BLACK WALNUT PROGENY AND CLONAL TESTS AT PURDUE UNIVERSITY

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Abstract.--The genetic improvement of Indiana black walnut at Purdue University has progressed along two important fronts: (1) progeny testing of superior tree selections, and (2) clonal development and testing of these selections.

Prior to clone bank flowering, half-sib testing of superior individuals comprised the bulk of our work. Nursery, one, two, and three-year-old outplanting results have shown surprisingly high inheritance, and genotypic and phenotypic correlations on growth parameters. In addition, family performance compared with the commercial check has been outstanding.

At this time the clonal program utilizes 106 grafted clones. Analysis of variance and heritability estimates on foliation date, defoliation date, height, diameter, sweep, crook, branch angle, branch number, and anthracnose resistance show striking differences.

Additional keywords: Juglans nigra L., heritability, height and diameter, foliation, form, half-sib, hardwood genetics.

The genetic improvement of black walnut (*Juglans nigra* L.) at Purdue University has progressed to the stage that significant information on inheritance and variation in certain traits from half-sib progeny and clonal tests is available. The program began in 1967 with a few black walnut selections of dubious quality. Today, 106 selections are included in the program. Selections are rated on a point system for straightness, apical dominance, and growth rate based on a modified system from that reported by Beineke and Lowe (1969). All selections are preserved by grafting in a clone bank at Purdue-Shidler Forest (Lowe and Beineke, 1969). Application of the clonal seed orchard approach is in the initial stages of development by the Indiana Division of Forestry and has many advantages over half-sib seedling orchards (Masters and Beineke, 1972).

HALF-SIB PROGENY TESTING

The function of our half-sib progeny testing was as a tool to confirm beliefs concerning the extent of genetic variation present in our selected population, and to gain knowledge about individual family performance. Since full-sib work on black walnut is not feasible in the wild population, it is a good approach while waiting for clone bank flowering.

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METHODS

The two half-sib tests reported in this paper were initiated, in 1969 and 1970. For both tests an attempt was made to collect open-pollinated seed from each parental selection that had seed available that year. In most cases, therefore, families were not replicated over years, however, both tests contained sources from southern Michigan to southern Indiana.

The seeds were sown in the Purdue-Shidler nursery, near Lafayette, Indiana, during the fall. The design was a randomized complete block with 3 blocks. After the first year in the nursery, the seedlings were outplanted to several different locations; remaining in the RCBD and arranged in 10-tree row plots.

The oldest study is four years old and because of its youth, total height, total diameter, and germination percent were the only traits evaluated.

RESULTS AND DISCUSSION

Mean family performance versus the commercial check [1/]

For the traits evaluated, we were interested in the ability of our selected families to perform in relation to the commercial stock available. Table 2 illustrates that in almost every test the performance of the selected families was superior to the commercial check. At high selection intensities the differences were great. The reason for these great differences is probably due to the high-grading of Indiana walnut, and that when exceptional individuals are located, their performance is much superior to the seed presently being used by Indiana nurseries.

Germination among families is quite variable, and since the commercial stock available today averages 50 percent, an increase in this trait alone would be a boon to walnut culture.

Given a 20 percent selection intensity, our selected families out-performed the commercial check showing a 29 percent and 51 percent increase for the 1969 and 1970 tests respectively (Table 1). Also, the mean for all selected families for the 1970 test had a germination percent higher than the commercial check.

Table 1. -- Germination percent

Test	Percent increase of best 20%	h^2	Reliability ratio
1969	29	.94	.39
1970	51	.84	.39

[1/] The commercial check is a random sample of the seed used for planting by the Indiana Division of Forestry nurseries.

Table 2. -- Performance of selected families in relation to the commercial check

Test	No. of families	Age	Intensity of selection 100% Ht.	100% Dia.	38% Ht.	38% Dia.	20% Ht.	20% Dia.
			- - - - - - - - - -	- Percent increase	- - - -	- - - -	- - - -	- - - -
Nursery:								
1969	13	1	6.3	8.1	33.3	17.8	38.0	20.2
Outplantings:								
Pike 1969	4	2	12.0	13.0	37.0	24.0	42.9	28.0
Stem 1969[a]	7	4	17.9	21.0	44.0	51.0	47.2	53.8
Orange 1969	12	4	10.9	5.1	20.4	16.3	25.4	21.6
Nursery:								
1970	18	1	3.6	1.8	18.9	14.8	26.3	18.1
Outplantings:								
Parke 1970	9	3	12.0	--	20.0	--	20.0	--
Flick 1970	8	3	0.0	0.0	10.6	3.0	13.5	4.9
Orange 1970	18	3	5.9	4.0	19.0	15.0	23.1	19.3

a/ Only 1 block remains

Half-sib analysis and heritabilities

All analyses of variance for the height and diameter parameters were statistically significant. Except for the 1969 nursery data for both height and diameter and the Orange, 1970 diameter; all were significant at the .01 level. Germination percent for both tests also proved significant at the .01 level.

Components of variance and heritabilities based on individuals, for all tests were estimated from the following mean square components:

Source of variation	EMS
Families	$\sigma^2_1 = \sigma^2_w + n\sigma^2_{RF} + bn\sigma^2_F$
Blocks	$\sigma^2_2 = \sigma^2_w + 1 - \frac{a}{A} n\sigma^2_{RF} + an\sigma^2_R$
F x B	$\sigma^2_3 = \sigma^2_w + n\sigma^2_{RF}$
Within	$\sigma^2_4 = \sigma^2_w$

where heritability, $h^2 = \dfrac{4\sigma^2_F}{\sigma^2_w + \sigma^2_{RF} + \sigma^2_F}$

standard error of $\sigma^2_F = s[\sigma^2_F] = \sqrt{\dfrac{2}{C^2}\left(\dfrac{M.S._1^2}{D.F._1 + 2} + \dfrac{M.S._3^2}{D.F._3 + 2}\right)}$

(Anderson and Bancroft, 1952)

Where C = coefficient of the σ^2_F component

Of interest in Table 3 are the heritability estimates and reliability ratios for the nursery and outplanting tests. The tests in Table 2 that were not included in Table 3 were left out because of circumstances making them unsuitable for statistical analysis.

In general, the narrow-sense heritabilities for total height and diameter show that selection for these traits is indeed useful and that beneficial gains can be expected. These heritabilities are comparable with those reported by Bey (1970), and Bey et. al. (1971). The reliability ratios are not as good as we would prefer, but in our opinion, they are reasonable. In future work, though, if the RCBD is used, greater precision will be realized by increasing block number. The data for germination percent also demonstrate that good gains can be expected in the future. Narrow-sense heritabilities for this trait were .94 and .84 for the 1969 and 1970 tests respectively. The reliability ratios for both heritability estimates were a respectable .39 (Table 1).

Table 3. -- Components of variance and heritabilities for height and diameter from two half-sib tests a/

Test	Age	Components σ_W^2	σ_{RF}^2	σ_F^2	$s[\sigma_F^2]$	h^2	Reliability ratio b/
Height							
Nursery:							
1969	1	.1259	.1490	.0893	.0561	.98	.63
1970	1	.1288	.0304	.0244	.0064	.55	.45
Outplantings:							
Orange 1969	4	1.1070	.0226	.1022	.0585	.33	.57
Orange 1970	3	.6612	.0114	.0629	.0300	.34	.47
Diameter							
Nursery:							
1969	1	.0039	.0018	.0010	.0007	.63	.66
1970	1	.0066	.0011	.0014	.0127	.61	.52
Outplantings:							
Orange 1969	4	.0880	.0045	.0127	.0067	.48	.52
Orange 1970	3	.0498	.0010	.0033	.0019	.25	.57

a/ In 1970, the 1969 test was coppiced due to extensive 17-year locust damage.

b/ The ratio $\frac{S.E.(\sigma_F^2)}{\sigma_F^2}$ was used to give a general indication of the reliability of heritability. If this ratio is greater than 0.50, reliability is low (Snyder, 1969).

Genotypic and phenotypic correlations

We decided to calculate genotypic and phenotypic correlations on the total height and diameter parameters to validate the relationship expected. The 1970 test was used as an example because the precision is the better of the two tests.

Table 4 illustrates, except for 1970 and 1971 height with 1970 diameter, that both genotypic and phenotypic correlations are very high. We offer no explanation for the two low correlations mentioned. Individually, the height and diameter correlations increased with age as expected since measurements of total height or diameter are the summation of annual effects. All correlations calculated increased with age. The important point to remember is that due to the high genetic correlations between height and diameter, selection for one characteristic would produce meaningful gains in the other.

Table 4. -- Genotypic and phenotypic correlations between height and diameter for the 1970 test

Characters	Genotypic correlations	Phenotypic correlations
1972 height with:		
1970 height	.84	.63
1971 height	.92	.73
1972 diameter with:		
1970 diameter	1.32	.82
1972 height with:		
1970 diameter	.90	.63
1972 diameter	.92	.85
1971 height with:		
1970 diameter	.21	.34
1972 diameter	.63	.44
1970 height with:		
1971 height	.97	.92
1970 height with:		
1970 diameter	.21	.34
1972 diameter	.67	.41

CLONAL TESTS

Measurements obtained from grafted black walnut at the Purdue-Shidler clone bank not only give some indication of our ability to select outstanding trees, but may be utilized directly if vegetative propagation on a large scale becomes a reality. Due to the high value of black walnut, the best clones could be offered for planting either as grafts or rooted cuttings, thus by-passing sexual reproduction and progeny testing by seedlings.

METHODS

The oldest grafts available for statistical analysis are four growing seasons old. Since the site is reasonably uniform, and due to differential grafting success, a completely randomized design was used for all traits and years. Only clones having at least three surviving, undamaged ramets were analyzed.

Analysis of variance provided the clone and error variance components as estimated from the mean squares for all variables following the approach of Mohn and Randall (1971). Broad-sense heritabilities were calculated using the formula $h^2 = \sigma^2 / \sigma^2 + \sigma^2$. Reliability ratios were calculated using the same formula as for the half-sib progeny tests (Table 3).

RESULTS AND DISCUSSION

Table 5 summarizes information for characteristics measured in the clonal tests.

Foliation and defoliation dates

The highest heritabilities and best reliability ratios were obtained for foliation and defoliation dates. Foliation date is of great importance in black walnut due to its susceptibility to late frosts. Frost often causes destruction of central stem tendency, thus a late foliator may escape damaging frost. Generally late foliators are early defoliators and have a short growing season. In spite of this short growing season, there was no indication of reduced growth. In fact, several of the late foliators and early defoliators were among the fastest growers. The correlation coefficient (r) between foliation date and height growth in 1971 was -0.02. Order of foliation was consistent from year to year with latest foliators invariably being late in subsequent years. This relationship was demonstrated by the high correlation coefficient (r = .80) between 1970 and 1971 foliation dates.

Height and diameter

Total height at age two had high heritability and good reliability. Potential for selection and breeding for height growth is indicated by these early estimates. Unfortunately, estimates from older material is lacking since wind damage, top pruning, and thinning removed many ramets and clones from possible analysis of growth parameters.

Table 5. -- Age, means, range of clone means, variance components, broad-sense heritabilities, and reliability ratios from a clonal test

Character	Year grafted	Age	No. clones	No. grafts	Test mean	Range of clone means	Clone variance	Error variance	h^2 a/	Reliability ratio
Foliation date (days) b/ **	1969	1	28	146	4.9	0.3 - 15.7	9.380	1.763	.84	.27
Foliation date (days) **	1969	2	27	143	17.6	1.7 - 25.7	22.140	3.707	.86	.28
Foliation date (days) **	69, 70,71	3,2,1	50	224	15.1	2.5 - 25.0	24.866	2.310	.92	.20
Defoliation date (days) c/ **	69,70	3,2	29	141	13.2	3.3 - 17.8	13.695	4.938	.73	.28
Total height (ft.) **	1969	2	27	141	8.2	6.4 - 10.9	1.026	0.839	.55	.31
Total diam. (DBH in.)*	1969	4	17	68	2.4	1.9 - 3.2	0.062	0.184	.25	.60
Sweep d/ **	1969	4	18	80	1.13	0.18- 3.38	0.268	0.630	.30	.51
Crook (no.) **	1969	4	17	76	1.12	0.33- 2.0	0.172	0.548	.24	.59
Branch angle (deg.)**	1969	2	27	138	56.2	44.8 - 66.0	13.838	55.982	.20	.49
Branch no. **	1969	1	28	147	7.9	3.7 - 23.3	12.025	17.571	.41	.34
Anthracnose resis. e/ **	69, 70,71	4,3,2	34	146	2.77	1.25- 4.50	0.430	0.366	.54	.29

a/ $\sigma_c^2 / \sigma_c^2 + \sigma_e^2$

b/ Days from first graft to show visible leaf
c/ Days from first graft to completely defoliate
d/ Deviation in feet from central stem per foot of total height x 10
e/ Rated 1 (outstanding resistance) through 5 (very poor resistance)

** Significant differences among clones at the 1% level
* Significant differences among clones at the 5% level

Heritability of diameter after the fourth year was relatively low. Nevertheless, they compared favorably with the narrow-sense heritabilities already discussed, and the findings of Mohn and Randall (1971) in cottonwood. Reliability of heritability for diameter was not outstanding, and probably reflected the same problems associated with height measurement in the older material. In addition, growth parameters are better analyzed by statistical designs other than the completely randomized design we were forced to utilize in this study. Growth of some clones has been excellent. In their fourth growing season, grafts grew as much as 6.7 feet in height and 1.3 inches in diameter at DBH. The largest four year old grafts measured 21.1 feet and 3.6 inches DBH.

Sweep and crook

Sweep and crook were not as highly heritable or reliable as was anticipated, however, method of measurement may have affected these variables. Number of crooks was counted subjectively by two independent observers and averaged for each tree. Sweep was measured objectively by the distance the terminal shoot deviated from the base of the tree divided by the height. However, differential effects of staking and pruning undoubtedly had an effect on heritability of both sweep and crook. Heritabilities for sweep (.30) and crook (.24) are high enough to produce meaningful gains in a breeding program. They compare favorably with heritabilities for form traits found in loblolly pine of the same age (Shelbourne and Stonecypher, 1971).

Branch angle and number

Branch angle apparently is not highly heritable in black walnut, even though there were significant differences among clones. A practical method of accurately measuring branch angle on the original selection could not be found, and it was hoped that branch angle could be measured on the grafts. However, branch angle apparently is affected more by leaf weight on the branch than any inherent branch angle as such. Branch angle can vary considerably from day to day and season to season on young trees.

Branch number, on the other hand, shows a relatively high heritability and is quite reliable. Number of branches is a good estimate of "bushiness" in black walnut since clones with many branches were those having a tendency to fork. One clone, not included in the study, never produces a branch during the first growing season after grafting, and branches produced the second year are slender and short, producing an ideal, compact crown.

Anthracnose resistance

Black walnut anthracnose (*Gnomonia leptostyla* (Fr.) Ces. and deN.) is a fungus disease affecting the leaves and fruit husk of the black walnut. It causes early defoliation, and therefore, presumably, decreases growth (Berry, 1964). In the 1972 growing season, optimum weather conditions for the spread of anthracnose, including high rainfall and humidity, and low temperatures occurred. On August 24, 1972, each graft regardless of age was rated subjectively on a scale from one to five with: 1 = few or no leaf lesions, no leaf fall; 2 = leaf lesions evident but only a few leaves fallen and no rachis fall; 3 = average -

leaf lesions and bronzing, some leaf and rachis fall; 4 = more leaf and rachis fall than in 3; 5 = serious defoliation and rachis fall, trees often nearly defoliated. Heritability was high and reliability good for anthracnose resistance, indicating that breeding for improved resistance to this serious disease is possible.

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