NATURAL VARIATION IN SPECIFIC GRAVITY AND FIBER LENGTH IN POPULUS TREMULOIDES CLONES[1]

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Natural variation in the specific gravity of wood and fiber length can be attributed to the effects of two factors genotype and environment. In most species the relative contribution of these factors cannot be estimated in natural stands. Aspen, however, very commonly reproduces vegetatively by means of root sprouts, establishing clones, i.e., colonies of trees of the same genotype Variation among trees in a clone is entirely due to the environment, while differences between clones are caused by both the genotype and the environment.

Van Buijtenen et a1. (1959) concluded on the basis of their study of variation in specific gravity and fiber length of aspen clones that the environment was more important than the genotype in the determination of each of these wood characteristics. Their estimates could, however, represent an overestimate of the environmental effects due to the large variation in stem diameter present not only between clones, but also within clones. Since their estimate of the genetic effects was based on the determination of environmental variance, this would then be underestimated.

The purpose of this study is to determine the effects of the environment on specific gravity and fiber length in trembling aspen (Pop ulus tremuloides Michx.). Four clones were selected for intensive study. Specific gravity and fiber length determinations were made on annual rings across the radius of the stem of trees selected from each clone. By this procedure the variation across the stem can be compared among the members of a clone to provide information on the environmental effects on these traits Information on the role of the geno type may be inferred from comparisons of clones. Reliable estimates of the genotypic component of phenotypic variance, however, can only be obtained from replicated field plantings.

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MATERIALS AND METHODS

Field Survey for Putative Trembling Aspen Clones.--Abandoned farmlands in the vicinity of Syracuse, New York, were surveyed for small discrete groups of trembling aspen, each potentially a clone. The shape of the clumps was to roughly approximate a cone in outline since it was thought that such a group would more likely represent a clone originating from a single seed in the invasion of these areas by pioneer species. The largest tree, whose crown formed the apex of the conical outline, would presumably be the "parent" tree with the smaller surrounding trees having arisen from sprouts from the "parent' tree root system. In this hypothetical model of a clone, the height of a given tree in relation to the other members of the group might be indicative of the time when it arose.

Selected Clones. The five putative clones selected for study and their locations are as follows:

Clone 1. Three Rivers Game Management Area, Baldwinsville, New York, approximately 13 miles northwest of Syracuse.
Clone 2. Albanese Lumber Co. land, Apulia Road, Jamesville, New York, approximately 8 miles south of Syracuse.
Clone 3. S. B. Silverborg Farm, Colton Road, Lafayette, New York, approximately 11 miles south of Syracuse.
Clone 4. S. B. Silverborg Farm, Colton Road, Lafayette, New York, approximately 150 yards east of Clone 3.
Clone 5. Alfred Novack Farm, Kirkville-Bridgeport Road, Minoa, New York, approximately 10 miles east of Syracuse.

A map was prepared for each putative clone showing the relative position of all trees with a minimum stem diameter of about 1.5 inches at a height of three feet above ground level. Height measurements were taken for each of these trees, and the sex conditions., i.e. 'male" (staminate flowers) or "female" (pistillate flowers), was determined for those that produced floral buds.

Since P. tremuloides is a dioecious species, members of a clone should ex hibit the same kind of flowers, either all male or all female. The sex condition was determined by the examination of floral buds in the laboratory using a dissecting microscope. The results demonstrated that putative Clone 1 was composed of at least two different clones since some of, the trees produced staminate flowers and others pistillate. This group was therefore discarded. The sex conditions of the trees in each of the other four clones were in concordance. Of the 17 trees in Clone 2, the 9 producing flower buds were all male. In Clone 3, 14 of the 15 trees that could be classified were male, and 13 of the 18 trees in Clone 1 were female. It should be noted that Clones 3 and 4 are only 150 yards apart, hence could represent fragments of a single clone. This difference in sex condition, however, removes this possibility. In Clone 5 only 7 of the 17 trees produced flower buds. These were all male.

In each of the remaining four putative clones, a sample of at least nine trees, representing the range in stem diameter and the distribution in the clone, was selected for analysis. Those with more than a 5 degree lean were not included, The numbers of trees sampled in each of Clones 2, 4, and 5 were ten, and in Clone 3, nine. It should be noted that the seven trees in Clone 5 classified for sex conditions were among the 10 sample trees analyzed.

A summary of the ranges in minimum diameter three feet above ground level, height and age of the maple trees to be analyzed is as follows:

Putative Clone	No. sample trees	Range in Min. diam. in inches 1/	Height in feet	Age in years 1/
2	10	2.0 to 7.8	16 to 38	17 to 36
3	9	1.7 to 5.1	16 to 50	9 to 18
4	10	1.4 to 7.1	17 to 52	5 to 29
5	10	2.0 to 6.5	19 to 40	6 to 11

1/ At a height of three feet above ground level.

Criteria for Establishing the Clonal Nature of Selected Groups. The two methods employed to establish that these groups of trees were indeed clones are (1) the sex condition which was just presented, and (2) similarities in leaf characteristics.

The leaf characteristics for comparisons include the petiole length and the blade tip angle, base angle, length, width and number of serrations per centimeter, The methods employed in the sampling measurement and analyses of these traits are similar to those described by van Buijtenen, Einspahr and Joranson (1959).

Leaf samples were selected as follows: Four branches were taken from the middle of the crown, one from each of the exposures, north, east, south and west. Eight leaves were then sampled from each branch, the second and fifth from the tip of each of the terminal four lateral twigs of each branch. The total leaf sample for each tree, therefore, is 32.

There were obvious differences in size among the leaves composing a sample which appeared to be due to differences in the amount of expansion of the blade. This, while not affecting the tip and base angle measures, would result in a large variation in blade length and width among the sample leaves of a tree. Since true differences between trees would be indicated in a statistical test by a greater variation between leaf samples than within the samples, a large heterogeneity due to this difference in size might obscure a real difference that might be present if one of the putative clones were composed of two or more clones. If a given blade shape, however, is a clonal characteristic and if this variation among the leaves of a sample does represent differences in blade expansion, the ratio of the length of the blade to its width should characterize a given blade shape and be constant for a clone. This ratio could then be used for comparisons between trees to establish the clonal nature of each of these groups.

The correlation of blade length and width was determined for the leaf samples from six trees selected at random from putative clones 2-5 to determine whether a relationship exists between these leaf blade measures. The values of the resulting correlation coefficients ranged from +0.879 to +0.956 and were statistically highly significant. It was concluded that a real relationship exists between these characteristics and that the use of this ratio, which minimizes variation within trees, would result in a more reliable comparison of the trees composing the putative clones.

Sampling of Wood and Laboratory Analysis Samples. A ten millimeter increment borer was used to obtain diameter cores of wood at a height of three feet above ground level. The occurrence of rot or branch stub at the three foot height necessitated sampling at heights of 4 feet 3 inches. In four trees, 3 feet 6 inches in one tree, 2 feet 9 inches in a sixth tree and 2 feet 6 inches in a seventh tree. The core was extracted across the minimum diameter of each tree at this height to reduce the possibility of including tension wood in the sample (Brown et al. 1949). Trees with less than a three-inch diameter at this height had to be felled and cross-section discs cut because of splitting caused by the large diameter of the borer. The samples were stored in a 0.5% solution of Dowicide G[1] under refrigeration to reduce deterioration by microorganism.

Each core was examined in the laboratory, and the width of each annual ring was measured using a calibrated dissecting microscope at a magnification of 15X. Each annual ring from the pith to the cambium from the small radius of the diameter core was then separated for specific gravity and fiber length determinations. Extensive rot in certain cases required use of the greater radius.

Specific gravity was calculated by the "maximum moisture content method" as outlined by Smith (1955). The density of the cell wall substances (Gso) of trembling aspen a constant necessary for the use of this method, was estimated according to the procedure described by Valentine (1961). This value was obtained for each of 19 annual rings selected at random from four sample trees, one from each of Clones 2 through 5. The mean of these values, 1.502 was then used in the calculation of specific gravity. In addition to this constant, the oven dry weight and completely saturated weight of the samples are required for this method Standard procedures were followed in obtaining these weights The entire annual ring was used as the wood sample for specific gravity determinations.

Wood samples composed of alternate annual rings from the pith to the cambium from two trees in each of the four clones were macerated for the fiber length measurements according to the Schedule No. 3 of Spearin and Isenberg (1947) using sodium chlorite and acetic acid. The samples of separated wood elements were stained with Bismark Brown and stored in 70% ethyl alcohol. A semi-permanent microscope slide mount was prepared for each sample of the stained cells mounted in glycerin jelly.

Fiber length measurements were made using a Seibert-Wetzlar projection microscope and a rule calibrated so that a direct reading in millimeters could be made from the projected image. One hundred fibers were selected at random for measurement from each miscoscope slide mount. The mean value for these fiber lengths is used as the unit of analysis for these data.

Statistical Analyses. Standard statistical procedures as given in Dixon and Massey (1957), Federer (1955), Snedecor (1957) and Steele and Torrie (1960) were used for the analyses in this study.

[1] Sodium pentachlorophenolate and a small percentage of other chlorinated phenolates.

RESULTS

Leaf Measurements Leaf measurements were tested by analysis of variance in a clonal, two-way classification by trees and by aspect for each type of measurement. The pooled values for the eight leaves from a given aspect were used as the unit of analysis to minimize the number of missing values that had to be estimated These missing data were due to a lack of branches for one of the exposures of the crown because of suppression by neighboring trees or to a deficiency of undamaged, intact leaves among those sampled from a given branch. The numbers of missing values in each classification include one in Clone 4, two in each of Clones 2 and 5, and five in Clone 3. Since three of the missing data in Clone 3 represented the north exposure, the analysis for this clone is based on the other three aspects leaving only two values to be estimated. It is assumed that the loss in sensitivity due to the decrease in numbers of samples would be less than that incurred by the estimation of three missing values, all from the same aspect of the crown of the trees concerned.

The leaf measure, numbers of serrations per centimeters is not used to test for differences between the leaf morphology of the trees. It is felt that these data are not too reliable because of irregularities in the general curvature of the leaf and frequent damage or disease of the margins which caused difficulties in determining this measure.

The results of the analysis for differences between the aspects within trees and between trees within clones for the remaining four traits are presented in table 1. None of the differences obtained between aspects within trees is statistically significant. Differences that are statistically significant are found, however, in eight of the 16 comparisons between trees of a clone, two for each of the leaf characteristics. The "Q" test was employed to determine the source of this large variation. The results of this test indicate that in only one case, petiole length in Clone 2, does one tree differ, at the 5% level of significance from all others in its clone. This particular tree, Tree H, also contributed to the variation in base angle--it differed from four other trees for this trait . It should be noted, however, that Tree H does not differ from the other members of its clone in blade length to width ratio or blade tip angle. In only two other cases does a given tree differ from one to four other trees in its clone for two characteristics, Tree F in Clone 3 and Tree C in Clone 4. Differences in these traits between each of these trees and the other six to nine members of their respective clones, it should be emphasized, are not significant.

To summarize these results, it should be noted that:

(1) In the tests for differences between trees in each clone, the variation is not significant for at lease one of the four traits
(2) In Clone 5, none of the differences is statistically significant.
(3) The variations which are significant can be attributed to one or two trees in the clone which differ from one to four other members of their particular clone, with the exception of Tree H in Clone 2 which differs in petiole length from the other nine trees.
(4) The vast majority of the large number of paired comparisons of trees within clones for each of the four traits, as revealed by the "Q" test, is not statistically significant.

Table 1.--Analysis of variance for leaf measurements between trees and aspects within clones.

Clone no.	Source of variation	D.f.	Mean square: Ratio blade length:width	Mean square: Blade tip angle	Mean square: Blade base angle	Mean square: Petiole length
2	Between trees	9	0.06	324.24*1/	955.88*3/	47.95**2/
	Between aspects	3	0.09	260.20	338.50	3.19
	Error	25	0.03	104.30	202.82	6.06
3	Between trees	8	0.21**3/	877.77*1/	1193.71	55.24
	Between aspects	2	0.03	44.43	320.36	38.35
	Error	14	0.04	262.42	656.70	34.63
4	Between trees	9	0.39**3/	46.29	1304.18**3/	61.89*1/
	Between aspects	3	0.14	115.10	872.28	7.06
	Error	26	0.09	687.85	272.97	18.70
5	Between trees	9	0.26	133.33	438.04	30.52
	Between aspects	3	0.11	31.71	163.63	9.97
	Error	25	2.62	141.85	398.10	11.62

* Significant at the 5% level of probability.
** Significant at the 1% level of probability.
1/ "Q" test shows one tree differed from three others in its clone.
2/ "Q" test shows one tree differed from all others in its clone.
3/ "Q" test shows two trees each differed from one to four other trees in the clone.

Table 2.--Analysis of variance for leaf measurements between clones and the mean leaf values for each clone.

Leaf measures	Source of variation	D.f.	Mean square	Mean leaf value in clone 2	3	4	5
Length-width	Between clones	3	9.00**	1.14	1.04 1/	1.02	1.29
	Between trees within clones	32	0.07				
Tip angle in degrees	Between clones	3	2753.03**	44.0	37.7	39.9	53.1
	Between trees within clones	32	186.87				
Base angle in degrees	Between clones	3	30091.99**	13.7	10.5	0.4	4.8
	Between trees within clones	32	317.60				
Petiole length in cm.	Between clones	3	164.00**	4.5	5.7	4.9	4.7
	Between trees within clones	32	15.71				

** Significant at the 1% level of probability.
1/ Underlined values indicate clones in which the differences are not significant at the 5% level of probability according to the "Q" test.

The mean of the pooled values of nine trees per clone was used to compare differences in leaf measurements between clones Nine trees were selected at random from Clones 2, 4, and 5 since there were only nine sample trees in Clone 3. This allowed differences between clones to be found by the "Q" test The results are presented in table 2. The mean value given for each of the leaf measurements is the clonal mean of the unit of analysis (the pooled value of the eight leaf measurements per aspect) divided by eight so that these measures are expressed as the average value per leaf.

The differences between clones for all four leaf measures are statistically highly significant The "Q" test results are also shown in this table, the underlined mean leaf values indicate the clones in which the difference is not significant at the 5% level. A comparison of the mean leaf values clearly reveals this lack of difference. It should be noted that among the six possible paired-comparisons of clones for each of three traits, only one is not significant. In the comparison of petiole length, however, none of the comparisons are significant except those involving Clone 3 This clone differs from the other three clones for this trait.

It is concluded on the basis of these tests and from the results of the classification of floral buds for sex conditions that each of these four "puta tive" clones is indeed a separate clone.

Specific Gravity. Specific gravity determinations for each annual ring from the pith to the cambium free of severe rot were obtained for each sample tree in each clone Because of the large numbers of these determinations, the results are presented in summary form. The general pattern of variation in specific gravity across the radius of the stem at the sample height, 36 inches above ground level, is as follows. The innermost ring or two has a high specific gravity. There is a rather sharp decrease in the successive rings, up to about a distance of 1.5 cm. from the pith. This is followed by large increases up to a distance of approximately 4 cm. Thereafter the rate of increase drops, so that the specific gravity values for successive rings to the cambium exhibit only slight if any increase.

A summary of the results obtained for the specific gravity determinations, rate of growth and age for each sample tree in each clone is presented in table 3 The left half of the table provides information based on the smaller radius of the wood core with the trees ranked from the highest mean specific gravity to the lowest. This value is based on the determinations obtained for the individual annual rings. A small number of rings that were included exhibited discoloration. The right half of the table shows the ranking of these same trees based on the mean specific gravity for groups of successive annual rings free of discoloration and rot that are common to all members of a given clone This second method for presenting these results is to minimize the effects on the mean specific gravity caused by variations in diameter of the trees as well as to eliminate rings showing discoloration. It should be noted that the segments are common for only eight of the ten tres in Clone 4 and seven of the ten in Clone 5 Though this does sacrifice trees in these two clones, it was felt that the larger common segment among the remaining trees allows for a more reliable comparison than would be the case for smaller segments common to all trees. The values given for the mean distance of the segments from the pith for each tree indicate the relative rates of growth of the trees in a given clone during this common period. It should be noted that there are a number of changes in rank of trees in each clone when common segments of the core are considered compared with results obtained from the entire radius of the core.

Table 3.--Summary of specific gravity and rate of growth determinations for trees in each clone based on individual annual ring analyses of wood samples that include (1) the minimum core radius and (2) groups of successive growth rings common to members of a clone.

		(1) Minimum radius of core				(2) Common segment of core				
Tree no.	Age	Radius in cm.	Mean rings per cm.	Specific gravity Mean	Specific gravity Range	Tree no.	Rings from pith	Distance 1/ Mean	Distance 1/ Range	Mean specific gravity
					Clone 2					
C	34	8.22	4.13	0.461	.417-.544	J	10-17	224	164-283	0.479
J	21	5.11	4.11	0.457	.414-.502	C	"	218	136-301	0.466
A2/	36	8.12	4.43	0.442	.400-.550	A2/	"	221	127-315	0.459
F	20	4.31	4.64	0.439	.395-.480	F	"	180	125-234	0.454
E	19	3.59	5.29	0.437	.402-.462	B	"	256	152-361	0.453
H	17	2.26	7.52	0.426	.376-.490	K	"	246	177-316	0.448
B	35	8.31	4.21	0.423	.359-.478	E	"	160	108-213	0.442
K	20	5.47	3.66	0.419	.369-.520	I	"	182	131-232	0.427
G3/	19	3.09	6.15	0.412	.364-.441	H	"	114	81-146	0.422
I	20	4.15	4.82	0.410	.356-.468	G3/	"	146	107-184	0.420
					Clone 3					
D2/	17	3.01	5.65	0.394	.348-.450	G	1-9	55	0-110	0.391
G	9	1.72	5.23	0.391	.354-.430	D2/	"	56	0-113	0.369
A	18	6.59	2.73	0.376	.335-.422	I	"	72	0-143	0.364
C2/	15	3.87	3.88	0.366	.324-.414	C2/	"	66	0-131	0.357
I	9	2.23	4.04	0.364	.335-.403	H	"	66	0-131	0.354
E2/	17	4.20	4.05	0.360	.308-.421	A	"	96	0-192	0.353
H	9	2.04	4.41	0.354	.337-.377	E2/	"	70	0-139	0.340
F2/	17	4.76	3.57	0.345	.298-.389	F2/	"	92	0-184	0.333
					Clone 4					
E	12	1.36	8.82	0.432	.361-.495	E	5-11	59	33-85	0.418
F	16	3.80	4.21	0.415	.372-.461	B	"	82	38-126	0.413
D	15	4.90	3.06	0.414	.387-.476	F	"	98	35-160	0.409
A	29	7.40	3.92	0.410	.366-.483	D	"	152	80-223	0.407
C	15	1.94	7.73	0.406	.376-.463	C	"	66	29-102	0.396
B	17	2.59	6.56	0.404	.381-.433	A	"	84	35-124	0.396
G	18	5.40	3.33	0.393	.333-.415	I	5-9	139	89-189	0.392
I	9	2.95	3.05	0.391	.366-.437	G	5-11	135	57-193	0.390
H3/	11	4.15	2.65	0.376	.337-.439	J	5	133	125-141	0.366
J	5	2.20	2.27	0.362	.346-.383	H3/	5-11	166	67-266	0.355
					Clone 5					
C2/	7	4.56	1.54	0.386	.337-.423	C2/	3-6	163	72-254	0.377
G	6	2.50	2.40	0.378	.355-.401	G	1-6	80	0-160	0.368
H	8	4.17	1.92	0.378	.352-.441	H	3-6	122	32-213	0.366
I	6	2.39	2.51	0.374	.336-.443	A	1-6	119	0-238	0.363
E	6	1.89	3.17	0.372	.343-.450	D	"	116	0-231	0.363
A	11	7.64	1.44	0.371	.342-.401	I	"	76	0-153	0.346
D	7	3.83	1.83	0.363	.338-.437	E	"	110	0-221	0.345
F	6	2.97	2.02	0.356	.328-.400	B	"	117	0-234	0.344
J2/	8	4.39	1.82	0.353	.323-.383	J2/	4-6	153	88-219	0.338
B	8	5.15	1.55	0.344	.314-.392	F	1-6	95	0-190	0.337

Footnotes on p. 33.

A multiple regression analysis was employed to determine whether the variation across the radius is related to (1) rate of growth as measured by ring width and (2) distance from the pith. Only the older trees in each clone which were free of rot and discoloration were used in this analysis with the following exceptions. In Clone 5 all trees were used and in Clone 4, because the only suitable trees showed signs of rot, the specific gravity values for affected rings were omitted from the analysis. A second regression analysis was completed on the same trees but omitting all high values for specific gravity that occur in the first few rings nearest the pith in Clones 3, 4, and 5. The results of these analyses are presented in table 4. It should be noted that all regression coefficients for density on distance are nonsignificant, but all are positive and increase in value in the second regression analysis The ring width to density coefficients are all significant at the 1% level of probability. In Clones 2 and 4, the values are positive; in Clones 3 and 5 they are negative.

In testing for differences in specific gravity between clones and between trees within clones, two different analyses were completed. The first was based on trees whose minimum radius in the sample core was 3.0 cm. or larger, and the segment of the core compared included the annual rings wholly or partially within a range of 1.5 to 3.0 cm from the pith. The mean values per tree were used to test for differences between clones. This analysis was performed because of the slight positive regression of density on distance from the pith. The second test was based on the annual growth rings produced during the same five year period, 1954 through 1958 The results of these two analyses are presented in table .It should be noted that the variation between trees within Clones 2, 3 and 4 is highly significant in both tests. The variation between clones, in each case, is also highly significant In neither analysis, however, is the variation between trees in Clone 5 significant.

Since members of a clone are genetically identical, differences between individuals should be entirely environmental. The variations in wood, such as the differences in specific gravity that occur across the radius of the stem, and the relationship between specific gravity and rate of growth, complicate the choice of wood samples for comparisons which reflect only the environmental effects. This is particularly difficult when the experimental material consists of natural clones in which there is not only a large variation in diameter and age, but also a large range in the environmental conditions within the clone Conclusions based on this kind of material therefore, must be general. Even though there was an attempt to minimize the effects of certain of these factors in analyzing the variation between trees within a clone, the differences are still large, indicating a strong environmental effect on specific gravity.

The variation between clones represents contributions due to the environment, genotype and the interaction of these factors. Since the four clones occur in three different areas, with, no doubt, rather large environmental differences between them, the statistically highly significant differences between clones is not surprising. Clone 3 and 4, however, occur approximately 150 yards apart on adjoining sides of an old field The environmental differences for these clones would be expected to be less than for any of the other

Table 3 footnotes.

[1]Linear measurements are given in units which multiplication by 0.01562 converts to centimeters

[2]Wood sample taken at 2'9" for Tree 2A, 4'3" for Trees 3C and 3F, 3'6" for Tree 5C and 2'6" for Tree 5J.

[3]Denotes a tree where the greater radius of the wood sample core was used in specific gravity determinations

five possible pair combinations of these four clones. A larger proportion of the variation between these two clones, therefore, would be expected to be due to the genotype and genotype-environment interaction.

Table 4.--Multiple regression analysis of specific gravity (X) on ring width (Y) in cm. and distance from the pith (Z) in cm.

The regression equation is: $\hat{X} = \bar{x} + b_{xz.y} (Z - \bar{z}) + b_{xy.z} (Y - \bar{y})$

Clone 2 Trees A, B, C, G, and I:

(a)1/ $\hat{X} = 0.395 + 0.0016 Z + 0.147 Y^{**}$

Clone 3 Trees A, D, E, and F:

(a) $\hat{X} = 0.371 + 0.0099 Z - 0.096 Y^{**}$

(b)2/ $\hat{X} = 0.338 + 0.0205 Z - 0.077 Y^{**}$

Clone 4 Trees A, C, D, F, and G:

(a) $\hat{X} = 0.389 + 0.0012 Z + 0.056 Y^{*}$

(b) $\hat{X} = 0.391 + 0.0017 Z + 0.060 Y^{*}$

Clone 5 All trees:

(a) $\hat{X} = 0.398 + 0.0004 Z - 0.060 Y^{**}$

(b) $\hat{X} = 0.348 + 0.0058 Z - 0.012 Y^{**}$

1/ (a) Using all rings in the wood sample core.
2/ (b) Omitting high values of specific gravity that occur in certain trees in the first few rings near the pith.
** Regression significant at 1% level of probability.
* Regression significant at 5% level of probability.
All ring width coefficients ($b_{xy.z}$) are significant at 1% level of probability.
Distance regression coefficients are too small to be significant.

Table 5.--Analysis of variance to test for differences between trees within clones and between clones in specific gravity of annual growth rings.

(1) Annual growth rings including the distance 1.5-3.0 cm. from the pith

Clone no.	Source of variation	D.f.	Mean Square
	Between trees within clones		
2	Between trees	8	0.0039**
	Within trees	64	0.006
3	Between trees	4	0.0085**
	Within trees	34	0.0007
4	Between trees	5	0.0032**
	Within trees	28	0.0005
5	Between trees	7	0.0003
	Within trees	18	0.0002
	Between clones		
	Between clones	3	0.0116**
	Within clones	27	0.0005

(2) Annual growth rings produced in 1954 through 1958.

Clone no.	Source of variation	D.f.	Mean Square
	Between trees within clones		
2	Between trees	9	0.0046**
	Within trees	40	0.0007
3	Between trees	7	0.0046**
	Within trees	32	0.0002
4	Between trees	7	0.0037**
	Within trees	32	0.0003
5	Between trees	9	0.0011
	Within trees	40	0.0007
	Between clones		
	Between clones	3	0.1035**
	Within clones	28	0.0007

**Significant at 1% level of probability.

The "Q" test was applied to the results obtained in the clonal comparison based on the 1954-1958 samples to determine which clones contributed to the large between clone variance. The use of this test requires the same number of individuals in each group, so that it was necessary to select two trees by random procedures to be eliminated from each of Clones 2 and 5, leaving eight trees, the number in Clones 3 and 4 The results indicate that the differences between only two of the possible six pair combinations of clones are not statistically significant, Clones 2 and 4 and Clones 3 and 5. It may be coincidental, but it should be noted that a positive relationship between ring width and specific gravity was obtained in the regression analysis for Clones 2 and 4, while this relationship was negative for Clones 3 and 5. This suggests that the role of the genotype may be important in contributing to clonal differences, perhaps acting through a control of the effect of rate of growth on the density of the wood Since the clones in each of these pairs are widely separated, the effect of the environment may be of lesser importance. This is supported by the significant difference in the comparison of Clones 3 and 4. Since the environ ment is quite similar for these clones, this difference may be largely genetic and genotype-environment interaction.

The relative importance of the environment, the genotype and the interaction of these factors on specific gravity in trembling aspen cannot be determined from this study. A reliable estimation of their respective contributions can only be obtained from an experiment with replicated field plots of several clones, The general results from this study, however, certainly indicate differences between clones in the genetic control of specific gravity, The large variation between trees within clones does demonstrate that the effect of the environment on this trait is considerable,?

Fiber Length. A multiple covariance analysis was employed to determine whether changes. In the mean fiber length for annual rings is related to the absolute distance of the ring from the pith and to ring width. In order to compare the changes in segments of the increment cores common to all eight trees, alternate rings from the pith to a distance of approximately 3 cm from the pith were used with the following exceptions. The wood samples of the first growth ring for tree D, Clone 3 and Tree G, Clone 5 and the eleventh growth ring for tree A. Clone 4 were either mislabeled or missing and so could not be included. In addition, all rings, numbers 2 through 6, were included for tree G, Clone 5 rather than just alternate ones. The result of this analysis is given in table 6 It should be noted that the variation among trees, adjusted for differences in distance from the pith and ring width, is statistically highly significant The large reduction in error sums of squares due to regression should be noted, a change from the unadjusted value of 0.837 to the adjusted value, 0.151 The analysis of the effect of each of the independent variables in this reduction is given at the bottom of Table 6 The results indicate that the effect of Y (distance from the pith) is highly significant, while that for Z (ring width) is non-significant. The independent variable, Z, was therefore dropped in further analyses, leaving the simple regression of fiber length (X) on distance from the pith (Y).

The results of the covariance analysis of fiber length adjusted for distance of the sample rings from the pith is presented in table 7. The component of the total variation attributable to differences among trees (adjusted) is again highly significant. This is not surprising since the group of eight trees includes two from each of the four clones, hence this could represent genetic as well as environmental differences. The within trees sums of squares was partitioned into the component attributable to deviations among the individual tree

Table 6.--Analysis of Covariance. Mean fiber length per annual ring (X) on distance from midpoint of the annual ring to pith (Y) and ring width (Z)

Source of variation	D.f.	Σy^2	Σz^2	Σyz	Σxy	Σxz	Σx^2	Adjusted X's D.f.	S.s.	M.s.
Total	49	193,734	5586	4886	401.31	1.07	1.131	47	.285	
Within tree (error)	42	185,284	2034	8305	354.91	19.20	0.837	40	.151	.00378
Among trees	7	8,450	3552	-3419	46.40	-18.13	-0.294	7	.134	.01914**

Reduction in error sums of squares due to Y and Z

Source of variation	D.f.	S.s.	M.s.
Regression on Y and Z	2	0.686	
Regression on Z	1	0.181	
Regression on Y after effects of Z removed	1	0.505	0.505**
Regression on Y	1	0.681	
Regression on Z after effects of Y removed	1	0.005	0.005
Error	40	0.151	0.00378

** Statistically significant at the 1% level of probability.

Table 7.--Analysis of Covariance. Mean fiber length per annual ring (X) on distance from the midpoint of the ring to the pith (Y)

Regression equation: $\hat{X} = \bar{x} + b_{xy} (Y - \bar{y})$

$\hat{X} = 0.713 + 0.00192 (Y - 98.6)$
(Y in microscope units; 64 units = 1 cm)

$\hat{X} = 713 + 0.123 (Y - 1.54)$ (Y in cm.)

Source of variation	Adjusted x's in mm.	b_{xy}1/ $X10^{-5}$	Adjusted X's D.f.	S.s.	Tests of significance M.s.	Value of F	D.f.
Total			48	0.300			
Within trees		192	41	0.156	0.0038		
Residual			34	0.121	0.0036		
Regr. Coeff.			7	0.035	0.0050	1.404	7.34
Among trees			7	0.144	0.0206**	5.421	7.41
					Differences, adjusted X's: $s_{\bar{d}}$	Value of t	
Tree 2C	0.678(5)2/	187	5	0.021	.030	2.467*	
2G	0.754(3)	199	8	0.036			
Tree 3A	0.779(1)	144	3	0.006	.035	0.057	
3D	0.777(2)	121	6	0.015			
Tree 4A	0.651(7)	204	5	0.006	.036	0.722	
4H	0.676(6)	168	3	0.033			
Tree 5A	0.612(8)	283	1	0.0004	.045	1.844	
5G	0.696(4)	276	3	0.004			

1/ Based on distance from pith in microscope units, 64 units = 1 cm.
2/ Numbers in parentheses indicate rank.
* Significant at the 5% level of probability.
** Significant at the 1% level of probability.

regression cefficients, and that due to deviations of the individual sample alues for each tree from the regression line for that tree. The result of the Ftest indicates that the variation among regression coefficients is random, that is the regression coefficients are homogeneous. This is indicated by the individual tree bs which are all positive and exhibit a relatively small range n value, 0.00121 to 0.00283. These compare with the within tree or error regression coefficient of 0.00192.

The regression equation for this analysis is also given in table 7. The first equation indicates the predicted changes in fiber length associated with changes in distance of the sample ring from the pith measured in microscope units. These units are read directly from the eyepiece micrometer in the dissecting microscope, with 64 microscope units equivalent to 1 cm. The second equation indicates the expected changes in fiber length in terms of distance from the pith in centimeters, hence is more obviously meaningful. The regression coefficient in this equation indicates an average increase in length of fibers of 0.123 mm. for each centimeter increase in distance from the pith in the segment of the radius included in this study, that is, up to about 3 cm. from the pith.

The difference between the adjusted mean values for fiber length (y) of the two trees from each clone was tested by a t-test to determine whether a "within clone" variation constitutes an important part of the difference among trees. The results are given at the bottom of table 7. It should be noted that only one difference is significant, that for trees C and G of Clone 2. Since the number of samples varies among the trees, a comparison of all 28 pair combination of the eight trees cannot be easily made. The comparisons, therefore, were restricted to ones of more obvious interest, those between members of the same clone. The result, though not definitive, certainly indicates that the variations between members of a clone are random.

This analysis suggests that variations in fiber length attributable to environmental factors are negligible. Since the rate of this change with distance, as indicated by the homogeneity of regression coefficients, is the same for all trees regardless of differences in the "age" of the samples (that is, number of annual rings from the pith), location and site of the clones, these changes appear to reflect an "aging" of the cambium which is more closely related to absolute distance from the pith than age in terms of years of growth. The negligible contribution to the reduction of error sums of squares attributable to the regression of fiber length on ring width shows that rate of growth does not, in itself, affect fiber length variations across the radius of the tree. If it were an important factor and if changes in fiber length were related to years of growth, one would expect heterogeneous regression coefficients. This is not the case.

The regression lines do, however, appear to differ in elevation, with the two members of the same clone having approximately the same elevation. This is indicated by the lack of difference between the adjusted mean fiber lengths of the two trees of each of three clones. The difference in the fourth clone, 0.066 mm, though significant, is not large. These results suggest that real differences exist between clones. The analysis of fiber length variations in three additional trees from each clone, which is now in progress, should clarify this result.

The differences between the trees in Clones 3 and 4, the clones located approximately 150 yards apart, should be noted. The adjusted mean fiber lengths for trees 3A and 3D are the largest in the group, while those for 4A and 4H are among the smallest. Since environmental differences between these clones would be less than between any other two clones, the effects of this factor on fiber length should be less than in any other comparison. The large observed differences, therefore, are no doubt largely due to genetic differences between these clones.

Van Buijtenen et al. (1959), on the basis of their analysis of variations in fiber length in trembling aspen clones, concluded that the genetic control of this trait was relatively unimportant. They estimated heritability., in the broad sense, to be 0.35, which indicates that the major part of the variation is attributable to environmental effects. The present tests do not support this conclusion, but rather indicate that environmental effects are unimportant. Though the comparisons are limited in the present experiment, it is felt that the results suggest that differences in fiber length between members of a clone are small and that real differences are present between clones.

A close look at the results reported by van Buijtenen et al. (1959). reveals a likely basis for the diametrically opposite conclusions from their results and those from the present experiment. Their use of a fiber length "index" as the unit of analysis per tree precludes a comparison of the variations across the radius of the stem in the several trees in each clone. This variation was measured in the present tests, and the comparison of the results represent a sensitive measure of the effects of the environment on fiber length in a clone. A more serious limitation is inherent in the fiber length "index" employed. This value was devised to reflect the amount of fiber present in the stem section analyzed and was constructed by weighting the mean fiber length values obtained for successive increment core segments of five annual growth rings each from the pith to the cambium according to the contribution to the volume of the stem section. If there were any appreciable variation in stem diameter among the trees, this would not only be reflected in differences in fiber length be tween trees since fiber length increases with diameter, but would be accentuated by this weighting. This may be somewhat less serious, than it appears, since in creases in length of fibers are small from approximately four to five centimeters from the pith to the cambium in trembling aspen (Valentine, unpublished results). Comparisons of trees with large diameters should, in fact, be quite reliable. The trees used in their study, however, were not large in diameter (average d.b.h. = 7.6 inches = 19.3 cm., or average radius of 9.7 cm.) and were quite variable. The variation in diameter between trees within clones (er or) was large, but even so, the variation between clones within areas was highly significant. The reported highly significant difference in fiber length between clones within areas most certainly reflects this large variation in diameter, as does the rather large error or within clone variance. It would appear that part of the large variation attributed to environmental effects (S2), the between trees within clone variance, was due to variations in diameter. The contribution of the genotype to fiber length variance according to their method of estimation was based on the partitioning of the between clones within areas variance into two components, genetic (S2) and environmental (S^2). The mean square was assumed to be equal to S^2 + 5S2. The validity of this procedure depends upon obtaining an unbiased estimate of S^2. Since this value is probably overestimated due to the effects of diameter, the component attributable to the genotype would be underes timated. This suggests that the differences between their result and that of this study are not as great as they appear.

SUMMARY AND CONCLUSIONS

Variations in specific gravity of wood and fiber length were determined for four clones of Populus tremuloides. Sex condition and leaf characteristics were used to establish the clonal nature of the nine or ten trees in each clone. The results show that changes in specific gravity of annual rings across the radius of the stem at a height of three feet above ground level are associated with rate of growth. In all four clones the regression coefficients for changes in specific gravity on ring width are highly significant but in two clones the coefficients are positive and in two clones, negative. In all clones slight positive regressions of specific gravity on distance from the pith are obtained, but *none* are statistically significant Differences in specific gravity between trees compared with the variations in common segments of annual rings from each tree are highly significant in three of the four clones. In one test, the segment employed included the annual rings from a distance of 1.5 cm to 3.0 cm. from the pith. In the second test, the growth produced in the years 1954 through 1958 was used. No differences were obtained between trees in either test for one of the clones. The variation between clones is highly significant in both tests.

Variations in fiber length across the radius of the stem is very closely related to distance from the pith. The rate of change is similar in the four clones. Differences in mean fiber length, adjusted for differences in distance from the pith in a covariance analysis, however, are significant. This appears to be due to differences between clones. Since only two trees per clone have been analyzed for fiber length, this difference is not clearly established by this study. Changes in fiber length are not related to rate of growth as measured by ring width.

Specific gravity of wood in trembling aspen is rather strongly influenced by environmental factors. Genetic differences between clones, however, are apparent. In contrast, fiber length appears to be little affected by the environment. The present results suggest slight differences between clones which are attributable to genetic differences.

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DISCUSSION[1]

GABRIEL - Dr. Mergen, did you notice any abnormality in meiotic division in your studies?

MERGEN - Yes, we observed different types of genetic chromosomal aberrations in both the pines and oaks, including chromosome breakage and bridges.

RUDOLPH - You mention in the early part of your presentation that the Brook haven report from last year, was it, showed a photograph of a tree that had been killed at 5 roentgens per year, I wonder if you didn't mean 5 roentgens per day?

MERGEN - The 1959 report showed a photograph of a tree that had been killed after an average exposure of 8 months per year for 5 years at a rate of not more than 8 roentgens per day.

BUCKINGHAM - One of your charts showed that there was a little increase in the height growth at low exposure but the overall showed a great decrease. Would that lead you to believe that a little exposure might be beneficial?

MERGEN - In some of the seed irradiation work we find a slight increase at low levels, with a decrease at the higher levels.

RAUP - Dr. Mergen, is this effective radiation carried down to the roots? In other words does it effect the sprouting habit of the oak?

MERGEN - For the pines we have seedlings that were killed and that sprouted, but in general the sprouting is decreased as a result of radiation. The large trees that had been killed did not sprout again. However, we have some seedlings that were subjected to radiation and where the tops died. There was some shielding effect of the primordia around the root collar by the soil and these seedlings were able to sprout. We had one seedling where the top was killed by radiation, but produced sprouts from the root collar, and within a few weeks produced a male flower with viable pollen on the largest sprout.

STAIRS - With the oaks, sprouting at the basal root collar is often increased due to irradiation damage of the upper portions of the tree. In another study I have some oak transplants in the gamma field at levels of chron ic irradiation ranging from lethal to approximately 25 r/day. Sprouting occurred from all levels which were severely damaged--even those which did not break dormancy following irradiation. These sprouts develop from a protected area below the soil surface and grow very rapidly. Such rapid growth allows them to reach considerable size before accumulating enough irradiation to limit growth. However, they do not form buds at the higher levels and growth is thus limited to the initial developmental stage. It is possible that this sprouting will continue for several growing seasons.

RAUP - This is the question I had in mind: does the ground itself, the soil, constitute a shield?

[1] Transcripts of the discussions were sent to each of the participants for editing with the specific request not to change the contents of their remarks.

MERGEN - It certainly does.

VALENTINE - A question to Mr. Stairs. I was wondering if the effects from the oaks could partly be related to polyploidy. I'm not sure of the chromosome conditions of the species that you referred to here.

STAIRS The chromosome complement in the oaks is 2N = 24 and is the same number as in the pines. No polyploidy was found in the investigated group.

GERHOLD- Dr. Mergen, You mentioned earlier some chromosomal abnormalities that showed up. Were these observations made on the large trees on the plot or on the seedlings that you grew from the seed?

MERGEN - This was done on large trees, mostly on chronically irradiated ones, and an trees in the acutely irradiated forest. All abnormalities refer to large trees.

GERHOLD - Did you have any observations on the seedlings that were grown from these irradiated seed?

MERGEN - The seedlings have been planted in a progeny test, and they will be subjected to flower induction studies, so we hope to have that information at a later date.

GERHOLD - One of our students, R. J. McMahon, competed a study last year using irradiated seed. His observations were made one to three months after germination, and he was not able to find any chromosomal bridges or fragments. These studies were made on root tips of the seedlings, but later on he examined some embryos at the early stages and he did find the abnormalities. Apparently these abnormalities were eliminated or repaired early in the development of the seedlings.

MERGEN - I guess I misunderstood your question. For the seedlings we collect the root tips after 21 days, fix them, and make smears. We found a lower number of mitotic divisions, and also occasional abnormalities during mitosis.

HUNT - I have a question for Dr. Valentine in regard to the two cases of positive correlation of ring width and specific gravity versus the two cases of negative correlation. Is this difference related to the sex of the clones, that is, does the correlation follow the sex?

VALENTINE - No, only one of the four clones was female, the others were male. The two clones in which the positive correlations were obtained, Clones 2 and 4, are male and female, respectively. The two in which the result was negative are both male.