POLLEN MATURATION AND EXTRACTION IN BLACK WALNUT

When to collect catkins and how to extract pollen free of contamination.

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Control-pollinated progeny tests are an integral part of progressive tree-breeding programs. Results from such tests are necessary to obtain information on general and specific combining ability, for the roguing of first generation seed orchards and for the development of second-generation seed orchards. In addition, the possibility of producing superior gene packages through wide-cross pollinations makes control-pollination an attractive procedure.

Forbes (*2*) and Beineke and Masters, (*1*) described techniques and results of control pollination in black walnut. In this paper we will report on phases of pollen maturation, collection, and extraction in black walnut. Studies reported in this paper were carried out on young black walnut grafts at Martell Forest near West Lafayette, Ind., and on a few large superior black walnut trees in the Lafayette vicinity.

Pollen Maturation

Black walnut is monoecious and wind pollinated. The staminate floral structure is best described as a grouping of 20 to 30 sessile stamens arranged in an elongated spike, raceme, or catkin (figure 1).

Catkins are first visible as axillary buds during late summer. They are borne on the maturing wood grown that summer and have a rough or lumpy surface compared to leaf buds, which have a scale like smooth covering with separations between the scales. In the early spring, catkins begin expansion and become much more prominent. In Indiana, they are approximately one-half inch long by April. Growth continues, and although catkins may reach a length of from 2 to 4 inches, they cannot be forced to shed pollen as long as they remain green in color. Several days before anthesis, the anthers begin to yellow and become "full bodied" in appearance (figure 1).

Figure 1.—*Staminate catkins of black walnut at anthesis.*

The shade of yellow varies, depending on pollen source; in most cases, however, the yellow color is pronounced at anthesis. When the yellow, full-bodied condition is reached, the catkin is ready to be picked and will dehisce in a matter of hours. Our experience has been that it is best to wait until a few catkins begin dehiscing to obtain maximum amounts of pollen.

Two environmental conditions, air temperature and relative humidity, affect pollen maturation. When air temperatures are high, pollen maturation is hastened; when they are cool, maturation is retarded. Extremely high relative humidity delays anthesis. In fact, if high relative humidity is prolonged during pollen maturation, the pollen becomes infected with molds and fungi.

Pollen maturation may vary within a tree. On grafts 5 to 10 feet tall, pollen matured at about the same time throughout the crown. On larger trees, however, several patterns of maturation were observed. In most clones, pollen developed in stages, maturing in the lower crown first. In a few clones, maturation took place first in the upper crown, while in other clones development seemed to occur at random throughout the crown.

Pollen Collection and Extraction

On small trees where only a few catkins were available, or when only a small amount of pollen was needed for testing purposes, mature catkins were picked individually from the tree and wrapped in aluminum foil. This method was very satisfactory, because the catkins of different sources could be kept separate and free from contamination, and most of the dehisced pollen was easily retrieved from the slippery surface of the foil. Larger lots of pollen were collected in paper bags. To obtain best extraction results using this method, only a small quantity of catkins should be placed in each bag in order to avoid heat and moisture buildup.

Insects, particularly thrips and aphids, occasionally infest the catkins of some clones. Pollen obtained from infested trees is usually difficult to extract and low in viability. We have solved this problem by dusting newly collected, severely infested catkins with pyrethrum. Pyrethrum apparently has no deleterious effect on pollen germination.

After collection, pollen was allowed to dehisce in the collection containers. The pollen collected in aluminum foil was opened for ventilation and covered with a small paper bag. A temperature of 27° C and a relative humidity of 40 percent was ideal for pollen dehiscence. This environment is best obtained in a growth chamber or other environmentally controlled room. If the catkins were collected at the mature stage of development, most of the pollen dehisced within 12 to 14 hours. Viability at room temperature was markedly decreased within 24 to 48 hours.

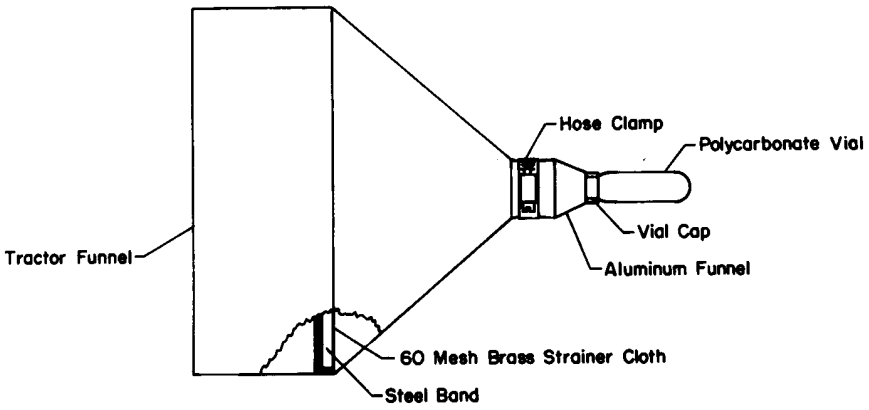

Figure 2.—*Black walnut pollen extractor.*

To extract small lots of pollen, the aluminum foil packet was agitated, freeing the pollen from the catkins; the pollen was then funneled directly into storage vials. A camel hair brush worked well for sweeping the pollen into the funnel. Because of the possibility of pollen contamination, this method should be used in a closed room that is free from viable pollen, or in a filtered airhood.

The following technique proved satisfactory on larger lots of pollen. An inexpensive and easily constructed pollen extractor was designed from a standard galvanized steel tractor funnel 10 inches in diameter (figure 2). A 60 mesh brass strainer cloth acting as a sieve was attached at the point of initial taper inside the funnel. A spring-steel band three-eighths of an inch wide was fastened as, a retainer for the strainer cloth with short metal screws from the inside of the funnel out. This assured a reasonably tight fit between the strainer cloth and the funnel walls. At the lower end of the tractor funnel, another small plastic or metal funnel was tightly secured in place with a radiator hose clamp. The small end of this funnel was the size needed to glue the threads from a cap of a pollen storage vial to the inside of the opening (figure 2).

The extractor should not be filled with catkins more than two layers deep if the catkins are to be dried in the extractor prior to actual extraction. If the catkins were dried in the paper sacks they were collected in, the catkins and dehisced pollen were poured and gently knocked from the sacks into the extractor. Again, because of the possibility of pollen contamination, all pollen handling should take place in a closed room that is free from viable pollen, or under a filtered air hood. Several layers of catkins could be extracted using the latter technique.

To enclose the top of the funnel extractor, a piece of Kraft paper was securely fastened over the top of the tractor funnel with a large rubber band. At the bottom of the small funnel, a pollen storage vial was screwed in place. The extractor was shaken vigorously to filter the pollen into the vial. This method provides a contamination-proof extraction system at a fraction of the cost of most installations. Extractors similar to this could be used to extract pollen of other tree species.

Literature Cited

1. Beineke, W. F., and C. J. Masters. 1976. Controlled pollination in black walnut. 10th Central States Forest Tree Improvement Conference, p. 66-72. Purdue University, West Lafayette, Ind.
2. Forbes, D. C. 1974. Black walnut control-pollination techniques. U.S. Dep. Agric. Tree Planters' Notes 25 (3): 9-11