

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

A STUDY OF THE GENETIC IMPROVEMENT OF QUAKING AND BIGTOOTH ASPEN
BY SELECTION, HYBRIDIZATION, AND THE EXPLOITATION OF POLYPLOIDY

Project 2412

Report Six

A Progress Report

to

LOUIS W. AND MAUD HILL FAMILY FOUNDATION

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SUMMARY

The studies carried out as part of Project 2412, although basic in nature, are closely related to the overall goals of the Institute's Aspen Genetics and Tree Improvement Program. The results of Project 2412 investigations form the fundamental basis for the more applied aspects of the Institute's program. The broad scope and the complex nature of the genetic improvement of forest trees makes it desirable to subdivide the work into a series of well-defined interrelated studies. The following is a very brief summary of progress made during the past year on the several investigations underway.

1. Selection of exceptional trees for use as parent trees continued during the past year. Outstanding among the trees located was a bigtooth aspen of superior form and unusual rooting ability.

2. Over 500 putative triploid trees were produced by crossing local origin diploid trees with a tetraploid of Swedish origin.

3. Colchicine treatment of cottonwood and bigtooth aspen was continued with a number of putative polyploids being recovered.

4. Studies dealing with the cytology of fertilization in aspen and cottonwood are nearing completion. The results promise to establish the most desirable time for colchicine treatments.

5. Growth chamber studies comparing nutritional requirements of aspen and aspen hybrids demonstrated differences between experimental materials in

response to increasing levels of essential nutrients and differences between materials in top-root ratios.

6. Preliminary trials with a growth-retardant chemical indicate such chemicals may be useful in controlling the size and in stimulating flowering of arboretum trees.

7. Forty-five experimental crosses were attempted in 1966. Twenty-nine parent trees were employed and major emphasis was placed on the production of bigtooth aspen crosses and bigtooth aspen hybrids suitable for "dry site" plantings.

8. Estimates of heritability based upon observations made on 25 full-sib families indicate moderate to strong genetic control over fiber length, specific gravity, extractives, and lignin.

INTRODUCTION

Land use information indicates that, although the total acreage in forest is not changing greatly, the quality of land available for forest production is decreasing. Large acreages of high-quality forest land are being taken out of production by highway, urban, and industrial development. Conversion to farm land and expanded recreation use of forest lands has further reduced the forest lands available for wood production. Even though a considerable acreage of land is being returned to forest production, usually this land is of low quality (eroded, depleted, rocky, steep, etc.) and the end result is an overall decrease in productive capacity.

Forestry is also facing other challenges. Population increases, projected increases in wood and paper consumption, and the recent upswing in woods labor problems have resulted in more and more organizations looking toward the use of genetically improved species, mechanized harvesting, fertilization, irrigation, and other methods of intensive forest management as ways of meeting future raw material requirements.

There are a number of genetic implications in the rapidly changing forestry picture. It appears that the so-called improved trees of the future should be selected and evaluated for their ability to grow satisfactorily on low-quality sites, and for their ability to respond to fertilization and irrigation. Equally important, improved trees should have the form and crown characteristics that enable them to grow in stands that lend themselves to future mechanized harvesting operations.

The report that follows includes a discussion of the progress made in the areas of selection, hybridization, and polyploid production. Also included

is a description of growth chamber studies aimed at early evaluation of the nutrient requirements and growth potential of experimental hybrids.

SECURING AND PROPAGATING DESIRABLE POLYPLOID AND
DIPLOID ASPEN AND COTTONWOOD

SELECTION--RECENTLY LOCATED OUTSTANDING TREES

Each year, 30 to 40 trees are measured and evaluated on the basis of wood, growth, and morphological characteristics. Those trees that meet the minimum standards for all characteristics and are outstanding in at least one or two characteristics are selected for use as parent trees in future hybridization and polyploid studies. A selection index system, employing a numerical rating method, is used. Trees that survive this initial selection are next checked for their flowering and crossing behavior and then evaluated on the basis of the quality and vigor of the progeny produced. Listed and described below are four of the better trees evaluated this past year and presently being tested for their crossing behavior.

Tree G-10-66

G-10-66, shown in Fig. 1, is a bigtooth aspen (Populus grandidentata), growing on a sandy, well-drained site near Nekoosa, Wisconsin. The tree is growing at a moderate rate of growth, has exceptional stem straightness, good natural pruning, low specific gravity, and good fiber length. This tree was originally selected for its exceptional stem straightness and, although it has not been evaluated as a parent tree, has shown exceptional rooting ability. Normally bigtooth aspen is very difficult to vegetatively propagate. However, over 90% of the root sprouts produced from G-10-66 rooted using standard greenhouse techniques. The tree is expected to be exceptionally useful in future

crossing work and as a clonal control material in the growth chamber and field trials. The growth, form, and wood quality data for this tree are as follows:

Total height--52 ft.
Height to 3 inch top--40 ft.
First live branch--25 ft.
Age--32 years
Stem straightness--very good
Natural pruning--very good
Form factor--86
Diameter at 4-1/2 ft.--8.6 inches

Diameter at 16-1/2 ft.--8.0 inches
Bark thickness at 4.5 ft.--0.22 inches
Crown diameter--12.5 ft.
Branch angle--75°
Number major branches--11
Specific gravity--0.325
Fiber length (age 30)--1.03 mm.

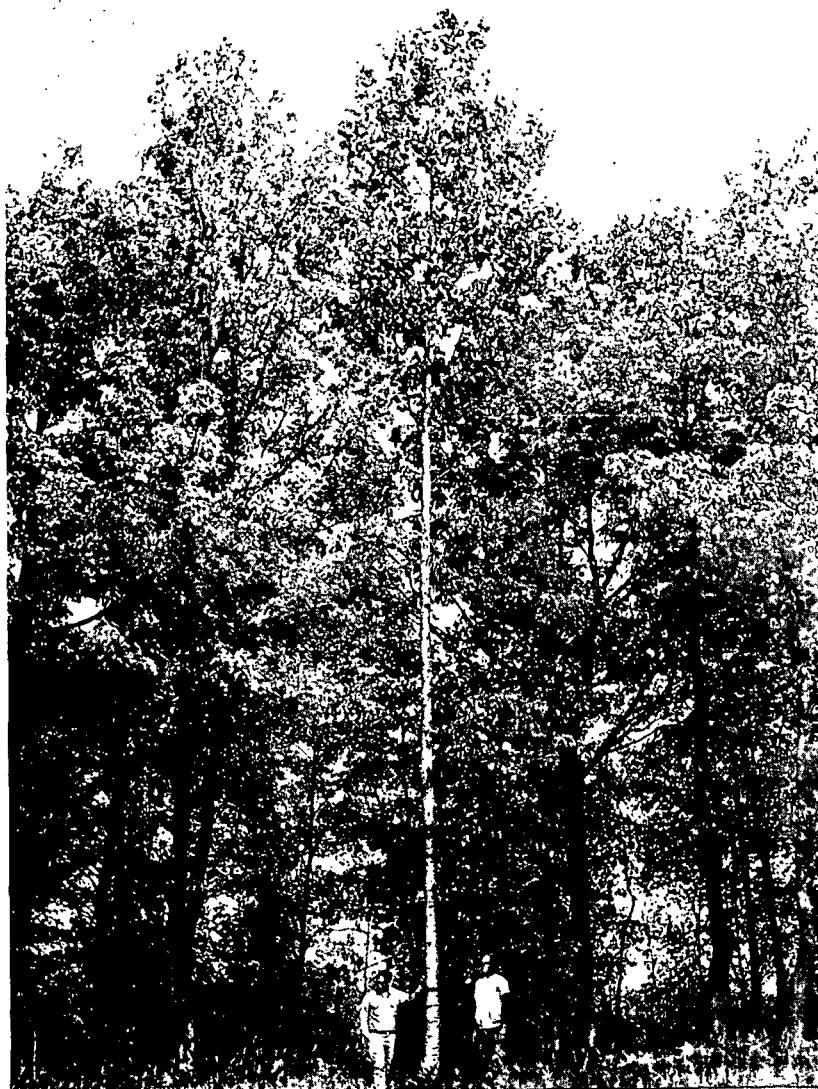


Figure 1. Tree G-10-66, a Bigtooth Aspen Growing On a Sandy, Well-Drained Site, Was Selected for its Superior Form. Propagation Studies Since Selection Have Revealed the Tree also Has Exceptional Rooting Ability

Tree G-6-67

This male, bigtooth aspen (P. grandidentata) was selected because of its satisfactory growth on sandy soils, its good stem straightness, and overall favorable appearance. The tree is located in Menominee County, about 6 miles north of Keshena, Wisconsin. The usefulness of this tree as a parent tree is being investigated in the 1967 crossing series. The following is a summary of the growth measurements and wood quality information.

Total height--87 ft.	Diameter at 4-1/2 ft.--16.1 inches
Height to 3 inch top--67 ft.	Diameter at 16.5 ft.--13.5 inches
First live branch--48 ft.	Bark thickness at 4.5 ft.--0.68 inches
Age--61 years	Crown diameter--18.2 ft.
Stem straightness--very good	Number major branches--14
Natural pruning--good	Branch angle--65°
Branch weight--fair	Form factor--79
Specific gravity--0.378	Fiber length (age 30)--0.998

Tree T-6-67

Tree T-6-67 is a male quaking aspen (P. tremuloides) that is part of an exceptional stand of aspen growing in the Porcupine Mountains of Upper Michigan. This tree is one of the older trees selected as a parent tree. It has good stem straightness, good natural pruning, and was selected because of its overall outstanding appearance and soundness despite its advanced age. Evaluation of this tree as a parent tree is underway and the preliminary results indicate it handles well in crosses and the next step will be the evaluation of the progeny. Tabulated below is a summary of growth and wood quality information available on this tree.

Total height--84 ft.	Diameter at 4.5 ft.--15.4 inches
Height to 3 inch top--66 ft.	Diameter at 16.5 ft.--13.3 inches
First live branch--53 ft.	Bark thickness at 4.5 ft.--0.41 inches
Age--74 years	Crown diameter--18.5 ft.
Stem straightness--good	Number major branches--8
Natural pruning--good	Branch angle--60°
Branch weight--good	Form factor--81
Specific gravity--0.364	Fiber length (age 30)--0.922

Tree D-5-67

This male cottonwood (P. deltoides) is growing near Brillion, Wisconsin and was selected because of its good stem straightness, good natural pruning, and relatively small crown. Figure 2 illustrates a foresters "eye view" of the crown size, natural pruning, and stem straightness of this tree. The tree is located on the edge of a low marshy area and is in a mixed stand of elm, willow, and cedar. Listed below is a description of the growth and wood quality data available on D-5-67.

Total height--97 ft.	Specific gravity--0.369
Height to 3 inch top--69 ft.	Diameter at 4.5 ft.--20.6 inches
First live branch--45 ft.	Bark thickness at 4.5 ft.--0.92 inches
Age--59 years	Crown diameter--16.2 ft.
Stem straightness--good	Number of major branches--10
Natural pruning--very good	Branch angle--30°
Branch weight--fair	Fiber length (age 30)--1.036

TRIPLOID ASPEN PRODUCTION

No new triploid aspen clones were located during the past year, and the production of triploid materials was confined to the vegetative propagation of previously discovered clones and the production of triploids by crossing local diploid female parents with a Swedish tetraploid aspen. Observations are continuing on the field plantings that contain triploid materials. As outstanding triploid individuals become evident, it is expected that the use of both vegetative and crossing techniques for the production of triploids will be increased.

Vegetative propagation of triploids was handled on a limited basis and the results are summarized in Table I. The trees produced are to be used for cytological studies and limited planting on Institute test areas.



Figure 2. This Forester's Eye-View of D-5-67 Illustrates the Small Crown, Good Stem Straightness, and Good Natural Pruning of This Male Cottonwood

TABLE I

NATURAL TRIPLOIDS VEGETATIVELY PROPAGATED IN 1966

Tree No.	Original Location	No. Surviving Individuals
T-2-56	Bruce Crossing, Mich.	87
T-9-59	Bruch Crossing, Mich.	9
T-38-59	Trout Creek, Mich.	49
T-1-62	Cornell, Wis.	89
T-2-65	Loman, Minn.	85

Seven experimental crosses were conducted using tetraploid pollen in an attempt to increase the numbers of triploids available to the program. Two crosses involved bigtooth aspen as female parents. Unfortunately, fresh pollen was not available from the Swedish tree and it was necessary at the last minute to use frozen one-year-old pollen. Table II summarizes the crosses that were attempted and the number of seedlings produced. The use of frozen pollen reduced the seed set and seedling production and, because there is some possibility that not all progeny produced are triploid, spot checking for chromosome number is planned. As Table II indicates, 582 presumed (putative) triploids were produced. Of particular interest are the crosses between bigtooth aspen and the Swedish-European aspen. Nowhere in the literature have triploids of this type been described. For additional details regarding these crosses the reader is directed to the section on Intraspecific and Interspecific Crosses.

TABLE II

SEEDS AND SEEDLINGS PRODUCED BY DIPLOID BY TETRAPLOID CROSSES

Cross No. ^a	Total No. Seeds	Total No. Seedlings
XT-Ta-8-66	506	121
XT-Ta-9-66	4,299	163
XT-Ta-10-66	439	80
XT-Ta-11-66	362	40
XT-Ta-12-66	289	23
XG-Ta-13-66	575	61
XG-Ta-14-66	633	<u>94</u>
	Total	582

^a

X = cross, G = P. grandidentata, T = P. tremuloides, Ta = P. tremula.

PRODUCTION OF HAPLOID ASPEN

Haploid aspen are trees that have one set of nineteen chromosomes instead of the normal two sets of nineteen. Details on the methods of production of haploids and the usefulness of such individuals were reviewed in Project 2412, Progress Report 4. Because of both the academic and practical usefulness of haploids, work on the production of haploid individuals is continuing. In 1964 the weakened pollen technique was investigated by treating pollen from Populus alba with electron irradiation and using the pollen in an experimental cross with quaking aspen. The principle involved is pollination without fertilization, i.e., the pollen applied serves only to stimulate the female gamete to develop into a haploid embryo. In an "alba x tremuloides" cross, when the normal diploid hybrid seed results, the seed will be large and the seedlings will have pubescent primary leaves. By discarding these large seeds and counting chromosomes of only the nonpubescent slow-growing individuals from the small-size seed, the maximum number of haploids should be recovered.

The results of the 1964 investigations produced one haploid individual, which, without treatment, developed into what we believe is a very desirable homozygous diploid individual. This individual will be very valuable in future breeding work. In 1965 the weakened pollen technique was repeated. A limited number of individuals were produced and none were found to be haploid. In 1966 the weakened pollen technique was again employed, with the modification that, in this instance, pollen weakened by heat treatment was used. The earlier work had employed pollen that had been weakened by irradiation from a linear-electron-accelerator.

The heat treatments that were tried in 1966 were very preliminary in nature and involved treating two samples of pollen; one for fifteen minutes at 100°C. and a second for thirty minutes at 100°C. The pollen from the two treatments was applied to three catkins and observations were made on the catkins. The three catkins pollinated with the "thirty-minute treatment" behaved as if they had not been pollinated and the catkins dried up and dropped off after three to four days. Two of the catkins pollinated with the "fifteen-minute treatment" behaved in a similar manner and dropped off after six to seven days. The third catkin remaining in the "fifteen-minute treatment" remained on for ten days but no viable seed was produced.

The preliminary results indicate that the treatments used were too severe. Plans for the coming year include the use of less severe heat treatments and the employment of a newly discovered chemical that, when applied to pollen, is reported to allow the pollen tube to grow but upsets the fertilization process. Properly used, such a chemical might result in the production of haploid individuals.

PRODUCTION OF TETRAPLOID ASPEN AND COTTONWOOD

For the mass production of triploid aspen, pollen from tetraploid male trees will be used to pollinate flowers from selected diploid female trees. Putative tetraploid seedlings were obtained in 1964 for quaking aspen and in 1965 for bigtooth aspen. Colchicine treatments were also applied to cottonwood in 1965, but chromosome counts were not available then. This year, cottonwood plants from the 1965 treatment were rechecked and the counts are reported.

Cottonwood - 1965

At 48 and 72 hours after pollination, 2 to 4 flower catkins of cross XD-40-65 were immersed in a solution of 0.3% colchicine for six hours. From each

of the 238 selected seedlings of this cross, leaf meristems were collected for chromosome counts. Descriptions of leaf anatomy were also recorded for each plant.

The reports of our two microscopists were in close agreement for the chromosome counts shown in Table III. Approximately the same number of putative triploids were recovered from both the 48 and 72-hour treatments, but tetraploids were found only among the early-treated material. Putative triploid and tetraploid plants will be retested next year and then outplanted in a nearby test area. There was no apparent correlation between the level of ploidy and leaf anatomy.

TABLE III

1965 COTTONWOOD CHROMOSOME COUNTS, XD-40-65

Hours ^a	Seed Mesh	No. Seedlings	Counts		
			<u>3n</u>	Intermed.	<u>4n</u>
48	20	54	4	13	10
	28	69	16	6	3
	40	<u>1</u>	<u>--</u>	<u>--</u>	<u>--</u>
		<u>124</u>	<u>20</u>	<u>19</u>	<u>13</u>
72	20	50	16	--	--
	28	62	1	--	--
	40	<u>2</u>	<u>--</u>	<u>--</u>	<u>--</u>
		<u>114</u>	<u>17</u>	<u>--</u>	<u>--</u>
		<u>238</u>	<u>37</u>	<u>19</u>	<u>13</u>

^a
Colchicine treatment after pollination.

Quaking Aspen and Cottonwood - 1966

On the basis of the cytological study conducted in 1965 (see next section on Cytology of Fertilization.....), flowers on cut branches of both quaking aspen and cottonwood were treated with colchicine in 1966. The cytological study indicated that, when material is pollinated and then placed in the growth chamber,

fertilization occurs about the second day in quaking aspen and the third day in cottonwood. The embryo begins to develop on about the third and sixth day, respectively, after pollination.

For both species, colchicine was applied both before and after the apparent time of fertilization. The objectives were to obtain (1) both triploids and tetraploids by the prefertilization doubling of chromosomes of one of the gametes, as well as (2) tetraploids only, by the doubling of the undivided zygote (fertilized egg).

During the treatments, material was collected for the cytological examinations reported in the next section. Pollination apparently failed in cottonwood because the catkins did not mature. Also, meristems were collected from quaking aspen seedlings too late in the summer and were physiologically unsuitable for chromosome counts. Next year the quaking aspen material will be recollected earlier in the season and colchicine treatments will probably be repeated on cottonwood, but at different times after pollination.

CYTOLOGY OF FERTILIZATION IN ASPEN AND COTTONWOOD

1965 Studies

In Report Four, preliminary results were given on the cytological study of fertilization in quaking aspen and cottonwood. A paper has recently been submitted to a scientific journal for publication giving the results of the completed analysis. A portion of that paper is presented on the following pages.

In our work, the primary objective is to produce tetraploids. Hence, colchicine should be applied during the one-celled zygote stage, which occurs from the time of fertilization of the egg until the first mitotic division of

the zygote. A cytological study was thus initiated to determine the rate of development after pollination for two species of Populus.

During April and May of 1965, three collections of five to seven branches each were made from one female quaking aspen tree growing in east-central Wisconsin. The branches were placed in a greenhouse with their cut bases immersed in ice water. The female flowers were receptive fourteen days after the first collection and progressively earlier for the second and third collections.

Catkins of the first collection were pollinated March 16 with pollen forced in the greenhouse. At periods of 2, 4, 6, 8, and 24 hours after pollination, whole catkins were placed in FAE, FPA, Graf III, and Bouin's fixative solutions. Individual capsules from the center of the catkin were then dehydrated in an ethanol--tertiary butanol series and embedded first in Parawax and finally in Tissuemat. Longitudinal, as well as a few cross and oblique sections, were cut, all at 10 microns. Mounted sections were stained in safranin and fast green during a xylene--ethanol schedule and made permanent by mounting with balsam in xylene.

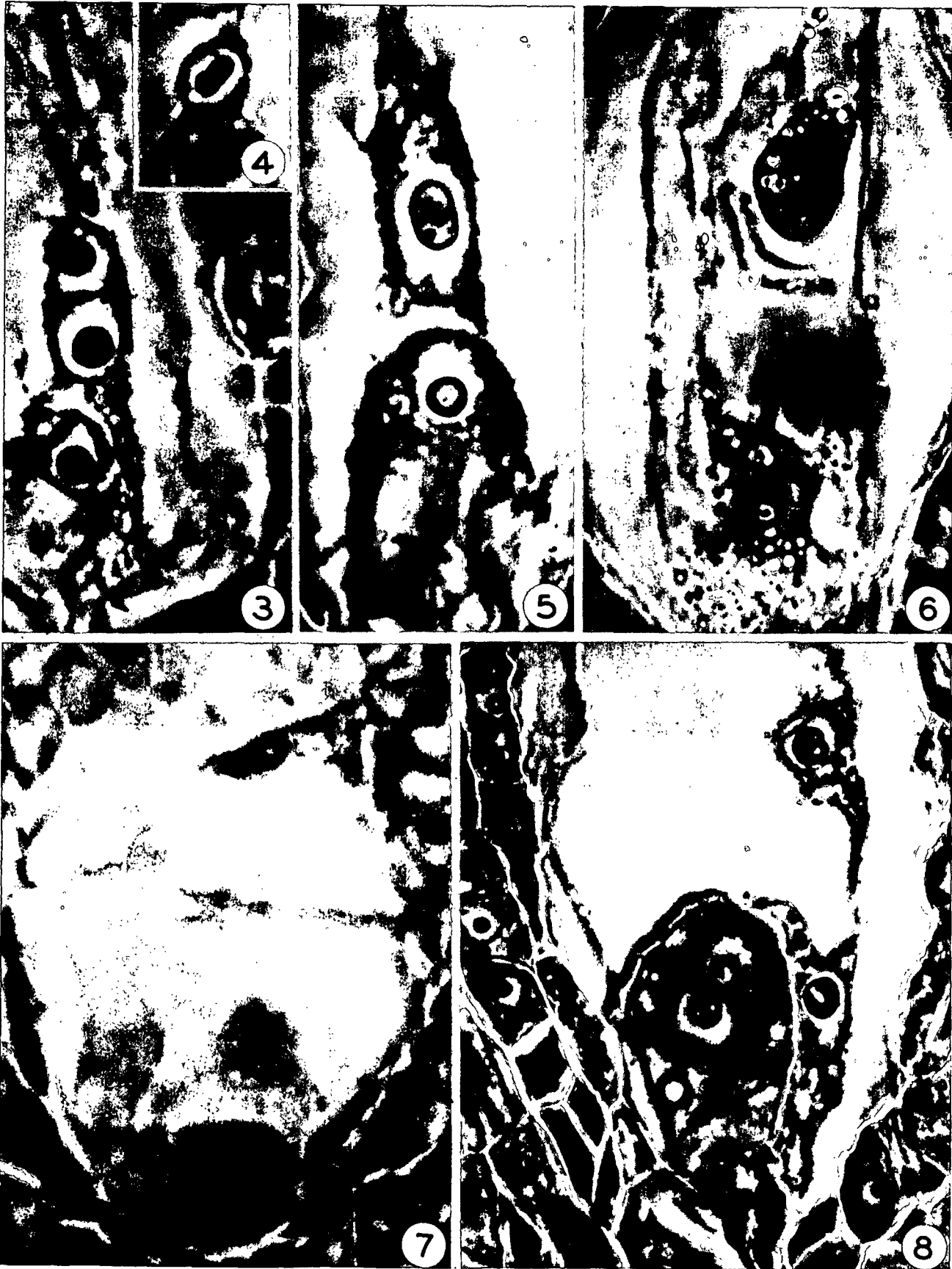
Catkins from the second collection were pollinated April 1 and collected daily in Graf III fixative for the first eight days, and thereafter in FAE fixative every 3 or 4 days until 18 days after pollination. Branches of the first two collections were left in the greenhouse. However, branches of the third collection were moved into a growth chamber after pollination on April 14. Catkins were placed in FAE fixative at half-hour intervals between five and eight hours after pollination, and thereafter 1 to 5 times daily until seed was shed on the twelfth day. Permanent slide preparations were made from 2 or 3 capsules per treatment. Photomicrographs were taken with Kodak 35 mm. Panatomic-X film in a phase-contrast Zeiss Photomicroscope.

Branches, bearing flower buds, were also collected from one female cottonwood tree in the same vicinity and forced in the greenhouse. After pollination on April 22, these branches were placed in the growth chamber. Material was collected in FAE fixative 6, 8, and 12 hours after pollination, then daily on days 1 through 12, and finally when seed was shed on the eighteenth day after pollination. Sections were prepared in the same manner described above.

At the time of pollination, both species had either two polar nuclei (Fig. 3) or one polar body (Fig. 5) in each mature megagametophyte. Figure 4 shows what appears to be the fusion of two polar nuclei seen in cross section. Receptivity was signaled by a faint pinkish blush in quaking aspen, and a yellowish translucent color in cottonwood, caused by the emerging stigmas.

In the quaking aspen flowers of the third collection, which was placed in the growth chamber after pollination, pollen germinated on the stigma at 6-1/2 hours, and double fertilization (Fig. 6) occurred between 30 and 48 hours after pollination. The zygote migrated into the micropylar cavity during the next 24 hours, followed shortly by the division of the polar body (Fig. 7) and the early development of the endosperm. The first transverse mitotic division of the zygote occurred between 54 and 72 hours after pollination (Fig. 8). Embryogeny then continued fairly rapidly, forming the mature embryo before the seed was shed on the twelfth day after pollination.

In contrast, aspen left in the greenhouse after pollination showed considerable variation in the rate of development. Some fertilization did occur as early as 8 to 24 hours in the first collection, but not until 48 to 72 hours in the second. Differences in the rate of development may have reflected differences in greenhouse temperatures. The times of fertilization were, respectively, about one day earlier and one day later than the 30 to 48 hours observed in the third collection.



The cottonwood material, unfortunately, was cut mostly in oblique sections, making a detailed analysis difficult. Fertilization probably occurred between 24 and 72 hours, and the endosperm was well developed by the fifth day. First division of the zygote apparently occurred on either the sixth or seventh day, because the young embryo was observed on the eighth day after pollination.

The placental epidermis hair cells on the funiculus divided longitudinally in aspen as early as 5-1/2 hours after pollination, but only after the fusion of the two polar nuclei. Hair cells were approximately one half the length of the ovules during fertilization, and almost as long as the ovules at the first division of the zygote. The onset of embryogeny was accompanied by the rapid elongation of both the ovules and the hair cells. Growth of the hair cells of cottonwood was difficult to follow, but may have been slightly faster than in aspen.

The results of this study indicate that in quaking aspen, which develops in the growth chamber, fertilization does not occur until 30 to 48 hours after pollination. Thus, colchicine applied to forced quaking aspen 24 hours after

Fig. 3-8. Populus tremuloides, ca. 2000X

- Fig. 3. Two Polar Nuclei Above the Egg Cell
- Fig. 4. Fusion of Polar Nuclei (Cross Section)
- Fig. 5. Polar Body Above, Egg Cell in Center
- Fig. 6. Fertilization. Egg Cell Out of Focus in Center
- Fig. 7. First Division of Polar Body. Egg Cell in Micropylar Cavity
- Fig. 8. First Transverse Mitotic Division of Zygote.
Note Development of the Endosperm

pollination should give both triploids and tetraploids by prefertilization chromosome doubling in the gametes. Treatment of the zygote 48 hours after pollination should give only tetraploids. The same results would be expected in cottonwood treated, respectively, on the second and fifth days.

The correlation between the relative length of the hair cells and the length of the ovules, during successive stages of development, appears to be fairly constant, at least in quaking aspen. It may be possible to use this characteristic in the greenhouse as a reliable indicator of developmental stages.

1966 Studies

As indicated in the previous section on tetraploid production, catkins of cottonwood failed to mature and chromosome counts were not available for quaking aspen leaf material. However, treated and untreated catkins of quaking aspen were collected every eight hours after the pollination of two treatments. Fixed and stained sections showed that colchicine was applied to T-3X (Table IV) just prior to fertilization and to T-4X just before the first division of the zygote (fertilized egg).

Thus, chromosome counts of this cross (to be checked next year), should have both triploids and tetraploids from Treatment T-3X but only tetraploids from Treatment T-4X.

TABLE IV
SCHEDULE OF TREATMENTS OF QUAKING ASPEN - 1966^a

Day	Control	T-3X	T-4X
1	0-Pollination	-Pollination	-Pollination
1	8		
2	24	0-Colchicine	
2	32	8	
3	48	24	0-Colchicine
3		32	8
4		48	24
4			32
5			48

^a

Treatments given to cottonwood were similar, except that the treatment and sampling interval was 24 instead of 8 hours.

TREE PHYSIOLOGY STUDIES

NUTRITIONAL REQUIREMENTS OF ASPEN HYBRIDS--
SUMMARY OF RESULTS*

Knowledge regarding growth and nutritional requirements of aspen hybrids in relation to the parent species used in producing the hybrids would be extremely valuable. Such information could be used in determining sites suitable for growing hybrids and predicting the relative growth advantage of the hybrids. One approach, and the one used in the studies described, is to use a sand culture technique, vary the level of nutrients, and compare the growth and nutrient uptake of the hybrids with the growth of seedlings of the parent species.

Project 2412, Progress Report Four, describes a sand culture technique that was devised to be run in the Biology Section growth chamber. Basically, the system employs growth containers containing silica sand. These containers are attached to pressurized carboys containing the nutrient solutions. A time clock activates a valve on a compressed air line which in turn causes the solution to be pumped into the growth containers. After five minutes the valve closes and the solution drains back into the carboys. The test seedlings are grown in the sand on this periodically fluctuating nutrient solution. One basic unit consists of a pressurized carboy and four growth containers. Each growth container is a replication and the four containers make up a single treatment. For each additional treatment an additional basic unit is added.

*Not all of the data for drawing the conclusions were available at the time the report was written. Plans are to prepare a separate Project 2412 progress report which will provide a complete description of work under way on the nutritional requirements of aspen hybrids.

The overall plan for the entire study consisted of running a series of five interrelated growth experiments. Light, temperature, day length, and relative humidity are held constant and in each of the five "growth chamber trials," the level of a different major soil nutrient is varied. Seed from four experimental crosses was used as a source of plant material. The full-sib progeny groups were started from seed in the sand-filled growth containers and the growth and nutritional status of the seedlings were measured after 40 days. The first type of hybrid aspen to be investigated using this procedure was the cross between quaking aspen (T) and European gray poplar (Ca). Table V lists the parentage of the four progeny groups used. It should be noted that experimental material two, cross XT-Ca-35-65, involves a quaking aspen as the female parent while experimental material three, XCa-T-8-65, is the reciprocal cross and involves a gray poplar as the female parent.

TABLE V
 PARENTAGE OF TEST TREES

Material Number	Type of Cross	Cross Number ^a	Parent Trees (female x male)
1	T x T	XT-36-65	T-12-58 x T-10-60
2	T x Ca	XT-Ca-35-65	T-12-58 x Ca-1-62
3	Ca x T	XCa-T-8-65	Ca-2 x XT-22-56, S-4
4	Ca x Ca	XCa-34-65	Ca-2 x Ca-1-62

^a

X = cross, Ca = P. canescens, T = P. tremuloides, the number indicates the cross number and year the cross was completed.

Olson's (1) combination of required elements was used in making up the nutrient solutions used in this study. The levels used by Olson were modified to meet the requirements of this investigation. Phosphorus was the element varied

in the first growth chamber run with levels of 0, 22, 43, 54, and 65 p.p.m. being employed. One series of levels of phosphorus was run in combination with the low level of the other essential nutrients and the second series was run using a medium level of the other nutrients. Both the "low series" and the "medium series" were handled in a single growth chamber trial. A similar procedure was used in each growth chamber trial with a different element being varied. Table VI presents the composition of Olson's low and medium solutions and the five levels of each element used in the growth chamber trials.

TABLE VI

COMPOSITION OF NUTRIENT SOLUTIONS IN P.P.M.

Nutrient	Olson's Low ^a	Olson's Medium ^a	Five Levels Used in Growth Chamber Trials				
			1	2	3	4	5
N	105	158	29	52	105	131	158
P	43	65	0	22	43	54	65
K	62	93	0	31	62	77	93
Ca	15	30	0	15	30	38	46
Mg	14	21	0	7	14	17	21

^a
Appropriate levels of micronutrients were included in the basic solutions.

Each of the growth containers contained four seedlings, one seedling of each of the four types of test materials. Growth on the complete nutrient solution is rapid and, at 40 days, it was not unusual to have seedlings that were 18 to 20 inches tall. After 40 days of growth, all surviving seedlings were washed from the growth containers and the green weight obtained for the tops and roots along with the oven-dry weight of the tops. Next, the oven-dry tops from four genetically similar seedlings grown on the same nutrient solution were combined and the tissue

ground in a Wiley mill. This ground tissue was used in determining the levels of N, P, K, Ca, and Mg in the seedlings produced by the various nutrient solution treatments. Instrumentation problems have delayed completion of the tissue analyses. The determinations have recently been completed and the data will be included in the proposed separate detailed report.

Growth Comparisons

One method of comparing the influence of varying levels of the various essential elements is to measure the total green weight of the plants produced. When such a comparison was made, considerable variation was encountered between individuals within progeny groups that had been treated alike. This, coupled with missing trees in some treatments, particularly treatments involving experimental material two, resulted in reduced usefulness of the results. Analysis of variance procedures were used to investigate differences between treatments and differences between growth of experimental materials. No statistically significant growth differences were obtained between materials grown on Olson's low level when compared with the Olson's medium level. This made it possible to combine the data for these two levels and handle as though one level had been used with a greater number of replications.

Nitrogen Growth Chamber Trial

The nitrogen growth chamber run was handled as described above. A total of ten treatments was employed. Five of the treatments involved nitrogen at 29, 52, 105, 131, and 158 p.p.m. used in combination with Olson's low level of other essential nutrients (Table VI). The other five treatments consisted of the above five levels of nitrogen used in combination with the medium level of other essential elements. Results of the growth information for the four experimental materials are

summarized in Fig. 9. As described in the introduction no statistically significant difference existed between the growth of the test trees on the two levels of Olson's solutions. Figure 9 shows the influence of varying levels of N on the average green weight of 40-day-old test trees. The plotted data are the combined average weight of seedlings from the low and medium nutrient solutions. The green weight differences between test materials are believed to be significant at the moderate levels of nitrogen. The nitrogen response curves indicate a rapid improvement in growth at low levels of N, and at leveling off followed by a decreased growth at the higher nitrogen levels. All test materials responded in a similar manner.

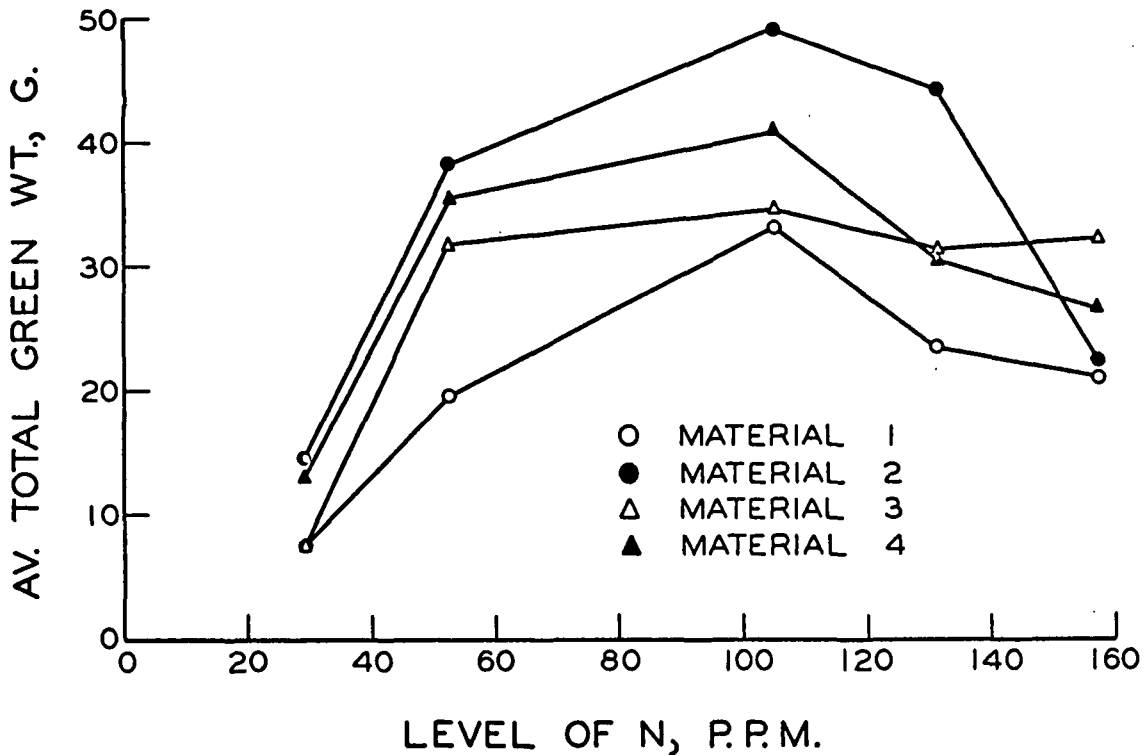


Figure 9. Differences in Average Green Weight of Trees Grown for Forty Days at Five Levels of Nitrogen

Phosphorus Growth Chamber Trial

The treatments in the phosphorus growth chamber trial were handled using a procedure similar to that described for nitrogen. Levels of phosphorus varied from zero to 65 p.p.m. and growth differences between Olson's low and Olson's medium levels were not significant. Figure 10 summarizes the influences of the varying levels of P on seedling growth. No growth was obtained at the zero level of P and growth of all test materials increased greatly as the result of the increase from zero to 22 p.p.m. Increasing levels of P above 22 p.p.m. failed to produce corresponding increases in growth. Nutrient uptake, although not completely summarized, is expected to provide information on differences between test materials in nutrient requirements. Missing trees in experimental test material two (T x Ca) contributed to the variability of the growth measurements of this material.

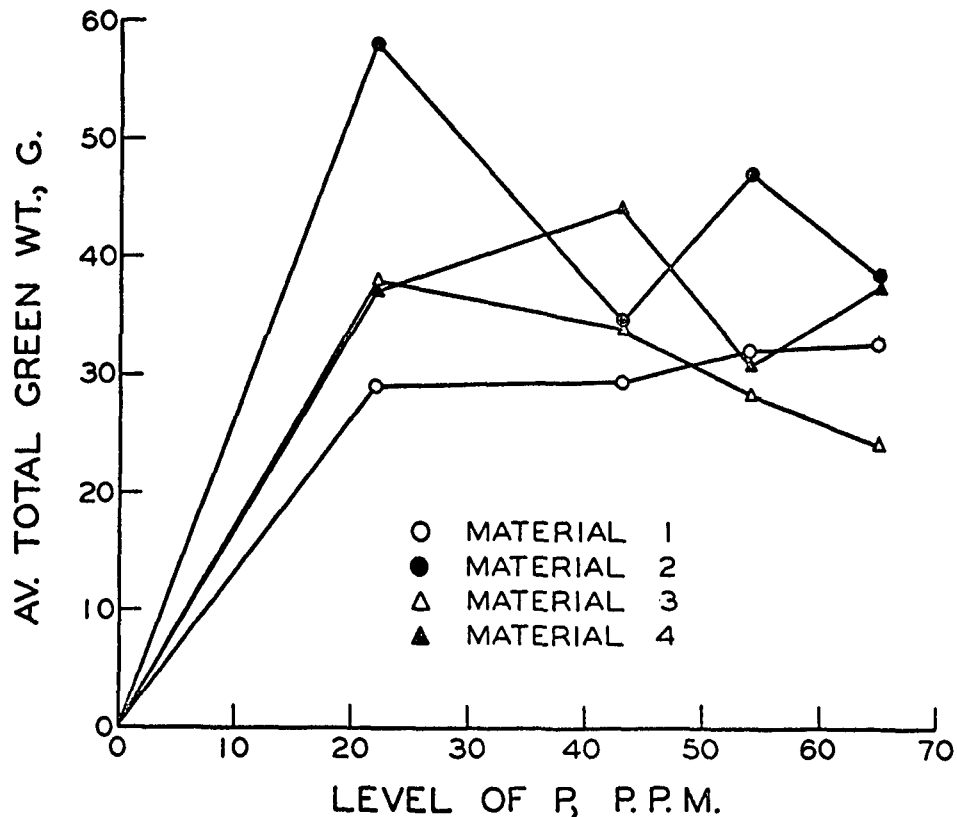


Figure 10. Differences in Average Green Weight of Trees Grown for Forty Days at Five Levels of Phosphorus

Potassium Growth Chamber Trial

Potassium levels were varied from zero to 93 p.p.m. and the experimental design of treatments was the same as described for previous runs. Figure 11 illustrates the response of the four test materials. The response of all four materials to increasing levels of K was statistically significant. At the highest level of potassium, response of test material two (T x Ca) was greater than the other test materials.

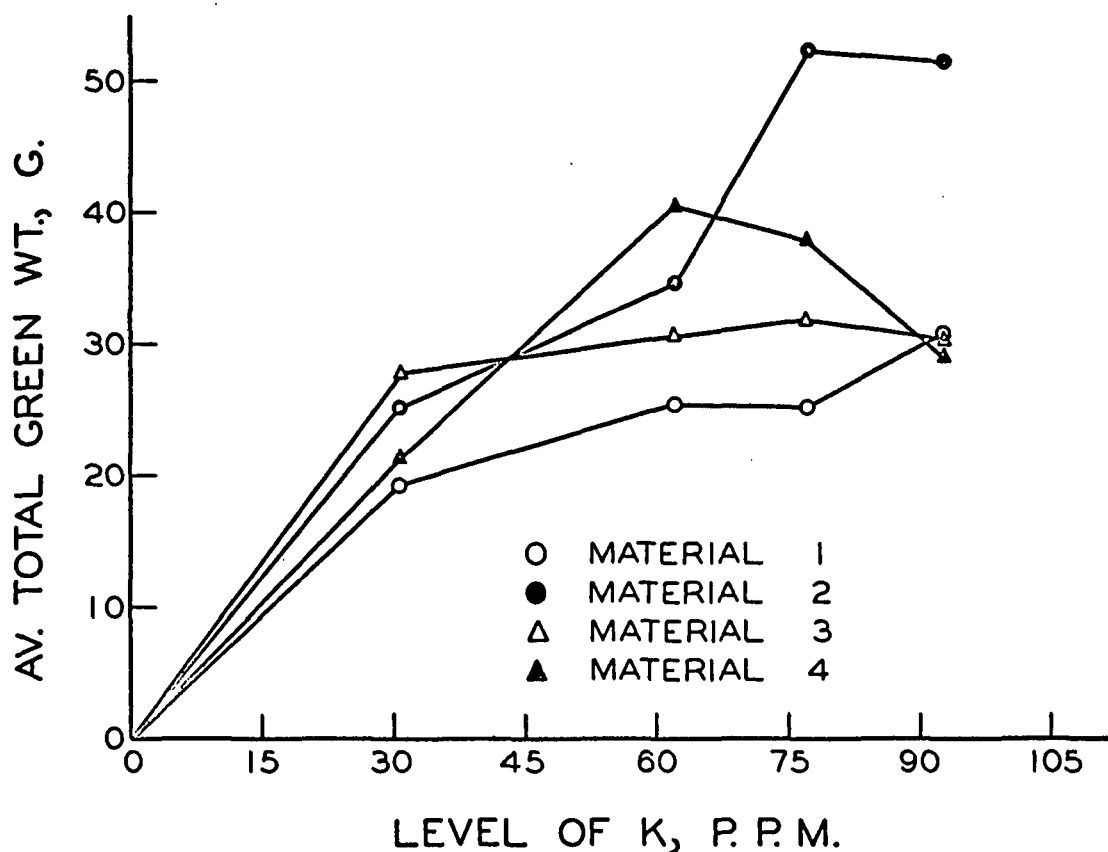


Figure 11. Differences in Average Green Weight of Trees Grown for Forty Days at Five Levels of Potassium

Calcium Growth Chamber Trial

Calcium levels were varied from zero to 46 p.p.m. and the earlier described experimental design for handling treatments was followed. Figure 12

shows the growth response obtained as the level of Ca was increased. Some growth was obtained for all test materials at the zero level of calcium. The response of the quaking aspen cross (T x T) to increasing levels of calcium was not quite statistically significant. The response of the other three materials was statistically significant. The average green weight of the four materials did not differ greatly at the highest level of calcium and it is expected that the level of calcium in the plant tissue may help clarify the question of whether differences between materials exist in calcium requirements.

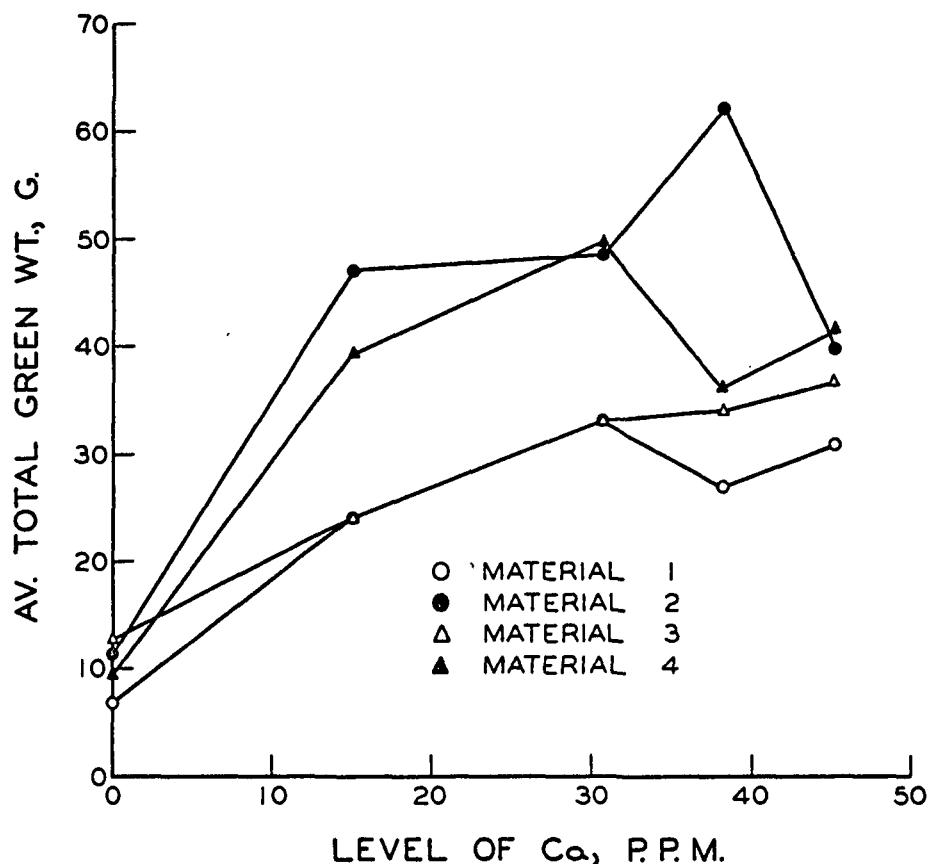


Figure 12. Differences in Average Green Weight of Trees Grown for Forty Days at Five Levels of Calcium

Magnesium Growth Chamber Trial

The standard procedure for the arrangement of treatments was followed and the response of the four test materials to varying levels of Mg followed a pattern very similar to that obtained for calcium. Figure 13 illustrates the results obtained. All test materials exhibited some growth at the zero level of Mg and materials one and three responded the least to increasing levels of Mg. Response for all materials was statistically significant. It is hoped that chemical tests on tissue samples will point out existing magnesium requirement differences.

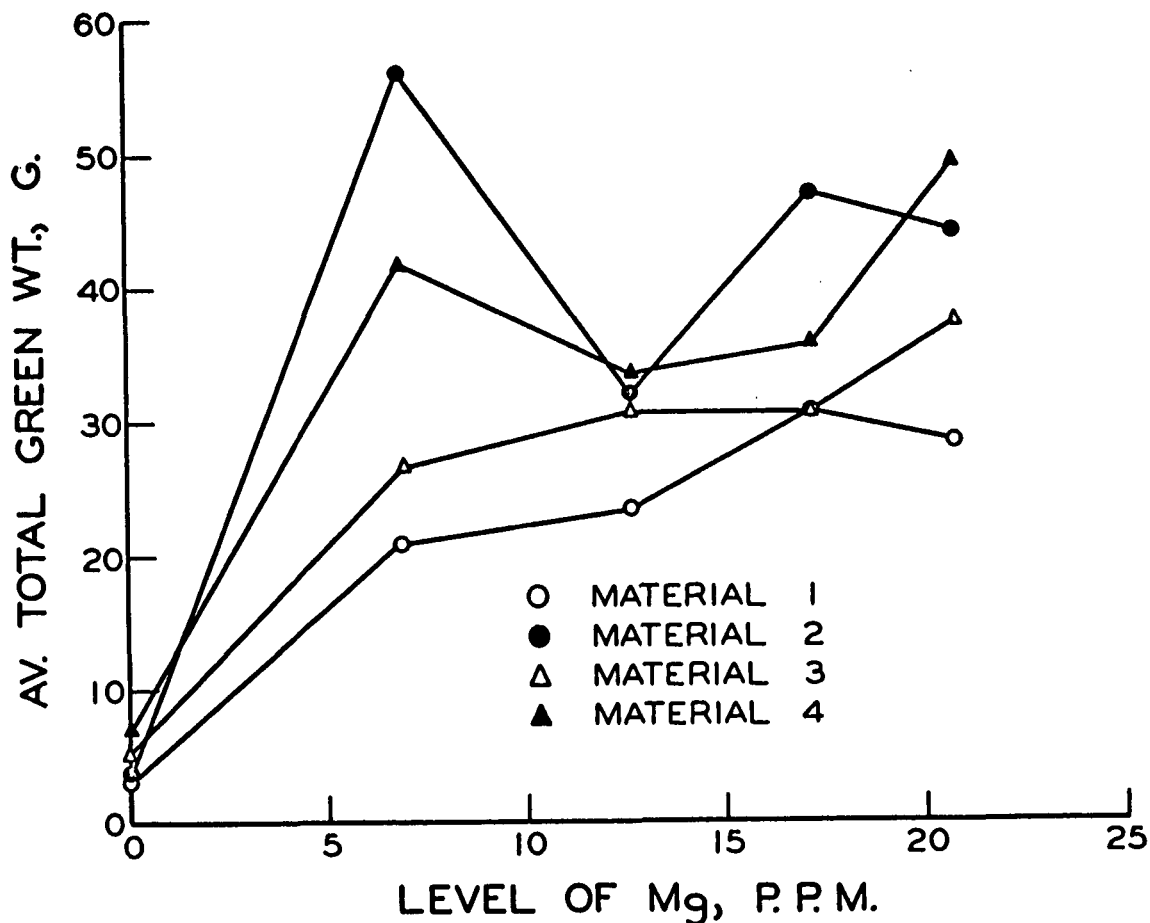


Figure 13. Differences in Average Green Weight of Trees Grown for Forty Days at Five Levels of Magnesium

Growth Differences Between Experimental Materials

The overall growth potential of the four types of experimental trees is of interest. One method of getting a rough picture of the relative early growth of each material is to average or combine, by test materials, the growth data for all experimental trials and plot the data over a relative level of limiting element in nutrient solutions. This means, for example, averaging for experimental material one, the green weight data for the zero level of potassium with the green weight data for the zero or very low levels for the other growth chamber trials. Figure 14 illustrates the growth curves obtained. Growth differences at the higher nutrient levels provide the most realistic picture of differences between test materials. Test materials two and four quite consistently had higher average green weights than materials one and three. Statistically significant differences were obtained between test materials.

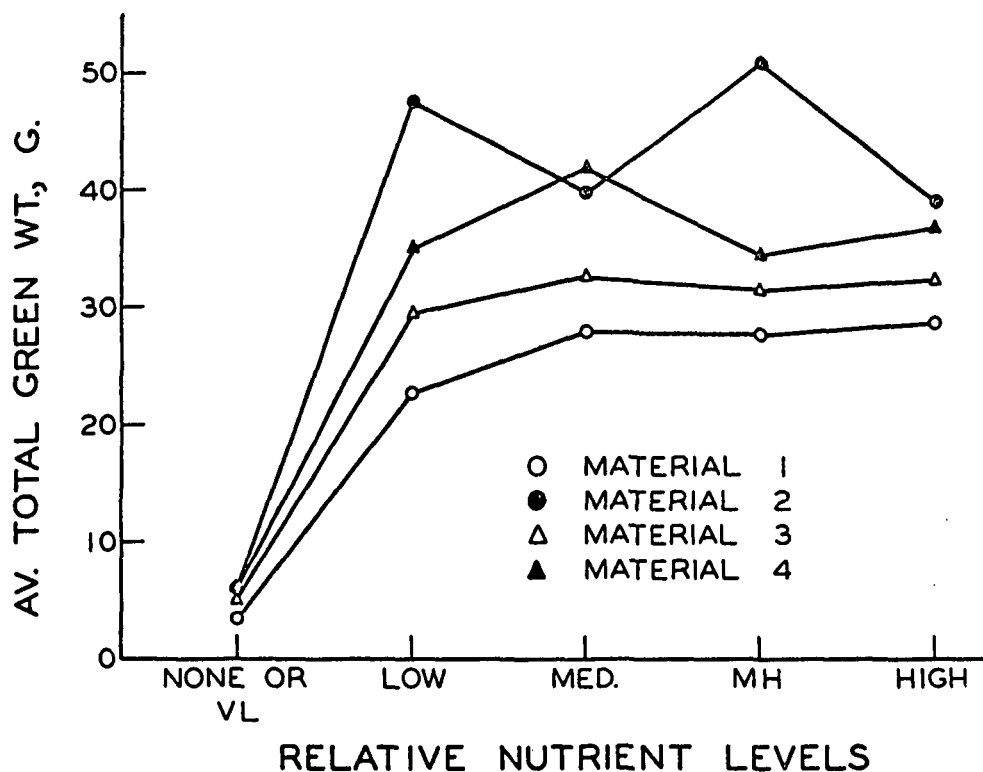


Figure 14. Differences in Green Weight of Trees Obtained by Averaging Data for All Trials and Plotting over Relative Nutrient Levels

Top-Root Ratio

Top-root ratios (T-R R) provide some insight into the root development of a species and in some instances appear to be related to the ability of the species to do well on adverse sites. The tops and roots from each experimental material were handled in such a way that ratios could be calculated for each treatment within each growth chamber trial. The T-R R varied considerably between treatments and there was no well-defined pattern within the growth chamber trials that was related to the level of the element being varied. Table VII summarizes the ratios obtained by type of experiment material and by growth chamber trial.

TABLE VII

TOP-ROOT RATIOS SUMMARIZED BY EXPERIMENTAL MATERIAL AND GROWTH CHAMBER RUN^a

Growth Chamber Run	Experimental Test Material				Average
	1	2	3	4	
N	1.33	1.08	1.42	1.41	1.31
P	1.09	1.00	1.54	1.32	1.24
K	1.20	0.92	1.31	1.22	1.16
Ca	1.15	0.98	1.31	1.40	1.21
Mg	1.50	1.16	1.62	1.35	1.41
Average	1.25	1.03	1.44	1.34	

^aTop-root ratios based upon all data except where, because of low nutrient levels, no growth occurred.

Growth of all four test materials was abnormal at the zero and/or very low treatment levels and top-root ratios are not very meaningful (see Appendix, Table XVII). At the medium and high nutrient levels of each experimental trial, the T-R R were fairly constant and large differences were obtained between the types of experimental trees. Figure 15, which was drawn using the overall average

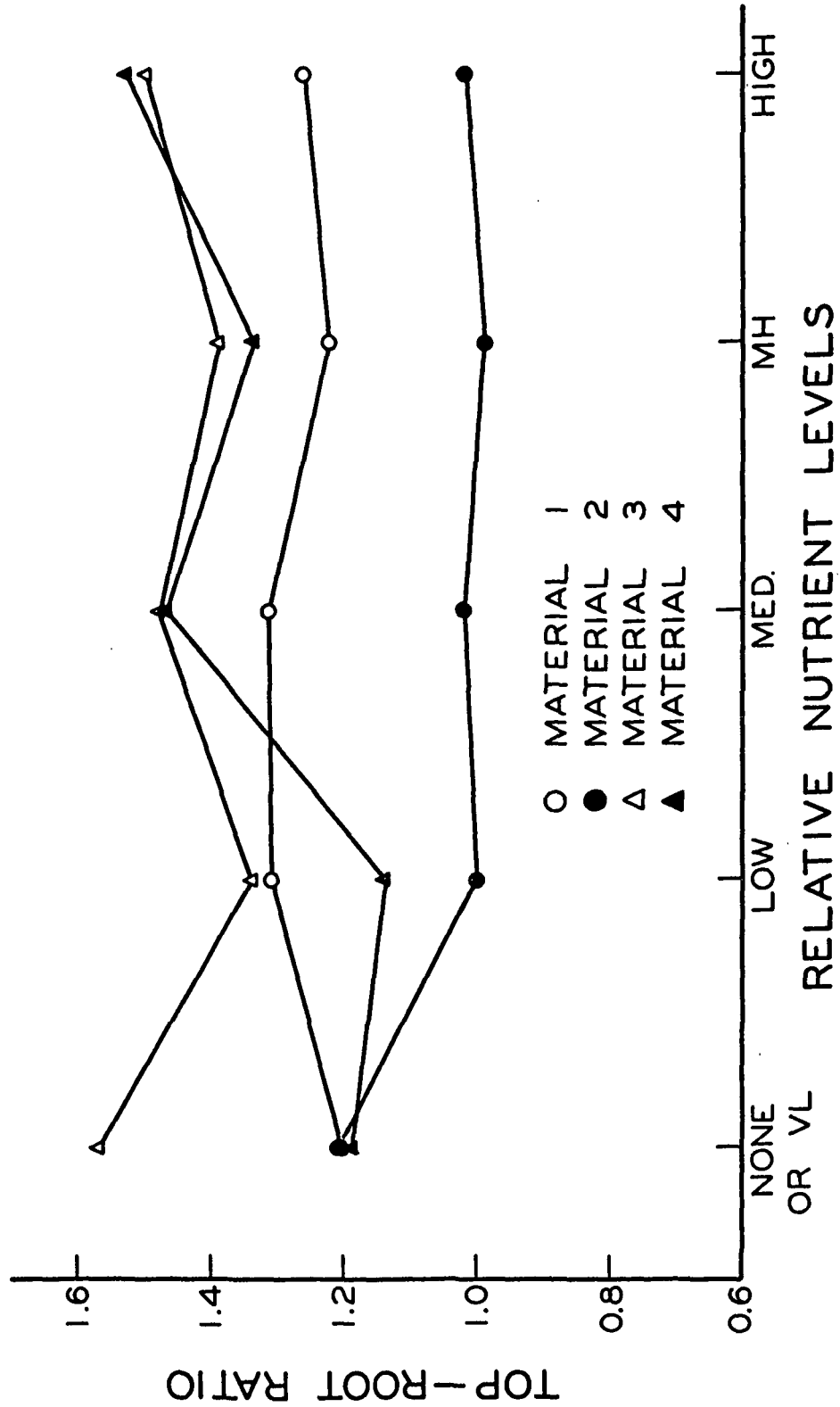


Figure 15. Top-Root Ratios Obtained by Averaging Data for All Trials. The Medium to High Nutrient Levels Give an Accurate Comparison of Differences Between Materials

top-root ratios, regardless of the nutrient element being varied, illustrates the differences obtained between test materials.

When an analysis of variance was run on the data included in Appendix Table XVII, with the none or very low treatments being excluded, the results indicated there were significant T-R R differences between experimental materials and between growth chamber trials. The P, Ca, and K, growth chamber trials produced the lowest ratios and the N and Mg trials had the highest T-R R. The N and Mg growth chamber trials were the last two trials in the series. It appears that the T-R R may be in part influenced by the element being varied and in part by the gradual decrease in light intensity that occurred over the nine-month period that the experimental trials were under way.

The differences between experimental materials appear to be real. Of the two so-called "parent species" test material one had the lowest ratio and material four the highest. Of the two hybrids, material two, as might be expected, was most like material one and consistently had the lowest (T-R R). The T-R R of materials three and four were very similar (see Table VII and Fig. 15).

CHEMICAL CONTROL OF GROWTH AND FLOWERING IN ASPEN

Introduction

The principal function of the selected trees in a breeding arboretum is to provide flowers for future crossing work. Considerable advantage could be obtained if the trees were kept small so the flowers were readily accessible and the trees stimulated to flower at an early age.

There is some evidence from experimental trials with flowers and horticultural crops that certain "so-called" growth retardants might both reduce the

growth and stimulate the flowering of aspen. The report that follows describes some preliminary work with Alar* (N-dimethyl amino succinamic acid), a chemical that has shown promise with several types of fruit trees.

Methods

Three different types of aspen trees were selected for treatment. The trees involved were located in the I.P.C. Greenville Arboretum and consisted of blocks of sixteen trees planted at a 9 x 9-foot spacing. Eight trees in each block were treated and the remaining were used as control trees. Table VIII briefly describes the types of materials treated.

TABLE VIII

TEST TREES USED IN ALAR TRIAL

Tree No.	Sex	Propagation Method	Remarks
AG-1-60	Bisexual	Rooted root sprouts	Rapid growing "alba x bigtooth" hybrid, field planted in 1962 and top pruned in 1964 and 1965.
T-32-57	Male	Grafts	Selected quaking aspen, field planted in 1962, several trees flowered in 1964 and 1965.
T-130-57	Female	Grafts	Grafts of selected quaking aspen field planted in 1962. No previous record of flowering.

Four levels of Alar (1.5, 1.0, 0.5, and 0.25%) were employed and each level was applied to two of the sixteen trees in a block. The chemical was applied as a foliar spray to the drip point. The treatments were applied a total of three times (June 1, July 1, and August 1) and measurements were made on growth and flowering. Treatment effects were checked by comparing the treated trees with control trees of comparable size.

*Available from the Chemical Division, U. S. Rubber Company, Naugatuck, Connecticut.

Results

Table IX summarizes the growth and flowering information for the three types of test materials. The results indicate that the higher treatment levels caused the greatest reduction in growth. The flowering which occurred on the T-32-57 grafts apparently resulted from the Alar treatments. The flowering results of T-130-56 are a bit misleading because the flowering listed under control trees was the result of flowering on only two of a total of eight nontreated trees. Four of the eight treated trees in this portion of the trial flowered.

TABLE IX
FLOWERING AND GROWTH REDUCTION OF ALAR-TREATED TEST TREES

Treatment Level, %	Terminal Growth, % reduction	Lateral Growth, % reduction	Total No. Flower Buds	
			Treated Trees	Control Trees
T-130-56				
1.5	50	18	3	0
1.0	38	25	0	38
0.5	16	14	10	18
0.25	0	0	6	0
T-32-57				
1.5	45	45	100	0
1.0	38	10	62	0
0.5	25	10	53	0
0.25	0	25	0	0
AG-1-60				
1.5	58	43	0	0
1.0	64	43	0	0
0.5	34	8	0	0
0.25	10	0	0	0

Examination of the foliage of the treated trees during the summer and just prior to leaf fall revealed that, for all test materials, the 1.5% Alar treatment caused numerous marginal and internal necrotic areas on the leaves, dieback of the growing tips, and early leaf drop. The 1.5% solution also caused considerable dropping of short twigs from the main stem and major branches on AG-1-60. Trees receiving the 1% treatment exhibited similar symptoms but were less severely affected. The trees receiving the two lower levels of Alar were normal in appearance and the reduction in growth was small.

The differences obtained between test materials in their reaction to the treatments is not surprising. Grafts can be expected to flower sooner than rapidly growing root sprouts. Also from observations made on natural stands it appears that male trees can be expected to flower more often and have larger numbers of flower buds than female trees. The results obtained are very tentative but do seem to indicate that Alar at levels between 0.5 and 1% may be useful in reducing growth and under certain circumstances will also cause stimulation of flowering.

CALLUS GROWTH IN ASPEN SEEDLINGS AS AN INDICATOR OF TREE GROWTH

1965 Studies

Mathes and Einspahr (2) observed a positive correlation between the rate of callus production on stem sections of Populus tremuloides, grown in vitro, and the rate of tree growth under natural conditions. In 1965, tests were begun to determine the relationship of callus growth on stem sections of seven-month-old seedlings and their subsequent natural growth as trees. The objective of this study is to develop a method of predicting tree growth on the basis of juvenile callus production.

On October 18, the top one foot of ten tall seedlings (averaging 5.3 feet in height) and ten short seedlings (averaging 2.0 feet) were collected from quaking aspen stock (XT-36-65) at the Greenville Nursery (Fig. 16). Individually, sections were cut into smaller pieces (5-cm. long) and sterilized for 10 minutes in Hi-Lex (5% NaOCl) with a few drops of Tween 20 added. After three rinses in sterile water, internodal segments 5-7 mm. long were cut and arranged on one dish each of the coconut-milk agar Medium 23, as well as on defined Medium W.

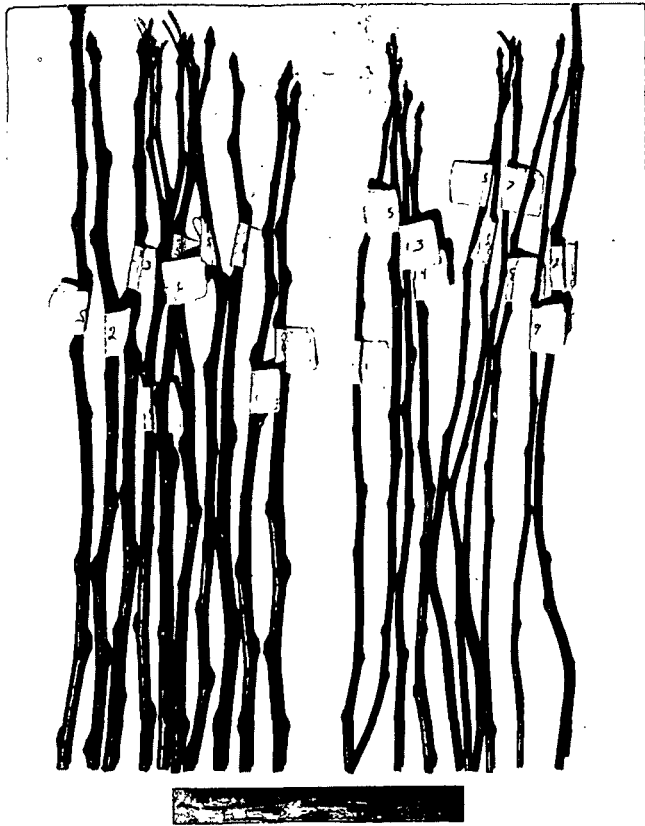


Figure 16. One-Foot Sections from Tall Seedlings (Left) and Short Seedlings (Right), Respectively, Averaging 2.0 and 5.3 Feet in Height

Loss from bacterial contamination was high. However, after two weeks, the four best segments per plate (Fig. 17) showed apparent differences in callus growth on segments from both tall and short seedlings. Each segment was weighed with and without callus, and the percent callus calculated (Table X). Although better callus growth was obtained on Medium W, similar proportionate growth was observed among segments grown on Medium 23.

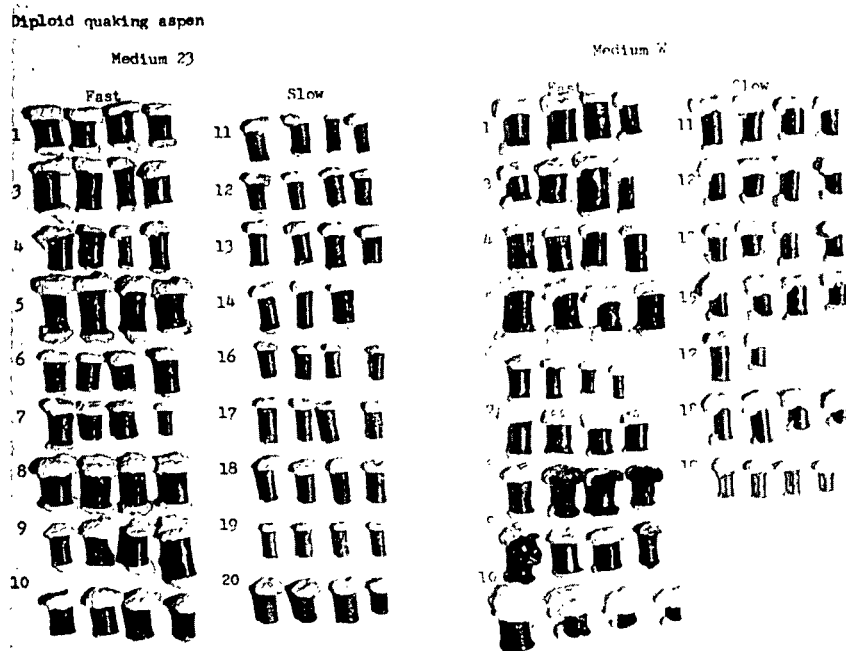


Figure 17. Two Weeks' Growth of Callus from Tall (Fast) and Short (Slow) Seedlings on Media 23 and W

TABLE X

PERCENT CALLUS PRODUCTION^a

		Two Weeks' Growth		One Week
		Medium 23	Medium W	Medium W
Tall	1	55	48	68
	2	--	--	74
	3	50	52	64
	4	30	34	55
	5	52	57	55
	6	38	40	--
	7	39	44	65
	8	52	49	51
	9	53	43	69
	10	49	74	57
Short	11	44	56	54 ^b
	12	42	71	64 ^b
	13	50	55	58
	14	31	--	49
	15	--	65	49
	16	29	--	43 ^b
	17	30	49	58 ^b
	18	53	74	58 ^b
	19	16	44	58 ^b
	20	40	--	53 ^b

^aC = callus weight, S = segment weight, % callus = (C/C+S) x 100.

^b-- Died during the winter of 1965-66.

On November 16 the same seedlings were again sampled, except that pieces were sterilized for 30 minutes and were placed only on Medium W. After four weeks, only one to three of the original ten segments per tree were free of infection. This time the differences in callus growth between seedlings were not so evident (after four weeks) as for the previous two-week trial. The twenty test seedlings were outplanted in the Greenville Test Area in 1966. Tree height will be recorded every 3-5 years and correlated with initial callus growth.

1966 Studies

Between October 13 and 27, juvenile plants from four species of Populus were lifted from the Greenville Nursery and brought to the greenhouse for measurements and collections of stem material. Twelve tall (4.8 feet) and 17 short (1.9 feet) seedlings were tested from Cross XT-2-66 of diploid P. tremuloides parentage. In addition, twelve seedlings each were tested from two clones of triploid P. tremuloides (T-2-56 and T-2-65), Cross XG-19-66 of P. grandidentata, Cross XD-34-66 of P. deltoides, and Cross XCa-23-66 of P. canescens.

Stem material was sterilized for 20-30 minutes, and 5-7-mm.-long segments were placed on agar Medium W. The ratios of callus/callus + segment will be calculated and reported next year. These ratios will then be correlated with future height measurements of the outplanted trees.

STUDIES OF NATURAL VARIATION

VARIATION AND HERITABILITY OF WOOD AND GROWTH CHARACTERISTICS OF FIVE-YEAR-OLD QUAKING ASPEN

Introduction

The long-term nature of forest genetics research emphasizes the importance of careful planning in the selection of wood, fiber, and growth characteristics to be stressed in an intensive tree improvement program. Satisfactory selection of properties to be emphasized can be made if the researcher knows those characteristics which influence the production or quality of the final product; the natural variation of the characteristics under consideration; the degree of genetic control, "heritability," that exists over these same characteristics; and the interrelationships, "correlations," that exist between the several selected characteristics.

Recent studies on species within the genus Populus indicate that considerable natural variation occurs in rate of growth, morphological characteristics, and wood properties. The study described was established to increase our knowledge regarding the variation and "heritability" of selected wood, fiber, and morphological characteristics believed to be important in the production of pulp and paper and provide important information on growth, wood, and pulp property interrelationships.

Methods and Materials

The heritability and correlation data presented are based upon measurements made on a total of 25 five-year-old quaking aspen (Populus tremuloides) full-sib families growing near Appleton, Wisconsin. These families were produced from control crosses made in the greenhouse and field planted in single, nonreplicated, 55-tree blocks in 1958, 1959, and 1960. The test site was a very uniform area having a silt loam texture, and a water table at a depth of 5-6 feet during the

growing season. A total of ten experimental crosses were field planted in 1958, six crosses in 1959, and nine crosses in 1960. The parent trees, although above average in general appearance, were not selected for any specific form or wood quality characteristic.

After the families had been grown in the fields for five years, the size and form of all surviving trees were measured and randomly selected trees were harvested for use in providing information on fiber length, specific gravity, and pulping characteristics. Twenty trees from each family were randomly selected as a source of specific gravity data. Ten of these were further sampled for fiber length information and, of the ten fiber length trees, five were randomly selected for use in obtaining pulping information.

The wood samples used were from a twelve-inch section taken at a height of 14 inches (14-26) above the ground. The samples contained five annual rings and were cut into a series of 1/2-inch-thick disks. The disks were used as a source of chips for the micropulping work and wedges for specific gravity and fiber length determinations. Specific gravity determinations were run in duplicate and the disks used for specific gravity determinations were also used as a source of fibers for the fiber length measurements. The fiber length data were based upon samples from the third, fourth, and fifth annual rings and the measurements consisted of measuring at least five hundred fibers for each growth ring. All fibers over 0.2 mm. including those cut, broken, and intact, were measured.

Information on the pulping potential of the experimental materials was obtained by micropulping chip samples of five randomly selected trees from each experimental cross. Duplicate determinations were made for each tree. The micropulping procedure used employed a kraft pulping system and a multiunit digester [van Buijtenen, et al. (3)]. The techniques used and the cooking conditions

employed are reported in detail in a recent paper by Gardner and Einspahr (4). The yield data presented are the percent yield of pulp and are based upon equivalent weights of wood in each digester. The permanganate number is a measure of the lignin remaining in the pulp after cooking. The adjusted pulp yield was calculated by subtracting the percent lignin in the pulp from the pulp yield. Zero-span tensile strength measurements were conducted on test handsheets using the procedure described by Wink and Van Eperen (5) and are interpreted as a measure of individual fiber strength. Alcohol-benzene extractives and percent lignin were determined using TAPPI Standard methods T 6 m-54 and T 13 m-54. Similar procedures were used to obtain "wood quality" data for parent trees using breast high increment core samples. Micropulping core samples were not available for all parent trees and thus limited the usefulness of the data.

Statistical procedures include calculating simple correlations and multiple regression information on the measured characteristics as well as the calculation of the heritability (h^2) estimates based upon progeny-parent tree regression and analysis of variance inner class correlations. Statistical procedures followed the guide lines of Snedecor (6) and the interpretation of results were based upon the procedures described by Falconer (7). The data and calculations were grouped by the year the progeny groups were planted and the heritability values are the average values based on these groups. Heritability estimates based upon interclass correlations are interpreted as estimating the upper limit of heritability while h^2 values based upon progeny-midparent regressions are considered to be valid estimates of narrow-sense heritability.

Summary of Results

Survival and growth of the 25 full-sib families were good and fairly large differences were obtained between families. Table XI summarizes growth

TABLE XI
HERITABILITIES AND WOOD, GROWTH AND PULPING CHARACTERISTICS
BASED UPON MEASUREMENTS OF 25 FIVE-YEAR-OLD FULL-SIB FAMILIES

Characteristic	Average Values Summarized by Year Planted			Heritabilities (h^2) ^c		
	1958 (10) ^a	1959 (6) ^a	1960 (9) ^a	Based on Interclass Correlations	Based on Progeny - Midparent Regression	
Total height, ft.	14.6	14.2	15.9	10.4-17.3	0.69	0.24
Diameter, 4-1/2 ft., in.	1.3	1.1	1.4	0.82-1.6	0.45	0.35
Natural pruning ^d	2.7	2.9	2.8	2.2-3.6	0.26	0.13
Stem straightness ^d	2.7	2.7	2.8	2.1-3.3	0.11	0.59
Crown diameter, ft.	6.6	6.9	6.4	5.0-8.1	0.42	0.29
Specific gravity, g./cc.	0.37	0.37	0.37	0.34-0.40	0.74	0.42
Fiber length, mm.	0.63	0.63	0.59	0.55-0.69	0.58	0.52
Lignin, %	18.5	17.9	17.8	17.1-19.9	0.58	--
Extractives, %	3.7	4.7	4.2	3.0-5.3	0.87	--
Adjusted pulp yield, %	47.9	47.0	46.2	45.2-49.4	0.63	--
Zero-span tensile, lb./in.	63.3	67.2	68.2	58.6-71.8	0.29	--

^aNo. progeny groups planted.

^bMean and range of 25 progeny groups.

^cValues based on interclass correlations are interpreted as setting the upper limits of heritability while values based upon progeny - midparent regression are valid estimates of narrow-sense heritability.

^dTrees ranked from 1 to 5, poor to very good.

and wood quality data and the average heritability estimates. The average total height of the experimental crosses ranged from 10.4 to 17.3 feet at the end of the fifth growing season. Moderately large differences were encountered in wood and fiber properties with specific gravity ranging from 0.343 to 0.402 g./cc. and average fiber length from 0.554 to 0.693 mm. Several experimental crosses show promise for use in pulpwood production and several individuals have been selected from the better families for use in future crossing work.

Simple correlations between tree growth, crown size, and wood and pulp properties pointed out the importance of crown size in obtaining satisfactory height and diameter growth. Fiber length was found to be strongly correlated with height and diameter growth while specific gravity was only weakly, if at all, influenced by rate of growth.

Multiple regressions calculated to investigate wood and pulp properties influencing pulp yield and fiber strength revealed that 62% of the variation encountered in pulp yield could be accounted for by the percent lignin, specific gravity, and fiber length of the wood samples used. Only 26% of the variation encountered in fiber strength (zero-span tensile strength) could be accounted for by the independent variables of specific gravity, permanganate number of the pulp, and formation of the handsheets. The fiber length, lignin, and tension wood content of the wood samples used in the pulping appeared to have little influence on fiber strength. The severity of the pulping process, as measured by the permanganate number, appears to have an important influence on fiber strength. Handsheet formation and wood specific gravity are known to be influenced by fiber dimensions. It appears that the addition of information on fiber width and cell wall thickness and/or fiber coarseness could be expected to improve the usefulness of the fiber strength multiple regression equation.

Narrow sense heritability estimates (\underline{h}^2) based upon interclass correlations and progeny - midparent regression information indicates: (1) moderate to good control over fiber length, specific gravity, extractives, and lignin; (2) limited genetic influence on fiber strength, crown size, and tree form; and (3) moderate possibilities for genetic improvement of height and diameter growth. The heritability estimates for pulping characteristics, because they were based upon full-sib interclass correlations are less reliable than the other \underline{h}^2 estimates.

INTRASPECIFIC AND INTERSPECIFIC CROSSING

The basic plan that has been followed in the Institute crossing program has been to select outstanding parent trees on the basis of form, rate of growth, and wood quality. Crosses are made using the selected parent trees, and, from the experimental crosses produced, the most promising crosses, selected on a basis of early vigor and uniformity, are evaluated in replicated field trials. After 8-12 years in the field trials, outstanding individuals within the crosses will be selected for future crossing work. As has been discussed in earlier progress reports, quaking aspen was the species that was first intensively studied and we are presently in the process of evaluating some of the earlier crosses. Bigtooth aspen and bigtooth aspen hybrids were next to receive major emphasis and the crossing program is nearing completion and evaluation work is under way. Cottonwood crosses, although made in limited numbers during the past three or four years, will receive additional emphasis in the coming three years.

The 1966 crossing program followed a pattern similar to that established in previous years. Crosses were made involving the three major Lake States species of Populus (quaking aspen, bigtooth aspen, and cottonwood) as parents. Major emphasis was placed on producing bigtooth aspen crosses and hybrids* well suited for growth on dry sandy sites. Quaking aspen crosses received the least attention in 1966.

Twenty-nine different parent trees including nine quaking aspen, seven bigtooth aspen, seven cottonwood, three European aspen, and three European gray

^aThroughout this report the term hybrid has been used to designate progeny produced as a result of crossing parents of two different species (interspecific). The term cross has been used when the parents were of the same species (intraspecific).

poplar were employed and a total of 45 crosses were attempted. In addition, five sources of open-pollinated bigtooth aspen seed and five sources of open-pollinated quaking aspen seed were produced for shipment to West Germany. Three sources of open-pollinated cottonwood seed were also produced for shipment to Turkey.

Table XII summarizes the parent trees utilized in the crossing program and Tables XIII and XIV provide additional information on crossing success, seedling size, and seedling production. Some seed produced in 1965 was used in producing seedlings in 1966 and Table XIV includes data on seedlings produced from such seed. Figure 18 illustrates the size of 1-0 seedlings produced in 1966.

QUAKING ASPEN CROSSES

A total of sixteen crosses were made in which either one or both parents were quaking aspen (T). Four crosses were made (XT-1-66 through XT-4-66) to evaluate the crossing behavior of two new parent trees and provide seedlings suitable for use in field evaluation work. Two quaking aspen crosses were made as part of studies on the production of polyploids. The majority of the remaining crosses involved quaking aspen female trees that were crossed with pollen from a Swedish tetraploid European aspen. The objective of the latter group of crosses was the production of triploid hybrids. Unfortunately, at the last minute, it was necessary to substitute frozen, one-year-old pollen for fresh pollen. Seed set and seedling production were greatly reduced and only a limited number of individuals were produced.

Several crosses were also made in which P. canescens (Ca) was one parent and quaking aspen (T) was the other. The seedlings produced as part of the "quaking aspen crosses" will be used in a replicated field trial and in limited numbers in plantings on company land.

TABLE XII

SUMMARY OF CROSSES AND LOCATION OF PARENT TREES

Cross No. ^a	Parents (female x male)	
XT-1-66	T-1-58 (Porcupine Mts., Mich.)	X T-10-60 (Porcupine Mts., Mich.)
XT-2-66	T-1-58 (Porcupine Mts., Mich.)	X XT-22-56, S-4 (Greenville, Wis.)
XT-3-66	T-29-63 (Thousand Island Lake, Mich.)	X T-10-60 (Porcupine Mts., Mich.)
XT-4-66	T-29-63 (Thousand Island Lake, Mich.)	X XT-22-56, S-4 (Greenville, Wis.)
XCa-T-5-66	Ca-2 (Czechoslovakia)	X T-10-60 (Porcupine Mts., Mich.)
XCa-T-6-66	Ca-2 (Czechoslovakia)	X T-20-60 (Alston, Mich.)
XTa-7-66	Ta-5, no. 13 (Appleton, Wis.)	X Ta-5, no. 2 (Appleton, Wis.)
XT-Ta-8-66	T-1-58 (Porcupine Mts., Mich.)	X Ta-10 (4n) (Ekebo, Sweden)
XT-Ta-9-66	T-29-63 (Thousand Island Lake, Mich.)	X Ta-10 (4n) (Ekebo, Sweden)
XT-Ta-10-66	T-20-56 (Watersmeet, Mich.)	X Ta-10 (4n) (Ekebo, Sweden)
XT-Ta-11-66	XT-22-56, S-5 (Greenville, Wis.)	X Ta-10 (4n) (Ekebo, Sweden)
XT-Ta-12-66	T-5-63 (Felch, Mich.)	X Ta-10 (4n) (Ekebo, Sweden)
XG-Ta-13-66	G-10-62 (Bonita, Wis.)	X Ta-10 (4n) (Ekebo, Sweden)
XG-14-66	G-9-63 (Bruce, Wis.)	X Ta-10 (4n) (Ekebo, Sweden)
XT-15-66	T-5-63 (Felch, Mich.)	X T-10-60 (Porcupine Mts., Mich.)
XG-16-66	G-12-60 (Black River Falls, Wis.)	X G-1-65 (Breed, Wis.)
XG-17-66	G-12-60 (Black River Falls, Wis.)	X G-2-66 (Hazelhurst, Wis.)

TABLE XII (Continued)

SUMMARY OF CROSSES AND LOCATION OF PARENT TREES

Cross No. ^a	Parents (female x male)	
XG-18-66	G-1-66 (Irma, Wis.)	X G-1-65 (Breed, Wis.)
XG-19-66	G-1-66 (Irma, Wis.)	X G-2-66 (Hazelhurst, Wis.)
XG-A-20-66	G-12-60 (Black River Falls, Wis.)	X A-1-65 (Czechoslovakia)
XG-A-21-66	G-12-60 (Black River Falls, Wis.)	X A-1-66 (Czechoslovakia)
XG-A-22-66	G-12-60 (Black River Falls, Wis.)	X A-1, #2 (Appleton, Wis.)
XG-Ca-23-66	G-12-60 (Black River Falls, Wis.)	X Ca-1-62 (Czechoslovakia)
XG-Ca-24-66	G-12-60 (Black River Falls, Wis.)	X Ca-1-65 (Czechoslovakia)
XG-Ca-25-66	G-1-66 (Irma, Wis.)	X Ca-1-62 (Czechoslovakia)
XCa-G-26-66	Ca-2 (Czechoslovakia)	X G-1-65 (Breed, Wis.)
XCa-G-27-66	Ca-2 (Czechoslovakia)	X G-2-66 (Hazelhurst, Wis.)
XCa-28-66	Ca-2 (Czechoslovakia)	X Ca-1-62 (Czechoslovakia)
XCa-29-66	Ca-2 (Czechoslovakia)	X Ca-1-65 (Czechoslovakia)
XT-30-66	T-16-56 (Greenville, Wis.)	X T-20-60 (Alston, Mich.)
XT-Ca-31-66	T-1-58 (Porcupine Mts., Mich.)	X Ca-1-62 (Czechoslovakia)
XT-Ca-32-66	T-1-58 (Porcupine Mts., Mich.)	X Ca-1-65 (Czechoslovakia)
XD-33-66	D-2-63 (Sherwood, Wis.)	X D-1-63 (Waupaca, Wis.)
XD-34-66	D-2-63 (Sherwood, Wis.)	X D-6-65 (Nichols, Wis.)

TABLE XII (Continued)

SUMMARY OF CROSSES AND LOCATION OF PARENT TREES

Cross No. ^a	Parents (female x male)	
XD-35-66	D-2-63 (Sherwood, Wis.)	X D-9-65 (Wittenberg, Wis.)
XD-36-66	D-2-63 (Sherwood, Wis.)	X D-2-66 (Navarino, Wis.)
XD-37-66	D-2-63 (Sherwood, Wis.)	X D-4-66 (Seymour, Wis.)
XD-38-66	D-8-65 (Nichols, Wis.)	X D-1-63 (Waupaca, Wis.)
XD-39-66	D-8-65 (Nichols, Wis.)	X D-6-65 (Nichols, Wis.)
XD-40-66	D-8-65 (Nichols, Wis.)	X D-9-65 (Wittenberg, Wis.)
XD-41-66	D-8-65 (Nichols, Wis.)	X D-2-66 (Navarino, Wis.)
XD-42-66	D-8-65 (Nichols, Wis.)	X D-4-66 (Seymour, Wis.)
XG-Ca-43-66	G-22-60 (Black River Falls, Wis.)	X Ca-1-62 (Czechoslovakia)
XG-Ca-44-66	G-64 (Wausau, Wis.)	X Ca-1-62 (Czechoslovakia)
XD-T-45-66	D-8-65 (Nichols, Wis.)	X T-Pollen Mixture

^aX = cross, A = P. alba, Ca = P. canescens, D = P. deltoides, G = P. grandidentata, S = selection, T = P. tremuloides, Ta = P. tremula.

TABLE XIII
SUMMARY OF 1966 CROSSES

Cross No. ^a	Type Cross ^b	No. of Catkins		Amt. Seed ^c	Seeds/Catkin ^c	Germ., % ^c
		Pollinated	Collected			
XT-1-66	C	32	31	1,720	53	99
XT-2-66	C	39	38	10,998	279	99
XT-3-66	C	31	18	856	11	40
XT-4-66	C	28	25	7,689	269	98
XCa-T-5-66	DS	109	30	189	0.6	34
XCa-T-6-66	DS	110	93	5,814	52	98
XTa-7-66		36	26	1,655	18	40
XT-Ta-8-66	P	30	30	506	8	46
XT-Ta-9-66	P	47	47	4,299	79	86
XT-Ta-10-66	P	21	17	439	4	20
XT-Ta-11-66	P	8	8	362	4	9
XT-Ta-12-66	P	28	22	289	0.3	3
XG-Ta-13-66	P	13	12	575	5	18
XG-Ta-14-66	P	10	10	633	17	27
XT-15-66	C	35	0	--	--	--
XG-16-66	DS	52	1	3	--	--
XG-17-66	DS	62	5	104	0.03	2
XG-18-66	DS	25	23	3,003	24	20
XG-19-66	DS	47	39	2,711	13	23
XG-A-20-66	DS	13	0	--	--	--
XG-A-21-66	DS	9	0	--	--	--
XG-A-22-66	DS	13	3	4	--	--
XG-Ca-23-66	DS	66½	15	1,083	0.49	3
XG-Ca-24-66	DS	25	0	--	--	--
XG-Ca-25-66	DS	40	18	467	5	42
XCa-G-26-66	DS	100	81	1,407	14	99
XCa-G-27-66	DS	100	91	12,965	128	99
XCa-28-66	DS	85	79	8,352	97	99
XCa-29-66	DS	75	54	1,035	3	24
XT-30-66	P	4	3	1,499	360	96
XT-Ca-31-66	DS	60	60	300	2	35
XT-Ca-32-66	DS	19	18	6	--	--
XD-33-66	B	15	13	1,971	16	12
XD-34-66	B	12	7	1,775	96	65
XD-35-66	B	14	9	22	0.02	2
XD-36-66	B	14	12	332	--	0
XD-37-66	B	15	5	353	2	8
XD-38-66	B	12	4	39	--	0
XD-39-66	B	11	10	965	--	0
XD-40-66	B	12	6½	191	1	7
XD-41-66	B	12	11	960	3	4
XD-42-66	B	12	11	928	3	4
XG-Ca-43-66	DS	13	13	2,982	37	16
XG-Ca-44-66	DS	3	3	226	--	0
XD-T-45-66	H	11	11	1,037	2	2.6

^aX = cross, A = *P. alba*, Ca = *P. canescens*, D = *P. deltoides*, G = *P. grandidentata*, T = *P. tremuloides*, Ta = *P. tremula*.

^bC = seed for semicommercial production, DS = dry site cross, B = crosses in black poplar group, P = polyploid cross, H = haploid cross.

^cAmount of seed, seeds/catkin pollinated and germination percent based upon 40 mesh and larger seed with the following exceptions: crosses 18, 19, 23, 25, 43, and 44 based upon 40 and 50 mesh seed; crosses 16 and 17 based upon 50 mesh seed.

TABLE XIV
SUMMARY OF 1966 SEEDLING PRODUCTION

Cross No. ^a	Total No. Seeds Planted	Total No. Plantable Seedlings Produced	No. Plantable _b Seedlings		Average Height ^c	
			Misc. Beds	Repl. Beds	All Seedlings	Plantable Seedlings
XCa-T-7-65	1500	259	159	100	3.6	3.6
XCa-T-8-65	2500	445	204	241	2.8	2.9
XCa-G-20-65	480	70	-	70	3.7	3.9
XG-23-65	4025	281	137	144	2.1	2.4
XG-24-65	1725	108	-	108	2.3	2.6
XG-32-65	1725	191	-	191	2.8	3.0
XG-33-65	1200	105	-	105	2.7	2.9
XT-Ca-35-65	3150	336	136	200	3.7	3.8
XD-37-65	520	160	160	-	3.7	3.8
XD-44-65	500	29	29	-	3.1	3.1
XT-1-66	1600	378	31	347	3.3	3.3
XT-2-66	2400	642	248	394	2.8	2.9
XT-3-66	835	216	-	216	3.2	3.2
XT-4-66	1200	275	-	275	2.8	2.9
XCa-T-5-66	210	49	49	-	3.7	3.9
XCa-T-6-66	2800	602	460	142	3.6	3.6
XTa-7-66	1555	80	80	-	2.5	2.6
XT-Ta-8-66	515	112	112	-	2.4	2.5
XT-Ta-9-66	4115	364	209	155	2.8	2.9
XT-Ta-10-66	445	68	68	-	2.4	2.7
XT-Ta-11-66	375	32	32	-	2.1	2.4
XT-Ta-12-66	290	16	16	-	1.6	2.0
XG-Ta-13-66	110	53	53	-	2.4	2.6
XG-Ta-14-66	215	84	84	-	2.6	2.8
XG-18-66	1415	201	-	201	2.9	3.1
XG-19-66	2005	106	-	106	2.1	2.2
XG-Ca-23-66	1110	26	26	-	1.8	2.3
XG-Ca-25-66	300	62	62	-	3.4	3.7
XCa-G-26-66	3100	584	123	461	3.7	3.8
XCa-G-27-66	2800	524	294	232	3.8	3.9
XCa-28-66	1600	440	440	-	3.2	3.2
XCa-29-66	800	154	-	154	4.4	4.4
XT-30-66	1500	108	108	-	2.2	2.5
XT-Ca-31-66	300	94	94	-	2.9	3.0
XD-33-66	545	246	246	-	3.4	3.4
XD-34-66	675	415	415	-	3.1	3.1
XD-37-66	440	25	25	-	3.4	3.4
XD-41-66	1500	5	5	-	3.1	3.1
XD-42-66	2220	23	23	-	4.1	4.1
XG-Ca-43-66	800	29	29	-	1.5	2.0

^aX = cross, A = *P. alba*, Ca = *P. canescens*, D = *P. deltoides*, G = *P. grandidentata*,
T = *P. tremuloides*, Ta = *P. tremula*.

^bNumber of plantable seedlings, 1.4 or larger in height and of satisfactory caliper.

^cAverage heights based upon seedlings in replicated seedbeds; when replicated beds were not available, miscellaneous seedbeds were measured.



Figure 18. Growth of the 1966 Seedbeds at the Greenville Nursery Was Good. Most of the Materials Shown Were Produced from Seed. The 1-0 Rooted Root Sprouts of AG-1-60 (Center), a Natural "Alba x Bigtooth" Hybrid, Averaged Seven Feet in Height

BIGTOOTH ASPEN CROSSES

Bigtooth aspen crosses (G) and bigtooth aspen hybrids* continued to receive a major amount of emphasis. The principal objective of the production of crosses utilizing bigtooth aspen as one or both parents is to find suitable genetic combinations that will do well on dry sandy sites.

Seventeen crosses were attempted in 1966 and of this number only seven produced seedling numbers in excess of fifty. Low seed production and reduced germination were responsible for the somewhat reduced number of individuals

*The term crosses has been used when both parents are the same species; G x G, T x T, etc. Hybrids are crosses in which the two parents are different species of Populus; G x Ca, G x A, Ca x G, etc.

available for field planting in 1967. Growth of the bigtooth aspen crosses was good with several of the "canescens x bigtooth" (Ca x G) crosses reaching average heights in excess of 3.5 feet.

The overall poor performance of the bigtooth aspen crosses is believed to be related to the reduced flowering of several of the best female trees and the resulting use of one untested tree and one tree (G-12-60) which has had below normal performance as is evident by past crossing records. Some modification of the methods used in handling the female branch collections may also have influenced seed size and seed production.

Eleven of the crosses involving bigtooth aspen and P. canescens parent trees were arranged in a modified diallel series in such a way that crossing compatibility and overall performance of four male and three female parent trees could be evaluated. Flowering behavior, seeds and plantable seedlings produced, and first-year seedling growth were used in the evaluation of parent trees. Table XV summarizes the results of these determinations. The overall results of this group of crosses are exceptionally poor. Only Crosses XCa-G-27-66 and XCa-28-66 produced near normal numbers of seeds. The female tree Ca-2, which had been evaluated earlier and was believed to be a good parent tree, turned out to be the best female in the series. G-12-60, a female from near Black River Falls, Wisconsin, performed very poorly and since it had below normal behavior in earlier evaluations, will be dropped as a parent tree. When the data on the male trees were compared, G-2-66 and Ca-1-62 were found to be the best two males. G-1-65, when evaluated in 1965, did much better and its poor performance was due in part to the below normal behavior of the two bigtooth aspen female trees used in the comparison.

TABLE XV
SEED AND SEEDLING PRODUCTION AND SEEDLING GROWTH
MODIFIED DIALLEL CROSSING SERIES

Female Parent Trees	Male Parent Trees			
	G-1-65	G-2-66	Ca-1-62	Ca-1-65
G-12-60	XG-16-66	XG-17-66	XG-Ca-23-66	XG-Ca-24-66
-- ^a	< 1	< 1	0.5	0
-- ^b	--	--	0.3	--
-- ^c	--	--	1.8	--
G-1-66	XG-18-66	XG-19-66	XG-Ca-25-66	--
-- ^a	24	13	5	
-- ^b	14	3	1.5	
-- ^c	2.9	2.1	3.4	
Ca-2	XCa-G-26-66	XCa-G-27-66	XCa-28-66	XCa-29-66
-- ^a	14	128	97	3
-- ^b	5	24	27	2.6
-- ^c	3.7	3.8	3.2	4.4

^aNumber viable seed produced per catkin pollinated.

^bNumber of plantable seedlings (1.4 feet plus) produced per catkin pollinated.

^cAverage height of all seedlings in seedbeds.

COTTONWOOD CROSSES

Several cottonwood crosses were handled successfully in 1965 using a modification of "cut-branch techniques." The modification involved bringing the branches containing flower parts into the greenhouse and placing the branches in an antibiotic solution and growing the developing catkins in a growth chamber under long days and fairly high light intensity.

During 1966 a modified diallel series of crosses involving two female and five male cottonwoods was established. A procedure using the antibiotic solution and branch collections forced in the growth chamber was employed. The crosses, although not entirely unsuccessful, did not produce the expected number of seeds. Table XVI summarizes the results obtained. Based upon the very limited

number of seedlings produced, D-2-63 appears to be the best female tree and D-6-65 the best performing male tree. During 1967, it is planned that the use of the antibiotic solution and growth chamber in the production of cottonwood seed will be evaluated further.

TABLE XVI

SEED AND SEEDLING PRODUCTION AND SEEDLING GROWTH
 MODIFIED DIALLEL CROSSING SERIES

Female Parent Trees	Male Parent Trees				
	D-1-63	D-6-65	D-9-65	D-2-66	D-1-63
D-2-63	XD-33-66	XD-34-66	XD-35-66	XD-36-66	XD-37-66
-- ^a	16	96	< 1	0	2
-- ^b	2	41	--	--	1.7
-- ^c	3.4	3.1	--	--	3.1
D-8-65	XD-38-66	XD-39-66	XD-40-66	XD-41-66	XD-42-66
-- ^a	0	0	1	3	3
-- ^b	--	--	--	1.0	2.8
-- ^c	--	--	--	3.1	4.1

^a Number of viable seed per catkin pollinated.

^b Number of plantable seedlings (1.4 feet plus) per catkin pollinated.

^c Average height of all seedlings in seedbeds.

PLANS FOR 1967

Four main areas of investigation will be emphasized by the Genetics and Physiology Group in 1967. These areas will include: (1) selection and hybridization of outstanding trees, (2) production of artificial polyploids, (3) studies of natural variations with special emphasis on wood quality, (4) tree physiology studies aimed at investigating the nutrient requirements of aspen hybrids and studies investigating ways of stimulating early flowering in aspen.

Selection and hybridization studies will continue to emphasize the production of trees for use in "dry site" plantings. Considerable effort will also be directed toward the production of cottonwood crosses suitable for use on "wet sites" in central and south central Wisconsin. Work will also be continued in the production of tetraploid cottonwood, bigtooth aspen, and quaking aspen for use in the mass production of triploids and triploid hybrids.

Studies of natural variation will again make up an important part of the overall program. Completed during the past year and reported in this report was the investigation into the "Variation and Heritability of Wood and Growth Characteristics of Five-Year-Old Quaking Aspen." Investigations, either just under way or in various stages of completion, include: (1) nature of within tree variation in specific gravity, (2) within tree variation in fiber length, and (3) natural variation of growth and wood properties of bigtooth aspen. Growth chamber studies on the nutrient requirements of aspen hybrids will be completed and evaluated during the coming year, prior to establishment of additional studies related to "physiology of establishing and growing of aspen on adverse sites."

PUBLICATIONS

PUBLICATIONS RELATING TO PROJECT 2412 SINCE MAY, 1966

1. Einspahr, Dean W., Benson, M. K., and Peckham, J. R. Variation and heritability of wood and growth characteristics of five-year-old quaking aspen. Genetics & Physiology Notes No. 1, Appleton, Wisconsin, The Institute of Paper Chemistry.

FUTURE PUBLICATIONS

1. Benson, M. K., Einspahr, D. W., and Schwalbach, D. E. Rooting of quaking aspen root sprouts. To be submitted to 'Tree Planters' Notes.
2. Benson, M. K., and Einspahr, D. W. Early growth of diploid, triploid and triploid hybrid aspen. Accepted by Forest Sci.
3. Einspahr, D. W., and Benson, M. K. Comparison of wood, fiber, and pulp properties of naturally occurring and artificially produced triploid aspen with diploid aspen. To be submitted to Tappi.
4. Einspahr, D. W., Benson, M. K., and Peckham, J. R. Geographic variation in growth and wood properties of quaking aspen. Submitted to Silvae Genetica.
5. Einspahr, D. W., and Benson, M. K. Management of aspen on ten to twenty-year rotations. Accepted by Journal of Forestry.
6. Mathes, M. C., and Einspahr, D. W. The chemical composition of quaking aspen tissue. Submitted to Canadian Journal of Botany.
7. Winton, L. L. Fertilization in forced quaking aspen and cottonwood. Accepted by Silvae Genetica.
8. Winton, L. L. Cytotechniques for aspen chromosomes. To be submitted for publication.
9. Winton, L. L. Estimating tree growth from callus production of juvenile aspen and cottonwood. To be submitted for publication.
10. Winton, L. L., and Einspahr, D. W. Colchicine-polyploids of quaking aspen. To be submitted for publication.

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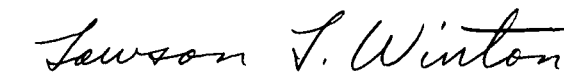
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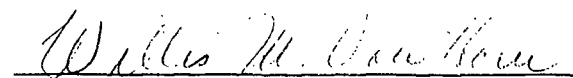
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APPENDIX

TABLE XVII

TOP-ROOT RATIOS^a

Growth Chamber Run	Experimental Test Material			
	1	2	3	4
N	1.15	1.34	1.46	0.98
	1.29	0.91	1.16	1.23
	1.30	1.18	1.78	2.00
	1.53	0.92	1.34	1.36
	1.40	1.04	1.38	1.48
P	--	--	--	--
	1.17	1.22	1.70	1.22
	1.16	0.90	1.41	1.14
	1.01	1.09	1.34	1.24
	1.01	0.80	1.68	1.68
K	--	--	--	--
	1.30	1.00	1.19	1.06
	1.12	0.98	1.28	1.08
	1.21	0.86	1.50	1.27
	1.18	0.86	1.30	1.47
Ca	0.70	0.98	1.64	1.65
	1.14	0.85	1.08	1.17
	1.50	0.98	1.32	1.59
	1.10	0.88	1.10	1.08
	1.32	1.20	1.42	1.50
Mg	1.76	1.30	1.60	0.93
	1.64	1.02	1.57	1.03
	1.46	1.06	1.59	1.54
	1.24	1.22	1.66	1.74
	1.40	1.22	1.70	1.51

^aValues listed within each cell in the two-way table are the top-root ratios obtained for the none or very low, low, medium, medium high, and high level of the nutrient being varied.