

Genetic Resistance to Anthracnose of Black Walnut

By D. T. FUNK, D. NEELY, and C. F. BEY¹⁾

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Abstract

Anthracnose incidence in 62 black walnut families was evaluated for 4 consecutive years. Year-to-year correlations for anthracnose incidence were positive but not especially consistent. Correlations for tree growth and anthracnose incidence were considerably lower than the year-to-year correlations, but they were negative. Comparisons of the eight fastest growing families and the eight families with lowest disease ratings showed only one family in common. Selecting solely for rapid growth would result in a 13 percent decrease in anthracnose incidence, while selecting solely for low anthracnose would result in a 7 percent increase in growth.

Key words: Leaf disease, selection potential, heritability.

Zusammenfassung

Für 62 Schwarznußfamilien (*Juglans nigra* L.) wurde in 4 aufeinanderfolgenden Jahren der Anthraknosebefall festgestellt. Die Korrelationen für den Anthraknosebefall waren von Jahr zu Jahr positiv, aber nicht übereinstimmend. Die Korrelationen zwischen Baumwachstum und Anthraknosebefall waren bedeutend niedriger als die zwischen den einzelnen Jahren, aber sie waren negativ korreliert. Vergleiche der acht raschwüchsigsten Familien und der acht Familien mit der niedrigsten Krankheitsrate zeigten nur bei einer Familie Übereinstimmung. Eine Selektion ausschließlich auf Schnellwüchsigkeit würde zu einem 13% niedrigeren Anthraknosebefall führen, während eine abschließliche Selektion auf niedrigeren Anthraknosebefall zu einer 7% höheren Wuchsleistung führen würde.

Introduction

Anthracnose, caused by the fungus *Gnomonia leptostyla* (FR.) CES. et DE NOT., is a widespread and serious leafspot disease of black walnut (*Juglans nigra* L.). Anthracnose causes spotting of leaves and fruit, and under favorable weather conditions, leaf infections can multiply rapidly and cause defoliation in July or August (BLACK and NEELY 1978). Walnut anthracnose has also been shown to be an indirect cause of "ambers", an undesirable darkening of walnut kernels that renders them unsuitable for culinary use (BERRY 1960). Severe infections provoke premature fruit drop or poor filling of nuts.

Fungicides will control anthracnose, but at least two applications during the growing season are required; furthermore, the most effective chemical, benomyl, is not presently registered for use on trees of fruit-bearing age (BERRY 1977). We have therefore begun to investigate genetic resistance to anthracnose in black walnut, and to select disease-resistant black walnut trees for use as parents in a seed orchard. Seed from this orchard would be expected to produce trees that grow faster and yield increased quan-

ties of nuts of improved quality, all without the need for fungicidal treatments.

Methods

In 1968 seed was collected from individual wild black walnut trees growing in the southern half of Illinois and in portions of Kentucky, Tennessee, Missouri, and Arkansas. The parent trees were required only to appear healthy and to be reasonably well-formed. The seed was sown in the Illinois Division of Forestry's Union Nursery, and in the spring of 1970, 1-year-old seedlings were outplanted at the Southern Illinois University Farm in Jackson County, Illinois. Six replications of five-tree-row plots were arranged in a randomized complete block design with 1.2 × 3.0-m spacing. After the fifth growing season, the plantation was thinned to leave the three tallest trees per plot. The progeny test will eventually be converted to a seedling seed orchard (BEY *et al.* 1972).

For our anthracnose-resistance investigation, we used trees of 62 families in three blocks of the plantation. The upland site is a former agricultural field that was cropped regularly prior to establishing the plantation. Site quality for black walnut is marginal, as indicated by already declining height growth. Only the three trees per plot remaining after thinning were used in this study.

Each year, at about the time disease buildup was highest (usually early August), each tree was evaluated for anthracnose incidence. In 1974, 1975, and 1977, the incidence of leaflets with one or more anthracnose-caused spots was visually estimated to the nearest 5 percent. In 1976, we estimated the proportion of the crown prematurely defoliated. In July 1978, electrical resistance (ER) of tissues in the cambial zone was determined using a Shigometer²⁾ (SHORTLE *et al.* 1977). Stainless steel electrode pins, oriented vertically, were pushed through the bark and slightly into the wood at a height of 1.37 m (areas immediately above or below pruning wounds were avoided). Two ER readings, separated by 90°, were taken and averaged. Tree height was measured to the nearest 0.5 foot (15 cm) after the 1972, 1974, 1976, and 1978 growing seasons.

Correlations were calculated for family and individual tree data. Family data were subjected to variance analyses and family heritabilities were calculated using the $h^2 = 1 - \frac{1}{F}$ formula of KUNG and BEY (1979), in which F is the variance ratio for families.

Results

Year-to-year correlations for estimates of anthracnose incidence were nearly all positive, but the values were not consistent (Table 1). Values based on individual trees proved to be stronger than those based on family means. Year-

¹⁾ Assistant Director, Northeastern Forest Experiment Station, Durham, New Hampshire; Plant Pathologist, Illinois Natural History Survey, Urbana, Illinois; and Principal Plant Geneticist, Southern Forest Experiment Station, Gulfport, Mississippi.

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²⁾ Shigometer Model 7950, Northeast Electronics, Concord, New Hampshire; Number 2E moisture detector electrodes in a number 552/A-106 retainer, Delmhorst Instrument Co., Boonton, New Jersey. Use of proprietary or firm names in this paper does not constitute endorsement by the U. S. Department of Agriculture Forest Service.

Table 1. — Correlation coefficients (r) for consecutive annual estimates of anthracnose incidence (individual tree basis)

Year	1975	1976	1977
1974 percent spotted leaves	0.44**	-0.01	0.43**
1975 percent spotted leaves	--	0.37**	.66**
1976 percent defoliation	--	--	.22**

** = r value significant at 0.9999 confidence level

Table 2. — Combined analysis of variance for leaflet spotting in 1974, 1975, and 1977

Source of variation	Degrees of freedom	Mean Square	F
Blocks (B)	2	465	2.68
Families (F)	61	729	4.20**
Error a	122	173	
Years (Y)	2	80,313	1218.90**
B x Y	4	482	7.31**
F x Y	122	133	2.03**
Error b	244	66	

** = F value significant at 0.9999 confidence level.

to-year correlations for percentage of spotted leaves were higher than those for percentage defoliation, with the highest value, $r = 0.66$, relating percentage of spotted leaves in 1975 and 1977. Both of these summers were epidemic anthracnose seasons, and the strong correlation between them suggests that estimates of genetic variation will be more consistent if made when the disease incidence is high.

Another indication of the effectiveness of evaluating disease susceptibility in epidemic years is that family heritability (h^2) estimates were also higher. For the years 1974 to 1977, h^2 estimates were 0.45, 0.77, 0.23, and 0.74, respectively. Note that 1975 and 1977 had markedly higher h^2 values, and that 1976, when defoliation was evaluated rather than leaf spotting, had the lowest. A combined analysis for 1974, 1975, and 1977 yielded an estimate of 0.76. The

Table 3. — Correlation coefficient (r) among estimates of anthracnose incidence, height growth, and Shigometer^R (ER) readings

Variable	INDIVIDUAL TREE BASIS			
	Height growth (2-year period)			ER
	1973-1974	1975-1976	1977-1978	
1974 spots	-0.02	-0.06	-0.04	0.04
1975 spots	-0.14**	-0.26**	-0.03	0.08
1976 defoliation	-0.10*	-0.30**	-0.12**	-0.05
1977 spots	-0.10*	-0.13**	-0.12**	0.17**
ER	-0.19**	-0.25**	-0.26**	--

FAMILY AVERAGE BASIS				
Variable	1973-1974	1975-1976	1977-1978	ER
1974 spots	0.03	0.00	-0.25*	0.15
1975 spots	-0.02	-0.24*	-0.04	0.15
1976 defoliation	0.18	0.00	-0.04	-0.02
1977 spots	-0.02	-0.06	-0.17	0.10
ER	-0.21	-0.32**	-0.08	--

* = r value significant at 0.95 confidence level

** = r value significant at 0.99 confidence level

analysis of variance (Table 2) provides the information needed for heritability calculation: F value for families = 4.20; $h^2 = 1 - \frac{1}{4.20} = .76$.

Correlations among tree growth, disease incidence, and ER readings were considerably lower than the year-to-year correlations for anthracnose (Table 3). As previously noted, height growth has slowed in the past few years, and correlations of total height with anthracnose estimates or Shigometer readings were poor. Height increment for the three most recent 2-year periods proved to be a better correlative statistic. As with the serial disease correlations, individual tree data provided the basis for more significant interrelationship than did family means (although the latter r values were occasionally higher, but still nonsignificant because of fewer degrees of freedom). In every instance, faster growth was negatively correlated with disease incidence and ER.

The only correlation between anthracnose ratings and ER that was statistically significant was positive (Table 3). This finding seems reasonable because low ER is considered to be an indicator of vigor (SHORTLE *et al.* 1977), and anthracnose symptoms are typically reduced in vigorous trees (FUNK *et al.* 1979).

Although the correlations between growth and disease were not strong, they were at least negative (less disease associated with greater growth). To assess the impact of this loose association on a selection program, we selected the eight fastest growing families for the 1977-1978 seasons, and the eight families with lowest leaf-spot ratings for the 1977 epidemic season; only one family was common to both groups. Their mutual characteristics are tabulated below:

Selection criterion	Leaves spotted	2-year height growth
	(percent)	(meters)
Low anthracnose	31.7	0.87
Rapid growth	45.0	1.37
Plantation average	51.8	.82

Five of the eight families selected for low disease incidence grew faster than the plantation average and the group exceeded the average-growth rate by 7 percent. All but one of the selected rapid-growing families had fewer than average leaf spots, with the group rating 13 percent lower (better) than average. Gains from the two approaches to selection are listed below:

Families selected for:	Percentage decrease in anthracnose	Percentage increase in growth
Low anthracnose	39	7
Rapid growth	13	67

In most black walnut breeding programs growth is of primary importance, and it should be advisable to select families on the basis of the single trait for rapid growth. For conversion of open-pollinated progeny tests to seedling seed orchards, within-family selection for anthracnose resistance might also be considered. A more thorough approach would be to assign a relative economic value to each trait and develop a selection index that considers the genetic and phenotypic variances and correlations for the two traits. A modified approach to simultaneous multi-trait selection could also be used (Bey *et al.* 1974). In the

modified approach, the best families are selected on the basis of single traits. It employs unequal weighting in favor of families that are superior for several traits and considers the correlation between traits for the selected families. For growth and anthracnose resistance, which is already partially reflected in the growth trait, the modified approach might easily be applied.

These findings will be of most use to breeders attempting to improve black walnut for both timber and nut production. Breeders strictly interested in timber production may find it more appropriate to emphasize growth and wood quality traits. However, because of the direct impact of anthracnose on both tree growth and nut quality and yield, multicrop breeders can hardly afford to pass up opportunities to improve disease resistance. We believe that the potential for making gains in both traits is attractive, and suggest that rating of anthracnose incidence be considered in genetic improvement programs for black walnut. Our procedures are simple to use, and ratings obtained in only one or two epidemic years should be sufficient for effective selection of resistant families or individuals.

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Estimation of Outcrossing in Ponderosa Pine, *Pinus ponderosa* Laws., from Patterns of Segregation of Protein polymorphisms and from Frequencies of Albino Seedlings

By J. B. MITTON¹, Y. B. LINHART¹, M. L. DAVIS¹ and K. B. STURGEON¹

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Summary

Six protein polymorphisms and one seedling character, albinism, were used to estimate the level of outcrossing in ponderosa pine, and to investigate several other aspects of the mating system. The six protein polymorphisms give a homogeneous set of estimates for outcrossing with a weighted average of 0.96, and this estimate is in good agreement with estimates based upon seedling albinism and with a previous estimate based upon one protein polymorphism. Row by columns contingency tests of progeny arrays from different seed trees detected no tree-to-tree heterogeneity in the mating system. For two of the six protein polymorphisms, estimates of allelic frequencies in the effective pollen pool differed significantly from the frequencies of adult trees in the population.

Key words: Outcrossing, ponderosa pine, protein polymorphism, albinism.

Zusammenfassung

Sechs Proteinpolymorphismen sowie ein Sämlingsmerkmal, (Albinismus), wurden benutzt, um den Grad der Fremdung innerhalb der Baumart *Pinus ponderosa* abzuschätzen und um einige andere Aspekte des Kreuzungsschemas zu untersuchen. Die sechs Proteinpolymorphismen erge-

ben in Bezug auf die Fremdung eine homogene Reihe von Schätzungen mit einem gewichteten Durchschnitt von 0,96. Sie stimmt mit den Schätzungen überein, die sich auf den Sämlingsalbinismus und einen Proteinpolymorphismus beziehen. Kontingenztests hinsichtlich der Nachkommenschaftsanordnungen aus verschiedenen Samenbäumen zeigten in dem Kreuzungssystem keine Heterogenität zwischen den Einzelbäumen. Für zwei der sechs Proteinpolymorphismen unterschieden sich die Schätzwerte hinsichtlich der Allelfrequenz in den Pollen-pools wesentlich von den Frequenzen der Elternbäume in der Population.

Introduction

The mating system is an important factor influencing several aspects of the genetic architecture of a population, most particularly the proportions of genotypes, the distribution of genetic variability, and the degree to which the genome is organized. Populations in predominantly outbred species are expected to have levels of heterozygosity approximating the expectations of the binomial square law, while populations in predominantly inbreeding populations are expected to exhibit a reduction in heterozygosity proportional to the degree of selfing. The mating system also influences genetic correlations among loci within a population. While outbred populations will exhibit independence between loosely linked and unlinked loci, in-

¹) Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, CO 80309, USA