

16. CONTROLLED POLLINATION TECHNIQUES

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As earlier speakers have dealt with some of the objectives to be achieved by controlled pollination and hybridization, we will now briefly discuss the means for attaining those ends.

In controlled pollination, loose, wind borne pollen must be prevented from reaching the female strobili or flowers at the time they are receptive and the desired pollen must be placed in contact with the flowers. As the essential ingredients in any tree breeding project are flowers, we might well begin with a hasty glance at the sexual structures of pines, the genus to which this discussion will be restricted.

While both are borne on the same tree, male and female strobili are separate structures. Both are found near bud tips, and although a few branches can be found bearing both sexes, usually they are produced on separate branches. The southern pines tend to produce male flowers in the lower portions of the tree crown and females toward the top. Flower buds begin forming during the late summer and fall, and buds of both sexes are easily recognizable by January. We now have a few slides showing flower development through the receptive stage.

Our first slide shows a female flower bud before the bud scales have started to peel back. This is the best stage to isolate or bag the flower.

In our next slide the flower has developed further and the small conelet is beginning to emerge from the bud. If a flower is bagged later than this stage, there is much danger of contamination by loose pollen.

This slide shows the flower fully emerged from the bud and at maximum pollen receptivity. When looking at an actual flower in this stage, an opening between scales to the axis is visible to the naked eye. This is the optimum stage for pollination.

The next illustration is of male flowers or catkins at maturity, just prior to pollen shedding. This is the stage when collecting should be done.

Now catkins are at the height of pollen discharge.

Flowering of slash pine is the earliest of our major southern pines. Early stages of slash flowers are very evident shortly after Christmas and female flowers usually reach maximum receptivity during the last half of January or by the first week in February. Both loblolly and longleaf pines flower during late February or during the first two weeks in March, which is the reason for natural

hybrid-Sonderegger pine. Shortleaf pine flowering, at least in Texas, occurs from mid-March into the first week of April. During some years there is a slight overlap of shortleaf and loblolly flowering.

Our next slide shows the female flower or conelet one year later. As you can see, it has not grown much. During this year the pollen has been growing toward the egg but fertilization has not yet occurred. During the second growing season the conelet starts to grow and fertilization finally takes place.

Pollen used in controlled pollination work is extracted from catkins collected at approximately the stage shown here. A simple test of pollen maturity is to squeeze a few catkins. If a thick yellow curd comes from the catkins, they are at collection stage. However, if too green when picked, mold can cause trouble during extraction. By daily observation, catkins can be collected after a few have started to shed and extraction will be much simpler.

If a very slight contamination is allowable, catkins may be placed in extractors in the condition collected. To be certain of purity, however, catkins may be washed free of foreign pollen.

Pollen is best extracted in a warm room at low humidity. However, a balance must be struck between ease of extraction and low pollen viability for too much heat and too low humidity will kill the pollen. Small lots are easier to handle without molding or excessive drying. The next slide shows the component parts of a simple extraction assembly designed by Phil Wakeley and Tom Campbell: eight pound paper bag, wire cage for catkins, plastic funnels, voile screen, adhesive tape.

The next slide shows this extractor assembled.

Now the extractor is in position.

Another simple method for extracting small lots of pollen, originated by Mergen, is with sausage casings. The bag is stapled closed with catkins inside. When pollen has shed a hole is cut in one corner and the pollen is strained into vial for storage.

Pollen will retain a high percentage of viability at room temperature for only three to 14 days. For this reason, cold storage is essential if many species are to be crossed. This also explains, along with flowering dates, why so much of the earlier hybridization of southern pines was done on shortleaf pine. To work on slash pine, for example, with pollen of any of the other species, storage for almost a whole year is necessary. The Texas Forest Service has tested various storage conditions and has found storage in vials with cotton plugs in a cold room held at 35 to 37 degrees F. best. The next best storage condition was found in home refrigerators.

As it is fruitless to climb a tree several times to bag flowers and then pollinate them with dead pollen, it is advisable to test viability of all stored pollen. A very simple test, called the hanging drop slide method, gives a good indication of pollen viability. Materials needed are hanging drop slides having a depression in the middle portion, cover slips, sugar, vaseline, water and low power microscope. Mix a little pollen in five percent sucrose solution; put one drop of the mixture on slide cover slip; put vaseline around the depression on the slide; lay the slide on the cover slip; with the cover slip sticking to the vaseline, flip the slide and the drop hangs in the depression. The pollen in the slides should be incubated at room temperature, approximately 70 degrees F., 24 to 48 hours. Observe the slide under low power microscope or even a good hand lens. The percentage of pollen grains germinating and producing pollen tubes is the indication of viability.

Now that we have collected and extracted pollen, stored it until needed and checked its viability, we are ready to start pollination. The material used to isolate female flowers must be porous and yet not open enough for loose pollen to get through. Workers in the South have found the canvas bags used on western species too heavy for our southern pines. Now widely used are synthetic sausage casings which allow enclosed branches to breath but cannot be penetrated by loose pollen. In this slide a bag is in place over female flowers. Needles are stripped off, the bag expanded and slipped over the branch and tied in place with wire twists. Cotton is placed around the branch at the point of tying, to close cracks. As indicated earlier, flowers are best bagged before the bud scales begin to peel back. We usually set an arbitrary limit of three flowers as a minimum for bagging on one branch and try to find branches with five or more flowers. It is very helpful to have a heavy copper wire with a brass hook brazed on the end for use in pulling limbs within reach and holding them there while working.

Numerous devices have been used for putting the desired pollen on female flowers when they reach the receptive stage. Sharp medicine droppers, hypodermic syringes with 16 gauge needles, and ear syringes with hypo needles are being used. The bag is punctured with the needle and a puff or two is usually sufficient to pollinate a bag if the pollen flows easily. The puncture is sealed with scotch tape.

After the flowers are well past the receptive stage as shown in the next slide, pollination bags are removed. A common practice is to slash them into shreds but leave the ripped bag tied to the branch. This serves as a flag to aid in relocating the conelets later. A long strip of durable cloth tied to the limb at this time also helps.

After the pollination bags are removed there is little to do except wait and hope. Squirrels, insects, disease, and wind damage are some of the hazards between pollination and cone collection 18 to 20 months later. A cloth bag placed over the conelets a year after pollination may help but we have had some injury following this practice. At least the bag prevents loss of seed if collection is delayed.

This has been a very hasty resume of pine pollination procedures, and I know many points have been skipped or touched very lightly. If there are any questions, Mr. Allen and I will try to answer them.