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PHYSIOLOGICAL GENETICS STUDIES OF POPULUS GRANDIDENTATA, POPULUS TREMULOIDES, AND THEIR HYBRID, POPULUS XSMITHII

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PHYSIOLOGICAL GENETICS STUDIES OF POPULUS GRANDIDENTATA, POPULUS TREMULOIDES, AND THEIR HYBRID, POPULUS XSMITHII

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ABSTRACT

PHYSIOLOGICAL GENETICS STUDIES OF
POPULUS GRANDIDENTATA, P. TREMULOIDES,
AND THEIR HYBRID, P. XSMITHII

BY

GREGORY LYNN REIGHARD

Populus tremuloides (trembling aspen) and *P. grandidentata* (bigtooth aspen) are sympatric Michigan species which occasionally hybridize. This study investigated the growth potential and physiological genetics of 206 families of *P. tremuloides*, *P. grandidentata*, and their hybrid, *P. Xsmithii*. Twenty *P. Xrouleauiana* backcrosses were also tested. Seven full-sib families were studied using gas-exchange and hydroponic systems to measure photosynthesis, respiration, root development, and carbon partitioning. In addition, eight cultural methods for planting aspen were evaluated.

The best planting method was placing the seedling root collars 15 cm below the soil surface and using the pre-emergent herbicide, simazine. The herbicides diuron and linuron were not effective in controlling weeds.

A progeny test was replicated over five Michigan sites to evaluate growth performance. Performance of trembling aspen families was above average at all sites and increased with latitude of plantation site. *P. Xrouleauiana* hybrids backcrossed to trembling aspen males were superior in growth at the three Lower Peninsula sites. Most *P. Xsmithii* families had growth rates below the mean.

Initial growth and survival of bigtooth aspen was poor at all plantations. Analyses of two nested mating designs showed that general combining ability was present in growth traits.

Trembling aspen leafed out earlier and dropped leaves later than bigtooth aspen. Hybrid phenology was intermediate. Morphological traits had both additive and non-additive genetic variance. For these traits, hybrids resembled the maternal parent. Hybrids suffered from shoot dieback, bud abortion, and adventitious sprouting. *Saperda inornata* and *Venturia tremulae* were the most serious insect and disease problems, respectively.

No significant differences were found for photosynthesis and dark respiration rates in the two species and *P.* X*smithii*. Whole plant photosynthesis and respiration were significantly correlated with leaf dry weight, shoot dry weight, and leaf area. Seedlings with high net photosynthetic rates and large leaf areas assimilated the most dry matter. Trembling aspen produced more primary roots and less secondary roots than bigtooth. *P.* X*smithii* produced slightly more primary and secondary roots than either species. Trembling aspen had a 50% smaller root to stem ratio than bigtooth. Hybrids had ratios similar to their maternal parents.

DEDICATION

To my grandfather, the late John Reighard, whose frugal lifestyle, devotion to hard work, and deep appreciation for agriculture and forestry inspired me to pursue and ultimately achieve an education in forest science.

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INTRODUCTION

Trembling aspen (*Populus tremuloides* Michx.) and bigtooth aspen (*P. grandidentata* Michx.) are sympatric species throughout Michigan. Hybridization between the species is infrequent under natural conditions because of prezygotic isolation barriers attributed to differences in flowering times (Heimburger 1936, Pauley 1956, Barnes 1961, and Pregitzer and Barnes 1980). Trembling aspen is a boreal species, and bigtooth aspen is a temperate mesic species (Fowells 1965). Trembling aspen exploits a wider range of sites in Michigan, but on sites with both species, bigtooth aspen outcompetes trembling aspen (Graham *et al*. 1963). Many diseases and insects attack both species (Davidson and Prentice 1968). However, bigtooth aspen is much less susceptible to *Hypoxylon* canker which causes the largest annual loss (8.5 million cubic meters) of aspen growing stock in the Lake States (Marty 1972). Abundant natural genetic variation in phenology, morphology, physiology, and wood properties has been found in trembling aspen by Van Buijtenen *et al*. (1959), Einspahr and Benson (1967), Barnes (1969), Cheliak and Dancik (1982), and many others. Although much less studied than trembling aspen, bigtooth aspen has also been reported to have large amounts of genetic variation (Barnes 1969, Okafo 1976).

Trembling and bigtooth aspen are the most important pulpwood species in Michigan (Blyth and Smith 1982). Volume production of natural stands at age 50 has been reported to be as high as 52 and 53 cords per acre for trembling and bigtooth aspen, respectively (Perala 1977). The demand for this resource is increasing as the forest products industry develops new technologies to use aspen for products (ie., particleboard, oriented-strand board, flakeboard, etc.) other than the traditional pulp, paper, and matchsticks. There are now a disproportionate number of old, low-quality aspen stands in Michigan (James Wadsworth, personal communication). To supplement the native aspen resource, Packaging Corporation of America is planting hybrid poplars in short-rotation fiber plantations. The use of aspens in these plantations has been avoided because genetically-improved planting stock is not readily available and economical establishment procedures have yet to be adequately developed.

To help resolve these economically-based problems, this study had three primary objectives. The first objective was to find a planting system based on herbicides which would successfully establish aspen on old-field sites.

The second objective was to improve the germplasm base of the planting stock. Even though extensive natural variation exists in Michigan populations of trembling and bigtooth aspen, genetic gain in growth rate cannot occur until the species have been progeny tested. A combination half-sib and full-sib progeny test was initiated to identify superior parents, families, and individuals. Furthermore, the two species were hybridized in an attempt to incorporate

Hypoxylon mammatum resistance into trembling aspen, to increase the site adaptability of bigtooth aspen, to diversify the breeding population, and to generate the non-additive genetic variance (heterosis) which has been reported in growth rates of white poplar x bigtooth aspen hybrids (Hall *et al*. 1982).

The third objective was to quantify the amount of additive and non-additive genetic variance in the two species and to determine how these types of genetic variance were expressed in the hybrid (*Populus Xsmithii* Boivin). Field measurements and controlled-environment studies using gas-exchange and hydroponic systems were used to meet this objective.

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CHAPTER 1

EFFECTS OF CHEMICAL WEED CONTROL AND SEEDLING PLANTING DEPTH ON SURVIVAL AND GROWTH OF ASPEN

(Accepted for Publication in Tree Planters' Notes)

ABSTRACT

Eight cultural treatments involving preemergent herbicides and planting depth were tested for effects on aspen growth and survival. Deep-planting aspen seedlings by placing the root collar 15 cm below the soil surface reduced injury from simazine. Simazine gave excellent weed control, whereas diuron and linuron were not as effective. Non-lethal herbicide damage that affected early seedling growth became insignificant as the trees grew older. Therefore, survival was more important than early growth as a criterion for evaluating herbicides for aspen establishment.

INTRODUCTION

Spawned by the energy crisis of the 70's, research interest in short-rotation tree plantations for biomass production continues to grow. Species of *Populus* are frequent choices for these plantations in the Lake States because of their fast growth and coppicing ability. Despite their popularity, inexpensive means of plantation establishment are lacking for most *Populus* species. Hybrid poplars, cottonwood, and aspen, along with most other hardwood species, need good site preparation and weed control in the first 2-3 years in order to be successfully established on abandoned fields (4, 13, 14). Cultivation is effective, but expensive. Chemical weed control is an alternative method that is less expensive, but it is more risky. Improved planting practices that include chemical weed control and exclude cultivation need to be developed and refined for all poplar species, especially the sensitive balsam poplars and aspens.

Much of the previous poplar research concerning preemergent herbicides has been directed at the cottonwood (Section Aigeiros), balsam poplars (Section Tacamahaca), and their hybrids. Herbicide research on aspen (*Populus grandidentata* Michx. and *P. tremuloides* Michx. of Section Leuce), however, has been limited. Since aspens are abundant and regenerate easily from established stands, there has been little past interest in planting them. With the recent upsurge of research on energy plantations, aspens are now being considered as a possible plantation species.

The work reported here tested the efficacy of the preemergent herbicides, simazine (2-chloro-4,6-bis (ethylamino)-s-triazine), diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), and linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) in controlling weed competition on an abandoned field in southern Michigan. These herbicides have been used previously in poplar plantations by numerous scientists including von Althen (11, 14), Dickmann, et al. (6), and Netzer and Noste (8). In conjunction with these herbicides, deep-planting of the seedlings and the use of plastic mulch were evaluated for protection from chemical injury. The purpose of these treatments was to find a system using chemicals that could be applied to planting aspens on abandoned farmland in Michigan.

MATERIALS AND METHODS

An agricultural field that had been idle for 25 years was chosen as a planting site. The field is located in Ingham County (S 6 T3N R1W), Michigan. The soil series, a Marlette fine sandy loam, is classified as a mixed, mesic Glossoboric Hapludalf. The soil properties and chemistry of the Ap horizon were analyzed by the Michigan State University Soil Testing Laboratory (table 1.1). The field was mowed in September, 1980 and sprayed with seven liters/hectare glyphosate in one-meter strips. The major vegetation cover at the time of spraying was quackgrass (*Agropyron repens* L. Beauv.).

Table 1.1. Soil properties of the Ap horizon of the field.

Soil Series	Texture (Class)	Organic Matter (%)	Clay (%)	pH	Cation Exchange Capacity (meq/g)
Marlette Fine Sandy Loam	Sandy Clay Loam	2.0	21.3	6.7	6

Nursery-grown, 1-0 seedlings of bigtooth aspen (Populus grandidentata Michx.) and quaking aspen (Populus tremuloides Michx.) that had been lifted in March and stored in a refrigerated room were planted May 8, 1981 in 5-7 tree plots in a randomized block design with seven replications. The trees were spaced 1.2 m within rows and 2.4 m between rows. There were eight plots (treatments) per replication. Each plot contained both species and was randomly assigned one of eight possible treatments (table 1.2). The doseage (2.8 kg/ha a.i.) for all chemical treatments was an arbitrary concentration that fell within the lower range of application rates commonly used for the three herbicides. Optimal herbicide doseages were not tested. Planting procedures consisted of making a slit down each sprayed row with a tree planter and then hand planting the trees in the slit. The root collars of all trees in each treatment plot were placed either 3 or 15 cm below the soil surface. In one treatment, a 30-cm-square black plastic mulch was placed around each tree.

On May 12th, all plots to receive a herbicide treatment were completely sprayed using a 9.5 liter Lofstrand (Model

1730) handsprayer that had been calibrated at 30-40 PSI. The control plots were hand cultivated. Rain (7.6 cm) fell on the test site from May 10th to May 15th. Rainfall was normal (18.5 cm) for May and June of 1981. The following April (1982) all plots were sprayed with 2.8 kg/ha a.i. simazine.

One month after the 1981 spraying, a subjective rating (table 1.2) of herbicide injury to each tree was taken. In September of 1981, weed control was evaluated for each plot. Tree heights were measured in the fall of 1981, 1982, and 1983. Diameters at 5 cm above ground level were measured in 1982 and 1983. Percent survival for each treatment was recorded for all three years.

Analysis of variance was performed on plot means for each trait except survival and weed control. Herbicide damage ratings were tested for normality before analysis. The survival and weed control data were not normally distributed, so Friedman's two-way classification test (10) was used to detect differences. An LSD test was applied to the treatment means for the 1981 height and herbicide damage data. Correlations between years were generated for treatment means of height and diameter. Non-parametric, rank correlations were calculated for the relationship of weed control to the treatment means for height and diameter.

Table 1.2. Effects of eight herbicide treatments on weed control, and aspen growth and survival.

Treatments			Phytotoxicity		Growth Data (Treatment Means)			
Chemical Herbicide (2.8 kg/ha)	Planting Depth (3 or 15 cm)	Other	Herbicide Injury[1] (aspen)	Weed Control[2] (%)	1983 Survival (%)	1981 Height (m)	1983 Height (m)	1983 Diameter[3] (cm)
Simazine	Regular		.67	87	62	.67	2.7	3.1
Simazine	Deep		.12	89	89	.72	3.0	3.5
Simazine	Regular	Plastic Mulch	.65	82	79	.74	2.7	3.2
Diuron	Regular		.69	59	77	.76	2.9	3.5
Diuron	Deep		.72	56	71	.75	3.2	3.5
Linuron	Regular		1.23	62	55	.69	2.8	3.1
Linuron	Deep		.92	62	61	.73	3.1	3.4
None	Regular	Cultivated	.00	100	98	.90	3.4	4.0
LSD (.05)			.35			.13		
Significance of F-value			**	**	*	*	n.s.	n.s.

[1]Injury rating was: 0 = no damage; 1 = yellow leaves; and 2 = yellow and black leaves.
[2]Percent of sprayed ground without live weeds.
[3]Diameter at 5 cm above ground surface.
*/** Significant at the 5 and 1 percent levels, respectively.

RESULTS

The control and one simazine (deep-planting) treatment had the least herbicide injury and the best weed control (table 1.2). The two linuron treatments had the most initial herbicide damage. Simazine gave better weed control than either diuron or linuron. Deep-planting reduced chemical injury on simazine plots but not on the diuron or linuron plots.

The control treatment had the highest survival in each of the three years. One simazine (deep-planting) treatment also had good survival. The two linuron treatments and one other simazine (regular-planting) treatment gave poor survival. Deep-planting increased survival on simazine plots but not on the diuron and linuron plots. Little mortality occurred after the first year; therefore, the survival rankings of the treatments remained unchanged throughout the three years.

First-, second-, and third-year heights and diameters were greatest in the control plots. The simazine (regular-planting) treatment generally gave the poorest height and diameter growth. In the second and third years, the deep-planted plots of simazine, diuron, and linuron grew more than the regularly-planted plots. This trend was also true for diameter except there were no diameter differences in the diuron plots for the third year. The seedlings that were deep-planted appeared to suffer no detrimental physiological effects from the placement of the root collar

15 cm below the soil surface. This observation agreed with Benson (4) who reported that deep-planting aspen 10 to 30 cm above the root collar did not create any adverse establishment effects.

Treatment means for height and diameter were significantly correlated at the 1 percent level between years 1981-82 and 1982-83 and at the 5 percent level for years 1981 and 1983 (table 1.3). Non-parametric, rank correlations between weed control and the growth data were low (r= .05 to .20) and non-significant at the 5 percent level of probability.

Table 1.3. Year-to-year correlations of the treatment means for height and diameter of the experimental aspen seedlings.

Years Correlated	Height	Diameter
	----- r[1] -----	
1981 with 1982	.84 **	———
1981 with 1983	.77 *	———
1982 with 1983	.97 **	.95 **

* / ** Significant at the 5 and 1 percent levels, respectively.
[1]Degrees of freedom = 7.

DISCUSSION

Three-year results of height and diameter growth for each treatment indicated that cultivation alone was superior to all chemical treatments, but the differences became insignificant after the first growing season. The smaller and less significant correlation (table 1.3) of the treatment means for height between years 1981 and 1983 indicate that first-year treatment differences were decreasing with time; e.g., the mean height of the poorest treatment increased from 74% (1981) to 79% (1983) of the mean height for the best treatment (table 1.2). The initial superiority in growth and survival of cultivated trees was attributed to the absence of both weed competition and phytotoxic effects from the chemical treatments, as well as increased soil aeration.

Simazine, diuron, and linuron are toxic to sensitive species of Populus at low concentrations. The principal means of uptake of these chemicals is by root absorption. Jaciw (personal communication) observed in Ontario that deep-planted white poplar-aspen hybrids were not damaged by simazine. Therefore, avoidance of herbicide contact with the root system is essential for establishing chemical-sensitive species such as the native aspens.

Roadhouse and Birk (9) found that simazine applied at a rate of 2.2 kg/ha a.i. on a cultivated field did not penetrate below the top 15 cm of soil during the first growing season. In addition, Weldon and Timmons (15) showed on a sandy clay loam and a loamy sand soil that diuron, when applied at rates of 2.2 and 4.5 kg/ha a.i., does not penetrate below 10 cm in the soil regardless of the amount of irrigation used. These findings suggest that deep-planting of aspen seedlings 15 cm below the soil surface should minimize herbicide contact with the root system. The simazine results here support this hypothesis. The one simazine (deep-planting) treatment had 89 percent survival after three years compared to 62 percent for the other simazine (regular-planting) treatment.

The reasons for the failure of the diuron and linuron deep-planting treatments to prevent herbicide injury may be because these two chemicals are more water soluble than simazine, and they also are foliar absorbed. Water solubility of simazine is 5 ppm (2), compared with 42 ppm for diuron and 75 ppm for linuron (1). The planting slit may have opened slightly because of soil shrinkage from evapotranspiration; the soil texture of the Ap horizon contained 21 percent clay, which increases the soil shrinkage properties (12). During May, heavy rains could have carried more of the more soluble herbicides, diuron and linuron, down into the slit compared with the highly insoluble simazine.

The other explanation for the increased damage in the diuron and linuron deep-planted treatments was that substituted urea herbicides, in contrast to simazine, are more readily absorbed by the foliage (1, 5, 7). At the time of the herbicide treatment, a few trees in each plot had new leaves just breaking through the bud scales. No attempt was made to cover the seedlings when each plot was sprayed because the spraying procedure was to simulate actual field application conditions. These early leafing seedlings suffered foliage injury and may have absorbed sufficient herbicide to kill them.

The effectiveness of plastic mulch in controlling chemical injury was intermediate when compared to the two simazine treatments. The plastic mulch (simazine) treatment had poorer survival than the deep-planting (simazine) treatment, but better survival than the regular-planting (simazine) treatment. Although the plastic lessened simazine injury, it did not prevent it. The main problem with the plastic was that it collected and pooled the herbicide spray and then funneled some of the chemical through the plastic at the hole around the root collar. The use of plastic mulch around individual stems to prevent herbicide damage should be reevaluated because of its cost in labor and materials as well as its uncertain effectiveness.

The good to excellent (82-89%) weed control produced by simazine compared to the moderate (56-62%) weed control exhibited by diuron and linuron was due largely to the chemistry of the herbicides. The low soil organic matter (2.0%) and the slightly acidic pH (6.7) of the Ap horizon were favorable for chemical activity of all three herbicides. However, under these conditions simazine gave better weed control partly because simazine-tolerant, late-season grasses did not invade the simazine plots in late summer. Simazine was probably more persistent in the soil because it was more insoluble and less volatile than diuron and linuron.

Achieving excellent weed control (75-100%) by using moderate to high application rates of chemicals such as simazine may not be advisable because of the increased risk of mortality. This was the case with the simazine plots, which averaged 86 percent weed control compared to 60 percent for the diuron and linuron plots. Despite having better weed control, simazine (regular-planting) plots had poorer survival and growth after three years than did the diuron (regular-planting) plots.

The absence of significant correlations between first-year weed control and the three years of growth data suggested that the initial weed control, which was as low as 35 percent in some plots, was sufficient to avoid serious growth inhibition from weed competition. This lack of correlation between weeds and growth agreed with Benson and Einspahr (3) who found that when greater than 50 percent of the vegetative cover was controlled, low survival or reduced tree growth resulted, presumably from chemical toxicity. They concluded that complete control of weeds is not necessarily a good criterion to judge the usefulness of herbicides. These results imply that prevention of herbicide injury while still achieving good survival and growth is a better strategy than trying to control all weeds. Therefore, on an average or better site, aspen can be successfully established even if weed control is 50 percent or less for the first growing season.

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CHAPTER 2

PROGENY TESTING OF NATIVE ASPENS AND THEIR HYBRIDS FOR BIOMASS PRODUCTION IN MICHIGAN

(Presented at the 1984 NEFTIC Meeting in Morgantown, WV.)

ABSTRACT

An aspen progeny test consisting of 206 families of trembling aspen (*Populus tremuloides*), bigtooth aspen (*P. grandidentata*), and their hybrids (*P.* X*smithii* and *P.* X*rouleauiana*) was planted on five Michigan sites and evaluated after two growing seasons. Growth performance of trembling aspen families when compared with all aspen taxa was above average and increased with the latitude of the plantation site. Backcrosses of trembling aspen males to white poplar-bigtooth aspen (*P.* X*rouleauiana*) females produced the fastest growing families at all Lower Peninsula plantations. Most hybrid aspen (*P.* X*smithii*) families had growth rates below the plantation means. Bigtooth aspen families had poor survival and growth at all plantations. Analyses of two nested mating designs showed that general combining ability (additive genetic variance) for height and diameter growth was present in the aspen population. Genotype x environment interaction was small at the family level.

INTRODUCTION

The aspens are fast-growing trees that have been studied by numerous poplar breeders, but unfortunately little progress has been made in improving the genetic base of the species. Large amounts of genetic variation in trembling aspen (*Populus tremuloides*) and bigtooth aspen (*Populus grandidentata*) have been reported (Pauley 1949, Einspahr and Benson 1967, Barnes 1969). This genetic variation provides an ample germplasm base for a tree improvement program. Furthermore, the ease of hybridization between the two species (Pauley 1956, Henry and Barnes 1977) permits the breeder to create additional variation in desired traits. The problem that geneticists have encountered when breeding aspen is how to quantify and "capture" the genetic variation found in the species. It has been difficult to establish test plantations for the species and to eliminate bias from genotype x environment interactions (Einspahr and Mohn, personal communication).

The objectives of this aspen progeny test were to: (1) quantify the genetic variation in the native aspen populations in Michigan, (2) create additional genetic variation by hybridizing the two native aspen species, (3) identify fast-growing aspen families for short-rotation biomass plantations, and (4) establish a progeny test on different sites in order to investigate genotype x environment interactions.

MATERIALS AND METHODS

Progeny Production and Mating Design

Seed and catkin-bearing branches of bigtooth and trembling aspen were collected from 43 counties in both peninsulas of Michigan during March and April of 1979 and 1980. Similar material from two putative white poplar-bigtooth aspen (*P.* X*rouleauiana*) hybrids located in the southern Lower Peninsula of Michigan was also collected. Controlled-pollinations were made using the cut-branch technique (Einspahr and Benson 1964). Progenies from the pollinations represented 48 half-sib and 66 full-sib families of bigtooth and trembling aspen. In addition, 72 full-sib families of hybrid aspen (*P.* X*smithii* = *P. grandidentata* X *P. tremuloides*) and 20 F_1 backcrosses of bigtooth and trembling aspen to the putative white poplar hybrid, *P.* X*rouleauiana* (*P. alba* X *P. grandidentata*), were produced.

The mating design used in the progeny test was a nested design (North Carolina Design 1) which later was reduced in size because of the failure of some crosses to produce sufficient seed. After adjustment for missing families, two nested designs were constructed from 46 of the 158 full-sib families that were well represented in the field tests. The first design had eight males (four each of bigtooth and trembling aspen) which were each crossed to two females of each species. Theoretically, random sampling of the population in the nested design would dictate that females be crossed only once, but in this test four females were used twice because of significant mortality in some families. The design comprised 32 families (8 males and 28 females) and was replicated six times in one plantation. The second design tested seven trembling aspen males which were each crossed with two different trembling aspen females. There were 14 families (7 males and 14 females) in this design, and they were replicated 16 times across three plantations.

Seed was sown in the nursery on May 26, 1981, and cultural procedures similar to those of Benson and Dubey (1972) were used to grow the seedlings. Insects were controlled with Orthene. The mean height for each family in the nursery was recorded before the seedlings were lifted the following March and placed in cold storage until the planting season started.

<u>Plantation Sites and Aspen Establishment Procedures</u>

The five plantation sites chosen for this study were abandoned agricultural fields. Three plantations are in the Lower Peninsula of Michigan and two are in the Upper Peninsula. The Lower Peninsula plantations are at Michigan State University's (MSU) Russ Experimental Forest (Cass Co., Lat. 42.0°N, Long. 86.0°W), MSU Water Quality Research Area (Ingham Co., Lat. 42.7°N, Long. 84.5°W), and Michigan Consolidated Gas Company's gas storage fields near the town of Six Lakes (Mecosta Co., Lat. 43.5°N, Long. 85.2° W). The soil textures of the Russ Forest, Water Quality, and Six

Lakes sites are a sandy loam, a fine sandy loam, and a sandy loam, respectively. Grasses and perennial weeds were the dominant vegetation on these sites.

The two Upper Peninsula plantations are at the Michigan State agricultural field station near Chatham (Alger Co., Lat. 46.3°N, Long. 86.9°W) and on Neebish Island (Chippewa Co., Lat. 46.3°N, Long. 84.2°W). The Chatham plantation is on a former alfalfa field which has a fine sandy loam soil texture. The Neebish Island plantation was a clover and timothy field with a heavy clay soil which was mottled at a depth of 20 cm. The soils at all plantations except Neebish Island were well-drained.

Site preparation for each of the five plantations consisted of mowing the existing vegetation with a rotary mower in August, 1981 and spraying seven liters/ha of glyphosate in one-meter-wide strips three to four weeks later. In April and May of 1982, the seedlings were planted at these five Michigan locations. The seedlings were machine-planted in two-tree plots with a spacing of 1.8 meters between trees within rows, and 2.4 meters between rows. The experimental design was a randomized block with six replications at each plantation. Following spring planting, 2.8 kg/ha a.i. of simazine was applied over the tops of the seedlings and onto the glyphosate-sprayed strips. The planting strips in the Water Quality and Six Lakes plantations were spot-sprayed once with glyphosate in July, 1982 to control invading grasses.

Data Analyses

Height, basal diameter, and survival of the families in all the plantations except Neebish Island were tallied in 1982 and 1983. The diameters were squared and then multiplied by the heights to give an index of biomass production (=biomass production index). Analyses of variance and correlations were calculated for all height, basal diameter, biomass production index, and other growth measurements. Family performances in all analyses were based on the plot mean of each family which was expressed in percent of the block (replicate) mean. Family and species performances within, and between geographical regions of Michigan were evaluated for trends and genotype x environment interactions. Heritability estimates for height and diameter were derived from analyses of variance of the first nested design. Male and female effects were tested and evaluated for the parents used in both nested designs. Age-age correlations were calculated for the relationship of first-year nursery height to the two-year-old plantation height data.

RESULTS AND DISCUSSION

Family Performance Among Plantations

Two-year survival of the families within each cross (taxon) is listed in Table 2.1 for the three best plantations. The Russ Forest and Neebish Island plantings were not listed because mortality was greater than 50% due to herbicide damage and drought. Survival increased with decreasing latitude of the plantations. Survival at the Chatham plantation was seven and 11 percent less than that at Six Lakes and Water Quality (East Lansing), respectively. The freeze-free period or growing season at Chatham is 100 days compared to 126 days at Six Lakes and 151 days at East Lansing (Mich. Dept. Agric. 1971). The shorter growing season and other environmental stresses significantly reduced the survival of *P. Xsmithii* families at Chatham. Survival of the two native aspens varied little across the three plantations.

Table 2.1. Two-year survival in percent for aspen taxa at three plantations.

Taxa (Female X Male)	Plantation Site		
	Chatham[1]	Six Lakes	Water Quality
Populus tremuloides X *P. tremuloides*	92	90	97
P. tremuloides X *P. grandidentata*	68	89	91
(*P. Xrouleauiana*) X *P. tremuloides*	84	88	99
P. grandidentata X *P. grandidentata*	72	73	75
P. grandidentata X *P. tremuloides*	72	86	87
(*P. Xrouleauiana*) X *P. grandidentata*	64	63	79
All Taxa Combined	79	86	90

[1]Replicates 4-6 were not included because of herbicide overdose.

Rapid juvenile growth is positively correlated with aspen survival (Pauley et al. 1963a, Hattemer and Seitz 1967, Melchior and Seitz 1966, Mohrdiek 1979a). The fastest growing families in this test also had the lowest mortality. Bigtooth aspen and the hybrids grew poorly on the Chatham site and consequently, suffered significantly higher mortality than the trembling aspen families. These initially slow-growing species and hybrids should not be planted in areas with severe climatic conditions because early mortality is likely to be high. However, if some of these families become fast growers once they are established, the early mortality can be compensated for by planting at higher densities.

The analyses of variance showed that differences in height, basal diameter, and biomass production index were significant ($P<.05$) for individual families and families-within-taxa at all plantations in years 1982 and 1983. The highest family means for two-year height and diameter at the best plantation (Water Quality) were 2.8 meters and 3.3 cm., respectively (Table 2.2). The height and diameter of the five top families when averaged over four plantations were 1.6 meters and 1.9 cm., respectively. Early growth of these families was comparable or greater than that reported for promising families of trembling aspen (Pauley et al. 1963b), hybrid aspen (Pauley et al. 1963c), triploid hybrid aspen (Benson and Einspahr 1967), white poplar-bigtooth hybrids (Johnson 1942), and white poplar-aspen trihybrids (Maynard 1977).

Table 2.2. Two-year growth performance of the best family and individual of each aspen taxon at the Water Quality plantation.

Aspen Taxon	Best Family (Mean) 1983 Ht[1] (cm)	Best Family (Mean) 1983 Diam[2] (cm)	Best Individual 1983 Ht (cm)	Best Individual 1983 Diam (cm)
Populus tremuloides	273	3.1	395	4.6
P. grandidentata	232	2.6	345	3.8
P. Xsmithii	275	3.3	435	5.6
(*P. Xrouleauiana*) X *P. tremuloides*	254	2.9	360	4.6

[1] Heights were measured to the nearest 5 cm.
[2] Diameters were measured at 5 cm above the soil surface.

To evaluate the growth potential of these families, the biomass production index, basal diameter squared times height, was used. Correlations of height to basal diameter within plantations ranged from .73 to .89 and were significant at the one percent probability level. Mohrdiek (1979a) summarized the findings of many *Populus* genetic studies and concluded that height is highly correlated with diameter for all poplars. Since the correlation between height and diameter is high in poplars, the biomass production index was considered a valid measure of biomass productivity.

The biomass production index of each taxon at four plantations is shown in Table 2.3. The number of families of each taxon that were among the top 25 families in biomass production index at each plantation are summarized in Table 2.4. The number of trembling aspen families in the top 25 increased with increasing latitude of the plantation site, while the number of hybrid and trihybrid families increased with decreasing latitude. Trembling aspen families comprised 19 of the 25 best families overall. Families of trembling aspen and the trihybrid were best in terms of biomass production index over all four plantations. The best family, a trihybrid, averaged 28% above the mean biomass production index for all families at age two.

Table 2.3. Biomass production index (diameter^2 X height) in % of plantation mean for five aspen taxa in four Michigan plantations.

		Taxon (Female X Male)				
Plantation	Latitude	AGxT[1]	TT[2]	TG,GT[3]	AGxG[4]	GG[5]
		%				
Chatham	46.3°N.	114	133	74	66	46
Six Lakes	43.5°N.	125	116	81	86	51
Water Quality	42.7°N.	123	116	94	74	61
Russ Forest	42.0°N.	160	108	76	86	76
Combined		122	120	84	77	56

[1](*Populus alba* X *P. grandidentata*) X *P. tremuloides*

[2]*P. tremuloides* X *P. tremuloides*

[3]*P. tremuloides* X *P. grandidentata* and the reciprocal cross

[4](*P. alba* X *P. grandidentata*) X *P. grandidentata*

[5]*P. grandidentata* X *P. grandidentata*

Table 2.4. Number of families of each taxon that comprise the 25 families with the highest biomass production indices at each plantation.

Taxon (Female X Male)	Plantation Site				
	Chatham	Six Lakes	Water Quality	Russ Forest	Combined
Populus tremuloides X *P. tremuloides*	20	16	12	11	19
(*P. Xrouleauiana*) X *P. tremuloides*	2	4	4	6	4
P. Xsmithii (plus reciprocal)	2	5	7	6	2
(*P. Xrouleauiana*) X *P. grandidentata*	-	-	1	1	-
P. grandidentata X *P. grandidentata*	1	-	1	1	-

Family x Site Interaction

Correlations between taxon performance (biomass production index) at each plantation with its respective performance over all plantations are listed in Table 2.5. The performance of individual families at each plantation site was significantly correlated with their average performance over the four sites (r= .68 to .77), although bigtooth aspen families at Six Lakes were an exception. The correlation data implied that although genotype x site interactions were present, they were not strong at the family level under the particular conditions of this test. These findings agree with those of Hattemer and Seitz (1967) who reported that family x site interactions for height data of hybrid aspen in Germany were non-significant. The results suggest that most aspen families had good correspondence in performance at two years across the environments tested. Therefore, the fast-growing genotypes expressed growth superiority in different environments.

Table 2.5. Correlations of taxon performance (biomass production index) at each plantation with mean performance of each taxon for all plantations.

Taxon (Female X Male)	Number of Families	Plantation Site[1] Chatham	Six Lakes	Water Quality	Russ Forest
		r			
Populus tremuloides X *P. tremuloides*	73	.63	.61	.62	.74
P. tremuloides X *P. grandidentata*	37	.73	.58	.86	.78
P. grandidentata X *P. grandidentata*	34	.68	-.05 ns	.70	.90
P. grandidentata X *P. tremuloides*	35	.31 *	.75	.58	.67
All Taxa Combined	179	.77	.74	.68	.71

[1]All correlation coefficients without asterisks are significant at the 1 percent level.
*Significant at the 5 percent level.
ns Non-significant at the 5 percent level.

Trembling aspen and trihybrid families had similar biomass production indices and exhibited growth rates that exceeded the mean at all plantations. However, trembling aspen families were superior in growth and survival to all other aspen taxa at Chatham (Upper Peninsula), whereas trihybrid families grew the best at the three Lower Peninsula plantations. Trembling aspen families did well in the Upper Peninsula because the species is more adapted to northern climates than the other taxa tested. Performance of trembling aspen families in relation to the other aspen taxa increased with increasing latitude of the plantation site.

The performance of trembling aspen families from site to site differed from those of bigtooth aspen and the trihybrid, P. Xrouleauiana X P. tremuloides. The P. Xrouleauiana backcrosses were not well adapted to the shorter growing season and colder temperatures at Chatham. The P. alba lineage in this cross probably originated in Central or Southern Europe and was less ecologically adaptable in the Upper Peninsula than the native aspens. This hybrid did perform well in the Lower Peninsula plantations, possibly because of the milder climate. All other hybrid taxa performed below the mean at the four sites. These hybrids may be late starters, or are more site specific since their performance varied markedly with site. Bigtooth aspen grew poorly at all sites, but growth was 65 percent better at the southernmost plantation (Russ Forest) than at the northernmost one (Chatham). In general, trembling aspen families grew best on northern Michigan sites, and hybrid and bigtooth aspen families grew better on southern Michigan sites.

The unidirectional clines in relative performance of trembling, bigtooth, and trihybrid aspen from northern to southern plantations were undoubtedly influenced by the latitude and climate of the planting site. Once the appropriate species or hybrid is chosen for a specific climatic region, family selection can then be based on the average performance for all plantations within that region. From this study, trembling aspen families would be selected for the Upper Peninsula and cold regions of the Lower Peninsula, whereas trihybrid families would be planted throughout the other areas of the Lower Peninsula.

Geographic Significance of Parent

Pauley *et al*. (1963a) found that *P. tremula* families from Central Europe grew faster than Northern European families in Weston, Massachusetts. In this test, the 20 best families overall were produced by 35 parents (17 females and 18 males) representing 26 widely dispersed counties. Families from the 206 in the progeny test that included a trembling aspen parent from northern Lower Michigan (above Lat. 43.8°N) grew two percent larger than families with Upper Peninsula trembling aspen parents, and five percent larger than families with southern Lower Michigan (below Lat. 43.8°N) trembling aspen parents. Families that had bigtooth aspen parents from southern Lower Michigan grew two to ten percent larger than families with bigtooth aspen parents from northern Lower Michigan and the Upper Peninsula. The best hybrid families (*P.* X*smithii*) were produced from parents of both species that were located in southern Lower Michigan. These families grew two to eight percent larger than did hybrids with parents from northern Lower Michigan and the Upper Peninsula.

Finding an existing natural aspen population (50-100 clones) with superior growth would increase the gain that would be achieved in the first breeding cycle. However, the clonal nature of aspen (Barnes 1966, Kemperman 1976) and its large genetic variability would necessitate a sample size so large that it would be impractical to progeny test. This study contained half-sib and full-sib progeny from 125 clones representing 43 counties. The number of families (206) used in this test was inadequate to estimate the within, and between stand variation of the two species. Furthermore, the failure of some 50 other clones to produce progeny reduced the efficacy of the mating designs to detect genetic variation.

Even though superior aspen stands were not identified, the general geographic location of the parents was important to the performance of the crosses. Okafo (1976) found that western Upper Peninsula sources of bigtooth and trembling aspen grow faster under greenhouse conditions than other Michigan sources. Other aspen researchers (Barnes 1959, Pauley _et al_. 1963a, Johnsson 1976, Melchior and Seitz 1966) have found that aspen parents from specific geographic regions produce the best progeny. In this study, the best bigtooth and trembling aspen parents came from northern and southern Lower Michigan, respectively. Since trembling aspen is a boreal species, the climate and soils of northern Lower Michigan are similar to the northern range of the species. Southern Lower Michigan, however, has soils and climate that are less representative of the boreal habitat of trembling aspen. Generally, Populus progenies from northern latitudes grow poorly at lower latitudes because the shorter photoperiod and warmer temperatures are thought to induce growth cessation (Pauley and Perry 1954). This trend was apparent in some families, but was relatively insignificant due to the large variation in phenology within each geographic population. However, only one of the four measured plantation sites was in the Upper Peninsula; consequently, the two percent lower growth rate of families with trembling aspen parents from the Upper Peninsula may partially be attributed to this latitudinal effect.

In contrast to trembling aspen, bigtooth aspen grows on drier sites and tolerates warmer temperatures. Bigtooth aspen appears to occur more frequently than trembling aspen on the farmland of Michigan's southern tier counties (Reighard, personal observation). The higher growth rates of bigtooth families from southern Michigan may be due to the commonly observed genetic trend that southern populations of many tree species sacrifice cold hardiness for faster growth rates (Wright 1976), or it may be that the longer leaf retention of the southern sources (Reighard, unpublished data) is an advantage because of autumn photosynthesis.

The best hybrid families had parents of both species from southern Lower Michigan. Barnes (1961) and Andrejak and Barnes (1969) have reported that natural hybridization is currently occurring between bigtooth and trembling aspen in southern Michigan. Due to this gene flow between the two species, the southern Michigan populations of these species probably share more genes than do their northern Michigan populations. This introgression may reduce the degree of chromosome non-homology in hybrid families that are produced from southern Michigan parents because many non-vigorous hybrids in the plantations suffered from a dysgenesis syndrome which included chlorophyll breakdown, bud abortion, and shoot or whole tree death. Pauley *et al.* (1963c) found a similar type of physiological breakdown when he reported that 39 of 41 progeny of a hybrid aspen family died at age eight from unknown causes. These physiological abnormalities and Peto's (1938) findings that hybrid aspen progenies commonly have chromosomal irregularities such as univalents and trivalents casts suspicion on the fitness of hybrid aspen over an entire rotation period.

Geographic Separation of Parent Stands

Certain fast-growing hybrid aspen progenies (ie. *P. alba* X *P. grandidentata* or *P. glandulosa*; *P. tremula* X *P. tremuloides*) have been produced by crossing Leuce poplars that were geographically and ecologically disjunct (Zsuffa

1973). Highly productive families have also been produced by hybridizing the sympatric species *P. alba* and *P. tremula* (Mohrdiek 1979b) and allopatric populations of *P. tremula* (Johnsson 1956). In this study, there were no apparent trends in the biomass production indices of the full-sib families of trembling and hybrid aspen in relation to the geographic distance between their parents (Table 2.6). However, there was a small decrease in the biomass production index of bigtooth aspen families with increasing geographic distance between the parents. This trend had a significant ($P<.05$) correlation of -.25.

Johnsson (1956) and Muhle Larsen (1970) have assumed that non-additive genetic variance (heterosis) was responsible for the growth superiority of hybrids between geographically isolated aspen species and populations. The absence of heterosis in the progeny that were produced by crossing geographically distant populations of trembling aspen may be attributed to gene flow occurring between the trembling aspen populations in Michigan. On the other hand, additive genetic variance (Mohrdiek 1980) has been reported in growth traits of aspen. If additive effects were indeed important in the performance of outstanding hybrid families, the variation within Michigan's aspen populations may have been equal to or greater than the variation between populations. The large genetic variation within aspen populations has been well documented by Barnes (1969), Cheliak and Dancik (1982), and many others. Therefore, maximum genetic gain could be achieved from selecting within one large, genetically tested population.

Table 2.6. Biomass production index in % of replicate mean of families-within-taxa in relation to the geographic distance between parents.

	Parental Taxon		
Distance (Km) Between Parents	*P. tremuloides* X *P. tremuloides*	*P. grandidentata* X *P. grandidentata*	*P. Xsmithii*
Open-pollinated	114	77	
1-175	132	50	84
176-325	121	46	90
326-650	130	45	76
Average	121	59	84

The slower growth of many *P.* X*smithii* families when compared to promising aspen hybrids such as *P.* X*rouleauiana* may be indirectly the result of the phenological isolation of bigtooth and trembling aspen (Pregitzer and Barnes 1980) over much of their Michigan ranges. This phenological barrier to introgression exerts upon each species a form of assortative mating which has been demonstrated by Gregorius (1980) to be a process that increases the rate of chromosome evolution via allelic mutations and ultimately leads to changes in chromosomal homology between two populations or species. The chromosomal irregularities in this hybrid were probably responsible for its inferior fitness in the progeny test. However, another explanation for the lack of vigor in *P.*

X*smithii* is that both parent species may have evolved under similar environmental conditions in eastern North America, and therefore, may be more alike genetically than other aspen species used to produce hybrids. We do not know why increased geographic distance between bigtooth parents reduced progeny performance, but sampling error due to the low number (34) of families analyzed cannot be ruled out.

Open-pollinated families of trembling aspen (Table 2.6) did not perform as well as full-sib families. The opposite was true for bigtooth aspen. The biomass production index of the open-pollinated families of trembling aspen was 13 percent below the mean biomass production index of its full-sibs. In contrast to trembling aspen, the open-pollinated families of bigtooth aspen grew 29 percent better than its full-sibs. Farmer and Barnes (1978), however, found that open-pollinated families of trembling aspen showed no more genetic variation than full-sib families. Since open-pollinated families usually have more than one male parent and full-sib families are fathered by one male parent, it would be expected that half-sib families would show more variation in phenotypic traits. We do not know why the number of pollen parents per family affected each species in a different manner.

Heritability and General Combining Ability

Height and diameter analyses of the two nested designs showed that the female-within-male variance component was significant ($P<.01$), but the male component was not.

Narrow-sense heritabilities for height (h^2=.31) and diameter (h^2=.39) were obtained from the variance components of the first nested design which included data from a single plantation. Heritabilities could not be obtained from the second design which included data from three plantations because of negative mean squares from insufficient observations in the sampling procedures.

The first nested design gave relatively large narrow-sense heritabilities (additive genetic variance) for height and diameter. The true heritability of these traits is much less because the genotype x environment interaction variance could not be partitioned out. Einspahr et al. (1967) reported similar narrow-sense heritibilities of .24 (height) and .35 (diameter) for full-sib families of trembling aspen. Likewise, their heritabilities were based on a single test plantation.

The nested design analyses show that selection of the female parent was important in the mating design. The choice of the male parent, however, was not found to be statistically important. This may be due to a strong maternal effect or experimental error. The latter is assumed because Mohrdiek (1979b) reported that both the maternal and paternal parents were important in crosses of P. tremula and P. tremuloides, and in the nested designs, the better males possessed good general combining ability (GCA). The top male in designs 1 and 2 had a general combining ability (GCA) of 18.5 and 7.9 units above the mean, respectively. In Germany, Hattemer and Seitz (1967) found the GCA of paternal aspen parents to be three to five times the GCA of the maternal aspen parents in diallel crosses. These analyses and the the narrow-sense heritability data suggest that mating designs that screen for additive genetic variance should be used to improve the breeding population of Leuce poplars.

Age-age Correlations

Age-age correlations between nursery height and the average two-year field height within each cross are presented in Table 2.7. Mohrdiek (1979a) found positive age-age correlations of .46 between years one and 20 and .83 between years nine and 20 for growth traits in aspen. After evaluating data from 36 aspen progeny trials, he recommended the eighth year as the earliest time to begin intensive selection. The age-age correlation in this study between nursery height growth and the height growth after two field growing seasons for all aspen families as a group was significant ($P<.05$) but somewhat low ($r=.48$).

Families of trembling aspen and the trihybrid had significant age-age correlations, in contrast to non-significant ones for bigtooth aspen families. Taxa with bigtooth aspen as the female parent were not significantly correlated, but the opposite was true for trembling aspen as the female parent. The age-age correlation of families with a trembling aspen female was less significantly correlated ($P<.05$) if the male parent was bigtooth aspen. One explanation for the data is that families of trembling aspen and the trihybrid were fast growers the first few years from seed, whereas bigtooth aspen was a slow grower for the first two years. In the second growing season in the field (three years from seed), bigtooth aspen began to grow as rapidly as trembling aspen. This initial slow start would explain why taxa with bigtooth aspen parents had low and non-significant age-age correlations at an early age. Hybrids containing European white poplar (*P. alba*) parentage had the largest age-age correlation of all the taxa and were the fastest growing in the nursery and the field plantings.

Table 2.7. Age-age correlations of the mean family height in the nursery with the mean family height averaged over four plantations.

Taxon (Female X Male)	Families Per Taxon	Correlation
		----------r----------
Populus tremuloides X *P. tremuloides*	59	.38 **
P. tremuloides X *P. grandidentata*	18	.51 *
P. grandidentata X *P. grandidentata*	14	.33 n.s.
P. grandidentata X *P. tremuloides*	19	.28 n.s.
(*P. Xrouleauiana*) X *Populus* species	17	.64 **
All Taxa	127	.48 **

*/** Significant at the 5 and 1 percent levels, respectively.

For short-rotation biomass plantations, selection within this progeny test at age three may be effective at the species level (ie., eliminate bigtooth families), but selection at the family level would only be partially effective until approximately year eight. By the eighth year, many families would be flowering (Valentine 1975). Therefore, the high age-age correlations for growth traits and precocious flowering found in the aspens might permit an eight-year breeding cycle which is considerably shorter than many other tree species.

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CHAPTER 3

Genetic Analyses of Fifteen Phenological, Physiological, and Morphological Traits in *Populus grandidentata*, *P. tremuloides*, and Their Hybrids

(Paper to be presented at the 1985 North Central Tree Improvement Conference at East Lansing, MI)

ABSTRACT

A combination half-sib/full-sib progeny test of *Populus grandidentata*, *P. tremuloides*, and their hybrids was planted at four locations in Michigan. Nine traits and susceptibility to six pests were examined. Phenological traits in *P. grandidentata* and *P. tremuloides* exhibited considerable additive genetic variance. *P. tremuloides* leafed out earlier and dropped leaves later than *P. grandidentata*. Hybrid phenology was intermediate. Date of leaf fall and the leaf area duration period were significantly correlated with growth performance. Morphological traits such as autumn leaf color, branchiness, and bud characteristics had both additive and non-additive genetic variance. For these traits, hybrids resembled the maternal parent more so than the paternal parent. Hybrids suffered from a number of disorders including shoot dieback, bud abortion, and adventitious sprouting. *Saperda inornata* and *Venturia tremulae* were the most serious insect and disease problems. Genetic resistance of a physiological nature to insects and diseases was not found. However, differences in susceptibility existed among families and species.

INTRODUCTION

The increasing reliance on artificial regeneration by the major forestry companies to produce high-quality wood fiber has expanded the market for genetically improved planting stock of commercially valuable tree species. Tree breeders have been somewhat successful in fulfilling the industry's needs by locating superior populations and families of many tree species through provenance and progeny testing and establishing seed orchards with these selected trees (Wright 1976). From the early southern pine seed orchards, maximum genetic gains above commercial nursery stock of 32% for loblolly pine (Weir 1983) and 45% for slash pine (Kossuth et al. 1982) have been reported. Second generation seed orchards have already been planted with seed produced from matings of superior first generation families. However, the original germplasm base would soon shrink if continued mating occurred between superior families or their progeny.

To maintain potentially important genes and to incorporate new ones, some geneticists diversify the gene pool of the initial breeding population by hybridizing races or species. Hybridization between species has been successful in increasing the genetic variation of important secondary traits (ie., foliage color, disease resistance, etc.) in the genera Picea (Kudray and Hanover 1980), Pinus (La Farge and Kraus 1980), and Populus (Zsuffa 1973). However, genes for some of these traits are lost when selection strategies emphasize only the average growth rate of each family. This is undesirable considering that secondary traits such as phenology (Nienstaedt and King 1970), cold-hardiness (Sakai and Weiser 1973), insect and disease resistance (Powers and Kraus 1983), crown architecture (Isebrands and Nelson 1982), and drought avoidance (Pereira and Kozlowski 1976) have been shown to significantly affect growth and survival of commercial tree species. Tree breeders often take the myopic view that mating the tallest trees will always give the most genetic gain in the shortest time. Instead, geneticists need to investigate the phenological, physiological, and morphological traits concurrently with growth rate so that secondary traits that affect growth rate can be identified. In addition, knowing the heritability and type of genetic variance of each trait facilitates the incorporation of its genes into the breeding population.

The objectives of this study were to investigate the relationship between three phenological traits and the two-year growth performance of 206 families of *Populus grandidentata*, *P. tremuloides*, and their hybrids in four Michigan plantations. In addition, interpretations of inter- and intraspecific inheritance patterns and genetic variance components of nine phenological, physiological, and morphological traits were made based upon either quantitative (nested design analyses) or qualitative (mean ranks) data. Lastly, resistance to six aspen pests was evaluated.

MATERIALS AND METHODS

Measurements

Nine traits were quantitatively measured in 1982 and 1983 in four Michigan plantations of a combination half-sib/full-sib aspen progeny test of *Populus grandidentata*, *P. tremuloides*, *P.* X*smithii*, and backcrosses to *P.* X*rouleauiana*. The plantation sites and progeny test have been previously described by Reighard (1984). The nine traits were time of leaf flush and leaf fall, leaf area duration, autumn leaf color, the number of primary branches, bud orientation from the stem, bud scale pubescence, shoot dieback, and the presence of root collar sprouts. In addition, six aspen pests were evaluated for damage to shoots and leaves: the "shepherd's crook" fungus (*Venturia tremulae*), an unidentified virus leaf spot, the poplar-gall beetle (*Saperda inornata*), the willow shoot sawfly (*Janus abbreviatus*), the poplar tentmaker (*Clostera inclusa*), and the whitetail deer (*Odocoileus virginianus*).

The nine traits and the leaf spot and willow shoot sawfly damage were measured or recorded at the East Lansing plantation (Ingham Co., Lat. 42.7°N, Long. 84.5°W). Deer browsing and the number of *Saperda* galls per tree were scored at the Six Lakes plantation (Mecosta Co., Lat. 43.5°N, Long. 85.2°W). Defoliation by the poplar tentmaker was tallied at the Decatur plantation (Cass Co., Lat. 42.0°N, Long. 86.0°W). Lastly, *Venturia*-infected shoots were counted in the Chatham plantation (Alger Co., Lat.

46.3°N, Long. 86.9°W) as well as at the Six Lakes and East Lansing test sites.

Leaf flush data were recorded as the number of days after May 1, 1983 until leaf flush occurred on the terminal leader. Trees were scored as leafed out when the first leaf had emerged from the terminal bud and was completely unrolled. The trees were scored every fourth day.

The leaf fall measurement in this study was equated with the loss of photosynthetically-active foliage. Leaf fall data were recorded as the number of days after September 1, 1982 until each tree had either lost 90% of its green leaves, or 90% of its leaves had changed to their autumn color. Trees were visited every seventh day. Leaf area duration was calculated by summing the days between the dates of leaf flush and leaf fall for each tree. Autumn leaf color of each tree was judged to be one of five colors: (1) yellow, (2) yellow-orange, (3) orange, (4) orange-red, and (5) red. If the leaves on a tree turned from green to brown or black, it was recorded as having no pigment coloration.

The number of primary branches originating from the central stem of each tree were counted. The center axes of the lateral buds on the terminal leader were scored as either appressed (parallel or inclined inward) or divergent (inclined outward) with respect to the axis of the terminal shoot. The terminal bud was scored as having either 0, <50, or >50 percent of its bud scales showing dense pubescence.

Shoot dieback from physiological dysgenesis was recorded in the spring of 1983. Also, the presence of root collar sprouts was noted. The amount of shoot dieback and sprouting on each affected tree was not recorded.

The virus leaf spot and *Venturia tremulae* diseases were scored for each tree as either present or absent during June, 1982. The number of terminal and sprout shoot tips girdled by the willow shoot sawfly were tallied throughout June and July of 1983. The number of *Saperda* galls per tree and the number of trees browsed by deer were counted in September, 1983. Also, in September, 1983 the number of trees of each species that were defoliated by the poplar tentmaker were recorded.

Statistical Analyses

Analyses of variance were calculated from plot means at the family and species level for the four leaf traits measured. Pearson correlations were calculated for the relationships between the leaf flush, leaf fall, and leaf area duration data. Correlations were also calculated for the association between these leaf characters and the growth measurements reported by Reighard (1984) for this progeny test. Narrow-sense heritabilities and general combining ability (GCA) were calculated from the leaf flush and leaf color data for a nested design (North Carolina Design 1) of 32 families (8 males, 28 females) from the East Lansing plantation.

The data collected from the other eleven characters in this study were not normally distributed according to the Kolmogorov-Smirnov one-sample test for normality (Hull and Nie 1981); therefore, Friedman's two-way classification test (Steel and Torrie 1960) for non-parametric data from a randomized block design was used to discern differences between species. Furthermore, Kendall's rank correlation for non-parametric data was used to detect the effects of branch number, *Saperda inornata*, *Venturia tremulae*, and deer browsing upon the height and diameter growth in the plantations.

RESULTS AND DISCUSSION

Leaf Phenology

Highly significant differences were found in phenology between and within the aspen species and hybrids (Table 3.1). Trembling aspen families flushed out earlier, retained their leaves longer, and in effect, had the longest growing season in comparison to the bigtooth and hybrid families. On the other hand, phenology of bigtooth aspen families was just the opposite. The hybrid families were midway between the parents.

The average time period for leaf flushing and leaf fall in all taxa was 12 days for leaf flush and 21 days for leaf fall. Although the hybrids were generally intermediate in phenology, some *P.* X*smithii* families either flushed later or dropped their leaves earlier than all families of both trembling and bigtooth aspen. The late flushing time

Table 3.1. Leaf flush, leaf fall, and leaf area duration in percent of replicate mean at the East Lansing plantation for the earliest and latest phenological families of *P. tremuloides*, *P. grandidentata*, *P. Xsmithii*, and *P. Xrouleauiana* backcrosses.

	Leaf Flush[1]			Leaf Fall[2]			Leaf Area Duration[3]		
Taxa	Earliest	Latest	$\bar{x}$	Earliest	Latest	$\bar{x}$	Minimum	Maximum	$\bar{x}$
	%								
P.tremuloides	43	111	80	89	126	107	97	112	104
(*P.Xrouleauiana*) X *P.tremuloides*	60	120	86	70	107	96	89	105	100
P.Xsmithii	61	256	118	66	107	96	83	105	98
(*P.Xrouleauiana*) X *P.grandidentata*	99	136	112	77	104	92	92	101	96
P.grandidentata	97	161	126	70	104	92	86	102	95
Significance of F-value			**			**			**

** Significant at the 1% level of probability.

[1] Mean value of all families (100%) = 14 days from May 1st (May 14th).

[2] Mean value of all families (100%) = 61 days from September 1st (October 31st).

[3] Mean value of all families (100%) = 170 days.

(3 weeks later than the mean for all families) of some hybrid families was probably due in part to abnormalities in the gene regulation of cytokinin production and translocation, since the production of cytokinin-like substances has been demonstrated to be associated with bud flushing in species of Populus (Domanski and Kozlowski 1968, Hewlett and Wareing 1973). The early leaf drop of some hybrid families (3 weeks earlier than the mean for all families) may result from a premature increase in ethylene production due to an imbalance between abscisic acid and growth promoter hormones. The leaf area duration of these aberrant families was as little as 29 days less than the plantation mean of all families.

Date of leaf flush of the families was significantly correlated at the 1% level with date of leaf fall ($r = -.38$) and the length of the leaf area duration period ($r = -.77$). Date of leaf fall was also significantly correlated ($P < .01$) with the leaf area duration period ($r = .86$). Early leafing families were more likely to drop their leaves later and thus have a longer leaf area duration period. Barnes (1969) reported similar findings for three trembling aspen clones in northern Michigan.

The early flushing of many trembling aspen families, when compared to the bigtooth families, may be due to greater sensitivity to temperature. Late winter and early spring temperatures are known to influence the physiological processes that control leaf flushing in many tree species

(Kramer and Kozlowski 1979). Genes that regulate hormone synthesis in the roots and buds of trembling aspen may require lower minimum temperatures than similar genes in bigtooth aspen before they become activated. Another hypothesis for the spring phenology differences in the aspens is that the breakdown of inhibitor hormones occurs more rapidly in trembling aspen as the temperatures warm above freezing. Since most P. Xsmithii families fell between the two species for this phenological character, additive genetic variance was assumed to be involved. However, the unusually late flushing times of some hybrid families could be the result of epistatic effects or genome incompatibility.

On the other hand, the leaf fall results suggest that bigtooth aspen and most hybrids were more sensitive than trembling aspen to decreasing photoperiod because they dropped their leaves earlier. These data implied that each species was sensitive to different environmental stimuli. However, if decreasing seasonal temperatures are more important than daylength changes, it would then seem logical that trembling aspen should drop its leaves later because of the hypothesis that gene activation of hormone synthesis occurs at lower temperatures in trembling aspen. A physiological adaptation to lower growing-season temperatures would be beneficial for a boreal species such as trembling aspen since cool nights are common throughout much of its native range.

The narrow-sense heritabilities calculated from the nested design data at the East Lansing plantation gave a heritability of 0.17 for leaf flush. Heritabilities from the leaf drop data could not be calculated because of negative mean squares from experimental error in the mating design. However, the female-within-male variance component was highly significant for both phenological characters, whereas the male component was statistically non-significant.

Despite the statistical insignificance of the male parents in the design, there was still a sizable difference in male effects (general combining ability) between the earliest and latest males for leaf flush. The general combining abilities (GCA) of the earliest and latest males for leaf flush averaged -23 and 30 percent from the mean, respectively. Gains could be made in breeding for either early or late flushing genotypes.

In addition to the large variation in phenology between and within the aspen species and hybrids, there was also a latitudinal or photoperiodic response shown by families from different latitudes at the East Lansing plantation (Table 3.2). Trembling aspen families from north of 44.2^{o}N latitude flushed up to 13% earlier than families south of this latitude. Leaf drop among trembling aspen families differed only in the Upper Peninsula (UP) sources which dropped their leaves three percent earlier than the rest of the families. The leaf area duration period for these UP families was also three percent less. In bigtooth aspen, the Upper Peninsula families flushed much later than the Lower Peninsula sources and dropped their leaves earlier and had shorter leaf area duration periods than Lower Peninsula sources. These short leaf retention periods undoubtedly contributed to the poor growth of UP bigtooth families in the southern Michigan plantations.

Table 3.2. Phenology differences in percent of replicate mean of trembling and bigtooth aspen half-sib families from different latitudinal regions in Michigan at the East Lansing plantation.

Region	Latitude	Species	Leaf Flush	Leaf Fall	Leaf Area Duration
			------------	%	------------
1	41.8-43.0°N	*P. tremuloides*	83	102	104
2	43.0-44.2°N	Do.	87	102	104
3	44.2-45.8°N	Do.	74	102	104
4	45.8-47.0°N	Do.	75	99	101
1	41.8-43.0°N	*P. grandidentata*	117	100	98
2	43.0-44.2°N	Do.	120	98	95
3	44.2-45.8°N	Do.	122	99	98
4	45.8-47.0°N	Do.	142	93	89

The early flushing of trembling aspen families from typically colder regions of Michigan (north of 44.2°N lat.) is probably an accelerated response to the temperature stimulus. In contrast, initiation of leaf senescence processes appears to depend more on a photoperiod stimulus because the northernmost families (north of 45.8°N) dropped their leaves early at the East Lansing site. However, temperature effects interacting with photoperiod cannot be ruled out. This "photoperiod" effect did not supercede the natural variation in leaf drop among families unless there was an approximate 3.8° (425 km) latitudinal difference between family origin and planting site. Pauley and Perry (1954) also found a similar effect to be present in *Populus trichocarpa* and *P. deltoides*. They reported significant correlations ($r = -.71$ to $-.89$) between latitude of the clone and date of height cessation at Weston, Massachusetts. Hence, discretion should be used when moving trembling aspen families more than 325 km (3°lat.) south of their native stand because growth cessation will occur earlier.

Leaf flush, leaf drop, and leaf area duration were significantly correlated with latitude of the parents of trembling aspen families (Table 3.3). In bigtooth aspen, however, leaf drop was the only phenological trait that was significantly correlated with the latitudinal origin of the parents. These correlations suggest the importance of photoperiod on leaf senescence in both species and the effect of temperature on leaf flushing in trembling aspen.

At the East Lansing plantation, early leaf flushing was significantly correlated with growth performance ($\text{diameter}^2 \times \text{height}$) of bigtooth aspen but not trembling aspen. Bigtooth families that flushed closer to the average date of the last spring frost (May 7th) grew slightly better than later-flushing bigtooth families. The three best and three worst bigtooth families flushed an average of 10 and 14 days after May 7th, respectively. Since trembling aspen naturally flushes earlier than bigtooth aspen, most (89%) of the trembling aspen families leafed out before the first bigtooth family flushed. The earliest flushing trembling aspen families flushed two days before May 7th, and all trembling aspen families had flushed before May 21st, the date that frost is likely to occur once every 10 years. Barnes (1969) hypothesized that when comparing the two species, late flushing by bigtooth may be a fitness advantage over trembling aspen on a given site. However, this theory could not be tested in the two-year-old plantations because insufficient time had elapsed for climatic conditions (early frosts) to affect tree growth.

Table 3.3. Correlations of three phenological traits of half-sib, full-sib, and combined trembling and bigtooth aspen families with the latitudinal origin of their parents.

Species	Family	Number of Families	Leaf Flush	Leaf Drop	Leaf Area Duration
			-----------r-----------		
P. tremuloides	Half-sib	32	-.32**	-.43**	-.26*
Do.	Full-sib	41	-.14	-.26*	-.18
Do.	Total	73	-.21**	-.33**	-.22**
P. grandidentata	Half-sib	13	.06	-.42*	-.20
Do.	Full-sib	21	-.02	-.40**	-.25
Do.	Total	34	.01	-.40**	-.24

*/** Significant at the 5 and 1 percent levels, respectively.

In general, aspen families at the East Lansing site that flushed early and dropped leaves late grew better at all plantations (Table 3.4). There was a north to south cline in the size of the correlation coefficients in Table 3.4. Northern plantations had higher correlations, possibly because the shorter growing seasons at these sites would favor families with phenologies that could use all of the growing season.

Table 3.4. Correlations of two-year growth performance of 179 bigtooth, trembling, and hybrid aspen families at each plantation with their respective phenological traits recorded at the East Lansing plantation.

Site	Species	Latitude	Frost-free Period (Days)	Leaf Flush	Leaf Fall	Leaf Area Duration
				-----	r[a/]	-----
Chatham	All	46.3°N	100	-.50	.45	.55
Six Lakes	All	43.5°N	126	-.43	.45	.53
E.Lansing	All	42.7°N	151	-.36	.48	.53
Decatur	All	42.0°N	158	-.27	.18	.23
Combined				-.55	.50	.61
				-----	r[b/]	-----
E.Lansing	*P.grandidentata*			-.36**	.25*	.43**
	P.tremuloides			-.13	.45**	.44**
	P.Xsmithii			-.21*	.25**	.35**

[a/] All coefficients were significant at the 1% level.

[b/] Coefficients significant at the 5% (*) or 1% (**) level.

The results from two-year growth data may not be valid over the long term because periodic late spring frosts are detrimental to early flushing families (Strain 1966). Furthermore, these families may have ranked differently if they had been scored for phenology at the other three plantations. However, if photoperiod and temperature are the environmental stimuli for aspen phenology, then the phenology rankings at the East Lansing site may be applicable to the phenological responses that would be found at the other sites.

Morphological Characters

Autumn leaf color within and between species and families at the East Lansing site varied from yellow to orange to red. Barnes (1969) reported that a range of autumn colors existed between aspen clones and species in Michigan. Significant differences (Table 3.5) were found between the species and hybrids. The autumn colors of most trembling aspen families in this test were predominantly yellow with some reds. Autumn coloration of bigtooth families was mostly yellow-orange or orange-red. *P.* X*smithii* families were largely yellow. The coloration of the hybrid families corresponded closely to that of their trembling aspen parent.

The narrow-sense heritability (additive genetic variance) for leaf coloration as calculated from the nested mating design data was 0.34. The male parent variance component was significant at the 10% level of probability

Table 3.5. Mean ranks from Friedman's two-way classification test for six morphological and physiological characters measured in 206 aspen and hybrid aspen families at the East Lansing plantation.

Taxa	Fall Color	Number of Branches	Bud Divergence	Bud Pubescence	Shoot Dieback	Basal Sprout
	Morphological Characters				Physiological Characters	
	Mean Ranks[1,2]					
P.grandidentata	6.0	1.0	5.2	5.0	2.3	2.4
P.grandidentata X P.tremuloides	3.0	2.5	3.5	2.8	4.2	5.8
P.tremuloides X P.grandidentata	3.0	3.5	2.5	2.2	5.3	4.5
P.tremuloides	2.3	5.2	1.0	1.0	1.7	2.3
(P.Xrouleauiana) X P.tremuloides	2.0	5.7	3.0	4.0	4.4	4.2
(P.Xrouleauiana) X P.grandidentata	4.7	3.2	5.8	6.0	3.3	1.8
Chi^2 Value	20**	25**	27**	30**	16**	21**

** Significant at the 1% level.

[1] Each mean rank is the ranking of non-parametric data for one trait of one taxon with respect to the data of the other taxa. These rankings are averaged across the number of replicates in the test (= mean ranks). ie., The mean rank of 6.0 for fall color in P. grandidentata signifies that this species always ranked sixth in the fall color data of every replicate.

[2] See Materials and Methods section for explanation of the scoring of each character.

and the female-within-male variance component was highly significant ($P<.01$). Autumn coloration in aspen appears to have non-additive (dominant effects) as well as additive genetic variance for genes that produce carotenes and xanthophylls (yellow-orange pigments) because the mean color scores for families with trembling aspen parentage were 2.3 (trembling X bigtooth), 2.3 (the reciprocal), 2.2 (white poplar hybrid X trembling), and 2.2 (trembling). In contrast, bigtooth families averaged 3.3, which is orange-red in color. Alleles for anthocyanin pigments might be either at the same loci as the carotenoid alleles but are recessive to these alleles or the anthocyanin genes are epistatically affected by the carotenoid genes.

The number of primary branches per tree after one growing season in the field (two years from seed) differed significantly between the species and hybrids (Table 3.5). Typically, trembling aspen produced five or more branches, whereas bigtooth aspen produced few or none. Branching of *P.* X*smithii* families were intermediate, but tended to favor the branch number of the maternal parent. Also, branch number in *P.* X*rouleauiana* backcrosses favored the aspen parent. Additive and maternal effects may be involved in bud release and the subsequent lateral branch formation in aspen during the first growing season in the field. In addition, both height ($r = .29$) and diameter ($r = .46$) were significantly correlated with branch number after the first field growing season. Therefore, branch number in young trees may be determined by both the species genotype and the physical size attained by the genotype (genotype x environment).

Significant differences between species and hybrids were found for bud orientation (Table 3.5). Bud divergence was essentially absent in trembling aspen and ubiquitous in bigtooth aspen, although there were exceptions. The variation between and within the P. Xsmithii families for bud orientation was very large. The mean of the hybrid families was approximately intermediate to the parent species, but the bud orientation within a hybrid family was more often like that of the maternal parent. Bud orientation in the P. Xrouleauiana backcrosses was influenced by the aspen parent, but generally resembled bigtooth aspen because P. alba has divergent buds. Non-additive genetic effects probably were involved because of the unpredictable segregation of this trait in the F_1 hybrid families.

Significant differences between species and hybrids were found for bud scale pubescence (Table 3.5). Noticeable pubescence on the bud scales was absent in trembling aspen, but was common in bigtooth aspen. Most P. Xsmithii families had little bud scale pubescence. Hybrid families with bigtooth aspen as the maternal parent had slightly more pubescence than the reciprocal hybrid. Since P. alba has a dense tomentum on its bud scales, the P. Xrouleauiana backcrosses with bigtooth aspen had more pubescence than bigtooth families, and with trembling aspen as the backcross parent, slightly less pubescence than bigtooth families. The gradual cline from P. tremuloides to P. Xrouleauiana in the amount of bud scale pubescence suggested additive effects were important.

Physiological Abnormalities

Significant differences existed between the species and hybrids in the occurrence of stem dieback and bud abortion (Table 3.5). Only a few individuals from the bigtooth and trembling families had some type of shoot dieback. However, this dieback was not uncommon in a number of hybrid families. Both P. Xsmithii crosses and the P. Xrouleauiana backcrosses had individuals with the disorder. The hybrid dysgenesis was probably the dieback syndrome that Heimburger (1940) found in P. Xsmithii during his early hybridization studies.

Significant differences in the frequency of root collar sprouting were present between the species and hybrids (Table 3.5). Many P. Xsmithii and (P. Xrouleauiana) X P. tremuloides families had from one to ten sprouts emerging from the root collar during the second growing season. Despite this abundant sprouting, the trees maintained actively-growing terminal leaders. The genetic regulation of endogenous hormones in these hybrids may have malfunctioned, thereby, releasing the adventitious and basal buds from inhibitor hormones. Hybrid families that developed basal sprouting in the second growing season often grew less in height than hybrid families without sprouts.

The young succulent shoot tips of these sprouts were strong sinks for photosynthate produced by the tree. These sprouts either became overtopped by the canopy and died or they continued to grow and divert photosynthate from the main stem.

Diseases, Insects, and Deer

An unnamed virus leaf spot described by Boyer (1962) was a severe problem for a few *P.* X*smithii* families. More hybrid families had this disease than did bigtooth and trembling aspen families. Boyer also found this to be true with hybrid aspen that he had inoculated with the virus. However, there were no significant differences between species and hybrids in incidence of this disease.

A serious fungal shoot pest was *Venturia tremulae*. It has been reported by Dance (1961) to attack only the young shoots of both bigtooth and trembling aspen. Dance observed that the time span of susceptibility to *Venturia tremulae* is about six weeks, beginning in early May. He reported that trembling aspen is more seriously injured than bigtooth aspen because bigtooth initiates shoot growth two weeks later; hence, the time during which reinfection can occur in bigtooth during the susceptible period is shorter.

In this study, there were also significant differences (Table 3.6) in infection rates between trembling, bigtooth, and hybrid aspen which supported Dance's findings. Trembling aspen was the most susceptible, whereas bigtooth was the least susceptible. The hybrids were intermediate, although hybrids with a trembling aspen parent were much more susceptible than the white poplar x bigtooth aspen hybrid.

Genes with additive effects, which were largely contributed by one species, *P. tremuloides*, appear to have influenced aspen susceptibility to *Venturia tremulae*. Some of these genes were probably the same ones or closely linked to those that controlled time of leaf flush, since Dance's work and the leaf flush data in Table 3.1 support this hypothesis. Resistance was found in some bigtooth families, whereas only differences in the degree of susceptibility to *Venturia* were found among trembling families.

Table 3.6. Susceptibility to *Venturia tremulae* in percent of the plantation mean for each species and hybrid at the Six Lakes plantation.

Taxa	Susceptibility in % of Plantation Mean	Total Number of Plots	Percentage of Plots Infected
P. grandidentata	31	72	8
(*P.* X*rouleauiana*) X *P. grandidentata*	35	32	9
(*P.* X*rouleauiana*) X *P. tremuloides*	88	51	22
P. grandidentata X *P. tremuloides*	98	102	25
P. tremuloides X *P. grandidentata*	93	97	23
P. tremuloides	120	315	30

The most destructive insect in the progeny test was the poplar-gall beetle, Saperda inornata. This borer has been reported by Nord et al. (1972) to be a common aspen pest in the Lake States. They found that open stands, such as young progeny tests, are highly susceptible to attack. Stunted growth, stem breakage, fungal decay, and Hypoxylon cankers were typical secondary effects of Saperda damage. At the Six Lakes site, families from all species and hybrids were heavily-infested. Two-year-old trees had as many as six galls on the main stem. Damage from stem breakage and secondary fungal pathogens was most pronounced on the dry, sandy Six Lakes site because tree vigor was low. On the better plantation sites (ie., Decatur), trees were vigorous enough so that stem damage from larval boring was minimized by production of sufficient xylem to prevent stem breakage.

There were no significant differences between species and hybrids in susceptibility to Saperda attack. However, (P. Xrouleauiana) X P. tremuloides hybrids averaged twice as many galls per tree as did P. grandidentata. Apparently it was no coincidence that the former taxon also had the fastest growing families and the latter the slowest growing ones. By age two, fast-growing trees had attained the 8-14 mm diameter range that Grimble et al. (1969) reported to be the preferred stem size for ovipositing Saperda beetles. Height ($r = .28$) and diameter ($r = .32$) were significantly correlated with the number of Saperda galls per stem. This does not necessarily infer that vigorous trees are more genetically susceptible but instead shows that larger trees support more larvae (galls). Vigorous trees, however, suffered less stem breakage from larval boring than did smaller trees.

The apparent lack of species resistance to *Saperda* and the significant damage that the larvae cause to young aspen plantations should be considered when selecting sites for genetic tests of aspen taxa. Poor to average aspen sites should be avoided if this insect is present unless a spraying and sanitation program is to be instituted.

Another shoot insect, the willow shoot sawfly (*Janus abbreviatus*), was a serious pest in the nursery and a lesser problem in the East Lansing plantation. The biology of this insect in the Lakes States has been described by Osgood (1962) and Graham *et al*. (1963). Shoot damage in the East Lansing plantation was greatest in the first growing season and decreased the following two years. In the second growing season 2.5 percent of all terminal shoots and 60% of all root collar sprouts were girdled and either killed or damaged. There was no preference for one taxon by the willow shoot sawfly. However, *P.* X*smithii* families suffered the most willow shoot sawfly damage because the abnormal root collar sprouting of these hybrids provided the sawflies with succulent sprouts for ovipositing. These families, which produced numerous root collar sprouts, should not be chosen for future breeding work.

The poplar tentmaker (Clostera inclusa) was yet another insect problem. The larvae of this moth defoliated 35 percent of the trees at the Decatur plantation in 1983. Entire cottonwood plantations in Mississippi have been completely defoliated in as little as three days by this notodontid (Stein and Oliveria 1979). Mortality and growth loss have been economically significant in these cottonwood plantations. The poplar tentmaker has been relatively unknown in Michigan but recently has been reported in Berrien, Cass, and St. Joseph counties in the southwestern part of the state. There were no differences in genetic resistance to the tentmaker among the aspen taxa at Decatur. Families with either trembling or bigtooth aspen as the maternal parent had 34 and 35 percent defoliation, respectively. If this insect becomes a problem in southwestern Michigan, chemical control has been proven to be effective (Coster 1973).

In addition to insects and diseases, deer damage was a common problem in the plantations. Browsing and fall rubbing by male deer damaged or killed the main stems of many trees. However, the correlation between browsing and tree height was very small ($r = .04$) and insignificant. Preference for a particular aspen taxon was not evident in this study. The importance of deer browsing in a plantation depends on tree vigor. Fast-growing trees usually escape deer browsing after two or three years. Slow-growing trees or trees planted on poor sites may be stunted indefinitely and become infected by secondary pathogens. Because deer repellents have not given promising results thus far in aspen plantings (Reighard, personal observation), planting superior genotypes and avoiding poor sites will lessen the impact of deer on aspen establishment and growth.

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CHAPTER 4

Comparative Physiological Studies of Photosynthesis, Shoot and Root Development, and Dry Matter Partitioning in *Populus grandidentata*, *P. tremuloides*, and *P.* X*smithii*

(To be Submitted to Forest Science)

ABSTRACT

Photosynthesis, shoot and root development, and dry matter partitioning were studied in *Populus grandidentata*, *P. tremuloides*, and their hybrid *P.* X*smithii* using a closed gas-exchange system, hydroponics, and a nursery. There were no significant differences among taxa in net photosynthesis and dark respiration rates. Whole plant photosynthesis and respiration were significantly correlated with leaf dry weight, shoot dry weight, and leaf area. Seedlings with high net photosynthetic rates and large leaf areas assimilated the most dry matter, but did not necessarily grow the tallest. *P. tremuloides* produced significantly more primary roots and less secondary roots than *P. grandidentata*. *P.* X*smithii* produced slightly more primary and secondary roots than either parent species. Rates of root growth in both species and the hybrid were similar after five weeks. *P. tremuloides* had a 50% smaller root to stem ratio than *P. grandidentata*. Hybrids had ratios similar to their maternal parents. The gas-exchange and hydroponic studies were not effective methods for predicting dry matter production of aspens grown under field conditions in the nursery.

INTRODUCTION

Aspens are fast-growing temperate tree species which have evolved metabolic processes that promote rapid juvenile growth. Numerous studies of key physiological traits have been conducted in aspens to elucidate which of these traits are reliable indicators of growth potential. Photosynthesis studies (Gatherum et al. 1967, Domingo and Gordon 1974, Okafo and Hanover 1978) have been the most frequently reported, although the results have not provided conclusive evidence that net photosynthesis is significantly correlated with growth rate. An inherent problem with photosynthesis studies has been that field conditions cannot be easily duplicated in the lab and even when they are approximated, the short-term nature of these gas-exchange measurements probably do not accurately represent the long-term gas-exchange processes in a natural environment.

In contrast to photosynthesis, other physiological traits such as leaf area, shoot and root growth, and carbon partitioning (Zavitkovski 1971, Okafo 1976) have been more helpful in defining the growth potential of aspen species. These physiological processes have not yet been investigated in comparative studies between aspen species and their hybrids. Furthermore, many of these traits have not been measured under both laboratory and field conditions. Understanding the relationship between plant physiological processes and dry matter production will provide the forest geneticist with important information concerning the feasibility of using indirect selection in physiological traits for biomass yield. Studying these traits in aspen species and their hybrids will give an indication of how similar genetically these processes are in the different parent species and therefore, aid in the development of inter- and intraspecific hybridization programs to improve biomass production.

The objectives of this study were to investigate photosynthesis, shoot and root development, and carbon partitioning in two sympatric but ecologically distinct aspen species (*Populus grandidentata* and *P. tremuloides*) and to determine how these interdependent physiological processes were expressed in their hybrid, *P. Xsmithii*. In addition, the importance of these processes relative to plant biomass production under both artificial and natural environments was evaluated.

MATERIALS AND METHODS

Photosynthesis Study

Seedlings of bigtooth aspen (*Populus grandidentata*), trembling aspen (*P. tremuloides*), and their hybrid (*P. Xsmithii*) were grown indoors in a growth frame for 12 weeks. These seedlings represented seven full-sib families. Each seedling was grown in a 6 x 6 x 27 cm polycoated paper plant band that had been filled with media consisting of 1:1:1 parts peat, perlite, and vermiculite. The bands were inserted in plastic milk cases with 25 bands to a case. One seedlot was sown per case. The root media was watered whenever the surface became dry, and the seedlings were fertilized once a week with Peters Soil Test Fertilizer (20-20-20) at a rate of 6 ml/l. Peters Soluble Trace Element Mix was applied once during week three at a rate of 0.3 ml/l.

Lighting for the seedlings came from cool white fluorescent bulbs (high output 800 mA) suspended one meter above the cases. The photosynthetic photon flux density (PPFD) was measured with a Lambda quantum sensor (Licor Inc. Model LI-1600, Lincoln, NE) at 0, 20, and 40 cm above the top of the plant bands. The PPFD data were collected in weeks 1 and 12 and then averaged. The average PPFD's at 0 (lower canopy), 20 (mid-canopy), and 40 cm (top canopy) over the 12-week growing period were 30, 42, and 55 μmol $s^{-1}m^{-2}$, respectively. The photoperiod was 18 hours light and 6 hours darkness. Air temperatures in the room during the growing period were $24^{\circ} \pm 4^{\circ}C$.

Twelve weeks after the sowing date, the five largest seedlings from each seedlot were selected for the experiment. The experimental design was a randomized block with each block or replicate representing a two-day time interval. There were five replicates with each replicate having seven trees (one tree per seedlot). The order of testing the seven trees within each replicate was random. Ten days were needed to take gas-exchange measurements on a total of 35 trees.

A schematic diagram of the plant gas-exchange system with its electronic components is presented in Figure 4.1. The volume of the system excluding the shunt pathway is 111 liters. Air was circulated by a belt-driven centrifugal blower (Paxton Corp., Santa Monica, CA) that had an airtight housing and seals. The air flow was directed through glass tubing which was connected by couplers with teflon sealing rings. The plant chamber and mist chamber were made of plexiglass. The thermometer and lithium chloride humidity sensors were housed in a plexiglass box. The shunts are copper T's with valves which regulated the air flow through the system. Air velocity in the plant chamber averaged 2.1 $m\ s^{-1}$. A radiator and heating coil that were enclosed in an insulated box regulated the relative humidity and air temperature of the circulating air.

System air temperature was monitored independently by three instruments. A digital centigrade thermometer was used to determine the current system temperature. A thermocouple attached to a Sargent recorder gave a temperature tracing to monitor temperature changes in the system. A thermistor was used to control a variable output voltage source that operated the heater element. System air temperature was maintained at $24^{\circ} \pm 0.5^{\circ}C$.

Relative humidity was monitored by an electronic hygrometer indicator (Hygrodynamics Inc., Model 15-3001, Silver Spring, MD). A narrow-range sensor head with eight temperature positions was used in conjunction with the

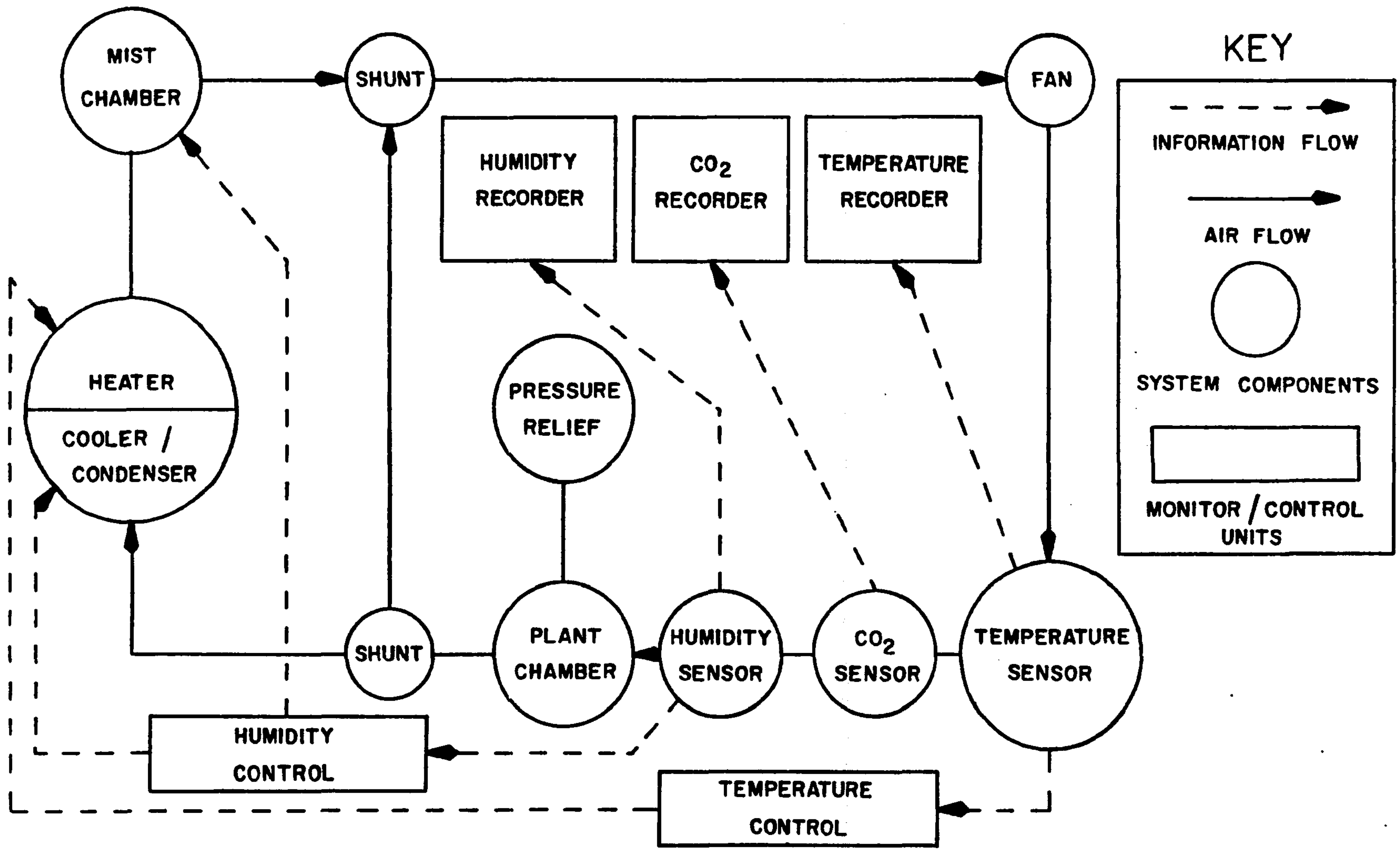

Figure 4.1. Schematic diagram of the plant gas-exchange system used in the photosynthesis study.

hygrometer indicator to obtain accurate readings of relative humidity within a specified temperature range. The relative humidity was traced on a Texas Instruments recorder. Deviations greater than 1% from 65% relative humidity were corrected by a humidifier/dehumidifier control circuit that operated the misting and cooling (radiator) units.

System air was pumped from the system and through two silica gel columns before entering an infrared gas analyzer (Beckman Instruments, Model 864) where carbon dioxide concentrations were measured. The carbon dioxide levels were traced on a Texas Instruments recorder. After passing through the analyzer, the air was returned to the system.

The light source for the plant chamber consisted of four 400-watt and one 1,000-watt metal halide bulbs (Sylvania Metalarc) attached to parabolic aluminum reflectors and suspended above and to the sides of the chamber. The photosynthetic photon flux density (PPFD) inside the plexiglass chamber at the bottom, mid-section, and top was 715, 765, and 1220 $\mu mol\ s^{-1}m^{-2}$, respectively. These readings were the average over the ten-day experimental period. The mean of these readings was 900 $\mu mol\ s^{-1}m^{-2}$.

Before inserting a seedling in the gas-exchange chamber, the seedling was watered and a collar made from a size 16 rubber stopper was placed around the base of the stem. The seam between the stem and stopper was sealed with Optosil (Unitek Corp., Monrovia, CA), a silicone-based molding material. The seedling was preconditioned to the light intensity and air flow inside the plant chamber for a period of 20 minutes before the gas-exchange system was sealed to room air.

Net photosynthesis of each seedling was determined from the time period that the plant needed to deplete the CO_2 concentration in the gas-exchange system from 340 ppm to 265 ppm. When the CO_2 concentration decreased to 265 ppm, the plant chamber was covered with black cloth and all lights were turned off. Dark respiration was determined from the increase in CO_2 levels in the system after 30 minutes of darkness. All data in ppm were converted to mg CO_2 $dm^{-2}hr^{-1}$.

Following the gas-exchange measurements, leaf area of each seedling was obtained by a LI-3000 Portable Area Meter (LiCor Inc., Lincoln, NE). Stem height was measured to the nearest centimeter. Leaves and stems were dried in an oven at 105°C for 48 hours. Oven-dry weight of the leaves and stems were weighed to 0.1 gram.

Two-way analyses of variance were calculated for data from each seedlot and species group. Pearson correlations were also calculated for relationships between the data.

Hydroponic Study

The hydroponic system used to study aspen root growth was modeled after an irrigation-type waterculture for root studies that was developed by de Stigter (1969) and later modified by Wood and Hanover (1980). Wood's system was slightly modified to avoid maintenance problems. The system was constructed in a glasshouse and supplemental lighting (100 μmol $s^{-1}m^{-2}$) from cool white fluorescent tubes was provided. The photoperiod was maintained at 16 hours. Temperature ranged from 20°C at night to 25°C in the day.

Seed from the seven seedlots used in the gas-exchange experiment were sown in 15 cm high plastic cases filled with 60% perlite and 40% peat. When the seedlings were five to seven cm tall, they were removed from the cases and their roots were gently washed with water and placed in 61 x 30 x 10 cm root trays that were oriented at a 20° angle from horizontal on a greenhouse bench. The trays were constructed from 2 mm PVC sheets and had removeable aluminum covers and false bottoms to avoid pooling of water around the roots. The nutrient solution for the system was prepared 2.5 times the strength used by Wood and Hanover (1980) and was changed once during week 4 of the 8-week experiment. Before placing seedlings in the tray, 1,150 ml of 1N HCl were added to the 230 liters in the system to lower the solution pH from 7.8 to 6.0. During the course of the experiment, acid was occasionally added to maintain the pH between 6.0-6.5.

The trays were connected to a 200-liter feeder tank by 1.9 cm I.D. rigid polyvinyl chloride (PVC) tubing which conducted the nutrient solution by gravity from the feeder tank to the trays and from the trays back to a 200-liter drainage tank. Each tray had three 0.9 mm I.D. trickle irrigation capillary tubes with bell weights (Chapin Watermatics Inc., Watertown, NY) connecting the feeder PVC tube with the top of the tray. These tubes drip-irrigated the cloth liner on which seedling roots grew. The cloth liner was 50% cotton and 50% polyester. The nutrient solution drained from the bottom of each tray into 1.9 cm I.D. PVC tubing which carried the solution to the drainage tank. When the drainage tank filled to the 120-liter level, a sump pump was activated and the nutrient solution was pumped up into the overhead feeder tank. The nutrient solution then flowed from the feeder tank and through a five micron filter which removed particulates. After passing through the filter, the nutrient solution entered the PVC tubing leading to the trays and the cycle repeated itself.

Root and shoot measurements began when the new white roots of the seedlings had reached 10-20 cm in length. Roots were referenced with stainless steel nuts placed at the root tips on the first day of each week that growth was to be measured. Measurements of root, leaf, and stem growth were taken once a week for five weeks. After the fifth week, the leaves, stems, and roots of all seedlings were harvested, oven-dried, and weighed. Leaf area was measured with a LI-3000 Portable Area Meter (LiCor, Lincoln, NE).

The experiment was a completely randomized design with three trees per seedlot. Data were analyzed by one-way analysis of variance. Correlations were calculated for the relationships between the data.

Nursery Study

On June 4th, 1983 seed from the seven seedlots used in the gas-exchange and hydroponic systems were sown in a nursery in a randomized block design with four replicates. The seedlings of the seven seedlots in each replicate were thinned to eight trees per square foot in July. The seedlings were fertilized with Peters Soil Test Fertilizer (20-20-20) six times during the summer. Benelate was applied once a week the first month to control damping-off fungi. Plictran was sprayed twice to control mites. Ten trees per seedlot in each replicate were randomly selected and harvested on September 27th. Height, caliper, dry weight, and bud number were measured. Dates of leaf flush, bud set, and leaf fall were recorded for another ten trees not harvested. Data were analyzed by two-way analysis of variance. Correlations were calculated for the data.

RESULTS AND DISCUSSION

Photosynthesis, Respiration, and Shoot Growth

Data from the photosynthesis experiment are listed in Table 4.1. There were no significant differences between bigtooth (*P. grandidentata*), trembling (*P. tremuloides*), and hybrid aspen (*P. Xsmithii*) in rates of net photosynthesis (P_N). The lack of significant differences between P_N rates of bigtooth and trembling aspen agreed with Okafo and Hanover (1978). Their P_N rates were similar to the ones found in this study. Hybrid aspen suffered no apparent physiological abnormalities in photosynthesis since their P_N

rates were equal to trembling aspen. However, large P_N rates of up to 15 mg CO_2 $dm^{-2}hr^{-1}$ reported for white poplar-bigtooth aspen hybrids (Domingo and Gordon 1974) were not observed in this study. The P_N rates of *P.* X*smithii* suggested that genes controlling the photosynthesis process were similar in *P. grandidentata* and *P. tremuloides*.

Table 4.1. Photosynthesis, respiration, leaf area, and plant dry weight data from the four aspen taxa in the photosynthesis study.

Character Measured	Taxa: GXG[1]	GXT[2]	TXG[3]	TXT[4]	F-Value Significance
Net Photosynthesis (P_N) (mg CO_2 $dm^{-2}hr^{-1}$)	5.7	6.3	6.3	6.3	n.s.
Whole Plant P_N (mg CO_2 hr^{-1})	61.1	73.4	70.5	58.2	*
Dark Respiration (R_D) (mg CO_2 $dm^{-2}hr^{-1}$)	1.3	1.2	1.2	1.5	n.s.
Whole Plant R_D (mg CO_2 hr^{-1})	13.7	13.5	13.4	13.3	n.s.
R_D/P_N Ratio (no units)	.24	.19	.20	.24	n.s.
Total Leaf Number (leaves > 2 cm)	13	19	19	31	***
Total Leaf Area (dm^{-2})	10.8	11.7	11.1	9.2	n.s.
Specific Leaf Area (dm^2g^{-1})	5.2	5.0	6.0	6.3	***
P_N per Leaf Dry Weight (mg CO_2 $hr^{-1}g^{-1}$)	30.2	34.8	37.8	39.6	n.s.
Total Leaf Dry Weight (grams)	2.1	2.4	1.9	1.5	**
Stem Dry Weight (grams)	0.6	0.6	0.7	0.6	n.s.
Whole Plant Dry Weight (grams)	2.7	3.0	2.6	2.1	**
Height (cm)	32	37	41	45	***

*/**/*** Significant at the 10, 5, and 1% levels, respectively.
[1] *Populus grandidentata* X *P. grandidentata* ($\bar{x}$ of 10 seedlings).
[2] *P. grandidentata* X *P. tremuloides* (mean of 5 seedlings).
[3] *P. tremuloides* X *P. grandidentata* (mean of 10 seedlings).
[4] *P. tremuloides* X *P. tremuloides* (mean of 10 seedlings).

On a whole plant basis, hybrids had significantly higher net carbon dioxide fixation than either of the two parent species. This was probably due to the larger leaf area in the hybrids. However, some of the lower leaves on each seedling had to be removed before inserting the seedling into the rubber stopper, so this hypothesis cannot be supported by the data.

Differences among aspen taxa in dark respiration per unit leaf area and total dark respiration per plant were non-significant in this study. Dark respiration rates have also been reported to be non-significant among aspen species (Okafo and Hanover 1978) and the black and balsam poplars (Luukkanen and Kozlowski 1972).

Specific leaf area was high, and significant differences existed between seedlings with either bigtooth or trembling aspen as maternal parents. The low light intensity of the growth frame and the absence of wind produced a shade-leaf morphology in the leaves. Since Okafo and Hanover (1978) found no differences in specific leaf area in these two species, specific leaf area (leaf thickness) in bigtooth aspen from this study was less affected by the artificial lighting than was trembling aspen. This was also evident in the hybrids with bigtooth maternal parentage.

Not surprisingly, trees with bigtooth maternal parentage also had significantly higher leaf dry weight. However, P_N per leaf dry weight was less in these trees.

Self-shading by the large leaves may have been a factor. In comparison, stem dry weight was similar in all species and hybrids. Shoot (stem + leaves) dry weight was significantly higher in bigtooth aspen and the hybrids. Despite having less leaf area, leaf dry weight, and shoot dry weight than bigtooth aspen and the hybrids, trembling aspen did possess the highest P_N per leaf dry weight and therefore, was the most photosynthetically efficient species.

Correlations between the gas-exchange data showed that P_N was significantly correlated at the 5% level with height (r=.39) but nothing else. P_N was positively correlated with leaf (r=.05), stem (r=.32), and shoot (r=.12) dry weight. Although small and non-significant, these positive correlations were contrary to the negative correlations reported by Okafo and Hanover (1978) for the relationship between P_N and dry matter production in bigtooth and trembling aspen.

Whole plant photosynthesis was significantly correlated at the 1% level with leaf weight (r=.67), stem weight (r=.69), shoot weight (r=.71), leaf area (r=.62), and specific leaf area (r=-.45). Total plant dark respiration was significantly correlated at the 5% level for leaf area (r=.34), leaf dry weight (r=.39), stem dry weight (r=.38), and shoot dry weight (r=.41). Dark respiration rates were significantly and inversely correlated at the 5% level (r=-.37) with leaf area.

These correlations suggest that aspen seedlings which have both high P_N rates and large leaf areas will assimilate the most dry matter. Applying this hypothesis to the data in Table 4.1, the two aspen hybrids should have produced more dry matter than the two parent species. Shoot biomass production by the hybrids was indeed equal or superior to the biomass produced by bigtooth and trembling aspen. However, it was not known how much root biomass was produced which is very important because of the clonal habit of aspens.

A problem with the P_N and leaf area hypothesis was that trembling aspen, which had the lowest dry weight, was also the tallest. The importance of P_N measurements for estimating field productivity is debatable. Gifford (1974) argued that high photosynthetic effiency becomes irrelevant by the time all other physiological processes have interacted with plant growth. Ceulemans _et al_. (1980) reported that the P_N measurement by itself was not significantly correlated with field productivity of six poplar clones. However, a later study (Ceulemans and Impens 1983) with 18 poplar clones showed P_N rates to be significantly correlated with first-year shoot length. In contrast, leaf area index (Anderson 1979) and leaf area (Isebrands and Nelson 1982) have been shown to be linearly related to poplar biomass production. Since rapid shoot growth is essential for intolerant species such as aspen, genes that control cell divisions in the peripheral (leaf) and apical (shoot) meristems may be considerably more important than genes which regulate CO_2 fixation (net photosynthesis).

Hydroponics: Plant Growth and Biomass Partitioning

Apical leaf production of hydroponically-grown seedlings varied little from week to week within each aspen species and hybrid (Fig. 4.2), but differed significantly between the four species and hybrids. Trembling aspen produced the most apical leaves per week and bigtooth aspen the least. Apical leaf production in the hybrids was intermediate, although leaf number more closely corresponded to the number of leaves of the maternal parent. The number of leaves (including branch leaves) and total leaf area were significantly higher in the trembling x bigtooth hybrids (Table 4.2) because this hybrid and trembling aspen averaged 7 and 6 branches, respectively, whereas branches were absent in bigtooth aspen and the bigtooth x trembling (GxT) hybrids. Therefore, genes regulating early branch formation were non-additive and have a maternal origin.

Although leaf area was significantly larger in trembling x bigtooth (TxG) hybrids, the correlation (r=.79) between hybrid leaf area and shoot dry weight was high but not significant (Table 4.3). However, leaf area of both bigtooth (r=.92) and trembling aspen (r=.88) were significantly correlated at the 1% level with shoot dry weight. Leaf area was also significantly correlated with root (r=.88) and plant (r=.94) dry weight for all species combined. Bigtooth and trembling also had slightly lower specific leaf areas (heavier leaves) than the hybrids.

Table 4.2. Shoot and root measurements of the four aspen taxa from the hydroponic study after week 5.

Character Measured	Taxa GXG[1]	GXT[2]	TXG[3]	TXT[4]	F-Value Significance
Number of Leaves	17	22	88	68	**
Leaf Area (dm^2)	31	57	86	52	**
Specific Leaf Area (dm^2g^{-1})	3.6	3.9	3.9	3.4	n.s.
Height (cm)	55	71	101	92	**
Number of Branches	0.0	0.2	7.2	6.2	*
Number of 1° Roots	12	16	17	15	n.s.
Mean Root Growth of Primary Roots (cm)	59	73	74	61	n.s.
Mean Number of 2° Roots Per 1° Root	204	212	227	148	**
Root to Stem Ratio	1.1	1.0	0.6	0.5	**
Root to Stem+Leaf Ratio	.29	.28	.21	.19	**

*/** Significant at the 5 and 1% levels, respectively.
[1] Populus grandidentata X P.grandidentata ($\bar{x}$ of 6 seedlings).
[2] P. grandidentata X P. tremuloides (mean of 3 seedlings).
[3] P. tremuloides X P. grandidentata (mean of 6 seedlings).
[4] P. tremuloides X P. tremuloides (mean of 6 seedlings).

Weekly height increment (Fig. 4.3) in all species and hybrids exhibited a normal growth curve. Height differences (Table 4.2) were significant at the 1% level, and total height was greatest in the TxG hybrids. In addition to the

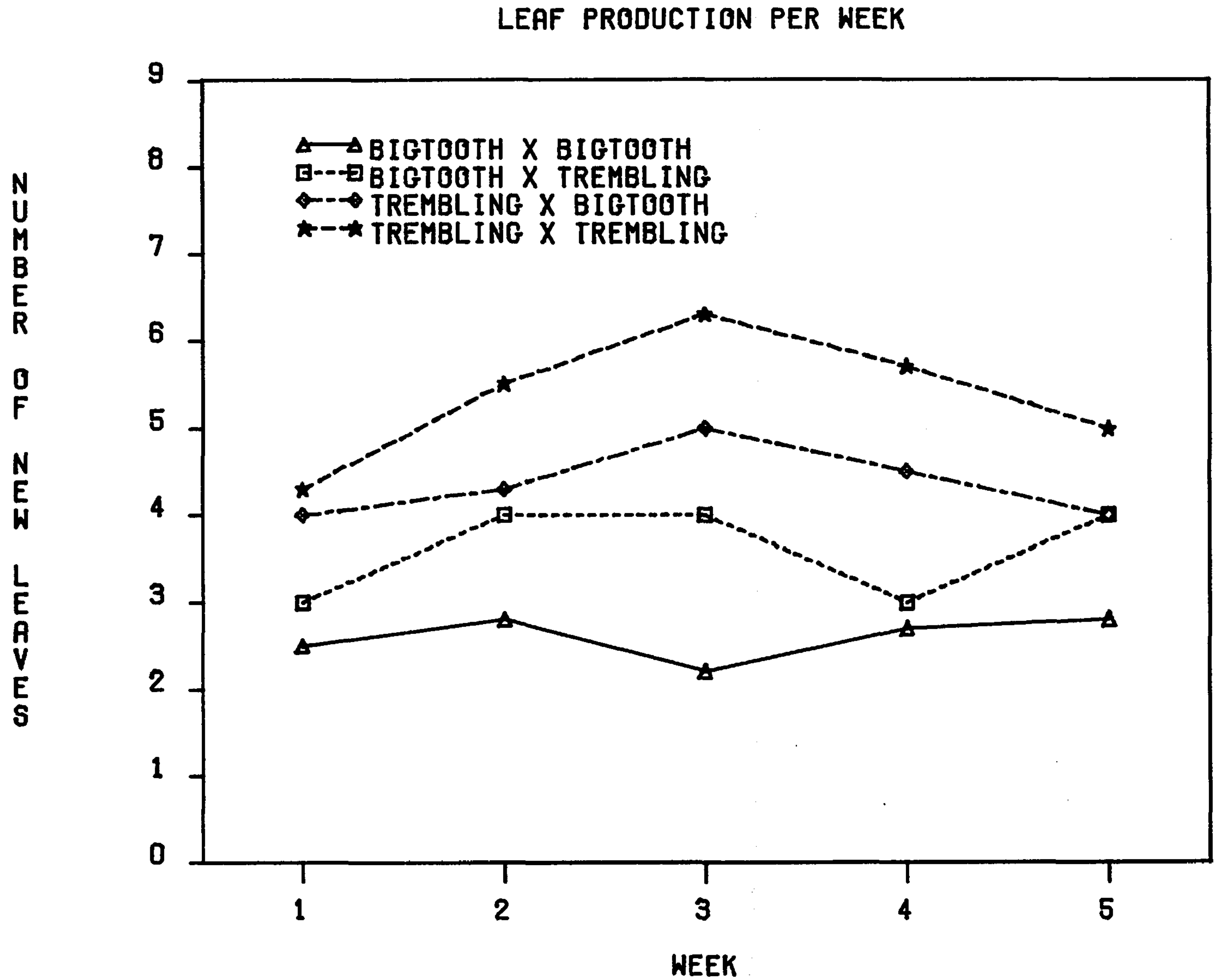

Figure 4.2. Weekly apical leaf production of hydroponically-grown aspen and aspen hybrids.

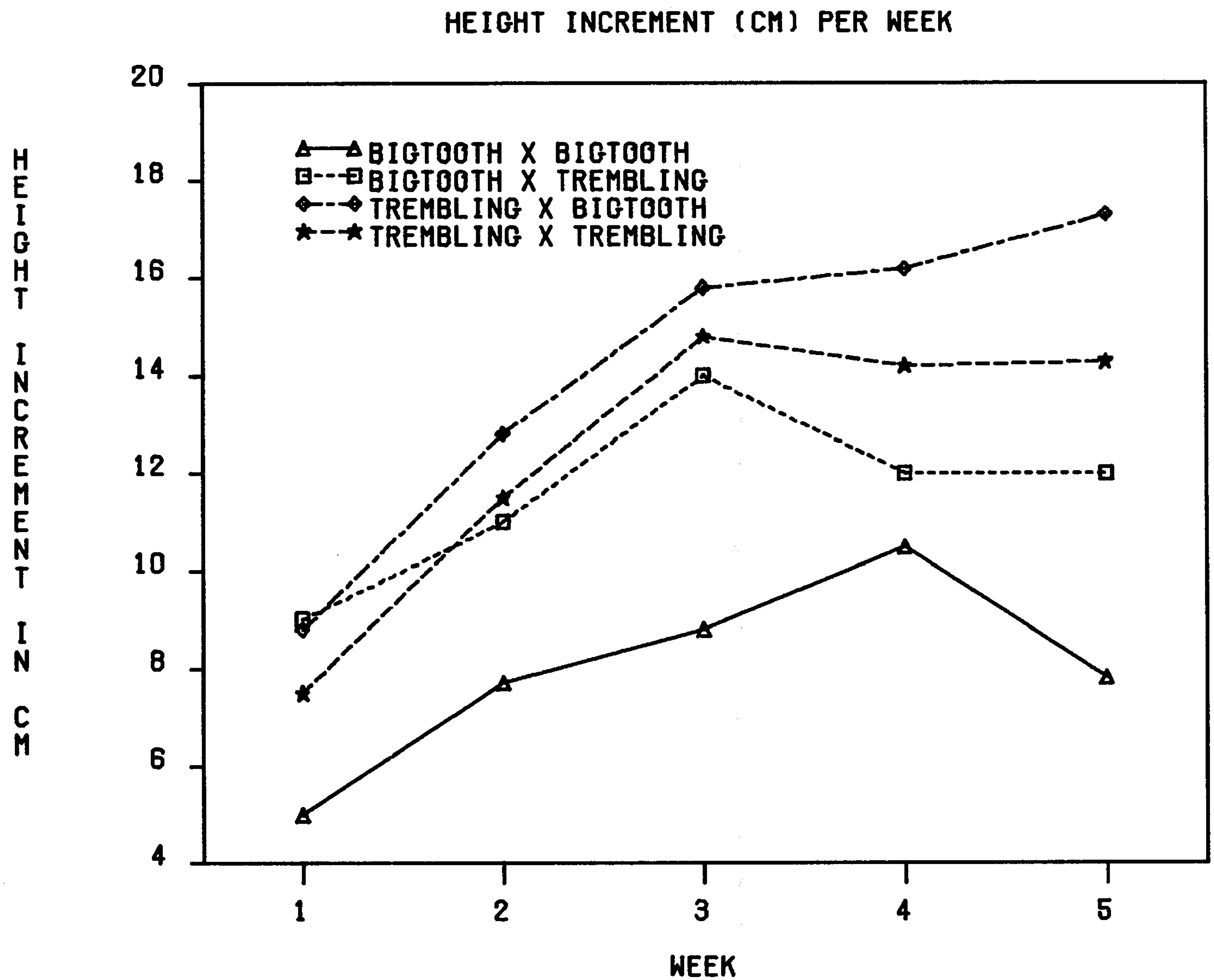

Figure 4.3. Weekly height increment of hydroponically-grown aspen and aspen hybrids.

heterosis in these hybrids, the GxT hybrids also grew better than what additive effects alone could explain. This non-additive genetic variance in height growth further supported the findings of large amounts of non-additive variance in the leaf number and leaf area data.

Primary root production was slightly greater in trembling aspen and the hybrids than in bigtooth aspen (Table 4.2). Genes for primary root initiation in trembling aspen appear to be dominant to those in bigtooth aspen. Weekly root growth (Fig. 4.4) of the primary roots was initially greater in the two hybrids, but by week 5, the rate of root growth in the hybrids had decreased to that of the two species. Root growth in the two aspen species remained stable over the five-week-period. There were no significant differences among the species and hybrids in total primary root growth after five weeks.

Table 4.3. Correlations between leaf area and plant dry weight of aspen seedlings grown in the hydroponic system.

	Leaf Area of Each Taxa			
Plant Biomass	*Populus grandidentata*[1]	*Populus Xsmithii*[2]	*Populus tremuloides*[3]	All Species[4]
	r			
Shoot Dry Weight	.92 **	.79 ns	.88 **	.88 **
Root Dry Weight	.92 **	.86 *	.91 **	.88 **
Total Dry Weight	.95 **	.84 *	.97 **	.94 **

*/** Significant at the 5 and 1% levels, respectively.
[1] d.f. = 6.
[2] d.f. = 9.
[3] d.f. = 6.
[4] d.f. = 21.

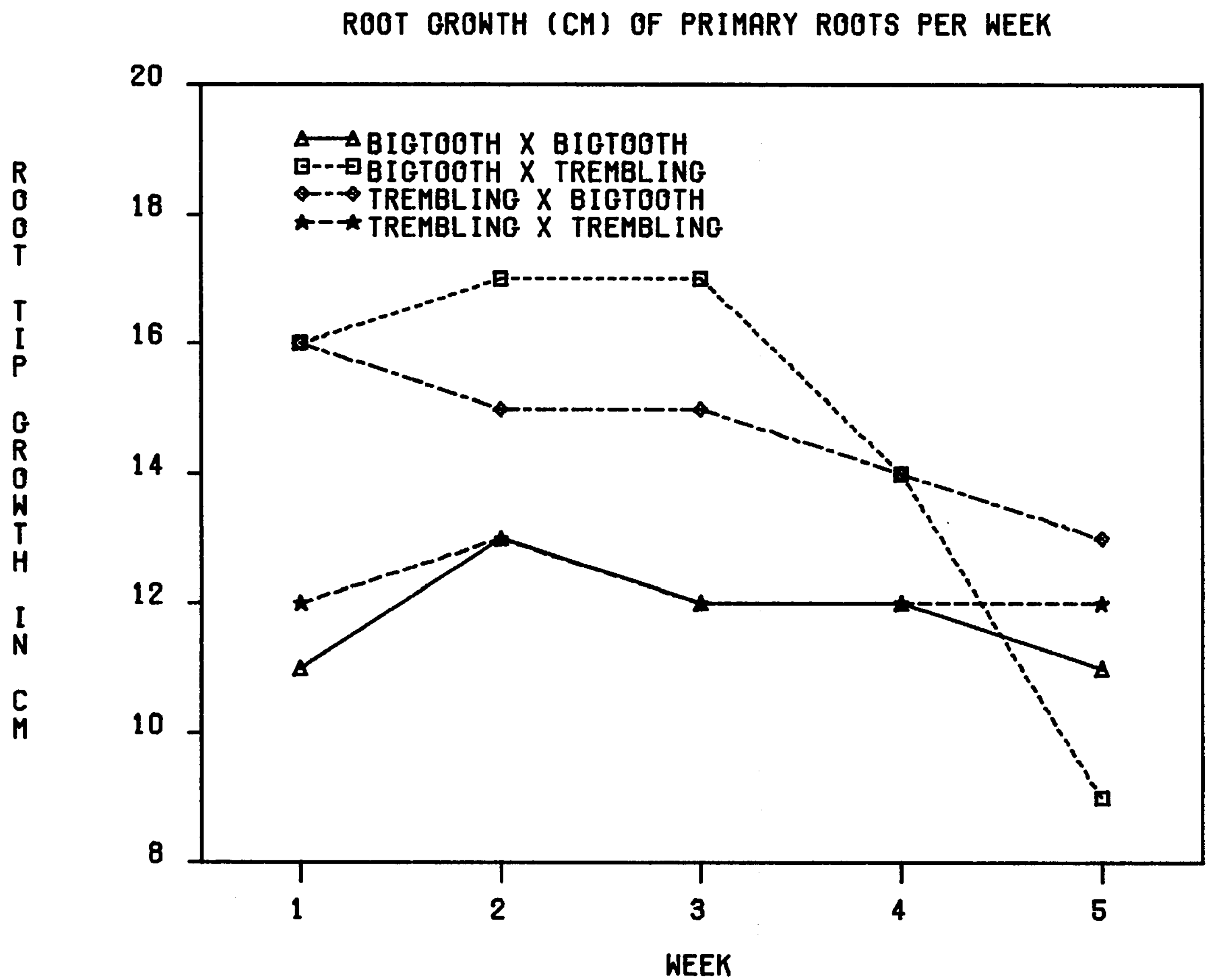

Figure 4.4. Weekly primary root growth of hydroponically-grown aspen and aspen hybrids.

Secondary root production (Table 4.2) was significantly greater in bigtooth aspen and the hybrids than in trembling aspen. In contrast to primary root production, genes for secondary root production in bigtooth aspen were dominant to those in trembling aspen. Secondary root production per week (Fig. 4.5) decreased from weeks 1 to 4 and then stabilized. The density or number of secondary roots per cm of primary root decreased over the five-week-period (Fig. 4.6) and began to level off in week 5. There were no significant differences among the species and hybrids.

The root data showed that trembling aspen produced more primary roots and less secondary roots than bigtooth aspen. However, root growth rates were the same in the two species. Okafo (1976) found similar root growth patterns in bigtooth and trembling aspen seedlings grown in a soiless media. After five weeks, both hybrids in the present study had produced slightly more primary and secondary roots than either bigtooth or trembling aspen, but root production in these hybrids had declined to the rates of the parent species by week 5.

Carbon partitioning (Fig. 4.7) between the stem, leaves, and roots was significantly different at the 1% level among bigtooth, trembling, and hybrid aspen, and the root to stem ratios and root to stem+leaf ratios (Table 4.2) were also highly significant. Bigtooth aspen and GxT hybrids partitioned equal amounts of carbon to the stem and roots. On the other hand, trembling aspen and TxG hybrids

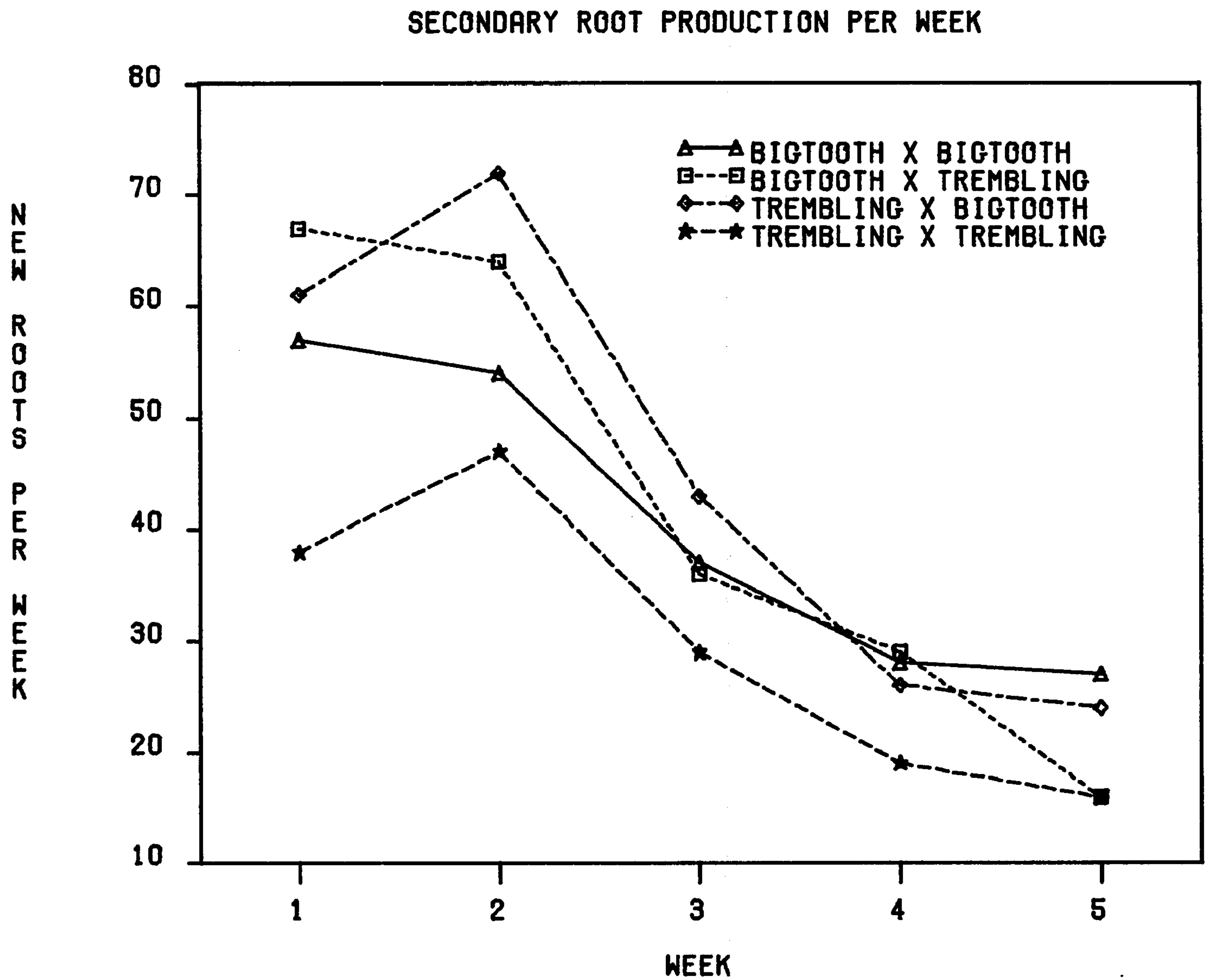

Figure 4.5. Weekly secondary root production of hydroponically-grown aspen and aspen hybrids.

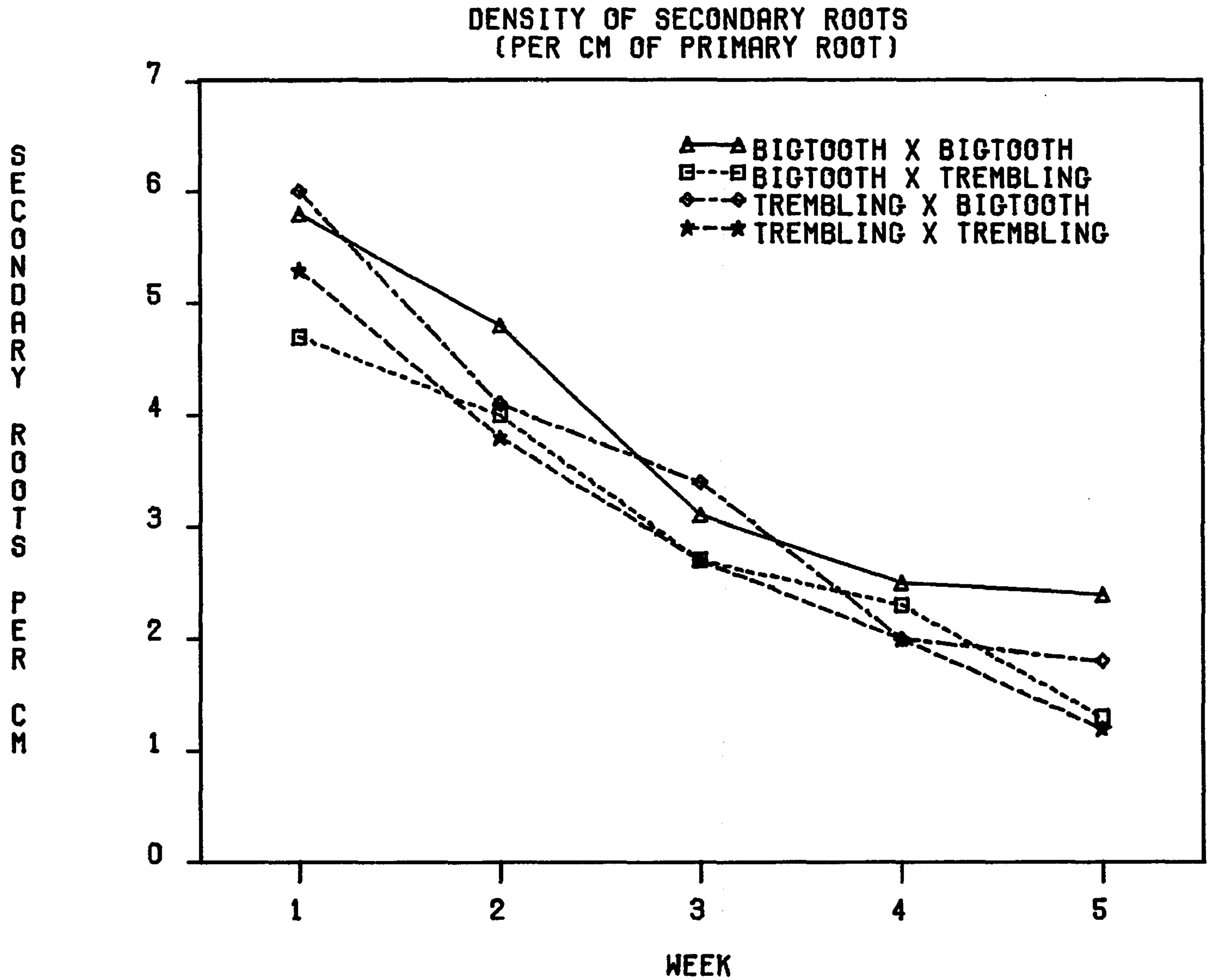

Figure 4.6. Number of secondary roots per cm of primary root of hydroponically-grown aspen and aspen hybrids.

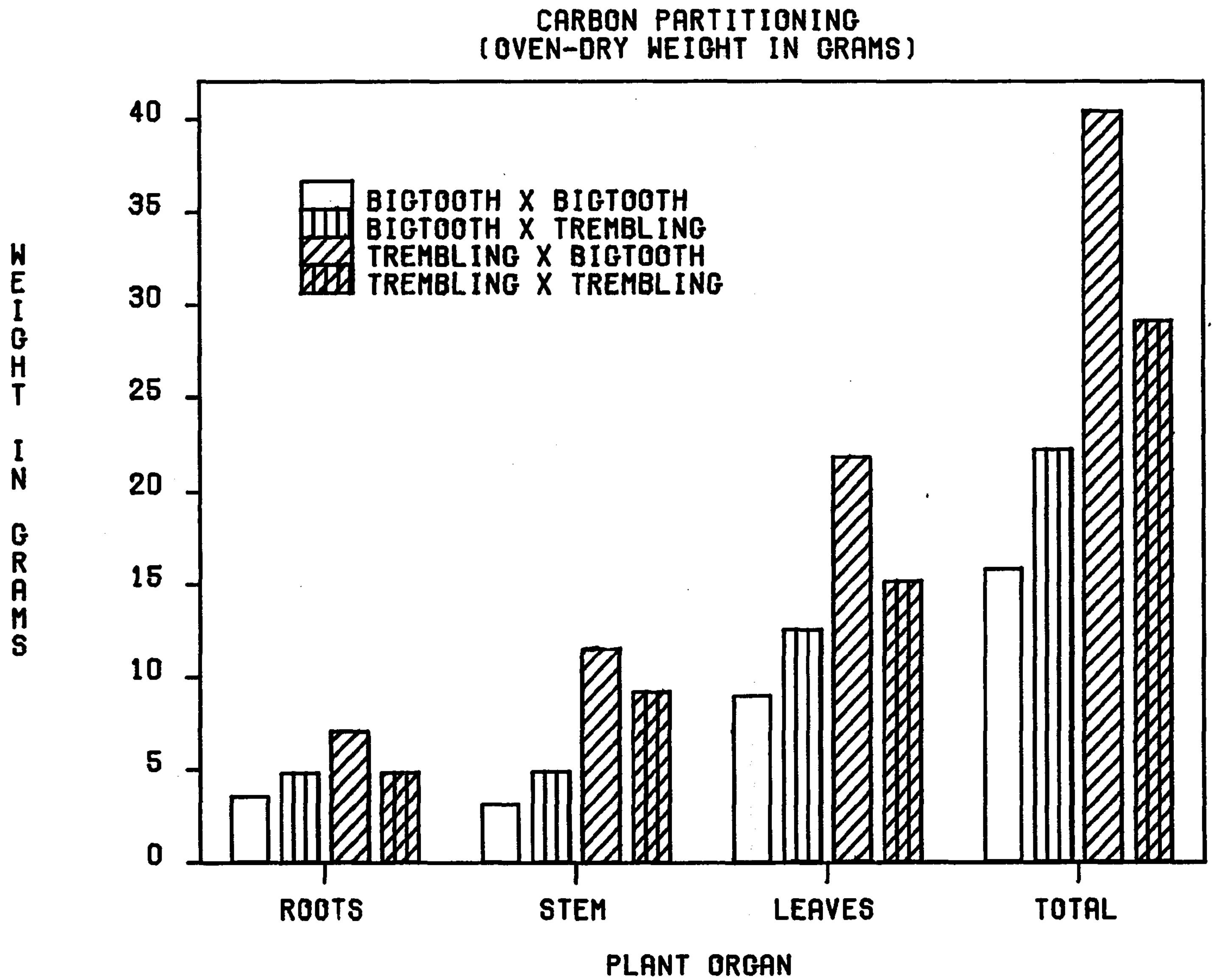

Figure 4.7. Comparative carbon partitioning between the roots, stem, and leaves of hydroponically-grown aspen and aspen hybrids.

partitioned twice as much carbon to the stem than they did to the roots. They also had five times as much dry matter in the shoot than the root. This same shoot to root ratio was 3.5 to 1 for bigtooth aspen and the GxT hybrids. The physiological processes causing translocation of photosynthate to the meristematic sinks appeared to be strongly influenced by maternal (cytoplasmic) effects in the hybrids.

The shoot to root ratios of trembling aspen and the TxG hybrids in this study were comparable to the ratios (6:1) reported by Gordon and Promnitz (1976) for field-grown poplars. A high shoot to root ratio is a desirable trait for intensive poplar culture (Dickmann 1975). If hydroponic studies could accurately detect genetic differences between dry matter partitioning patterns in species or clones, early screening for high shoot to root ratios would be possible. The precision of this test system, however, will depend upon how the genotype x environment interaction affects carbon partitioning in each species or clone.

Differences in the amount of non-additive genetic variance for dry matter production were found in the two hybrids. The TxG hybrids had the largest dry matter biomass, partly because of overdominant effects (heterosis). Under the near optimum growing conditions of the hydroponic system and glasshouse, non-additive genetic variance was expressed in many leaf, stem, and root traits in these hybrids. Non-additive effects were also evident in the GxT hybrids, although no overdominant effects were present. The intermediacy of plant dry weight for the GxT hybrids in relation to the dry weights of bigtooth and trembling aspen indicate additive genetic effects were also involved in carbon assimilation within the hybrids.

Nursery Growth Data

The height growth rankings of the aspen species and hybrids grown in the nursery were identical to those of the indoor physiology studies (Table 4.4) except that the TxG hybrids exhibited no heterosis. Stem dry weight was significantly correlated at the 1% level with height (r=.86) and caliper (r=.91). Excluding caliper, the hybrids were intermediate (additive effects) to the parent species in the growth traits measured, although small maternal effects were present.

Table 4.4. Mean growth data of 40 seedlings for each of four aspen taxa grown in the nursery for one year.

Taxa	Height (cm)	Caliper (mm)	Bud Number	Stem Dry Weight (grams)
Populus grandidentata	57	6.4	7.4	34.5
P.grandidentata X P.tremuloides	67	7.3	9.6	52.5
P.tremuloides X P.grandidentata	74	7.3	11.1	59.0
P.tremuloides	82	7.2	15.3	60.5
Significance of F-Value	**	*	**	**

*/** Significant at the 5 and 1% levels, respectively.

Phenology data (Table 4.5) again demonstrated the intermediacy of the hybrids. However, growth cessation (bud set and leaf fall) in the GxT hybrids was strongly influenced by the bigtooth parent. These non-additive effects were influenced by changes in environmental stimuli and therefore, would not be evident in uniform environments such as those in the physiological studies.

Table 4.5. Mean phenology data of 40 seedlings for each of four aspen taxa grown in the nursery.

	Phenology Characters[1,2]				
Taxa	Leaf Flush (Days)	Bud Set (Days)	Leaf Fall (Days)	Leaf Area Duration (Days)	Stem Elongation (Days)
Populus grandidentata	10	136	187	177	126
P. grandidentata X *P. tremuloides*	8	136	185	177	128
P. tremuloides X *P. grandidentata*	6	140	190	184	134
P. tremuloides	5	146	206	201	141

[1] All phenology characters were significantly different among aspen taxa at the 1% level.

[2] Days to leaf flush, bud set, and leaf fall were based on May 1st as the first day.

Conclusions

Many shoot and root traits in the hybrids from the photosynthesis and hydroponic studies exhibited non-additive genetic variance. Non-additive variance was not as pronounced in the nursery-grown hybrids. The controlled environments appeared to enhance the non-additive effects which probably contributed to the differences in the species rankings for plant dry weights over the three studies. A large portion of this growth variation might be attributable to environmental lighting because trees from the photosynthesis study had "shade-leaves", in contrast to trees with "sun-leaves" in the hydroponic and nursery studies. This situation probably confounded the results.

The results from these three studies do indicate that height growth differences between aspen species can be evaluated early with basic physiological studies; however, using specific physiological processes (ie., net photosynthesis) to screen for potential growth rate in aspens was not found to be effective. The utility of these basic studies to select fast-growing trees was probably dependent upon the amount of additive and non-additive genetic variance inherent in each trait. Field tests of P. grandidentata, P. tremuloides, and P. Xsmithii have shown additive effects for height growth, but non-additive effects for specific morphological and physiological traits (Reighard 1984). Non-uniform (natural) environments may neutralize or reduce epistatic and cytoplasmic effects, thereby, making it difficult to use data from controlled environmental studies for projecting species growth potential in natural environments. Before designing physiological experiments to determine growth potential in aspens, a thorough understanding of how environmental conditions affect gene expression should be gained.

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CONCLUSIONS AND RECOMMENDATIONS

Successful establishment of plantations of aspen (*Populus tremuloides*, *P. grandidentata*, *P. Xsmithii*) on old-field sites in Michigan was achieved when good weed control without excessive herbicide damage were combined. Deep-planting aspen seedlings 15 cm above the root collar was not deleterious and reduced herbicide damage. However, at some plantations, herbicide injury, deer browsing, and *Saperda* damage significantly affected survival and growth during the first two years. More research on the toxicity of pre-emergent herbicides to Section Leuce species and soil-herbicide interactions in non-agricultural soils are needed. Furthermore, aspen should not be planted on sites where seedlings are not vigorous enough to outgrow deer and *Saperda* damage within two to three years.

Significant genetic variation in growth rate was found between and within families of *P. tremuloides*, *P. grandidentata*, and the hybrids. The 20 best families (top 10%) averaged 25% above the mean for both height and diameter growth after two growing seasons in the field. Age-age correlations between ages one and three were moderate but significant. Therefore, if short-rotation plantations are the objective, some gain in yield may be possible by selecting the best families and individuals. However, Upper Peninsula trembling and bigtooth aspen families planted in southern Lower Michigan stopped growth earlier than Lower Peninsula families because of ecotypic differences associated with photoperiod and temperature. These ecotype differences (genotype x environment interaction) between planting sites should be considered if the geographic distances are greater than 325 km.

Additive genetic variance was found for height, volume, and other growth-related traits in the aspen progeny test. Mating designs (ie., North Carolina Design 2) that identify additive effects would be useful and further clarify inheritance patterns. Most non-additive genetic variance was found in morphological traits. Hybridization of *P. tremuloides* and *P. grandidentata* did not produce heterotic progeny except in the hydroponic system. Future species hybridization studies in the aspens should first concentrate on selecting parents with good combining ability (additive genetic variance) for important economic traits before implementing the hybridization phase.

The photosynthesis and hydroponic studies indicated that the growth performance of the hybrids ranged from average to superior when compared with the parent species. The field studies, however, showed that most hybrids were no better than intermediate in growth to trembling and bigtooth aspen. These early results suggest that *P.* X*smithii* does not promise to be a commercially valuable hybrid. In contrast, the growth performance of the trihybrid (*P.* X*rouleauiana* X *P. tremuloides*) and unpublished data of the F_1 *P.* X*rouleauiana* hybrid strongly suggest that these hybrids should be emphasized in future hybridization studies. To develop a breeding program using these hybrids, more germplasm of both *Populus alba* and *P. grandidentata* is necessary because of the relatively narrow genetic base for both species in the current progeny tests. In addition, controlled matings between selected families of trembling aspen should be initiated because of the excellent growth of this species in all plantations. A trembling aspen improvement program would be particularly useful for sites that are ecologically unsuitable for *P.* X*rouleauiana* hybrids. The quantity of genetic variation, the ease of controlled pollinations, and the inherent early flowering in the aspens provide ample mechanisms for producing genetically-improved seed sooner than most Michigan tree species.