ARTIFICIAL HYBRIDIZATION IN THE GENUS ULMUS

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Species hybridization plays an important part in the forest tree improvement work being carried on at the State University College of Forestry at Syracuse University.

Nearly all of the species in the genus Ulmus growing in the Northeastern United States are attacked by Dutch elm disease (Ceratocystis ulmi). With this fact in mind, the primary objective of this hybridization study was to produce plant material which might be fully, or at least to a certain degree, resistant to the Dutch elm disease. The study was conducted by making both intraspecific and interspecific crosses.

The program described in the following pages is a continuation of work done in the genus Ulmus by Dr. F. U Klaehn in 1958, and J. A. Winieski in 1959.

Literature Review

The genus Ulmus contains 18 species which are scattered throughout Eastern North America, Europe, and Asia (Harlow and Harrar, 1937; Smith and Nichols, 1941). According to Johnson (1939) and Smith and Nichols (1941), there are three natural hybrids within the genus and these are: U. x hollandica (U campestris x effusa), Huntington elm (U. glabra x montana), and (U. arbuscola x pupumila).

Artificial hybridization in elm was first done by Klotzch in 1854 when he successfully crossed U. campestris with U. effusa (Johnson,1939; Smith and Nichols, 1941). The hybrid obtained was found be more vigorous in growth than the parents. Since that time, many hybridization studies in elm have been carried on in Europe and in the United States. The following inter-specific crosses have been reported to have been made with success (Smith and Nichols, 1941; Johnson, 1939); U. wilsoniana x japonica, U. glabra x americana. U. americana x laevis, U. x laevis. U. glabra x carpinifolia, U. x holandica x japonica).

The cross between U. wilsoniana and U. japonica was found to be more vigorous in growth and more resistant to insect attack than U. japonica (Smith and Nichols, 1941).

Most of the species in the genus Ulmus flower in the early spring, but two species, U. parviflora, Chinese elm, and U. serotina, from the south central United States flower in autumn (Sax, 1933). According to Smith and Nichols (1941), all the species in the genus Ulmus are self-fertile and strongly protogynous, The authors did not provide data to support their statement that all elms are self-fertile; however, it is generally believed that all elms, with perfect flowers, are quite self-sterile Studies made by Winieski (1960) indicated that American elm is highly self-incompatible.

Method and Materials

To effect artificial hybridization in the greenhouse, elm branches averaging one to two inches in diameter and about two to four feet long, and with a sufficient number of flower buds, were collected from elm trees ranging from 26 to 65 feet in height. Four clones were obtained from Cornell Plantation in Ithaca, New York, from four American elm trees which have been isolated by Dr. Welch of Cornell University as more or less resistant to the Dutch elm disease. These clones are as follows R23-45, R19-2, R22-37, and R14-1-4. Eleven additional clones from nine different elm species which were suggested by Dr. Klaehn of Syracuse, were collected from Highland Park in Rochester, New York. The susceptibility of these clones to disease was not known. The elm clones and the tree numbers used in the hybridizations were U. wilsoniana #3692, U. glabra #3670, U. laevis #3607, U. japonica #3693 and 3686 U. carpinifolia #3651 and 3697, U. x hollandica, #3639, U. thomasi #1 and #2, U. alata #W384, and U. americana R14-1-4, R23-45, and R19-2.

The forcing of pollen was conducted in the greenhouse using the technique suggested by Schreiner (1938) and also used by Winieski (1960). Each clone was placed in a separate half gallon ceramic crock filled with cold water, and isolated from the other clones to prevent natural cross pollination. When the flower buds were about to open, these were covered with paper bags, leaving a few to serve as a control. The necks of the bags were sealed by being tied over cotton wool which was wrapped around the stem to prevent the entrance of pollen and to keep the bags from slipping., The flowers were not emasculated as the species are believed to be self -incompatible. Pollen from each clone was collected and kept in a separate glass vial stoppered with a plug of absorbent cotton. A pollen germination test was made by growing the pollen on a ten percent agar solution.

When the female flowers became receptive, the bags were removed and the female flowers pollinated. Each bag was replaced immediately after pollination, and some flowers in other bags were left unpollinated to serve as a bagged control, In applying the pollen, separate artists? brushes were used for each pollen source. These were sterilized with alcohol between dips into the pollen. A total of 45 interspecific crosses and six intraspecific crosses were made. Three days after pollination, all the bags were removed to allow seed formation. To supplement the nutrient requirements of the branches. White's solution, a formulation of vitamins, and organic and inorganic nutrients, was added to the water every week. Blocks of ice were added to the water every morning to keep down algae formation. The water was changed weekly, and the lower ends of the branches were cut to prevent impaired conduction due to algae formation.

Twenty one days after the date of pollination, the mature seeds were collected, counted and checked under light to determine the number of filled and empty seeds. A cutting test was also done as a check, and the filled seeds were planted in the nursery. The seeds germinated in about 12 days and the percentage of germination determined.

Results

Shown in table 1 are the dates of pollen collection, pollination, removal of bags, and date of seed collection. It was found that the species U. americana, abra, laevis, thomasi, and japonica were protandrous, that is, the male elements of the flower developed two to four days before the female elements were receptive. U. wilsoniana, alata, and hollandica were the reverse of this they were metandrous. One, U. carpinifolia, reported to be protandrous, was metandrous rather than protandrous, The female flowers were receptive for about three days. The percentage of artificial pollen germination was very low; less than one percent germination was obtained in four of the species. This low pollen germination result was probably due to the pollen germination technique used.

Table 1.-- Seed obtained from successful crosses and germination percentages

Cross no.[1]	Pollen collected	Pollination	Removal of bags	Seed collection	Seeds matured	Seed germination
	Date	Date	Date	Date	Number	Percent
4	4/6/60	4/8/60	4/11/60	5/2/60	49	37.5
16	"	"	"	"	10	0.0
18	4/7/60	"	"	4/30/60	21	15.0
19	"	"	"	5/2/60	21	30.0
32	4/6/60	"	"	"	5	0.0
60	4/9/60	4/9/60	4/12/60	"	7	83.3
101	4/10/60	4/10/60	4/13/60	"	4	66.6
138	4/7/60	4/11/60	4/14/60	"	8	1.4
148	4/10/60	"	"	"	3	50.0
183	4/7/60	4/10/60	4/13/60	"	31	0.0
187	4/9/60	"	"	"	26	0.0
188	"	4/11/60	4/14/60	"	52	0.0
191	4/10/60	"	"	"	49	0.0
195	4/9/60	4/10/60	4/13/60	"	38	0.0
				Totals	324	33.6

[1] All flowers were isolated by bagging on 4/6/60

Of the 51 intraspecific and interspecific crosses attempted, a total of 324 mature seeds were obtained from 14 successful crosses (table 1). Five of these crosses were intraspecific within the American elm clones. They produced 1.06 seeds of which 27 percent germinated. Nine interspecific crosses produced a total of 218 putative hybrid seeds. Eight of these interspecific crosses were not known to the writer to have been reported and these were U. americana x thomasi , U. japonica x americana , U. japonica x U. xhollandica, U. xhollandica x americana, U. xhollandica x laevis, U. xhollandica x glabra, U. xhollandica x carpinifolia, U. xhollandica x t homasi.

The ninth successful interspecific cross was between U., glabra and U. carpinifolia. This cross has been reported. The five interspecific crosses with U. hollandica as the female tree, produced many seeds but all of them were empty, The remaining four crosses produced a total of 22 seeds, and out of these, 13 produced seedlings.

Out of 35 bagged unpollinated controls only one produced seeds and these seeds did not germinate (table 2). This indicates that the species are self-incompatible. Many seeds were obtained from the unbagged controls This was expected because the use of a brush made it difficult to prevent the unbagged controls from being contaminated during the pollination of the flowers that were bagged U. thomasi produced many flowers, but they all dropped during the handling wilsoniana and U. alata were later dis carded because of insufficient number of flower buds.

Table 2.--Seeds from crosses and from bagged and unbagged controls

Clones	Mature seeds	Empty seeds	Filled seeds	Seeds planted	Seeds germinated	
	Number	Number	Number	Number	Number	Percent
R 23-45 × R 19-2	49	--	49	48	18	37.5
Bagged control	--	--	--	--	--	--
Unbagged control	23	-	23	20	8	40.0
R 14-1-4 × R 23-45	10	--	10	9	0	0
× R 22-37	21	--	21	20	3	15.0
× R 19-2	21	--	21	20	6	30.0
Bagged control	--	--	--	--	--	--
Unbagged control	--	--	--	--	--	--
R 22-37 × R 14-1-4	5	--	5	4	0	0
Bagged control	--	--	--	--	--	--
Unbagged control	--	--	--	--	--	--
R 19-2 × thomasi #2	7	--	7	6	5	83.3
Bagged control	10	--	10	10	0	0
Unbagged control	8	8	--	--	--	--
U. glabra × carpinifolia #3651	4	--	4	3	2	66.6
Bagged control	--	--	--	--	--	--
Unbagged control	--	--	--	--	--	--
U. japonica × R 22 #3686	8	--	8	7	5	71.4
×hollandica	3	--	3	2	1	50.0
Bagged control	--	--	--	--	--	--
Unbagged control	2	--	2	2	1	50.0
U. ×hollandica × R 23-37	31	31	--	--	--	--
× glabra	26	26	--	--	--	--
× laevis	52	52	--	--	--	--
× carpinifolia	49	49	--	--	--	--
× thomasi #2	38	38	--	--	--	--
Bagged control	--	--	--	--	--	--
Unbagged control	25	25	--	--	--	--
U. japonica #3693						
Bagged control	--	--	--	--	--	--
Unbagged control	18	--	18	18	9	50.0
U. laevis						
Bagged control	--	--	--	--	--	--
Unbagged control	3	3	--	--	--	--
U. carpinifolia #3697	--	--	--	--	--	--
Bagged control	--	--	--	--	--	--
Unbagged control	7	4	3	3	1	33.3
Totals	420	236	184	172	58	30.0

The number of mature seeds obtained from the crosses together with the seeds from the bagged and unbagged controls, are shown in table 2. Out of a total of 420 mature seeds, 172 were found to be filled after testing the seeds under light. The seeds started to germinate about 12 days after planting. The total percentage of germination was 30; however, this low germination was due partly to severe drought conditions in the nursery beds.

Discussion and Conclusion

All the 196 putative hybrid seeds which resulted from the five interspecific crosses with U. hollandica as the female trees were empty. This may be due to the fact that U. hollandica is a hybrid. The cross U. hollandica x japonica has been reported to have been made with success, hut in this study it was only the reciprocal cross that was successful. This cross produced mature seeds that germinated. Of the nine interspecific crosses that produced mature seeds in this study, only the cross, U. glabra x carpinifolia is known to the writer to have been previously reported.

Out of 35 bagged unpollinated controls, only one produced mature seeds. This may have resulted from contamination. This indicates that the species used in this study are highly self-incompatible. The use of the artist brush in pollinating the flowers made it difficult to prevent contamination of the unbagged flowers. The use of a small dropper or a pipette as suggested by Schreiner (1938) can prevent such contamination.

Each clone consisted of two to four branches of elm placed in a crock, It was found that in many of the clones from one to three of the branches wilted and the flowers dropped. This may have been due to poor water and nutrient uptake and the condition of the branches at the time of collection; another contributing factor may have been due to the position of the branches on the ortet (mother tree) from which they were collected.

The results of this study are not complete for the nature of the seedlings. Their growth characteristics and their resistance to disease is yet not determined. However, it demonstrates the possibility of producing elm trees in the greenhouse with good growth characteristics and resistance to Dutch elm disease by means of artificial intra- and inter-specific hybridization.

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DISCUSSION

LANDIS. I would like to ask the first speaker how the average person in the field can determine the thickness of bark in relation to stem?

SANTAMOUR. The use of anatomical selection criteria in wild stands is a possibility, but I don't believe it would have wide application. The main difficulty is in obtaining-comparable material from different trees. We dug roots from hybrid poplars that were 5 or 6 years old, and found that it was extremely difficult to find roots of a 1/4 inch to 1/2 inch diameter on roots of different trees. Some required almost complete excavation. The main application of anatomical criteria would appear to be in selection of nursery stock, whether of pedigreed or unknown origin, for outplanting and testing. The procedure of determining bark percent itself is relatively simple. You just take the root, measure the diameter outside bark, diameter inside bark, calculate the bark percent, and there you have it.

LANDIS. Are these side roots?

SANTAMOUR. In the work we have done it hasn't mattered whether it's a side root or main root, just as long as the diameter is fairly constant.

GABRIEL. Did you find any evidence of root grafts in your plantation?

SANTAMOUR. In the larger plantations that we examined we found no evidence of root grafts mainly because we did not have the perseverence to follow the roots all through the entire plantation. The tests in which we worked were 100-tree plots in the crop-tree clonal test and sometimes we would have to follow the roots entirely into the next plot to get a root of the right diameter for investigation. This investigation on large trees as I said was merely to establish the stability of this particular characteristic. On the one-year old trees of course it was very easy (well, not very easy as Harry Kettlewood can tell you) to dig out the roots and get usable material.

GABRIEL. We found root grafts in the seedling maples that we have been growing.

SANTAMOUR. Could these be easily detected?

GABRIEL. Yes.

SANTAMOUR. That may be a possibility, but if you can detect the root graft certainly it's no problem in collection criteria.

GABRIEL, What was the percentage of albinos in the selfed progenies you studied?

LESTER, I am not certain that the chlorophyll deficiency reported here can be considered as an albino condition. However, 21 percent of the progeny from selfing exhibited a chlorophyll deficiency.

GABRIEL, The reason I am interested in albinism is that we are finding albinos in sugar maple and I thought it would be interesting to compare percentage of occurrence between aspen and maple,

MERGEN. It is difficult to raise aspen seedlings because they are very susceptible to damping-off diseases. I would like to ask Mr. Lester what his feelings are on the mechanism of sex determination in aspen. Is the sex in aspen determined by a genetic mechanism or is it a physiological mechanism that determines the differentiation of the primordia into a male or female flower?

LESTER. I think this should come under the realm of speculation, but in the work done by others the transmission of lability in sexual expression appears to be a genetic mechanism while the actual expression of sex may be environmentally influenced.

LITTLEFIELD. I realize that this is probably going to expose my ignorance, but I was brought up to believe that application of high nitrogen fertilizers had the effect of stimulating vegetative growth at the expense of flower and fruit production. This seems to be a reversal of that principle, or was I wrong in the first place?

STEPHENS. This has been a conception that has been held for a long time, and I think it first received its greatest impact on the thinking of physiologists and other people from the work of Kraus and Kraybill back about 1914. They demonstrated quite markedly with tomato plants and to a lesser extent with apple seedlings, that increased applications of nitrogen fertilizer tended to bring about lush vegetative growth at the expense of reproductive growth, and they further postulated the carbon-nitrogen ratio theory which is an extension of Kleb's earlier Kohlen-Nahrstoff, the carbon-nutrient ratio in a material. It appears for trees, and it is true for many species, that this does not hold true. There is no single carbon-nitrogen ratio that one could say is extremely favorable for flowering since with a given ratio you could have very wide variation in absolute levels within the tree. Now you will recall that at the highest level of nitrogen application on the 22-year-old trees there was a drop in the cone production. However, as far as diameter growth is concerned there has been no significant increase. I don't have all the data yet on leader extension for this year, but there has been no apparent increase. The reason for increased floral production in trees with increased additions of nitrogen is not really apparent yet. It is not really known what it brings about in the plant. This is the ultimate basis of the problem. Orchardists for many years have been fertilizing their trees, not only with nitrogen fertilizer, but complete fertilizer and getting good results. But still this idea that heavy loads of nitrogen will bring about vegetative growth persists, I might add that I neglected to mention that the 14-year-old trees in this particular study this year did not produce any flowers at all although they previously did produce a few in 1958.

MERGEN. I might mention also that before we set up the flower stimulation studies with slash pine trees in Florida, we made a thorough review of the literature and consulted various scientists in the field of orchard tree management. On the basis of this we made up a recipe that was very high in phosphorus, low in nitrogen, and average in potassium. We decided on a 3-18-6 formulation. We assumed that phosphorus would bring about the stimulation, and that nitrogen would promote vegetative growth. Therefore we also included a high nitrogen content fertilizer (10-10-10). The results were quite different than anticipated. The high nitrogen fertilizer induced flowering in young trees and also promoted a greater number of flowers on older trees, The higher levels of the 3-18-6 formulation worked only as well as the low levels of the high nitrogen fertilizer.

LITTLEFIELD I have another question which may be a little out of order in that it probably is in the field of arboriculture rather than genetics but it does have to do with tree flowers I promised an acquai ntance of mine that I would get the answer among this group of rather distinguished people here who know something about tree flowers. It appears that people have this problem particularly with silver maple because of its profuse seed production which causes a first-class nuisance The question has arisen as to whether there has been developed any spray which can be applied to in hibit flower production in silver maple. Does anybody know anything bout that?

MERGEN. I don't know of any specific reports with maple) but there are many reports on the application of hormone sprays to drop the flowers of hardwood trees. It is common practice in fruit tree orchard management to use a thinning spray on the trees while they are flowering to inhibit the development of a portion of the flowers. This is being done with apple) pear and plum trees if the flower crop is larger than is desired. I am sure that a similar spray could be used on maple flowers.

BUCKINGHAM. Is there any information as to whether or not the topping of white pine increases flower production?

STEPHENS. I dont know. This has been contemplated; we haven't done it yet. There have been reports in the last year mainly from the Pacific Northwest Forest Experiment Station where this was contemplated several years ago. I followed it up and found that it had been done neither by the Northwest Station nor by the Weyerhaeuser Forestry Research Center who had also contemplated it. I don't know the reasons against it It has been tried here in Connecticut on the white spruce seed orchards. I don't think it is late enough yet to tell whether it is going to have any effect or not.

POST. Do you have an idea of what the total available nitrogen was in the treated trees? In other words, what was the available nitrogen in the soil in addition to the fertilizer which you applied.

STEPHENS. I have no data on this at present.

POST. This will be part of your study, will it not? Why did you think that the desirable level of application was around 100 pounds?

STEPHENS. The response was plotted over the range of fertilizer applications and the optimum from a freehand curve appeared to occur at about 100 pounds of nitrogen per acre. I wouldn't say 100 pounds per acre of nitrogen for all conditions. It just happened to be in this particular case.

FORD. You have a very interesting study. Do you have any plans to find out what is in the cones? You know that there is work being done on earliness of cone collection, that is, the time that the seed is matures and in addition they have introduced the factor of cold treatment and light. I wonder if you are going to follow through and find out what you will get inside those cones?

STEPHENS. It is not contemplated at this time to follow through to that degree. I am working on this alone and I find that there is a limit to my capabilities.