DIAGNOSING LOW SEED AND CONE YIELDS FROM CONTROLLED POLLINATIONS OF SOUTHERN PINES

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Abstract.--Rapid improvement of southern pines requires efficient and effective controlled pollinations, but many controlled crosses produce few cones and low yields of filled seeds per cone. Using the known causes of seed failure and cone mortality, a method for relating these causes to problems with controlled pollinations has been developed. This paper describes diagnostic observations of seed failure and cone mortality and procedures to improve the seed yields from controlled pollinations.

Keywords: Tree breeding, pollen viability, pollen storage, Pinus taeda L.

INTRODUCTION

Controlled pollinations are used by southern pine tree breeders to produce seeds for progeny testing. Success with controlled pollination requires experience and knowledge of the reproductive process. Techniques and equipment vary among tree breeders, but the basic methods are similar for loblolly pine (Pinus taeda L.), slash pine (P. elliottii Engelm.), shortleaf pine (P. echinata Mill.) and Virginia pine (P. virginiana Mill.). The objective is to produce adequate quantities of filled seed so that progeny tests can include equal numbers of seedlings from each mating. Timely selection of the advance generations, therefore, depends on efficient controlled pollinations.

DIAGNOSTIC OBSERVATIONS

In controlled pollinations, low-quality pollen, poor timing, and poor bag installation can cause seed or cone losses but so can insects or fungi. Pinpointing the causes of losses requires careful observations. In the following discussion, the necessary diagnostic observations are described and the probable causes related to the controlled pollination process are discussed. For a general reference on controlled pollination and pollen extraction, storage and testing see "Pollen Management Handbook" (Franklin (ed), 1981) and "Advances in Pollen Management" (Eramlett et al. (eds) 1993). Seed and cone losses from insects are described in "Seed and Cone Insects of Southern Pines" (Ebel et al. 1975).

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First-year aborted ovules

Large numbers of ovules often are aborted during the first year of development in cones that have been control pollinated (Table 1). They appear as small remnants of the ovule rather than developed seeds (Bramlett et al. 1977). One cause is the lack of at least one viable pollen grain in the pollen chamber of the ovule. When ovules in control-pollinated flowers do not have adequate pollen, the most likely reason is that pollen was not applied at the time of peak female receptivity. Open-pollinated flowers are exposed to wind-born pollen for extended periods, but the tree breeder applies pollen for only a few seconds. It is thus imperative that the pollen reaches the micropyle during the pollination process. In a timing study on loblolly pine flowers, Bramlett and Matthews (1983) reported that the average number of pollen grains per ovule was not significantly different from wind-pollinated flowers when the control pollinations were completed within 2 days of maximum flower receptivity (stage 5) or within a 4-day window with flower stages from

Table 1.--Diagnostic observations, probable causes and procedures that may reduce losses of ovules and seeds from controlled pollinations.

Diagnostic observation	Probable causes	Procedures to reduce losses
A. First-year aborted ovules (> 20%)	1. Inadequate pollination	. Check timing of pollen application . Check amount of pollen applied . Check pollinator for distribution in bag . Avoid pollinating wet bags
	2. Low pollen viability	. Complete in vitro germination test . Check collection, extraction and storage procedures
	3. Insect damage	. Check pest management program for seedbug protection
B. Second-year aborted ovules (> 10%)	1. Insect damage	. Check pest management program for seedbug protection
	2. Developmental Problem	No known treatment. Possibly related to water stress (clonal)
C. Empty seeds (> 25%) per cone or 15% of total seed)	1. Low pollen vigor	. Check pollen extraction and storage procedures.
	2. Embryonic lethal alleles	. Check parents for relatedness
	3. Seedbug damage	. Check pest management program
	4. Fungal damage	. No known treatment (clonal)

4.5 to 5.5. Control pollinations that were earlier or later than this period had statistically less pollen per ovule and an increasing percentage of unpollinated ovules per cone.

When there is a wide range of developmental stages of the individual flowers within a given bag, multiple pollinations of that bag are needed. For example, if flower stages range from 3.0 to 5.5, a second or even a third visit are needed to get adequate pollen into each flower. Multiple pollinations, however, are not efficient or beneficial if flowers are only two days apart in receptivity stage.

Distribution of pollen to the individual ovules is the function of the pollinator. The cyclone pollinator distributes pollen within the bag very effectively (Matthews and Bramlett 1981), but other pollinators, including a syringe and needle, a plastic wash bottle, and a camel's hair brush, also can be effective. From my experience, the type of pollinator is not as important as the correct timing of the pollination. Any of several available pollinators can give good results. Tree breeders should avoid pollinating bags that have water droplets condensed on the inside of the pollination bag.

For continued ovule development, at least one pollen grain must germinate in the ovule and begin growth through the nucellus tissue. If pollen does not germinate, the ovule aborts. Matthews and Bramlett (1986) looked at the effect of the percent viability of pollen on the eventual seed set. The development of filled seed is a function of the average number of pollen grains per ovule. The pine pollen chamber can hold up to 7 pollen grains. A normal seed will develop if just one of these grains germinates properly. Thus, with good pollen distribution per ovule, serious reductions in the number of ovules with viable pollen do not occur until pollen viability is 50% or lower. Even pollen with relatively low viability can produce reasonable filled seed yields. However, for high seed yields, tree breeders should use pollen with high viability.

To compensate for low pollen viability, the number of flowers pollinated should be increased rather than increasing the amount of pollen applied per bag (Eramlett et al. 1985). Filled seed yields from controlled pollinations can be increased by increasing the quantity of pollen applied per bag, but adequate seed set can be achieved with relatively low pollen amounts per bag (Bramlett 1977, Matthews and Bramlett 1986). If the pollen supply is plentiful I recommend applying 1.00 cc of pollen per bag with the cyclone pollinator (Matthews and Bramlett 1981). If the pollen supply is limited, the cyclone pollinator can be successfully used to deliver as little as 0.25 cc per bag. If less than 0.25 cc of pollen are available per pollination, a camel's hair brush should be used.

The third cause of first-year aborted ovules is the feeding of the seedbug Leptoglossus corculus (Say). Second-stage nymphs of this insect feed on southern pine conelets and penetrate the ovules. This damage halts ovule development and the net result is an aborted ovule that is indistinguishable from one caused by a lack of viable pollen. Insect damage can be reduced by a reliable pest management program.

It is important to distinguish insect damage from pollination problems. To estimate the number of first-year aborted ovules due to other causes, wind-pollinated cones can be collected and the aborted ovules counted. If the wind-pollinated cones also have large numbers of first-year aborted ovules, then it is a safe bet that insects are a major cause of the damage.

Second-year aborted ovules

As far as we know, ovule abortion during the second year of development is not related to pollination (table 1). Seedbugs are the only confirmed cause. These ovules begin enlargement in the second year but abort before the seedcoat is fully developed. Typical second-year aborted ovules are flattened or sunkened. They may be as large in outline as a mature seed. They frequently are filled with resin that exudes from the damaged ovule. If seed orchards have an adequate pest management program, second-year aborted ovules are rare.

Small hardened second-year ovules are sometimes found in mature cones. The cause of this seed loss has not been determined, but in most cases it accounts for less than 1% of the total seed potential. Occasionally, certain clones will have a large number of these second-year aborted ovules but the cause and cure are unknown.

Empty seeds

When the number of empty seeds in control pollinated cones exceeds 25 per cone or the proportion is greater than 15%, the loss is large enough to indicate problems that could be corrected. Unfortunately there are at least four causes of empty seeds in pines (table 1). Insects are the major cause of empty seeds in unprotected orchards or in natural populations of pines. The damage is caused by feeding of the seedbugs, L. *corculus* and *Tetyra bipunctata* (H.-S.) and by seed worms (*Laspeyresia* spp.). Adequate protection of seed orchards is currently provided by insecticide applications. Breeding orchards should also be protected at the same level to prevent losses of control-pollinated seeds. Small breeding orchards, especially those without buffers, may require adjacent areas to be treated to insure adequate protection.

Fungi are also known to cause empty seeds in pines, but no preventative fungicide applications are available to reduce the losses. Fortunately, the average loss to fungi is low, but losses in some individual clones may be substantial.

Embryonic lethal alleles can cause empty seeds. On the average, an individual pine has 10-12 recessive embryonic lethal alleles. Apparently there are a large number of independently segregating lethal alleles in the pine population. Thus, the likelihood that two individuals will have matching heterozygous lethal alleles is very low. However, with any degree of inbreeding, and in particular with self-pollination, there is a relatively high probability that a homozygous combination of lethal alleles will occur at one or more loci. Individual embryos that have homozygous lethals abort soon after fertilization. If only one fertilization occurs in the ovule, embryonic abortion results in an empty seed at cone maturity. However, because pines are polyembryonic, empty seeds occur only in ovules where all the embryos in a given ovule have aborted. Therefore embryonic lethal alleles are not a major cause of empty seeds when controlled pollinations are completed among unrelated parents. When controlled pollinations are made among related parents, some reduction in filled seed yields should be expected.

If you have good insect protection, no fungal problems, and no inbreeding and you still get a large number of empty seeds, the probable cause is low pollen vigor. We first became aware of this problem in loblolly pine when we tested storage methods for pollen (Matthews and Bramlett 1983). We noted that pollen stored in a desiccator produced similar numbers of fully developed seeds as pollen stored under vaccum or as fresh pollen. The number of filled seeds, however, was greatly reduced after pollinations with the desiccator-stored pollen. In vitro germination tests of the pollen prior to controlled pollinations indicated that the viability of the desiccator-stored pollen was as good as the vacuum-stored or fresh pollen. Pollination with the viable pollen stimulated the ovules to develop archegonia and fully developed seedcoats, but the pollen apparently was not vigorous enough to complete fertilization. Without fertilization, the development of the gamephyte stopped and the result was an empty seed.

We confirmed these results in a similar test using different pollen lots (Bramlett and Matthews 1991). Thus, low pollen vigor shows up as an increase in the number of empty seed and apparently is associated with deterioration during storage. Tree breeders who observe large numbers of empty seeds should check their pollen storage procedures for possible reductions in pollen vigor. Jett et al. (1993) have described the currently recommended procedures for pollen extraction, testing and storage.

Conelet survival

Only two causes of conelet losses may be attributed directly to the pollination procedure (Table 2). First, if conelets do not receive adequate pollen, they may abort. Just how many pollen grains are required for the female parent to retain conelets is not known. Some clones will hold unpollinated cones to maturity without producing any viable seeds. In other clones, poor pollination causes some abortion of conelets after pollination.

The second pollination-related loss of conelets is from damage caused by the bag. The developing female flowers are delicate structures and any buffeting from wind or rubbing against the bag (particularly sausage casing) can cause light to severe damage. When the damage is severe, the conelet usually dies.

All other causes of conelet mortality are unrelated to the pollination process. These causes include damage from insects, fungi, freezing temperatures, and high winds. Tree breeders should take adequate measures to minimize these losses but total elimination is not possible. A reasonable goal is to keep conelet losses to less than 10 percent.

Small mature cones

When pine flowers are not adequately pollinated, the mature cone is noticeably smaller than open-pollinated cones on the same tree (table 2). Size is reduced because the cone scales associated with unpollinated ovules are stunted. If small cones are observed after controlled pollinations, pollen quantity, distribution, or viability should be checked for improvements.

Table 2.--Diagnostic observations, probable causes and procedures to check to reduce losses of cones from controlled pollinations.

Diagnostic observation	Probable causes	Procedures to reduce losses
A. Conelet survival (< 90%)	1. Insect damage	. Check pest management program
	2. Bag damage	. Check bagging procedure
	3. Inadequate pollination	. Check timing of pollen application . Check application amount pollen applied . Check pollinator for distribution in bag
	4. Low pollen viability	. Complete in vitro germination test . Check collection, and storage procedure
	5. Freezing temperatures	. Avoid frost pockets
	6. Fungi	. Avoid branches with pitch canker
	7. Wind	. No known prevention
B. Small mature cones (25% smaller than open-pollinated cones.	1. Inadequate pollination	. Check timing of pollen application . Check application amount pollen applied . Check pollinator for distribution in bag
	2. Low pollen viability	. Complete in vitro germination test . Check collection, and storage procedure
C. Cone Survival (< 75% of flower crop)	1. Insects	. Check pest management program
	2. Fungi	Reduce pitch canker in orchard

Poor cone survival (year 2)

Poor cone survival during the second year is not related to the pollination procedure (table 2). Insect damage is the most likely cause of second-year cone mortality, but fungi are also known to cause cone mortality. Squirrels can be a problem in some orchards. If these problems are reducing cone and seed yields from controlled pollinations, the pest management program should be evaluated to reduce cones losses.

METHODS TO ASSESS POLLINATION PROBLEMS

Many causes of reduced seed and cone yields in seed orchards have been identified. It is important to know which ones are caused by poor or incomplete controlled pollination. Perhaps the simplist way is to collect at least five open-pollinated cones from the same tree for comparison. The control-pollinated cones should be extracted first. If the seeds are extracted in a bulk lot, the total number of seed should be divided by the total number of cones. When insect-damaged cones are in the cone collection, estimate the amount of seed each damaged cone contributed (for example 25%-75%).

Next, separate filled from empty seeds, and count the number of filled seed and empty seed per cone. Air blowers or flotation systems are satisfactory for separating the seeds. Estimate the number of aborted ovules by substracting the total number of developed seeds per cone from the seed potential. If you do not have seed potential data for each clone, use 155 for loblolly, 170 for slash and 90 for shortleaf and Virginia pine (Bramlett et al. 1977).

If seed yields are 80-100 [1/] filed seeds per cone and the cone survival is also high (> 75%), the controlled pollination should provide adequate seed for progeny testing. If seed yields do not meet expectations, compare control-pollinated and open-pollinated and seed yields per cone. Of all the diagnostic observations, the number of first-year aborted ovules and the percent of empty seeds per cone are the most revealing. You do not want to try to correct a pollination problem if in fact you have insect or other causes of seed losses. If the open-pollinated cones have fewer first-year aborted ovules or a lower percentage of empty seeds, the difference between the control- and open-pollinated cones is an estimate of the "pollination effect."

The next step is to examine the suspected causes in tables 1 and 2 for the diagnostic observation. It is quite likely that several causes are contributing to the loss. Then, you must decide whether the loss is large enough to justify corrective action. In general, losses associated with poor pollination can be corrected.

CONCLUSION

There is no apparent biological reason why tree breeders cannot produce an average of 80-100 filled seeds per cone and 75% cone survival from controlled pollinations in loblolly or slash pine. To achieve these goals, care must be taken in the collection, extraction, processing, and storage of pine pollen. High-quality pollen must be correctly applied to bagged female flowers. And the flowers, conelets, and cones must be protected to maturity. If these goals can be consistently achieved, tree breeding can rapidly move forward with progeny testing and continued breeding for future generations of genetically improved southern pines.

[1/] For loblolly and slash pine; use 40-50 filled seeds for shortleaf and Virginia pine.

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