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INSTITUTE OF
PAPER CHEMISTRY
Appleton-Wisconsin

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

Project 2412

Report Four

A Progress Report

to

LOUIS W. AND MAUD HILL FAMILY FOUNDATION

May 2, 1966

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

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SUMMARY

1. Selection of exceptional trees from natural stands and progeny groups continued. Outstanding among the many trees that were examined were a female cottonwood and a naturally occurring hybrid clone from east central Wisconsin.

2. Growth measurements, fiber measurements, and other wood quality determinations confirm the superiority of the newly reported triploid quaking aspen clone growing near International Falls, Minnesota.

3. The new colchicine treatment technique which involves treating newly formed embryos was applied to cottonwood and bigtooth aspen. Preliminary chromosome counts indicate that promising results can be expected.

4. Preliminary cytological observations indicate fertilization of quaking aspen occurs three to five days after pollination. After fertilization there is a short resting period followed by rapid cell division. Cottonwood fertilization and embryo development follow a similar pattern but proceed at a somewhat slower rate.

5. Analysis of the "quaking aspen geographic variation data" confirmed the earlier reported south to north trend of decreasing specific gravity. Fiber length and fiber strength determinations, although differing significantly between areas, did not exhibit a well-defined geographic trend.

6. Forty-seven experimental crosses were attempted during the 1965 crossing season. Thirty-two parent trees were employed and major emphasis was placed upon the production of bigtooth aspen and bigtooth aspen hybrids suitable for "dry sites."

7. A comparison of the early growth and wood properties of diploid and triploid aspen demonstrated the superiority of triploid hybrids over natural triploids and diploid aspen in rate of growth, fiber length, and specific gravity.

INTRODUCTION

The basic objectives of Project 2412 are to discover ways of increasing the per acre production of usable wood and to improve the quality of the wood produced. Previous progress reports discuss the tree improvement techniques to be employed and the importance of being informed regarding heritability, natural variation, and final product requirements when selecting wood, morphological, and biochemical characteristics for genetic improvement.

During the past year considerable information has been obtained in the areas described above and a sound foundation from which can be launched further investigations is being established. The report that follows covers the period from January 1, 1965 to December 31, 1965 and describes the work under way with several species of Populus in the areas of selection, hybridization, and polyploidy. Of major interest has been the progress made and the increased knowledge obtained in the areas of natural variation, heritability of wood and fiber properties, fertilization and embryo development, and the cytology of polyploid production.

SECURING AND PROPAGATING DESIRABLE POLYPLOID AND DIPLOID ASPEN

SELECTION--RECENTLY LOCATED OUTSTANDING TREES

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

Tree D-8-65

D-8-65 is a female eastern cottonwood, *P. deltoides*, growing on a sandy poorly drained soil east of Nichols, Wisconsin in Outagamie County. The tree has been selected for its growth and form (see Fig. 1) and is being evaluated as a parent tree in both the 1965 and 1966 crossing series. Growth, form, and wood quality information is as follows:

Total height--85 feet	Diameter at 4.5 feet--18.9 in.
Height to 3 inch top--70 feet	Diameter at 16.5 feet--15.6 in.
First live branch--51 feet	Bark thickness at 4.5 feet--1.03 in.
Age--49	Crown diameter--25.5 feet
Stem straightness--good	Number of major branches--15

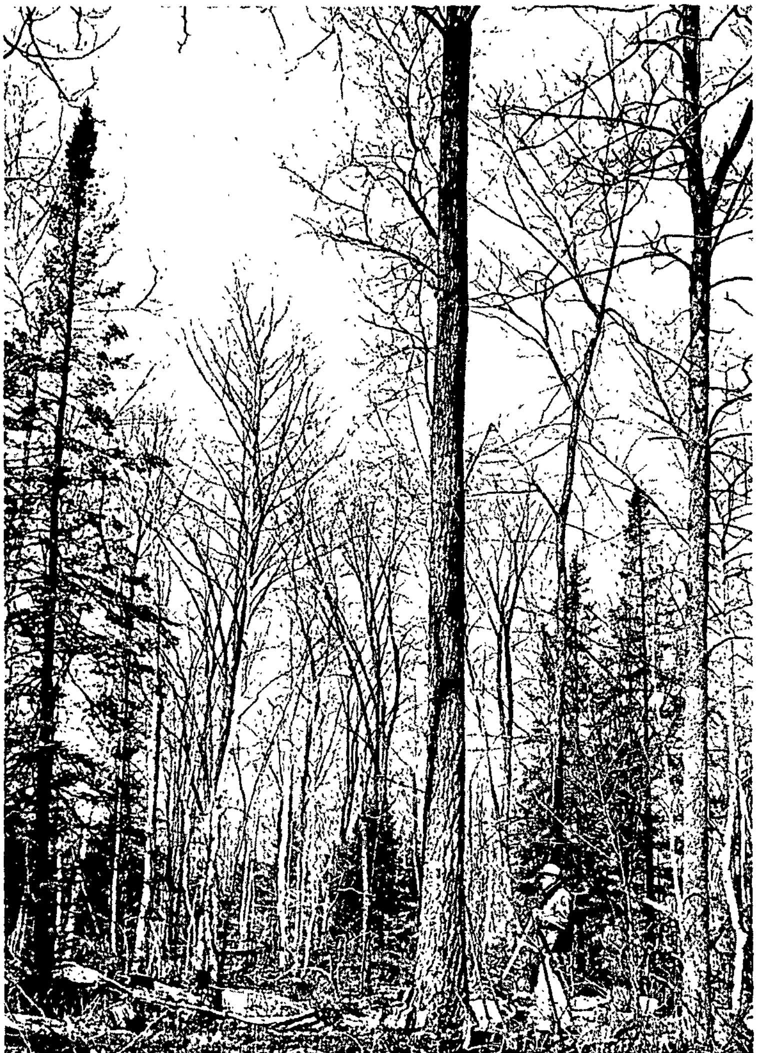

Figure 1. A Cottonwood (D-8-65) Selected for its Growth and Straightness. This Tree is Being Evaluated as a Parent Tree in Both the 1965 and 1966 Crossing Schedules

Natural pruning – fair

Branch angle – 70°

Branch weight – good

Form factor – 78

Specific gravity – 0.339

Fiber length (age 30) – 0.996

Tree D-2-66

This is a male P. deltoides which is growing on a sandy poorly drained soil a few miles north of Navario, Wisconsin. The tree was selected for its good form and rate of growth. Its usefulness as a parent tree is being evaluated in the 1966 crossing series. Information pertaining to its growth, form, and wood quality is listed below:

Total height – 71 feet

Diameter at 4.5 feet – 14.3 in.

Height to 3 inch top – 50 feet

Diameter at 16.5 feet – 11.2 feet

Height to first live branch – 34 feet

Bark thickness at 4.5 feet – 0.50 inch

Age – 31

Crown diameter – 13.8 feet

Stem straightness – good

Number of major branches – 10

Natural pruning – good

Branch angle – 60°

Branch weight – good

Form factor – 72

Specific gravity – 0.342

Fiber length (age 25) – 0.866

Tree G-2-66

This male bigtooth aspen, P. grandidentata, has been selected because of its growth and performance on a dry sandy soil. The tree shows better growth and form than the surrounding clones (see Fig. 2). The usefulness of this tree as a parent is being tested in the 1966 crossing series. It is located a few miles south and west of Hazelhurst, Wisconsin, in Oneida County on Owens-Illinois, Inc. land. The following is a summary of the measurements and wood quality information.

Figure 2. This Male Bigtooth Aspen (G-2-66) was Selected Because it Had Better Growth and Form than the Other Clones in the Area. The Location is a Typical Dry Sandy Site of Northwestern Oneida County

Total height – 76 feet
Height to 3 inch top – 51 feet
First live branch – 39 feet
Age – 53
Stem straightness – good
Natural pruning – fair
Branch weight – fair
Specific gravity – 0.389

Diameter at 4.5 feet – 15.2 inches
Diameter at 16.5 feet – 13.4 inches
Breast height bark thickness – 0.37 inch
Crown diameter – 22.0 feet
Number of major branches – 16
Branch angle – 75°
Form factor – 83
Fiber length (age 30) – 0.763

Tree AH-4-66 and Clone

Tree AH-4-66 is believed to be a naturally occurring hybrid between P. alba and P. grandidentata. The tree was recently discovered in a lowland woodlot a few miles south of Seymour, Wisconsin, in Outagamie County. The tree is a male and is a member of a clone of eight older trees and several suckers (1-5 years) growing out to 70 ft. from the older trees (see Fig. 3). This clone will be vegetatively propagated and tested to evaluate its usefulness on a variety of sites. Listed below is the growth, form and wood quality information available on tree AH-4-66. This tree is a little below the clone average in dimensions but illustrates how trees of the clone would perform in a dense stand.

Total height – 90 feet
Height to 3 inch top – 75 feet
Height to first live branch – 35 feet
Age – 56
Stem straightness – good
Natural pruning – fair
Branch weight – fair
Specific gravity – 0.351

Diameter at 4.5 feet – 17.8 inches
Diameter at 16.5 feet – 15.2 inches
B.h. bark thickness – 0.44 inch
Crown diameter – 15.0 feet
Number of major branches – 10
Branch angle – 60°
Form factor – 81
Fiber length (age 30) – 0.980

Figure 3. A Recently Discovered, Naturally Occurring 56-Year-Old Hybrid Clone Located in Northern Outagamie County Near Seymour, Wisconsin. The Parents of This Clone are Believed to be European Silver Poplar and Our Native Bigtooth Aspen. The Average Diameter of the Clone at 4.5 Feet is 18.2 Inches and Individuals in the Clone are over 100 Feet Tall

DISCOVERY AND PRODUCTION OF TRIPLOID ASPEN

Triploid quaking aspen* are of interest because it has been demonstrated that triploids have longer fibers and grow faster than normal diploid trees. Each year a limited amount of time is spent searching for trees of unusual size and rate of growth in an effort to locate additional triploid clones**. Although no new triploid clones were located in 1965, several additional trees from the triploid quaking aspen clone growing near International Falls, Minnesota, were sampled and described. As reported in Progress Report Two, chromosome counts confirmed that tree T-1-65 was a triploid. This tree was a member of a group of 30-35 trees and was reported to the Institute by Mr. Arthur Ennis of the Mando Division of Boise Cascade Corporation. The fast growth of this clone was first noted in 1960 when a one-tenth acre plot revealed the trees were approximately 42 years old, 11.9 inches in diameter, had a basal area of 250 square feet and a volume of 110 cords per acre. The trees were growing on a gray calcareous silt loam located topographically on a bench above the Little Fork River. Nothing was done with the clone from 1960 to 1964 at which time Mr. Ennis discussed the possibility of the clone being triploid with Winton and Einspahr of the Institute staff. By 1964, the stand volume had increased still further and the largest tree in the group had reached a diameter of 20.01 inches at d.b.h. and a total height of approximately 102 feet. Figure 4 illustrates the size and form of several of the clone members in 1965.

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* Triploid trees are individuals that have three sets instead of the normal two sets of hereditary units in each cell nucleus. The normal diploid or 2n chromosome number is 38, the triploid or 3n number is 57 and the tetraploid number of 4n is 76.

** A clone is a group of genetically identical individuals derived from a single individual by asexual reproduction. In natural stands of aspen, clones have been produced from a root system of a single individual by root suckering — usually after fire, cutting or similar injury.

Figure 4. Triploid Aspen, T-4-65, is 98 ft. Tall, 13.8 in. in Diameter and 46 Years Old. This Tree is a Member of a Triploid Clone Growing Near International Falls, Minnesota

TABLE I

SUMMARY OF GROWTH AND WOOD PROPERTIES OF INTERNATIONAL FALLS TRIPLOID QUAKING ASPEN

Tree No.	Total Height, ft.	Diam. 4-1/2 Ft., in.	Age, yr.	Sp. Gr., g./cc.	Age 30 Av. Fiber Length, mm.	Chromosome Count	Fiber Strength,[a] lb./in.
T-1-65	98	13.3	45	0.372	1.24	3n	64.9
T-2-65	88	13.6	45	0.388	1.14	3n	-
T-3-65	101	12.7	45	0.366	1.13	3n	-
T-4-65	98	13.8	46	0.377	1.05	3n	-
T-5-65	88	13.7	50	0.346	0.94	2n	-
T-20-65	87	12.2	45	0.381	0.97	-	-
N. Wis.[b]	57	9.5	45	0.383	0.86	2n	65.2

[a] Zero-span tensile strength is interpreted as a measure of fiber strength.
[b] Values estimated from Aspen Geographic Variation Study and based upon nonselected normal diploid aspen.

Root and wood samples were secured from four trees of the clone and the results of the measurements made on these trees are shown in Table I. Measurements from two northern Minnesota diploid clones and average values for diploid clones in northern Wisconsin have been included in Table I for comparison purposes. Examination of the data on the four trees indicates that the triploid clone combines the attributes of rapid growth, satisfactory specific gravity and fiber strength, and the increased fiber length. The only unsatisfactory characteristic of the trees in this clone is the poorer than average natural pruning that is evident in Fig. 4. Branch stubs lower the quality of the wood produced and often serve as place of entry for wood-decaying organisms. At present, rooted individuals from this clone are being propagated for use in a future experimental planting.

PRODUCTION OF TETRAPLOID ASPEN AND COTTONWOOD

Tetraploid aspen are trees that have four sets of hereditary units (chromosomes) instead of the normal two sets. Most tetraploids are slow growing but are useful because, when they are used as a parent in a "tetraploid x diploid" cross, they contribute two sets of chromosomes and the diploid contributes one set. As a result a high percentage of the progeny produced from a "tetraploid x diploid" cross are triploids. Well-adapted local origin tetraploids would be of considerable value in the mass production of triploids.

As Project 2412, Progress Report Two, described, colchicine* treatment of germinating seed, colchicine treatment of newly formed embryos and hybridization

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*Colchicine, a strong alkaloid poison derived from the plant Colchicum autumnale, induces the doubling of chromosome numbers in plants.

using polyploid parents are three possible methods of producing tetraploids. During the past three years all three of the above techniques have been used. Progress Report Two reported the promising results obtained when newly formed embryos of quaking aspen were treated with colchicine. Theoretically, the sooner the colchicine can be applied after the fertilized egg (zygote) begins to divide, the fewer cells it will be necessary to treat and the better are the chances that the entire embryo and resulting seedling will be tetraploid. The mechanics of handling the treatment are relatively simple. The female catkins are forced in either the greenhouse or growth chamber and when the flowers are receptive pollen is applied. At various intervals after pollination, the catkins are immersed in a 0.3 percent solution of colchicine for 6 hours. Several "after pollination intervals" are used because cytological studies under way have as yet not tied down the exact time when the zygote begins to divide. After treatment the catkins are allowed to mature and the seeds produced are separated by size and germinated on special seed germination saucers. The seedlings are transplanted when one-half to three-fourths inch tall; and when 3 to 5 inches tall meristematic leaves are removed, fixed, and stored for chromosome counts.

During 1965, colchicine was applied to pollinated female catkins of bigtooth aspen and cottonwood and the information that follows describes the results obtained.

Bigtooth Aspen

Two colchicine-treated bigtooth aspen crosses were made in 1965: XG-21-65 and XG-26-65. The female catkins that were treated were on one-year-old grafted scions, and usually the three catkins making up a single treatment were located on a single scion. The pollinated catkins were immersed in a 0.3 percent solution of colchicine for 6 hours at 6, 12, and 18 hours after pollination. The catkins of cross XG-26-65 were quite short and could not be completely covered by the colchicine solution. Wrapping the top of each catkin with colchicine-moistened cotton was moderately successful in improving the coverage. Table II summarizes the treatment data and results. No 20-mesh seeds were recovered and all putative tetraploids obtained were from 40-mesh and 50-mesh seeds. Eight seedlings from cross XG-26-65 and eleven from XG-21-65 gave consistently high counts of 76 $\pm$ 9, indicative of tetraploidy. In addition, 23 and 14 seedlings respectively, from the two crosses had counts in which some of the cells were tetraploid and other cells were believed to be either diploid or triploid. These seedlings will be rechecked in the spring of 1966 and the best tetraploids and triploids will be propagated.

Cottonwood

Two crosses were made in 1965 which were treated with colchicine. Cross XD-41-65 was attempted with flowers on grafted scions. Colchicine was applied to the catkins at 6, 12, 18, or 24 hours after pollination, and the coverage was estimated to be good. Soon after treatment, the catkins began to drop off as though they had not been pollinated and no catkins remained alive long enough to produce seed. The cross was repeated using the "cut-branch technique" and a new cross number was assigned (XD-40-65). The material was forced in the growth chamber and, when the catkins were receptive, the branches were moved into the greenhouse and their bases kept in an antibiotic solution.*

*An antibiotic solution developed by Dr. J. G. Berbee, University of Wisconsin.

TABLE II

1965 TREATMENTS WITH COLCHICINE OF NEWLY FORMED EMBRYOS OF BIGTOOTH ASPEN

Hours After Polli- nation	Catkins		Seeds		Germi- nation, %	Seedlings		Ploidy Levels						
	Polli- nated	Collected	Mesh[a]	No.		Total	Counted[b]	2	I[c]	3	I	4	2+3[d] misc.	2+4[d] 3+4
XG-26-65 (G-64 x G-1-58)														
6	3	2	40	129	87	87	57	–	6	28	7	2	4	10
			50	37	73	28	20	–	–	11	2	1	2	4
12	3	2	40	51	81	39	30	–	1	6	11	3	1	8
			50	27	56	13	10	–	–	1	6	2	–	1
										46		8		23
XG-21-65 (G-10-62 x G-1-58)														
6	3	3	40	37	84	30	20	–	–	5	4	3	–	8
			50	9	78	6	6	–	–	1	3	1	–	1
12	3	3	40	2	50	1	1	–	–	1	–	–	–	–
			50	4	75	3	3	–	–	1	1	–	–	1
18	3	4[e]	40	58	81	38	25	–	3	10	8	–	–	4
			50	12	58	7	7	–	–	–	–	7	–	0
										18		11		14
										64		19		37

[a] Seed retained on standard 40 and 50-mesh screens.

[b] Rapid growing normal appearing seedlings were discarded.

[c] Intermediate.

[d] Seedlings in which some cells gave 2n, some 3n and some 4n counts.

[e] Includes one apparently normal catkin.

After pollination the branches were held in the greenhouse and colchicine was applied at 48 or 72 hours after pollination. After the colchicine treatment was completed the branches were returned to the growth chamber until the seed was mature. Table III summarizes the treatment applied and Table IV indicates the amount of seed produced and provides a partial listing of chromosome counts obtained. Meristems have been collected from 238 selected seedlings* of this cross and the chromosome counting is nearing completion. Preliminary counts indicate some putative tetraploids have been produced and higher than expected numbers of triploids have resulted from the treatment. In addition, the size, shape, and margins of the leaves of the two to four-inch selected seedlings were described. When the chromosome counts are completed, correlations will be attempted between leaf anatomy and level of ploidy. Future plans in this area of study will include plans to produce tetraploids among other species of Populus by the colchicine treatment of newly formed embryos.

PRODUCTION OF HAPLOID ASPEN

Haploid aspen are aspen that have one set of chromosomes instead of the normal two sets that occur in each cell nucleus. As explained in earlier reports, aspen cells contain two sets of 19 chromosomes or a total of 38. For each chromosome in one set of 19 there is an equivalent or homologous chromosome in the second set. The members of a pair of like chromosomes correspond in size and shape and have groups of genes in the same linear sequence that control the same set of characteristics. Theoretically, a tree should be able to grow with just one set of chromosomes. It is of academic as well as of practical interest,

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*The seedlings selected were either slow growing or had abnormal leaves or abnormal cotyledons.

to determine whether haploid quaking aspen can be produced. After haploid production is achieved, the next step would be to attempt to double the chromosome number back to the normal 38; and in this way produce an individual in which the homologous chromosomes are identical not only in size and shape, but also genetically identical. Such a homozygous individual would be extremely valuable for tree improvement research, since it would breed true by seed.

TABLE III

COLCHICINE TREATMENTS APPLIED TO
COTTONWOOD CROSS XD-40-65

Treatment Number	Type of Treatment
T-1	Colchicine at 0.3% + Tween 20 applied 48 hours after pollination. Treatment applied for a period of six hours. Entire catkins treated. Two catkins treated.
T-2	Same treatment as treatment no. 1 with the exception that only the lower half of the catkins were treated. Two catkins treated.
T-3	Colchicine at 0.3% + Tween 20 appled 72 hours after pollination. Treatment for a period of six hours. Entire catkins treated. Total of two catkins treated.
T-4	Treatments identical with treatment T-3 except that only the lower 1/2 of the catkins were treated. Total of two catkins were treated.

TABLE IV

SEEDS, SEEDLINGS AND CHROMOSOME COUNTS
OBTAINED ON COTTONWOOD CROSS XD-40-65

Colchicine Treatment	Catkins Pollinated	Catkins Collected	Seed Produced Mesh[a]	Seed Produced No.	Germination, %	Seedlings Counted[b]	No. Putative Polyploids[c] 4n	No. Putative Polyploids[c] 3n
T-1	2	2	20	302	94	38	10	3
			28	466	88	44	6	24
			40	56	2	0	–	–
T-2	2	2	20	83	93	15	2	11
			28	125	83	25	0	23
			40	24	0	0	–	–
T-3	2	2	20	196	93	27	0	20
			28	398	88			
			40	21	10			
						Counts Not Completed		
T-4	2	1	20	172	95			
			28	59	76			
			40	7	0			

[a] Seed retained on standard 20, 28, and 40-mesh screens.

[b] Seedlings on which chromosome counts were made were individuals that were either slow growing, had abnormal leaves, abnormal cotyledons, or short thickened stems.

[c] The number of putative polyploids is believed to be higher than normal for treatment T-1 and these individuals are being rechecked.

The weakened pollen technique was investigated by treating pollen from Populus alba with electron irradiation and using the pollen in an experimental cross with quaking aspen. The principle involved is pollination without fertilization, i.e., the pollen applied serves only to stimulate the female gamete to develop into a haploid embryo. In such a cross, when the normal diploid hybrid seed results, the seed will be large and the seedlings will have pubescent primary leaves. By discarding these large seeds and counting chromosomes of only the nonpubescent, slow-growing individuals from the small-sized seed, the maximum number of haploids should be recovered.

The results of the first attempt at the production of haploid aspen were discussed in Project 2412, Progress Report Two. One seedling was produced in which at least a portion of the plant was haploid. This individual was vine-like when small and originally was believed to be entirely haploid. Further checking, however, revealed that a new rapidly growing shoot near the base of the seedling was diploid and the slow-growing portion was still haploid (see Fig. 5). The nature of the "ploidy" involved was further complicated when the slow-growing portion that had twice before been reported as haploid began to grow rapidly. A check of this new rapidly growing tissue revealed that this portion of the plant was now diploid also. The origin of this chimera (part haploid and part diploid) is not clear. One possibility is that the seedling originally was haploid and, sometime during development, one of the haploid cells divided abnormally giving rise to a diploid cell. Subsequently, this diploid cell, because of its normal chromosome number, produced a vigorous shoot that finally outgrew the haploid portion of the plant. This same sequence of events is believed to have occurred in the slow-growing confirmed haploid portion of the plant, thus producing by chance the homozygous individual that was desired. Cytological studies are anticipated to clarify the character and origin of this plant.

Since this first trial, additional treatments of a similar nature have been undertaken, seedlings produced, and chromosome counting is in progress.

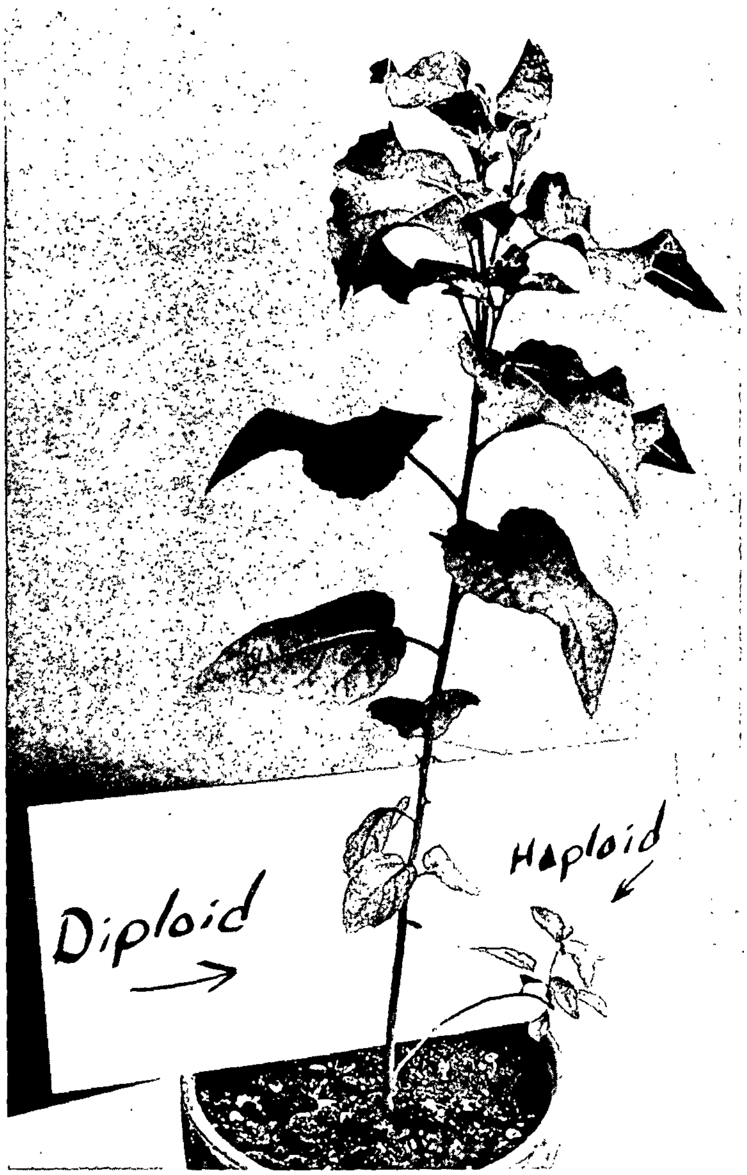

Figure 5. A Part Diploid and Part Haploid Seedling Produced Using the "Weakened Pollen" Technique

Future plans in this area of investigation also include efforts to (1) vegetatively propagate the two diploid portions of the haploid described above, and (2) repeat the weakened pollen work in an effort to produce additional haploids.

CYTOLOGICAL STUDY OF FERTILIZATION AND EMBRYOLOGY IN ASPEN AND COTTONWOOD

The mass production of triploid quaking aspen requires the crossing of tetraploid and diploid parent trees. Some putative tetraploids have been produced at the Institute by the application of colchicine solutions to presumably newly-formed embryos in flowers forced in the greenhouse (see PRODUCTION OF TETRAPLOID ASPEN AND COTTONWOOD). In addition to tetraploids, apparent triploids and aneuploids (irregular sets of chromosomes) have also been reported. The recovery of these unexpected polyploids led to speculations as to their origin. If colchicine is applied after fertilization and before the first division of embryony, the whole plant should be tetraploid. However, if applied before fertilization, colchicine may only double the one chromosome set in either the male or female gametes, resulting in triploid or aneuploid seedlings after fertilization. In 1965, a study was initiated to determine cytologically the time after pollination that (1) fertilization occurs, and (2) the first division of embryony begins. These data, along with known times of colchicine application, would permit a better insight as to the origin of different levels of ploidy.

Three treatments of trembling aspen were made and one of cottonwood. Material was forced in the greenhouse, as well as in the growth chamber. Collections were made at specified hours or days after pollination for each treatment and the material was fixed in several different solutions for later embedding in paraffin. Minor problems in technique were studied, and approximately 600 permanent slides were prepared and are now awaiting examination. Details of treatments and collections will be given next year, after all the results are analyzed. Preliminary results, based on a superficial examination of selected slides, show that fertilization probably occurs in trembling aspen forced in the greenhouse 3-5 days after pollination. Cottonwood appears to lag one or two days behind aspen. Development is speeded up by 1-3 days in both species when branches are forced and flowers mature in the growth chamber.

Figure 6 shows a cross section through a trembling aspen capsule in which several ovules are presented. Figure 7 is a close-up microphotograph of one ovule within the capsule. Figure 8 is an enlargement of the megagametophyte (central light area) within the ovule, showing the two guard cells (bottom), the egg cell (center), and the polar nucleus (top). After the pollen tube enters the megagametophyte (at the bottom between the guard cells) one of the two 1*n* male gametes will fertilize the 2*n* polar nucleus to form the 3*n* endosperm. The other gamete will fertilize the 1*n* egg to form a 2*n* zygote – later to divide and become the embryo, and still later, the seedling. Development in the ovule (until it is shed as a seed) has been arbitrarily divided into ten easily recognized stages. One of the objectives of our analysis will be to determine overlapping frequencies of different stages of development within the same capsule and within the same catkin. Of particular interest is the apparent correlation between the relative length of ovular hairs and the stages of development. If, for example, the interval between fertilization and the start of embryo division is associated with hairs 1/2-3/4 the length of the ovule, the time of application of colchicine could be determined quite rapidly by simply cutting open capsules and examining the ovular hairs with a hand lens. Future plans will include the sophistication of techniques, as well as calibrating the chronology of ovular development in other species of *Populus*.

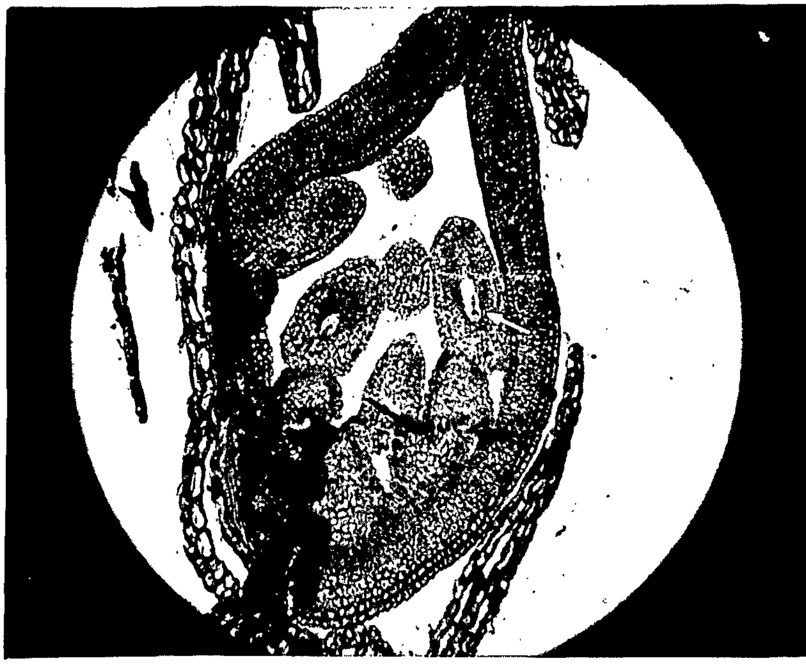

Figure 6. Capsule of Trembling Aspen, Six Hours After Pollination, Showing Enclosed Ovules (100X)

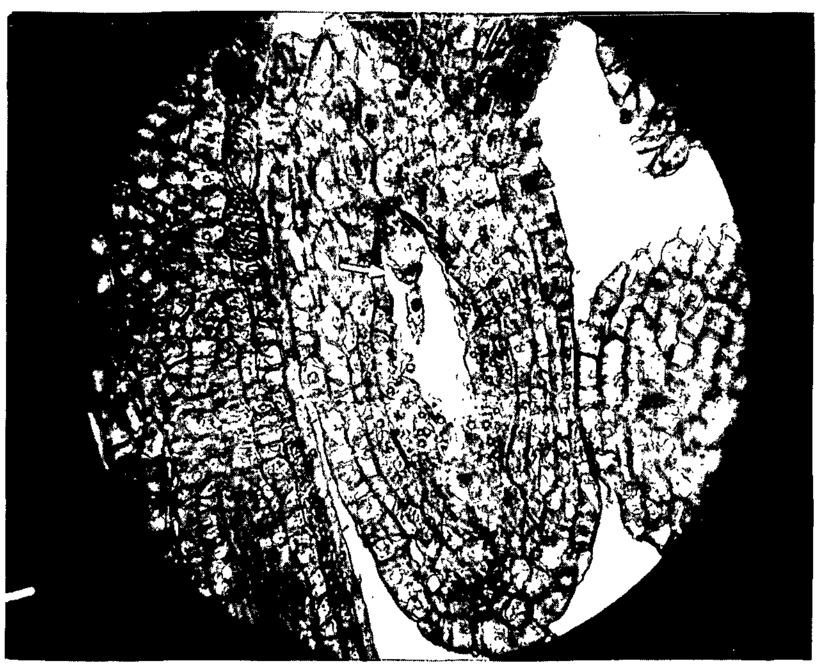

Figure 7. An Ovule, Enlarged from Fig. 6, Showing the Central, Light-Colored Megagametophyte (400X)

Figure 8. Megagametophyte, Showing Two 1n Guard Cells (Bottom) One 1n Egg Cell (Center), and the 2n Polar Nucleus (Top) (1000X)

TREE PHYSIOLOGY STUDIES

NUTRITIONAL REQUIREMENTS OF ASPEN HYBRIDS

One question that immediately arises when hybrid progeny groups are produced concerns the nutritional requirements of the newly produced materials. Will they do well on sandy, low fertility sites? Are their requirements higher, lower, or the same as the parent species? There are several approaches available that can be used to answer these questions. One approach, and the one used in this study, is to use a sand culture technique, vary the level of the major soil nutrients, and compare the growth of the hybrids with the growth of seedlings of the parent species.

A sand culture technique was devised similar to that described by Swan (1), in an effort to look into the nutritional requirements of aspen hybrids. Basically, the system has four growth containers containing sterile silica sand. These containers are attached to a common pressurized carboy containing the nutrient solution. A time clock activates a valve on a compressed air line which in turn causes the solution to be pumped into the four growth containers. After five minutes the valve closes and the solution drains back into the carboy. The test seedlings are grown in the sand on this periodically fluctuating nutrient solution. One basic unit consists of a pressurized carboy and four growth containers (replications) and makes up a single treatment. For each additional treatment an additional basic unit is added. In the study under way ten treatments were employed and this then resulted in the use of ten carboys and 40 growth containers.

The overall plan for the entire study consists of running a series of five interrelated growth experiments in the Biology Section's growth chamber.

Light, temperature, day length, and relative humidity are held constant and in each of the five experiments the level of a different major soil nutrient is varied. Seed from four experimental crosses was used as a source of plant material. These full sib progeny groups are started from seed in the sand-filled growth containers and the growth and nutritional status of the seedlings are measured after 40 days. Figure 9 is a view of the growth chamber showing the instruments used to control environmental conditions within the growth chamber (left) and the growth containers and nutrient carboys (right). The seedlings are 35 days old and are part of the growth chamber run in which the level of phosphorus was being varied.

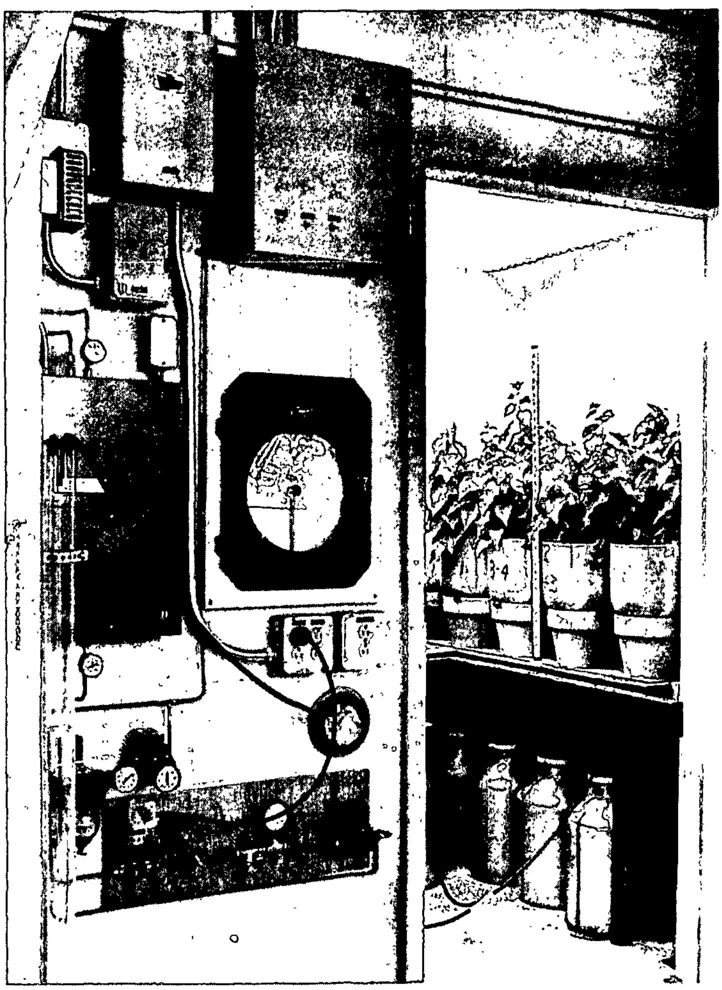

Figure 9. A View of the I.P.C. Growth Chamber Showing the Control Instruments (Left) and the Growth Containers Above and the Nutrient Carboys Below (Right). The Aspen Seedlings Shown Were Seeded 35 Days Earlier

The first type of hybrid aspen to be investigated using this procedure was the cross between quaking aspen (T) and European gray poplar (Ca). Table V lists the parentage of the four progeny groups used. It should be noted that cross XT-Ca-35-65 involves a quaking aspen as the female parent while XCa-T-8-65 is the reciprocal cross which in this case involves the gray poplar as the female parent.

Olson's (2) combination of required elements was used in making up the nutrient solutions used in this study. The levels used by Olson were modified to meet the requirements of this investigation. Phosphorus was the element that was varied in the first growth chamber run with levels of 0, 22, 43, 54, and 65 p.p.m. being employed. Potassium was the element that was varied in the second growth chamber run and in this case 0, 31, 62, 77, and 93 p.p.m. were the levels used. At the writing of this report, the growth chamber runs in which calcium, magnesium, and nitrogen were the elements being varied have been completed. Table VI presents the levels employed of the above elements.

Each of the growth containers contained four seedlings, one seedling of each of the four types of test materials. Growth on the complete nutrient solution is rapid and, at 40 days, it is not unusual to have seedlings that are 18 to 20 inches tall (see Fig. 10). After 40 days of growth, all surviving seedlings are washed from the growth containers and the green weight obtained for the tops and roots along with the ovendry weight of the tops. Next, the ovendry tops from the four genetically similar seedlings grown on the same nutrient solution are combined and ground in a Wiley mill. This ground tissue is used in determining the levels of N, P, K, Ca, and Mg in the seedlings produced by the various nutrient solution treatments. The tissue samples from the growth chamber

TABLE V

PARENTAGE OF TEST TREES

Cross Number[a]	Parent Trees (female x male)[b]		
XT-36-65	T-12-58	X	T-10-60
XT-Ca-35-65	T-12-58	X	Ca-1-62
XCa-T-8-65	Ca-2	X	XT-22-56, no. 36
XCa-34-65	Ca-2	X	Ca-1-62

[a]X = cross, Ca = P. canescens, T = P. tremuloides.

[b]For information on parent tree locations, see Table XVIII of the section on Intraspecific and Interspecific Crossing.

TABLE VI

COMPOSITION OF NUTRIENT SOLUTIONS IN P.P.M.

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

Figure 10. Growth of Aspen Test Trees in a Sand Culture System At Varying Levels of Magnesium. Olson's Low Level of N, P, K, and Ca (see Table VI) was Used with (Left to Right) 0, 7, 14, 17, 21 and 0 p.p.m. Magnesium

runs for phosphorus, potassium, and calcium are ready for analysis and the chemical analysis is expected to get under way by the first of May. Samples from the remaining growth chamber runs will be available for analysis by June 1. The growth data obtained is being tabulated and readied for computer processing. It is hoped that the results will point up any nutrient requirement differences that may exist between the test materials under investigation. A complete analysis of the data will be available for the next annual report. Upon completion of the crossing work presently being conducted in the growth chamber, a second series will be established using bigtooth aspen and bigtooth aspen hybrids as the test trees being investigated.

STUDIES OF NATURAL VARIATION

GEOGRAPHIC VARIATION OF QUAKING ASPEN IN WISCONSIN AND UPPER MICHIGAN

Introduction

Natural variation and degree of genetic control over existing variation are important factors which must be considered when selecting characteristics to emphasize in a tree improvement program. In the spring of 1963 a study of geographic variation in quaking aspen was initiated and the field sampling work completed during the summers of 1963 and 1964. The objectives of this study were to increase existing knowledge of the natural variation of wood, fiber, and growth characteristics and accumulate data needed for establishing base lines for judging growth and "wood quality" of quaking aspen. A preliminary description of this study was given in Progress Report Two.

Experimental Methods

Study areas were established in five geographic locations within the states of Wisconsin and Upper Michigan (Fig. 11) to investigate the existence of geographic trends and provide data for establishing "base lines" for judging the relative quality of selected trees. Five stands were measured in each geographic area and three trees in each of three clones were sampled in each stand. This sampling procedure resulted in a total of forty-five trees being measured in each geographic area. The stands sampled ranged in average age from twenty-three to forty-four years and were limited in location to medium and light-textured upland soils. Measurements were restricted to the dominant and codominant trees of each clone. Information taken on each experimental plot included: (1) age, form, and

GEOGRAPHIC VARIATION STUDY AREAS

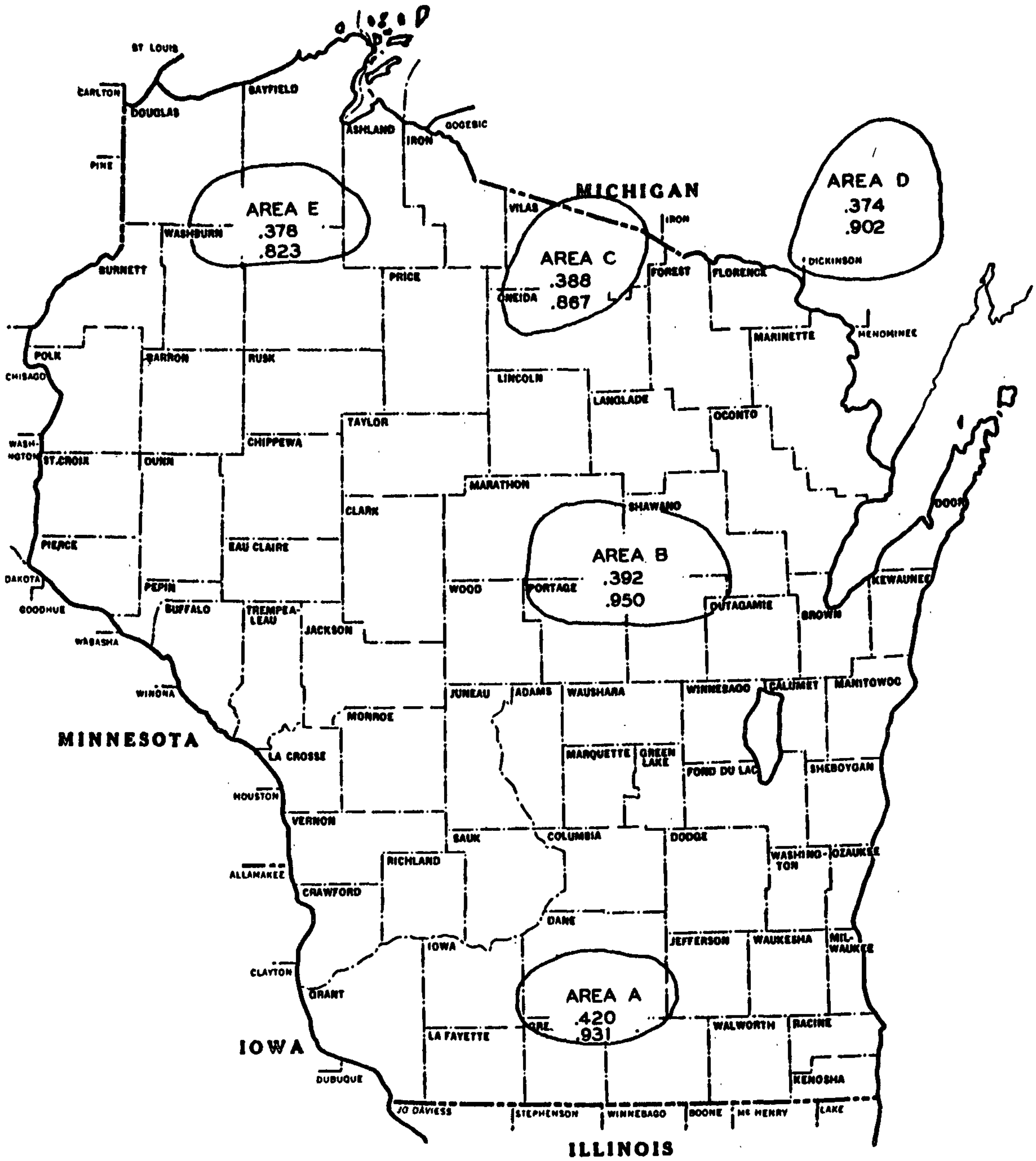

Figure 11. Illustrated are the Specific Gravities and Fiber Lengths of Study Areas Sampled in the Quaking Aspen Geographic Variation Study

rate of growth information; (2) specific gravity and fiber length based on four 10-millimeter increment cores; (3) soil and other site information based on soil samples taken from the "A" and "B" horizons; and (4) pulping information based on the micropulping of 4 to 6, 10-millimeter increment core samples per tree. Pulpin information (fiber strergth, pulp yield, and permanganate number) was based upon just one stand in each geographic area.

Tree growth was measured in terms of age, height, diameter at breast height, and form factor, and the corresponding tree volume in cubic feet was based upon the form factor table of Mesavage (3). The crown volume was calculated using the crown diameter and length of live crown and assuming the shape of the crown was cone. Examination of breast height increment cores provided the age determinations.

Specific gravity and fiber length data were obtained by taking appropria measurements on four 10-millimeter breast height increment cores taken at right angles from each other. Core volumes were obtained by using a calibrated incremen core borer and measuring the length of the saturated cores. The specific gravity values are expressed as grams of dry weight per cubic centimeter of green volume. Fiber measurements on all areas with the exception of three clones in Areas B and C were obtained by treating the last ten annual rings from a minimum of two increment cores with sodium chlorite and acetic acid [Spearin and Isenberg (4)]. The fiber lengths of 400-500 fibers were measured on this ten-year increment core segment. All fibers 0.3 mm. and longer including cut, broken, and intact fibers were measured by projecting the fibers on a ground glass screen at a magnification of fifty times. Fiber lengths were adjusted to age thirty by plotting the fiber length and age information on a previously prepared fiber length-age curve for quaking aspen. Three clones in Area B and three clones in Area C were part of a previous study and the fiber length at age thirty for the trees in the earlier study was obtained by dividing the increment cores into five-year age intervals, compositing portions of equal age and macerating according to the techniques cited above. Eighty fibers were measured for each interval, the fiber length-age curve for each tree prepared, and the age thirty fiber length obtained from this curve.

Soil pits were dug at each clone location, the soils of the upper twenty-four to thirty inches described, and samples of the "A" and "B" horizons taken for laboratory examination. The soil texture (percent sand, silt, and clay) was obtained by the use of the hydrometer method as described by Bouyoucos (5). Soil samples were submitted to the University of Wisconsin Soil Testing Service and information on the acidity and levels of essential soil nutrients (N, P, K, Ca, and Mg) were thus obtained. The data on soil nutrients presented are weighted average levels based upon nutrient levels and thickness of the A and B horizons. If multiplied by the factor of four, the values presented would be approximately equal to the total available soil nutrient in the upper twenty-four inches of the soil.

The pulping data presented were obtained by using a micropulping procedure that employs a kraft pulping system and a multiunit digester [van Buijtenen, et al., (6)]. This procedure makes possible the simultaneous pulping of seven small samples and the use of a sample size as small as fifteen grams dry weight. The techniques used and the cooking conditions are reported in detail in a recent paper by Gardner and Einspahr (7). The yield data presented are the percent yield of pulp and are based upon equivalent weights of wood in each digester. The permanganate number is a measure of the lignin in the pulp after cooking. Zero-span tensile strength measurements were conducted on test handsheets using the procedures described by Wink and Van Eperen (8) and are interpreted as a measure of individual fiber strength.

Field sampling was so arranged that an analysis of variance of the form outlined in Table VII [Snedecor's hierarchical classification (9)] was used to examine the sources of variation for the growth and wood quality data. A slight modification of the illustrated form of analysis was used in examining the soils and pulping data. Duncan's new multiple range test (10) was used to examine area means more closely when significant between area differences existed. In addition to the analysis of variance, a number of correlations were calculated relating tree growth, wood, and soil properties. Space does not permit a complete discussion of this phase of the work and information is presented on only a selected number of correlations. Specifically omitted were the relationships obtained between soil texture and available soil nutrients.

TABLE VII

OUTLINE OF ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sum of Squares	Mean of Squares	Mean of Squares is an Estimate of
Total	224	"	"	
Areas	4	"	"	$\sigma^2 + 3\sigma_c^2 + 9\sigma_s^2 + 45\sigma_A^2$
Stands, within areas	20	"	"	$\sigma^2 + 3\sigma_c^2 + 9\sigma_s^2$
Clones, within stands	50	"	"	$\sigma^2 + 3\sigma_c^2$
Trees, within clones	150	"	"	σ^2

Results

Tables VIII and IX provide a summary of the most useful tree growth, wood quality, and soils data collected for each geographic area and give the reader a feel for the variability encountered. Tables X and XI provide brief summaries of the results of the correlations and the analyses of variance calculations carried out on the above data. The multiple range test data in Table XII provide information indicating statistical differences between areas.

Soils Data

The soils supporting quaking aspen stands on the upland sites in the five geographic study areas varied considerably from area to area and stand to stand within areas (Table VIII). The soils were fairly uniform in Areas A and D and varied the greatest in Area B. An analysis of variance of a form similar to that illustrated in Table VII was used to examine the soil property variation. The differences between clones within stands were quite small and contributed only a minor amount to the overall variation. Highly significant (1% level) differences were obtained between areas and stands within areas in soil texture, pH, P, Ca, total exchangeable bases, and "A" horizon thickness. The statistical analysis of organic matter data indicated that there were highly significant differences between stands within areas while between area differences were significant only at the 5% level.* Potassium and magnesium were found to have highly significant differences between areas but there were no significant differences between stands within areas.

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*Highly significant indicates significant at the 1% level of probability while the term "significant" is used to indicate values significant at the 5% level of probability. NS is used when the correlation coefficients fall below 5% level of probability.

TABLE VIII

SUMMARY OF SOILS DATA

Area	Stand	Sand, %	Silt, %	pH	OM, lb./acre ÷ 1000	Available, lb./acre P	K	Ca	Mg	"A" Horiz. Thick., in.	Site Index
A	1	41.4	45.8	5.5	14	61	168	947	347	3.2	46
	2	43.1	40.6	5.9	12	34	138	1367	557	4.2	47
	3	49.6	43.8	7.4	22	32	113	3520	543	8.0	47
	4	55.1	31.0	5.6	10	71	133	1363	550	4.0	49
	5	43.0	44.5	6.6	16	51	159	2003	793	10.0	53
	Av.	46.4	41.1	6.2	14.5	50	142	1840	558	5.9	48.4
B	1	70.1	21.7	5.0	20	33	65	457	115	2.9	51
	2	69.7	20.7	5.3	20	73	90	847	165	2.0	54
	3	54.4	37.8	5.0	34	17	73	533	93	1.0	56
	4	32.9	48.0	5.0	19	10	82	823	232	4.6	67
	5	84.1	9.4	4.7	54	58	52	320	57	5.0	54
	Av.	62.3	27.5	5.0	29.4	38	72	596	132	3.1	56.4
C	1	77.7	17.4	5.1	11	138	58	373	95	2.8	53
	2	88.4	7.3	5.4	11	152	98	427	83	1.9	53
	3	62.8	32.5	4.9	36	17	72	407	60	0.8	58
	4	75.9	16.2	5.2	20	90	50	427	35	2.0	61
	5	76.5	15.6	5.6	20	75	63	1293	73	3.8	55
	Av.	76.2	17.8	5.2	19.4	94	68	585	69	2.3	56.0
D	1	91.7	7.0	5.7	10	224	80	323	83	1.4	44
	2	92.8	4.5	5.3	12	191	85	257	73	1.8	51
	3	85.5	11.5	5.4	14	141	83	310	77	2.8	52
	4	67.9	24.4	6.4	16	37	93	1053	217	2.5	50
	5	86.8	10.1	5.7	14	134	88	673	70	2.3	52
	Av.	84.9	11.5	5.7	13.1	145	86	523	104	2.2	49.8
E	1	82.1	13.5	5.3	11	157	70	412	78	2.7	48
	2	64.9	26.8	5.0	13	163	85	603	88	0.8	41
	3	88.7	8.8	4.9	13	130	80	317	58	3.7	43
	4	79.3	17.9	5.3	14	64	78	400	103	1.9	49
	5	45.5	49.3	5.4	18	94	108	730	143	3.3	44
	Av.	72.1	23.3	5.2	14.0	122	84	492	94	2.5	45
Grand av.		68.4	24.2	5.5	18.1	90	91	807	192	3.2	51.1

TABLE IX

SUMMARY OF GROWTH, WOOD & PULPING DATA

Area	Stand	Age, yr.	Ht./Age, ft./yr.	D.B.H./Age, in./yr.	Vol./Age, cu. ft./yr.	Cr. Vol./Age, cu. ft. x 10^3	Form Factor	Sp. Gr., g./cc.	Fiber Length, mm.	Fiber Strength, lb./in.	Pulp Yield, %
A	1	34	1.45	0.19	0.16	7	74	0.433	0.909		
	2	31	1.58	0.20	0.16	20	76	0.417	0.917		
	3	35	1.47	0.22	0.17	17	73	0.397	0.943	60.8	51.2
	4	25	1.78	0.27	0.19	28	74	0.424	0.978		
	5	44	1.42	0.17	0.18	8	78	0.427	0.906		
	Av.	34.0	1.54	0.210	0.172	16	75.1	0.420	0.931		
B	1	35	1.62	0.22	0.20	22	78	0.400	0.984		
	2	35	1.68	0.22	0.24	31	80	0.398	0.952		
	3	36	1.71	0.24	0.33	40	83	0.391	0.926	56.0	52.4
	4	23	2.48	0.31	0.27	35	75	0.387	0.943		
	5	33	1.72	0.21	0.20	27	80	0.383	0.943		
	Av.	32.7	1.84	0.241	0.246	31	79.1	0.392	0.950		
C	1	41	1.51	0.17	0.18	19	79	0.382	0.815		
	2	35	1.63	0.22	0.21	41	74	0.391	0.821		
	3	40	1.60	0.21	0.26	27	80	0.375	0.808	64.4	51.8
	4	37	1.83	0.26	0.37	44	80	0.393	0.982		
	5	30	1.85	0.26	0.22	34	75	0.402	0.910		
	Av.	36.8	1.68	0.226	0.251	33	77.5	0.389	0.867		
D	1	44	1.20	0.17	0.13	24	76	0.382	0.890		
	2	40	1.49	0.19	0.14	33	77	0.385	0.912		
	3	30	1.75	0.22	0.17	36	77	0.369	0.898	67.7	51.2
	4	41	1.43	0.18	0.17	27	78	0.365	0.892		
	5	34	1.67	0.22	0.18	32	78	0.372	0.918		
	Av.	37.8	1.51	0.197	0.160	30	77.1	0.375	0.902		
E	1	42	1.34	0.18	0.17	18	75	0.376	0.744		
	2	36	1.27	0.18	0.13	13	76	0.369	0.724		
	3	42	1.19	0.20	0.16	27	74	0.387	0.874	63.4	51.2
	4	37	1.45	0.20	0.16	28	72	0.379	0.857		
	5	38	1.28	0.19	0.15	22	76	0.380	0.915		
	Av.	39.2	1.31	0.188	0.154	22	74.7	0.378	0.823		
Grand av.		36.1	1.58	0.212	0.196	26	76.7	0.391	0.895	62.5	51.6

TABLE X

SUMMARY OF CORRELATION COEFFICIENTS INFORMATION BY GEOGRAPHIC AREAS[a]

Variables		Areas A	B	C	D	E	Combined
Tree Vol./Age	Ht./Age	NS	(5)	(1)	(1)	(1)	0.560(1)
	Form Factor	(1)	(5)	(1)	NS	(1)	0.455(1)
	Crown Vol./Age	NS	(1)	(1)	(1)	(1)	0.583(1)
	D.B.H./Age	(5)	(1)	(1)	(1)	(1)	0.694(1)
	Fiber Length	NS	NS	(1)	NS	NS	0.267(1)
	OM	NS	NS	NS	NS	NS	0.331(1)
	P	NS	NS	NS	NS	NS	-0.299(1)
	Site Index	(5)	(5)	(1)	(1)	(1)	0.779(1)
Ht./Age	Crown Vol./Age	(1)	NS	(1)	(1)	NS	0.477(1)
	D.B.H./Age	(1)	(1)	(1)	(1)	(1)	0.781(1)
	Fiber Length	(5)	NS	NS	NS	NS	0.321(1)
	Age	-(1)	-(1)	-(1)	-(1)	-(1)	-0.735(1)
	Clay	NS	(1)	NS	NS	NS	0.400(1)
	P	NS	NS	NS	NS	NS	-0.329(1)
	Site Index	NS	(1)	(1)	(1)	(1)	0.857(1)
Form Factor	Age	NS	(1)	(1)	NS	NS	0.164(5)
	Site Index	NS	NS	NS	NS	NS	0.384(1)
Crown Vol./Age	D.B.H./Age	(1)	(1)	(1)	(1)	(1)	0.629(1)
	Age	-(1)	NS	NS	-(1)	NS	-0.256(1)

Note: See end of table for footnote.

TABLE X (Continued)

SUMMARY OF CORRELATION COEFFICIENTS INFORMATION BY GEOGRAPHIC AREAS[a]

Variables		Areas A	B	C	D	E	Combined
Crown Vol./Age	Silt	-(1)	NS	NS	NS	NS	-0.296(1)
	K	NS	NS	NS	NS	NS	-0.365(1)
	Mg	NS	NS	NS	NS	NS	-0.360(1)
	Total Ex. Bases	NS	NS	NS	NS	NS	-0.324(1)
	Site Index	NS	NS	NS	(1)	NS	0.580(1)
D.B.H./Age	Fiber Length	NS	NS	(1)	NS	(1)	0.336(1)
	Age	-(1)	-(1)	-(1)	-(1)	NS	-0.684(1)
	Clay	NS	(1)	NS	NS	NS	0.318(1)
	Site Index	NS	(1)	NS	(1)	NS	0.655(1)
Sp. Gravity	Fiber Length	NS	NS	(5)	(5)	(5)	0.318(1)
	Sand	NS	NS	NS	NS	NS	-0.307(1)
	Clay	NS	NS	NS	NS	NS	0.405(1)
	K	NS	NS	NS	NS	NS	0.432(1)
	Ca	NS	NS	NS	NS	NS	0.343(1)
	Mg	NS	NS	NS	NS	NS	0.541(1)
	Total Ex. Bases	NS	NS	NS	NS	NS	0.415(1)
	"A" Horiz. Th.	NS	NS	(5)	NS	NS	0.341(1)
Fiber Length	Age	-(5)	NS	NS	NS	NS	-0.246(1)
	Clay	NS	NS	(5)	NS	NS	0.344(1)
	P	NS	NS	NS	NS	NS	-0.357(1)
	Site Index	NS	(5)	NS	NS	NS	0.287(5)

[a] The correlation coefficients are not listed on an area by area basis, only the level of significance is given. NS = not significant, (1) = significant at the 1% level, (5) = significant at the 5% level.

TABLE XI

SUMMARY OF F VALUES USED IN EXAMINING VARIATION BETWEEN CLONES, STANDS WITHIN AREAS AND AREAS[a]

Source of Variation	D.F.[b]	Age	Ht./Age	D.B.H./Age	Tree Vol./Age	Crown Vol./Age
Areas	4	1.36(NS)	4.38(5)	2.19(NS)	6.13(1)	4.54(1)
Stands within areas	20	5.43(1)	3.95(1)	4.29(1)	2.80(1)	1.80(1)
Clones within stands	50	11.5(1)	8.66(1)	4.99(1)	3.89(1)	2.99(1)

Source of Variation	D.F.[b]	Form Factor	Sp. Gr.	Fiber Length	Yield, %	Zero-Span Tensile Strength
Areas	4	3.21(5)	15.4(1)	4.47(1)	1.09(NS)	6.54(1)
Stands within areas	20	2.24(5)	0.0(NS)	4.39(1)	–	–
Clones within stands	50	1.43(5)	2.37(1)	3.03(1)	1.65(NS)	2.04(NS)

[a] F values provide information on the existence of significant difference between areas; stands within areas and clones within stands. NS = not significant, (1) = significant at the 1% level, (5) = significant at the 5% level.

[b] The degrees of freedom for Yield, % and Zero-Span Tensile Strength are: Areas – 4, Stands Within Areas – 0, and Clones Within Stands – 10.

TABLE XII

SUMMARY OF MULTIPLE RANGE TESTS[a]

Height Growth, ft./yr.		Volume Growth, cu. ft./yr.		Specific Gravity, g./cc.	
B	1.84	C	0.251	A	0.420
C	1.68	B	0.246	B	0.392
A	1.54	A	0.172	C	0.389
D	1.51	D	0.160	E	0.378
E	1.31	E	0.154	D	0.375

Fiber Length, mm.		Fiber Strength, lb./in.	
A	0.950	D	67.7
B	0.931	C	64.4
D	0.902	E	63.4
C	0.867	A	60.8
E	0.823	B	56.0

[a]Methods used are those described by Duncan (10). The area means that are joined by the line at the right do not differ statistically (5% level of probability).

The soils data were compared with the site index information. The percent clay and exchangeable bases which were correlated with the percent clay were the soil factors which contributed most to site differences. Examination of the correlations* between soil properties, growth, and wood quality factors (Table X)

*All possible correlations were run between the variables listed in Tables VIII and IX. The correlations were run on an area-by-area basis and then again using all data combined. All "soil characteristic by soil characteristic" correlations have been omitted and only those correlations in which a highly significant correlation was obtained using the combined data and/or at least two of the area correlations were highly significant are presented in Table X.

indicated that the clay content and the level of phosphorus appeared to be the soil properties that have the most influence on tree growth and wood quality. The clay content within geographic areas did not vary greatly and this resulted in nonsignificant within-area correlations. When the data for all areas were combined, height growth, diameter growth, specific gravity, and fiber length increased with the percent clay content. The level of phosphorus was negatively correlated with the percent clay and positively correlated with the percent sand. As a result of this relationship, there occurred a somewhat unexpected negative correlation between the level of phosphorus and such properties as tree volume growth, height growth, and fiber length.

Tree Growth

Height growth, diameter growth, tree volume growth, crown volume growth, and form factor were the growth characteristics compared. Height growth varied considerably between clones within stands, between stands within areas, and as the analysis of variance calculations reveal (Table XI), highly significant differences were obtained for all levels of comparisons including between area differences. Area B contained stands having the youngest average age and was the area that had the most rapid height growth; Area E, the area with the oldest stands, had the slowest height growth. Volume growth and diameter growth had patterns of variation very similar to height growth with the exception that between area differences for diameter growth were not statistically significant. Form factor also followed a similar pattern of variation with the exception that, for all levels of comparison, the variation was less pronounced and the "F" values were significant at only the 5% level of probability.

Highly significant correlations were obtained between tree volume growth and height growth, diameter growth, form factor, crown volume growth, fiber length, and site index. Also of interest was the often reported negative correlation between the age of the stands and height and diameter growth.

Wood and Fiber Properties

Specific gravity and fiber length at age thirty were determined for forty-five trees in each area. Table IX illustrates the between-area and within-area variation encountered. Examination of the fiber length data indicates there are highly significant differences between clones within the stands, between stands within the areas and between areas. Despite these differences, no well-defined geographic trends in fiber length were evident. Areas A and B, which had soils with the highest amount of clay and the lowest levels of sand had the longest average fiber length. The average fiber length for the northern areas suggests there is an east-west fiber length trend. The multiple range test for the area means also indicates that the area means presented differed significantly. Variation between the stands within Area C, however, seems to indicate that this trend may be a chance occurrence. The highly significant fiber length – tree growth correlations* and the height growth – soils correlations suggest that the east-west fiber length trend may be due to soil differences. The highly significant F value for clones within the stands indicates genetic variation and the importance of genetic influences on geographic trends. Fiber length (age 30) was correlated with height and diameter growth and factors influencing tree growth, thus suggesting the possible overriding influence of local site quality and genetic factors on geographic trends.

* Fiber length was significantly correlated with height growth, diameter growth, and tree volume growth.

Specific Gravity

The specific gravity of the aspen stands studied varied considerably between clones within the stands while the variation between stands within the areas was relatively small. The analysis of variance calculations (see Table XI for F tests results) indicated there were highly significant differences between areas and between clones within stands and no significant difference between stands within areas. For specific gravity there appears to be a south-to-north trend of decreasing specific gravity and the multiple range test of treatment means (Table XII) indicated Area A (specific gravity 0.420) represented one population, Area B (specific gravity 0.392), and Area C (specific gravity 0.389) were a second population and Areas D (specific gravity 0.375) and E (specific gravity 0.378) were from a third population. Specific gravity, although not correlated with soil properties on a within-area basis, was correlated when all of the data were combined and a larger overall variation in soil properties and specific gravity values was involved. Soils no doubt contribute to the geographic trend noted in specific gravity but the fact that the soils differ from stand to stand within geographic areas without a complementary significance between stand variation in specific gravity suggests that other climatic factors are in operation. The lack of specific gravity — tree growth correlations suggests less dependence of specific gravity on growth rate and site quality than seems to be the case for fiber length.

Fiber Strength*, Pulp Yield and Permanganate Number

Increment core samples from one stand in each geographic area were pulped using the discussed micropulping procedure. Although the data do not permit

*Zero-span tensile strength measurements, as indicated earlier, are interpreted as a measure of fiber strength and the two terms have been used interchangeably in this report.

testing for stand-to-stand variation within areas, a measure of within-stand and between-area differences was obtained. Zero-span tensile strength was found to differ significantly (1% level) between areas but not between clones within areas. Yield of pulp failed to differ significantly either between clones within stands, or between geographic areas. The multiple range test of the area means for fiber strength indicated fiber strength values were similar for Areas C, E, and A and the significant differences between areas resulted from Area B and Area D differences. No geographic trends appeared to exist in fiber strength values.

The data on the forty-five trees that were pulped were combined and all possible simple correlations were calculated between fiber strength, pulp yield, fiber length, specific gravity, tree volume growth, diameter growth, height growth, crown volume growth, and age. Table XIII summarizes all significant correlations between fiber strength, pulp yield, and permanganate number and the other growth characteristics listed. The correlations between the several growth characteristics described above were of the same magnitude as listed earlier in Table X and were not repeated in Table XIII.

Zero-span tensile strength was found to be negatively correlated with pulp yield, specific gravity, diameter growth, and volume growth. These results are in agreement with an earlier study on triploid quaking aspen [Einspahr, <u>et al</u>., (<u>11</u>)], and suggest the necessity for independent selection of trees with high fiber strength within those trees exhibiting rapid growth and satisfactory specific gravity. Absent is the earlier reported fiber length — fiber strength correlation (<u>11</u>). The nature and method of obtaining the age thirty fiber length values apparently resulted in the anomalous results described.

TABLE XIII

SIGNIFICANT CORRELATIONS OF PULPING DATA

Variables		*r* Values[a]
Zero-Span Tensile	Pulp Yield	-0.489(1)
	Permanganate No.	-0.437(1)
	Specific Gravity	-0.385(1)
	D.B.H./Age	-0.295(5)
	Tree Vol./Age	-0.384(1)
Pulp Yield	Tree Vol./Age	0.323(5)
Permanganate No.	Tree Vol./Age	0.377(5)

[a]Correlation coefficients followed by the levels of significance 1 = 1% level, 5 = 5% level.

Base Lines for Judging Tree Growth and Wood Quality

Standards or "base lines" with which to compare wood properties and growth characteristics of individual trees are necessary if the forest geneticist is to properly select individual trees to be used in tree improvement work. One of the objectives of this study was to provide data that could be used in establishing base-line information for quaking aspen. Of particular interest was the establishment of base lines for comparing the specific gravity, fiber length, fiber strength, and growth rate of selected trees with the average of the population from which the trees were selected. The analysis of variance and the comparisons of geographic area means revealed that for a single property or characteristic, several base lines were required because of the differences between geographic areas. Calculation of the base-line curves for all important growth, wood and fiber properties are under way and three examples are included for illustration purposes. The procedures used consisted of plotting the selected property over age, calculating the least squares regression line for the data and then calculating the statistical zones of one and two standard deviations above and below the mean value. The data in the illustrations required the use of the linear regression procedure, while for certain data curvilinear regression calculations may be required. When significant linear or curvilinear relationships do not exist, a mean value for the population and the standard deviation of the mean can be used for comparison purposes.

Figures 11, 12, and 13 illustrate the base-line curves for height growth, specific gravity, and age thirty fiber length. The solid line is the average for the data and the dashed lines are one and two standard deviations above and below the average data. By plotting the information for an individual tree over the age of the tree an indication is obtained of how this tree ranks in relation to the other trees in the population.

When zero-span tensile strength was plotted over age, it was obvious that the age range of the trees used was extremely narrow (32-44 years) and that satisfactory age-related base lines could not be established. Considerable zero-span tensile strength variation existed between trees of the same age and these data are an example in which the area mean values and the standard deviation from the mean should be used as a basis of comparison. Zero-span tensile strength values for Areas D, C, and E were considered to be from the same population and a common mean and standard deviation were calculated for use as a base line.

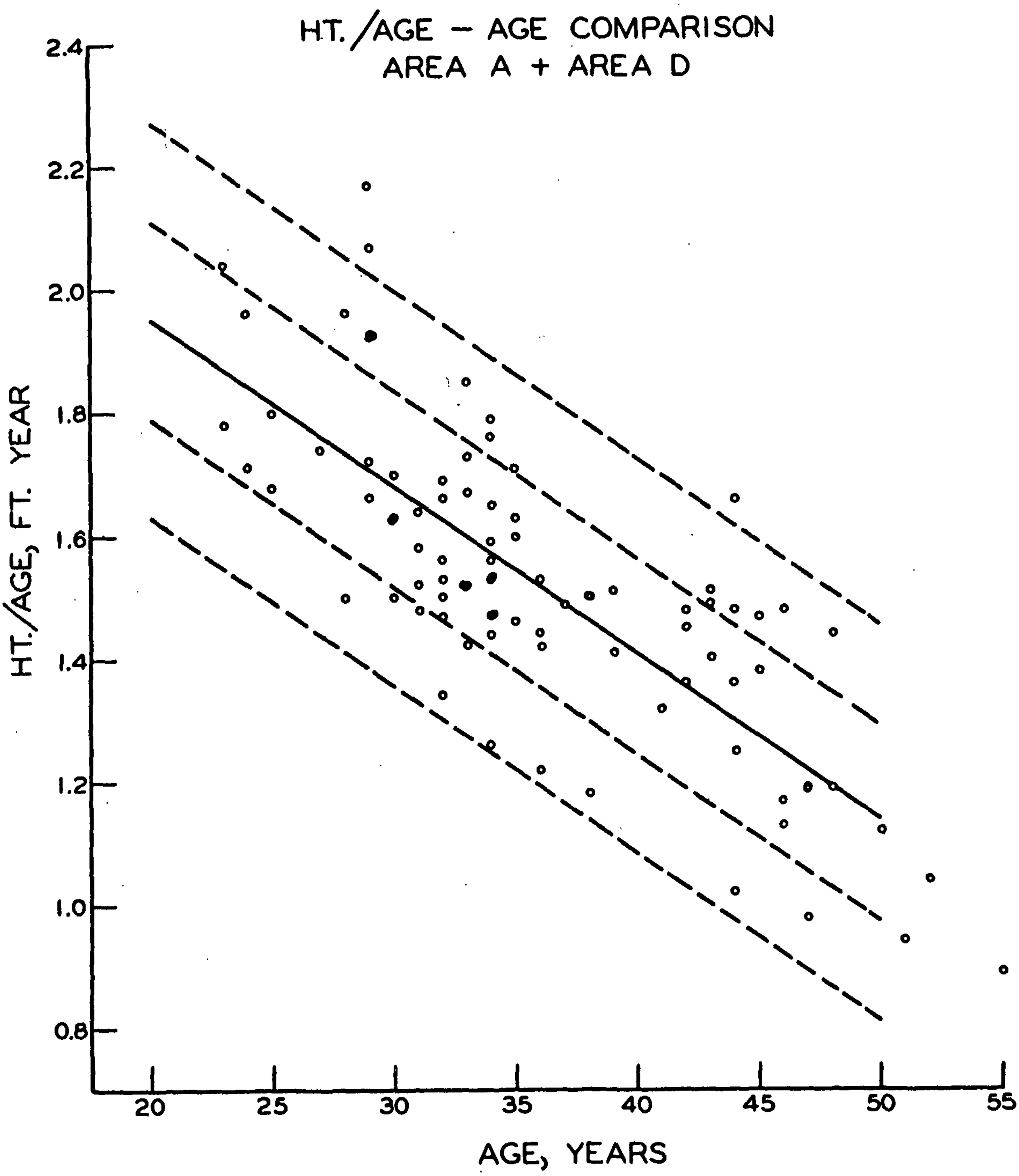

Figure 11. Height Growth Base-Line Values for Quaking Aspen in Areas A and D. The Solid Line is the Average Height Growth and the Dashed Lines Are One and Two Standard Deviations Above and Below the Average Height Growth (Ht./Age)

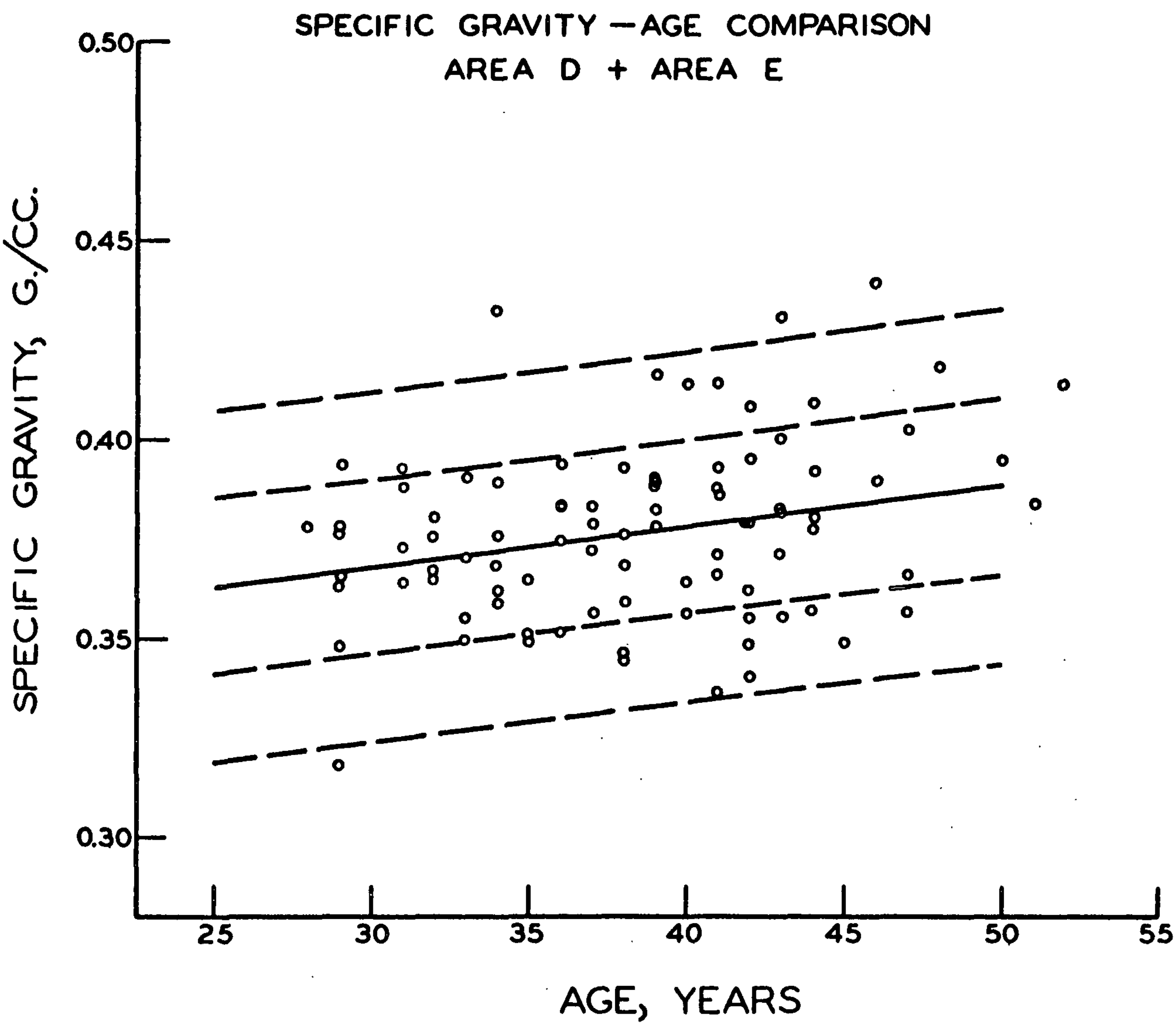

Figure 12. Specific Gravity Base-Line Values for Quaking Aspen in Areas D and E. The Solid Line is the Average Specific Gravity. The Dashed Lines Are One and Two Standard Deviations Above and Below the Average Values

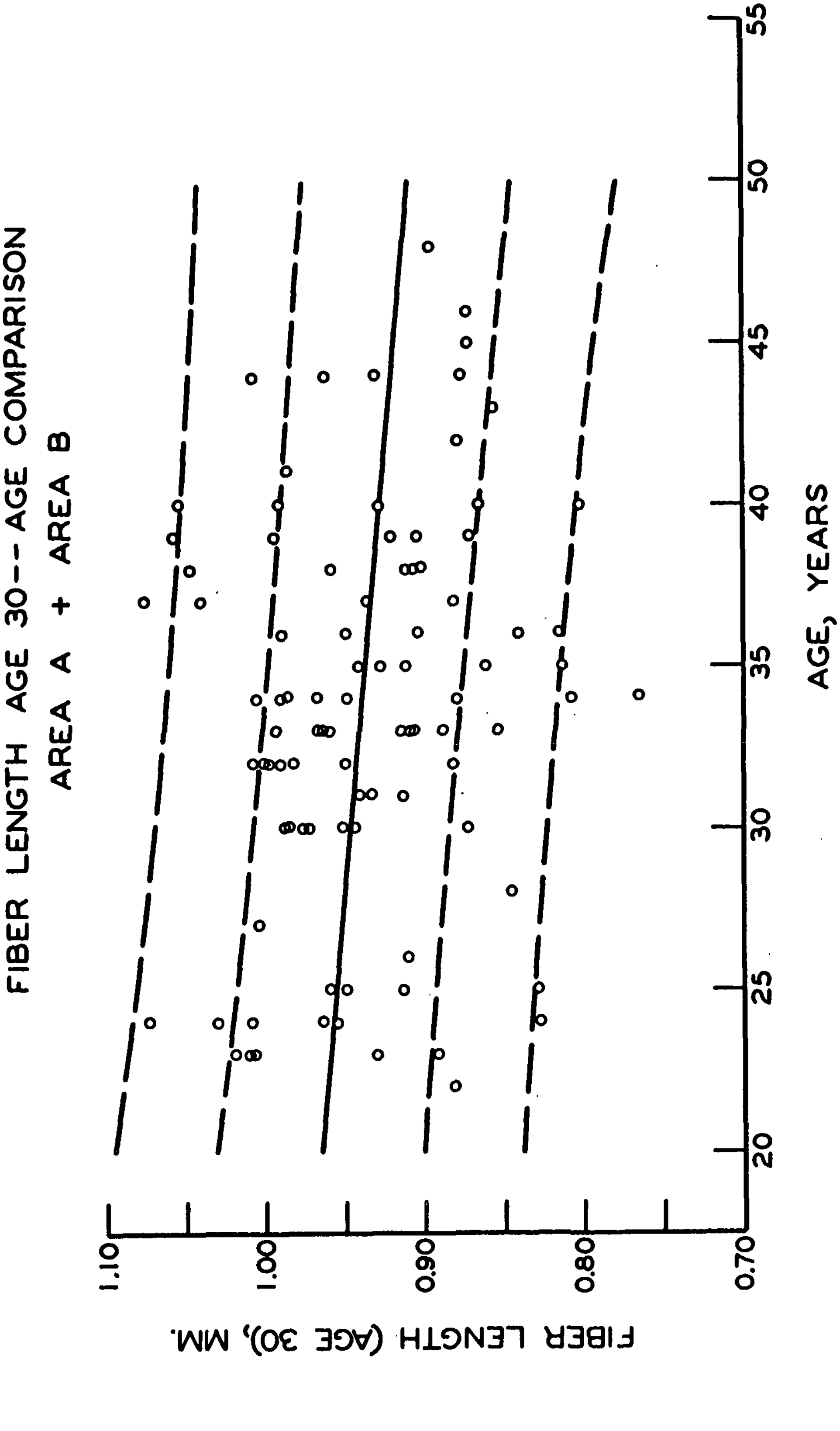

Figure 13. Fiber Length at Age 30 Base-Line Values for Quaking Aspen in Areas A and B. The Solid Line is the Average Fiber Length and the Dashed Lines Are One and Two Standard Deviations Above and Below the Average Values

Summary and Conclusions

Natural quaking aspen stands in five geographic areas were measured and sampled and soils data taken as part of the experimental procedure. The results of a statistical comparison of the data indicated there existed a well-defined south-to-north trend of decreasing specific gravity. No geographic trend was evident for fiber length, fiber strength, pulp yield, or any of the tree growth characteristics.

The percent clay and the exchangeable bases which were correlated with the percent clay were the soil factors that appeared to have the most influence on tree growth. Fiber length was correlated with height and volume growth and several soil properties. The lack of geographic trend in fiber length seems to have resulted from the overriding influence of genetic and local site factors. The positive correlation between fiber length and specific gravity and fiber length and height and volume growth simplifies the selection of these important properties for genetic improvement. The negative correlation of fiber strength with specific gravity, tree volume growth, and diameter growth suggests the need for independent selection for this property.

The data collected on growth and wood quality provided suitable information for establishing "base lines" for judging the potential of selected trees. The negative age 30 fiber length – age trend, although significant at only the 6 to 7% level of probability seems to indicate that the curves used to adjust fiber length to age 30 were overcorrecting for the older age trees. When unadjusted fiber length – age correlations for Areas A and B (data from these two areas were used in the age 30 fiber length "base-line" illustration) were calculated, there was a highly significant positive correlation between fiber length and age.

HERITABILITY OF WOOD AND GROWTH CHARACTERISTICS OF QUAKING ASPEN

Heritability information, when used in conjunction with information on natural variation makes possible reliable decisions regarding the growth, morphological, and wood properties which can most successfully be modified through tree improvement work. Experimental Trial VII was established to provide heritability information on wood and fiber properties as well as information on the inheritance of growth and morphological characteristics. The study involves a total of 25 quaking aspen crosses. These crosses were carried out in the Institute's greenhouse and 55 progeny from each cross have been planted in single blocks on a uniform site at the Greenville Test Area.

Project 2412, Progress Report 2 described in some detail the measurement data taken on Experimental Trial VII, and this description will not be repeated. All pulping and all growth, wood, and fiber measurement data for this trial have been completed and it is hoped that the analysis of the data can get under way soon after the first of the year. Because of the large amount of measurement data involved, the results of this study will be reported in a separate progress report.

EARLY GROWTH AND WOOD PROPERTIES OF DIPLOID AND TRIPLOID ASPEN

Laboratory, greenhouse, and growth chamber studies relative to selection, hybridization, and polyploidy represent one phase of the overall aspen genetics and tree improvement program. This phase is supported by the Louis W. and Maud Hill Family Foundation. The field testing and evaluation of improved materials represents a second and very necessary aspect of the overall program. This phase of the program is supported by the Aspen Genetics and Tree Improvement Group which is composed of nine pulp and paper companies. Because the results obtained from the field testing work are helpful in providing a more complete picture of the progress being made, the following summary of a five-year-old field trial is presented.

Summary

A replicated Experimental Trial of aspen was established near Eagle River, Wisconsin, in the spring of 1959. Three different types of aspen materials were used in the study including two vegetatively propagated triploid quaking aspen clones, two triploid hybrids grown from seed, and two seedling sources of diploid quaking aspen. Early growth and survival of the test materials were good with survival ranging from 92 to 100% and average height growth for each of the materials exceeding 2.5 feet per year. Figures 14 and 15 illustrate the size and form of two of the test materials. After the materials had grown in the field for five years, the plots were carefully measured and the trees in three of the four replications were cut to obtain information on the wood quality and sprouting ability of the test materials. In addition to growth and form measurements, the wood samples taken were used to evaluate the specific gravity, fiber length, and overall pulping potential of the test trees. Listed below are the major results obtained.

1. Height and diameter growth differences were obtained between test materials with the triploid hybrids exhibiting the most rapid growth.

2. Differences in tree form and natural pruning were also evident with the triploid clones ranking high in these characteristics.

Figure 14. Three Progeny From the Diploid Test Material 12-58 that Illustrate the Size and Form of Selected Diploid Aspen After Five Years in the Field

Figure 15. The Triploid Hybrid 14-58 Pictured Above Had the Best Average Height Growth (18.9 ft. After Five Years) of Materials Tested. The Tree Being Examined is the Largest Tree in the Entire Trial with a Total Height of 28 Feet and a Diameter at 4-1/2 Feet of 3.2 Inches

3. The two triploid clones were found to be free of Hypoxylon pruinatum and injury by the root-girdling Agrilus horni, two primary causes of reduced growth and mortality in plantings and natural stands.

4. Encouraging fiber length differences were discovered with the two triploid hybrids having the longest fibers, the triploid clones ranking second, and the diploid sources having the shortest fibers.

5. Significant differences were also obtained between test materials in specific gravity. The two triploid hybrids had the highest specific gravity and the two triploid clones along with one of the diploid sources had equally low specific gravity.

6. Differences between test materials in zero-span tensile strength were not significant and zero-span tensile strength was found to be negatively correlated with specific gravity.

7. Lignin, extractives, and pulp yield were similar for the six test materials and the differences obtained were not significant.

8. Simple correlations were calculated between growth, wood, fiber, and pulp properties. Of particular interest were the correlations between height growth and fiber length (positive), zero-span tensile strength and specific gravity (negative) and the increasing evidence of the lack of any correlation between rapid growth and specific gravity.

INTRASPECIFIC AND INTERSPECIFIC CROSSING

The 1965 crossing program followed a pattern similar to that established in previous years. Three major types of crosses were attempted. These included crosses and hybrids* involving quaking aspen as parent trees, crosses and hybrids involving bigtooth aspen as parent trees and crosses involving cottonwood as parent trees. Major emphasis was placed on producing bigtooth aspen crosses and hybrids well suited for growth on dry sandy sites and cottonwood crosses that would be useful on low moist areas of central and southern Wisconsin.

Thirty-two different parent trees, including 12 quaking aspen, 7 bigtooth aspen, 7 cottonwood, and 3 European gray poplar were employed and a total of 47 crosses were attempted. Considerable effort was also expended in improving crossing and handling techniques for eastern cottonwood. Table XIV summarizes the parent trees utilized in the crossing program and Tables XV and XVI provide additional information on crossing success, seedling size, and seedling production. Figure 14 illustrates typical 1-0 seedling size.

QUAKING ASPEN CROSSES

Quaking aspen crosses, as discussed in past reports, received major emphasis during the early phases of the project crossing program. Differences in performance of these early crosses are developing and outstanding individuals within the crosses are beginning to show up. Because of the large number of past quaking aspen studies only a moderate amount of work was done in this area during

*Throughout this report the word 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 XIV

SUMMARY OF CROSSES AND LOCATION OF PARENT TREES

Cross No.[a]	Parents (female x male)		
XT-Ta-1-65	T-20-56 (Watersmeet, Mich.)	X	Ta-10 (4n) (Ekebo, Sweden)
XT-2-65	T-1-58 (Porcupine Mts., Mich.)	X	T-44-60 (Ralph, Mich.)
XT-3-65	T-1-58 (Porcupine Mts., Mich.)	X	XT-22-56, no. 36 (Greenville, Wis.)
XT-Ta-4-65	T-1-58 (Porcupine Mts., Mich.)	X	Ta-10 (4n) (Ekebo, Sweden)
XT-5-65	XT-22-56, no. 50 (Greenville, Wis.)	X	XT-22-56, no. 36 (Greenville, Wis.)
XT-Ta-6-65	XT-22-56, no. 50 (Greenville, Wis.)	X	Ta-10 (4n) (Ekebo, Sweden)
XCa-T-7-65	Ca-2 (Czechoslovakia)	X	T-44-60 (Ralph, Mich.)
XCa-T-8-65	Ca-2 (Czechoslovakia)	X	XT-22-56, no. 36 (Greenville, Wis.)
XT-Ta-9-65	T-80-57 (Alston, Mich.)	X	Ta-10 (4n) (Ekebo, Sweden)
XT-Ca-10-65	T-1-58 (Porcupine Mts., Mich.)	X	Ca-3-65 (Czechoslovakia)
XT-Ca-11-65	T-49-60 (Ralph, Mich.)	X	Ca-3-65 (Czechoslovakia)
XCa-12-65	Ca-2 (Czechoslovakia)	X	Ca-3-65 (Czechoslovakia)
XG-A-13-65	G-64 (Wausau, Wis.)	X	A-1-65 (Czechoslovakia)
XG-Ca-14-65	G-64 (Wausau, Wis.)	X	Ca-3-65 (Czechoslovakia)
XG-Ta-15-65	G-10-62 (Bonita, Wis.)	X	Ta-10 (Ekebo, Sweden)

Note: See end of table for footnote.

TABLE XIV (Continued)

SUMMARY OF CROSSES AND LOCATION OF PARENT TREES

Cross No.[a]	Parents (female x male)		
XG-A-16-65	G-10-62 (Bonita, Wis.)	X	A-1-65 (Czechoslovakia)
XG-Ca-17-65	G-9-63 (Bruce, Wis.)	X	Ca-3-65 (Czechoslovakia)
XA-18-65	A-2-61 (Maple, Ontario)	X	A-1-65 (Czechoslovakia)
XG-Ca-19-65	G-64 (Wausau, Wis.)	X	Ca-1-62 (Czechoslovakia)
XCa-G-20-65	Ca-2 (Czechoslovakia)	X	G-3-58 (Tippy Dam, Mich.)
XG-21-65	G-10-62 (Bonita, Wis.)	X	G-1-58 (Porcupine Mts., Mich.)
XG-22-65	G-10-62 (Bonita, Wis.)	X	G-3-58 (Tippy Dam, Mich.)
XG-23-65	G-10-62 (Bonita, Wis.)	X	G-8-63 (Hiles, Wis.)
XG-24-65	G-10-62 (Bonita, Wis.)	X	G-1-65 (Breed, Wis.)
XG-26-65	G-64 (Wausau, Wis.)	X	G-1-58 (Porcupine Mts., Mich.)
XG-27-65	G-64 (Wausau, Wis.)	X	G-3-58 (Tippy Dam, Mich.)
XG-28-65	G-64 (Wausau, Wis.)	X	G-8-63 (Hiles, Wis.)
XG-29-65	G-64 (Wausau, Wis.)	X	G-1-65 (Breed, Wis.)
XG-30-65	G-9-63 (Bruce, Wis.)	X	G-1-58 (Porcupine Mts., Mich.)
XG-31-65	G-9-63 (Bruce, Wis.)	X	G-3-58 (Tippy Dam, Mich.)

Note: See end of table for footnote.

TABLE XIV (Continued)

SUMMARY OF CROSSES AND LOCATION OF PARENT TREES

Cross No.[a]	Parents (female x male)		
XG-32-65	G-9-63 (Bruce, Wis.)	X	G-8-63 (Hiles, Wis.)
XG-33-65	G-9-63 (Bruce, Wis.)	X	G-1-65 (Breed, Wis.)
XCa-34-65	Ca-2 (Czechoslovakia)	X	Ca-1-62 (Czechoslovakia)
XT-Ca-35-65	T-12-58 (Clintonville, Wis.)	X	Ca-1-62 (Czechoslovakia)
XT-36-65	T-12-58 (Clintonville, Wis.)	X	T-10-60 (Porcupine Mts., Mich.)
XD-37-65	D-3-64 (Greenwood, Wis.)	X	D-1-63 (Waupaca, Wis.)
XD-38-65	D-3-64 (Greenwood, Wis.)	X	D-2-64 (Bruce, Wis.)
XD-39-65	D-3-64 (Greenwood, Wis.)	X	D-6-65 (Nichols, Wis.)
XD-40-65	D-8-65 (Nichols, Wis.)	X	D-1-63 (Waupaca, Wis.)
XD-41-65	D-8-65 (Nichols, Wis.)	X	D-1-63 (Waupaca, Wis.)
XD-42-65	D-8-65 (Nichols, Wis.)	X	D-2-64 (Bruce, Wis.)
XD-43-65	D-8-65 (Nichols, Wis.)	X	D-6-65 (Nichols, Wis.)
XD-44-65	D-8-65 (Nichols, Wis.)	X	D-9-65 (Wittenberg, Wis.)
XT-A-45-65	T-12-58 (Clintonville, Wis.)	X	A-1, no. 12 (Czechoslovakia)
XT-46-65	T-29-57 (Bruce Crossing, Mich.)	X	T-2-56 (Bruce Crossing, Mich.)
XT-47-65	T-29-57 (Bruce Crossing, Mich.)	X	T-124-56 (Bruce Crossing, Mich.)
XD-0-48-65	D-7-62 (Nichols, Wis.)	X	Open pollinated
XD-0-49-65	D-8-65 (Nichols, Wis.)	X	Open pollinated

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

TABLE XV

SUMMARY OF 1965 CROSSES

Cross No.[a]	Type Cross[b]	No. of Catkins Pollinated	No. of Catkins Collected	Amt. Seed[c]	Seeds/Catkin[c]	Germ., %[c]
XT-Ta-1-65	C-P	50	41	370	6	81
XT-2-65	C	45	32	3,830	81	95
XT-3-65	C	41	41	7,200	163	93
XT-Ta-4-65	C-P	58	55	20	0.3	95
XT-5-65	C	6	6	150	22	88
XT-Ta-6-65	C-P	15	15	480	33	94
XCa-T-7-65	C	49	42	5,790	115	97
XCa-T-8-65	C	50	47	13,150	255	97
XT-Ta-9-65	C-P	70	62	2,610	33	89
XT-Ca-10-65	C	10	10	no seed	–	–
XT-Ca-11-65	C	28	25	no seed	–	–
XCa-12-65	C	25		no seed	–	–
XG-A-13-65	DS	14	12	1,310	70	76
XG-Ca-14-65	DS	46	10	no seed	–	–
XG-Ta-15-65	DS	16	12	370	5	21
XG-A-16-65	DS	10	6	330	14	43
XG-Ca-17-65	DS	8	8	no seed	–	–
XA-18-65	DS	5	4	710	76	54
XG-Ca-19-65	DS	37	16	110	1	45
XCa-G-20-65	DS	50	39	4,610	83	89
XG-21-65	DS	16	16	2,240	108	77
XG-22-65	DS	11	11	11,120	887	88
XG-23-65	DS	21	21	13,710	618	96
XG-24-65	DS	9	8	14,320	1,511	96
XG-25-65	no cross	–	–	–	–	–
XG-26-65	DS	35	0	–	–	–
XG-27-65	DS	43	43	6,740	143	91
XG-28-65	DS	38	9	370	12	95
XG-29-65	DS	69	32	4,760	62	90
XG-30-65	DS	8	8	2,600	303	93
XG-31-65	DS	5	5	280	50	88
XG-32-65	DS	9	9	8,020	826	95
XG-33-65	DS	7	7	6,880	839	88
XCa-34-65	C		66	13,530		78
XT-Ca-35-65	C	87	86	15,870	398	94
XT-36-65	C	68	66	8,970	262	100

Note: See end of table for footnotes.

TABLE XV (Continued)

SUMMARY OF 1965 CROSSES

Cross No.[a]	Type Cross[b]	No. of Catkins Pollinated	No. of Catkins Collected	Amt. Seed[c]	Seeds/Catkin[c]	Germ., %[c]
XD-37-65 (Growth Chamber)	B	16	10	1,880	24	20
XD-37-65 (Greenhouse)	B	15	14	3,310	24	11
XD-37-65 (Bottle Graft, Gr. Chamber)	B	5	1	490	–	–
XD-38-65 (Bottle Graft, Gr. Chamber)	B	5	3	1,660	276	83
XD-39-65 (Crown Veneer Graft)	B	4	1	260	–	–
XD-41-65 (Greenhouse no. 3)	B	9	6	3,420	68	18
XD-41-65 (Gr. Chamber no. 5)	B	8	2	510	22	34
XD-41-65 (Gr. Chamber no. 7)	B	8	2	290	0.4	1
XD-42-65 (Growth Chamber)	B	13	8	1,810	84	60
XD-43-65 (Growth Chamber)	B	12	4	140	6	47
XD-43-65 (Bottle Graft, Greenhouse)	B	7	1	230	–	–
XD-44-65 (Growth Chamber)	B	9	9	1,020	59	52
XD-44-65 (Bottle Graft, Greenhouse)	B	6	1	490	–	–
XT-A-45-65 (Medium)	H	9	3	30	2.1	65
XT-A-45-65 (High)	H	10	9	100	2.7	25
XT-46-65	P	14	4	no seed	–	–
XT-47-65	P	5	5	no seed	–	–

[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, DS = dry site cross, B = crosses in black poplar group, P = polyploid cross, H = haploid cross.

[c] Amount of seed, no. viable seeds/catkin pollinated and germination % based upon 40 mesh seed for all "T", "T x Ta", "Ca x T", "T x Ca", and "Ca" crosses; upon 20+ mesh seed for XA-18-65; upon 28 mesh and up for all "D" crosses; and on both 40 and 50 mesh seed for the rest.

TABLE XVI

SUMMARY OF 1965 SEEDLING PRODUCTION

Cross No.[a]	Total No. Seeds Planted	Total No. Seedlings Produced	No. Plantable Seedlings[b] Misc. Beds	Repl. Beds	Average Height[c] All Seedlings	Plantable Seedlings
XT-Ta-1-65	370	152	–	101	2.8	2.8
XT-2-65	3320	1465	795	405	3.4	3.5
XT-3-65	9920	1760	953	397	3.0	3.0
XT-Ta-4-65	20	9	–	6	2.0	2.4
XT-5-65	150	42	–	30	2.0	2.1
XT-Ta-6-65	520	183	–	112	1.9	2.1
XCa-T-7-65	3320	1290	656	444	3.4	3.4
XCa-T-8-65	6820	1100	539	411	3.1	3.1
XT-Ta-9-65	2480	750	255	345	2.6	2.7
XG-A-13-65	1310	240	–	218	2.9	3.1
XG-Ta-15-65	370	21	–	16	2.8	2.9
XG-A-16-65	330	41	–	34	4.3	4.3
XA-18-65	710	87	–	75	3.6	3.7
XG-Ca-19-65	110	5	–	5	2.6	2.6
XCa-G-20-65	3600	335	–	289	2.8	2.9
XG-21-65	2230	185	20	130	2.0	2.3
XG-22-65	10180	555	182	218	2.2	2.4
XG-23-65	5450	765	314	236	1.8	2.1
XG-24-65	5500	500	203	147	2.0	2.3
XG-27-65	1500	245	–	166	1.5	1.9
XG-28-65	370	62	–	30	1.4	1.8
XG-29-65	3000	177	34	91	1.8	2.1
XG-30-65	1950	280	22	178	2.3	2.5
XG-31-65	280	56	–	45	1.8	2.0
XG-32-65	4420	575	255	195	1.7	2.0
XG-33-65	3120	295	87	113	1.7	2.0
XCa-34-65	1740	770	69	531	3.2	3.2
XT-Ca-35-65	3500	800	349	301	3.4	3.4
XT-36-65	3150	770	219	381	3.1	3.2
XD-37-65	6620	345	–	345	4.0	4.0
XD-38-65	1430	354	–	351	3.4	3.4
XD-41-65	5360	353	–	350	3.6	3.6
XD-42-65	1920	401	–	395	3.2	3.2
XD-43-65	150	21	–	21	1.9	1.9
XD-44-65	1100	303	–	281	2.3	2.4
XT-A-45-65	140	20	(Only abnormal seedlings saved)			

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

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

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

Figure 16. The 1965 I.P.C. Seedbeds With 1-0 Bigtooth Aspen Seedlings in the Foreground and 1-0 Bigtooth Aspen Hybrids and European Gray Poplar Seedlings in the Background

the past year. Crosses one through eleven and Crosses 35, 36, 45, 46, and 47 involve quaking aspen (T) as one or both of the parents and the overall success of these crosses was slightly less than usual. Several factors appear to be involved in the reduced success of the quaking aspen crosses. In crosses one, four, six, and nine the female parents were Lake States diploid aspen (2n)* and the male parent was a tetraploid European aspen (Ta) growing in Sweden. The pollen from the Swedish tetraploid and the pollen from a European gray poplar

*Diploid refers to individuals having the normal two sets (2n) of chromosomes, triploids are individuals containing three sets (3n), tetraploids are individuals having four sets (4n) and polyploid is a general term that refers to individuals having more than the normal two sets of chromosomes. Haploids (1n) are individuals with one set of chromosomes, 1/2 the normal two sets.

(Ca-3-65) growing in Czechoslovakia arrived in very poor condition and the low seed production apparently resulted from poor pollen quality. Experimental Crosses 45, 46, and 47 were attempted in an effort to produce haploids and/or polyploids and the reduced seed and seedling production was expected because of the type of cross involved. Seed production on the other quaking aspen crosses was near normal. The seedlings produced, particularly the "XCa-T" and "XT-Ca" crosses, will be used in the Institute test plantings and cooperator plantings.

BIGTOOTH ASPEN CROSSES

The bigtooth aspen crosses and bigtooth hybrids continued to receive a major amount of emphasis with 20 crosses being attempted (Crosses XG-A-13-65 through XG-33-65). Genetic combinations accomplished in this rather general group include crosses between bigtooth aspen parents and hybrids between bigtooth aspen (G), and European white poplar (A), European gray poplar (Ca), and the previously mentioned tetraploid European aspen (Ta-4<u>n</u>). The principal objective of the crosses utilizing bigtooth aspen as one or both parents is to find suitable combinations that will do well on dry sandy sites. Seed production and seed germination of bigtooth aspen crosses are usually quite variable and average less than the quaking aspen crosses. With the exception of one or two crosses where the pollen quality was poor, seed production and seed quality were above average. Seedbed production of bigtooth aspen seedlings was below average and apparently resulted from improper ventilation of the seedbeds on one or two very warm days just after the seed had begun to germinate.

The bigtooth aspen crosses XG-21-65 through XG-33-65 were arranged in a modified diallel series and used to evaluate the crossing compatibility and the overall performance of four male and three female bigtooth aspen parent trees.

Flowering behavior, seeds, and plantable seedlings produced, and first-year seedling growth were the basis of evaluation. Table XVII summarizes the information used in the evaluation of the parent trees. Of the three female trees being evaluated, G-10-62 and G-9-63 had been evaluated earlier and again both rank higher than the other trees with which they had been compared. When the data on the male trees being evaluated were compared, G-1-65 and G-8-63 appeared to be the best two males and G-1-58, which did poorly in an earlier comparison, was the least satisfactory. Growth of the bigtooth aspen crosses and the bigtooth aspen hybrids was satisfactory and although seedbed production was lower than normal, sufficient numbers of most crosses are available for field testing.

COTTONWOOD CROSSES

Difficulties in bringing reasonable numbers of cottonwood female catkins through to maturity continue to hamper large-scale production of cottonwood seedlings. Pollination of catkins that are developing on newly grafted scions has been the most successful technique to date. In 1965 this method was less successful than in previous years and led to the decision that additional work on the techniques of handling cottonwood crosses should be undertaken. Basically, the approach that was investigated was a modification of the very successful "standard cut branch" technique* employed in the aspen crossing work.

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*The "standard cut branch" technique employed involved placing the freshly collected branches in a vase of water, clipping off a small section of stem and changing the water every day. When ice water was used in the vase, clipping, and changing the ice water were placed on an every other day basis.

TABLE XVII

SUMMARY OF SEED AND SEEDLING PRODUCTION AND SEEDLING GROWTH
MODIFIED DIALLEL CROSSING SERIES

Female Parent Trees	Male Parent Trees			
	G-1-58	G-3-58	G-8-63	G-1-65
G-10-62	XG-21-65	XG-22-65	XG-23-65	XG-24-65
--[a]	108	887	618	1511
--[b]	9	40	69	117
--[c]	2.0	2.2	1.8	2.0
G-64	XG-26-65	XG-27-65	XG-28-65	XG-29-65
--[a]	no seed	143	12	62
--[b]	--	22	1	3
--[c]	--	1.5	1.4	1.8
G-9-63	XG-30-65	XG-31-65	XG-32-65	XG-33-65
--[a]	303	50	826	839
--[b]	33	10	90	67
--[c]	2.3	1.8	1.7	1.7

[a]Number of viable seeds 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.

The standard cut branch technique had been tried earlier and the new work included the use of an antibiotic solution to maintain the branches in a good condition and the use of the newly completed growth chamber to speed catkin development. Table XVIII summarizes the preliminary results obtained when the branches and catkins of Cross XD-41-65 were treated in a variety of different ways. These results seem to indicate that the University of Wisconsin antibiotic solution* has some promise and the other phases of this investigation

*Antibiotic solution developed by Dr. J. G. Berbee, University of Wisconsin.

TABLE XVIII

RESULTS OF HANDLING TECHNIQUES TRIED ON CROSS XD-41-65

Solution	Treatment[a]	Location	Catkins Polli-nated	Catkins Collected	Time for Seed Develop., days	Total Number Seeds Produced[b]	Germ., %	Viable Seeds /Catkin Pollinated
Ice water	None	Greenhouse	7	0	--	--	--	--
Ice water	Buds removed	Greenhouse	6	0	--	--	--	--
Antibiotic soln.[c]	Buds removed	Greenhouse	9	6	38	3421	18	68
Ice water	None	Growth chamber	5	0	--	--	--	--
Antibiotic soln.[c]	None	Growth chamber	8	2	24	511	34	22
Antibiotic soln.[c]	Foliar spray	Growth chamber	5	0	--	--	--	--
Modified antibiotic soln.	None	Growth chamber	8	2	21	287	1	0.4

[a]Branch collections clipped periodically and either none, vegetative buds removed or foliar spray applied to leaves.

[b]Total of 20 and 28-mesh seed only. 40-Mesh seed excluded because of very low germination.

[c]Formula obtained from Dr. J. G. Berbee, University of Wisconsin.

Plans for investigations in the area of tree physiology include completion of the present investigation under way on the nutrient requirements of "P. canescens x P. tremuloides" type hybrids and the expansion of the work to include studies of the nutrient requirements of "P. canescens x P. grandidentata" type hybrids. These latter studies involve the use of sand culture techniques and will be conducted in the growth chamber.

Plans and experimental investigations under way in the area of biochemical characterization of aspen clones and aspen hybrids will be handled in a separate report.

indicate the removal of vegetative buds and the use of the growth chamber could be expected to speed catkin development and increase seed production. Additional work with this technique is planned.

Eight cottonwood crosses were completed and, as Table XV indicates, a number of the crosses were split and handled part in the greenhouse and part in the growth chamber. As a result of the use of the growth chamber, satisfactory numbers of cottonwood seedlings were produced in five out of the six crosses attempted (Table XVII). Plans for outplanting these progeny groups are indefinite pending the location of an appropriate test site.

PLANS FOR 1966

No major changes are planned in 1966 with regard to the emphasis of the work under Project 2412 with the exception that additional tree physiology studies are planned. The five main areas of investigation will include: (1) selection and hybridization, (2) production of artificial polyploids, (3) studies of natural variation with special emphasis on "wood quality", (4) tree physiology studies aimed at investigating the nutrient requirements of aspen hybrids, (5) biochemical characterization of aspen clones and aspen hybrids.

Selection and hybridization studies will continue to emphasize the production of trees suitable for use in so-called "dry site" plantings. Continued effort will also be directed toward the production of cottonwood crosses suitable for use on "wet sites" in central and north central Wisconsin. Production of polyploids, particularly tetraploids for use in the eventual mass production of triploid cottonwood and triploid bigtooth aspen, will be continued. Cytological studies of fertilization and embryo development will receive continued emphasis because of the importance of this basic information in the production of tetraploids.

Several studies of natural variation either under way or in various stages of completion include: (1) the heritability of wood and growth characteristics of quaking aspen, (2) nature and causes of within tree variation in specific gravity, (3) natural variation of growth and wood properties of bigtooth aspen. In the quaking aspen heritability study all data have been collected and only the analysis of the data and a write-up of the report remains to be completed. Studies on Items 2 and 3 are just getting under way and only a minor amount of field work has been completed. Time permitting, additional field sampling for the latter two studies will be undertaken this coming summer.

Plans for investigations in the area of tree physiology include completion of the present investigation under way on the nutrient requirements of "*P. canescens* x *P. tremuloides*" type hybrids and the expansion of the work to include studies of the nutrient requirements of "*P. canescens* x *P. grandidentata*" type hybrids. These latter studies involve the use of sand culture techniques and will be conducted in the growth chamber.

Plans and experimental investigations under way in the area of biochemical characterization of aspen clones and aspen hybrids will be handled in a separate report.

PUBLICATIONS

PUBLICATIONS RELATING TO PROJECT 2412 AND ALLIED PROJECTS SINCE MAY, 1965

1. Einspahr, D. W. Colchicine treatment of newly-formed embryos of quaking aspen. Forest Sci. 11:456-9(Dec., 1965).

2. Mathes, M. C., and Einspahr, D. W. Comparison of tree growth and callus production in aspen. Forest Sci. 11:165-73(Sept., 1965).

FUTURE PUBLICATIONS

1. Benson, M. K., Einspahr, D. W., and Schwalbach, D. E. Rooting of quaking aspen root sprouts. To be submitted to Tree Planters' Notes.

2. Benson, M. K., and Einspahr, D. W. Early growth of triploid aspen and triploid aspen hybrids. Submitted to Forest Science.

3. Einspahr, D. W., and Benson, M. K. Comparison of wood, fiber and pulp properties of naturally occurring and artificially produced triploid aspen with diploid aspen. To be submitted to Tappi.

4. Einspahr, D. W., Benson, M. K., and Peckham, J. R. Geographic variation in growth and wood properties of quaking aspen. To be submitted to Silvae Genetica.

5. Winton, L. L., and Einspahr, D. W. Fertilization and early embryology of forced quaking aspen and cottonwood. To be submitted to Silvae Genetica.

6. Winton, L. L. Cold-water Feulgen technique for aspen chromosomes. To be submitted to Stain Technology.

ACKNOWLEDGMENTS

The authors of this report are indebted to Donald Grady and Delmar Schwalbach for their assistance with the field measurements and the wood-sampling aspects of the program. The authors also wish to acknowledge the help of Mrs. Marianne Harder and Mrs. Dorothy Olson for their assistance in carrying out the fiber length and specific gravity measurements and to acknowledge the work of John Bachhuber, Mrs. Harder, and Mrs. Olson in handling the computer problems. Thanks also go to the members of the Technology Section for their contribution to the pulping phases of the program and to W. Wink and members of the Paper Evaluation Section for making the zero-span tensile strength measurements.

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