NOTE

Heartwood production in a 35-year-old black walnut progeny test

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Abstract: A 35-year-old black walnut (*Juglans nigra* L.) progeny test was evaluated for growth and production of heartwood. The test trees, which were open-pollinated progeny of select females in seven states, were planted on a good-quality, uniform site in Wabash County, central Indiana, U.S.A. Increment cores were used to estimate the amount of heartwood at 1.3 m above ground level. There were significant differences among open-pollinated families (α = 0.10) for both area of heartwood and percent area of heartwood. Narrow-sense heritability estimates for these traits were moderate (0.40 and 0.27), indicating opportunity for gain from selection. Faster growing trees had more heartwood and a higher percentage of heartwood area in cross section. Genetic correlations indicated that the rate and amount of heartwood formation is closely related to diameter growth.

Résumé : La croissance et la production de bois de cœur ont été évaluées dans un test de descendance de noyers noirs (*Juglans nigra* L.) âgés de 35 ans. Les arbres testés, qui sont issus de la pollinisation libre d'arbres femelles sélectionnés dans sept états, ont été plantés sur un site homogène de bonne qualité dans le comté de Wabash, dans le centre de l'Indiana aux États-Unis. Des carottes ont été utilisées pour estimer la quantité de bois de cœur à 1,3 m au-dessus du niveau du sol. Il y a des différences significatives (α = 0,10) entre les familles à pollinisation libre tant pour la surface de bois de cœur que pour le pourcentage de la surface occupée par le bois de cœur. Les estimations de l'héritabilité au sens strict pour ces caractères sont modérées (0,40 et 0,27), indiquant qu'il serait possible d'obtenir un gain par la sélection. Les arbres à croissance rapide ont plus de bois de cœur, et une plus forte proportion de bois de cœur en section radiale. Les corrélations génétiques indiquent que le taux de formation et la quantité de bois de cœur sont étroitement reliés à la croissance en diamètre.

[Traduit par la Rédaction]

Introduction

There has been speculation that faster growing black walnut (*Juglans nigra* L.) genotypes being developed for short-rotation intensive management may produce substantially less heartwood than wild trees (Nelson 1976; Szopa et al. 1980; Phelps and Chen 1989). This is a concern, because the value of the heartwood of black walnut far exceeds the value of the sapwood. Although little information is available concerning environmental factors that influence heartwood color and quantity, considerable variability appears to exist among unrelated trees in even-aged stands (Nelson 1976).

Very little data exists on the genetics of heartwood formation in walnut, probably because there are comparatively few walnut plantations containing identifiable families and because existing sampling techniques for heartwood injure trees and can destroy their value as timber. Rink (1987) evaluated 10-year old trees from an open-pollinated progeny test and estimated the "narrow-sense family heritability" for heartwood area to be 0.56. Rink also reported a very highly significant positive phenotypic correlation (r = 0.48) between heartwood area and diameter at 1.3 m above ground level (DBH). The phenotypic correlation between sapwood area and diameter (r = 0.73) was greater than that for heartwood and also very highly significant, indicating that gains in heartwood area from selection on diameter might be accompanied by even larger increases in sapwood area. Estimates of the heritability of heartwood diameter in Scots pine (*Pinus sylvestris* L.) were in the range of 0.3–0.5 (Fries and Ericsson 1998; Ericsson and Fries 1999), but the genetic similarities or differences between hardwoods and softwoods for heartwood formation are not known.

Observations of the variability of heartwood area in trees being harvested for timber led us to speculate that the genetic regulation of heartwood formation in mature black walnut might be different than for 10-year-old trees (Rink 1987) and that estimates from mature trees growing in additional locations were warranted.

Materials and methods

Plantation establishment and maintenance

The trees in this study were planted in 1963 and 1964 at Salamonie River State Forest near Lagro, Wabash County, Indiana, as a part of USDA Forest Service black walnut

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progeny study CG-369 (NC-1402). The site was uniform with respect to soil type, with good drainage and very little slope. The original vegetation on the site was second-growth beech–maple mixed-hardwood forest typically compatible with good walnut growth. The site, including a 23-m isolation strip, was cleared and sprayed with herbicide. Five adjacent, homogeneous blocks were laid out in an area of about 76 × 137 m with about 3 m between trees and about 5 m between rows. Black walnut seeds cannot be stored from year to year, and walnut tends to bear in alternate years, so seeds from open pollinations (putative half-sibs) were harvested from 15 select parent trees in the fall of 1961, from 13 parents in 1962, and from 14 parents in 1963. The seeds were germinated and grown at the Jasper-Pulaski State Nursery at Medaryville, Ind. In 1963, 1964, and 1965, 1–0 stock from each family was outplanted to the study site. Four-tree plots were randomly planted in each of the five blocks. No border trees were planted. In 1972, survival of the original 840 trees averaged 50%. The stand was thinned in the winter of 1992–1993, with the best two trees per plot retained. Minnesota and Tennessee sources were removed at that time because of poor performance. About 35% of the original planted trees remained after this thinning. The plantation was mowed annually from 1977 until the mid-1990s.

Sampling method

For this study, we identified 10 families from the 1963 planting year and 12 families from the 1964 planting year that had two surviving open-pollinated sibs in each of at least three blocks. The 22 families represented parent trees from seven states: Illinois (1 family), Indiana (11), Iowa (1), Kentucky (1), Michigan (2), Missouri (2), and Ohio (5). A total of 175 trees were evaluated, a mean of 7.9 trees per family. There are no established seed or breeding zones for black walnut, and no firm relationship between seedling source and performance has been established (Bey 1973). Diameter outside the bark at 1.3 m above ground level (diameter at breast height, DBH) was measured, and an increment core was taken at the same height. The cross-sectional area of the tree (XSA) was defined as the area of a cross section with the bark removed taken at 1.3 m above ground level. The amount of sapwood in a tree was determined by measuring the increment core from the cambium layer inward to the start of the heartwood. The diameter of heartwood was calculated by subtracting the bark and sapwood depth from DBH. Percent area of heartwood (PAOH) was AOH/XSA expressed as a percentage. PAOH was used as a means to evaluate and remove the effect of diameter growth on the amount of heartwood.

Statistical analysis

Analysis was performed using the SAS System for Windows release 8.1 (SAS Institute Inc., Cary, N.C.).[1] The UNIVARIATE procedure was used to obtain simple statistics and to determine if variables were normally distributed. Based on these results, the variable “area of heartwood” (AOH) was $\log_e$ transformed for all analyses. Estimates of variance components and intraclass correlations were made using PROC VARCOMP. Families were nested in years for all analyses. The significance of family(year) effects was determined for each dependent variable using the mean square (MS) for (family × block(year)) as the error term. The intraclass correlation, t, was estimated as $(V_{\mathrm{F(y)}}/(V_{\mathrm{F(y)}} + V_{\mathrm{B \times F(y)}} + V_{\mathrm{e}}))$, where $V_{\mathrm{F(y)}}$, $V_{\mathrm{B \times F(y)}}$, and V_{e} were the family(year), block × family(year), and error variances, respectively. The narrow-sense heritability (h^2) was estimated as $4t$ (Falconer 1981). Standard errors of the heritabilities were calculated as follows: $4[2(1 - t)^2(1 + (k - 1)t)^2/k(k - 1)(N - 1)]^{0.5}$, where k was the coefficient for family variance taken from the type III expected MS output by PROC GLM, and N was the number of families (Becker 1984). Analysis of covariance (ANCOVA) was performed using the general linear models (GLM) procedure. Each dependent variable was tested in a preliminary analysis to determine if the covariate × family interaction was significant. If the interaction was not significant ($\alpha = 0.05$), the slope of the GLM regression was considered nonheterogeneous and the covariate was added as the first independent variable in the model (Cody and Smith 1997). The ANCOVA was then performed and the effect of open-pollinated families tested as described above for the GLM.

Spearman correlations were calculated on an individual tree basis. Genetic correlations (r_{g}) were calculated using the sum of traits method (Stonecypher 1992) after the mean and SD of the variables were standardized using PROC STANDARD. PROC GLM was used to test the significance of the effect of year, block, and family(year). The standard errors of the genetic correlations between traits x and y were calculated as $\sigma_{r_{\mathrm{g}}} = [(0.71)(1 - r_{\mathrm{g}}^2)(\sigma_{h_x^2}\sigma_{h_y^2}/h_x^2 h_y^2)^{0.5}]$ (Falconer 1960). Gain from selection within the study population was calculated using the following relationship: response = selection differential × h^2 (Falconer 1981).

Results

The growth of the trees in this study was typical of black walnut in Indiana: on average, trees increased their diameter by about 0.6 cm/year. As suggested in the original (1962) study plan, the site was fairly uniform with respect to growth but less so with respect to the heartwood variables: the contribution of blocks to the total phenotypic variance was 0.0, 2.0, 4.0, and 7.0% for DBH, XSA, AOH, and PAOH, respectively. The block effects were significant for AOH and PAOH ($\alpha = 0.10$), but not DBH or XSA (Table 1). The site did not strongly favor the growth of families that originated in Indiana, since the top five families for DBH were from four different states. Planting year did not have a significant effect on DBH ($F_{[1,20]} = 1.12$, $P \leq 0.30$), XSA ($F_{[1,20]} = 1.27$, $P \leq 0.27$), or AOH ($F_{[1,20]} = 2.1$, $P \leq 0.16$), but the effect of planting year on PAOH was significant ($F_{[1,20]} = 4.0$, $P \leq 0.06$). Trees planted in 1963 had, on average, a larger DBH, XSA, AOH, and PAOH, than those planted in 1964, but the range and variability of family means for each of the traits were similar for both planting years (Table 2). Whatever variability was introduced by staggering the planting year, it was relatively small compared with other effects by the time

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[1] The use of trade names is for the information and convenience of the reader and does not imply official endorsement or approval by the U.S. Department of Agriculture or the Forest Service of any product to the exclusion of others that may be suitable.

Table 1. Mean squares, family intraclass correlation, estimate of narrow-sense heritability, and F statistics for family and block for two growth and two heartwood traits of black walnut.

	Mean square[b]						Family		Block	
Trait[a]	Family (F)[c]	Block (B)	B × F	Error	t^d	h^{2e}	$F_{[20,65]}$	P	$F_{[4,68]}$	P
AOH	0.44	0.68	0.29	0.21	0.10	0.40±0.22	1.52	0.10	2.38	0.06
DBH	4.2	4.4	3.5	2.7	0.070	0.28±0.24	1.26	0.24	1.21	0.32
PAOH	80	261	51	44	0.068	0.27±0.25	1.58	0.086	5.16	0.001
XSA	727	860	487	368	0.096	0.38±0.22	1.49	0.12	1.77	0.14

[a] AOH = ln(area of heartwood) (cm^2); DBH, diameter at breast height (cm); PAOH, percent area of heartwood; XSA, cross-sectional area (cm^2). For details, see Materials and methods.

[b] Degrees of freedom were 20, 4, 63, and 81 for family(year), block, family(year) × block, and error, respectively.

[c] Expected mean squares for family(year) = $V_e + 1.7V_{B\times F(y)} + 7.0V_{F(y)}$.

[d] Intraclass correlation for families.

[e] Narrow-sense, individual-tree heritability.

Table 2. Family mean simple statistics for comparison of families planted in 1963 and 1964.

	1963				1964			
Trait[a]	Mean[b]	Minimum	Maximum	CV	Mean[c]	Minimum	Maximum	CV
AOH	4.93	4.49	5.44	8.7	4.78	4.36	5.23	8.4
DBH	22.4	19.4	27.7	10	21.5	19.2	24.9	8.1
PAOH	46.7	41.5	50.3	6.3	44.4	36.9	52.0	8.7
XSA	333	258	532	23	302	235	367	15

[a] AOH = ln(area of heartwood) (cm^2); DBH, diameter breast height (cm); PAOH, percent area of heartwood; XSA, cross-sectional area (cm^2). For details, see Materials and methods.

[b] Mean of 10 families containing a total of 86 individuals.

[c] Mean of 12 families containing a total of 89 individuals.

Table 3. Spearman phenotypic correlations, with significance levels given in parentheses (above diagonal), and genetic correlations (±SE) (below diagonal) among growth and heartwood traits.

	XSA	DBH	AOH	PAOH
Cross-sectional area (XSA)		0.99 (<0.0001)	0.95 (<0.0001)	0.21 (<0.004)
Diameter at breast height (DBH)	1.0		0.94 (<0.0001)	0.20 (<0.008)
Area of heartwood (AOH)	0.92±0.07	0.98±0.02		0.47 (<0.0001)
Heartwood area percent (PAOH)	0.70±0.29	0.99±0.01	1.1	

the trees were about 35 years of age, since planting year accounted for only 5% of the total phenotypic variance for DBH, 2% of the variance for XSA, 3% of the variance for AOH, and 5% of the variance for PAOH.

There were large differences among families (Table 2) and individuals (data not shown) for heartwood and growth characteristics, indicating that selection for improvement of these traits may be successful under better experimental conditions. Family effects for XSA, AOH, and PAOH were statistically significant at the $\alpha = 0.10$ level. Narrow-sense heritabilities for these traits were in the range of 0.25–0.35. There were no significant family(year) × block interactions for any of the heartwood or growth variables.

Correlations between easily obtainable measures of tree growth (such as DBH) and the amount of heartwood in a tree are important, because there are no methods for directly measuring the heartwood volume of a standing tree and because the available sampling method (increment cores) is destructive. Phenotypic correlations between the two variables used to evaluate tree growth (DBH and XSA) were essentially unity ($r = 0.99$; Table 3), and the phenotypic correlations between DBH and XSA and each of the heartwood variables (AOH and PAOH) were very similar. The trees in this study had very strong, positive, and significant phenotypic correlations between tree growth (DBH, XSA) and AOH. There was a very similar phenotypic correlation between growth and amount of sapwood (not shown). The phenotypic correlation between XSA and PAOH was low ($r = 0.21$) but positive and significant, indicating that the larger diameter trees in this study tended to have proportionately more heartwood than sapwood. The phenotypic correlation between AOH and PAOH was moderate ($r = 0.47$), positive, and very highly significant. Genetic correlations among the traits were extremely high (Table 3); most notably, the genetic correlations between PAOH and AOH and between PAOH and DBH ($r_g = 0.99$). In this study, growth (DBH and XSA) and the amount of heartwood (AOH, PAOH) were reflections of what was genetically the same character.

Despite the very high genetic correlation between growth and heartwood traits, analyses of covariance showed significant ($\alpha = 0.10$) family effects for the heartwood variables AOH and PAOH ($F_{[20,66]} = 1.56$, $P = 0.09$ for both) after the effects of DBH were removed. There were no significant family effects for PAOH when AOH was used as a covariate.

Discussion

It is not clear from these results if heartwood formation is genetically distinct from growth. Genetic correlations (Table 3) indicate the traits are fundamentally related, at least in 35-year-old walnut trees, but genetic correlations are particularly sensitive to sampling error and are biased by epistasis. Analysis of covariance showed that when DBH was removed, family effects for AOH and PAOH were significant, so it seems likely that some genetic component of these traits is distinct from growth.

There were important differences between the phenotypic correlations among growth and heartwood variables reported by Rink (1987) and those reported here (Table 3). Specifically, Rink found that the phenotypic correlation between AOH (Rink's variable HW) and DBH was significant but only moderate (r = 0.48). Rink found no correlation between DBH and PAOH (Rink's variable HWA%). Both these phenotypic correlations were significant in this study, and the correlation between AOH and DBH was very high (r = 0.94). Differences in the results from the two studies may be attributable to the age difference between the two stands, the small size of both populations, and the availability of only one location in both studies. In addition, although the population described in this study was similar in size (175 individuals) to that described by Rink (134 individuals), the trees in Rink's study were the smallest in the population, i.e., the trees being thinned out of a stand, whereas the trees described here were the more vigorous trees remaining after a thinning in 1992.

These results indicate that selection for faster growing black walnut genotypes does not require a sacrifice in heartwood area or percent heartwood area. The estimated narrow-sense heritabilities for AOH and PAOH were sufficiently high that gains for these traits should be possible in the early generations of mass selection. The five fastest growing individuals (2.8% of population) had a mean DBH 48% greater than the population mean, a mean AOH 32% greater than the population mean, and a mean PAOH of 47.2%, slightly higher than the population mean (45.0%). Because DBH, AOH, and PAOH were positively correlated in this population, the top five individuals for DBH were predicted to produce progeny superior to the population mean by 13% in DBH, 12.5% in AOH, and 1.0% in PAOH. An increase in AOH is equivalent to an increase in yield, since heartwood is the valuable commodity in black walnut timber. The value of an increase in PAOH is less direct, but assuming a tree growing at 0.84 cm/year in diameter with a rotation size of about 50 cm DBH, a 1% increase in PAOH translates into a 1% decrease in rotation age. The value of increasing PAOH increases as trees increase in diameter.

Selection for heartwood traits is complicated by the necessity for destructive sampling for AOH and because the age–age correlations among 10-year-old, 35-year-old, and rotation-age trees for AOH and PAOH are not known. In addition, several factors created bias in the heritability and family effects estimates (Table1). First, estimates were biased, because the study was maintained in only one location, and we could not estimate family × site effects. This is especially important for walnut, which is considered very sensitive to site. Second, the open-pollinated families used in the study may have contained some full-sibs and even possibly seeds from self-pollinations. As a result, the families may have had a greater covariance than would be found in true half-sib families, and the heritability estimates (Table 1) may be upper bounds. Finally, because of the biological constraints described in the Materials and methods, families were nested within planting year, so planting year × family effects could not be estimated. It is probably not instructive to relate the heritability estimate for AOH reported here (0.33) to that of Rink (1987), for reasons described above, and because Rink used a different method of calculation and a different type of heritability.

The genetic and phenotypic correlations reported here are evidence that in general, factors that contribute to rapid tree growth in walnut also increase the AOH. Perhaps vigorously growing trees produce more of the polyphenols typically found in heartwood. Rink (1987) and Hillis (1975) suggested a relationship between heartwood formation and "stress". The data presented here do not strongly support this connection because the most vigorous trees had the highest AOH and PAOH. While there were significant block effects for AOH and PAOH, it is not clear what environmental factor(s) contributed to this effect.

Black walnut trees in well-managed plantations can grow at twice the rate of the trees in this study. It will be important to determine if PAOH can be improved in trees growing at this faster rate.

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