U. S. FOREST SERVICE

RESEARCH NOTE NC-101

NORTH CENTRAL FOREST EXPERIMENT STATION, FOREST SERVICE—U.S. DEPARTMENT OF AGRICULTURE
Folwell Avenue, St. Paul, Minnesota 55101

GEOGRAPHIC VARIATION FOR SEED AND SEEDLING CHARACTERS IN BLACK WALNUT

ABSTRACT. — Geographic variation in 480 1-year-old families of black walnut was studied. For most characters, the pattern of variation was clinal.
OXFORD: 176.1 *Juglans nigra*: **165.52:232.12**

Black walnut (*Juglans nigra* L.) grows naturally from Minnesota to Texas and from Kansas to the East Coast. Within this area, the mean annual rainfall ranges from about 25 to 70 inches, temperature from 45 to 67 degrees, and the frost-free season from 140 to 280 days (USDA 1965). The wide geographic range and diverse conditions over which walnut is found, coupled with the increased and continuing demand for high-quality logs, has justified starting a black walnut genetics project. These first-year results from a range-wide seed source study indicate that wide genetic diversity does exist in the species.

METHODS

In fall 1965, black walnut seeds were collected[1] from 97 natural stands ranging from southern Texas to southern Minnesota, and from eastern Pennsylvania to western Iowa. Elevation varied from 27 m. in Maryland to 884 m. in North Carolina.

All seeds were shipped to Ames, Iowa, where they were washed and culled. A 25-seed sample from each parent tree was randomly selected and weighed. The wet seeds were stored in plastic bags at 2° C. for 6 months.

In May 1966, the seeds were planted in nursery beds at Ames, Iowa. A randomized complete block design was used. Each of six blocks contained 480 plots or families from 78[2] sources (stands). Number of families per stand varied from two to twelve. Four seed (1- x 1-foot spacing) were planted in each plot.

The color of the new leaves was recorded from July 28 to 30, 1966. The following rating and coding scheme was based on the amount of red pigment visible to the eye in leaves less than 15 cm long:

Code	*Color of new leaves*
1	All new leaves red
2	New leaves mostly red, but a small amount of green visible
3	New leaves pinkish (about halfway between all green and all red)
4	New leaves mostly green, but still a hint of pink or red
5	All new leaves green

Insect damage was observed in June and July. In late June, a number of seedlings had growing tips missing; in each case an insect was found rolled in one of the young leaves near the tip. Possibly the fruit tree leaf roller (*Archips argyrospila* (Wlkr.)) was responsible for eating the terminal bud and adjacent new leaves, thereby causing multiple-stemmed seedlings. Other insects seen chewing on the walnut leaves were the white-marked tussock moth (*Hemerocampa leucostigma* (A. and S.)), velvetbean caterpillar (*Anticarsia gemmatalis*), and the walnut caterpillar (*Datana integerrima* (G. and R.)). Insect damage was assessed for each plot on August 9 by counting the total number of leaves apparently chewed by insects and then calculating the average number of chewed leaves per plant.

---

[1] *The seeds were collected by many cooperators and their assistance is gratefully acknowledged.*

[2] *Nineteen sources were excluded because they did not have at least two families with seeds with specific gravity of 1.00 or greater.*

On August 10 the average leaf angle of the upper mature leaves was estimated ocularly for each plot. The degrees of leaf angle away from the vertical were coded as follows:

Code	*Degrees*
1	Less than 30
2	30-40
3	41-50
4	51-60
5	Greater than 60

The height and the longest leaf of the tallest seedling in each plot were measured on August 11-12. These data were used in computing the height/leaf-length ratio.

Data of leaf drop was recorded for each plot as follows: In plots with only one seedling, that seedling must have lost all of its leaves or all except one. In two-, three-, and four-tree plots, all trees except one must have lost all leaves or all but one.

Total height of the tallest seedling in each plot was measured at the end of the growing season. The stem diameter of the same seedling was measured 2.5 cm. above the ground line.

The trees with multiple stems in each plot were counted at the end of the growing season. Since the total number of seedlings per plot varied from one to four, a percentage of the trees with multiple stems was recorded and transformed to $\arcsin\sqrt{\text{percent}}$ before analysis.

The significance of the data for each character was evaluated by analysis of variance, (AOV), using blocks 1, 2, and 3. Tukey's ω-procedure was used for multiple comparisons (Steel and Torrie 1960). Because black walnut stem height and diameter are influenced by seed weight (Chase 1947), height and diameter values were adjusted for differences in the seed weight, using standard covariance analysis procedure. The adjusted values were used in the AOV. A critical value was determined, using a 10-percent level of significance for all seedling characters. Where sources differed, an attempt was made to relate the differences to latitude, longitude, altitude, or a combination of these variables. From these analyses, models of the pattern of variation were suggested. Continuous and discontinuous patterns were explored.

After the model which seemed to best explain the pattern of variation for each character was established, it was tested with data from sources in blocks 4, 5, and 6. Where the data suggested that variation was discontinuous, a boundary was delineated and an analysis of variance was used to determine if differences between populations were significant.

Where the data suggested that the variation pattern was continuous, blocks 4, 5, and 6 were used to test the significance of the regression model. If the regression for these blocks was significant, the data for all blocks were combined for the best estimate of the slope.

The Pattern of Variation

Characters Not Related to Latitude

There were no significant differences at .05 level in average seed weight among sources. This supports Wright's (1954) finding of no correlation between geographic origin and seed size. The average seed weight for 456 families from 69 sources was 22 grams per seed.[8] The average seed weight varied from 12 to 30 grams for sources and from 5 to 40 grams for families. In 59 percent of the sources, the average seed weights per family within the same source differed 10 grams or more.

Seven sources differed significantly in leaf color from many of the remaining 71 sources. The seven sources in which the new leaves tended to be red were in the southeastern part of the range at altitudes varying from 146 to 884 m. It appeared that the southeastern population was distinct from the rest of the population for this character. All sources from North Carolina, South Carolina, Georgia, Alabama, Mississippi, and Tennessee (except one western source) were grouped as one population and tested against all the other sources. The data from blocks 4, 5, and 6 showed the two populations as being different for new leaf color.

Although there were significant differences among sources, there was no apparent pattern of variation for frequency of insect damage or for leaf angle.

Characters Related to Latitude

There were a large number of differences among sources for height/leaf-length ratio, leaf drop, multiple stem frequency, adjusted stem diameter, and adjusted stem height. Model searching followed by linear regression analysis revealed that these characters followed a clinical pattern with latitude. Sources from the south had a higher height/leaf-length ratio, later leaf drop, higher multiple stem frequency, larger diameter, and were taller (fig. 1).

Variance Components, Coefficients of Variation, and Heritability

An analysis of variance was run, using all six blocks in the experiment, to estimate the variance

[8] *Only sources that contained at least four families were used in the analysis.*

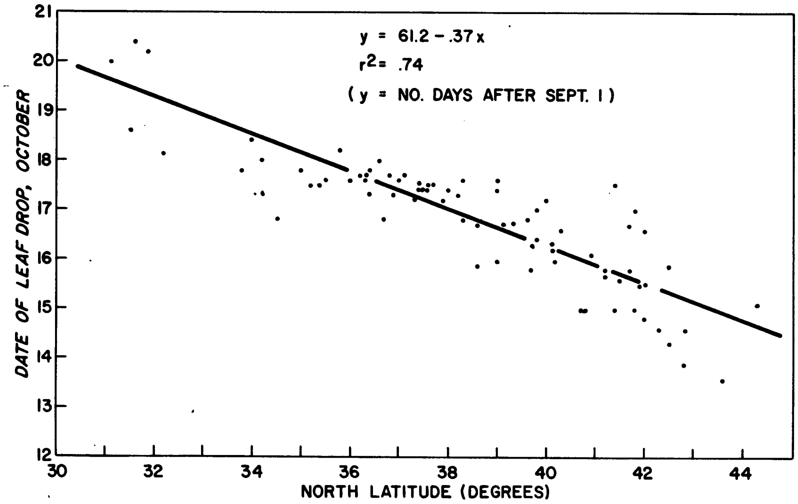

Figure 1. — The pattern of leaf drop was continuous with latitude.

components for each character. The within-source (family) variances ($\sigma_F{}^2$) and the among-source variances ($\sigma\, s^2$) were computed as:

$$\sigma_F^2 = \frac{\text{Family MS - (Blocks x Family MS)}}{\text{Number of Blocks}}$$

$$\sigma_S^2 = \frac{\text{Source MS - (Blocks x Source MS)}}{\text{Number of Blocks}}$$

Both family and source variance components were then computed as a percentage of the Block x Family MS (table 1).

For the eight seedling characters studied, the average variance percentage for families and sources was 7.9 and 49.6 respectively. Source variance exceeded the family variance for each character. Variances were computed among families regardless of source.

The coefficient of variation (CV), was computed for each character. The values ranged from 11.3 percent for leaf drop to 186.7 percent for multiple stem frequency. CV for all other characters, except insect damage (50.4 percent), fell between 15.1 and 26.2 percent.

The narrow-sense family heritability (H) for seedling characters was computed from the partitioned variances as follows:

$$H = \frac{\sigma_F^2}{\frac{\sigma_e^2}{b\ \text{(Blocks)}} + \sigma_F^2}$$

The family component refers to families within sources.

Heritability values for seedling characters ranged from .13 for leaf drop to .49 for adjusted stem diameter (table 1). Leaf color and adjusted stem height were comparatively high, .45 and .44 respectively. Heritabilities for other characters varied from .18 to .32.

Correlation Among Characters

Using the data from all six blocks, the correlation coefficients (r values) among all characters were computed (table 2). Leaf color was negatively correlated with all other characters. In terms of trends, red-leafed sources from the southeastern United States grow faster, lose their leaves later, have shorter leaves (relative to stem height), and have a higher frequency of multiple stems.

Insect damage was positively correlated with leaf-drop date, height/leaf-length ratio, multiple stem frequency, stem diameter, and stem height. This may be partially due to the fact that the larger plants from southern sources had more leaves to be chewed on than did the smaller plants.

Leaf angle is negatively correlated with the height/leaf-length ratio, stem diameter, and stem height. By selecting for trees with a wide leaf angle, we are concomitantly selecting for smaller trees.

Date of leaf drop is positively correlated with insect damage, height/leaf-length ratio, frequency of multiple stems, stem diameter, and stem height. Although date of leaf drop is generally related to the onset of dormancy, the low correlation between leaf drop and height and diameter growth indicates that there are also other factors responsible for the observed differences in height and diameter growth.

DISCUSSION AND CONCLUSIONS

The pattern of variation for most of the characters studied for black walnut was continuous. Variation in several characters was associated with latitude. Evidently climatic, biotic, and edaphic factors that are associated with latitude are major forces of natural selection.

Table 1.—*Family and source variance (as percent of Block x Family variance), mean for all sources, coefficient of variation, and heritability for eight characters studied*

Character	Family variance	Source variance	Mean for all sources	Coefficient of variation	Heritability value
	Percent	Percent		Percent	
Leaf color	13.7	52.6	3.44 (rating)	15.1	0.45
Insect damage	6.3	28.3	3.32 leaves per plant	50.4	.27
Leaf angle	7.8	25.8	2.99 (rating)	18.6	.32
Height/leaf-length ratio	7.2	71.4	99.48 (ratio)	20.2	.30
Leaf drop	2.4	73.8	16.94 (October date)	11.3	.13
Multiple stem frequency	3.6	18.2	10.47 (arcsin $\sqrt{\text{percent}}$)	186.7	.18
Adjusted stem diameter	16.2	53.2	7.72 mm.	20.1	.49
Adjusted stem height	13.0	73.6	35.19 cm.	26.2	.44

Table 2.—*Correlation coefficients between eight characters*

Character	a	b	c	d	e	f	g	h
a - Leaf color	X							
b - Insect damage	-.02	X						
c - Leaf angle	-.03	.00	X					
d - Date of leaf drop	-.08	.06	.00	X				
e - Height/leaf-length ratio	-.06	.27	-.07	.20	X			
f - Multiple stems	-.09	.17	.07	.15	.15	X		
g - Adjusted stem diameter	-.07	.41	-.10	.04	.46	.17	X	
h - Adjusted stem height	-.09	.41	-.09	.07	.69	.22	.82	X

Data from this study support Emerson (1906) and Wright (1954), who found that southern sources of black walnut hold their leaves later in the fall. Emerson reported that in Nebraska, southern sources suffered more winter injury, but that trees from as far south as Georgia, Alabama, and South Carolina were able to survive the much colder climate of Nebraska for at least 8 years. Ellis (1925) reported that black walnut seedlings of west-central Illinois origin thrived for 60 years in Vermont, approximately 200 miles north of their origin. Lotbiniere (1920) reports that a walnut plantation in Quebec, Canada (about 200 miles north of natural range), grew to 55 feet in height and 7 inches in diameter in 37 years.

It appears that we may be able to make some genetic gains by using southern sources of seed. Outplantings of this study have been established in 11 states and will be valuable for making seed source recommendations in the future,

LITERATURE CITED

Chase, S. B. 1947. Eastern black walnut germination and seedbed studies. J. Forest. 45:661-668.

Ellis, Z. H. 1925. The farthest north in U.S. nut culture. N. Nut Growers Ass. Rep. 16:28-30.

Emerson, R. A. 1906. The relation of early maturity to hardiness in trees. Nebr. Agr. Exp. Sta. Annu. Rep. 19:101-110.

Lotbiniere, A. J. de. 1920. The walnut plantations at Point Platon. Can. Forest. J. 16(7):325-329.

Steel, G. D. and Torrie, J. H. 1960. Principles and procedures of statistics. 481 p. New York: McGraw-Hill Book Co.

USDA Forest Service. 1965. Silvics of forest trees of the United States. U.S. Dept. Agr., Agr. Handb. 271, 762 p.

Wright, J. W. 1954. Preliminary report on a study of races in black walnut. J. Forest. 52:673-675.

CALVIN F. BEY
Plant Geneticist
Carbondale, Illinois
(Laboratory maintained in cooperation with Southern Illinois University)

1970