DEVELOPMENT OF SEXUAL AND VEGETATIVE ORGANS ON DETACHED FOREST TREE BRANCHES CULTURED IN THE GREENHOUSE¹

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Experiments on the relation of nutrient solutions to development of seeds on detached branches of *Populus grandidentata* showed distilled water to be superior to solutions of Hoagland's, sucrose, or Hoagland's combined with sucrose, or to a peat humus infusion.

Greenhouse conditions simulating outdoor conditions in mid-May (temperature varying from 50°F. at midnight to 70°F. at noon, 15 hours of light, and about 60% relative humidity) proved highly favourable to the development of flowers and seeds on detached branches of *Populus* and other forest trees cultured in water.

Data are presented indicating the time at which various species of forest trees pass from a state of true dormancy to one of imposed dormancy (imposed by the unfavourable winter environment). For most species it appeared that this transition occurred between the middle of November and the first of December.

Greenhouse phenological records showed that the time required for flower development decreases progressively from the onset of imposed dormancy.

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INTRODUCTION

In 1924 when Stout, McKee, and Schreiner commenced, in *Populus*, the first comprehensive hybridization work within a forest tree genus, it was necessary to climb high into large trees or to work on roof tops in order to effect interspecific cross pollination. (6). In 1935 von Wettstein-Westsheim (7) published details of a technique by which flower-bearing branches cut from male and female poplars of the desired species were cultured in the greenhouse through stages necessary for cross pollination to seed maturity on the part of the maternal parent. The culture solution was tap water. The following year Al'benskii and Delitsina (1) reported successful results in *Populus* using a similar technique, but with Knopp's solution as the culture medium. When interspecific hybridization in *Populus* was undertaken on a comprehensive scale by the author in 1938 it soon became apparent that there was a need for greater knowledge of some of the phenomena relating to the "detached branch" method of hybridization. The information sought included especially the following:

1. A means of increasing the efficiency of the method in terms of final yield of mature seed, which was very low (even in intraspecific crossing) in a number of species, notably *P. grandidentata* and *P. tremuloides*. The logical approach was considered to be (a) augmentation of stored nutrients of the branch by nutrient culture solutions; and (b) the provision of optimum light, temperature and humidity conditions for the developing flowers and seeds.

2. An indication of the earliest time during winter dormancy at which proper development could be obtained in detached branches, in order that the greenhouse crossing season could be extended to a maximum. In designing studies on this problem it was considered worthwhile to include a wide range of forest tree materials to determine: (a) which species could be carried to the length of maturing seed in the greenhouse, i.e., would be adaptable to the method; and (b) which species would produce viable pollen on detached branches, and at what time during the dormancy period.

3. The compilation of a phenological calendar for the species being used in the hybridization program, especially in *Populus*, which would show the period of time required under greenhouse conditions to produce pollen or stigmatic receptivity by flowering branches of different species collected at different times during the dormant period.

The present paper reports the results of the studies undertaken to obtain information on the problems stated above.

GENERAL METHODS

The procedures used in obtaining deliberately imposed experimental conditions are described in the pertinent sections following. But it is useful here to describe the standard procedure generally followed in the practical breeding work—almost entirely in *Populus*—which forms the basis upon which experimental conditions were built.

Flower-bud branches were cut from the trees, usually as large branches, brought immediately to the greenhouse, trimmed to smaller, straighter branches usually two to three feet long, and placed in groups of three or four in two-quart jars filled with tap water—the jars being flushed every few days by water pressure from a hose. This procedure was standard for all experiments, except for obvious departures in the culture solution and size-of-branch experiments in Sections 1 and 3 respectively.

During the first four winters of greenhouse hybridization, temperatures were maintained at either 60° or 70°F. (the lower proving the better), relative humidity was held at approximately 50% through the agency of moist peat on the floor, and illumination was by natural sunlight augmented on dull days by electric lighting. This procedure was standard until it was superseded in November, 1941, by that outlined in Section 3 following.

In all cases of electric lighting, one 500 watt bulb under a parabolic reflector was suspended four feet above each square yard of bench space.

EXPERIMENTAL

1. THE EFFECT OF NUTRIENT SOLUTIONS ON SEED PRODUCTION IN

Populus grandidentata

Branches having numerous flower buds were cut from a single female tree of *P. grandidentata* on 21 February, 1940, brought to the greenhouse and distributed as uniformly as possible among 28 2-quart jars containing seven nutrient solutions (considering distilled water as such) arranged in a four-replicate randomized series.

The nutrient solutions are given in Table 1. Hoagland's solution was made up according to the procedure given by Eaton (2) in which iron, boron, manganese, zinc and sodium chloride are added to the base nutrient solution. The peat-humus infusion was made by mixing 1.25% (by weight) air-dry sedge peat with distilled water. Chemical analyses of the sedge peat have been reported by Farrar and Grace (3). The pH of the infusion was 5.6. Distilled water was used in making up and maintaining levels in all solutions. All solutions were renewed on 14 March.

As female flowers became receptive they were fertilized with viable pollen of the same species. The greenhouse was accurately controlled at 60°F. and maintained at about 50% relative humidity. The experiment was terminated on 30 March.

In tabulating results on relative seed set it was found convenient to use a seed capsule index, which took into account the original number of catkins, the number of capsule-bearing catkins, and the average number of capsules per catkin which actually bore seed. It gives approximately the proportion of seeds obtained from the material cultured in each solution. The results are presented in Table 1.

It was concluded that distilled water is definitely better as a culture medium than any of the other solutions tested, and that the data do not afford any obvious leads to further studies.

Table 1. Effect of culture solutions on the development of catkins and seed capsules on detached branches of

P. grandidentata (female)

Culture Solution	Total No. of catkins ¹	Percentage of catkins elongating	No. of replicates (out of 4) producing seed capsules	Seed capsule index
Distilled water	345	96.5	4	171
1/10 Hoagland's (H/10)	320	79.4	1	12
Hoaglands (H)	420	93.6	1	12
1.0% sucrose (S)	358	88.3	3	36
H/10 + 1.0% S	373	95.4	2	32
H + 1.0% S	331	81.0	0	0
Peat-humus infusion	362	81.2	1	0

¹Total number of catkins was determined by the chance occurrences of flower buds on the selected branches, and was in no way influenced by culture solutions.

2. THE EFFECT OF "NATURAL" DIURNAL VARIATION IN LIGHT, TEMPERATURE AND HUMIDITY

The failure to demonstrate beneficial effects from nutrient solutions led to a new attack on the problem of nutrition in detached flower branches,

the governing principle being the provision of optimum environmental conditions for flower and seed development in order that the most efficient use might be made of the nutrients already stored in the branch. The criterion used as a guide to these optimum conditions was the normal outdoor conditions prevailing during the flowering and seed-setting period for *Populus* spp. (roughly, the month of May).

The temperature in the greenhouse (equipped with the Taylor temperature control system) was uniformly varied between 50°F., at midnight, and 70°F., at noon. The control was very close except occasionally where bright, warm mid-day sunshine gave temperatures up to 80°F.

The lights were automatically switched on during the hours just before sunrise providing the necessary addition to natural sunlight to give 15 hours of continuous illumination—approximately equal to the length of day during May in this latitude.

Moist peat on and under the benches gave an average relative humidity of about 60%, simulating with perhaps some exaggeration the humidity conditions of the forest in May.

This procedure has been used since November, 1941, and has, it is felt, largely overcome the more serious difficulties which attended greenhouse culture of detached flower branches from female trees of certain *Populus* species.

3. GENERAL OBSERVATION ON DETACHED BRANCHES OF *POPULUS* IN THE GREENHOUSE

During the course of the greenhouse hybridization work, differences in productivity of different types of branches, otherwise similarly treated, were noted systematically. The following points may be taken as reasonably established facts:

1. Large female branches, three to five feet long, generally were able to sustain a higher proportion of catkins to seed, maturity than smaller branches, which tended to drop developing catkins prematurely.

2. Small male branches, about two feet long, produced pollen more quickly than larger branches.

3. Branches with bright, living bark were superior to those with dull scurfy bark in sustaining developing seed capsules to seed maturity. Heimbürger has suggested (4) the use of branches from young trees.

4. TRUE AND IMPOSED DORMANCY IN FOREST TREES

A study was undertaken to determine the time at which trees of different species pass from a state of true dormancy to one of imposed dormancy, the latter being a state in which the tree is no longer truly dormant but where growth cannot start because of unfavourable environmental conditions (5). The experiment involved semi-monthly collections of branches bearing flower and leaf buds and of cutting wood (current season's growth) from 17 species of forest trees. The detached branches were cultured as outlined in Section 2 above. Results are given in Table 2.

For most of the species studied it would appear that the transition from

true to imposed dormancy occurred between the middle of November and the first of December. It is also apparent, from the calculations made on the difference in the time required for a given organ to develop in material collected at the beginning of imposed dormancy and in material collected one month later, that some form of sexual and vegetative development occurs soon after the breaking of true dormancy.

The usefulness in greenhouse hybridization of the data compiled in Table 2 is quite obvious (see Introduction). These data may also prove useful in indicating critical stages bearing on vegetative propagation experiments.

5. PHENOLOGICAL OBSERVATIONS ON DETACHED BRANCHES OF FOREST TREES IN THE GREENHOUSE.

During six "greenhouse seasons" records have been kept on the length of time required by detached branches of various species to produce mature flowers (determined by pollen dehiscence in the male, and by apparently receptive stigmas in the female). It was found that species within a genus varied considerably in the time required; and that in all species (*Populus acuminata* somewhat of an exception) the time decreased as the materials were collected successively later in the winter.

A consolidation of these records is presented in Table 3.

An outstanding feature of the data in Table 3 is the relatively constant rate of decrease in time to flowering as the collection date is advanced toward spring. Results of the preceding experiment (see Table 2) show that this decrease starts with the breaking of true dormancy and, as may be judged from Table 3, continues throughout the period of imposed dormancy. These facts indicate that a slow but constant development occurs in the flower buds during the period of imposed dormancy, which for most species extends onwards from mid-November or early December (see Table 2).

The data in Table 3 have been used extensively in planning the time schedules underlying control of coincidental flowering in the opposite sexes of different species used in greenhouse hybridization.

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TABLE 2.

Greenhouse development of leaves, flowers, roots and seeds on branches of forest trees collected at semi-monthly intervals between November 1, 1941, and February 2, 1942

SPECIES	Earliest collection capable of producing organ, and time required for organ to develop.						Reduction in the time required to develop in collection made 31 days later					
	leaf			flower†			mature anther			root		
	date coll.	no. of days	date coll.	no. of days	date coll.	no. of days	date coll.	no. of days	date coll.	no. of days	date coll.	no. of days
ACER NEGUNDO†	D16	52	D16	34	—	42	D16	60	—	—	D16	75
ACER NEGUNDO‡	D16	50	—	—	D16	42	—	—	—	—	—	—
ACER SACCARUM	J1	47	D1	50	D1	50	J16	54	—	—	—	—
BETULA LUTEA	J1	45	N16	45	J16	50	D16	60	—	—	—	—
BETULA PAPYRIFERA	D1	42	N16	35	D1	53	D1	46	—	—	—	—
FRAXINUS LUTEA†	D1	38	D1	22	—	—	D1	32	—	—	—	—
FRAXINUS LUTEA‡	D1	38	—	—	D1	37	—	—	—	—	—	—
LARIX KAEMPFERI	D1	43	N16	26	N16	20	N16	30	—	—	—	—
LARIX species	N1	40	D1	22	—	—	D1	35	—	—	—	—
PICEA ABIES	J1	43	J1	30	—	—	J1	41	—	—	—	—
PINUS STROBUS	—	—	J1	61	—	—	—	—	—	—	—	—
POPULUS GRANDIDENTATA†	D16	39	D1	30	—	—	D1	38	—	—	—	—
POPULUS GRANDIDENTATA‡	D16	37	—	—	D1	30*	—	—	—	—	—	—
POPULUS TREMULOIDES†	D16	38	N16	10	—	—	D1	32	—	—	—	—
POPULUS TREMULOIDES‡	D16	37	—	—	D1	30*	—	—	—	—	—	—
QUERCUS ALBA	D16	65	D16	65	—	—	D16	75	—	—	—	—
TILIA AMERICANA	D16	65	—	—	—	—	—	—	—	—	—	—
SALIX DAPHNOIDES†	D1	37	N16	12	—	—	D1	27	—	—	N1	34
SALIX DAPHNOIDES‡	N16	30	—	—	D1	25*	—	—	—	—	N1	30
SALIX PENTANDRA†	D1	60	D1	55	—	—	—	—	—	—	N16	36
SALIX PENTANDRA‡	D16	46	—	—	—	—	—	—	—	—	N16	40
ULMUS AMERICANA	D16	34	D1	11	D1	11*	D1	22	—	—	—	—
ULMUS PUMILA	N1	45	D1	11	D1	11*	D1	20	—	—	—	—

NOTE—N denotes November, D December, J January; the figures following denote day of month.

† denotes male

‡ denotes female

* viable seed produced.

T A B L E 3

Number of days in the greenhouse required to produce pollen dehiscence or stigmatic receptivity in detached branches of forest tree species collected during the months January to April, inclusive, 1938 to 1945.

Species	Sex	January		February		March		April	
		No. of collections	Av. No. of days	No. of Collections	Av. No. of days	No. of Collections	Av. No. of days	No. of Collections	Av. No. of days
POPULUS GRANDIDENTATA	male	2	18.0±2*	6	13.8±1	1	11	1	5
P. GRANDIDENTATA	female	2	18.5±1	3	13.7±1	1	10	1	5
P. TREMULOIDES	male	3	17.7±2	1	11	1	8	1	5
P. TREMULOIDES	female	4	13.3±2	2	8.0±1	2	8.0±0	2	6.0±1
P. TREMULOIDES AUREA	male	4	12.8±6	2	6.0±0	1	6	1	3
P. TREMULOIDES AUREA	female	2	10.5±2	2	7.5±1	1	5	2	4.0±2
P. ALBA	female	3	12.0±2	8	10.0±3	1	11	—	—
P. DELTOIDES	male	4	15.0±3	2	12.5±1	2	9.0±1	1	4
P. CANESCENS	male	3	15.0±1	4	12.0±2	1	10	1	6
XP. EUGENEI	male	2	18.0±4	1	14	1	13	1	11
P. ACUMINATA	female	1	22	1	23	2	19.5±1	—	—
SALIX DAPHNOIDES	male	1	19	1	18	1	15	1	8
S. DAPHNOIDES	female	1	17	1	17	1	13	1	7
S. PENTANDRA	male	2	28.5±2	2	30.0±2	2	15.5±1	—	—
S. PURPUREA	female	1	16	1	14	1	10	1	7
BETULA LUTEA	male	2	46.5±1	1	38	1	24	1	7
B. LUTEA	female	1	50	1	40	1	29	1	7
B. PAPYRIFERA	male	2	28.0±2	1	22	1	13	1	6
B. PAPYRIFERA	female	1	34	1	25	1	13	1	5
ULMUS AMERICANA	male	1	11	1	11	2	10.0±1	1	6
U. AMERICANA	female	1	10	1	8	1	7	1	5
U. PUMILA	male	2	8.0±0	1	7	1	5	1	5
U. PUMILA	female	2	7.5±1	1	6	1	5	1	5

* The plus or minus value when added to or subtracted from the average gives the extreme range of individual variations.