

Black walnut

controlled-pollination techniques

Black walnut breeders need to know which existing controlled pollination techniques used with other species work with black walnut and which ones need major adaptation or complete development. This article covers a practical study that tested a standard pollen gun used in pine breeding: stigma receptivity (early or late after flower opening): two bags-synthetic sausage casing and Terylene paper: and methods of pollen collection, transportation, and storage.

Materials

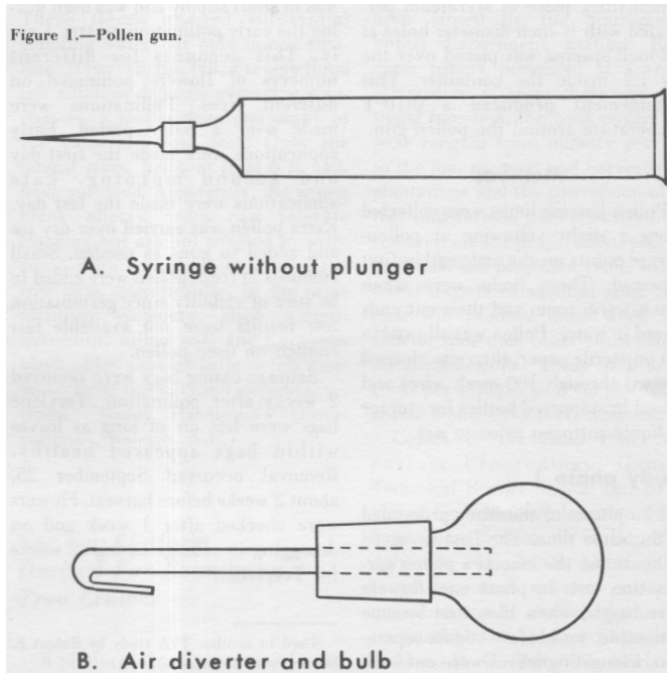
Walnut flowers are occasionally found singly but more often in two's and three's, rarely in four's. A No. 12 size paper bag will usually enclose two groups for a total of four to six flowers that will produce the same number of nuts.

Black walnut pollen is free-flowing and wind-carried: it resembles pine pollen to the eye and works well in pine pollen guns.

Two ramets of each of two clones served as females and two others as males. Males and females were selected on the basis of simultaneous pollen and flower production. All four were found in an old TVA orchard composed of trees selected for nut quality.

Terylene bagging is a cloth-like paper that is permeable to water and air. Synthetic sausage casing allows some air transfer but will hold water. It also allows a much higher heat

buildup inside the bag than does Terylene and provides no shading.



A typical pollen gun is shown in figure 1. Several workers including Crane et al (1). Serr and Forde (3) have used variations of the hypodermic needle to inject pollen into bags. Ours is an exact copy of those used in the University of Florida pine tree improvement program. Piece A is half filled with pollen: then B is inserted into A. Squeezing the bulb forces

diverted air into the pollen chamber which causes a stream of pollen-filled air to be ejected through the needle. This gun should always be used with the bulb lower than the needle to prevent ejection of unnecessary amounts of pollen. Graham (2) found that small, rather than large, amounts of pollen gave best results with Persian walnut.

1 Staff forester (geneticist). Division of Forests, Fisheries, and Wildlife Development Tennessee Valley Authority.

Other materials used included cotton and Twistems for closing bag ends around flower-bearing limbs, string for lifting bagged limbs above the horizontal to prevent water filling, and a Styrofoam chest filled with dry ice for field pollen transport. This chest had 2-inch thick walls and was about 8 inch x 10 inch x 10 inch deep, large enough to hold two pollen guns and a block of dry ice. It is a standard container used to transport drugs and can be obtained from drug houses. A 2-inch thick piece of styrofoam perforated with ¼ inch diameter holes at a 2-inch spacing was placed over the dry ice inside the container. This arrangement produced a 0-10°F temperature around the pollen gun.

Methods

Pollen-bearing limbs were collected when a slight yellowing at pollenrelease points on the male catkin first appeared. These limbs were taken into a warm room and their cut ends placed in water. Pollen was allowed to fall on sterile paper, then was cleaned (passed through 100 mesh wire) and placed in stoppered bottles for storage in liquid nitrogen prior to use.

Study phase 1

Two phases of the study, proceeded at the same time. The first involved pollinations, the second a pollen germination test. In phase one, flowers were bagged when they first became noticeable, well before stigma separation. Elongating leaves were cut back

about halfway to prevent their filling bags. Table 1 shows treatments. Except for pollen 3 on tree 1, all flowers received pollen just after stigma separation, and half were again treated after full stigma elongation. The strategy here was to be sure that viable pollen was on stigmatic surfaces when they were receptive. According to Graham (2) it should be before the dry appearance associated with full elongation. Pollen 3, stored in liquid nitrogen for over a year,² was in short supply and was used during the early pollination on tree 1 only. This accounts for different numbers of flowers pollinated on different trees. Pollinations were made over a 5-day period. Early applications were made the first day and second morning. Late applications were made the last day. Extra pollen was carried over dry ice and added to guns as needed. Small amounts of fresh pollen were added to be sure of viability since germination test results were not available fast enough on used pollen.

Sausage casing bags were removed 2 weeks after pollination: Terylene bags were left on as long as leaves within bags appeared healthy. Removal occurred September 25, about 2 weeks before harvest. Flowers were checked after 1 week and on debagging in sausage casing (2 weeks for Terylene).

² Used in another TVA study by Robert E. Farmer, soon to be concluded.

Study phase 2

Phase two included the pollens used for phase one pollinations (type 1) and other samples from the same pollen parents but not used in field pollinations (type 2). This second type was kept at 0°F overnight during testing and placed outside during the day at air temperature inside a bare transfer container (no dry ice) that was covered, but with the lid ajar. Samples were collected from both pollen groups at the end of 1, 2, 3, and 5 days. Germination above 30 percent was considered good; 10-30 percent was rated fair.

Results and Discussion

After 2 weeks, about half of all flowers in sausage casing bags on tree 1 had dropped: nearly all had dropped on tree 2. None had dropped in Terylene bags on tree 1, but over half had dropped on tree 2. None matured in sausage casing bags on either tree. Slightly less than half of all flowers bagged with Terylene matured on tree 1, but only one matured on tree 2. Pollens showed no difference in ability to effect fertilization. Biggest differences observed were between bag types and between mother trees.

Obviously, sausage casing is not an acceptable bagging material for walnut if bags are left on for a week or more. Terylene seems ideal. Lack of heat buildup due to increased air movement and the shading effect is the probable reason for Terylene's

TABLE 1.—Flowers pollinated by bag type, time of pollination, pollen, and mother tree.

Pollen	Sausage Casing				Terylene			
	Early Pollination		Late Pollination		Early Pollination		Late Pollination	
	Tree 1	Tree 2	Tree 1	Tree 2	Tree 1	Tree 2	Tree 1	Tree 2
1	4	6	4	6	4	6	4	6
2	4	6	4	6	4	6	4	6
3	8	X	X	X	8	X	X	X

"x" equals no pollination