a very small number of pollen grains are found in the pollen chamber. Because only those pollen grains in the pollen chamber are capable of producing seed, the effectiveness of the controlled pollination can be evaluated by examining the pollen chambers and recording the number of pollen grains present. Matthews and Blalock (1981) have described the procedures for making the pollen chamber count and this method has been a valuable tool to quantify the effectiveness of both controlled- and wind-pollinated pines. By using the pollen count technique, the timing of pollen application has been found to be more flexible than once thought. It appears that 2 days before or after maximum receptivity (Stage 5) are nearly as effective as pollination at stage 5 (Bramlett and Matthews 1983).

The pollinator used to supply the pollen can be an important part of the seed yield and many types of pollinators have been successfully used (Bramlett and O'Gwynn 1981). Any device that effectively delivers the pollen to the ovules can be used and may include a camel's-hair brush, wash bottle, syringe, or cyclone pollinator. The quantity of available pollen may influence the choice of pollinator. For very limited amounts of pollen, the camel's-hair brush is effective. When pollen is abundant, the cyclone pollinator uses high volumes of air and pollen to completely distribute pollen to all flowers within the bag.

Best results have been achieved by using the cyclone pollination with 1 cc of pollen applied per bag. Normally, one application at the correct stage of flower development is adequate, but up to three pollen applications per bag may be used when flower development within the bag is widely divergent.

FREQUENCY DISTRIBUTION OF POLLEN WITHIN THE OVULES

In several controlled pollination experiments we have quantified the pollen catch per ovule. From these studies, the pollen distribution approaches but does not equal the average number of pollen grains per ovule for wind-pollinated flowers. In the data presented in Figure 1, four quantities of pollen 0.25, 0.50, 1.00, and 2.00 cc were applied to three separate female clones in the Georgia Forestry Commission's Arrowhead Seed Orchard. Conelets were collected 10 to 14 days after pollination and approximately 1,500 ovules were observed for pollen counts within the pollen chamber. An analysis of variance of the data set indicated no statistical differences for the mean pollen count among the 0.25, 0.50, and 1.00 quantities of pollen. These three quantities were combined in the frequency distributions shown in Figure 1A and had a mean value of 2.22 pollen grains per ovule. In this distribution 12 percent of the ovules had zero pollen grains. If an ovule has no pollen grains or has pollen grains that are not viable, the ovule aborts soon after pollination and no seeds are formed. If the ovule has at least one viable pollen grain, however, the ovule continues development and forms a mature seed coat unless the normal development is disrupted by destructive agents. In Figure 1B, the pollen counts for ovules pollinated with 2.0 cc of pollen had a mean value of 2.78 pollen grains per ovule and lower frequencies of ovules with 0, 1, or 2 pollen grains. Thus, the percentage of ovules with at least one viable pollen grain would be expected to increase with increasing mean values and consequently a low-frequency distribution of ovules with 0, 1, or 2 pollen grains per ovule. For example, in Figure 1C, the frequency distribution for wind-pollinated ovules illustrates a mean value of 3.97 with small numbers of ovules with 0, 1, or 2 pollen grains per ovule.

Regardless of the distribution frequency of the pollen grains in the ovules, the probability that some ovules will have no viable pollen grains increases as the viability decreases. Thus, for each distribution function, the empirical pollination effectiveness can be calculated by summing the probabilities of ovules with at least one viable pollen grain for each pollen count class.

DEVELOPMENT OF A PREDICTIVE MODEL

If the pollen viability and distribution are known, a probability model can be developed for varying quantities of pollen. For example, with 50 percent viable pollen and the pollen distribution as shown in Figure 1A, the combined probabilities would give 60 percent of the ovules with at least one viable pollen grain. This ratio of ovules with one or more viable pollen grains to the total number of ovules per cone (seed potential) has been termed the pollen effectiveness (PE) (Bramlett 1981). Therefore, the maximum predicted seed yield for pollen lots with 50 percent viability and 2.2 pollen grains per ovule would be PE x SEED POTENTIAL or 0.60 x 160= 96 developed seeds per cone. Obviously the actual yield of developed and filled seeds would be lower than the maximum as will be discussed later in the paper.

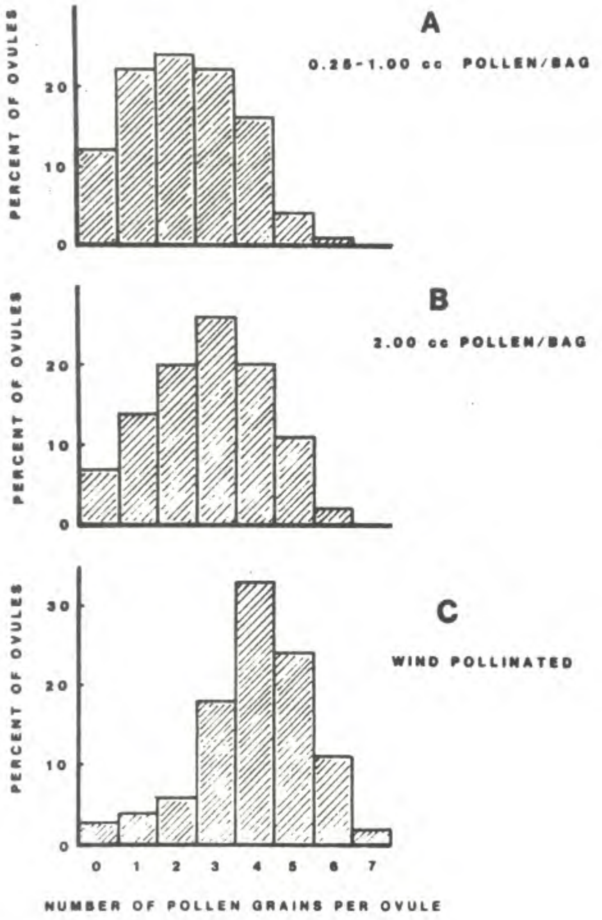

Figure 1.--Number of pollen grains per ovule for loblolly pines following controlled pollinations and wind pollinations (A) Controlled pollinations with 0.50 to 1.0 cc of pollen per bag (B) Controlled pollination with 2.0 cc of pollen per bag (C) Wind pollinations.

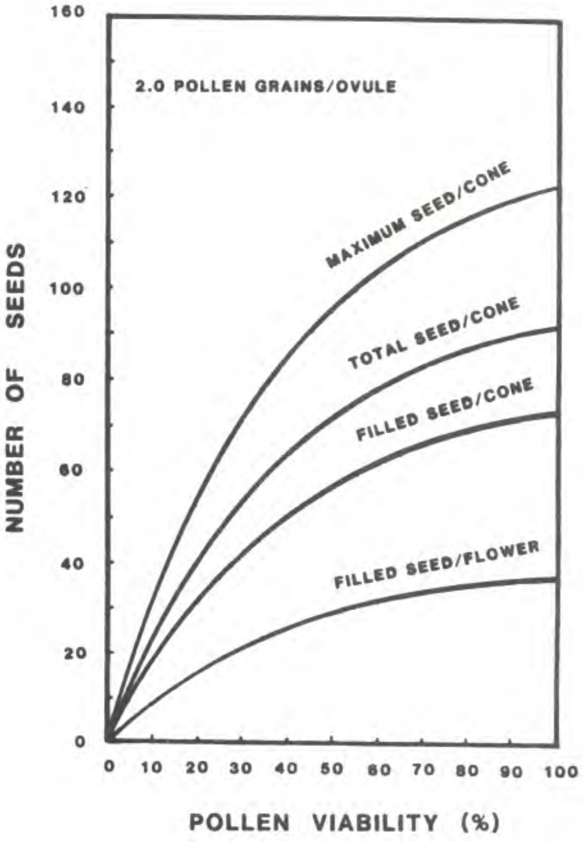

Figure 2.--Predicted maximum seed yield per cone, total developed seed per cone, filled seed per cone, and filled seed per flower for controlled pollinations in loblolly pine. The predicted values are generated from a model with 2.0 pollen grains per ovule and a seed potential of 160 ovules per cone and are shown for varying pollen viabilities.

The next development of the general predictive model was derived from pollen count data collected from a pollination timing study in 1982. In this study, pollen was applied to loblolly pine (*Pinus taeda* L.) flowers at varying female flower development stages (Bramlett and Matthews 1983). Pollen application before or after the date of maximum receptivitiy reduced the mean number of pollen grains per ovule. Using this data set a nonlinear predictive model was derived by fitting the observed data with a function described by Richards (1959):

$$\text{Pollination effectiveness} = a \times (1-e^{(-b \times \text{VIAB})})$$

$$\text{Where: } a = 8.0402 + (53.4169 \times \text{MPC}) - (7.7432 \times (\text{MPC}^2))$$

$$b = 0.00737 + (0.0087 \times \text{MPC})$$

with VIAB = Viability of applied pollen
and MPC = mean pollen count per ovule

From this predictive equation, values of pollination effectiveness were generated for the values of the mean number of pollen grains per ovule (MPC) ranging from 0.50 to [1]4.0 (Table 1). The range of MPC are realistic values that have been observed in controlled pollination studies. Expected values in controlled pollinations average between 2.0 to 3.0 pollen grains per ovules. Counts approaching [1]4.0 have been observed with heavy applications of pollen but would generally be expected to be the upper limit of average number of pollen grains per ovule. Counts below 1.0 occur when low quantities of pollen are applied or when the pollen is not applied at the correct stage of flower development.

Table 1.--Predicted pollination effectiveness and apparent pollination effectiveness for loblolly pine at varying pollen viabilities.

Mean Pollen Grains/Ovule	Pollen Viability (%)				
	10	25	50	75	90
	--------Pollination effectiveness--------				
0.5	0.036	0.083	0.146	0.192	0.214
1.0	0.080	0.178	0.297	0.376	0.411
2.0	0.184	0.388	0.596	0.708	0.749
3.0	0.281	0.559	0.801	0.906	0.938
4.0	0.337	0.638	0.860	0.937	0.956
	----Apparent pollination effectiveness----				
0.5	0.027	0.062	0.110	0.144	0.160
1.0	0.060	0.133	0.223	0.282	0.308
2.0	0.138	0.292	0.447	0.531	0.562
3.0	0.211	0.419	0.601	0.680	0.704
4.0	0.253	0.478	0.645	0.703	0.717

PREDICTED SEED YIELD FROM CONTROLLED POLLINATIONS

The PE value can be transformed to seed yields by multiplying PE value by seed potential. In Figure 2, the predicted number of developed seed per cone for a mean pollen count of 2.0 and varying pollen viabilities is illustrated. The highest values are the maximum seed per cone that could be expected with the given parameters (Maximum Seed/Cone, Fig. 2). However, not all of the ovules with one or more viable pollen grains per ovule will continue development and form a seed coat. Numerous causes may exist but one primary problem is insect damage to the ovule resulting in ovule abortion before seed coat formation.

If the pollen grains have not been counted in the pollen chambers, the apparent pollination effectiveness (APE) can be measured as the ratio of the total developed seed to the seed potential (Total Seed/Cone, Fig. 2). APE approximates PE when insect losses are minimal. From seed yield data when varying quantities of pollen were applied to three loblolly pine clones, APE was 75 percent of the pollination effectiveness as determined by the pollen count per ovule data. Thus the APE for a given tree could be expected to average 0.75 x PE (Table 1).

A second factor in the reduction of seed yield is that not all of the developed seeds are filled. Again, several causes of empty seed are known but insect damage and embryonic abortion are principal reasons that only a percentage of the total seeds are filled and thus capable of germination.

The values in Table 1 for APE can be converted to predicted filled seed yields per cone by expanding to the seed potential for loblolly pine and then adjusting for the percentage of total seeds that are filled. Although the percentage of filled seeds may vary from year to year depending on the level of insect protection and other factors, an average of 80 percent filled seed is a reasonable number to use for seed orchard controlled pollinations. Thus the predicted filled seeds per cone would equal:

Filled seed (FS) = APE x SP x PFS
APE = Apparent pollination effectiveness
SP = Average seed potential for loblolly pine (160)
PFS = Percent filled seed (0.80)

Values of predicted filled seeds per cone for varying viabilities and pollen distribution functions are shown in Table 2. As expected, the seed yields in Table 2 indicate that low seed values are associated with low mean pollen counts per ovule. These low mean values could result from inadequate distribution of pollen to the ovule or if flowers were not pollinated at the proper time. More important, low values of filled seed would also be expected when the viability is reduced. For example, with a mean value of 2.0 pollen grains per ovule, predicted filled seed yields with 90 percent viable pollen are four times the yields when using 10 percent viable pollen. In Figure 2, yield of filled seed per cone can be compared with the maximum seed and total developed seed per cone.

Table 2.--Predicted number of filled seeds per surviving cone for control-pollinated loblolly pine at varying levels of pollen viability.

Mean Pollen Grains/Ovule	Pollen Viability %				
	10	25	50	75	90
	filled seed				
0.5	3	7	14	18	20
1.0	7	17	28	36	39
2.0	17	37	57	67	71
3.0	27	53	76	87	90
4.0	32	61	82	89	91

NUMBER OF FLOWERS TO POLLINATE

The final factor for controlled pollination guidelines to be both effective and efficient is to know the number of flowers to pollinate based on the viability of the pollen source. As stored pollen viability can be determined in _vitro_ before installation of pollination bags, guidelines are needed to adjust the number of bags to use per cross based on the expected seed yield. Currently 144 seedlings are required for outplanting each cross in the N.C. State Tree Improvement program (Talbert et _al._ 1981). To ensure adequate seedlings, approximately 300 filled seeds per cross are needed.

The number of flowers required to provide 300 filled seed per cross are estimated in Table 3. The table gives four levels of cone survival. Rarely would 100 percent of the flowers pollinated reach cone maturity. Based on experience, more reasonable survival rates are 50 to 75 percent of the flowers pollinated. In Figure 2, the number of filled seeds per pollinated flower are presented for 2.0 pollen grains per ovule at varying pollen viabilities. Survival rates of 25 percent indicate serious problems involving insects or other factors including poor pollination techniques. With pollen viability of 75 to 90 percent and two or more pollen grains per ovule, less than 10 flowers would be required to produce the 300 seeds for progeny testing. This number may be lower than is currently being used for operational breeding programs. Obviously, it is important to be sure that adequate seed are available, and the numbers of flowers given in Table 3 should be considered as minimum requirements. Also Table 3 illustrates the guidelines to follow for loblolly pine. Other species could be adjusted based on pollen counts per ovule and differences in the seed potential.

Table 3.--Minimum number of loblolly pine flowers to pollinate to produce 300 filled seed per cross, at varying levels of pollen viability.

Cone Survival	Mean Pollen Grains/Ovule	Pollen Viability %				
		10	25	50	75	90
		----------------no. of flowers------------------				
25 percent	0.5	400	172	86	67	60
	1.0	172	71	43	34	31
	2.0	71	33	22	17	17
	3.0	45	23	16	17	14
	4.0	38	20	15	14	14
50 percent	0.5	200	86	43	34	30
	1.0	86	36	22	17	16
	2.0	36	17	11	9	9
	3.0	23	11	8	9	7
	4.0	19	10	8	7	7
75 percent	0.5	134	57	29	22	20
	1.0	57	24	15	12	11
	2.0	24	11	8	6	6
	3.0	15	8	5	5	5
	4.0	13	7	5	5	5
100 percent	0.5	100	43	22	17	15
	1.0	43	18	11	9	8
	2.0	18	9	6	5	5
	3.0	12	6	4	5	4
	4.0	10	5	4	4	4

ADDITIONAL REMARKS

The theoretical model for the effectiveness of controlled pollinations with varying levels of pollen viability indicates that tree breeders sacrifice a large amount of efficiency when low pollen viabilities are used. However, our model indicates that only a very few female flowers per cross are required to produce adequate seeds when correct timing, adequate distribution of pollen within the bag, and highly viable pollen are used.

One of the untested assumptions of the model is that the ratio of developed seeds to the maximum percentage of ovules with at least one viable pollen grain would be the same regardless of the pollen viability. This assumption may not be valid. Some preliminary work indicates that when pollen viability is low, that pollen vigor may also be reduced. Pollen vigor is a loosely defined term that currently is not measured *in vitro.* The effects of low pollen vigor are reduced percentages of filled seed per cone. This apparently is a result of pollen that germinates on the nucellus but does complete fertilization. Thus ovules pollinated with low-vigor pollen could have had relatively high in vitro viability but rather low filled seed yields per cone.

Table 3.--Minimum number of loblolly pine flowers to pollinate to produce 300 filled seed per cross, at varying levels of pollen viability.

Cone Survival	Mean Pollen Grains/Ovule	Pollen Viability %				
		10	25	50	75	90
		--no. of flowers--				
25 percent	0.5	400	172	86	67	60
	1.0	172	71	43	34	31
	2.0	71	33	22	17	17
	3.0	45	23	16	17	14
	4.0	38	20	15	14	14
50 percent	0.5	200	86	43	34	30
	1.0	86	36	22	17	16
	2.0	36	17	11	9	9
	3.0	23	11	8	9	7
	4.0	19	10	8	7	7
75 percent	0.5	134	57	29	22	20
	1.0	57	24	15	12	11
	2.0	24	11	8	6	6
	3.0	15	8	5	5	5
	4.0	13	7	5	5	5
100 percent	0.5	100	43	22	17	15
	1.0	43	18	11	9	8
	2.0	18	9	6	5	5
	3.0	12	6	4	5	4
	4.0	10	5	4	4	4

ADDITIONAL REMARKS

The theoretical model for the effectiveness of controlled pollinations with varying levels of pollen viability indicates that tree breeders sacrifice a large amount of efficiency when low pollen viabilities are used. However, our model indicates that only a very few female flowers per cross are required to produce adequate seeds when correct timing, adequate distribution of pollen within the bag, and highly viable pollen are used.

One of the untested assumptions of the model is that the ratio of developed seeds to the maximum percentage of ovules with at least one viable pollen grain would be the same regardless of the pollen viability. This assumption may not be valid. Some preliminary work indicates that when pollen viability is low, that pollen vigor may also be reduced. Pollen vigor is a loosely defined term that currently is not measured *in vitro.* The effects of low pollen vigor are reduced percentages of filled seed per cone. This apparently is a result of pollen that germinates on the nucellus but does complete fertilization. Thus ovules pollinated with low-vigor pollen could have had relatively high *in vitro* viability but rather low filled seed yields per cone.

The number of flowers to pollinate per cross does not indicate how many bags should be used. Frequently large numbers of flowers can be enclosed within one bag. The danger is that a broken branch would be disastrous. Therefore, a minimum number of bags should be five or six. Also, when large numbers of flowers per bag are used, the flower development may extend over several days, requiring more than one pollination per bag.

Finally, the number of flowers required to complete a cross can be used in two ways. The first way is increase the effectiveness and efficiency of the breeding program. The second way is to evaluate current breeding results. If large numbers of flowers are required to complete each cross, this would appear to be a warning signal that one or more of the several components of pollination are not favorable. Pollen viability would be the most likely item but pollen distribution and flower mortality could also be part of the low efficiency. Taking corrective action should mean that seed yields increase and the breeding program becomes both more effective and efficient.

LITERATURE CITED

Bramlett, David L. 1981. Effectiveness of wind pollination in seed orchards. In: E.C. Franklin (ed.) Pollen Management Handbook. Agric. Handb. 587. Washington, DC: U.S. Dep. of Agric. Forest Serv. p. 10-14.

Bramlett, David L., and Frederick R. Matthews. 1983. Pollination success in relation to female flower development in loblolly pine. In: Proc. 17th South. For. Tree Improv. Conf. June 6-9, 1983; Athens, GA. p. 84-88.

Bramlett, David L., and Claude H. O'Gwynn. 1981. Controlled pollination. In: E.C. Franklin (ed.) Pollen Management Handbook. Agric. Handb. 587. Washington, DC: U.S. Dep Agric. For. Serv. p. 44-51.

Goddard, R.E. and F.R. Matthews. 1981. Pollen Testing. IR E.C. Franklin (ed.) Pollen Management Handbook. Agric. Handb. 587. Washington, DC: U.S. Dep. of Agric. For. Serv. p. 40-43.

Matthews, F. R. and T. E. Blalock. 1981. Loblolly pine pollen grain counts by ovule dissection. In: Proc. 16th South. For. Tree Improv. Conf. May 27-28, 1981, Blackburg, VA: p. 276-278.

Matthews, F. R., and D. L. Bramlett. 1985. Pollen quantity and viability affects seed yields from controlled pollinations of loblolly pine. South. J. Appl. For. (In Press).

Richards, F.J. 1959. A flexible growth function for empirical use. J. Exp. Bot. 10(29): 290-300.

Sprague, J.R. and E.B. Snyder. 1981. Extracting and drying pollen. In: E.C. Franklin (ed.) Pollen Management Handbook. Agric. Handbk. 587. Washington, DC: U.S. Dep. of Agric. For. Serv. p. 33-36.

Talbert, J. T., F. E. Bridgwater, and C. C. Lambeth. 1981. Genetics Testing Manual, N.C. State Univ.-Ind. Pine Tree Improv. Coop., Raleigh, N.C., p. 37.