BLACK SPRUCE POLLEN HANDLING

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ABSTRACT.--The Minnesota Department of Natural Resources began an applied black spruce breeding program in 1986. Over two seasons, pollen has been collected from 54 clones. The range of pollen germination rates is 13-81 %, averaging 47 %. In 1987, 29 controlled crosses were made according to a 5-parent disconnected diallel mating design. Collection, testing, and storage procedures are described. Plans for advanced generation breeding are discussed.

Additional Keywords: *Picea mariana*

INTRODUCTION

At some point in a genetic tree improvement program it becomes necessary to control ancestry of breeding stock, if continuous long term gain is desired. It is moot when this point should occur in the life of a program, either immediately or at some later time. The fact remains that programs planning for future improvement cannot enter advanced generations with half-pedigreed breeding stock. The deleterious effects of selfing are well-documented (Franklin, 1969). The use of "unknown relatives" in a production seed orchard is also likely to depress expected genetic gain in future generations.

To avoid this situation, conifer tree improvement programs rely upon controlled mating to create fully pedigreed breeding populations. At some arbitrarily chosen point in time, the very best unrelated individuals are selected to mass produce improved stock for reforestation, usually in a seed orchard. This process is both labor and time consuming, thus those programs that minimize the generation interval usually have the highest rates of return.

A serious bottleneck in applied breeding programs is pollen availability. Both grafted trees and seedlings usually produce female strobili before males. Once male production begins, it is often difficult to collect enough pollen from many different trees to complete crosses quickly. If pollen collection can be accelerated more crosses can be made in less time, leading to a reduction in the generation interval.

The Minnesota Department of Natural Resources (DNR) began advanced generation breeding in black spruce (*Picea mariana* (Mill.) B. S. P.) in 1986. This paper will discuss black spruce pollen handling, the breeding program, and steps taken to accelerate breeding.

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POLLEN HANDLING

Male strobili. The trees available for breeding in the DNR black spruce tree improvement program are located on five separate sites (Figure 1). Four of these sites constitute an open pollinated progeny test planted in 1978. There are 40 first generation selections spread across these sites. The individual trees are 12-20 feet tall at present. The fifth site is located near the village of Sturgeon Lake, and contains about 150 clones grafted in the mid-to-late 1970's. These trees are 5-15 feet tall and have been designated as a breeding orchard.

On young, open grown black spruce trees, male strobili are borne in the lower to middle crown. Male buds are easily identified in the early spring, being the first buds to swell. The male strobili become very conspicuous upon breaking bud, being crimson red to purple in color. The strobili are borne singly, or in clusters of two to many. Trees with a heavy male "flower" crop often produce clusters of strobili at the apices of the previous year's growth.

To ensure prompt, maximum recovery of pollen, black spruce male flowers should be collected when they exude a small amount, or none, of yellow fluid when squeezed. In 1986, male flowers at Sturgeon Lake started to mature about May 15. During the extremely warm and dry spring of 1987, pollen collection began April 30.

It is not uncommon for the male flowers on the south side of a tree to be ready to pick, while those on the north are not. In this case, the flowers should be picked only from the southern exposure. Dry, warm, windy weather at the time of male flower development speeds maturation. Conversely, cool and damp weather slows maturation. During changeable weather, repeated trips to the breeding orchard are necessary to pick trees that are ready.

Male strobili are easily collected by rubbing a thumb over the flower. It is important to collect flowers only; needles and other organic debris make extraction difficult. In the field, male flowers are placed in a Kraft lunch bag, no more than one inch deep. The clone number is written on the outside of the bag, which is stapled shut.

The Sturgeon Lake breeding orchard is only 12 miles from General Andrews Nursery, the base of operations. Collected male strobili are processed immediately. However, where distance does not permit rapid processing, flowers should be kept cool.

Pollen extraction. To facilitate pollen extraction, a forced air system was built at the nursery in early 1986. The system consists of a series of funnels connected to a squirrel cage blower. Male flowers are placed in the funnels and the pollen falls into jars suspended below. The entire system is contained in a separate room, kept dry and warm with a dehumidifier. A steady air stream into the jars dries the pollen to the proper moisture content (7 - 10 %) so that it can be used immediately or stored. The forced air system is described in more detail by Sprague and Snyder (1981).

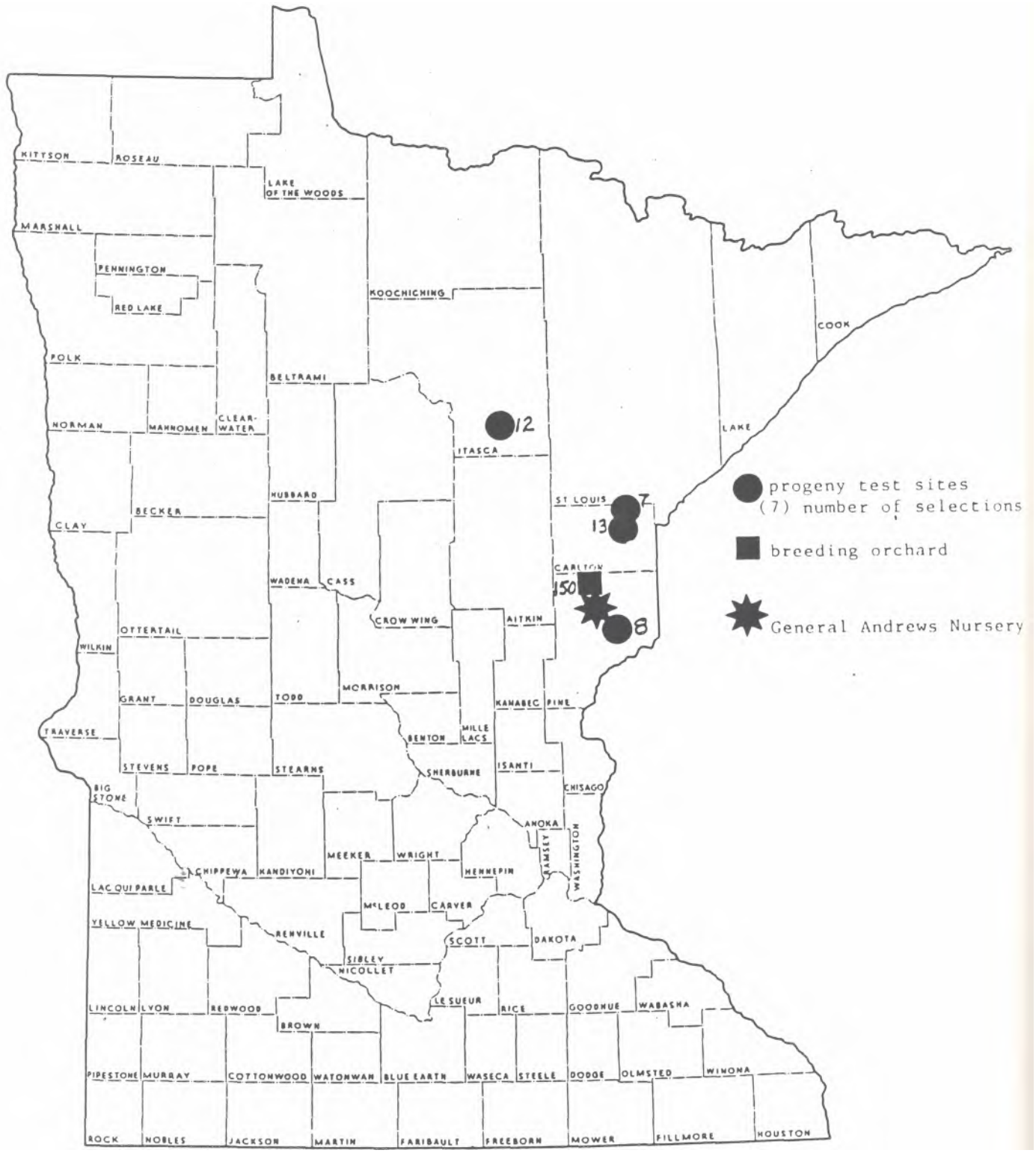

Figure 1. Location of black spruce trees available for advanced generation breeding.

Once male flowers are placed in a funnel, useable pollen is available within 48 hours. In 1986, the system contained 12 funnels and produced pollen from 25 separate clones in one week. The system was expanded to 22 funnels in 1987, producing pollen from 36 clones.

Pollen storage. Proper pollen storage is critical. After extraction, the pollen is placed in sterile, dry vials properly labelled with clone number and date. The vials are stoppered and sealed with Parafilm. Sealed vials are then placed in plastic freezer boxes, also sealed with Parafilm. The boxes are placed in a manual defrost refrigerator at + 2 to 4 degrees Celsius. To date, pollen stored in this manner has kept for one year with no loss of viability.

Pollen testing. Once pollen is collected, dried, and stored it is important to test for viability. Germination rates are used to gauge how much pollen to use in a particular controlled cross. The amount of pollen to use is inversely proportional to it's germination rate. In a breeding program using a pollen mix, germination rates are used to determine the proportions of each pollen in the mix. In this way, each pollen can make a nearly equal contribution to the cross.

To reduce eyestrain and make the tests more accurate, only 10 clones are set up for testing at one time. Black spruce pollen germinates in 36-48 hours, so a test series is not started after Wednesday. Tests are run in a warm room. The higher the temperature the faster the pollen germinates.

The germination media is simply a 3 % sucrose solution. Karo Syrup or unpasteurized honey in distilled water works well. Sterile test tubes are filled one half way with the sucrose solution. A small amount of pollen is sprinkled in each test tube, one clone per tube. The amount of pollen is critical. Too much pollen will cause clumping and molding as it germinates. Each test tube is accurately labelled. The test tubes are agitated frequently, at least three times per day.

After 48 hours the pollen is counted. A drop of Fuschin stain is added to each tube making the pollen grains easier to see. Using an eyedropper, the pollen is agitated again by squirting the solution in the test tube. Germinated pollen sinks to the bottom of the test tube; squirting mixes the pollen together for more accurate reading. This is shown in Table 1. One test was run on eight clones with a sample taken from unmixed test tubes. A second test was run with a thoroughly mixed sample.

Table 1. Pollen germination rates of eight black spruce clones, taken from unmixed and mixed samples.

Clone No.	Germination Rate (%) Unmixed	Mixed
68	4	41
172	14	42
223	3	36
278	15	49
297	3	35
303	9	39
309	22	62
335	10	46

To prepare the test, two small drops of stained solution are placed on a clean microscope slide and covered with a cover slip. At least 200 pollen grains using two tally whackers. One keeps the count of total grains while the other counts the germinants. Pollen grains in which the pollen tube is at least as long as the grain is wide are counted as germinated. Germination rate is simply:

$$\frac{\text{count of germinants}}{\text{total count}} \times 100$$

Results. The results to date are very encouraging. Using the procedures as described, a total of 61 pollen lots have been collected from 54 different clones. Germination rates range from 13-81 %, averaging 47 %. The pollen has also proven to be quite hardy. In late December, 1986, the refrigerator used for storage accidentally became unplugged. This went unnoticed for two weeks, during which time the inside temperature rose to 24 degrees Celsius. Despite this seemingly disastrous incident, only two of the 25 lots were killed outright, and six had reduced germination. Such harsh treatment is not recommended, but it is satisfying to know that proper drying and storage saved the loss of an entire year's work.

At this point, it is unknown how long black spruce pollen can be stored under these conditions. The DNR is not equipped to set up such exotic treatments as freeze-dried vacuum storage for the long term. However, in an applied program such long term storage is unnecessary. If the refrigerated pollen keeps for 3-5 years, controlled crossing should proceed without delay.

BREEDING STRATEGY

Planning for the long term, multi-generation improvement of black spruce, the DNR has decided to develop two fully pedigreed populations. The parent stock is comprised of 40 first generation selections located in four progeny

test sites, and 150 clones located in a breeding orchard (Figure 1). Based on the origin of the parent trees, these 190 trees have been divided into northwestern and northeastern populations of about 90 trees each. These populations are based on the Lake States Seed Zones (Figure 2) (Rudolf, 1956). While this subdivision may seem arbitrary, it is based on valid climatic data. It also creates populations of more manageable size. Future progeny test data should determine the validity of these populations.

The plan for second generation improvement calls for the establishment of one control pollinated seedling seed orchard for each population, supported by separate full sib progeny tests. The seed orchards will be established and managed intensively from the outset as orchards only. The progeny tests similarly will be designed and managed for the single purpose of progeny testing.

A 5-parent disconnected diallel has been chosen as an appropriate mating design for this breeding strategy (Figure 3). This design allows good estimates of general and specific combining ability, while maintaining reasonable numbers of unrelated families. With 90 parents there will be 18 unrelated families in a population. The amount of crossing is fairly heavy, although the flower biology of black spruce makes the task simpler. First, mature seeds are produced in one year. Second, flowering is fairly consistent year to year. Third, it is common to pollinate many female flowers in a single isolation bag. Given the close proximity of the breeding orchard to the pollen facilities, travel time is minimized. Thus, it should be possible to complete the mating design fairly quickly.

The black spruce breeding program began in 1986 with the collection of 25 pollen lots. In 1987, 36 lots were collected and 29 specific crosses were made. A major bottleneck in the breeding program had been availability of pollen. Now that significant pollen is available, breeding should accelerate. The target date for completion of this stage of controlled breeding is 1992. At that time there should be about 180 full sib families per population.

	1	2	3	4	5	6	7	8	9	10 . . .n
1		X	X	X	X					
2			X	X	X					
3				X	X					
4					X					
5										
6							X	X	X	X
7								X	X	X
8									X	X
9										X
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n-1										

Figure 3. 5-parent disconnected diallel

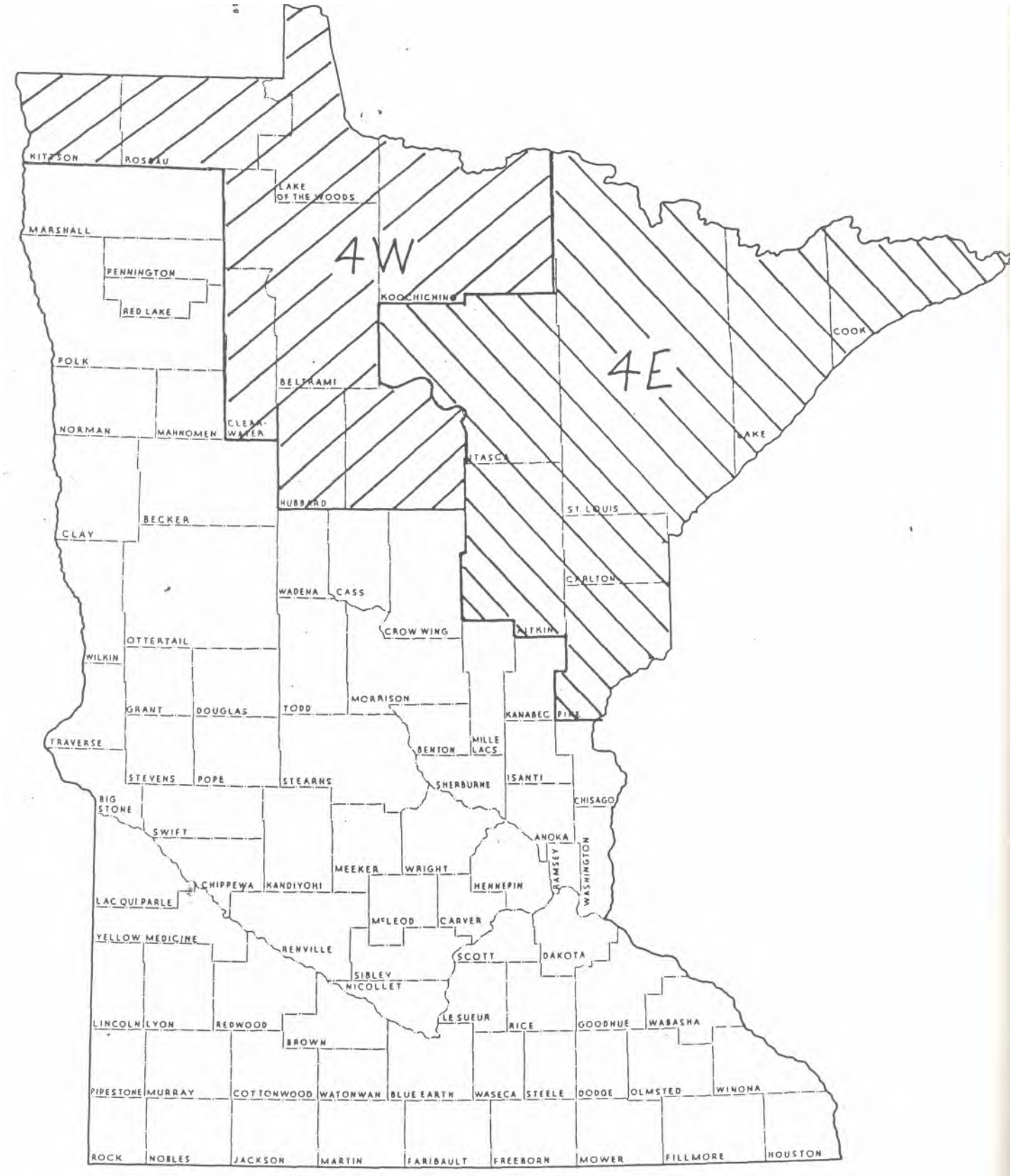

Figure 2. Black spruce breeding populations.

SUMMARY

Applied tree improvement programs cannot make long term continuous genetic gain until ancestry of breeding stock is controlled. A major hindrance in controlled breeding is pollen availability. The Minnesota DNR has set up pollen extraction and storage facilities at General Andrews Nursery. Using these facilities, applied breeding was started in black spruce in 1986. Pollen is dried to the proper moisture content so that it can either be used immediately or stored. Pollen testing methods have been developed yielding good results. Pollen has been stored under refrigeration for one year without a loss of viability.

With an efficient pollen collection system in place, controlled breeding can move forward at a quick pace. Two years into an eight year cycle, pollen has been collected from 54 of 190 clones. In the second year, 29 controlled crosses were made according to a 5-parent disconnected diallel mating design.

LITERATURE CITED

Franklin, E.C. 1968. Inbreeding as a means of genetic improvement of loblolly pine. in proc. 10th Southern Forest Tree Improvement Conference. June 17-19. Houston, TX. pp. 107-115.

Sprague, J.R. and E.B. Snyder. 1981. Extracting and drying pine pollen. in Pollen Management Handbook. [E. C. Franklin, editor]. USDA Forest Service Agriculture Handbook No. 587. pp. 33-36.