TECHNIQUE OF MASS PRODUCING JACKII POPLAR SEED UNDER GREENHOUSE CONDITIONS

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INTRODUCTION

Jackii poplar (Populus Jackii) as we recognize it in nature is a hybrid resulting from a cross between an eastern cottonwood mother (Populus deltoides Marsh.) and a balsam poplar father (Populus balsamifera L.). The resulting F_1 progeny of this cross has the form of a cottonwood with slightly ribbed shoots, oval petioles, pink main vein, heart-shaped leaves and elongated buds. These modified cottonwood features appear to be determined by the dominant genes of the female cottonwood. In the reciprocal cross of these two species, the female balsam poplar asserts the dominant role as the F1 progeny more closely resemble the balsam poplar mother than the eastern cottonwood father having such balsam poplar features as form of a balsam poplar with rounded stems and petiole, ovate to almost lanceolate leaves, green main vein and elongated buds. However, when Jackii are back crossed with deltoides then the deltoides genes appear dominant whereas when Jackii is back crossed with balsam poplar, the balsam poplar features appear to prevail. Lastly, when Jackii is crossed with Jackii, balsam poplar features appear to be somewhat more pronounced than the eastern cottonwood characteristics.

Many of the Fl progeny of the deltoides mother x balsamifera father appear to inherit such desirable forestry features as hybrid vigor, apical dominance, white heartwood, frost hardiness, site adaptability, high rooting capacity and are rarely damaged by mice, rabbits and deer. Because of these valuable traits, and because this hybrid will grow at least 100 miles north of the eastern cottonwood range, it is now being recommended for planting in our bottomland reforestation program in preference to pure eastern cottonwood. In addition it has the added advantage of originating from parents which are already programed for a wide range of conditions in southern Ontario.

Since this hybrid rarely occurs in nature, it was decided to try and mass produce this tree on a commercial basis by the controlled crossing of plus-tree parents of both species on severed branches rather than by the limited method of producing seed from bench grafted stock and at the same time to select from such large populations any outstanding seedlings that might occur for future propagation and reforestation purposes. For instance a healthy eight-foot (2.4 m) branch is capable of producing from 50,000 to 66,000 seedlings based on 100 seed catkins per branch consisting of from 33 to 44 capsules per catkin of which each capsule contains 20 seeds.

This program was started in the spring of 1973 and has been evolving ever since as new information comes to light.

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METHODS AND MATERIALS

Parent Trees.--The breeding stock used in these trials have consisted of two female and one male Populus deltoides with dbh's of 26" (66 cm), 32" (81 cm), 34" (86 cm), respectively and a height of almost 100' (30 m) which were crossed with one male and one female Populus balsamifera, each with a dbh of about 26" (66 cm) and a height of about 90' (27 m). Both species were also crossed with a female and two male Populus Jackii with a dbh of 6" (15 cm), 18" (45 cm), and 20" (51 cm) and a height of 40' (12.2 m), 80' (24 m), and 85' (26 m), respectively.

This series of crosses produced ten hybrids rather than sixteen as neither of the eastern cottonwood nor balsam poplar selections were crossed intraspecifically (Table 1) and no viable seed was produced from the remaining three potential crosses,

Table 1.--Showing crosses made between P. deltoides, P. balsamifera and P. Jackii which produced viable seed.

Female \ Male	D 34	B 6	J 7	J 13
D 35	-	D x B	D x J	-
D 38	-	D x B	-	D x J
B 5	B x D	-	B x J	B x J
J 16	J x D	J x B	J x J	-

Legend:

D 34 - P. deltoides, tree #34
D 35 - " " " #35
D 38 - " " " #38
B 5 - P. balsamifera " #5
B 6 - " " " #6
J 16 - P. Jackii " #16
J 7 - " " " #7
J 13 - " " " #13

Flower Branches.--Male and female branches of all three species were used for the production of pollen and seed respectively. The criterion for the selection of the branches on the dormant plus trees is based on their size, position on tree and on the abundance of flower buds. The selected branches are then shot down from the plus trees with a high velocity 225 Savage rifle. The male branches should be brought into the greenhouse in the first week of February, one week before the female branches to ensure that there will be an abundance of pollen when the female flowers become receptive.

To induce rapid flowering, the fresh branches are brought into the greenhouse where the temperature is held as closely as possible to 70°F and the relative humidity at 40 percent or more. Each branch is placed in fresh water which is changed once every two days and the bottom one to two inches of each branch is cut off using pruners once every four days. This is to ensure that the conducting tissue of each branch is not blocked with bacteria or algae preventing the free entrance of fresh water into the branch, slime forms on the immersed portion of the branch it is wiped off at the time of changing the water. The branches are also examined for insects and if any are observed in the egg, larvae, purpal or adult stages, they are sprayed with an appropriate spray. For instance many of the branches are often partially covered with colonies of oyster shell scale which can be easily eliminated with an emulsive mineral oil. Again, just before the female branches come into flower they are automatically sprayed with Malathion to eliminate a Lepidoptera caterpillar which is generally present and which otherwise will destroy the developing flowers and ultimately the seed.

Collection of Pollen.--The male flowers of deltoides, balsamifera and Jackii generally begin to shed their pollen at about 14, 10 and 11 days respectively from the time they are put in the greenhouse. The pollen of each species is collected by placing the dehising flowers into covered paper trays and shaking them carefully to remove the free pollen. The unclean pollen is then placed on smooth writing paper where it is cleaned by simply tilting the paper at a slight angle and tapping it with a finger causing the lighter floral portions of the flower to slide off the paper leaving the pollen behind. The cleaned pollen is then placed in numbered glass vials, corked with absorbent cotton and put in a dessicator in the freezing portion of a refrigerator.

Breeding Procedure.--Prior to pollination, each female branch is separated with black painted bands into sections with about the same number of floral catkins in each. The compartments on each branch are identified by painting the name of the male tree which will be used to pollinate the receptive flowers in each of the sections.

The Populus balsamifera and Populus Jackii flowers take about 14 days in the greenhouse to become receptive whereas the Populus deltoides flowers take from 19 to 24 days.

The act of pollination simply consists of brushing the appropriate pollen on to the receptive flowers in each of the designated sections. This procedure is repeated once a day for three days to ensure maximum fertilizations. To prevent the pollen from one cross contaminating the unfertilized flowers of another cross, each section is separated from the adjoining sections with thin plastic sheets. They remain in situ until fertilization is completed.

Seed Branches.--Immediately following pollination every effort is made to not only simulate nature but to create artificial conditions which will speed up the maturation of the seed. This is accomplished by maintaining the temperature differential of 70 ° F in the daytime and 65 ° F at night and

particularly by controlling the lighting so that there are two simulated nights and two simulated days in every 24-hour period. For instance normal darkness is permitted from 5 p,m. to 10 p.m, then the lights are automatically turned on from 10 p.m. to 2 a.m, which is then followed by normal darkness and remaining so until daylight. The establishment of two dark and two light periods in every 24-hour day was done on the basis that this technique would reduce the time of seed maturation from nine weeks to four or five weeks. This time reduction was considered essential as it is doubtful if a severed branch could survive nine weeks under the artificial conditions so described,

Studies in 1973, 74, and 75 have indicated that a completely healthy cottonwood or Jackii branch of from eight to ten feet high can produce viable seed within five to six weeks after pollination providing it is exposed to an intermittent mist. However, at the same time a balsam poplar branch of the same dimensions can produce viable seed within four weeks time without misting. In fact misting appears to be detrimental as the catkins and shoots shrivel before the seed is mature. Dr. B. Dance identified one bacteria and two fungii on the diseased shoots. However, it was not determined whether they were causal agents. Dr. Carpenter, of the University of Guelph analysed the bacteria and found it was sensitive to terramycin, auromycin, and streptomycin. He felt that any one of these three bacteriacides might be used to effectively eliminate this bacteria. Dr, B, Dance suggested that Benomyl be used against the two fungii.

Harvesting the Seed.--The seed catkins are collected when the seed capsules start to dehise. They are placed in small covered paper boxes and allowed to open fully. Those that do not fully open are manually opened. The fluffy seed is then placed between two fine soil screens and is subjected to blasts of air which separate the fluff from the seed. The seed is then collected at the bottom of the tray, put in vials which are then labelled, placed in a dessicator and stored in the freezing unit of the refrigerator until required for sowing.

Sowing the Seed.--All seed lots are given a limited germination test prior to being sown directly in tubes or in flats in the greenhouse. They germinate in 2 to 4 days. The resulting seedlings are kept moist and are fertilized with RX15 once every two weeks. Two weeks before transplanting, the young seedlings are hardened off in a coldframe.

Transplanting.--The tubed or free seedlings when they are from one inch to four inches (2.5 cm to 10.1 cm) in height are transplanted six inches (15 cm) apart and three feet (0.8 m) between the nursery rows preferably between the last two weeks of May and the first week of June. They are kept moist and fertilized with a complete fertilizer once every two weeks until the middle of July. Most of the seedlings in 1973 were transplanted to the nursery in the third and fourth week of May and by September over 90 percent of the seedlings were from 3 feet to 6 feet (0.9 m - 1.5 m) in height. However, the 1974 Jackii progeny were not only smaller at time of transplanting but also they were not transplanted into the nursery until the first week of June and for these two reasons they only attained half the height of the 1973 seedlings. This difference

in height permitted the 1973 progeny to be outplanted in the spring of 1974 whereas the 1974 seedlings will require two growing seasons and will not be planted until the spring of 1976.

RESULTS

Experimental Tests and Results for 1973.--Male and female branches of P. balsamifera and P, deltoides ranging from 8 feet to 10 feet (2.4 m to 3.1m in height were brought into the greenhouse on the 14 of February. The male branches of each species were put into water and produced pollen in 10 to 12 days time. The female branches of the aforementioned species were also put in water under mist. They became receptive in 8 to 16 days respectively producing immature but viable seed in 32 days after pollination which is from 40 to 48 days respectively from the start of the test. It was noted at the time that over 90 percent of the seed capsules of both species shriveled up within 18 days of pollination and produced no seed at all.

The viable seed was immediately treated, sown in trays on 26 March, followed by germination within two to four days. On the 15 May, the seedlings were put in a cold frame where they remained until they reached a height of from two to four inches (5 cm to 10 cm) and were transplanted into the nursery between the 23 May and the 6 June, There was excellent survival and growth in the nursery. A September tally indicated 90 percent survival giving a total of 1100 seedlings of the deltoides mother x balsamifera father cross. Over 96 percent of these were between 3 feet and 6 feet (0.9 m and 1.8 m) tall. However, the reciprocal cross of these two species produced only 39 seedlings which were uniform in size but only averaging 2,2 feet (0.7 m) in height. The leaves of these seedlings in the first growing season were very narrow resembling willow leaves.

Experimental Tests and Results for 1974.--The program was greatly expanded in 1974 by reciprocally crossing Populus deltoides, Populus balsamifera and Populus Jackii hybrids. Branches were brought into the greenhouse between the 15 and 21 February. Surplus branches of each selection were stored in the cold room at 1 ° celsius for later breeding studies.

The male branches from one balsamifera, two Jackii and one deltoides produced pollen in 8, 10, 11, and 14 days respectively from the time they were put in the greenhouse. The flowers of the female branches of one balsamifera, one Jackii and two deltoides were receptive on 13, 13, 20, and 24 days respectively from the time they were brought into the greenhouse.

Immature but viable seed from the balsam poplar, the Jackii and one of the deltoides was collected in 35 days, and the other deltoides in 43 days after pollination.

A series of indicator tests were run in conjunction with seed production namely the effects of misting and not misting, of spraying and not spraying anti dessicants, fertilizing and not fertilizing the water with a complete fertilizer and evaluating water absorption by removing and not removing a 4 to 6 inch (3.6 cm to 4.1 cm) strip of bark from the basal portion of the treated branches on seed production.

Although the seed branches were generally seriously decimated by either some physiological disorder or by an unidentifiable bacteria and two fungii, or by all three factors, a sufficient number of viable seed was produced from each of the seven crosses (Table 2) to be able to conduct an analysis of some of the genetical features characterising each of the crosses,

The 1974 seedlings were planted too late that year for outplanting in 1975 and consequently will not be outplanted until the spring of 1976.

Table 2.--The number of Jackii seedlings transplanted from each cross into the nursery and their survival up to the end of the first growing season.

Cross	June (1974)	September (1974)
D35 x B 6	374	297
D38 x J13	182	162
B 5 x J13	232	210
B 5 x J 7	8	8
J16 x D34	84	75
J16 x B 6	3	1
J16 x J 7	35	24
TOTAL:	918	777

Experimental Tests and Results for 1975.--The program was reduced somewhat in 1975 by concentrating the tests on what appeared to be the most important variables effecting seed production namely disease control and size of seed branch.

This year the male branches of balsamifera, Jackii and deltoides were brought into the greenhouse on the 21 February approximately one week before the female branches of balsamifera and deltoides. They produced pollen in 8, 11, and 14 days respectively. The pollen was stored in vials in a deep freeze at a temperature of -10 celsius where it remained until required for pollination.

Two lots of female branches were brought into the greenhouse at two different periods. The first lot consisted of large branches of one balsamifera and two deltoides timber selections ranging in height from 8 feet to 10 feet (2.5 m to 3.1 m). These branches were subdivided into breeding sections and 15 flower buds on each branch were marked with ribbon to indicate which flower spurs were to be sprayed with a bactericide (Terramycin), a fungicied (Benomyl) and with a mixture of both chemicals. These were applied in spray form once very five days during the entire develomment of the flower and seed. Results indicated that both chemicals were ineffective at the rates applied as all flower catkins on both deltoides selections wilted and fell off. Even the leaves of both selections were only partially developed by the end of the test. However, the balsamifera branch regardless of whether it was

sprayed or not with biocides developed normal flowers and seed catkins producing viable seed within 23 days after pollination, These good results were accomplished without using a mist or any other treatment.

It was also noted that the leaves of this species developed normally following pollination being one quarter developed within 17 days and fully developed in 30 days after being brought into the greenhouse. The branch even started to develop roots. The flower buds of the two deltoides females started to open in 7 days and were receptive in 11 and 14 days respectively, However, all catkins shriveled on the large branches within 18 days of pollination regardless of whether they had been treated or not with a biocide. Furthermore, the leaf buds did not develop or if they did they only partially unfurled.

The second lot of branches consisting of both large branches from 8 feet to 10 feet (2.6 m to 3.1 m) and small branches from 3 feet to 5 feet (0.9 m to 1.5 m) from one deltoides selection only were brought into the greenhouse on the 15 April. Half of the large and half of the small branches were put in water and the other half in wet sand. The large branches produced seed catkins in both the water and in sand containers and again they shriveled up within 18 days of pollination. The leaf buds again did not react normally but only partially opened. The small branches on the other hand produced normal seed catkins and leaves in that sequence, However, again the capsules did not develop on those in the water but one branch in sand which had rooted within three weeks, produced two normal seed catkins containing mature viable seed which was threshed 31 days after pollination.

CONCLUSIONS

Initial results of the assorted tests conducted since 1973 have indicated the great possibility of mass producing superior seedlings from selected strains of Jackii poplar by the controlled crossing of plus trees using the severed branches of deltoides mothers x balsamifera fathers under greenhouse conditions.

Observations indicated that the best seed production occurred on those branches which developed normally and remained healthy under greenhouse conditions, i.e., the normal enlargement of the seed capsules and the unfurling of the leaves following pollination and finally the development of minute adventitious roots within three weeks of pollination.

Therefore, better results may be expected if branches are pre-rooted prior to pollination and if all adverse physiological, entomological, and pathological factors are eliminated during seed development. This may well be accomplished by pre-rooting the seed branches and the application of the appropriate biocides in conjunction with a temperature ranging from 65 to 70 F, two simulated nights and two simulated days every 24-hour period at a relative humidity of not less than 40 percent.

Jackii poplar plus tree.