ARTIFICIAL HYBRIDIZATION AND GRAFTING METHODS WITH ULMUS AMERICANA

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Since 1957, a study of artificial hybridization and grafting methods using the American elm, Ulmus Americana, has been under way at the State University, College of Forestry at Syracuse University, Syracuse, New York. This project was added to the existent forest-tree improvement program of the Silviculture Department to (1) perfect the hybridization method of using cut-off elm branches in the greenhouses (2) determine the best possible method of grafting to be used with U. americana; (3) provide hybrid seed and material of U. americana that is more or less resistant to the fungus Ceratocystis ulmi, causal agent of the Dutch elm disease.

Selection of Material

Selection of U. americana to be used in the project was based on resistance to the fungus C. ulmi. To date, 12 selections have been made and worked with in the greenhouse at Syracuse.

In 1957, three American elms in the city of Syracuse that had had the Dutch elm disease and apparently recovered, were selected by Dr. Fred Klaehn, after a Dutch elm disease survey done by Dr. Howard Miller. In 1959, the project was expanded to include one more American elm in Syracuse and eight from the Cornell Plantation in Ithaca, New York. All of the Ithaca material, selected for the project by both Dr. Klaehn of Syracuse and Dr. Welch of Cornell, had been inoculated between three and six times with spores from a combination of some 40 strains of C. ulmi and had survived. This material was selected from among the remains of a mass inoculation and selection program for resistant American elms being conducted by Dr. Welch, Plant Pathologist at Cornell.

Hybridization

Method and materials.--The method used to accomplish the artificial hybridization in the greenhouse is the same as that used by Von Wettstein with aspen in 1930 and later by Smith and Nichols with elm in 1941. The account of several interspecific elm crosses done by Smith and Nichols as well as Von Wettstein's method is described in "Svensk Vaxtforadling", Skogsvaxterna, 1951, pp. 800-804. At Syracuse, we used the method successfully for intraspecific crosses in U. americana. Simply, the hybridization method consists of forcing dormant elm branches to flower in water-filled crocks in the greenhouse, collecting and storing pollen, and applying it to the female elements of other elm flowers when they are receptive.

Between February and April, dormant branches of U. americana (1/2"- 3/4" in diameter, and 2'-4' long) were collected and brought into the greenhouse. There, at greenhouse temperature (average 700°F,) each selection was placed in a half gallon ceramic crock filled with cold water (40° F.). To keep the water cold and prevent the formation of algae that would impair stem conduction, ice was added to the crocks daily, The water was changed twice a week and at that time three drops of Whites Solution was added to each crock.

(White's Solution is a liquid formulation of vitami ns, minerals, and organic and inorganic nutrients that is used at the College for keeping excised seed embryos alive).

Within two to six days after being brought into the greenhouse, the flower buds on the dormant branches began to grow and produced panicles of perfect elm flowers. Translucent waterproof paper bags were placed over the flowering branches to aid in the collection of pollen. Pollen was collected from the bags and stored in glass vials in a refrigerated desicator. On the average, in about two to four days after pollen collection, the pistils were receptive and the crosses accomplished by applying pollen to them with artist paint brushes. Immediately after pollination9 the flowers were isolated again with paper bags to prevent contamination by loose pollen. At the end of two days the bags were removed and the seed allowed to develop. In all of the hybridization work, care was taken to prevent contamination, from the isolation of selections in separate greenhouse compartments, to isolation bags on branches, to using separate pollen brushes for each pollen source and sterilizing brushes with alcohol between the application of pollen and dips in the pollen source.

In cases where there were too many flower buds per branch, some of them were removed so that only about 15 to 20 buds were left to develop on the average 1/2" branch. At the time of isolation with paper bags for pollen collection, a branch of each selection was bagged as a control, and one of each selection was left unbagged in each compartment as a check for pollen contamination.

Results in 1958.--Of six intraspecific crosses tried in 1958, only one produced seed that germinated in the seedbed; 65 seeds of cross #307 x #303 were collected from cut branches and produced 13 seedlings. Now at the end of their second growing season, 12 of these seedlings measure about 26" in height and are very uniform in their appearance. One of these seedlings measures only about ten inches in height after two growing seasons and is believed to be a product of inbreeding. It is generally believed that American elm, with perfect flowers, is quite self-sterile. In the work done at Syracuse, all the selections exhibited protandry, that is the male elements of the flowers developed 2-4 days before the female elements of the same flowers were receptive. Further proof that U. americana is self-sterile lies in the fact that although 27 seeds were produced in a bagged control in 1959, none of them produced seedlings in the seedbed.

Results in 1959.--A total of 1907 hybrid seeds were produced in 16 different intraspecific crosses in U. americana in the greenhouse at Syracuse in 1959. Only ten of these crosses produced seed that germinated in the seedbed. Two hundred-fifty-one of the 1907 seeds produced seedlings this spring for a 16.1 percent germination. The overall low germination percentage may be explained in part by a test conducted at the College by Mr. Dick Neu in which pollen of one source resulted in zero percent germination. He attributes the result to the weakened condition of the tree due to the Dutch elm disease.

In examining the data collected, there appears to be a close correlation between the time of branch collection and pollination and the success of the crosses made.

1. Of 12 crosses made on March second and third of this year, seed of only six produced seedlings (50%), while all four crosses made on April ninth produced seed that resulted in seedlings (100%).

2.Branches collected in February and early March forced flowers in an average of six days and produced mature seed in from 19 to 22 days after the pollination date. Branches collected in early April forced flowers in an average of two days and required only 11 days to produce mature seed after pollination.

3. Germination percentage of seed produced in March was 9.2% (104 seedlings from 1135 seeds) as compared to a germination percentage of 17.7% (137 seedlings from 772 seeds) of seeds produced in April.

More success can be expected, when attempting to produce hybrid elm seed by forcing dormant branches in the greenhouse, if the work is done as close to the natural flowering time as possible. In Central New York, more seedlings will result from seeds produced in early April than from seeds produced in February and early March.

Grafting

Grafting methods.--Based on experience with different grafting methods used to preserve elm material in 1958, a test was designed in 1959 to determine which of three grafting methods is most successful with U. americana. The bottle, side and cleft grafting methods were chosen to be tested on the same 12 selections made for hybridization purposes. In this way the material would also be preserved and concentrated for future work.

All grafts were spring greenhouse grafts, with the root stocks growing and the scion material dormant or just beginning to bud out. All root stocks were 3-year-old seedlings of U. americana that had been potted for one year. Scion material was one-, two- or three-year-old wood, used fresh or stored outside in snow filled plastic bags when necessary. Scions were secured to root stocks with grafting rubber bands and brushed with grafting wax. To minimize the loss due to individual technique, half of each selection was grafted by a different man.

After all grafting was completed, the root stocks of all grafts were gradually trimmed back beginning five weeks from the time of grafting. The water in the bottle grafts was changed each week and the end of scion material cut back each week for the first three weeks to insure water conduction. During the winter months, to keep the water cool and retard algae formation, icicles were picked from the greenhouse roof and placed in the bottle grafts. Side and cleft grafts required no extra care besides watering. All material was grafted between February 20 and April 11, 1959.

Results in 1959.--In measuring the success of the different grafting methods, all grafts that had not formed buds for next years growing season were considered to be dead. In the work done in 1958, hybridization was accomplished on those grafts that produced flowers. However, where all the seed was allowed to develop on grafts, there was little or no bud formation for the following season and most grafts died. So in 1959, all flower buds were removed from scion material and the hybridization work done on separate branches. The last check of grafted material was made on August 6, 1959.

At that time all the root stock material had been cut back to the graft union and the extension of scions into bottles of the bottle grafts had been removed for six weeks. The following tabulation summarizes the success as recorded on August 6, 1959.

Method	Grafts	Grafts alive with buds	
	Number	Number	Percent
Bottle	76	62	81.6
Side	39	25	64.1
Cleft	34	17	50.0
	149	104	69.8

Bottle grafting proved to be the most successful (81.6%) of the three methods tested, followed by side grafting (64.1%) and cleft grafting More success was observed in all methods where the scion material was either one- or two-year wood in good condition. It should be mentioned that the lower percent of success recorded with the side graft method was partially due to the use of poorly stored scion material on one selection, UaOn4C.

As a sidelight to the grafting test, it may be of interest to note that 12 attempts were made to graft material from the Cristine Buisman elm, U. carpinifolia, on root stock of U. americana. Six of these heteroplastic grafts were successful (50.0%), and again the bottle grafting method proved to be the best. Other heteroplastic grafting combinations done at Syracuse that have been successful include the Bea Schwarz elm and the Chinese elm (U. parvifolia), both on root stocks of the American elm.

Summary

Because the American elm is quite self-sterile and because it is capable of producing mature seed within a very short period after flowering, it was possible to successfully adapt Von Wettstein's greenhouse method of hybridizing cut branches of this species, We believe that we have shown the importance of timing both in the collection of material and in the pollination.

In testing the preservation of U. americana material by grafting, we have shown that although the bottle graft method is most successful, it requires quite a bit more care after grafting than either the side graft or cleft graft methods. Perhaps where scion material is abundant the side graft method would produce the most success per unit of effort. But where material may be scarce or of poor quality, the bottle graft method will probably produce the greatest percentage of successful grafts.

We have in the seedbed and greenhouse at Syracuse some 264 hybrid American elm seedlings whose parents have shown more or less resistance to the Dutch elm disease fungus (C. ulmi). In two years this material will be tested for resistance in close cooperation with Cornell University. Whether or not this material will prove to be of value is unknown at this time, but all parental material has been preserved as part of the project. We do believe that when and if an American elm is found or developed that is resistant to the Dutch elm disease, the results of this project will be of great value in attempting to mass produce material either by hybridization or by vegetative propagation through grafting, air layering, or cuttings.