CROSSING EASTERN COTTONWOOD IN THE GREENHOUSE

R. E. FARMER, JR., AND W. L. NANCE[1]
Southern Forest Experiment Station
Forest Service, U. S. Department of Agriculture
Stoneville, Mississippi

Eastern cottonwood (*Populus deltoides* Bartr.) is the subject of breeding programs designed to develop planting stock with potential for rapid growth, desirable wood properties, and pest resistance (4). Techniques for making controlled crosses within the species are essential to breeding, and their development constitutes an early phase of genetics research. This paper will describe techniques that are being used in the lower Mississippi Valley.

Controlled crossing techniques for the dioecious *Populus* species were developed in Europe and have been used there for several decades. The aspens (Section Leuce) may be crossed by placing bases of dormant branches in water after chilling requirements are met, forcing these branches in greenhouses, and pollinating the female flowers. Fresh pollen is obtained by similarly forcing male flowers. Fertilized catkins are matured on the branches in three to four weeks. This procedure, originally developed for *Salix*, was first used for *Populus* by Wettstein-Westersheim (11) and has been adapted for the American aspens by Einspahr and Benson (1), Johnson (7), and others.

While black poplars (Section Aigeiros) can be crossed by these means, their larger catkins with sometimes longer maturation time require more nutrition than is supplied by cut branches. Consequently, branches bearing female flowers are bottle-grafted to supporting rootstocks. The technique, as used in Europe, has been generally described by Muhle Larsen (8) and Schreiner (10). We have modified it for cottonwood. In brief, female scions are bottle-grafted to potted juvenile stock in mid-autumn. After chilling, flower buds on these grafted scions are forced and pollinated in the greenhouse. This procedure obviates climbing sometimes remotely situated trees.

METHODS AND RESULTS

Grafting

Cottonwood scions bearing female flower buds may be bottle-grafted to vigorously growing stock in the fall or in late winter immediately prior to forcing. Fall grafting may be advantageous because a functional graft is established before forcing and the concomitant depletion of the scions' food reserves. Also, fall grafting is done before the busy crossing season.

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[1]The techniques described here were developed while the authors were on the staff of the Southern Hardwoods Laboratory, which is maintained at Stoneville, Mississippi, by the Southern Forest Experiment Station in cooperation with the Mississippi Agriculture Experiment Station and the Southern Hardwood Forest Research Group. Farmer is now Plant Physiologist, Tree Improvement Section, Division of Forestry Development, TVA, Norris, Tennessee. Nance is presently a graduate student at Michigan State University, East Lansing.

Scions are collected and grafted in late September or early October. At these times trees are still fully foliated in the lower Mississippi Valley, but both vegetative and flower buds are set on sexually mature trees. Male and female flower buds are readily distinguished in September (2). Scions taken in August or early September are easily grafted but tend to renew apical growth and drop flower buds soon after grafting. In a preliminary test with scions collected in late summer, 66 percent of the successfully grafted scions lost all flower buds. In 1965 and 1966, when grafts were made later in the season, 12 and 17 percent dropped buds.

Scions are shot from trees with rifles and cut to approximately 20 cm. in length. Each scion should have an apical bud and one to six flower buds. Leaves are removed before grafting. Stocks are potted seedlings or cuttings about 1 cm. in diameter at the base. An ordinary approach graft is made, and the scion's base is placed in a container of water (6). The graft is bound with string and covered with grafting tape; no wax is used. With the exception of the incision essential to making the graft, the stock's stem is left intact.

Grafts may be stored in a greenhouse or a covered lath house. Under lath house conditions in central Mississippi, grafts made early in October establish unions in two to three weeks, i. e., before freezing weather begins. During winter, potted grafts are kept in a covered lath house so that unions will not be damaged by freezing rain.

In 1966, unions were achieved with 98 percent of the 271 attempted grafts; mortality of scions from 10 individual trees varied from 0 to 7 percent. Although scion mortality was low prior to forcing, flower bud drop made 17 percent of the grafts unsuitable for crossing. The tree-to-tree range in scion loss due to bud drop was 3 to 43 percent. Twenty-two percent of the grafts with unions proved unsuitable for crossing either because flower development was poor or because the union failed during greenhouse forcing. Fifty-nine percent of the attempted grafts flowered normally after forming effective unions, and were used in crossing; success with individual trees ranged from 20 to 81 percent.

Forcing and Flower Development

In central Mississippi, mature cottonwood chilled out-of-doors remains physiologically dormant until mid-February (3). Material forced before then takes several weeks or more to resume growth. On the average, male trees flower before females (i.e., require less chilling) but there is wide tree-to-tree variation in flowering time (5) which is under strong genetic control (12). Given this information, we have developed a reasonably successful procedure for forcing flowers.

Male branches are collected in early February and placed in aerated water under long-day greenhouse conditions. Bases of branches are trimmed twice weekly. Temperature is 75-

85°F. and relative humidity is 60-80 percent. Anthesis begins in one to two weeks, depending upon the individual tree; pollen is shed three to five days after catkins appear. By making two or three collections at approximately weekly intervals, one can obtain a continuous supply of fresh pollen during the several weeks of crossing.

Bottle-grafted female scions are brought into the greenhouse a few days after male branches are collected. At this time the stock is pruned back to approximately one inch above the graft. Catkin growth, which begins in two to three weeks, is usually rapid. In 1967 growth was completed in six to eight days, and final length of catkins varied from 5.5 to 21.5 cm. Catkin length on trees generally ranges from 10.0 to 20.0 cm. in central Mississippi; most of the catkins on grafts attained lengths within this range. Flowers mature from the base to the apex of catkins. Flowers at the base are receptive within two days after budbreak.

Pollination and Capsule Development

Crossing in greenhouses is done in late February and early March, before cottonwood begins flowering out of doors. Individual males are assigned to separate greenhouses or specially designed pollination chambers in a single greenhouse. Potted scions of females to be crossed with individual males are usually forced in the greenhouses assigned to designated males. When the smaller pollination chambers are used, female scions are moved to the chambers when anthesis begins and are taken out after flowers are no longer receptive.

We have used fresh pollen in making most crosses, since it is easily obtained. If pollen dispersal appreciably precedes female anthesis, however, pollen can be stored for a few weeks in desiccators at 10-25 percent relative humidity and 35-40° F. We have not tried longer storage.

Pollinations begin during the second day of catkin growth and are repeated on three successive days so that all flowers will be pollinated. Pollen is dusted over catkins with a small camel's hair brush.

In 1967, 160 scions were pollinated, and 51 percent were fertilized; success with scions from individual trees ranged from 0 to 83 percent. Incompatibility seemed to be the reason for failure with some crosses. Some variation in results was also associated with location of scions; crosses were generally more successful in greenhouses with a northern exposure than in pollination chambers.

Unfertilized capsules abscised within a week after catkins completed growth. Fertilized capsules completed enlargement within five weeks after budbreak (range 17 to 35 days). Final capsule diameter for individual catkins ranged from 4.8 to 6.7 mm and averaged 5.8 mm. Capsules on trees in natural stands have similar dimensions (5-7 mm) in central Mississippi. Fertilized capsules on a grafted scion are shown in figure 1.

Figure 1. Maturing cottonwood fruit on bottle-grafted cottonwood scion.

SEED MATURATION, DISPERSAL, AND GERMINATION

The first viable seed is obtained in mid-May from crosses made in late February or early March. During this two- to three-month period, mortality of catkins may be high; in 1967, 27 percent of the fertilized catkins died. A common cause of mortality may be graft failure resulting from insect infestation. Grafting tape should be removed from grafts shortly after growth resumes in the greenhouse, since numerous insects may be sheltered by it. A regular spraying schedule is essential. Some necrosis of individual capsules, apparently related to fungal or bacterial infection, has been noted.

Seed dispersal in 1966 and 1967 extended from mid-May until August, a range similar to that observed in natural stands (5). This wide range is due mostly to differences in maturation time of individual females, although some single catkins have been observed to disperse seed for a month. Each capsule contains 40 to 60 seeds.

Seed is collected as capsules dehisce, then cleaned by air (9) or by hand and germinated in sub-irrigated peat pots filled with a loam potting mixture. After approximately two weeks peat pots are transferred to nursery beds.

On the average, germination of seed from controlled crosses has been appreciably less than that for seed from natural stands. In 1967, germination of fresh seed from individual crosses varied from 14 to 88 percent; mean germination was 48 percent.

CONCLUSIONS

The method described above is providing sufficient full-sib progeny for testing, but seeds are produced on a relatively small percent of the grafts attempted. The technique is therefor expensive even though a few successfully matured catkins produce many seeds. However, average success is somewhat misleading, since the tree-to-tree range has been considerable; several females were complete failures and very good results were obtained with others. Some causes of crossing failure with individual trees may be unrelated to the technique; i.e., incompatibility is likely. Flower bud drop and other causes of loss before forcing reduce the method's utility for some trees. Spring grafting may therefore be more suitable for some females.

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MODERATOR CANNON: Mr. John Roller will you come forward and speak to us on "Open Field Propagation"?

OPEN FIELD PROPAGATION

JOHN ROLLER
Cartwright Nurseries
Collierville, Tennessee

LAND PREPARATION

The preparation of the land for open field propagation is very simple, as we practice it at Cartwrights. It consists of deep plowing, eighteen to twenty four inches deep, or sub-soiling. After this, a disc is run over the land as many times as is necessary to break up any clods and get it in good working condition. If necessary, we use a land leveler to level the field, or to give a smooth slope, but we prefer to use only a harrow as the land leveler packs the soil more than we like. After this, rows are spaced about thirty inches apart and are opened to a depth of four to six inches. The cuttings are stuck into these little furrows rather than on bedded rows.

CUTTING PREPARATION

The timing of the cuttings is governed by the weather conditions that we have that year. We like to start taking them when they are in the "summer dormancy" stage. In the Memphis area the usual starting time varies from about August 20, until early September. The cuttings planted in this type operation include all of the Pfitzer varieties, Andorra, *excelsa stricta,* Irish, *densa glauca, procumbens,* Sargents, Von Ehron and all sabinas, in fact, about any juniper that can be rooted in the greenhouse. However, *scopulorum* and most *virginiana* do not root well by this method. We think that Pfitzer juniper roots better if planted later than the other junipers and is usually the last juniper we stick. We, sometimes, delay taking part of them until we are sticking the broadleaf cuttings, which is usually mid-November. Broadleafed varieties propagated in this manner are the usual ones that are normally produced from hardwood cuttings, euonymus varieties, abelia, privet. lonicera, crape myrtle and others. We do not plant these until they have gone into dormancy, usually in November.

To take our cuttings, crews in the field use eight inch pruning shears. We like our cuttings to be about eight inches long. We start cutting at the end of the limb, trimming off the tender tip, and then cut just as far down the limb as we can