THE CONTROLLED POLLINATION OF EASTERN COTTONWOOD IN THE GREENHOUSE

By

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THE CONTROLLED POLLINATION OF EASTERN COTTONWOOD

IN THE GREENHOUSE

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CHAPTER I

INTRODUCTION

Eastern cottonwood (Populus deltoides Bartr.) has been recognized as a species which can increase future wood production. It grows along river bottoms much farther west than most other desirable fast-growing hardwoods. Good pulping and growth characteristics make it a desirable species from a short rotation standpoint, as pointed out by Farmer (6). Cottonwood can be reproduced vegetatively allowing the production of large amounts of genetically identical material.

A breeding program will be necessary to fully utilize the potential of the species under intensive management. In 1966, Farmer (6) outlined the status of cottonwood improvement research in the lower Mississippi river valley, and concluded that cottonwood genetic improvement should be an essential part of research leading to intensive cottonwood culture in the region. This research should be based on silvicultural characteristics as well as the natural variation of the species.

As cottonwood genetic research continues, the need arises for crossing techniques which can be easily applied. The objective of this study was to develop and evaluate two methods of obtaining cottonwood control-pollinated progeny in the greenhouse.

Advantages of Making Controlled Crosses in a Greenhouse Versus Natural Stands

Making controlled crosses in a greenhouse has two major advantages: (1) The crossing can be performed under a controlled environment, and (2) The parent trees need only be visited once, to shoot out the cuttings, compared to four to five times if crossing were performed in natural stands. Also, the parent trees must be climbed at each visit if crosses were made in the natural stand.

Flower Morphology and Phenology

Figure 1 illustrates cuttings containing male and female flower buds. The male buds, shown at left, are much larger and are quite curved, while the female buds, shown at right, are much smaller, straighter, and resemble leaf buds.

Some trees are very difficult to sex on the basis of buds alone The buds on some male trees may look very much like females, and vice-versa. Through trial and error, it was discovered that the carpellate flower of the female and the cup-like disk of the staminate flower of the male (Figure 2) can be readily distinguished by removing the bud scales on a dormant bud and examining with a hand lens. It may be possible to determine sex early in the dormant season, however, in this study the first buds were not examined until the end of January.

Figure 2 illustrates a single staminate flower and a staminate catkins, and a single pistillate flower and a pistillate catkin. Illustrated also are the flower parts which can be examined easily in the field by the use of a hand lens.

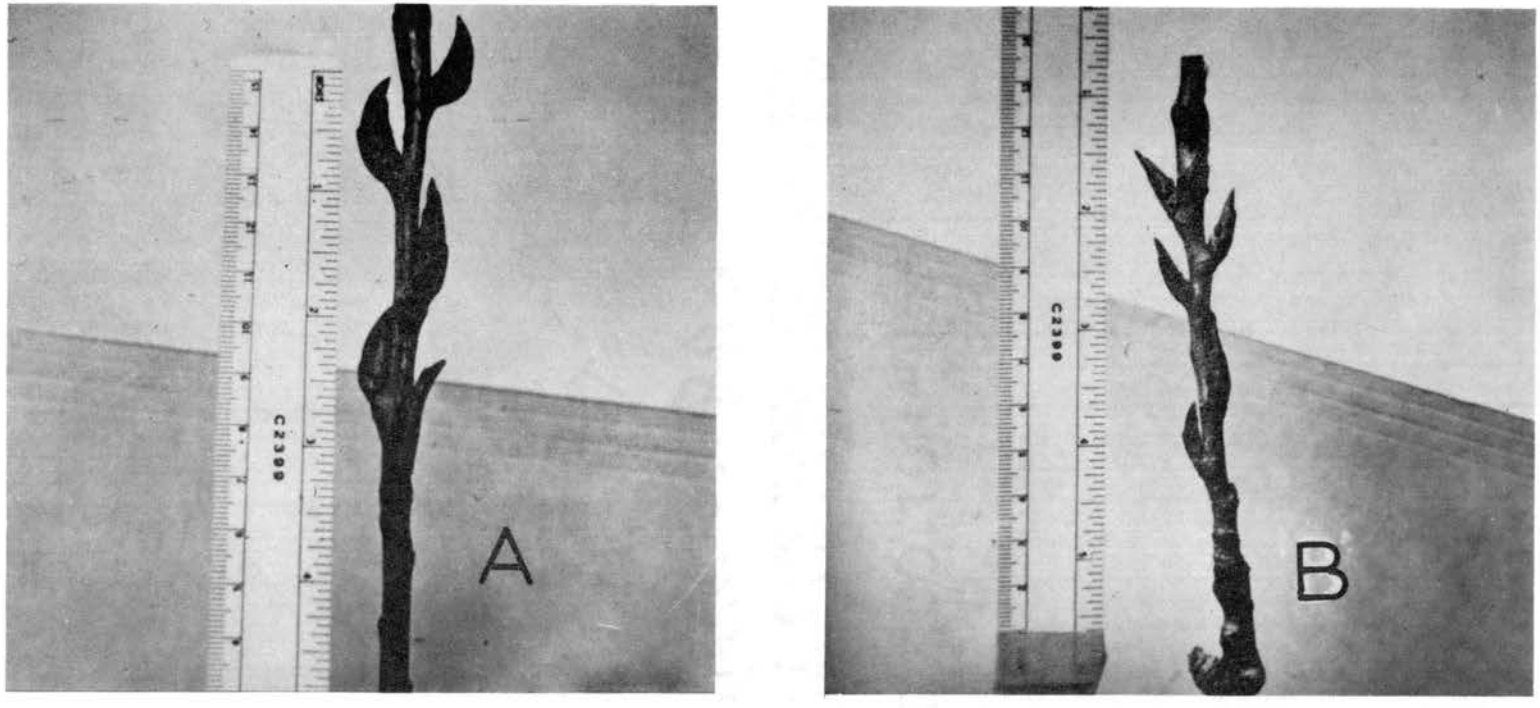

Figure 1. The Photograph on the Left (A) Shows a Cutting with Male Flower Buds. The Photograph on the Right (B) Shows a Cutting with Female Flower Buds.

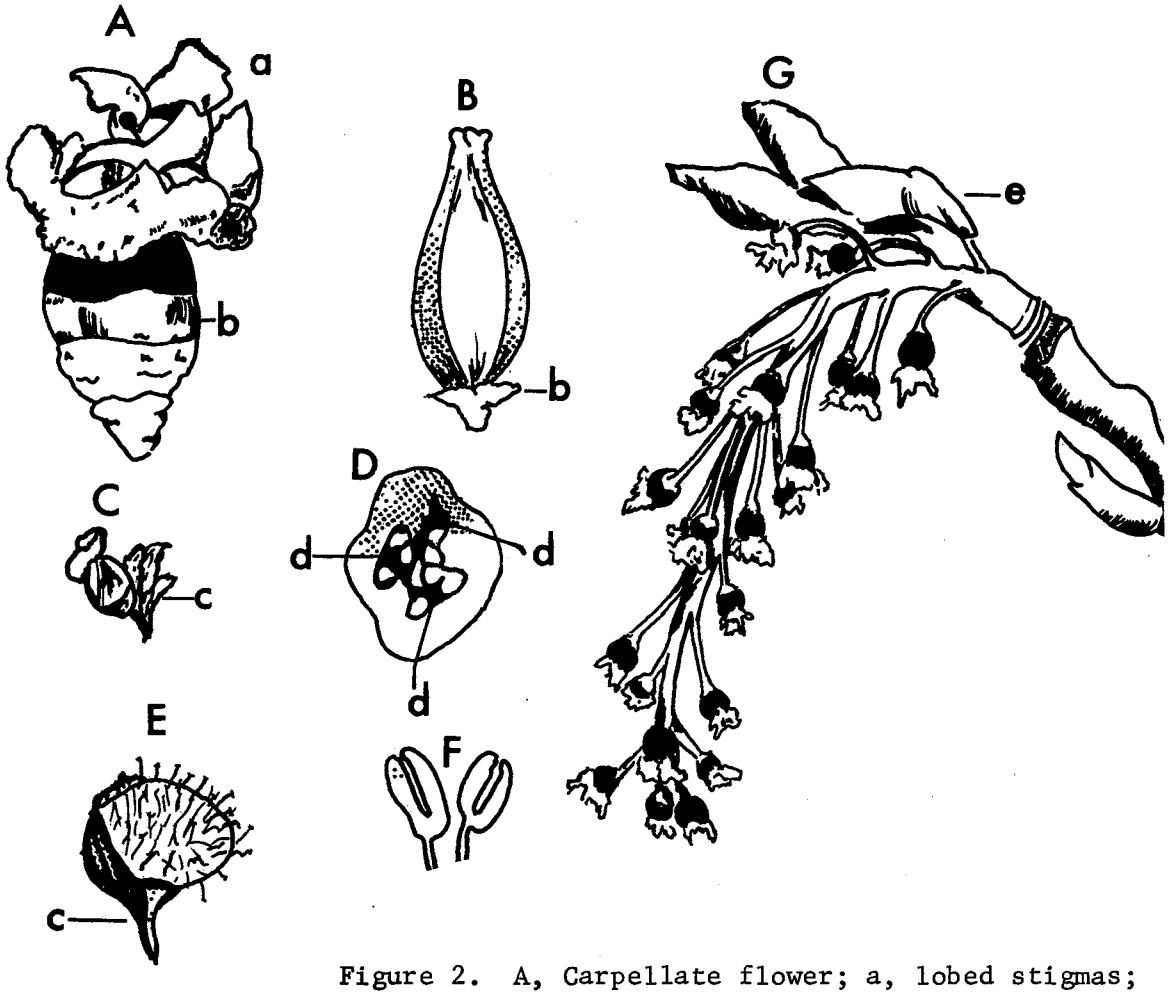

Figure 2. A, Carpellate flower; a, lobed stigmas; b, cuplike disk; B, the late stage of the three-carpellate gynoecium; C, carpellate flower with subtending bract (c); D, transverse section of ovary, with three parietal placentas (d) which correspond to the three carpels; E, cup-like disk of a staminate flower, with filaments of stamens, and subtending bract (c); F, dorsal and ventral aspects of the stamens; G, the female catkin as it appears on stem just after pollination; H, male catkin just after it comes out of the bud and before pollen is shed; e, the old bud scales. The figures were drawn with the aid of Johnson (9) and Vines (11).

The cold requirements of the flowering buds were studied by Farmer (4), who observed that the buds would force* any time after December 15. Cold storage studies revealed that cuttings stored at 39 degrees F after being taken from the ortet in late September would also force any time after December 15. Similar observations were made in Canada by Johnson (10).

The variation in time of flowering in the lower Mississippi valley was studied by Farmer (5), indicating that the natural flowering sequence in males began between March 5 and 10, and lasted about one month. Initiation of female flowering began between March 8 and 12, and also lasted about one month. Most of the variation in flower initiation was found to be between trees rather than within trees.

Techniques of Greenhouse Crossing

A method for maintaining healthy greenhouse-pollinated catkins was developed by Bergman and Lantz (1). Cuttings were placed in cooled distilled water to discourage the growth of algae and fungi which could inhibit the conduction of water within the stem. In another study, by Johnson (10), distilled water was found to be superior to solutions of Hoaglands, sucrose, Hoaglands combined with sucrose, or to a dampened peat bed for successfully growing catkins of Populus grandidentata Michx. to maturity.

A technique of grafting female scions onto potted rootstock was developed by Farmer (8) as a method for providing food to sustain

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*Throughout this paper, "forcing" or "forced" will be defined as: inducing flower buds to flower by use of controlled temperature and/or day length.

greenhouse-pollinated catkins to maturity. Male cuttings were collected in early February and forced in one to two weeks using aerated water. Long-day greenhouse conditions, with temperature and humidity ranges of 75 to 85 degrees F and 60 to 80 percent respectively were maintained during forcing. Pollen was collected and stored in desiccators at 35 to 40 degrees F for up to one month with good pollination success. A few days after the males were collected, grafted female scions were brought into the greenhouse and forced in two to three weeks using natural daylength with temperatures between 75 and 85 degrees F.

Cottonwood Seed Dispersal and Germination

Cottonwood seed dispersal in natural stands occurred between mid-May and late July in the lower Mississippi valley (5). Most of the variation in time of seed dispersal occurred from tree to tree. Farmer (8) reported seed dispersal in the greenhouse occurring from mid-May until late July, with most of the variation from tree to tree. The variation in number of seeds per capsule ranged from 40 to 60, while germination percentages ranged from 14 to 88 with a mean of 48.

CHAPTER II

MATERIALS AND METHODS

Selection of the Parent Trees

On January 11, 1971 the parent trees for this project were selected from a band of 10 to 20 year old cottonwood along the Cimarron river west of Ripley, Oklahoma. A north-south base line, approximately two hundred yards in length, was established through the center of the 150 feet wide band. Starting in the middle of the base line, the first three male trees and first five female trees were selected from both north and south directions along the line. A total of sixteen trees were selected (only trees old enough to produce flower buds were taken). Cuttings with flower buds were shot from each tree and forced to flower in a germination chamber to verify the sex of the ortet. A 14 hour light period and 12 hour dark period at 80 and 60 degrees F respectively were used during forcing.

Forcing the Male Flowers for Collection of Pollen

Five cuttings, each with at least two flower buds, were taken with a rifle from each of the six male trees on March 5, 1971. Seven inch nursery cans in the greenhouse were filled with a one to one, peat to sand mixture. The cuttings were planted at a depth of seven inches leaving the flower buds a minimum of three inches above the level of

the mixture. Flower buds on each cutting were reduced to two. The cuttings were watered daily by hand and the greenhouse temperature was maintained between 50 and 70 degrees F. The buds began to open on March 13, 1971, and in seven days all had opened. Two days after anthesis (flower opening), the pollen began to dehisce. A plastic bag was placed over the mature catkin and both were shaken until all loose pollen had fallen. The bag was then removed, and the pollen was placed in a vial. This type of collection procedure was performed twice daily on the mature catkins until all pollen had shed.

The pollen from all clones was mixed and stored in a vial which was stoppered with cotton and placed in a larger container with crystals of silica gel. The container was covered securely and stored in a refrigerator at 35 to 40 degrees F. A similar method was used by Farmer (8) as a means of reducing moisture content.

Attempts were made to test the pollen by germinating in Petri dishes using distilled water, pure sucrose, and pure sucrose diluted with distilled water as media but only small buds on the pollen grains were observed, with no pollen tube growth.

Collecting and Planting of the Female Cuttings

On March 25, 1971, ten cuttings were shot from each of the ten female ortets. Care was taken to obtain cuttings from the upper crown to insure the existence of flower buds.

Five cuttings from each tree were planted in seven-inch nursery cans which were filled with a one to one, sand and peat mixture (Figure 3). All cuttings were watered daily.

Figure 3. Cuttings With Flower Buds in Sand and Peat Mixture

The remaining five cuttings from each tree were placed in one-quart plastic cups filled with distilled water, as suggested by Johnson (10). The water was circulated through the plastic cups continuously using the system shown in Figure 4. A lid was placed on each cup with a hole large enough for the cutting. The lids were painted silver, in an effort to reflect sunlight and keep the water cool, thus hindering fungi growth, as noted by Bergman and Lantz (1). The bottom one-quarter inch of each cutting was cut off weekly, to minimize xylem blockage at the base of the cuttings.

Anthesis and Pollination of the Female Flowers

The temperature in the greenhouse was maintained between 60 and 80 degrees F while the flower buds were opening. No artificial light was used. As the flower buds started to open, all but the largest and most healthy two flowers on each cutting were removed.

Pollination was accomplished using the stored pollen-mix as soon as anthesis had occurred. A photograph of the pollinator is shown in Figure 5. Each flower was pollinated in the morning and afternoon until the stigmas abscised approximately two days after anthesis. The pollinations lasted about one week until all flowers had opened.

Seed Processing

A catkin was assumed to be mature when the first capsule opened and dispersed "cotton". The catkin was then taken from the cutting, the number of capsules was counted, and the catkin was placed in a 4 by 10 inch paper envelope to dry. After all capsules had dehisced, the "cotton" was taken out of the envelope and the seed was separated using

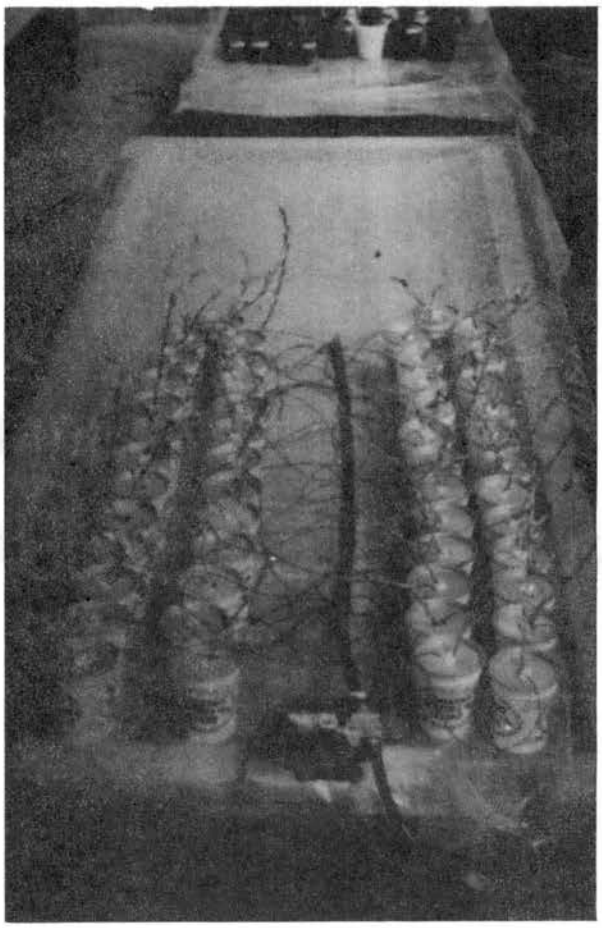

Figure 4. Table Holding Cuttings With Flower Buds in Circulated Distilled Water

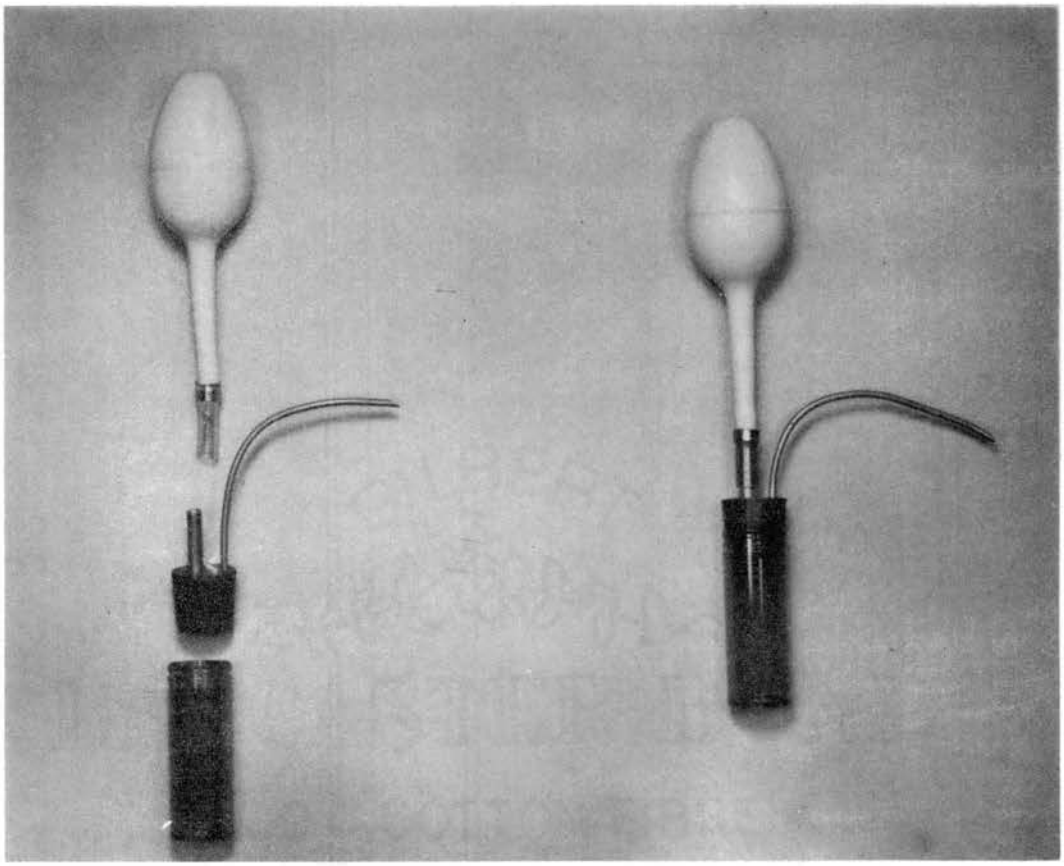

Figure 5. The Pollinator Used to Control-Pollinate the Female Flowers

a "micro-thresher" (a blender which has been slowed to several hundred revolutions per minute, plus an additional blade on a six inch extension which was added above the standard blade). As the "cotton" rotated inside the blender, the seed was thrown out and away from the fiber and deposited at the base of the pitcher. The seeds taken from each capsule were counted, and the average number of seeds per capsule was calculated by dividing the total number of seed per catkin by the number of capsules for that catkin. Two randomly selected 50-seed lots were counted, put in separate vials, and stored in a refrigerator at 35 to 40 degrees F for later use in the germination study.

On May 12, 1971, mature catkins were taken from five of the ortets. The other ortets had previously dispersed part or all of their seed, making it impossible to collect complete catkins. These open pollinated catkins were processed in the same manner as those from the greenhouse croses. The capsules on five catkins from each tree were counted. Two randomly selected 50-seed lots were taken from each tree and refrigerated at 35 to 40 degrees F for later use in the germination test.

A randomized block design with two replicates was used for testing germination. A double germination chamber calibrated for a 14 hour light period and a 10 hour dark period at 85 and 65 degrees F respectively was used during the test. One 50-seed lot was randomly placed in each chamber (replicate). All catkins taken from the greenhouse crosses were represented in the test by 50-seed lots, as were five open-pollinated parent trees from the natural stand. The study was continued until all germination ceased, and the ungerminated seeds were checked for filled embryos by crushing the seed.

CHAPTER III

RESULTS AND DISCUSSION

Cuttings in Water

The female flowers on the cuttings which were placed in circulated distilled water developed vigorously at first, but gradually dried out resulting in only two catkins which survived to disperse seed (Figure 6). The seed dispersed only after their catkins dried out on the cutting. Shortly after the catkins were fully expanded, the leaf buds opened and the cuttings leafed out. After the catkins died, the cuttings stayed alive until the experiment was terminated in mid-June. None of the cuttings placed in water developed root systems.

The water remained clean during the entire experiment, with no evidence of development of fungi or algae. About one-half gallon of distilled water per day was added to the system because of plant use and evaporation.

Cuttings in Sand and Peat Mixture

As shown in Figure 6, the cuttings in the sand and peat mixture maintained a much higher percentage of living catkins than those in distilled water. Most of the flowers appeared healthy during development and through pollination. The few individual flowers which died, did so periodically from pollination to maturity for no apparent reason.

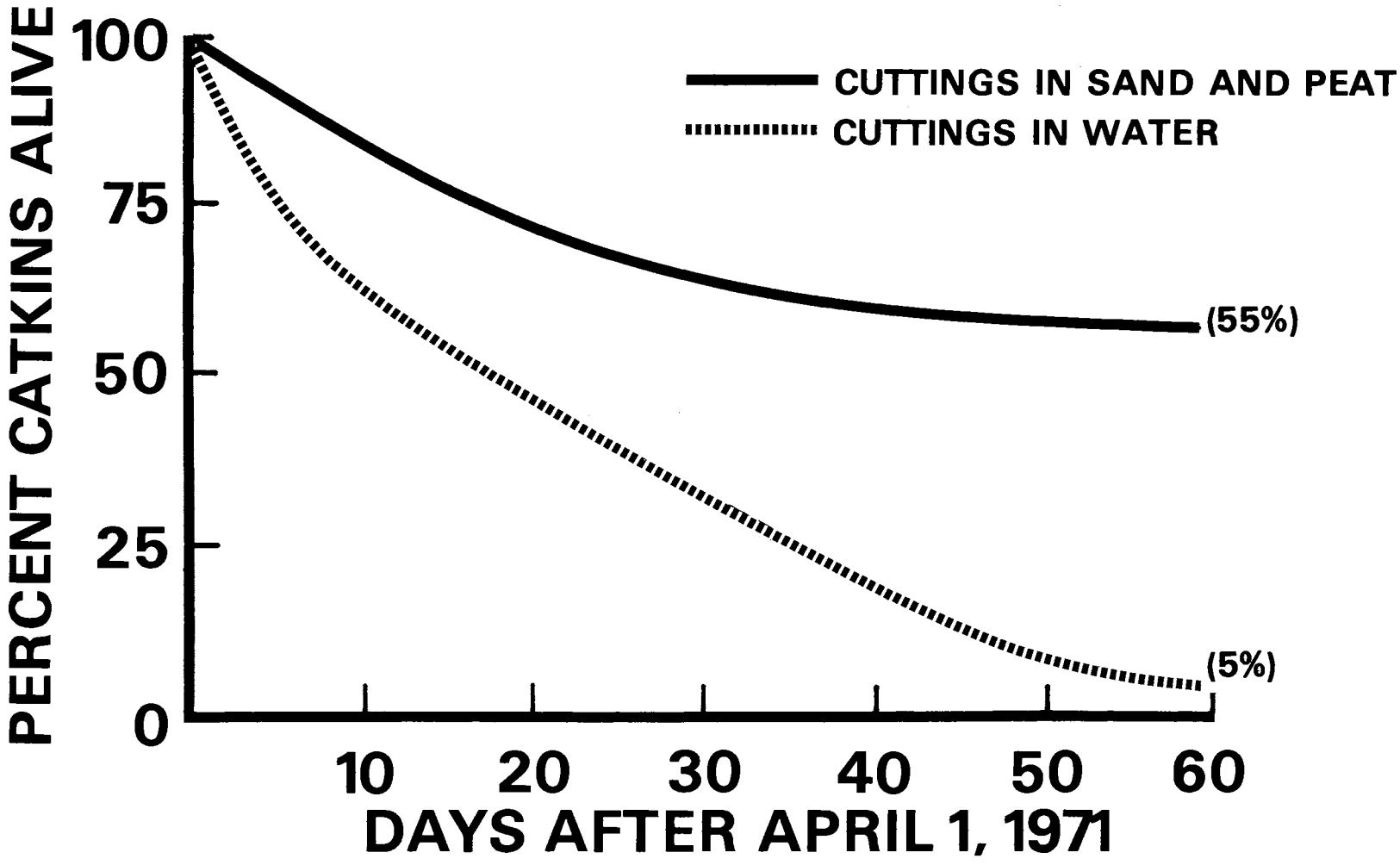

Figure 6. Comparison of Survival of Catkins for the Two Culture Methods. The Last Points on the Curves Represent the Total Percent Catkins Which Were Harvested

The cuttings apparently rooted at the time of flowering, as roots were observed protruding from the bottom of the cans shortly after the cuttings leafed out (a few days after pollination). New leaf, shoot, and root growth continued virogously until the experiment was terminated in Mid-June. Figure 7 shows a cutting with leaves and roots shortly after the catkin was harvested. All cuttings containing flower buds rooted. These results differed from those of Farmer (7), who found the use of indolebutyric acid essential, and the removal of flower buds as desirable to promote good rooting.

The Time of Flowering and Seed Dispersal

Figure 8 shows the time of flowering and seed dispersal for the greenhouse cuttings and the ortets in the field. Similar field observations were made by Farmer (5), however the natural sequence started two weeks earlier in the lower Mississippi valley.

The female buds in the greenhouse were forced before the flowers in the field opened naturally to prevent contamination from outside pollen. Male flowers were forced before the females in order that a sufficient amount of time to collect pollen would be assured before the females were forced. A similar technique was used by Farmer (8) when making controlled crosses in the greenhouse.

In the field, the first clones to initiate flowering were males (Figure 8). The flowering for both sexes lasted about one month with most of the variation being between trees and very little within trees as the flowering for an entire tree took only 3 to 5 days. Similar observations were made by Farmer (5) when studying natural variation in flowering time in the lower Mississippi valley.

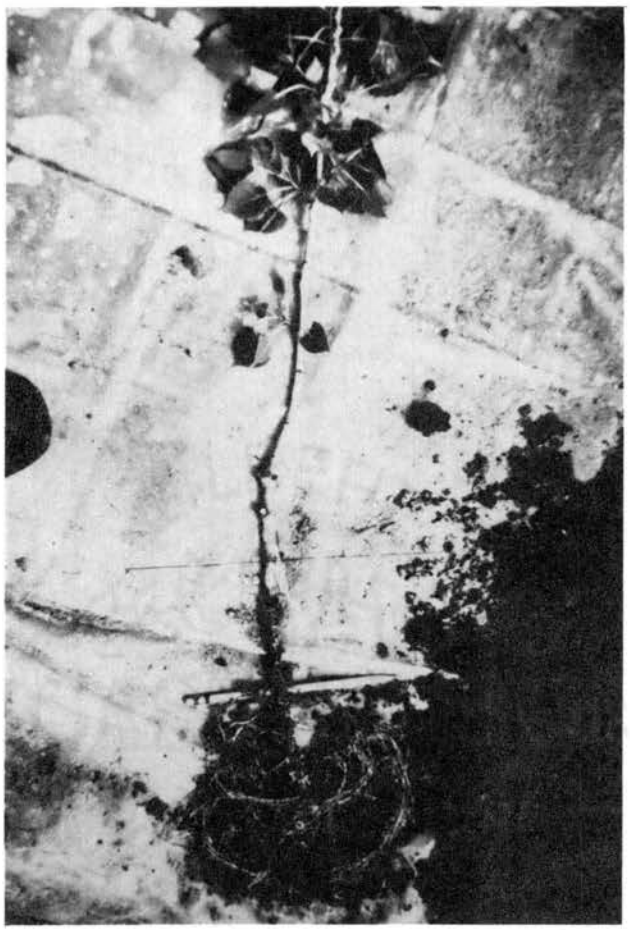

Figure 7. A Cutting With Leaves and Roots Shortly After a Single Catkin Was Harvested

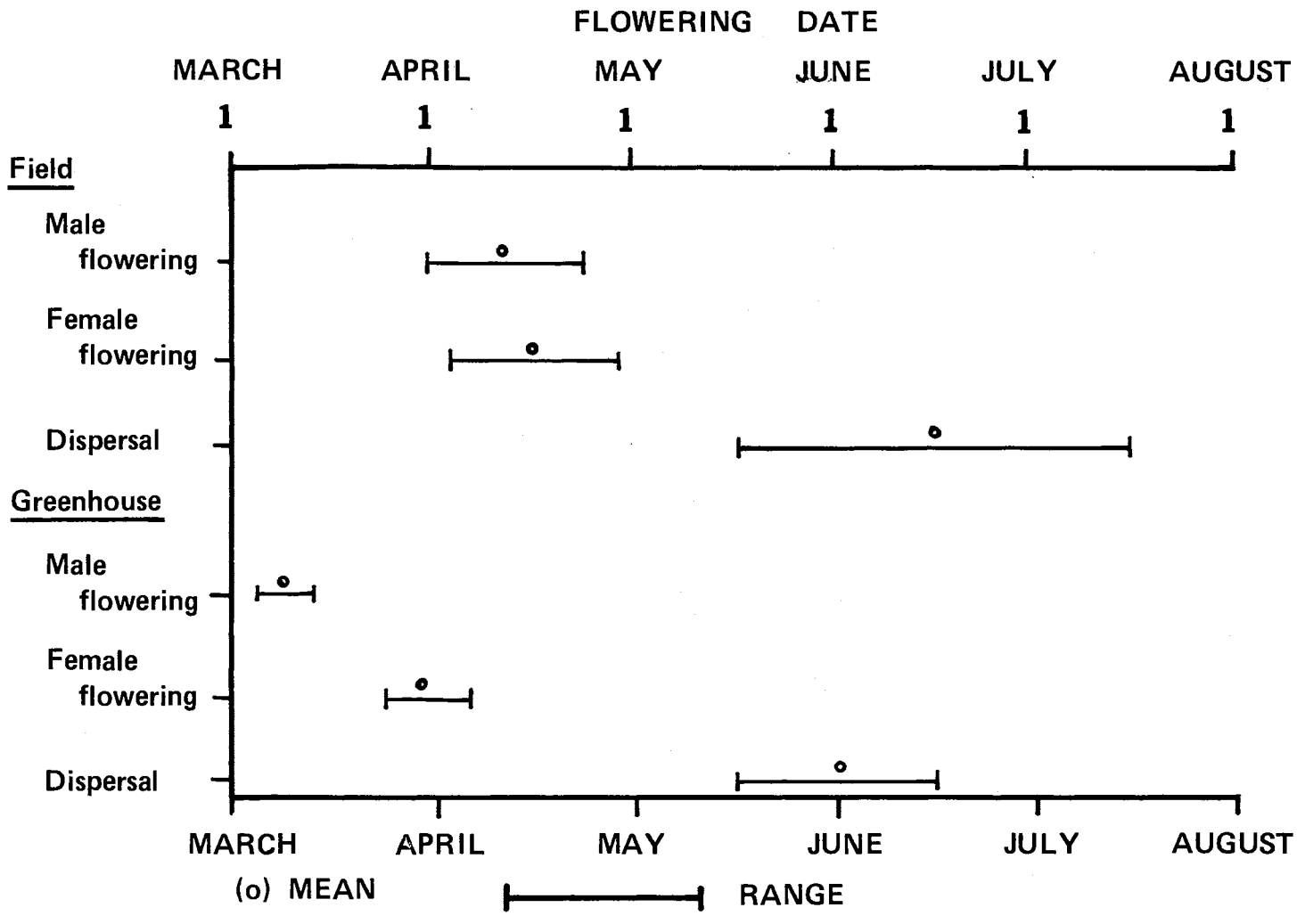

Figure 8. Time of Flowering and Seed Dispersal (Mean and Range) for the Greenhouse Cuttings and the Ortets in the Field

Catkin Development

Two days after pollination, the gynoecia started to enlarge, separating the stigmas from the remaining female flower parts. After two weeks, the capsules had reached their full diameter. Figure 9 illustrates two catkins grown in the sand and peat mixture, as they appeared three weeks after pollination.

The mortality of pollinated catkins in the sand and peat mixture and the distilled water was 45 and 95 percent respectively (Figure 6). This difference could be the result of the ability of the cuttings in the sand and peat mixture to carry on photosynthesis because of the development of leaves and root systems, while the cuttings in water had only stored food to support the growing catkin. There were no indications of nutrient deficiencies from observations of the leaves uf cuttings in water. It is possible that a floral preservative containing sugar might have improved the supply of food for the cuttings in water. The mortality of the catkins in this study was higher than the 27 percent reported by Farmer (8) using the grafted scion technique.

Seed Dispersal and Processing

Two catkins were collected on May 20, 1971 from the flowers pollinated on cuttings in water. These catkins, which contained immature seeds, were the last catkins left on the cuttings in that group. All other catkins died before their seeds were mature enough for their capsules to dehisce.

The capsules started to dehisce on catkins from cuttings in the sand and peat mixture on May 25, 1971. The capsules on the apex of the catkins opened first and the others dehisced basipetally.

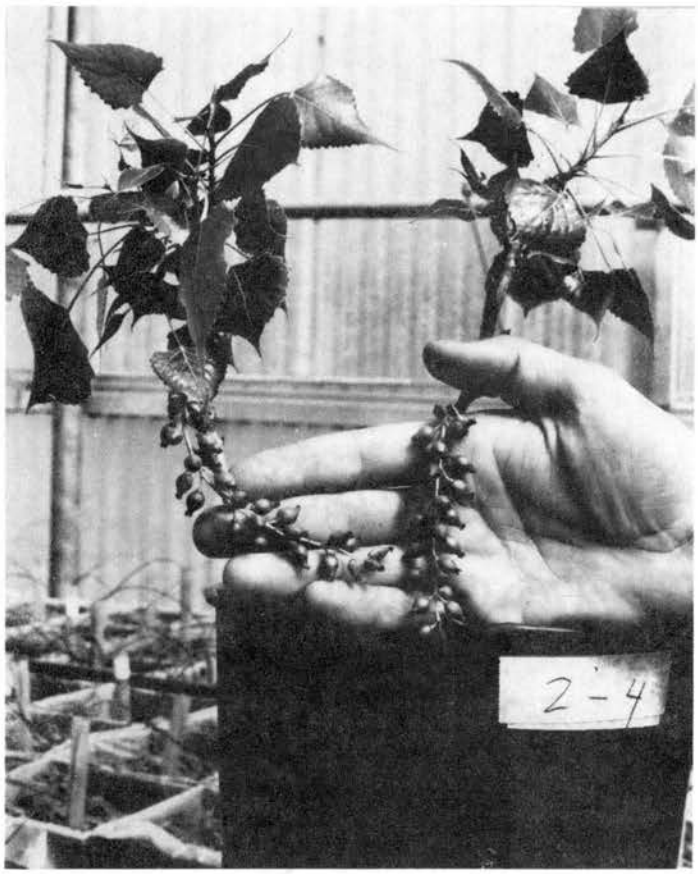

Figure 9. These Catkins Are Shown Three Weeks After Pollination. The Cutting Was in a Sand and Peat Mixture.

To prevent loss of seed, the entire catkin was removed from the cutting as soon as the first capsule opened. For this study, it was assumed that all the seeds in a catkin were mature when the first capsule dehisced. This method was used to collect as much seed as possible for an accurate seed count. It is possible, however, that all the seeds in a catkin were not mature when the first capsule opened. The seed dispersal lasted about three weeks (Figure 8) in the greenhouse, until all catkins had matured.

The seed dispersal started in the field about the same time as in the greenhouse and lasted much longer (Figure 8). In Mississippi, Farmer (8) reported greenhouse dispersal lasting from May thru late July, the same time as in the field. Farmer's parent trees must have represented the entire range of variation in seed dispersal for that region, whereas the trees in this study represented only a small segment of the variation in seed dispersal for northern Oklahoma.

The seed processing, as previously described in the Materials and Methods section, worked fairly well. The only problem encountered was in drying the catkins. One week in the drying envelope was necessary for the catkin to dry sufficiently for the capsules to dehisce. This was not desirable because the seeds within the capsules which opened first could dry out before the remaining capsules have opened. Cottonwood seeds have a very thin seed coat (11). Under the low humidity of an air-conditioned building, the seeds could dry out, causing poor germination or death. A more desirable collection method would be to break the capsules open immediately after the catkin is picked. The "cotton" could then be extracted using forceps, left to dry for not more than one day, and then separated from the fiber and stored

immediately. This would prevent any seeds from drying longer than one day.

Comparisons of the number of capsules per catkin, number of seeds per capsule, and total number of seeds per catkin were made between the greenhouse pollinated progeny and the open pollinated progeny from the ortets, using paired "t" tests. The results of these tests are shown in Table I. In every comparison the over-all mean for the greenhouse progeny is larger than that for the open pollinated progeny. The difference in the number of capsules per catkin was significant at the .10 level. The difference in the number of seed per capsule was not significant, while the difference in the total number of seeds per catkin was significant at the .01 level. The total number of seeds per catkin was calculated by multiplying the mean number of capsules per catkin by the mean number of seeds per capsule, for each clone.

TABLE I

THE RESULTS OF PAIRED "t" TESTS COMPARING CLONAL MEANS OF OPEN AND GREENHOUSE-POLLINATED PROGENY ON THE BASIS OF NUMBERS OF CAPSULES PER CATKIN, NUMBERS OF SEED PER CAPSULE, AND THE TOTAL NUMBER OF SEED PER CATKIN

Item	Clone Number					Mean	Difference	Calculated "t"	Significance Level (α)
	2	5	10	11	16				
Number of capsules per catkin									
open*	13	18	21	17	17	17.2	- 5.4	- 2.7386	.10
greenhouse** . .	25	26	20	20	22	22.6			
Number of seed per capsule									
open	20	26	24	20	18	21.6	- 0.2	- 0.0634	N.S.
greenhouse . . .	21	20	16	22	30	21.8			
Total number of seeds per catkin									
open	260	468	504	340	306	375.6	-117.4	-14.3171	.001
greenhouse . . .	525	520	320	440	660	493			

*The mean for the open-pollinated clones was based on five catkins.

**All catkins collected from each greenhouse-pollinated clone (the number ranged from 3 to 5) were counted to obtain the clonal means.

Seed Germination Test

Two days after the initiation of the germination test, nearly all of the live seeds had germinated. The remaining seeds were left in the germinator for one week, then crushed to check for filled embryos. None of the ungerminated seeds contained filled embryos. Table II shows the average germination percent of each tree, for both open and greenhouse-pollinated progeny. Similar results were shown by Farmer (8), who reported a range of 14 to 88 percent, with a mean of 48 percent.

TABLE II

THE MEAN GERMINATION PERCENTAGE OF EACH CLONE FOR BOTH OPEN AND GREENHOUSE-POLLINATED SEED

Clone*	Greenhouse	Open
1	45.75	--
2	25.25	18.00
5	8.62	1.00
10	57.16	52.50
11	92.25	35.00
12	46.50	--
13	90.50	--
14	49.30	--
16	67.67	92.50

*Clone 15 is ommitted since leaf buds were mistaken for flower buds on all cuttings from this clone.

Two analyses of variance were made with these data. Included in the first analysis were clonal means and germination percentages from all ramets included in the greenhouse progeny. The second analysis used clonal mean germination percentages for the open-pollinated progeny as well as greenhouse-pollinated progeny, including only the clones that were in common. The arc-sin transformation was made on all data. The first analysis is shown in Table III. The "F" tests show the difference between clones significant at the .05 level, and the difference in ramets within a clone significant at the .001 level.

TABLE III

ANALYSIS OF VARIANCE FOR THE ARCSINE OF THE GERMINATION PERCENTAGE BY REPLICATIONS, CLONES, REPS X CLONES AND RAMETS IN CLONES FOR GREENHOUSE PROGENY ONLY

Source	d.f.	S.S.	M.S.	Significance Level (α)
Total	55	33,291.684		
Replications	1	6.71		
Clones	8	18,886.08	2,360.76	.05
Reps x clines	8	475.00	59.375	N.S.
Ramets in clones	19	12,890.99	678.473	.001
Error	19	1,032.854	54.36	

The results from the second analysis are shown in Table IV. The "F" tests show the difference between clones significant at the .05 level, and the interaction between clones and technique significant at the .05 level. These results indicated no significant difference in germination percentage between the seed produced by controlled pollination in the greenhouse, and the seed produced by natural pollination in the field.

TABLE IV

ANALYSIS OF VARIANCE FOR THE ARCSINE OF THE GERMINATION PERCENTAGE BY REPLICATIONS, CLONES, TECHNIQUES, AND CLONES X TECHNIQUES

Source	d.f.	S.S.	M.S.	Significance Level (α)
Total	19	8,936.383		
Replications	1	0.3057		
Clones	4	6,664.211	1,666.053	.05
Technique*	1	643.440	643.440	N.S.
Clones x technique	4	1,189.013	297.253	.05
Error	9	439.383	48.820	

*Open vs. greenhouse pollinations.

The significant difference between clones which was seen in the first analysis is still evident, however. The significant interaction between clones and techniques may be explained by referring to Table II, page 24. The seed produced in the greenhouse had a higher germination percentage than the seed produced in the field for every clone except clone 16. The inconsistency in the reaction of this clone to different environments apparently resulted in a significant interaction. An interaction, if large enough, could mean that some clones may be difficult to cross in the greenhouse because at some point in the crossing procedure they may not be compatible with the technique being used. The temperature and humidity sensitivity of some clones may cause them to react unfavorably to the greenhouse crossing technique.

CHAPTER IV

SUMMARY AND CONCLUSIONS

This investigation was designed to develop and evaluate two methods of obtaining cottonwood control-pollinated progeny in the greenhouse.

The method which involved placing cuttings with flower buds in circulated distilled water was not satisfactory. There apparently was not enough stored food to maintain a catkin containing twenty or more capsules to maturity. This method could be satisfactory for a cutting containing male flower buds, because as early as two days after flowering the pollen would be mature. It is possible that a floral preservative containing sugar might have improved the supply of food for the catkin.

The cuttings placed in a peat and sand mixture did quite well. Over fifty percent of the catkins lived to maturity, with at least two catkins harvested from each cross combination.

When comparing catkins produced by greenhouse controlled crossing and open pollination in the field, those produced in the greenhouse had significantly more capsules per catkin and significantly more total seeds per catkin. There was no significant difference in the number of seeds per capsule between the two methods.

The germination test showed that there was a significant difference in seed germination percentage between clones, and between ramets

within a clone, but no significant difference in seed germination between the field and the greenhouse.

In summary, therefore, this procedure can be an effective method to cross-pollinate and grow cottonwood catkins to maturity in the greenhouse. The method is also much simpler to implement than controlled-crossing in the natural stand.

Many questions need to be answered in order to simplify and increase the usefulness of this greenhouse-crossing technique.

1. How long can cuttings be stored without impairing the ability of the flower buds to open?
2. How long can pollen be stored without loss of viability and how can it be easily tested?
3. What is a usable technique for isolating female cuttings so that many cross combinations can be made in the same greenhouse at the same time?
4. If effective storage techniques are developed, how many successful crossing sequences could be made in a greenhouse in one year using the same equipment (chambers, pots, etc.) for each sequence?
5. What technique is most desirable for collecting, processing, and storage of seed such that optimum germination percentages can be obtained?

These questions must be answered before the optimum utilization of this technique can be realized.

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