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

Project 2412

Report Eight

A Progress Report

to

LOUIS W. AND MAUD HILL FAMILY FOUNDATION

May 20, 1968

THE INSTITUTE OF PAPER CHEMISTRY

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SUMMARY

Investigations under way during the past year include a wide variety of studies aimed at developing improved aspen that can be used to increase the annual per acre yield of usable wood. Work continued on developing and locating outstanding polyploid and diploid trees. Discovery of a hybrid aspen that in nineteen years reached a diameter of 19.6 inches and a height of 70 feet confirmed the growth potential of such hybrids under ideal growing conditions.

Summaries of growth chamber studies designed to evaluate the growth and nutrient requirements of hybrid aspen are presented. The hybrids tend to be intermediate in nutrient requirements. One of the hybrids exhibited hybrid vigor, i.e., the hybrid exceeded the growth of the parent species.

Wood property variation in Populus was investigated by tabulating data from 494 parent trees and 753 five- and six-year-old progeny. Variation in specific gravity, fiber length, fiber strength, pulp yield, lignin, and extractives were investigated. Comparisons between parent trees and five- and six-year-old trees emphasized the favorable wood property position of young aspen.

Preliminary specific gravity, moisture content, and bark measurements made on medium and small-size aspen stems demonstrated fairly consistent specific gravity differences between wood and bark. The differences obtained suggest reasons for the success of the flotation procedure being used to separate chips and bark. Moisture content varied with the season, the wood having the highest moisture content during the dormant season and the bark the highest content during the growing season.

The 1967 crossing program was one of the largest ever undertaken. A total of 69 crosses, employing 39 parent trees, were attempted. Emphasis was divided between a series of cooperative quaking aspen and European aspen crosses scheduled for planting in the Lake States and West Germany and the production of bigtooth aspen crosses and bigtooth aspen hybrids suitable for dry site plantings.

INTRODUCTION

When the growth rate of native forests are compared on a region by region basis, Lake States Forestry has reason to be concerned. Data from the United States Forest Service publication "Timber Trends" indicates the average growth for commercial forest lands in the Lake States area is only 22 cubic feet per acre per year compared to 42.3 cubic feet per acre per year for the south Atlantic states and 46.8 cubic feet per acre per year for the Pacific northwest region. There are, however, a number of bright spots in the Lake States forest production picture, one of which is the indispensable nature of hardwood fibers in papermaking. The expanded use of hardwood fiber in the production of books, magazines and tissue papers and increased use of hardwoods for corrugating board has led to the prediction that hardwood use will quadruple by the year 2000. This suggests that in the next 33 years hardwood use will increase to 3.3 billion cubic feet and at this new level will amount to 40% of the total domestic roundwood production.

The northern forests, and the Lake States region in particular, could be in a position to supply a large portion of this predicted demand if proper forestry practices are instituted. Aspen, because it regenerates easily, grows on a wide variety of upland soils, grows in stands well adapted to mechanical harvesting, and has satisfactory wood properties even at relatively young ages, could logically be expected to be a major future source of hardwood pulp. Land use trends and mechanization of harvesting operations serve to emphasize the need for improved varieties that will do well on light-textured upland sites and that will respond to such intensive forestry practices as fertilization and irrigation. The report that follows describes the progress made during the past year in selection, hybridization and polyploidy; all approaches aimed at producing improved materials that will help meet our ever-increasing requirements.

SECURING AND PROPAGATING DESIRABLE POLYPLOID AND DIPLOID ASPEN AND COTTONWOOD

PRODUCTION OF HAPLOID AND POLYPLOID* ASPEN

Haploid Production

Introduction

Haploid trees are trees that have a single set of chromosomes instead of the normal two sets. Such individuals are of interest because, when by chemical means the chromosome number is doubled back to the normal number, the newly produced diploids will have two sets of chromosomes that are genetically identical. Because such "homozygous" individuals will breed true for all genetically controlled characteristics, they are extremely valuable to a breeding program.

The report that follows describes studies under way on the use of heat-treated pollen in the production of haploids. The principle involved is "stimulation without fertilization." It is believed that the heat treatment will weaken the pollen to the extent that the pollen nucleus will not fertilize the egg but the presence of the pollen and pollen tube will stimulate a small percentage of the unfertilized eggs (haploid embryo) to develop into haploid individuals. Figure 1 shows pollen from a normal diploid quaking aspen prior to treatment. Also under way as part of this study and not reported in this report are investigations on ways of evaluating pollen viability. Pollen germination tests using sucrose-agar medium and the tetrazolium test for viability are the approaches being investigated.

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*See glossary for information on terminology used in this section.

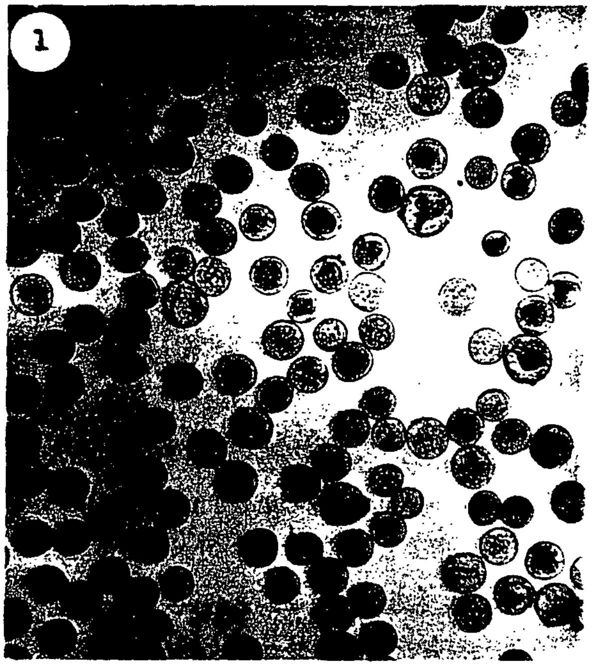

Figure 1. Untreated Diploid Quaking Aspen Pollen T-13-58, 220X

Heat-Treated Pollen

Kopecky (1) left pollen of Populus alba in a closed, humid test tube in the greenhouse for one week, and used the weakened pollen to produce 3.2% haploids from among 0.2% of the viable seed. We produced weakened pollen with dry heat, using one "Penetray" infrared lamp, suspended 11 inches above the bench top. This was directed down through the top of a tripod having foil wrapped between two of the three spaces between the legs. On top of three rubber stoppers on the bench top, a watchglass containing pollen maintained a temperature of 50°C. By lowering the lamp to the top of the tripod, a temperature of 100°C. was reached.

Pollen from T-13-58 was given 50°C. for 0, 0.2, 1, 5, and 10 minutes, as well as 100°C. for 10 seconds. Some of the pollen was tested with tetrazolium*; and after 1.5 hours, pollen heated at 50°C. for one minute or less was pink, but color was faint or colorless with treatments of 5 and 10 minutes. About half of the pollen receiving 100°C. was purple. A second test was made at one-minute intervals. When tested with tetrazolium, pollen grains turned from pink to purple betwee the two and three minute treatments, and grains were all purple that received 100°C. for 10 and 30 seconds. The pollen stuck slightly to the watchglass, however, when given 5 minutes of 50°C. heat. Dehydration, in this case, may have meant death.

Bioassay of Heat-Treated Pollen (XT-65-67)

Quaking aspen pollen (T-10-60) that received 0, 1, 2, 3.5, 4, and 5 minutes of 50°C. heat was applied to two female catkins of T-80-57 per treatment on March 11, 1967. Twelve days later, catkins ranged from 80-100 mm. in length for the 0-2 minute treatments, but decreased in size to 20-80 mm. for longer treatment times. The number of capsules per catkin also decreased from 150-190 to 7-160 between the 3.5 and 4-minute treatments (Table I). One capsule was collected from each treatment, fixed in FAE solution, aspirated for 30 minutes, dehydrated, embedded in paraffin, sectioned at 10 µm., stained in fast-green safranin, and mounted in balsam. Normal embryos were observed for treatments receiving up to 4 minutes of heat (Fig. 2), but all flower organs were aborted after 5 minutes of heat (Fig. 3). Seeds from all surviving catkins were recovered and planted, and are described later as cross XT-65-67. On the basis of this test, the critical time necessary to weaken but not kill quaking aspen pollen was between four and five minutes.

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*Used 0.5% solution of 2,3,5-triphenyltetrazolium chloride to check pollen viability. Pink and red colors indicate viable pollen, purple inconclusive and colorless indicates nonviable pollen.

TABLE I

SUMMARY OF HAPLOID CROSS XT-65-67 (T-80-57 x T-10-60)

Treatment[a] Number	Treatment[a] 50°C., min.	Catkins Number	Catkins Length, mm.	Catkins Av. Number Capsules	Seed Mesh	Seed Number	Seed Germ., %	Seedlings Post Thinning	Seedlings Selected
1	0	2/2[b]	112	193	40	470	—	--	--
			85	150	50	109	85	88	5
2	1	2/2	97	203	40	308	97	273	3
			83	180	50	82	91	77	3
3	2	2/2	99	183	40	54	85	47	1
			88	190	50	17	71	12	3
4	3.5	2/2	105	180	40	322	93	292	18
			47	90	50	125	80	104	9
5	4	2/1	62	7	40	12	92	11	1
			19	53					
6	5	2/2	80	168	40	16	94	14	1
				165	50	5	100	5	1
						1520		923	45

[a]Duration of heat treatment.

[b]Number catkins pollinated/number collected.

Figure 2. Normal Embryo from Cross with Quaking Aspen Pollen Treated for Four Minutes at 50°C., 63X

Figure 3. Abnormal Embryo from Cross with Pollen Treated for Five Minutes at 50°C., 63X

Heat-Treated Canescens Pollen

Stored pollen of Populus canescens was treated for 3, 3.5, 4, 4.5, and 5 minutes at 50°C. Pollen began to stick to the watchglass after 4.5 minutes. In tetrazolium, pollen receiving 4 minutes or less of heat were pink and spherical; but at 4.5 and 5 minutes, grains were shriveled and pink or red. An interesting photomicrographic study was made of this series of pollen, showing different markings on grains from different treatments, but this pollen was not used to pollinate female catkins.

Heat-Treated Alba Pollen (XT-A-69-67)

The use of heat-treated pollen from Populus alba was originally proposed for this study by Einspahr, so that hybrids (diploids) would be easily recognized by their leaf pubescence. This pollen was in short supply in 1967, but 2-3 ml.

were given 50°C. heat for each of the treatments of 3.5, 4.0, 4.25, 4.5, and 5.0 minutes. Pollen A-3-67 had been stored in the refrigerator over $CaCl_2$ and was shriveled, but did not stick to the watchglass until after receiving 5.0 minutes of heat. This series of treated pollen was used for the main cross XT-A-69-67. A small amount of each treated pollen was tested with tetrazolium, but the results were not conclusive. Pink grains were occasionally found in untreated pollen, and sporadic pink grains were observed for up to and including the 4.5-minute treatment. However, all pollen was colorless in tetrazolium after receiving 5.0 minutes of heat. An extra series of treatments showed that after 6, 10, or 30 minutes of heat, pollen grains decrease in size. However, with increasing heat from 0-4.25 minutes, the main change in the pollen was the reduction of the size of the nucleus. At 4.5 and 5.0 minutes, the nucleus was almost full again, but may have been damaged by the heat treatment.

Pollination, Seed Recovery, and Seedling Growth

The cross XT-A-69-67 was made in the greenhouse in April, 1967, and seed collected, sorted, and planted in flats. Some of the treated and untreated catkins receiving treated P. alba pollen were dipped in different concentrations of indole-3-acetic acid (IAA) before and after the stigmas dried up to stimulate apomixes. After thinning out the obviously normal seedlings and those with pubescence, the remaining plants were individually potted. The crossing and survival data are summarized in Table II.

Chromosome Counts

Expanding leaves (1-cm. long) were pretreated in saturated paradichlorobenzene for four hours at 3°C., then fixed for 24 hours each in 1:3 acetic-acid-ethanol with and without 5% $FeCl_3$. Leaves were hydrolyzed for 10 minutes at

TABLE II

SUMMARY OF CROSS XT-A-69-67 (T-16-56 x A-3-67)

Treatment[a]			Number	Seed		
Number	50°C., min.	IAA, p.p.m.	Catkins	Mesh	Number	Germ., %
7	0	--	2/1[b]	40	27	11
				50	12	0
8	2	--	2/1	28	10[c]	10
				40	89[c]	24
				50	14[c]	0
9	3.5	--	--	28	403	--[d]
				40	1857	--[d]
				50	494	1
				60	4	0
10	4	--	20/19	28	303	--[d]
				40	1186	--[d]
				50	248	1
11	4	H_2O	2/1	--	--[c]	--
12	4	10 IAA	2/2	40	167	16
				50	39	5
13	4	100 IAA	2/0	--	--	--
14	4	1000 IAA	2/1	40	48	6
				50	20	0
				60	1	0
15	4.5	--	20/14	28	266	--[d]
				40	741	--[d]
				50	353	0
				60	3	0
16	6	--	2/1	40	9	11
				50	2	0
					6296	

[a]Duration of heat treatment and level of IAA. Some catkins were dipped in water a… (H_2O) or in a water solution of indole-3-acetic acid (IAA), shown in p.p.m., after stigmas dried up on pollinated capsules.

[b]Number of catkins pollinated/number collected.

[c]Seeds of Treatments 8, 11 were mixed.

[d]Seed not seeded because of large size.

60°C. in 2% acetocarmine, then left in the same stain until squashed in 45% acetic acid within 1-2 days.

From the 45 surviving plants of Cross XT-65-67, 37 samples have been examined and include 20 diploids or aneuploids, no haploids, and the rest uncountable. Of the 51 selected seedlings from Cross XT-A-69-67, only 10 samples have been examined and all have been diploid. In both crosses, the most aberrant seedlings were examined first, and the remaining uncounted seedlings all appear to be normal diploids. The most promising putative haploid, from the standpoint of phenology, was seedling number 31 from Cross XT-65-67. This stunted and spindly plant is shown in Fig. 4 with the normal diploid plant number 32. Seedling 31 grew from a 40-mesh seed from the pollen treatment receiving 3.5 minutes of heat. Other aberrants in Cross XT-65-67 were seedlings 4, 11, 16, 22, 26, and 27. Aberrants in Cross XT-A-69-67 were 38, 46, 47, 51, 58, 60, 61, 62, 65, and 70.

Polyploid Production

A small pilot study was made in 1967, to produce triploids and tetraploids from a cross between diploid and triploid quaking aspen parents. Pollen from the triploid T-2-56 was placed on receptive catkins of T-16-56 to give the Cross XT-68-67. The number of seeds obtained were 28-mesh (318), 40-mesh (1133), and 50-mesh (13). All seeds were planted in the greenhouse; and after thinning out phenologically-normal seedlings, 159 plants from 28-mesh seeds were individually potted and left in the greenhouse, and all but 75 plants from the other two seed sizes were lined out in one bed in the nursery.

Figure 4. Abnormal (Left) and Normal-Appearing Seedling from 3.0-Minute Heat-Treated Pollen. Both Plants Were Diploid

In August, plants in the greenhouse were separated into seven phenological classes, ranging from stunted aberrants to normal plants. Small leaves from most of the plants were collected from 1-4 times, and samples from most of the aberrant plants have been examined cytologically. Aneuploidy was common, but no triploids or tetraploids have yet been found. The majority of the larger plants appear to be normal, and selected sampling indicates these are probably diploid. About 20 of the plants had characteristic deeply-serrate and lanceolate leaves, but these also are apparently diploids or aneuploids. In October, the plants in the nursery were examined, and 16 were marked for potting and examination in the spring. Several short thick-stemmed seedlings and some tall plants that still had their leaves were marked. These two classes were the best choices for possible tetraploids and triploids, respectively.

Discussion

Haploids, triploids, or tetraploids have not yet been identified among the surviving plants of the 1967 crosses. All leaf samples have not been examined, but the most likely aberrant plants were tested, some several times, and there were no positive results. The number of seedlings produced in each cross may have been too small, since the frequency of haploid and polyploid production in other plants by similar methods is exceptionally small. Other pollen-weakening agents are now being tried for haploid production, and the number of catkins per treatment have been increased. The development of dependable methods to determine pollen viability, either by germination or by the tetrazolium test, would greatly facilitate the production of haploids. Diploid by triploid crosses require no treatments and appear to be an inexpensive way to produce tetraploids if the criteria for seedling selection can be narrowed. A refinement we are trying to develop is the mechanical recovery of the large pollen grains (presumably unreduced) from triploid pollen and the use of such pollen on diploid females, in order to increase the frequency of polyploids.

CYTOLOGICAL INVESTIGATIONS

The results of the 1967 cytological investigations can be termed only a partial success. A successful study was initiated to determine the long-range variation in pollen development, both among and between locally grown aspen species. On the other hand, a very cool spring and summer caused slower growth in our plant material, resulting in little progress in the improvement of cytotechniques. The poor physiological condition of the plants also reduced the number of cells with countable chromosomes in several haploid and polyploid crosses. Even with the new phase-contrast microscope, at least 60-70% of the sampled plants could not be evaluated for ploidy level. Fortunately, the unidentified material was heeled-in for the winter, and will be recollected this spring.

Natural Microsporogenesis in Quaking Aspen

Male flowers of *Populus tremuloides* and *P. deltoides* pass the winter in the spore-mother-cell stage, then complete the development and shedding of pollen the following spring [Nagaraj (2)]. Pollen can be forced in the greenhouse any time after dormancy is broken (early winter) until it is shed naturally in the spring. However, if special treatment is to be given before pollen is formed, e.g., irradiation or chemical treatments, then male flowers must be collected before the first division of meiosis* occurs and treatments must be given during the very short period between metaphase and anaphase I. The purpose of this study is to determine the year-to-year variation in the time of meiosis and natural pollen shedding in several species of aspen in the Appleton area.

Male buds and catkins were collected from one tree (T-13-67) of quaking aspen (*P. tremuloides*), located near the Institute, from March 9 to April 14, 1967. With few exceptions, catkins were collected daily for the first 19 days, and were either placed in one of three fixatives or frozen intact. Catkins were also collected before and after pollen was shed on April 5. Weather conditions were recorded during each collection.

*Meiosis — specialized nuclear cell divisions prior to the formation of gametes (either egg or sperm). Usually the first division reduces the chromosome number one-half and the second division results in the production of four cells with half the original number of chromosomes.

Meiosis was already under way when collections began, in the initial form of long thin chromosomes in the leptotene stage (Fig. 5). This stage lasted for another 10 days, until the chromosomes doubled in the zygotene stage. Within the next four days, the chromosomes contracted greatly into 19 pairs of chromosomes at metaphase (Fig. 6), followed quickly by the first and second anaphase divisions of meiosis on March 25. Each of the four cells derived from each spore-mother-cell developed into one pollen grain having one set of chromosomes (haploid). The male buds were essentially unchanged in size during the early stages of meiosis (Fig. 7a, 7b), but started to expand two days before division, from 10-12 mm. to 13 mm. in diameter (Fig. 7c). After division (Fig. 7d), the catkins elongated rapidly from 25-28 mm. to 30-60 mm. at the start of pollen shedding (Fig. 7g), reaching 60-70 mm. at full elongation by April 12.

If the response in this one tree is typical, male buds apparently begin to swell slightly a few days before meiotic division. Swelling of the buds and the rapid conclusion of meiosis were also associated with an increase in the maximum daily temperature from 30-40° to 45°F. Division took place in 1967 on the first warm day of the year when a high of 54°F. was recorded. Pollen was shed 11 days after the completion of meiosis. This year, it is hoped that the dates of meiotic division can be determined for many local species by collecting male buds for several days after the first noticeable swelling occurs. If this method proves to be satisfactory, routine tests will be made yearly to estimate the variations due to species and local weather conditions. This knowledge will permit closer control of experiments designed to obtain large amounts of unreduced pollen for polyploid production.

Figure 5. Aspen Microsporogenesis. Early Meiotic Leptotene Stage

Figure 6. Metaphase Preceding Anaphase I

7

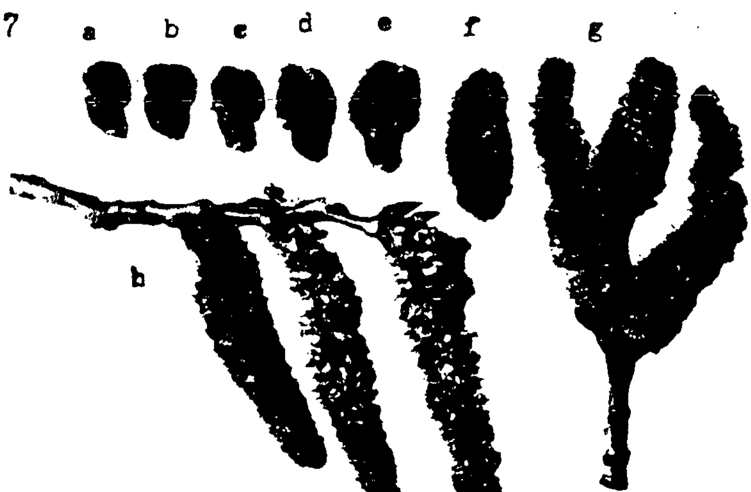


Figure 7. Swollen Male Buds at Leptotene (b), Zygotene (c), Metaphase (d), Pollen Shedding (g), and Full Elongation (h)

Physiological Condition of the Material

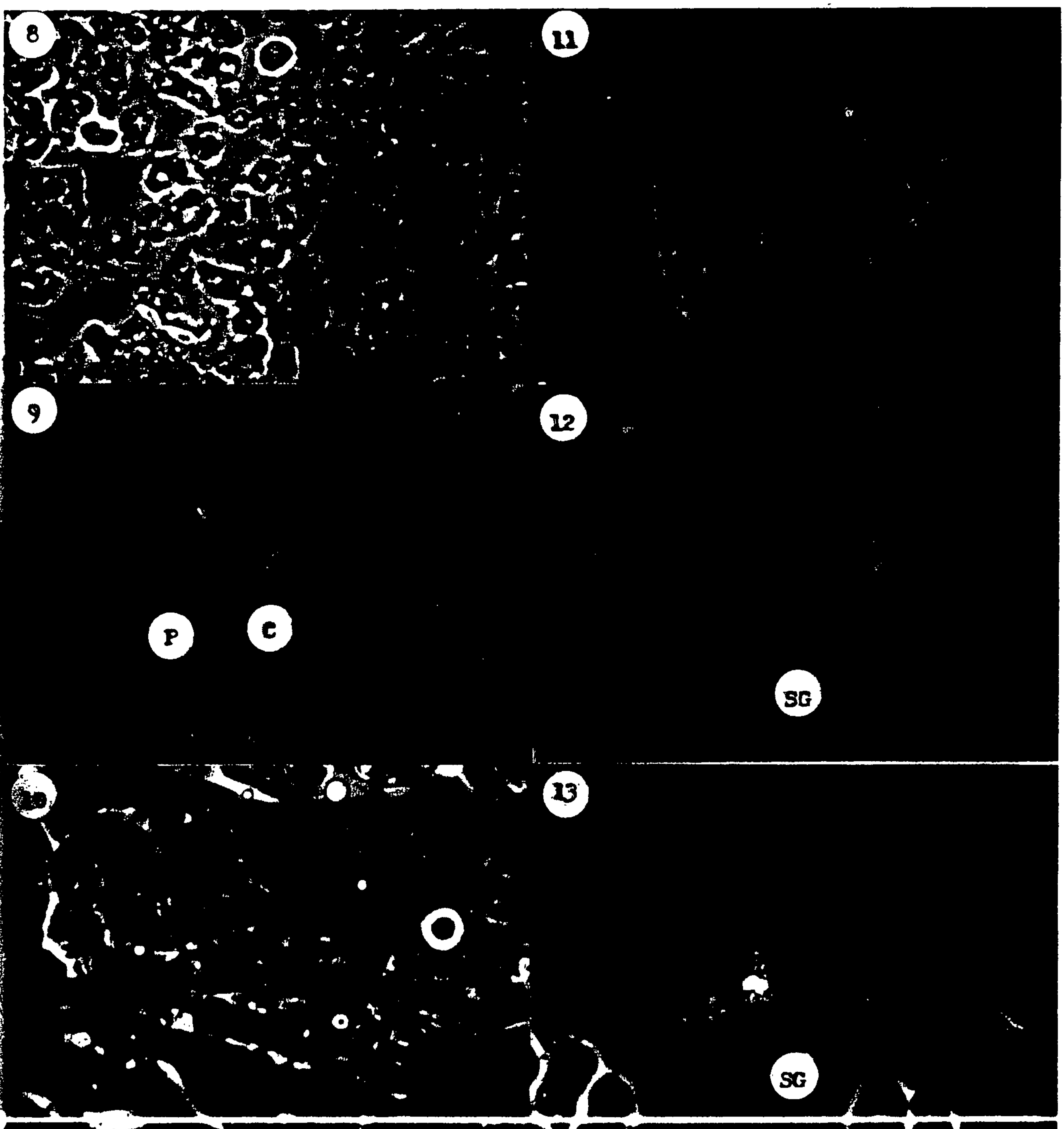
Since the start of chromosome counting at the Institute, problems have developed in the areas of optics, techniques, and material. Now that we have a phase-contrast microscope, we have no more problems with optics and it is possible at last to separate the problems of technique from those of material. For most routine counting, our present method of staining in acetocarmine [van Buijtenen (3)] is satisfactory, and improvements are required only for detailed studies of chromosome morphology. Our main problem now is with the material.

In past years we have noticed that, at best, only a few cells are usually found in each leaf-tip squash in which the chromosomes can be counted. Sometimes no countable cells are found. In April and May of 1967, daily weather records were kept during early collections, in an effort to determine the relationship between cell division and the environment. Small growing leaves were collected from greenhouse forced 1-0 cottonwood seedlings, after a variety of sunny or overcast days having different temperature ranges. The best cell division occurred on the first warm day after a week of cool overcast days in mid-April. In later collections, under similar conditions, not many dividing cells were found.

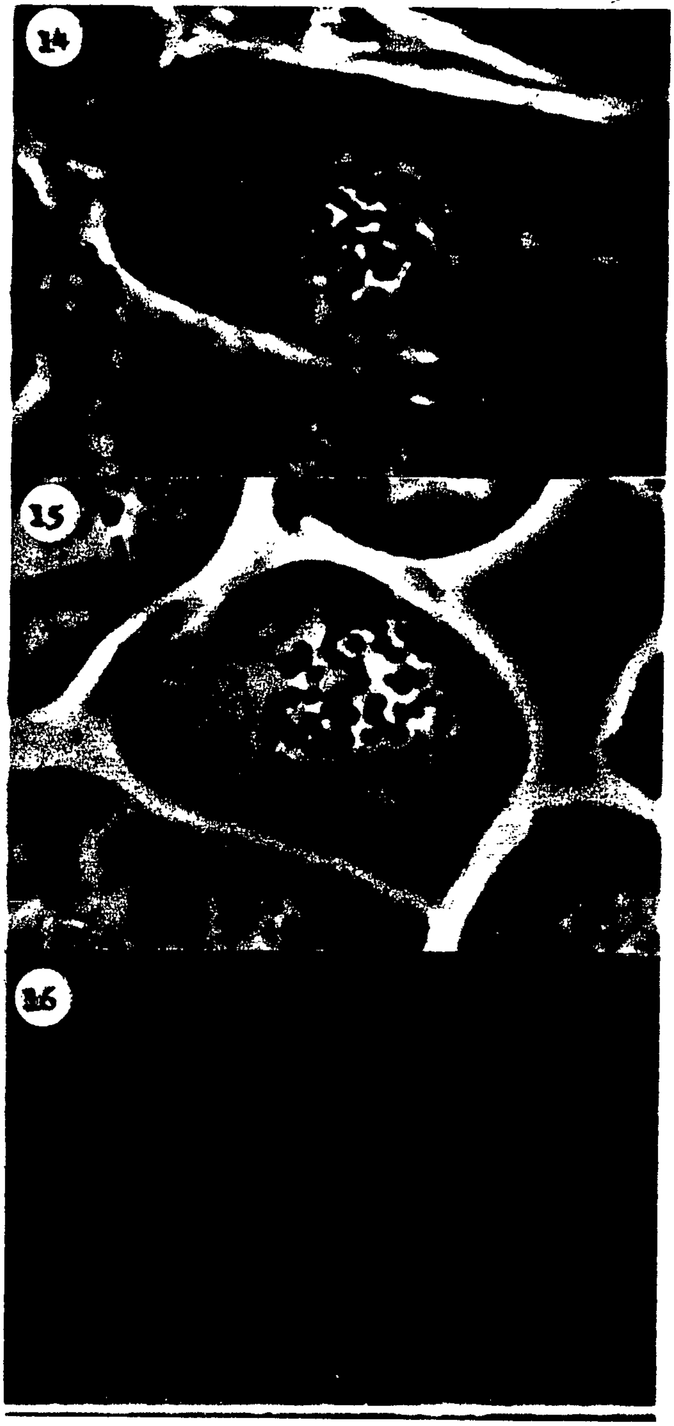
During July and August, nine rather elaborate test collections were made from triploid quaking aspen root-sprouts grown in the greenhouse. The main variables were in the pretreatment of the leaves, testing water vs. para-dichlorobenzene, three hours vs. overnight, sunlight vs. room light vs. darkness, and 10° vs. 23° vs. 27°C. A great deal of work was done and a great amount of variability was observed among the collections.

One difficulty was that the stain technique was too good, giving very clea cytoplasm in the cells and allowing many cell inclusions to be observed (Fig. 8). The second difficulty was that during the cool summer, cell division was taking place at a much reduced rate. The result was that spherical chromosomes (Fig. 9C) were often confused with angular cell inclusions (Fig. 9P). These small dark bodies would sometimes enlarge greatly throughout the day (Fig. 10) and diminish again at night. When left in the sun during pretreatment, they would sometimes clump together at the periphery of the cells. Both mitochondria and chloroplasts were destroyed by the staining procedure, so the best guess was that the dark bodies were starch grains. Proof came when one squashed cell was observed that showed clear spherical plastids (Fig. 11) containing 1-6 dark, angular starch grains (Fig. 12SG). After the starch grains were identified, they could occasionally be seen in an intact cell clustered in their invisible plastids (Fig. 13SG). Cells undergoing divisic had few if any starch grains (see Fig. 14-16).

Chromosomes were scarce in this study, and in material collected from the haploid and polyploid production crosses. Numerous collections were required from the same plant to provide one leaf with countable cells. The blame was placed on the poor growing season. Amid the dearth, however, good counts were obtained from many plants, and the best chromosome complement was observed from a Swedish-produced tetraploid plant of European aspen growing in the greenhouse. Four sets of 19 chromosomes, for a total of 76, are seen in Fig. 16. Previous photos of diploid (Fig. 14) and triploid (Fig. 15) quaking aspen are included for comparisons.



Figures 8-13. Cellular Starch Grains. Small Dark Bodies (Fig. 8, 220X) Generally Angular (Fig. 9P, 880X), Were Sometimes Confused with Spherical Chromosomes (Fig. 9C). These Bodies Grow Larger During the Day (Fig. 10, 880X), and Were Later Identified as Starch Grains (SG) in Clear Plastids (Fig. 11, 12, 13; 880X, 2200X, 880X)



Figures 14-16. Aspen Chromosomes. Diploid (Fig. 14) and Triploid (Fig. 15) Quaking Aspen, and Tetraploid (Fig. 16) European Aspen, Respectively, Containing 38, 57, and 76 Chromosomes (Magnifications 2200X)

SELECTION - RECENTLY LOCATED OUTSTANDING TREES

Selection work continued during the past year at about the same pace as in previous years. The trees selected were evaluated on the basis of wood, growth, and morphological characteristics. A selection index system employing a numerical rating was used and the trees that survived the initial selection are presently being checked for their flowering and crossing behavior. The final evaluation of the trees will be made on the basis of the quality and vigor of the progeny produced. Considerable emphasis was placed on the selection of desirable cottonwood and 2 of the 4 trees described below are cottonwood growing in east central Wisconsin.

Tree D-7-68

D-7-68, shown in Fig. 17, is a male cottonwood (Populus deltoides) that is growing on a low moist site near Black Creek, Wisconsin. The tree was selected for its good form and above average rate of growth. The tree is growing near a drainage ditch in a mixed stand of elm, basswood, and white birch. A description of the growth and wood quality data available on D-7-68 is listed below.

Total height - 113 ft.	Specific gravity - 0.365
Height to 3 in. top - 96 ft.	Diam. at 4.5 ft. - 21.4 in.
First live branch - 53 ft.	Bark thickness - 0.75 in.
Age - 55 yr.	Crown diameter - 28 ft.
Stem straightness - good	Number of major branches - 10
Natural pruning - good	Branch angle - 45°
Branch weight - fair	Fiber length (age 30) - 1.12 mm.

Figure 17. The Outstanding Form of Male Cottonwood D-7-68 is Readily Seen by Comparing the Selected Tree with Nearby Check Trees

Tree D-6-68

This young male cottonwood (P. deltoides) is growing on a public hunting ground in Oconto County. The tree was selected for its rapid growth, small crown, and above average form. The tree is growing on a moist sandy site near Lake Michigan (Green Bay). Although not exceptional in form, the tree has been growing approximately 3 feet in height each year and has a better than average diameter growth. Tabulated below are the growth and wood quality data for D-6-68.

Total height – 93 ft.
Height to 3 inch top – 71 ft.
First live branch – 71 ft.
Age – 32 years
Stem straightness – fair
Natural pruning – good
Branch weight – fair

Specific gravity – 0.360
Diameter at 4.5 ft. – 11.8 inches
Bark thickness – 0.4 inch
Crown diameter – 16 ft.
Number of major branches – 4
Branch angle – 45°
Fiber length (age 30) – 1.08 mm.

Tree G-5-67

This male bigtooth aspen (P. grandidentata) was selected because of above average form, good growth (considering the soils and site on which it was growing), above average fiber length and very high fiber strength. The tree is growing on a loamy sand soil in Waupaca County near Rural, Wisconsin. The tree has a very small crown and long fibers at a very young age. The usefulness of the tree as a parent tree is being investigated in the 1968 crossing program. The following is a summary of the growth measurements and wood quality information.

Total height – 75 ft.
Height to 3 inch top – 60 ft.
First live branch – 47 ft.
Age – 40 years
Stem straightness – fair
Natural pruning – fair
Branch weight – good
Specific gravity – 0.360

Diameter at 4.5 ft. – 10.8 inches
Bark thickness – 0.35 inch
Crown diameter – 9.6 ft.
Number of major branches – 8
Branch angle – 45°
Fiber length (age 30) – 0.98 mm.
Fiber strength – 81.8 lb./in. (V. high)

Tree A-H-4-67

A-H-4-67 is believed to be a natural hybrid between P. alba and P. grandidentata and was selected for further study because of its outstanding rate of growth.

Found in a private yard in Norway, Michigan (see Fig. 18), the tree had apparently been planted here at age 2 by the occupant who found the tree growing near a clone of P. alba. The fiber length of A-H-4-67 is average and its wood specific gravity is slightly better than average. As a 19-year-old tree it has exceptional dimensions, 70-feet tall and 19.6 inches D.B.H., which apparently resulted because the tree was growing on a drain field of a septic system. The growth conditions involved are a most significant point because they indicate the potential available via the intensive management procedures of fertilization and irrigation. The tree is presently being propagated and plans are to evaluate its growth with other hybrids developed by the Institute's tree improvement program. Summarized below are the growth and wood properties of this unusual tree.

Total height – 70 ft.
Age – 19

Specific gravity – 0.400
Diameter at 4.5 ft. – 19.6 inches
Fiber length (age 18) – 0.856 mm.

Figure 18. Mrs. Bjornson of Norway, Michigan, Standing Next to the Stump of the Tree Which 17 Years Earlier She Had Planted in Her Yard

TREE PHYSIOLOGY STUDIES

GROWTH AND NUTRIENT REQUIREMENTS OF HYBRIDS BETWEEN POPULUS CANESCENS AND POPULUS TREMULOIDES — SUMMARY

The growth and nutrient requirements of hybrids between European gray poplar (*P. canescens*) and quaking aspen (*P. tremuloides*) were investigated by comparing the growth and nutrient uptake of the two hybrid progeny groups with the performance of "parent species" seedlings.

The study undertaken employed a time-clock-controlled sand culture system and the entire study was conducted in a growth chamber that provided constant temperature, humidity, day length, and light quality conditions. A standard nutrient solution was used and a series of five interrelated growth chamber trials were conducted in which the influence of varying the level of a single major nutrient was investigated. Nitrogen, phosphorus, potassium, calcium, and magnesium were the nutrient elements investigated.

Green weight and dry weight measurements revealed that there were significant growth differences between the four types of experimental trees. Seedlings from one of the hybrid crosses grew more rapidly than the seedlings of the two "parent species" crosses. Chemical determinations made on the tops of the test trees demonstrated that there were significant between material differences in the uptake of K, Mg, and P.

Varying the level of the five major elements resulted in significant differences in the uptake of N, P, K, Ca, and Mg. In general uptake by the seedlings was low at the low treatment levels, increased to moderate levels and then remained

constant at the medium high and high treatment levels. Evidence was also obtained that indicated varying the level of certain elements influenced the utilization of the other elements.*

GROWTH AND NUTRIENT REQUIREMENTS OF HYBRIDS BETWEEN POPULUS CANESCENS AND POPULUS GRANDIDENTATA — SUMMARY

The growth and nutrient requirements of hybrids between European gray poplar (P. canescens) and bigtooth aspen (P. grandidentata) were investigated by comparing the nutrient uptake and growth of the two hybrid progeny groups with the performance of the "parent species" seedlings.

The procedures used were the same as for the previously described study involving European gray poplar and quaking aspen. Five growth chamber trials were conducted in which the influence of varying the level of a single major nutrient element was investigated. As in the previous work, nitrogen, phosphorus, potassium, calcium, and magnesium were the elements investigated. Green weight and dry weight measurements demonstrated that there were significant growth differences between the four types of experimental trees and significant differences in growth as the result of varying the level of N, P, K, Ca and Mg. The bigtooth aspen test material grew the slowest and responded the least to the increases in nutrient levels. The European gray poplar cross had the most rapid growth and the highest nutrient requirements. Seedlings of the two hybrid progeny groups were intermediate in rate of growth and nutrient requirements.

*A complete description of the study is presented in Project 2412, Progress Report Seven.

Chemical determinations made on the tops (leaves plus stems) suggests that only minor differences exist in the nitrogen requirements of the four types of test materials with material two (one of the hybrids) having the highest level of nitrogen uptake. There were no significant differences between materials in phosphorus and potassium uptake. There were fairly large differences between test materials in the uptake and presumed requirements of calcium and magnesium. The European gray poplar parent species had the highest uptake and the bigtooth aspen parent species had the lowest uptake.

Varying the level of the five major elements resulted in significant differences in the uptake of the N, P, K, Ca, and Mg. Use by the seedlings was low at the low treatment levels, increased to moderate levels and then remained constant at the two higher treatment levels. Data were also obtained that indicated the uptake of certain elements influenced the use and overall requirements of the other major nutrient elements. A complete description of this work is being prepared and will be presented as Project 2412, Progress Report Nine.

CHEMICAL CONTROL OF GROWTH AND FLOWERING IN ASPEN

Introduction

Breeding arboretum trees are maintained to provide a source of flowers for future crossing work. As discussed in previous work, considerable advantage can be obtained if the trees are kept small so the flowers are readily accessible and the trees stimulated to flower at an early age.

Alar* (N-dimethyl amino succinamic acid), a chemical that has shown promise as a growth retardant and stimulant to flowering when used on fruit trees

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

was tried on aspen growing in the Greenville Arboretum. The report that follows describes the second-year results of the Alar treatments applied to aspen.

Methods

Six different sources of aspen were selected for treatment in 1967. The trees involved were located in the I.P.C. Greenville Arboretum and consisted of blocks of 16 trees planted at a 9 x 9 foot spacing. Six trees in each block were treated and the remaining trees were used as control trees. The treatment levels used were 1.0, 0.75, and 0.50%. Table III briefly describes the types of materials treated. It should be noted that the first three materials listed received Alar treatments in 1966. The 1966 investigation employed Alar levels of 1.5, 1.0, 0.5, and 0.25% and each level listed was applied to two of the 16 trees in each block. Table IV summarizes the results of the first-year (1966) treatments. Based upon the results obtained in 1966, the 1.5% treatment was dropped, the 1.0 and 0.5% treatments were repeated, and the 0.25% treatment was increased to 0.75%. The chemical was applied as a foliar spray to the drip point. 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 the control trees of comparable size. Flowering was evaluated by comparing the treated trees with the average number of flower buds produced by six to eight control trees.

Results

Table V summarizes the growth and flowering information for the three materials treated in 1966 and retreated in 1967 (top part of the table) and for the three materials that were treated for the first time in 1967.

TABLE III

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-130-56	Female	Grafts	Grafts of selected quaking aspen field planted in 1962. No previous record of flowering.
T-30 Control	Female	Grafts	Selected parent tree, grafted 1961, field planted 1962. No prior flowering.
T-33-57	Male	Grafts	Selected parent tree, grafted 1960, field planted 1962. Some flowering in 1962.
T-1-58	Female	Grafts	Rapid growing tree of outstanding form, grafted in 1960, field planted in 1962. Scattered flowering in 1962 and 1966.

TABLE IV

FLOWERING AND GROWTH REDUCTION OF ALAR-TREATED TEST TREES — 1966 MEASUREMENTS

Treatment Level, %	Terminal Growth, % reduction	Lateral Growth, % reduction	Total No. Flower Buds: Treated Trees	Total No. Flower Buds: 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

Looking at growth reduction of the 1.5% treatment level, this treatment was applied three times in 1966 and was discontinued in 1967 because of excessive injury to the treated trees. Observations and measurements made in 1967 indicated the treated trees had recovered in part from the treatment but still were not growing as rapidly as the control trees. Looking next at the 1% treatment level, terminal growth was reduced by 65 to 85% and growth of lateral branches was reduced by 25 to 75%. The 0.75% Alar treatment reduced terminal growth by 52 to 86% and growth of the laterals by 16 to 64% while the 0.5% Alar level resulted in a 50 to 85% reduction in growth of the terminals and 0 to 58% reduction in growth of the lateral branches. Some leaf curl and early leaf drop was noted in the trees treated at the 1% level.

Comparing the 1966 growth reduction figures for the 1.0 and 0.5% level of Alar (Table IV) with the reduction obtained after the second year of treatment, makes it appear that there is an additive effect of the treatments because of the greater growth reduction obtained during the second year of treatment. Based upon these somewhat preliminary growth measurements, it appears that if routine treatment of arboretum trees is considered it may be desirable to skip the treatment every third year.

Considering the influence of the chemical treatments on flowering, the results are less consistent and appear to be strongly influenced by the sex of the trees, the age of the material, and the method of propagation. Looking at the two male clones (T-32-57 and T-33-57), the Alar treatment appears to have both decreased growth and increased the flowering. There is, however, little relationship between treatment level and flower bud production. When the flowering of the three female clones (T-130-56, T-30 Control and T-1-58) are compared, even greater inconsistencies are evident in the flowering. The T-30 control trees were grafted in 1961 and were small in size and although the treatments reduced growth by 52 to 77%, flowering was not stimulated. The grafts of T-130-56 were of moderate size and those of T-1-58 were moderate to large and were one to two years older than the T-30 control grafts. Growth of the terminal and lateral shoots of these two female trees was reduced. Some flower bud formation seemed to have been stimulated but the results were inconsistent and not related to the treatment levels. Growth of the bisexual hybrid (AG-1-60) was greatly reduced by the 1% level of Alar; however, like the T-30 control trees, no flowering occurred on either the treated or nontreated trees. The bisexual nature and the root sprout origin could be the reasons for the lack of flowering response.

Looking at the overall results, the Alar treatments consistently reduced growth but the differences between the treatment levels were small. The treatments stimulated early flowering on the two male clones. However, the differences between treatment levels were minor and not consistent. Flowering was stimulated less in the case of the female clones, while no flowering occurred at all on the bisexual hybrid. Pollination work using the flowers produced as a result of the treatments demonstrated that both the male and female flowers were normal. A comparison of the percentage of treated trees that flowered (regardless of treatment level) indicates that 40% of the treated trees flowered compared to 17% of the nontreated control trees. The use of the 0.75% level of Alar on a "spray two years and skip one year" basis is recommended.

CALLUS INITIATION OF JUVENILE ASPEN AS AN INDICATOR OF TREE GROWTH

For the past two years, stem terminals have been harvested from 1-0 nursery stock, sterilized and cut into small segments, then placed on a nutrient agar medium to produce callus tissue. The purpose has been to determine the relationship between the rate of callus production on juvenile stem segments and the rate of growth of the same plant later on as a tree. In the first study in 1965, ten tall and ten short seedlings of diploid quaking aspen (XT-36-65) were tested. The callus production after both two and four weeks is summarized in Report Six (May, 1967). The average height at planting (spring, 1966) was 3.1 feet for the tall and 1.0 foot for the short seedlings. All short seedlings died during either the first or second year in the field, but the average total heights for the tall seedlings in the fall of 1966 and 1967, respectively, were 3.8 and 4.1 feet. No correlation was found between callus production and height growth during the first two years.

In the fall of 1966, tall and short diploid quaking aspen stems (XT-2-66) were again tested; as well as tall bigtooth aspen (XG-19-66), cottonwood (XD-34-66), gray poplar (XCa-28-66), and sprouts of two triploid quaking aspens (T-2-56, T-2-65). All cottonwood material was lost from fungus infection on two attempts, and bigtooth callus did not develop well in the dark. Callus production was good on all other tissue, which was selected and subcultured monthly, and eventually gave rise to several clonal strains of stock callus tissue. This callus tissue is now being used in differentiation experiments, aimed at producing independent plants from callus tissue, and possibly from single cells.

During the callus production tests in 1966, measurements were made on ten segments from each seedling, including the final wet weight of callus plus segment (after two weeks of growth), the segment after removal of the callus, and the length and diameter of each segment. The weight of callus was estimated by difference, and the percent of callus calculated for each segment. Percentages were transformed to arcsin functions and used to calculate comparisons of variability for the quaking aspen data only. These data will be fed into a computer program, for correlations with height-growth data taken from field-planted trees at 5-year intervals.

Table VI shows the ranking of each plant by the average percent of callus production, as well as giving plant height and segment measurements. Figures 19 and 20 show typical callus production for tall and short quaking aspen. In the spring of 1966, sampled seedlings planted in the nursery averaged 3.2 feet for the tall and 0.5 foot for the short plants. When measured the following November, plants averaged 4.0 and 1.4 feet, respectively, with average height differences of 0.7 for the tall and 0.9 for the short plants. The plantings were rototilled during the summer, so the greater growth from the smaller seedlings may have been due only to

TABLE VI

PERCENTAGE OF CALLUS AND AVERAGE MEASUREMENTS FOR DIPLOID QUAKING ASPEN XT-2-66 IN 1966

Plant Number	Percent Callus	Significance[a]	Plant Height, cm.	Stem Segments, mm. Diameter	Length	D x L	Volume
			Tall				
42	63.4 ± 0.3		147	3.7	7.3	27.0	84.8
50	52.4 ± 0.7	F	147	4.1	7.4	30.3	95.3
45	51.7 ± 0.8		148	4.1	7.8	32.0	100.5
41	46.0 ± 0.7		142	5.1	8.2	41.8	131.4
49	43.9 ± 0.1		141	4.0	7.3	29.2	91.7
48	39.7 ± 2.0	I	141	3.9	8.8	34.3	107.8
43	39.0 ± 1.0		150	4.0	7.6	30.4	95.5
47	37.7 ± 2.3		141	4.5	7.0	31.5	99.0
46	34.9 ± 0.7		147	3.6	7.1	25.6	80.3
51	30.0 ± 0.8	S	145	4.0	8.0	32.0	100.5
44	25.1 ± 0.5		147	4.2	7.2	30.2	95.0
			Av. = 145 cm.				
			Av. = 4.8 ft.				
			Short				
53	65.6		62	3.0	8.6	25.8	81.0
64	62.5		54	3.1	7.9	24.5	76.9
67	61.5		56	3.2	7.8	25.0	78.4
59	59.1	F	72	3.0	7.7	23.1	72.6
65	59.1		53	2.3	6.8	15.6	49.1
60	55.8		70	3.7	7.8	28.9	90.7
66	54.7		53	2.5	6.7	16.8	52.6
54	52.0		62	3.6	7.4	26.6	83.7
55	49.0		60	2.5	7.7	19.2	60.5
58	45.0		56	2.4	8.4	20.2	63.3
57	41.1		68	3.1	8.8	27.3	85.7
68	40.7	I	53	3.4	7.1	24.1	75.8
69	34.9		53	3.0	8.5	25.5	80.1
56	33.8		47	2.4	8.7	20.9	65.6
62	28.6	S	53	3.1	6.5	20.2	63.3
61	21.4		57	3.7	6.8	25.2	79.0
			Av. = 59 cm.				
			Av. = 1.9 ft.				

[a] Duncan's multiple range test (4) was used to examine differences in percent callus. Values bordered by a common line are not significantly different at the 5% level of probability.

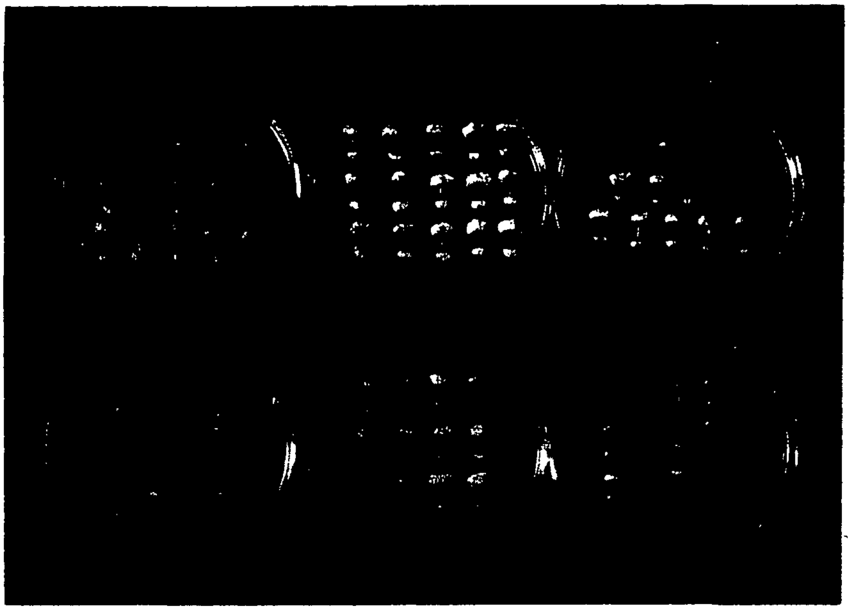


Figure 19. Callus Growth of Tall Quaking Aspen Seedlings (L. to R.) 41, 42, 43 (Top) and 44, 45, 46 (Bottom)

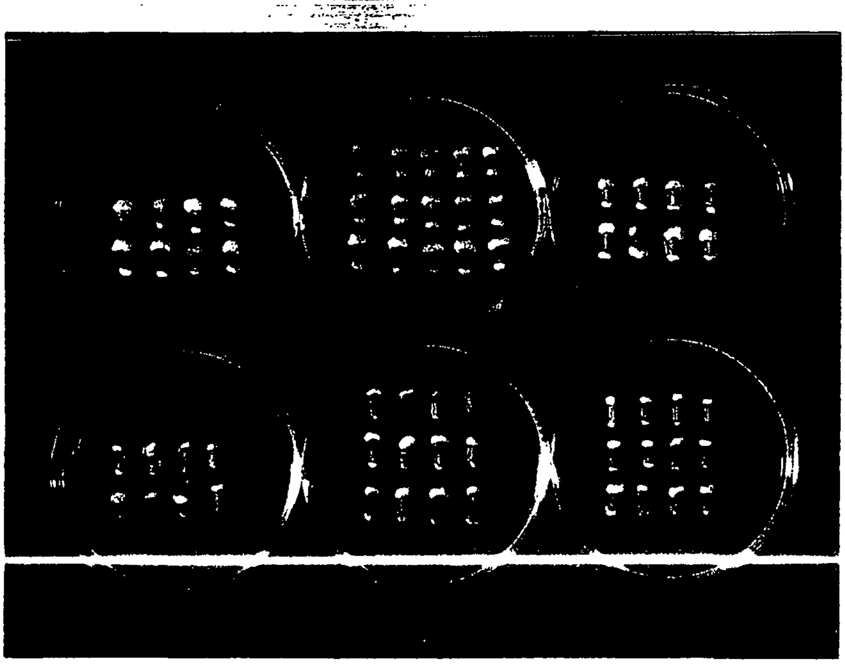


Figure 20. Callus Growth of Short Quaking Aspen Seedlings (L. to R.) 53, 54, 55 (Top) and 56, 57, 58 (Bottom)

a more advantageous root/top ratio. Much variation was observed in new growth, particularly among the short plants. These growth differences are plotted in Table VII along with the rankings of callus production per plant. Lines are drawn to connect the same plants, but show no correlation between juvenile callus production and the first year of growth in the field. One confounding factor may be that perhaps not all of the plants resumed growth at the terminal bud, so that height differences between the times of planting and measuring are not meaningful. A more important consideration, however, may be that the time necessary to adjust to the environment is different for each individual plant. For this reason, correlations will not be attempted until fifth-year measurements are made. A corrective measure would be to cut back all plants to the same height next spring, so that, in five more years, both the total height and the current increment will both be meaningful. This would also allow measurements and correlations to be made during the same year with plants tested this past fall of 1967 and scheduled to be field planted next spring.

In the fall of 1967, seedlings were tested from the three "parent species" and four hybrid crosses listed below.*

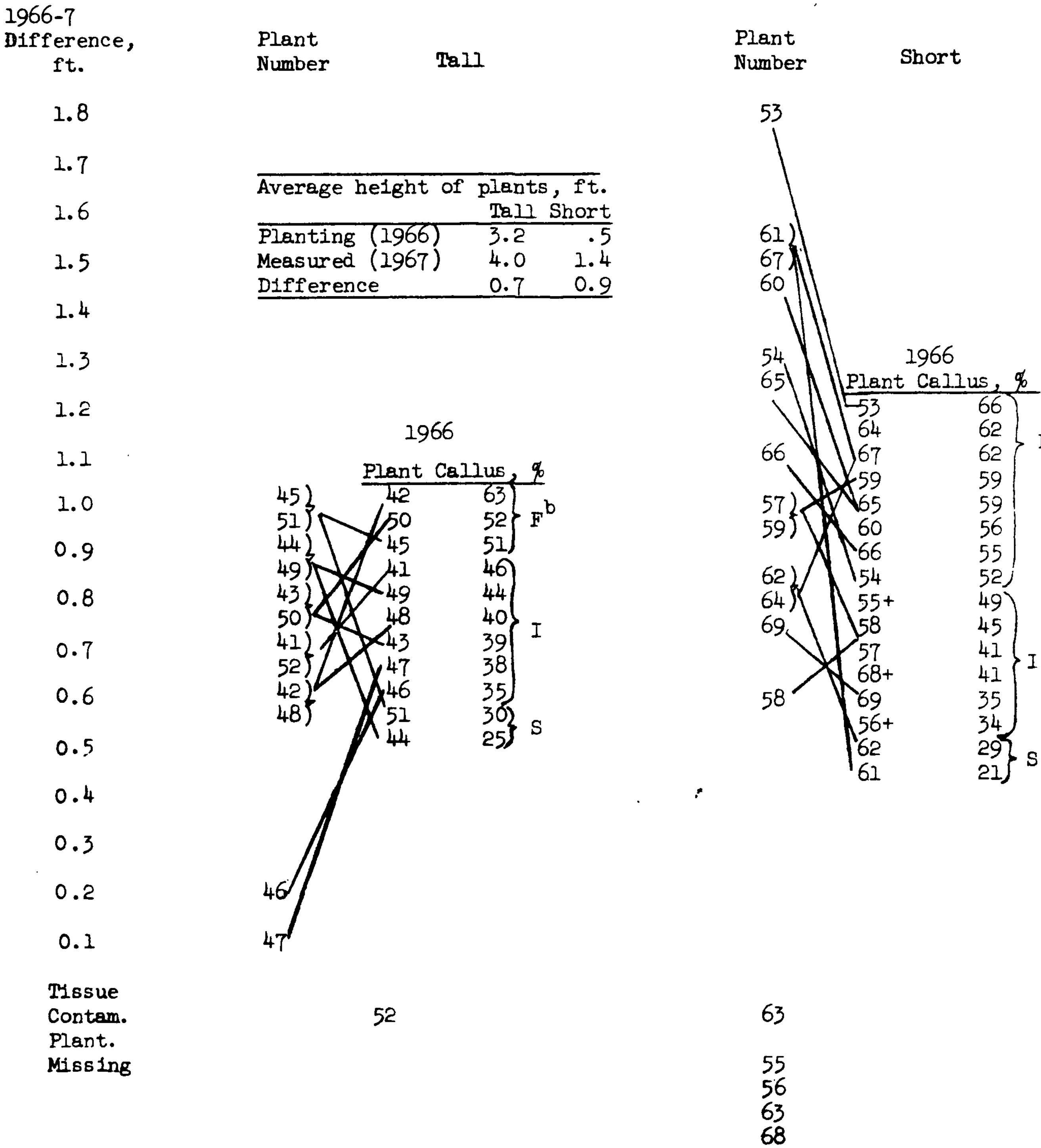
1.	XT-7-67	(T-5-67 x T-20-60)
2.	XT-Ca-33-67	(T-5-67 x Ca-1-62)
3.	XCa-T-26-67	(Ca-2 x T-20-60)
4.	XG-42-67	(G-64 x G-5-67)
5.	XG-Ca-50-67	(G-64 x Ca-1-62)
6.	XCa-G-38-67	(Ca-2 x G-5-67)
7.	XCa-36-67	(Ca-2 x Ca-1-62)

Twelve tall and fourteen short seedlings were lifted from the nursery from one cross on any one day, and taken to the greenhouse the same evening. On the following day, seedlings were measured and tagged and the top 18 inches harvested.

*See Table XIII for a more complete description of the crosses listed.

TABLE VII

COMPARISONS OF 1966 CALLUS PRODUCTION WITH THE FIRST YEAR SEEDLING GROWTH OF DIPLOID QUAKING ASPEN XT-2-66[a]



Average height of plants, ft.	Tall	Short
Planting (1966)	3.2	.5
Measured (1967)	4.0	1.4
Difference	0.7	0.9

[a] Average heights before harvesting were 4.8 feet for the tall and 1.9 feet for the short 1-0 seedlings.

[b] Fast (F), intermediate (I), slow (S).

The terminal 4-6 inches were discarded and the remaining 10-12-inch section was used for the callus production study. Each section was cut into smaller 2-inch sections, and all sections from one seedling were sterilized together in one 250-ml. flask in Hi-lex bleach. Sections were sterilized for 30 minutes from tall plants and for 20 minutes from short plants. After three rinses in sterile water, 15 segments, 7-10 mm. long, were cut and distributed among three plates of agar medium per seedling. Plates were left in a humid incubator at 27°C. for three weeks, then segments were weighed with and without callus, and the percentage of callus per segment determined. Some loss of material occurred from fungus contamination, but weighings were not complete by the time of this writing. In late November, all sampled plants heeled-in for the winter, as well as all plants now in the field from the 1966 test were cut back to 8-inch tops. After all plants are in the field this spring, growth will be measured every year; but correlations will only be attempted with the juvenile callus data for each five-year measurement of total height and current increment.

In the past, we probably discarded short trees that became stunted by overcrowding; but which, in reality, may have possessed superior genetic growth-potentials that would have shown up in the field. Unfortunately, most tree selection is now made from external or phenotypic characters, with the hope that superior phenotypic characters reflect superior genotypes. Mathes and Einspahr (5) showed that high callus production on branch segments from 5-year-old trees was positively correlated with fast growth. This present study was based on their hypothesis that high callus production on stem segments from juvenile plants may also indicate a fast growth potential. If so, we will have a predictive test for a genetic trait which would enable us to detect superior trees from among all the progeny of any particular cross. Not only would this facilitate our tree improvement program, but would also contribute to our knowledge of aspen genetics.

STUDIES OF NATURAL VARIATION

WOOD PROPERTY VARIATION IN POPULUS

Introduction

One of the major goals of Project 2412 has been the improvement of wood properties important to pulp and paper quality. During the past five years, a number of selected trees were evaluated and a number of studies have been completed in which wood, fiber, and pulp properties were obtained for individual trees. Comparable sampling and measurement techniques were employed in the several studies involved with the result that a tabulation of these data makes possible comparisons not previously available.

The report that follows summarizes wood property measurement data for several species of *Populus* and for both natural and artificially produced aspen hybrids. Although the nature of the data prevents any rigorous statistical treatment of the information, the tabulation provides a useful comparison between native species of *Populus* and the younger-aged plantation grown hybrids.

Materials and Methods

The older trees used in this comparison were trees growing in natural stands and were either trees selected for use as parent trees because of form and rate of growth or randomly located trees that were being evaluated in heritability studies or studies of natural variation. Ten-millimeter, breast high increment cores were used as a source of wood from the older trees. A minimum of two cores were used in making the specific gravity and fiber length measurements and additional cores were taken when micropulping information was determined.

The data on five- and six-year-old trees were from plantation-grown trees that had been field planted as 1-0 stock and had grown in test plantings for a total of five or six years. These trees were cut and the wood samples used in the evaluation work were disks or appropriate sized wedges from disks. The wood samples were located sixteen to thirty-two inches above the ground and contained only those annual rings that developed after field planting.

Specific gravity determinations were run in duplicate and the wood samples used for specific gravity were also used as a source of fibers for the fiber length measurements. The fiber length data for the five- and six-year-old trees were obtained on a ring-by-ring basis for the last three growth rings. The fiber length measurements reported were based upon measurements on approximately 500 fibers from the fifth annual ring. All fibers 0.3 mm. and longer including those cut, broken, and intact were measured.

The age 30 fiber length measurements for the older trees were obtained by dividing the ten-millimeter increment cores into five-year intervals, measuring 400 to 500 fibers per five-year interval, plotting the fiber length – age curve, and taking the age 30 fiber length value from the curve. For trees that were less than 30 years old, the fiber length for the last ten-year interval was determined and the fiber length age information was adjusted to age 30 using a previously prepared fiber length – age curve. All fibers 0.3 mm. and longer including those cut, broken, and intact were measured.

Information on pulp yield and fiber strength was obtained by micropulping small chip samples prepared from increment core or disk samples. Duplicate determinations were made upon the five- and six-year-old trees while single determinations were available for all other trees. The micropulping procedure used employed a kraft pulping system and a multiunit digester [van Buijtenen, et al. (6)]. The techniques used and the cooking conditions employed are reported in detail in a paper by Gardner and Einspahr (7). The yield data presented are the percent yield of pulp and are based on equivalent weights of wood in each digester. Zero-span tensile strength measurements were conducted on test handsheets using the procedure described by Wink and Van Eperen (8) and are interpreted as a measure of individual fiber strength. Alcohol-benzene extractives and percent lignin were determined on wood samples using TAPPI standard methods T 6 m-54 and T 13 m-54.

Discussion of Results

Tables VIII and IX summarize the results of the wood quality evaluation work with aspen and cottonwood carried on over the past ten years. Table VIII summarizes specific gravity, fiber length, and fiber strength measurements while Table IX presents data on pulp yield, lignin, and extractives. The numbers in parentheses in Tables VIII and IX indicate the number of trees measured to obtain the mean and range information presented. The greatest amount of evaluation work has been done with specific gravity and the least number of measurements involve lignin and extractive determinations. Comparison of data from wedges from young trees with increment core data from older trees will tend to reduce slightly the differences between the two types of measurements. The reason is that the core samples weight the center of the tree more than the exterior portion with the result that the wood samples in this instance are more nearly alike (high in proportion of young wood) than usual.

TABLE VIII

WOOD AND FIBER PROPERTIES OF LAKE STATES GROWN
POPULUS AND POPULUS HYBRIDS

Species[a]	Age, yr.	Specific gravity, g./cc. Average	Specific gravity, g./cc. Range	Fiber length, mm.[b] Average	Fiber length, mm.[b] Range	Zero-span tensile, lb./in. Average	Zero-span tensile, lb./in. Range
P. alba hybrids	18-30	0.392 (2)[c]	0.389-0.394	1.10 (2)	1.00-1.21	--	--
	31+	0.358 (4)	0.334-0.375	0.99 (2)	0.98-1.00	72.2 (1)	
P. deltoides	18-30	0.363 (3)	0.357-0.372	1.10 (2)	1.05-1.15	--	--
	31+	0.352 (10)	0.336-0.374	0.95 (8)	0.82-1.05	66.7 (5)	59.0-75.2
P. grandidentata	18-30	0.363 (2)	0.341-0.385	0.97 (3)	0.94-0.99	--	--
	31+	0.378 (31)	0.325-0.433	0.97 (40)	0.77-1.18	72.9 (5)	67.7-81.8
P. tremuloides (3n)	31+	0.391 (34)	0.318-0.447	1.23 (33)	1.02-1.37	56.9 (23)	48.6-65.9
P. tremuloides	18-30	0.390 (123)	0.310-0.456	0.97 (124)	0.76-1.20	55.3 (5)	49.3-61.4
	31+	0.390 (285)	0.331-0.476	0.93 (283)	0.62-1.19	60.5 (66)	49.4-76.7
P. tremuloides	5	0.370 (554)	0.311-0.450	0.67 (305)	0.46-0.94	65.5 (153)	56.2-75.4
P. tremuloides (3n)	5	0.362 (72)	0.342-0.394	0.65 (48)	0.55-0.76	61.8 (24)	56.6-66.5
P. tremuloides x P. tremula (3n)	5	0.403 (70)	0.347-0.481	0.75 (48)	0.53-0.88	61.0 (24)	46.9-77.2
P. tremuloides x P. sieboldii	6	0.345 (9)	0.318-0.366	0.72 (4)	0.64-0.85	61.2 (4)	57.2-64.2
P. tremuloides x P. davidiana	6	0.340 (22)	0.291-0.374	0.74 (8)	0.70-0.84	63.5 (9)	55.2-68.1
P. alba x P. grandidentata	6	0.380 (5)	0.344-0.418	--	--	--	--
P. grandidentata x P. davidiana	6	0.345 (12)	0.312-0.374	0.76 (4)	0.69-0.81	63.7 (3)	60.8-67.2
P. grandidentata x P. alba	6	0.373 (9)	0.324-0.441	0.73 (4)	0.67-0.78	59.0 (4)	55.8-60.4

[a] The P. alba hybrids are naturally occurring hybrids in which the female parent is P. alba and the male parent is believed to be P. grandidentata. 3n indicates trees having three sets of chromosomes (triploids).

[b] Fiber length based on age 30 for mature, natural stand trees, and age 5 for plantation grown trees.

[c] Number in parentheses indicates number of individuals used to obtain the mean.

TABLE IX

PULP AND CHEMICAL PROPERTIES OF LAKE STATES GROWN POPULUS AND POPULUS HYBRIDS

Species[a]	Age, yr.	Pulp Yield, %		Lignin, %		Extractives, %	
		Average	Range	Average	Range	Average	Range
P. alba hybrids	18-30	--	--	--	--	--	--
	31+	51.5 (1)[b]		20.4 (1)		3.8 (1)	
P. deltoides	18-30	--	--	--	--	--	--
	31+	51.2 (5)	50.5-52.7	22.4 (5)	22.0-22.9	2.1 (5)	2.0-2.2
P. grandidentata	18-30	--	--	--	--	--	--
	31+	51.2 (5)	50.0-52.8	20.3 (5)	20.0-20.6	3.0 (5)	2.7-3.3
P. tremuloides (3*n*)	31+	55.1 (24)	51.8-59.0	18.3 (24)	16.2-19.9	3.14 (24)	2.38-4.00
P. tremuloides	18-30	51.0 (3)	49.7-52.6	--	--	--	--
	31+	51.8 (61)	47.8-54.4	19.2 (1)		3.5 (1)	
P. tremuloides	5	48.8 (153)	43.6-52.4	18.1 (153)	15.7-20.4	4.64 (153)	2.70-16.7
P. tremuloides (3n)	5	48.2 (24)	46.6-50.2	17.2 (24)	16.6-17.9	6.32 (24)	4.98-7.90
P. tremuloides x *P. tremula* (3*n*)	5	48.8 (24)	46.8-50.4	17.2 (24)	16.3-18.2	5.38 (24)	4.76-6.37
P. tremuloides x *P. sieboldii*	6	48.0 (4)	47.2-48.9	19.3 (4)	18.4-19.8	4.56 (4)	4.30-4.93
P. tremuloides x *P. davidiana*	6	49.3 (9)	48.0-51.2	18.3 (8)	18.0-18.8	4.64 (8)	4.34-5.18
P. alba x *P. grandidentata*	6	--	--	--	--	--	--
P. grandidentata x *P. davidiana*	6	49.3 (3)	48.4-49.8	17.6 (4)	17.2-18.4	4.90 (4)	4.65-5.40
P. grandidentata x *P. alba*	6	50.1 (4)	49.2-50.7	18.0 (4)	16.9-19.2	4.45 (4)	3.93-4.94

[a] The *P. alba* hybrids are naturally occurring hybrids in which the female parent is *P. alba* and the male parent is believed to be *P. grandidentata*. 3*n* indicates trees having three sets of chromosomes (triploids).

[b] Number in parentheses indicates number of individuals used to obtain the mean.

Specific Gravity

Specific gravity, because of its influence on pulp yield and pulp properties, and because it is influenced by cell wall thickness, relative proportion of late wood, proportion of thin-walled vessels, and the presence of reaction wood, is of interest from the tree improvement point of view. The specific gravity data presented were based upon measurements made on a total of 1245 trees. The majority of trees measured were either mature quaking aspen or five-year-old plantation-grown quaking aspen. Because of the numbers of individuals involved, these data make reliable standards of comparison. Looking first at the mature trees, there appears to be no significant difference between the diploid quaking aspen "age groups" or between diploid and triploid quaking aspen. Bigtooth aspen, specific gravity data although based on fewer trees, seems to indicate that this species may have a slightly lower specific gravity than quaking aspen. It also appears that the specific gravity of cottonwood and the older P. alba hybrids are lower than quaking aspen. Comparison of the younger aged trees (five and six years old) with the mature tree specific gravity of 0.390, suggests that only moderate decreases have resulted despite the relatively young age of the trees involved.

The young triploid hybrids (P. tremuloides x P. tremula triploid) had the highest average specific gravity of the trees evaluated and the hybrids between P. tremuloides and P. davidiana had the lowest specific gravity. Also of interest is the fairly wide range in values reported which suggest that the genetic improvement of specific gravity appears to be quite promising.*

*Broad sense heritability for specific gravity in aspen has been estimated to be about 0.4 [Einspahr, et al. (9) and van Buijtenen, et al. (10)].

Fiber Length

Fiber length influences a number of pulp strength characteristics and particularly in the short fiber hardwoods, longer average fiber length is generally accompanied by higher tear resistance, and to a lesser extent increases in burst, tensile, and fold (11). Because of the importance of fiber length, parent trees and progeny groups have been evaluated and minimum standards established.

Using the fiber length of diploid quaking aspen (Table VIII) as a standard of comparison, it appears that bigtooth aspen, quaking aspen, and cottonwood have similar age 30 fiber lengths. Triploid quaking aspen has a fiber length that is approximately 28% longer than the native diploid species mentioned above. The fiber length data for *P. alba* hybrids are too limited to judge adequately the fiber length of this material. Preliminary measurements indicate the fiber length is at least as good as that of the native quaking aspen.

The age five fiber lengths are as expected, less than the age thirty fiber measurements. Using the quaking aspen diploid average fiber length as a standard of comparison, the several aspen hybrids all had average fiber lengths that exceeded that of the diploid quaking aspen.

An earlier reported comparison of the age five fiber length of the triploid hybrids confirmed the fiber length superiority of this type of material over diploid quaking aspen [Einspahr, *et al.* (12)]. Despite the restricted number of measurements on the other hybrids, the fifth-year fiber length of several of the materials suggest that these hybrids can be expected to have fiber lengths that are longer than those of the native aspen. It is also of interest that the fifth-year data reported are approximately equal to the fiber length averages reported for mature maples. Pulping work with these young aspen and aspen hybrids indicate they will yield pulps satisfactory for the types of papers presently being made from quaking aspen.

Zero-Span Tensile Strength

Zero-span tensile strength is a difficult measurement to make but when properly handled gives an average fiber strength value for the fibers being tested. Zero-span tensile strength has been shown to be related in a positive manner to the more conventional paper tests of burst, tear, and tensile strength and has the advantage that it can be made on a very limited amount of pulp. Because the absolute values obtained are influenced by cooking conditions, sheet formation, and other processing variables it is necessary for fairly large differences (8 to 10 lb./in.) to exist between the averages presented in order for the differences presented to be meaningful.

Based upon earlier experience [Gardner and Einspahr (7)] with this test it seems unlikely that the zero-span tensile strength differences presented for the five- and six-year-old trees are statistically significant. Comparing the fiber strength of the older trees, it appears that bigtooth aspen and possibly cottonwood can be expected to have fiber strength values greater than diploid quaking aspen. A comparison of the young trees with the older trees reveals that the five- and six-year-old trees have higher zero-span tensile strength values, although it is doubtful that the differences presented are statistically significant.

Lignin, Extractives, and Pulp Yield

Because of the interrelationships between levels of lignin, extractives, and yield of pulp, these factors are considered together and the data summarized in Table IX. Differences between types of materials in the above properties were not large with the exception of the older triploid quaking aspen. The older triploid quaking aspen had the highest average pulp yield and relatively low levels of lignin and extractives. The five- and six-year-old trees had two to three percent lower pulp yield than the older bigtooth and quaking aspen. This reduced yield apparently resulted from the higher extractive levels present in the younger-aged trees. Lignin levels in the younger trees were one to two percent less than the older trees but despite this lower level of lignin the overall pulp yields were less. The pulp yield, extractive and lignin levels for the younger-aged trees are surprisingly uniform considering the differing genetic parentage. The somewhat reduced overall natural variation in the above chemical properties of aspen suggest that only limited gains can be expected if these properties are emphasized in tree improvement work.

POLLEN SIZE VARIATION IN POPULUS

Introduction

Average pollen size and pollen size distribution is of interest because it appears that it could be useful in characterizing species, identifying hybrids and determining levels of polyploidy for male trees within the genus *Populus*. With these possibilities in mind a series of preliminary measurements were made to determine how useful such information might be. Little was known about measurement procedures and the influence storage might have on pollen size. The information that follows describes the preliminary measurements made using a photographic technique and compares these results with several pollen measurements made using the Coulter counter.

Methods and Materials

Table X lists the several sources of quaking aspen (*P. tremuloides*) and European aspen (*P. tremula*) male trees on which pollen measurements were made using the photographic measurement procedure. This procedure consists of making up a microscopic slide containing a dilute suspension of pollen. Three microphotographs each containing approximately 100 to 150 pollen grains were taken of the slides and a positive transparency* made from the negatives obtained. The measurements were made directly on the transparencies using a scale graduated in millimeters. Transparencies were 4-1/2 x 6-1/2 inches in size and the magnification on the transparencies was 200X. The data was so recorded that it provided information on pollen size distribution and average pollen diameter. Comparisons between the three transparencies made from a single pollen sample indicated that the measurement procedures produced highly reproducible results. The procedure used in making measurements using the Coulter Counter will be discussed in a later paragraph.

Results

The first series of measurements made, after the reproducibility of the procedures had been established, were measurements to examine the influence that pollen storage has on pollen size. To accomplish this, pollen samples of a diploid quaking aspen (T-13-58) were measured one week, two months, and 13 months after collection. Storage was over calcium chloride in a conventional refrigerator (approximately 40°F.). Data from this series of measurements are presented in the top section of Table X and demonstrate an apparent decrease in average pollen diameter with storage. Scattered shriveled pollen grains were noted on the transparencies for the pollen samples that had been stored for 13 months.

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*Transparencies were used because they are less subject to dimensional changes during drying and later storage than conventional photographic prints.

TABLE X

SUMMARY OF PRELIMINARY POLLEN MEASUREMENTS
PHOTOGRAPHIC PROCEDURE

Tree no.[a]	Ploidy Level[b]	Storage Time, months	Av. Pollen Diam., µm.	Range of Pollen Diam., µm.	Shape of Curve[c]
T-13-58	2n	1/4	32	25-40	Moderate – Single peak
T-13-58	2n	2	31	20-40	Moderate – Single peak
T-13-58	2n	13	29	20-40	Moderate – Single peak
Ta-10	4n	1	40	30-55	Low – Single peak
Ta-1	3n	1	32	22-42	Moderate – Single peak
Ta-5	2n	1	30	15-38	Sharp – Single peak
T-6-61	2n	1	27	20-35	Sharp – Double peak
XT-32-56, no. 53	2n	1	31	22-40	Sharp – Single peak
XT-22-56, no. 36	2n	1	34	20-45	Low – Single peak
XT-7-58, no. 26	2n	1	30	22-38	Sharp – Single peak
T-2-56	3n	1	34	22-42	Moderate – Single peak
T-124-56	3n	1	32	20-42	Moderate – Single peak

[a]T = P. tremuloides, Ta = P. tremula, XT indicates the tree was from a P. tremuloides cross.

[b]Ploidy level designates chromosome number (2n = diploid, 3n = triploid and 4n = tetraploid) of the vegetative cells.

[c]Pollen size distribution curve obtained by plotting percent pollen over pollen diameter.

The influence of polyploidy on pollen size is of particular interest to the program. Available at the time this work was being conducted were diploid (2n), triploid (3n), and tetraploid (4n) pollen of European aspen (P. tremula). Samples of these pollens were measured and the results are shown in Table X. Based upon these measurements, it was apparent that the average pollen size increased with degree of ploidy. Plotting the pollen size distribution for the three levels of ploidy (Fig. 21) further emphasized the differences involved. The pollen size curves for the 2n and 3n pollen were quite similar while the curve for 4n pollen differs both in shape

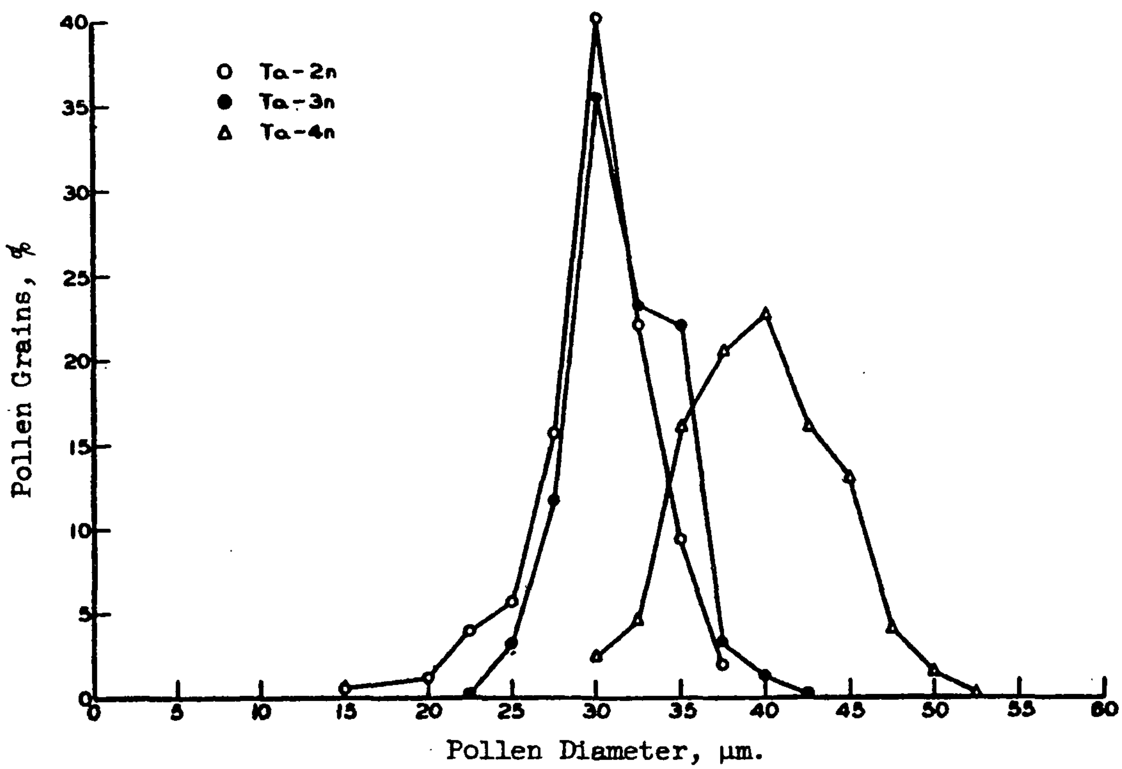

Figure 21. Pollen Size Distribution Curves for Diploid (2n), Triploid (3n) and Tetraploid (4n) European Aspen

(low broad curve) and, because of the larger pollen diameter, was displaced to the right of the 2n and 3n curves. These data suggest that pollen measurements might be used to identify pollen from tetraploid trees. Also, it is fairly well established that triploid pollen can be expected to obtain about 2% unreduced pollen which when applied to normal trees will produce tetraploids. Considering the ploidy level/size relationship established, it appears that pollination using the large size fraction of triploid pollen would very likely yield a higher than normal proportion of both triploids and tetraploid progeny.

Table X also presents measurements made on diploid and triploid quaking aspen. Considerable variation was encountered in the average diameter and type of curve formed by the pollen size distribution data for diploid quaking aspen, although in most instances a curve very similar to that formed by the diploid European aspen was obtained. The differences between 2n and 3n quaking aspen are small and, like the differences shown for European aspen, may not be great enough to be useful in distinguishing these two levels of ploidy. Additional data are needed to clarify this point.

<u>Coulter Counter Measurements</u>

Approximately one year after the pollen measurements were started, the Institute obtained the use of a Coulter Particle Counter. This is an electronic device which quite rapidly measures particle size in the 1 to 1000-µm. diameter range by the displacement of an electrolyte within an aperture. As the particle being measured is passed through the aperture, the ion flow across the aperture is momentarily impeded, causing a voltage drop and making possible the counting of the particle. Since the voltage drop for spherical particles is primarily governed by the relation of particle volume to aperture size, it is possible not only to count the particle but to determine its volume. The analysis circuits of the counter allow the choice of a given particle size threshold and the counting of all particles which exceed this threshold. Varying the threshold allows the generation of an accumulative distribution curve of particle size. Analysis of a single sample takes approximately one-half hour.

The capabilities of the Coulter Counter appear to be well suited to our measurement work and, if sufficiently reliable, could be expected to reduce the time spent on measuring samples to about 1/5 the time involved in the photographic procedure.

With this in mind, a number of pollen samples were prepared and average pollen diameter and pollen size distribution curves prepared. Several minor technical difficulties have been encountered and when these have been worked out, it appears that the instrument will greatly facilitate pollen size investigations. Table XI presents some of the preliminary measurements made with the Coulter Counter. One of the technical problems encountered was the possibility that dispersing the pollen in an electrolyte would change the volume of the pollen. By measuring the pollen immediately after dispersing and after 15 minutes in the electrolyte, the decrease in average diameter that resulted was less than 1 μm. Although the decrease is small, it should be considered in establishing a standard measurement procedure.

TABLE XI

SUMMARY OF PRELIMINARY POLLEN MEASUREMENTS
WITH THE COULTER COUNTER

Tree No.[a]	Ploidy Level[b]	Storage Time, months	Av. Pollen Diam., μm.	Range of Pollen Diam., μm.	Shape of Curve[c]
T-13-58	2n	2-1/2	28.5	12-63	Moderate single peak
T-20-60	2n	2-1/2	26.7	16-50	Sharp twin peaks
T-46-60	2n	3	26.3	12-40	Sharp twin peaks
T-2-56	3n	1	30.3	16-50	Moderate flat-topped curve
T-36-56	3n	1	29.0	16-63	Sharp single peak
T-124-56	3n	1	25.8	20-50	Sharp single peak
Ta-1-67	2n	3-1/2	25.9	16-40	Sharp single peak
Ta-2-67	2n	3-1/2	27.9	16-40	Sharp single peak
D-6-65	2n	1	26.0	20-50	Sharp single peak
D-2-66	2n	1	24.6	12-50	Moderate flat-topped curve
D-5-67	2n	1	24.2	20-40	Moderate twin peaks
A-3-67	2n	2	25.9	20-50	Sharp single peak
Ca-1-62	2n	3-1/2	29.6	16-50	Moderate twin peaks

[a] A = P. alba, Ca = P. canescens, D = P. deltoides, T = P. tremuloides, Ta = P. tremula.
[b] Ploidy level designates chromosome number (2n = diploid, 3n = triploid).
[c] Pollen size distribution curve obtaining by plotting % pollen over pollen diameter.

Stored pollen was available for measurement for three of the pollens that had been measured earlier by the photographic method. The average diameter of the tree pollens (T-13-58, T-2-56, and T-124-56), as measured by the Coulter Counter, ranged from 3 to 6 µm. lower than the measurements made using the photographic method. There were also some minor differences in the shape of the pollen distribution curves. Additional comparisons appear desirable and plans call for the use of a standard fresh pollen and with this pollen careful investigations will be made into: (1) the photographic procedure vs. the Coulter Counter method, (2) the influence of the electrolyte on pollen size, and (3) pollen size changes with storage time. The preliminary measurements obtained to date suggest that the Coulter Counter could be very useful and that the information on pollen size and the shape of the pollen size distribution curves may be useful tools in hybridization and polyploid work.

SPECIFIC GRAVITY, BARK, AND MOISTURE CONTENT VARIATION IN QUAKING ASPEN STEMS

Before the natural advantages of aspen, mentioned in the introduction of this report, can be fully appreciated and more sophisticated aspen management concepts can be put into common use several areas must be investigated. One of these areas is the possibility of using small diameter aspen stems in papermaking.

The first objection usually expressed to the small diameter stem concept was, "What will you do about the bark?" The second was, "What is the papermaking quality of small stems?" Project 2412 work has involved several studies of wood quality of small diameter aspen stems and the work is summarized in an earlier section of this report titled "Wood Property Variation in Populus." The bark problem has been investigated through other projects at the Institute. Dr. Kremers of the Institute staff has demonstrated the possibility of enzyme digestion of bark. A flotation process for separating wood and bark has also been investigated. In this technique rough aspenwood was chipped and the chips put through a vat of water. The wood floated and the bark sank. No attempt was made to maximize the efficiency of the separation and it appears that the process could be modified to meet varying company standards.

Midmonthly collections of aspen were taken from a clone near Greenville, Wisconsin to determine seasonal differences in success of the flotation process. Halfway through a year's planned midmonthly collections from natural stands, henceforth called "wild aspen," midmonthly collections from a nine-year-old plantation were added. At that time it was also decided to determine some physical properties of the wood and bark to better understand why the procedure was successful. The following is a summary of that data.

Trees taken from the wild aspen were limited to a maximum diameter of 8 inches at 4-1/2 feet because of the chipper size. Disks of normal wood and bark were taken at 6-foot intervals up the tree starting at one-half foot and ending at the interval nearest the 1/2-inch top diameter. From these disks the following information was taken:

(1) Percent bark (bark/bark + wood) — by volume green weight and ovendry weight.
(2) Percent moisture content (moisture loss/green weight) — for bark, wood and whole tree.
(3) Percent heartwood (heartwood/total wood) — by volume and ovendry weight.
(4) Specific gravity (grams per cubic centimeter) — for bark, wood, sapwood, and heartwood.

When the tree was cut, moisture content samples were taken and sealed in individual polyethylene bags so moisture content figures would represent field conditions. Moisture content, specific gravities, volumes, and ovendry weights were determined by use of TAPPI Standard T 18 m-53 "off the balance" techniques. Heartwood was determined by a soap-solution-air-stream technique. A soap solution was applied to one face of the disk and the disk placed over an air stream. The air penetrated the sapwood but could not pass through the heartwood and the soap bubbles made it possible to locate the sapwood-heartwood boundary.

The range and average: % bark; % moisture content; % heartwood; and specific gravity of bark, total wood, heartwood, and sapwood of both the wild aspen and the plantation trees are given in Table XII. More detailed data from this study are given in the tables of the Appendix. While this was not a study in depth of the various properties, there are certain generalizations that can be made that have a bearing on future investigations regarding the intensive management of aspen. In interpreting the data it is well to remember: (1) The wild aspen are from one stand at one area and were codominant because of chipper limits on wood diameter. (2) The plantation trees are from the same area, represent two crosses and usually were the inferior trees of the crosses. The range of percent bark was larger for the plantation trees than those from the wild trees. The average percent was about the same for both types of trees with 17-18% bark by volume or 21-24% by ovendry weight. The data for both types of trees indicated the percent bark increases up the stem of the tree. This does not mean that the percent of bark is always inversely proportional to the stem diameter. The data obtained also give some indication that when stems of the same diameter are compared, older trees usually have a greater bark thickness and as a result a greater percent bark.

TABLE XII

ASPENWOOD AND BARK DATA

Characteristic	Wild Aspen[a] Range Min.	Wild Aspen[a] Range Max.	Wild Aspen[a] Average	Plantation Aspen[b] Range Min.	Plantation Aspen[b] Range Max.	Plantation Aspen[b] Average
% Bark by:						
Volume	16	19	17	13	24	18
Green Wt.	20	23	21	13	28	22
Ovendry Wt.	23	26	24	15	29	21
% Moisture Content						
Bark	35 Jan.	42 June	38	40 Oct.	54 June	47
Wood	45 June	51 Mar.	48	41 Aug.-Sept.	55 Mar.	47
Whole Tree	44 June	48 Feb.-Mar.	46	41 Sept.	53 Mar.	47
% Heartwood						
Volume	10	20	15	0	10	2
Ovendry Wt.	9	20	14	0	9	2
Specific Gravity, g./c.c.						
Bark	0.569	0.668	0.628	0.348	0.525	0.429
Wood	0.397	0.441	0.416	0.318	0.459	0.388
Heartwood	0.388	0.417	0.400	--	--	--
Sapwood	0.411	0.426	0.419	--	--	--

[a] Based on 6 trees taken from a natural stand near Greenville from Jan. 1967 - June 1967. Average tree size was 43 feet tall and 5.7 inches d.b.h. at an average age of 30 years.

[b] Based on 19 trees taken from a 9-10-year-old plantation at Greenville from Jan. 1967 - Oct. 1967. Average tree size was 26 feet tall and 2.6 inches d.b.h.

Moisture contents of both types of trees were greatest for bark and wood samples taken from the upper stem. There were not enough data from the "wild aspen" population to speculate on a 12-month moisture content trend. The plantation trees include data from 19 trees taken over a 10-month period. These data seem to indicate a trend of higher moisture content in the wood than in the bark during the dormant season. A rather abrupt reversal of this order occurs at the time of leaf flush and holds until leaf fall and the start of another dormant season.

The percent heartwood data for the wild trees averaged 15% by volume and 14% by ovendry weight. For the younger-aged plantation trees the percent heartwood averaged 2% both by volume and ovendry weight with a data spread of 0 to 9.8%.

The average specific gravities for the wild tree samples when ranked from high to low fall as follows: bark -0.628, sapwood -0.420, total wood -0.416 average, and heartwood -0.400 average. The data for the plantation trees show a smaller difference between the specific gravity for bark (average 0.429) and for total wood (average 0.388). Specific gravities for heartwood and sapwood were compared only for those plantation trees with heartwood. In contrast to the wild tree data, the plantation tree average specific gravity for the heartwood (average 0.369) was higher than that of the sapwood (average 0.352). This apparent conflict actually fits in with other specific gravity data, taken by the Genetics and Physiology Group, which indicate specific gravities in aspen start out high for the first few years, drop off, and eventually rise to the normal high.

These minimal data, in addition to providing information that indicate why the flotation procedure works, indicate certain areas that should be considered in selecting and breeding trees and in the developing of management plans for aspen that is to be grown on short rotations. Areas of consideration include:

(1) Desirability of selecting and breeding for low percentage of bark,

(2) Feasibility of manipulating the environment to minimize the percentage of bark in young stems,

(3) Advantage gained in harvesting young stands when moisture content is low,

(4) Desirability of selecting and breeding for high specific gravity for aspen that is to be harvested at young ages, and

(5) Possibilities of environmental manipulation of specific gravity.

INTRASPECIFIC AND INTERSPECIFIC CROSSING

Each year the crossing investigations make up an important part of the Project 2412 program. Quaking aspen crosses and quaking aspen hybrids* have been planted on a wide variety of sites and geographic areas. As more and more measurement data become available, the usefulness of quaking aspen becomes increasingly evident. Bigtooth aspen crosses and bigtooth aspen hybrids have been produced in fairly large numbers the past four years. Several types of bigtooth hybrids show considerable promise. The crossing work involving bigtooth aspen will be reduced for the next few years pending evaluation of the materials of this type presently being field tested. Cottonwood crossing investigations are just well under way and a number of progeny and clonal tests are planned.

Despite efforts to reduce the total number of crosses, thirty-nine different parent trees including seventeen quaking aspen, four European aspen, two European gray poplar, eight bigtooth aspen, and eight cottonwood were employed as parent trees and a total of sixty-nine crosses were attempted. Part of the reason for the large number of crosses was that sixteen crosses were conducted at the Institute as part of a cooperative study conducted with Dr. Melchior of Schmalenbeck, West Germany.

*Throughout 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).

Table XIII summarizes the parent trees utilized in the crossing program and Tables XIV and XV provide additional information on crossing success, seedling size, and seedling production. Seed production was high in certain types of crosses and fairly large amounts of seed were stored for use in 1968. Not all of the seedling produced were grown in the I.P.C. nursery. Figure 22 illustrates the September condition of aspen hybrids growing in the Kimberly-Clark nursery at Norway, Michigan.

QUAKING ASPEN CROSSES

A total of thirty-nine crosses were made in which either one or both parents were quaking aspen (T). The number of crosses were larger than originally planned because of the availability of several unusual sources of pollen acquired as part of the cooperative program. Sixteen of the crosses (XT-8-67 through XT-Ta-23-67) were part of the cooperative crossing program described above. Two crosses (XT-1-67 and XT-2-67) were made to check on the influence of the Alar growth-retardant chemical on flowering, pollen production, and seed set of flowers produced by the treated trees. The majority of the remaining crosses were either hybrids between quaking aspen and European gray poplar for use on "dry site" plantings or quaking aspen crosses. Seed production of most crosses was good and with the exception of the several crosses in which pollen was shipped in from Europe, total seeds available were adequate to meet requirements.

BIGTOOTH ASPEN CROSSES

Bigtooth aspen crosses (G) and bigtooth aspen hybrids continued to receive considerable emphasis in 1967. Seventeen crosses were conducted in which either one or both parents involved were bigtooth aspen. The majority of the crosses were made in an effort to obtain genetic combinations that would do well on dry sandy sites.

TABLE XIII

SUMMARY OF CROSSES AND LOCATION OF PARENT TREES

Cross No.[a]	Parents (female	x	male)
XT-1-67	T-16-56 (Greenville, Wis.)	X	T-32-57 (Eagle River, Wis.)
XT-2-67	T-130-56 (Bruce Crossing, Mich.)	X	T-20-60 (Alston, Mich.)
XT-3-67	T-1-58 (Ontonagon, Mich.)	X	T-20-60 (Alston, Mich.)
XT-4-67	T-1-58 (Ontonagon, Mich.)	X	T-6-67 (Ontonagon, Mich.)
XT-5-67	T-5-63 (Norway, Mich.)	X	T-20-60 (Alston, Mich.)
XT-6-67	T-5-63 (Norway, Mich.)	X	T-6-67 (Ontonagon, Mich.)
XT-7-67	T-5-67 (Ontonagon, Mich.)	X	T-20-60 (Alston, Mich.)
XT-8-67	Clone 5 (Wausau, Wis.)	X	T-28-63 (Watersmeet, Mich.)
XT-9-67	Clone 5 (Wausau, Wis.)	X	T-12-67 (Ontario, Canada)
XT-10-67	T-16-56 (Greenville, Wis.)	X	T-28-63 (Watersmeet, Mich.)
XT-11-67	T-16-56 (Greenville, Wis.)	X	T-12-67 (Ontario, Canada)
XT-12-67	T-12-58 (Clintonville, Wis.)	X	T-28-63 (Watersmeet, Mich.)
XT-13-67	T-12-58 (Clintonville, Wis.)	X	T-12-67 (Ontario, Canada)
XT-14-67	T-5-61 (Ontonagon, Mich.)	X	T-28-63 (Watersmeet, Mich.)
XT-15-67	T-5-61 (Ontonagon, Mich.)	X	T-12-67 (Ontario, Canada)
XT-Ta-16-67	Clone 5 (Wausau, Wis.)	X	Ta-1-67 (Schmalenbeck, Germany)
XT-Ta-17-67	Clone 5 (Wausau, Wis.)	X	Ta-2-67 (Schmalenbeck, Germany)
XT-Ta-18-67	T-16-56 (Greenville, Wis.)	X	Ta-1-67 (Schmalenbeck, Germany)
XT-Ta-19-67	T-16-56 (Greenville, Wis.)	X	Ta-2-67 (Schmalenbeck, Germany)
XT-Ta-20-67	T-12-58 (Clintonville, Wis.)	X	Ta-1-67 (Schmalenbeck, Germany)
XT-Ta-21-67	T-12-58 (Clintonville, Wis.)	X	Ta-2-67 (Schmalenbeck, Germany)
XT-Ta-22-67	T-5-61 (Ontonagon, Mich.)	X	Ta-1-67 (Schmalenbeck, Germany)
XT-Ta-23-67	T-5-61 (Ontonagon, Mich.)	X	Ta-2-67 (Schmalenbeck, Germany)
XTa-24-67	Ta-5 no. 13 (Appleton, Wis.)	X	Ta-1-67 (Schmalenbeck, Germany)
XTa-25-67	Ta-5 no. 13 (Appleton, Wis.)	X	Ta-2-67 (Schmalenbeck, Germany)
XCa-T-26-67	Ca-2 (Czechoslovakia)	X	T-20-60 (Alston, Mich.)
XCa-T-27-67	Ca-2 (Czechoslovakia)	X	T-44-60 (Ralph, Mich.)
XCa-T-28-67	Ca-2 (Czechoslovakia)	X	T-46-60 (Ralph, Mich.)
CaT-29-67	Ca-2 (Czechoslovakia)	X	T-6-67 (Ontonagon, Mich.)
XT-Ca-30-67	T-80-57 (Alston, Mich.)	X	Ca-1-62 (Czechoslovakia)
XT-Ca-31-67	T-1-58 (Ontonagon, Mich.)	X	Ca-1-62 (Czechoslovakia)
XT-Ca-32-67	T-5-63 (Norway, Mich.)	X	Ca-1-62 (Czechoslovakia)
XT-Ca-33-67	T-5-67 (Ontonagon, Mich.)	X	Ca-1-62 (Czechoslovakia)
XT-Ta-34-67	T-80-57 (Alston, Mich.)	X	Ta-4-67 (Czechoslovakia)
XT-Ta-35-67	T-1-58 (Ontonagon, Mich.)	X	Ta-4-67 (Czechoslovakia)
XCa-36-67	Ca-2 (Czechoslovakia)	X	Ca-1-62 (Czechoslovakia)

[a] See end of table for footnote.

TABLE XIII (Continued)

SUMMARY OF CROSSES AND LOCATION OF PARENT TREES

Cross No.[a]	Parents (female x male)			Cross No.[a]	Parents (female x male)		
XCa-G-37-67	Ca-2 (Czechoslovakia)	X	G-1-67 (Armstrong Creek, Wis.)	XTa-54-67	Ta-5, no. 13 (Appleton, Wis.)	X	Ta-4-67 (Czechoslovakia)
XCa-G-38-67	Ca-2 (Czechoslovakia)	X	G-5-67 (Rural, Wis.)	XD-55-67	D-1-67 (Deer Creek, Wis.)	X	D-2-67 (Deer Creek, Wis.)
XCa-G-39-67	Ca-2 (Czechoslovakia)	X	G-6-67 (Menominee Co., Wis.)	XD-56-67	D-1-67 (Deer Creek, Wis.)	X	D-5-67 (Brillion, Wis.)
XCa-G-40-67	Ca-2 (Czechoslovakia)	X	G-8-63 (Hiles, Wis.)	XD-57-67	D-1-67 (Deer Creek, Wis.)	X	D-7-65 (Nichols, Wis.)
XG-41-67	G-64 (Wausau, Wis.)	X	G-1-67 (Armstrong Creek, Wis.)	XD-58-67	D-4-67 (Black Creek, Wis.)	X	D-2-67 (Deer Creek, Wis.)
XG-42-67	G-64 (Wausau, Wis.)	X	G-5-67 (Rural, Wis.)	XD-59-67	D-4-67 (Black Creek, Wis.)	X	D-5-67 (Brillion, Wis.)
XG-43-67	G-64 (Wausau, Wis.)	X	G-6-67 (Menominee Co., Wis.)	XD-60-67	D-4-67 (Black Creek, Wis.)	X	D-7-65 (Nichols, Wis.)
XG-44-67	G-2-67 (Millston, Wis.)	X	G-1-67 (Armstrong Creek, Wis.)	XD-61-67	D-6-67 (Brillion, Wis.)	X	D-2-67 (Deer Creek, Wis.)
XG-45-67	G-2-67 (Millston, Wis.)	X	G-5-67 (Rural, Wis.)	XD-62-67	D-6-67 (Brillion, Wis.)	X	D-5-67 (Brillion, Wis.)
XG-46-67	G-2-67 (Millston, Wis.)	X	G-6-67 (Menominee Co., Wis.)	XD-63-67	D-6-67 (Brillion, Wis.)	X	D-7-65 (Nichols, Wis.)
XG-47-67	G-7-67 (Mountain, Wis.)	X	G-1-67 (Armstrong Creek, Wis.)	XD-64-67	D-7-67 (Appleton, Wis.)	X	D-6-65 (Nichols, Wis.)
XG-48-67	G-7-67 (Mountain, Wis.)	X	G-5-67 (Rural, Wis.)	XT-65-67	T-80-57 (Alston, Mich.)	X	T-10-60 (Ontonagon, Mich.)
XG-49-67	G-7-67 (Mountain, Wis.)	X	G-6-67 (Menominee Co., Wis.)	XT-66-67	T-11-67 (Ontario, Canada)	X	T-12-67 (Ontario, Canada)
XG-Ca-50-67	G-64 (Wausau, Wis.)	X	Ca-1-62 (Czechoslovakia)	XT-67-67	T-11-67 (Ontario, Canada)	X	T-28-63 (Watersmeet, Mich.)
XG-Ca-51-67	G-2-67 (Millston, Wis.)	X	Ca-1-62 (Czechoslovakia)	XT-68-67	T-16-56 (2n) (Greenville, Wis.)	X	T-2-56 (3n) (Bruce Crossing, Mich.)
XG-Ca-52-67	G-7-67 (Mountain, Wis.)	X	Ca-1-62 (Czechoslovakia)	XT-A-69-67	T-16-56 (Greenville, Wis.)	X	A-3-67 (Czechoslovakia)
XG-Ca-53-67	G-9-63 (Bruce, Wis.)	X	Ca-1-62 (Czechoslovakia)				

[a] X = cross, A = P. alba, Ca = P. canescens, G = P. grandidentata, D = P. deltoides, T = P. tremuloides, Ta = P. tremula.

TABLE XIV

SUMMARY OF 1967 CROSSES

Cross No.[a]	Type Cross[b]	No. of Catkins Pollinated	No. of Catkins Collected	Amt. Seed[c]	Seeds/Catkin[c]	Germ., %[c]
XT-1-67	TP	20	15	603	45[d]	97[d]
XT-2-67	TP	26	24	4,785	270[d]	99[d]
XT-3-67	C	260	257	51,158	195	99
XT-4-67	C	220	220	75,887	341	99
XT-5-67	C	102	69	338	-[e]	-[e]
XT-6-67	C	97	83	436	-[e]	-[e]
XT-7-67	C-TP	202	159	91,263	420[d]	98[d]
XT-8-67	C	15	12	13,146	868	99
XT-9-67	C	10	7	6,691	662	99
XT-10-67	C	45	39	11,944	255	96
XT-11-67	C	30	30	16,019	529	99
XT-12-67	C	11	8	6,518	581	98
XT-13-67	C	23	22	13,916	478	79
XT-14-67	C	22	22	10,450	456	96
XT-15-67	C	14	9	3,672	226	86
XT-Ta-16-67	C	23	23	18,165	774	98
XT-Ta-17-67	C	20	20	540	-[e]	-[e]
XT-Ta-18-67	C	30	26	16,515	468	85
XT-Ta-19-67	C	30	27	4,605	152	99
XT-Ta-20-67	C	14	13	6,171	375	85
XT-Ta-21-67	C	17	14	861	-[e]	-[e]
XT-Ta-22-67	C	27	24	9,273	337	98
XT-Ta-23-67	C	12	12	354	-[e]	-[e]
XTa-24-67	C	30	28	3,054	33	32
XTa-25-67	C	30	0	-[e]	-	-
XCa-T-26-67	DS	185	136	28,703	154	99
XCa-T-27-67	DS	171	84	25,116	145	99
XCa-T-28-67	DS	197	159	45,655	229	99
XCa-T-29-67	DS-TP	221	171	31,275	182	99
XT-Ca-30-67	DS	320	303	120,180	377	94
XT-Ca-31-67	DS	130	124	33,073	252	99
XT-Ca-32-67	DS	258	142	4,013	13	84
XT-Ca-33-67	DS	162	162	48,369	104	35
XT-Ta-34-67	C	52	41	103	-[e]	-[e]
XT-Ta-35-67	C	52	35	21	[e]	-[e]
XCa-36-67	DS	63	56	9,059	137	95
XCa-G-37-67	DS	355	240	53,152	141	94
XCa-G-38-67	DS	344	324	56,004	161	99
XCa-G-39-67	DS	383	354	91,956	223	93
XCa-G-40-67	DS	343	280	140,130	392	96

See end of table for footnote.

TABLE XIV (Continued)

SUMMARY OF 1967 CROSSES

Cross No.[a]	Type Cross[b]	No. of Catkins Pollinated	No. of Catkins Collected	Amt. Seed[c]	Seeds/Catkin[c]	Germ.,
XG-41-67	DS-TP	83	59	11,758	118	75
XG-42-67	DS-TP	83	66	12,862	105	84
XG-43-67	DS	52	34	13,097	242	96
XG-44-67	DS	21	7	1,299	59	95
XG-45-67	DS	19	9	3,819	187	93
XG-46-67	DS	16	15	6,430	386	96
XG-47-67	DS	16	0	-	-	-
XG-48-67	DS	13	0	-	-	-
XG-49-67	DS	10	0	-	-	-
XG-Ca-50-67	DS	40	33	11,001	253	92
XG-Ca-51-67	DS	15	7	518	31	90
XG-Ca-52-67	DS	10	0	-	-	-
XG-Ca-53-67	DS	51	51	18,917	364	98
XTa-54-67	C	37	0	-	-	-
XD-55-67	B-TP	16	6	617	20	51
XD-56-67	B-TP	6	3	279	20	42
XD-57-67	B-TP	16	12	2,962	72	39
XD-58-67	B-TP	14	11	82	-[e]	-[e]
XD-59-67	B-TP	16	8	491	3	9
XD-60-67	B-TP	17	4	520	-[e]	-[e]
XD-61-67	B-TP	42	34	1,309	9	39
XD-62-67	B-TP	15	10	91	-[e]	-[e]
XD-63-67	B-TP	36	19	1,189	31	95
XD-64-67	B-TP	30	27	4,531	59	39

[a]X = cross, A = P. alba, Ca = P. canescens, D = P. deltoides, G = P. grandidentata, T = P. tremuloides, Ta = P. tremula.

[b]C = seed for semicommercial production, TP = seed for tree physiology studies, DS = dry site cross, B = crosses in black poplar group.

[c]Amount of seed, seeds/catkin pollinated and germination percent based upon 40-mesh and larger seed for all crosses except for "G" crosses which were based on 50-mesh seed and crosses 50 and 51 which were also based on 50-mesh seed.

[d]Crosses used in tree physiology studies and data presented is based on control treatments.

[e]Too few seeds to allow laboratory germination test to be run.

TABLE XV

SUMMARY OF 1967 SEEDLING PRODUCTION

Cross No.[a]	Total No. Seeds Planted	Total No. Plantable Seedlings Produced	No. Plantable Seedlings[b] Misc. Beds	Repl. Beds	Average Height[c] All Seedlings	Plantable Seedlings
XT-3-67	1500	615	-	615	2.3	2.4
XT-4-67	1500	439	-	439	2.1	2.3
XT-7-67	1500	391	-	391	2.4	2.5
XTa-24-67	2950	683	-	683	2.2	2.3
XCa-T-26-67	1500	649	-	649	2.7	2.8
XCa-T-27-67	4500	1475	951	524	3.2	3.2
XCa-T-28-67	2000	678	45	678	2.7	2.8
XCa-T-29-67	1500	370	-	370	2.8	2.9
XT-Ca-30-67	5500	725	434	291	2.5	2.6
XT-Ca-31-67	5500	1225	801	424	2.6	2.8
XT-Ca-32-67	1800	73	-	73	1.7	2.1
XT-Ca-33-67	4500	134	-	134	2.9	3.1
XCa-36-67	5500	1475	429	1046	2.9	3.0
XCa-G-37-67	1650	575	91	484	2.9	2.9
XCa-G-38-67.	4500	1375	894	481	3.3	3.3
XCa-G-39-67	2750	725	354	371	2.9	3.0
XCa-G-40-67	1500	475	45	430	2.9	2.9
XG-41-67	4000	325	179	146	1.8	2.1
XG-42-67	5500	725	448	277	1.5	1.8
XG-43-67	5500	525	313	212	1.4	1.7
XG-44-67	850	255	-	255	2.0	2.3
XG-45-67	1650	225	16	209	1.5	1.9
XG-46-67	1800	332	-	332	2.2	2.4
XG-Ca-50-67	4400	675	390	285	3.0	3.1
XG-Ca-51-67	520	168	-	168	2.4	2.8
XD-56-67	260	93	93	-	1.8	2.0
XD-57-67	2640	188	188	-	3.0	3.2
XD-61-67	1220	84	84	-	2.5	2.6
XD-63-67	1030	387	387	-	2.2	2.5
XD-64-67	2290	209	209	-	2.3	2.5

[a]X = cross, Ca = P. canescens, G = P. grandidentata, D = P. deltoides, T = P. tremuloides, Ta = P. tremula.

[b]Number of plantable seedlings, 1.4 or larger in height and of satisfactory caliper.

[c]Average heights based upon seedlings in replicated seedbeds; when replicated beds were not available, miscellaneous seedbeds were measured.

Figure 22. The Fall Condition of 1-0 Hybrid Aspen Seedbeds at the Kimberly-Clark Corporation Nursery in Norway, Michigan

Sixteen of the crosses involving bigtooth aspen and European gray poplar were arranged in such a way (modified diallel series) that the crossing compatibility and the over-all performance of four male and four 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 XVI summarizes the results of these determinations. Two of the female trees (G-2-67 and G-7-67) and three of the male trees (G-1-67, G-5-67, and G-6-67) were being evaluated for the first time. Of the female trees, G-2-67 behaved normally and produced seedlings of satisfactory size. G-2-67 compared favorably with Ca-2 and G-64 which, based on past performance, are considered to be good female trees. All of the bigtooth male trees performed satisfactorily with G-6-67 having the best seed and seedling production. Seedling growth varied between crosses with each of the males siring crosses which were above average in seedling growth. Also of particular interest is the growth advantage the hybrid seedlings (Ca x G and G x Ca crosses) exhibit when compared with seedlings produced in the bigtooth aspen crosses. Growth chamber studies have confirmed the growth advantage of hybrids of this type.

TABLE XVI

SEED AND SEEDLING PRODUCTION AND SEEDLING GROWTH
MODIFIED DIALLEL CROSSING SERIES

Female Parent Trees	Male Parent Trees			
	G-1-67	G-5-67	G-6-67	Ca-1-62
G-64	XG-41-67	XG-42-67	XG-43-67	XG-Ca-50-67
--[a]	118	105	242	253
--[b]	8	33	36	36
--[c]	1.8	1.5	1.4	3.0
G-2-67	XG-44-67	XG-45-67	XG-46-67	XG-Ca-51-67
--[a]	59	187	386	31
--[b]	19	26	74	11
--[c]	2.0	1.5	2.2	2.4
G-7-67	XG-47-67	XG-48-67	XG-49-67	XG-Ca-52-67
--[a]	--	--	--	--
--[b]	--	--	--	--
--[c]	--	--	--	--
Ca-2	XCa-G-37-67	XCa-G-38-67	XCa-G-39-67	XCa-36-67
--[a]	141	161	223	137
--[b]	44	52	54	46
--[c]	2.9	3.3	2.9	2.9

[a] Number viable seed produced per catkin pollinated.
[b] Number of plantable seedlings (1.4 feet plus) produced per catkin pollinated.
[c] Average height of all seedlings in seedbeds.

COTTONWOOD CROSSES

During the past two years modifications of the "cut branch technique" have been investigated in an effort to develop a routine procedure for crossing cottonwood. The modifications involved bringing branches containing flower buds into the greenhouse and growth chamber and placing the branches in a number of solutions containing different combinations of sucrose, captan, and streptomycin. Some of the treatments were conducted in the greenhouse while others were handled in the growth chamber under conditions of long days and high light intensity. The results were not conclusive but seemed to indicate that some sucrose was necessary to carry the catkin through to maturity. When, however, excessive amounts of sucrose were used, growth chamber seed production was reduced. This apparently resulted because of excessive internal moisture stresses that developed under long days and high light intensity. Two different sucrose levels are suggested for the two different environmental conditions.

During 1967 a total of ten cottonwood crosses were conducted and this involved the use of eight parent trees. Nine of the crosses were arranged in a modified diallel series which was designed to evaluate three male and three female trees. All of the trees were newly selected parent trees. Table XVII summarizes the seed and seedling production, and seedling growth information for the nine crosses involved. Four of the crosses received several "cut branch" treatments and only the flowers growir in the greenhouse on the 1/2 standard sucrose plus antibiotics were used in this comparison. Based upon seed and seedling production per catkin pollinated, the male tree D-7-67 and the female D-1-67 were the most prolific and best-performing parent trees. When the crosses were examined from the viewpoint of seedling growth, the crosses produced by these two parent trees produced seedlings having satisfactory rates of growth.

TABLE XVII

SEED AND SEEDLING PRODUCTION AND SEEDLING GROWTH
MODIFIED DIALLEL CROSSING SERIES

Female Parent Trees	Male Parent Trees		
	D-2-67	D-5-67	D-7-67
D-1-67	XD-55-67	XD-56-67	XD-57-67
--[a]	25	18	85
--[b]	5	16	12
--[c]	2.7	1.8	3.0
D-4-67	XD-58-67	XD-59-67	XD-60-67
--[a]	--	2	--
--[b]	--	--	--
--[c]	--	--	--
D-6-67	XD-61-67	XD-62-67	XD-63-67
--[a]	2	--	3
--[b]	2	--	3
--[c]	2.5	--	2.2

[a]Number of viable seed produced per catkin pollinated.
[b]Number of plantable seedlings (1.4 ft. plus) produced per catkin pollinated.
[c]Average height of all seedlings in seedbeds.

PLANS FOR 1968

During the coming year the emphasis of the program will be directed toward three main areas of investigation. These areas will include: (1) production of improved trees by selection, hybridization and polyploidy; (2) tree physiology studies; and (3) studies of natural variation with special emphasis on "wood quality. In addition to the above three main areas of investigation, work will be undertaken to develop a dry weight table for small aspen stems.

Selection and hybridization studies will continue to emphasize the production of cottonwood crosses and cottonwood clones suitable for use on "wet sites" in central and north central Wisconsin. The number of "dry site" crosses will be reduced and replacing this work will be a series of crosses between quaking aspen and European aspen which will be established in an effort to determine the growth potential of this type of cross <u>versus</u> diploid quaking aspen and triploid hybrid aspen. Production of polyploids, particularly triploids will be continued with the mass production of triploid cottonwood and triploid bigtooth aspen as major goals of this phase of the program.

Additional growth chamber studies are to be undertaken in the continuing program aimed at determining the nutrient requirements of aspen hybrids. Providing growth chamber space is available, additional studies relating to "physiology of the establishment and growth of aspen on adverse sites" will also be undertaken.

Studies of natural variation make up an important part of the overall Project 2412 program. Progress in this area during the past year was not as rapid as hoped for due to a shortage of experienced field personnel during this past summer. Investigations either 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, (3) natural variation of growth and wood properties of bigtooth aspen, and (4) volume measurements of ten to twenty-year-old aspen sprout stands.

PUBLICATIONS

PUBLICATIONS RELATING TO PROJECT 2412 SINCE MAY, 1967

1. Benson, M. K., and Einspahr, D. W. Early growth of diploid, triploid, and triploid hybrid aspen. Forest Sci. 13, no. 2:150-5(1967).

2. Einspahr, D. W., Benson, M. K., and Peckham, J. R. Geographic variation in growth and wood properties of quaking aspen. Silvae Genetica 16, no. 3:106-12 (1967).

3. Einspahr, D. W., Benson, M. K., and Peckham, J. R. Wood and pulp properties of five-year-old diploid, triploid, and triploid hybrid aspen. Tappi 51:72-5(1968).

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. Einspahr, D. W., van Buijtenen, J. P., and Peckham, J. R. Pulp characteristics of ten-year-old loblolly pine selected for extreme wood specific gravity. To be submitted to Silvae Genetica.

3. Einspahr, D. W., Benson, M. K., and Peckham, J. R. Wood property variation in <u>Populus</u>. To be published in Proceedings of the Eighth Lake States Forest Tree Improvement Conference.

4. Einspahr, D. W., and Benson, M. K. Management of aspen on ten to twenty-year rotations. Accepted by Journal of Forestry.

5. van Buijtenen, J. P., Einspahr, D. W., and Peckham, J. R. Micropulping loblolly pine grafts selected for extreme wood specific gravity. Submitted to Silvae Genetica.

6. Winton, L. L. Rooting of liquid-grown aspen callus. Accepted by American Journal of Botany.

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10. Winton, L. L., and Einspahr, D. W. Colchicine-polyploids of quaking aspen. To be submitted for publication.

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APPENDIX

TABLE XVIII

TREE SIZE, AGE AND COLLECTION MONTHS

Identification Number	Month Collected	Tree Height, ft.	Tree D.B.H., inches	Age
		Wild Aspen		
T-4-67	January	38	4.1	21
T-10-67	February	52	7.3	33
T-14-67	March	36	5.4	32
T-15-67	April	39	5.4	24
T-16-67	May	52	7.0	36
T-21-67	June	43	5.0	35
		Plantation Trees		
XT-22B-56-S-6	January	27	2.3	9
XT-22B-56-S-7	February	30	3.0	9
XT-22B-56-S-8	February	30	3.1	9
XT-22B-56-S-9	March	18	2.7	9
XT-22B-56-S-10	March	27	2.7	9
XT-22B-56-S-11	April	31	2.6	9
XT-22B-56-S-12	April	25	3.0	9
XT-22C-56-S-13	May	22	2.5	9
XT-22C-56-S-14	May	28	2.9	9
XT-22B-56-S-15	June	28	2.4	9
XT-22B-56-S-16	June	22	2.4	9
XT-22A-56-S-17	July	21	2.0	9
XT-22B-56-S-18	July	25	2.6	9
XT-0-42-56-S-1	August	25	1.9	9
XT-0-42-56-S-2	August	29	2.9	9
XT-0-42-56-S-3	September	26	2.7	10
XT-0-42-56-S-4	September	26	2.8	10
XT-0-42-56-S-5	October	26	2.3	10
XT-0-42-56-S-6	October	24	1.9	10

TABLE XIX

WILD ASPEN DATA[a]

Bark, %

Sample Location, feet	Volume						Green Weight						Ovendry Weight					
	Feb.	March	April	May	June	Av.	Feb.	March	April	May	June	Av.	Feb.	March	April	May	June	Av.
4.5 b.h.	-	16	15	15	16	16	18	20	18	17	22	19	21	25	23	19	23	22
0.5 stump	-	-	16	20	17	17	20	18	22	16	18	19	22	22	19	18	18	20
6.5	12	17	13	13	16	14	18	20	19	17	22	19	22	24	24	21	23	23
12.5	15	19	15	15	16	16	21	24	19	18	23	21	25	29	24	21	24	25
18.5	16	21	17	16	19	18	20	27	21	20	25	22	24	30	26	24	25	26
24.5	18	23	20	17	19	19	23	26	24	24	24	24	27	31	29	26	25	28
30.5	24	30	24	19	20	23	27	35	27	23	27	28	32	42	32	26	28	32
36.5	28		33	23	20	26	36		37	27	32	33	40		42	31	33	37
42.5	40			30		35	43			32		38	47			36		41
48.5				42		42				45		45				43		43
Weighted tree av.[b]	16[c]	19[c]	16	17	17		21	22	21	20	23		25	26	24	23	24	

Moisture Content, %

Sample Location, feet	Bark						Wood						Tree					
	Jan.	Feb.	March	April	May	June	Jan.	Feb.	March	April	May	June	Jan.	Feb.	March	April	May	June
4.5 b.h.	25	36	36	34	38	40		48	51	50	46	43		46	48	47	45	42
0.5 stump	35	37	34	36	37	40		46	46	22	42	42		44	44	25	41	42
6.5	30	38	37	34	37	41		52	52	51	50	44		49	49	48	47	43
12.5	38	39	38	37	39	42		52	53	54	50	45		49	49	51	48	45
18.5	39	38	46	38	40	43		50	52	54	52	44		48	50	50	49	44
24.5	42	39	42	40	40	47		50	55	53	48	48		47	51	51	46	47
30.5	45	37	42	42	43	48		50	56	54	52	49		46	51	51	50	49
36.5		39		45	44	51		49		56	54	53		45		52	51	52
42.5		44			47			52			55			49			52	
48.5					57						54						56	
Weighted tree av.[b]	35	38	38	37	39	42		50	51	46	49	45		48	48	45	47	44

See end of table f r footnote.

TABLE XIX (Continued)

Specific Gravity

Sample Location, feet	Bark							Wood						
	Jan.	Feb.	March	April	May	June	Av.	Jan.	Feb.	March	April	May	June	Av.
4.5 b.h.	0.667	-	0.6[illegible]5	0.665	0.637	0.606	0.640	0.419	-	0.478	0.425	0.410	0.410	0.428
0.5 stump	0.629	-	0.6[illegible]6	0.616	0.587	0.601	0.608	0.458	-	0.499	0.490	0.466	0.490	0.481
6.5	0.710	0.766	0.6[illegible]9	0.785	0.630	0.590	0.687	0.421	0.412	0.459	0.418	0.399	0.417	0.421
12.5	0.607	0.636	0.6[illegible]1	0.664	0.619	0.586	0.626	0.386	0.389	0.388	0.396	0.409	0.387	0.392
18.5	0.589	0.624	0.578	0.640	0.617	0.587	0.606	0.388	0.318	0.414	0.400	0.387	0.408	0.386
24.5	0.528	0.603	0.557	0.595	0.587	0.499	0.563	0.388	0.397	0.400	0.392	0.399	0.386	0.394
30.5	0.457	0.594	0.515	0.548	0.580	0.478	0.529	0.398	0.452	0.391	0.388	0.402	0.389	0.403
36.5		0.597		0.500	0.557	0.417	0.518		0.410		0.396	0.393	0.379	0.395
42.5		0.501			0.448		0.474		0.435			0.389		0.412
48.5					0.310		0.310					0.384		0.384
Weighted tree av.[b]	0.638	0.667[c]	0.6[illegible]15	0.668	0.603	0.569		0.414	0.397[c]	0.441	0.421	0.408	0.417	

Sample Location, feet	Heartwood						Sapwood					
	Jan.	Feb.	March	April	May	June	Jan.	Feb.	March	April	May	June
4.5 b.h.	0.402	-	0.430	0.381	0.399	0.447	0.432	-	0.429	0.420	0.420	0.439
0.5 stump	0.447	-	0.438	0.427	0.367	0.474	0.485	-	0.524	0.486	0.478	0.461
6.5	0.401	0.362	0.415	0.375	0.375	0.373	0.411	0.402	0.417	0.424	0.398	0.399
12.5	0.401	0.357	0.421	0.370	0.393	0.376	0.408	0.413	0.406	0.405	0.409	0.398
18.5	0.373	0.397	0.380	0.381	0.379	0.403	0.398	0.493	0.395	0.392	0.396	0.404
24.5		0.381	0.416	0.384	0.395	0.375	0.388	0.399	0.401	0.391	0.400	0.371
30.5		0.475			0.391	0.419	0.398	0.398	0.391	0.388	0.387	0.382
36.5		0.457			0.397			0.399		0.396	0.382	
42.5								0.435			0.389	
48.5											0.384	
Weighted tree av.[b]	0.407	0.384[c]	0.417	0.388	0.399	0.401	0.423	0.426[c]	0.435	0.423	0.411	0.414

[a]Data shown are for individual trees by height of sample in tree and month tree was cut. For information on procedures involved, see the "Specific Gravity, Bark, Moisture Content Variation in Quaking Aspen Stems" section of this report.

[b]Data weighted by the square of the radius of the sample following Smalians formula; b.h. samples not included in average.

$$Y = \left(\frac{B_1 + B_n}{2} + B_1 + B_2 \ldots\right) L$$ (Chapman & Meyer Forest Mensuration, McGraw-Hill, 1949).

[c]Weighted average obtained using approximation for missing data.

TABLE XX

PLANTATION ASPEN DATA[a]

Moisture Content, %

Sample Location, feet	Bark											Wood										
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Av.	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Av.
4.5 b.h.	47	45	48	48	51	53	49	47	41	42		-	56	55	54	47	48	40	40	41	47	
0.5 stump	45	39	40	42	43	45	45	42	38	34		-	44	46	48	45	47	41	39	37	40	
6.5	48	46	49	49	52	56	50	48	43	42		-	49	54	54	53	50	42	42	42	48	
12.5	49	51	54	51	54	56	54	54	47	47		-	50	54	52	52	50	43	43	43	48	
18.5	49	51	53	51	56	62	54	57	49	49		-	50	58	50	50	53	41	46	44	50	
24.5		51	55	53	60	67		58	55	52		-	50	57	52	49	56		48	50	48	
Weighted tree av.[b]	47	49	47	48	50	53	49	48	43	41		-	49	52	51	50	49	41	42	41	46	

Sample Location, feet	Tree										
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Av.
4.5 b.h.	-	55	54	53	48	46	42	41	41	46	
0.5 stump	-	43	45	46	44	47	42	40	38	38	
6.5	-	48	54	53	53	52	44	43	42	46	
12.5	-	49	54	52	52	52	46	45	44	48	
18.5	-	50	57	50	52	56	45	49	46	50	
24.5	-	50	56	52	53	59		52	51	49	
Weighted tree av.[b]	-	49	51	50	50	51	43	43	42	44	

Bark, %

Sample Location, feet	Volume											Green Weight										
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Av.	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Av.
4.5 b.h.	-	-	11	14	18	16	16		16	18	16	-	13	15	17	20	20	21	23	18	20	19
0.5 stump	-	-	24	18	22	21	21	24	21	27	22	-	21	21	21	24	24	27	30	25	29	25
6.5	-	12	17	14	15	14	18	18	17	19	16	-	16	15	16	18	19	22	23	17	21	19
12.5	-	12	18	16	17	18	22	17	17	21	18	-	17	19	19	22	23	19	23	21	22	22
18.5	-	16	22	21	23	24	25	23	21	25	22	-	22	22	25	29	30	34	28	26	28	27
24.5	-	22	26	29	33	30		42	25	29	30	-	30	35	32	41	23		43	35	41	35
Weighted tree av.[b]	-	13	16	16	19	18	20	20	19	22		-	19	16	19	22	22	25	26	21	25	

Sample Location, feet	Oven Dry Weight										
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Av.
4.5 b.h.	-	15	17	18	18	18	18	21	18	22	18
0.5 stump	-	22	23	22	25	24	25	28	25	31	25
6.5	-	17	17	17	18	18	19	21	17	23	19
12.5	-	16	19	19	21	21	25	20	20	23	20
18.5	-	22	25	24	26	26	29	24	24	29	25
24.5	-	29	36	32	36	18	-	38	33	38	32
Weighted tree av.[b]	-	18	18	20	22	21	23	24	23	26	

Specific Gravity

	Sample Location, ft.						
	4.5 b.h.	0.5 stump	6.5	12.5	18.5	24.5	Weighted Tree Av.[b]
Bark	0.440	0.506	0.406	0.376	0.348	0.298	0.429
Wood	0.377	0.448	0.366	0.366	0.369	0.376	0.388

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[a] % data are averages of 2 trees for each month but January which was one tree. Specific gravity data are averages for 19 trees. For information on procedures involved see the "Specific Gravity, Bark, Moisture Content Variation in Quaking Aspen Stems" section of this report.

[b] Data weighted by the square of the radius of the sample following Smalians formula; b.h. samples not included in average.

$$V = \left(\frac{B_1 + B_n}{2} + B_1 + B_2 + \ldots\right) L$$ (Chapman & Meyer, Forest Mensuration, McGraw-Hill, 1949).

GLOSSARY

Bisexual. Having both functional male and female reproductive organs in the same flower, or in the case of Populus, a tree having both male and female flowers.

Catkin. A scaly spike of usually unisexual flowers, as in Betula and Populus.

Chromosome. A microscopic, usually thread- or rodlike body carrying the units of inheritance (genes). The chromosomes are the primary constituents of the cell nucelus but are individually distinguishable only during nuclear division.

Chromosome number. The number or complement of chromosomes characteristic of a species. The number of sets must also be specified, thus in Pinus the chromosome number may be expressed as "$\underline{n}$ equals 12" or as "$2\underline{n}$ equals 24," depending on whether sex cells or vegetative cells are observed.

Chromosome set. The chromosomes inherited as a unit from one parent. Most eggs or sperm carry only one set. A set usually includes one of each kind of chromosome characteristic of the species.

Clone. A group of plants derived from a single individual (ortet) by asexual reproduction. All members (ramets) of a clone have the same genotype and consequently tend to be uniform.

Colchicine. A poisonous chemical (an alkaloid) used for multiplying or modifying the chromosomes or genes in an effort to create new types of plants.

Cross. As used in the Aspen Genetics Program the term applies to progeny produced by mating trees of the same species (intraspecific).

Cytology. The study of the cell, i.e., its structure, function, development, and reproduction in relation to growth, differentiation, and heredity.

Diploid. Having two sets of chromosomes in the nucleus – indicated by "$2\underline{n}$." One-half of the chromosomes are contributed by the female parent, one-half by the male parent. Many higher organisms are diploid except for their sex cells and associated tissue.

Gene. The smallest unit that can be shown to be consistently associated with the occurrence of a specific genetic effect. The genes are ultramicroscopic and act as if linearly arranged at fixed places (loci) on the chromosomes. Each gene interacts with other genes and the environment to produce within the cell certain physiological effects that control the development of one or more characters of an individual.

Genotype. An individual's hereditary constitution, expressed or hidden, underlying one or more characters; the gene classification of this constitution expressed in a formula. The genotype is determined chiefly from breeding behavior and ancestry.

Haploid. Having the reduced chromosome number ($\underline{n}$), i.e., having one set of chromosomes in the nucleus. This is normal in sex cells, which have only half of the number of sets occurring in diploid ($2\underline{n}$) vegetative cells.

Heritability. A measure of the relative degree to which a character is influenced by heredity as compared to environment. The heritability (in the narrow sense) of a character in a population is the fraction of the total variation that is contributed by transmissible (additive) genetic differences, i.e., it is the ratio of genotypic variance to phenotypic variance. High heritability indicates that an individual's phenotype is indicative of its genotype and that differences in environment will cause little modification, i.e., that genetic control is high.

Heterosis. Hybrid vigor; the increased vigor of a hybrid as compared to the better parent. Heterosis is at a maximum in the $\underline{F1}$ generation.

Heterozygosity. Presence in an organism of different members of the same allelic set, i.e., both the dominant and the recessive gene. For example, an Aa plant is heterozygous whereas AA and aa plants are homozygous. A heterozygous individual characteristically does not breed true and is known as a hybrid with respect to the genes in question.

GLOSSARY (Continued)

Homozygosity. Presence of identical alleles, either both dominant or both recessive, as for example AA or aa. A homozygous individual breeds true when mated with the same genotype for the character(s) in question.

Hybrid. As used in the Aspen Genetics Program the term applies to progeny produced as the result of mating trees of different species (interspecific).

Hybrid vigor. Same as heterosis.

Inbreeding. Intercrossing or selfing related organisms. This procedure, especially if carried out for a number of generations, exposes undesirable recessive characters and "fixes" desirable ones, i.e., renders them true-breeding.

Interspecific. Between species; e.g., interspecific hybridization is the production of offspring by cross-pollinating one species with another.

Intraspecific. Within a species; e.g., intraspecific hybridization is the production of offspring by cross-pollinating one individual of a species with pollen from another individual of the same species.

Mutation. A sudden variation from the ancestral phenotype, due to gene or chromosome changes. If the cause can be demonstrated as a chromosome change, the mutation is preferably referred to by the specific phenomenon involved, e.g., a change in structure (aberration) or number (ploidy). Although mutations are infrequent, and usually recessive and harmful, they are the raw material of evolution and plant breeding.

Nucleus. The cell part made up chiefly of the chromosomes.

Ortet. The one plant from which members of a clone were originally derived.

Phenotype. (1) The demonstrable characteristic(s) of an organism; the product of the interaction of the genes of an organism with the environment. (2) Individual(s) described on the basis of demonstrable characteristics. Similar phenotypes do not necessarily breed alike.

Ploidy. The chromosome situation with respect to number of sets, e.g., two sets (diploid), or variation from full sets (aneuploid).

Polyploid. Having three or more times the haploid number of chromosome sets in its cells. A cell or organism having three sets ($3\underline{n}$) is called triploid; four sets ($4\underline{n}$) tetraploid.

Progeny test. Evaluation of the breeding value of parents by suitable comparisons among their offspring.

Ramet. An individual member of a clone.

Reciprocal cross. The repetition of a cross where the sexual function of the parents is reversed, i.e., female A x male B is the reciprocal of female B x male A.

Selection. Artificial selection is the propagation by man of organisms possessing desired characteristics. The aim generally is to improve the population or gain knowledge of its hereditary potentials. Natural selection is part of the evolutionary process resulting in the survival of the "fittest," i.e., of the best adapted individuals.

Sibs (siblings). Offspring, irrespective of sex, from the same parents but from separate fertilizations. Full sibs have both parents in common, half-sibs, only one in common.

Sprout. Vegetative shoot arising from the stump or roots. Root sprouts may also be designated as suckers.

Suckers. Vegetative shoots arising from subterranean roots or stems.

Vegetative propagation. Propagation of a plant by asexual parts, as in budding, dividing, grafting, rooting, and air layering. Hereditary characteristics of the resulting clone (ramets) are identical with those of the original plant (ortet).