CONTROLLED POLLINATION IN BLACK WALNUT[1]

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Abstract.--Controlled pollination of black walnut (Juglans nigra L.) has been accomplished at Purdue University. Six different bagging materials have been tested over a five year period and of these only "Terylene" and "Pollen Tector" bags have produced seed set. However, low seed yields are a continuing problem. Only 908 control-pollinated seeds have been obtained from 3782 flowers pollinated or 24 percent success. Excessive pollination (i.e., large quantities of pollen per pollination) was found to be detrimental since only 8 percent of flowers receiving large quantities of pollen developed nuts vs. 66 percent for the controls. Pollen dilution might increase seed set in the future. Methods of identifying control-pollinated nuts after they fell from the tree were investigated. Of the five techniques attempted, husk chipping with a pocket knife was superior.

Keywords: black walnut, Juglans nigra, control-pollination, bagging, breeding, seed set, seed identification.

INTRODUCTION

As selected trees in improvement programs approach the age of sexual reproduction, the desire of the zealous tree breeder to initiate controlled-pollinations becomes overwhelming. Most of us succumb, and in the case of black walnut (Juglans nigra L.), due to its peculiarities, we continue to attempt new techniques and approaches to better our meager results.

The two major problems in obtaining sufficient control-pollinated seed in black walnut for progeny testing involve the fact that only one to five pistillate flowers develop per flower cluster and low seed set per flower cluster is the norm. In order to obtain sufficient seedlings to analyze in a progeny test a great number of pollination bags must be attached per tree.

In spite of the drawbacks to controlled pollination in black walnut, control-pollinated tests are considered necessary to obtain information on general and specific combining ability, to determine rogueing sequence in first generation seed orchards, and to provide individuals of known parentage for inclusion in second generation seed orchards. Superior gene packages through wide-cross pollination and crosses which due to dichogamy could not naturally occur are additional reasons for controlled-pollination.

This paper reports on progress to date in bagging and controlled-pollination in black walnut over a five-year period near Purdue University, West Lafayette, Indiana. Problems associated with black walnut are probably not unique and in initiating wide-crosses in other NC-99 species, the same problems may be encountered.

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METHODS

Pistillate Flower Isolation

Black walnut trees used in the bagging trials included original selections in West Lafayette and grafts aged 3 to 8 years old at the Purdue-Martell Forest 10 miles west of West Lafayette (Beineke 1974 and Beineke 1975).

Isolation of the pistillate flower was accomplished using six standard techniques including (1) dialysis tubing, (2) kraft paper bag, (3) viscose sausage casing, (4) cotton batting, (5) "Pollen-Tector" paper bags[1] and (6) the "Terylene" white, nonwoven fiber bag with plastic window.

Bags were attached to the old wood, adjacent to the current year's growth, allowing at least 4 inches for terminal expansion after the bag was in place. In preparation for bagging, the buds and catkins were removed from the portion of the old wood that would be inside the bag, and the leaves located nearest the flower were removed leaving a total of 3 to 4. Remaining leaves were cut in half to prevent additional elongation. Leaf material and buds were removed to reduce congestion inside the bag.

Leaf removal may have reduced leaf surface to the point that the control-pollinated nuts were often noticeably smaller than open-pollinated nuts. In 1976 leaf removal was not attempted prior to bagging. No real hinderance to pollination was experienced, and in fact, leaf expansion helped to extend the sides of the bag and bagging time was reduced. Control-pollinated nuts in 1976 were approximately the same size as open-pollinated nuts.

Cotton was wrapped around the branch at the point of the bag attachment, and, in using the "Terylene" bag a wire expander was necessary due to frequent collapse of the bag during storms. The expander was made from 14 AWG. solid tinned copper wire constructed to look like a halo with a 4-inch diameter circle, and a stem for support. This wire is very malleable and easy to work with. To attach the expander, the stem of the expander was wrapped securely around the old wood portion of the stem, positioning the halo above the flower. The stem wire of the expander was bellied over the flower to provide maximum bag expansion. The bag was then tightly secured to the old wood with a twistem.

Occasionally it was necessary to bag a flower on a very slender branch. By securing the bagged flower to a larger branch with a twistem, sufficient support was provided. Occasionally, twistems were used to secure terminally located laterals parallel to the main branch enabling both the terminal and lateral flower clusters to fit in one bag. This often allowed up to 12 flowers to be included in a single bag.

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[1] Available from Carpenter Paper Co. of Iowa, 106-110 S.W. 7th Street, DesMoines, Iowa 50302.

Pistillate Flower Receptivity and Pollination

Bags must be attached before the female flower is receptive which is sometimes difficult to determine. In Persian walnut (*Juglans regia* L.) Wood (1934) and Wellington (1931) found that the flower occasionally can be pollinated when the stigmas are just beginning to show division. Therefore, the bag should be attached as soon as the female flower becomes visible to avoid all chances of contamination.

At the other extreme, if the glandular surface of the stigmas begin drying to the point that areas of necrotic tissue appear before pollination, little nut set will occur. The period of maximum receptivity is approximately mid-way between the two bounds. It is at this point that the flower looks fully developed. The stigmas are full and spread with their surfaces convoluted; and they appear fresh and moist. This period usually lasts from two to six days.

When considering isolation prior to controlled pollination, the best policy, therefore, is to isolate the flower soon after it is visible, and to leave the flower in isolation until the stigmas dry and develop small brown dots or the developing nutlet reaches the diameter of a dime.

Usually bags were attached 7 to 10 days prior to maximum receptivity and removed 10 to 14 days after pollination. Weather plays a large role in determining these time intervals.

Catkins were collected when they began to show yellowing just before pollen dehiscence. Pollen was extracted utilizing a large funnel in an enclosed system.

The pollen delivery system used an 18-gauge needle attached to a 10 cc disposable plastic syringe, with a small diameter glass tube connected to a rubber bulb and inserted inside the syringe barrel to provide pollen agitation and a metered pollen flow.

Excessive Pollination

One factor that may increase nut set is a reduction in the quantity of pollen per pollination. Flower abscission due to excessive pollination has been reported in Persian walnut (Kavetskaya and Tokar 1963).

In a small study at Purdue University in 1976, extra pollen was shaken onto 64 unbagged female flowers with a camel hair brush. One hundred additional flowers on the same trees were flagged and left to open-pollinate.

Nut Identification

Aluminum or cloth tags identified crosses while the fruit was attached to the tree, however, the fruit of black walnut must remain attached to the

tree until an abscission layer forms, and nut development is not complete until this occurs. Therefore, "picked" nuts generally will not germinate satisfactorily.

Screen wire bags proved too cumbersome and time consuming to attach. In addition the nuts dried rapidly after abscising into the bag, reducing nut germination. However, screen wire bags did discourage all squirrel pilferage.

The following five treatment combinations were tried to determine if some method of marking the nut would identify nuts after they fell from the tree:

1. Spray-paint color code.
2. Ballpoint pen or pencil.
3. Magic Marker color code.
4. Magic Marker inscription.
5. Husk chipping with a pocket knife.

RESULTS

Controlled Pollination

Dialysis tubing, viscose sausage casing, and cotton batting bags produced no nut set (Table 1). Reasons for lack of seed set may have included excessive heat buildup, moisture retention, and total collapse on wetting. Kraft paper bags did produce acceptable nut set, but the bags were easily punctured and holes developed in all kraft bags so that the pollen source was of doubtful origin (Table 1).

The advantages of the "Terylene" bag include the plastic window which allows observation of flower development and manipulation of the pollination needle inside the bag. The white non-woven fiber material reflects heat and allows moisture and air exchange. The bag is light weight and holds up reasonably well in wind and rain especially with the wire expander. Its disadvantages include high costs, general unavailability, necessity of the wire expander, and doubtful protection against outside pollen contamination if reused.

The "Pollen-Tector" bag, which is made of rigid, heavy, waterproof paper, was used successfully in 1976. Its advantages were: low cost, easy availability, no wire expander required, and due to low cost, it can be discarded after one season's use. In fact the only disadvantage of the "Pollen-Tector" bag was the lack of a window. However, sufficient pollen reached enough flowers to provide results as good as those obtained with the "Terylene" bag (Table 1).

Only the "Terylene" and "Pollen-Tector" bags produced successful seed set (Table 1). Over a five year period, 3,782 black walnut flowers were control-pollinated using "Terylene" and "Pollen-Tector" bags and 908 plantable

Table 1.--Comparison of pollination bags for black walnut.

	Dialysis tubing	Sausage casing	Cotton batting	Kraft paper	Terylene	Pollen-Tector
Bags attached (number)	20	40	65	10	913	232
Seed set (number)	0	0	0	5	658	250

Table 2.--Control-pollination in black walnut 1971-1976.

Year	Pollination[a] bags placed (No.)	Flowers pollinated (No.)	Seed set (No.)	Flowers pollinated (%)	Seed set per bag (No.)	Flowers pollinated per bag (No.)
1971	187	502	155	31	.83	2.7
1972	347	900	143	16	.41	2.6
1974[b]	189	828	192	23	1.06	4.4
1975	190	564	168	30	.88	3.0
1976	232	988	250	25	1.08	4.3
Total	1145	3782	908	24	.80	3.3

[a] Includes only Terylene and Pollen-Tector Bags.

[b] Control-pollination in 1973 was a failure due to late frosts.

seeds were obtained for an average 24 percent success rate (Table 2). Total bags placed numbered 1,145 or approximately 0.8 seed per bag. Flowers pollinated per bag varied from 2.6 to 4.4 per year and averaged 3.3 for all years (Table 2).

Excessive Pollination

Only 8 percent of the 64 flowers having extra pollen brushed on developed while 66 percent of the 100 open-pollinated flowers produced nuts.

The implication is clear that in order to obtain maximum nut set, excessive pollination must be avoided. Various techniques must be tested to obtain carefully metered pollination such as dilution with talc, dead walnut pollen, or dead pollen of other species.

Nut Identification

Painting proved unsatisfactory because the husk was killed. Ballpoint pen or pencil was deemed too time consuming, and attempts to color code with Magic Markers proved unsatisfactory because it was too difficult to distinguish between colors. The last two treatment combinations, Magic Marker inscription and husk chipping, worked the best. However, if the husk turned brown before the nut fell or was collected, the Magic Marker was usually obscured by brown husk color. Husk chipping not only leaves a permanent visible mark but is easy to do.

Husk chipping was done in September, because nut size was at its maximum. A specific code was established for each cross utilizing a pattern of 2, 3, or 4 small superficial chips in the husk. One chip was found to be unsatisfactory due to occasional single chips by other agents.

CONCLUSIONS

Even though controlled pollination was successfully accomplished in black walnut, the meager numbers of seed produced demonstrate that any full-scale control-pollinated progeny test using present methods is beyond our resources. The major use of control-pollinated progeny will be as a source of new combinations of genes for inclusion in second generation seed orchards. In addition it is possible that specific crosses might produce trees of such superior virtues that they would be propagated clonally.

Pollen-Tector bags are the only bags available at the present time that successfully produced control-pollinated seed.

Excessive pollination was implicated as the cause of the low seed set usually obtained in black walnut. Pollen dilution may solve this problem and increase seed set.

Since walnuts abscise and fall from the tree, the identity of the cross becomes a problem. This was solved by removing a small chip from the nut husk with. a pocket knife. Combinations of numbers and patterns of chips can serve to identify different crosses on the same tree.

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