VARIATION IN LEAF MORPHOLOGY IN BLACK WALNUT (Juglans nigra L.) AND ITS POSSIBLE ROLE IN PHOTOSYNTHETIC EFFICIENCY

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The photosynthetic efficiency or net productivity of trees and forests is determined by an integration of genetic and environmental factors which affect the photochemistry, biochemistry, physical diffusion of CO2 into chloroplasts, and activities of nonphotosynthetic plant tissues. Photosynthetic efficiency also involves the ultimate translocation of products derived from CO_2 assimilation into the plant tissues or organs that comprise yield, usually the stem or bole in forest trees (Zelitch, 1971). Photosynthetic efficiency is affected by such diverse plant and site factors as:

1. Properties of leaves such as stomatal numbers and behavior; response of the mesophyll cells to irradiance, reflectance and transmittance properties; effect of temperature on dark respiration and photorespiration; and their physical resistances and carboxylation characteristics.
2. Plant architecture including total leaf area, leaf distribution along the stem, and the angle of leaf elevation from the horizontal.
3. Duration of photosynthesis, changes of photosynthesis with leaf size and age, the efficiency of CO_2 assimilation, the rapidity with which the leaf area enlarges to absorb the available irradiation, plant height in mixed stands, photosynthesis by organs other than leaves, and the efficiency of transport of photosynthate to tissues of economic importance.
4. Soil factors such as soil moisture, nutrient availability, and CO_2 released in soil respiration.
5. Ambient climatic factors such as air temperature, wind speed, CO2 concentration, relative humidity, angle of sun, and nature of radiation.

During photosynthesis a CO2 concentration gradient exists between the ambient air and that of the chloroplasts. The rate of net photosynthesis is determined by the size of this gradient and by a series of resistances to the diffusion of CO2 (Figure 1). The resistances to diffusion are both inside and outside the leaf. Some are physical in nature and are related to an interaction of environmental and plant factors. Others are mostly biological in nature.

A great deal of attention has been given to the study of the biochemistry of photosynthesis and respiration, and several examples of interspecific and intraspecific variations have been reported. Zelitch and Day (1968) reported genetic differences in photorespiration of tobacco. Bjorkman, *et al.* (1970 reported differences in photorespiration within the genus *Atriplex*. Carpenter and Hanover (1972) found great variation in rates of net photosynthesis, dark respiration, and photorespiration between individual trees of black walnut and honeylocust. Honeylocust seedlings exhibited remarkable superiority in CO2 uptake over black walnut seedlings on a leaf-area basis. However, black walnut, because of its larger leaf area, had much higher rates of CO_2, uptake on a whole seedling basis. Much of the intraspecific variation also appeared to be related to leaf morphology and total crown area.

Relatively few attempts have been made to study leaf morphology in relation to photosynthetic efficiency. EI-Sharkawy and Hesbeth (1964) found differences in stomatal size and frequency in some crop plants which appeared to affect photosynthetic rate. Holmgren (1968) found significant differences between ecotypes of *Solidago virgaurea* L. Leaf thickness may be an important factor in deciduous leaves (McClendon, 1962). It is not unreasonable to expect that stomatal size and distribution as well as other apsects of leaf morphology may show similar variations between ecotypes of the same species. In 1972 we made a preliminary study of variation in leaf morphology in black walnut.

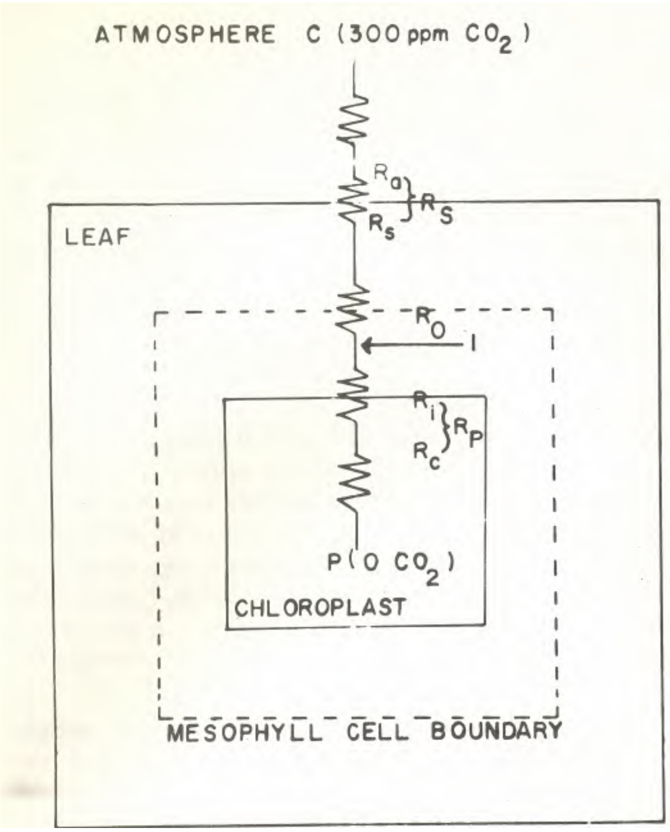

Figure 1. Diagrammatic representation of the relation between net photosynthesis and respiration in a leaf (from Waggoner, 1969). Photosynthesis is shown as a flux of CO_2 caused by the higher concentration of CO_2 (300 ppm) in the atmosphere and the zero concentration at some point within the chloroplast. Total respiration, I, joins the main photosynthetic current outside the chloroplast and thus diminishes net CO_2 uptake. The current of CO2 assimilation is opposed by a series of resistors including Ra in the air, Rs the stomata, Ro at the cell boundary, Ri inside the cell, and Rc caused by limitations of photochemistry and biochemistry on the carboxylation within the chloroplast. Ra and Rs are sometimes combined as Rs; Ri and Rc are taken together as Rp.

METHODS

In March, 1972, one-year-old black walnut seedlings were planted in 5-gallon plastic pots containing an equal mixture of soil, sand, and peat. These seedlings were from open-pollinated, single-tree seed collections made by the U.S. Forest Service and grown in Carbondale, Illinois, in 1971. Seven geographic origins of seed were represented, as follows:

Alabama, Marshall County
Georgia, Rabun County
Illinois, Carbondale
Kentucky, Logan County
Louisiana, Winn County
Michigan, Cass County
Texas, Houston County

A single seedling was planted in each pot, and the pots were placed in an open field in a randomized block design. Each of the five blocks contained seven pots, one from each geographic source. Location of this work was in Lexington, Kentucky.

In late September, 1972, total leaf area was measured with a planimeter, and samples were collected for determinations of stomatal size and distribution and chlorophyll content. Stomatal distribution and size were determined from SEM photomicrographs. A magnification of 135X was used to determine stomatal distribution (Figure 2). The magnification was increased to 680X to study stomatal apertures (Figure 3). Because width of stomatal pores varies greatly during stomatal movement, only lengths were measured to provide an index of stomatal size. Chlorophyll concentrations were determined by the method of Strain and Svec (1966). A single compound leaf was extracted in 80-percent acetone, and chlorophyll *a* and *b* levels were determined with a colorimeter at wavelengths of 649 and 665 min, respectively.

Figure 2. Photomicrograph of lower epidermal surface of black walnut leaf showing trichomes and stomates (X 135).

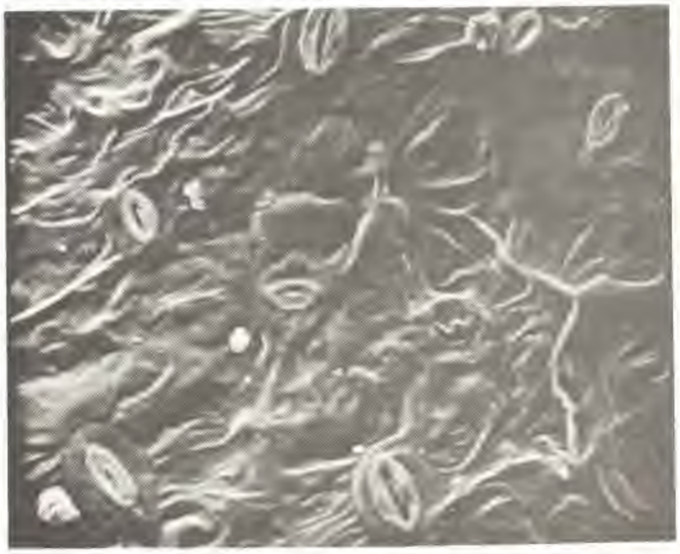

Figure 3. Photomicrograph of lower epidermal surface of black walnut leaf (X 680).

RESULTS AND DISCUSSION

Distribution and Number of Stomates

Stomates from all seven source were limited to the lower epidermal surface. Stomatal means varied from 13,809 per cm.^{2} in the Louisiana source to a high of 35,428 per cm.^{2} in the Illinois source, as follows (in ascending order):

Source	*Stomatal mean per cm.^{2}*
Louisiana	13,809
Kentucky	22,667
Texas	24,381
Michigan	27,905
Alabama	28,571
Georgia	32,381
Illinois	35,428

These values are considerably lower than those previously reported for black walnut (Meyer, *et al.*, 1960). Only the Louisiana source showed signficiant variation from the other source means. No geographic trends were observed in stomatal distribution.

Stomatal Size

Stomatal size does not appear to have been previously reported for black walnut. Variation between the seven sources was found to be (ascending order) as follows:

Source	*Stomatal Length (microns)*
Texas	12.9
Georgia	13.2
Kentucky	13.4
Alabama	14.0
Louisiana	14.4
Illinois	14.9
Michigan	15.1

There was some evidence of a north-south trend. Smallest stomates, 12.9µ, were observed in the Texas source while the largest stomates were observed in the Illinois and Michigan sources. Stomatal sizes reported here for black walnut are reasonably close to those previously reported for other deciduous forest trees (Meyer, *et al.*, 1960). Much larger stomates have been observed in annual plants which exhibit higher rates of net photosynthesis than woody plants.

Total Leaf Area

Total leaf surface area, lower epidermis only, varied from a low of 771 cm.^{2} in the Alabama source to a high of 2,489 cm.^{2} in the Illinois source. These values for all seven sources were as follows:

Source	*Leaf area per seedling (cm.^{2})*	*Mean leaf area in cm.^{2} per compound leaf*
Illinois	2489	144
Michigan	1285	116
Georgia	1377	109
Alabama	771	106
Kentucky	1034	92
Louisiana	1369	84
Texas	1092	78

There was no observable geographic trend. Note from the above, however, that, when leaf area is expressed as an average per compound leaf, a north-south trend is indicated. Southern sources had a greater number of compound leaves and thus a smaller average area than northern sources with fewer compound leaves.

Chlorophyll Concentration

Leaves of ecotypes which retain high chlorophyll concentrations in the fall would appear to have an advantage in photosynthetic efficiency over leaves from other ecotypes which become chlorotic at an early date. There were visual differences between the sources in this study as leaf abscission began. Table 1 shows the actual variation in chlorophyll content in the seven sources.

Table 1. Chlorophyll content of leaves of widely separated sources of black walnut at the end of the growing season

	Chlorophyll Content (µg/cm^2)		
Source	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Louisiana	.026	.043	.069
Kentucky	.024	.040	.064
Texas	.022	.035	.057
Illinois	.019	.031	.050
Alabama	.018	.026	.044
Georgia	.013	.026	.039
Michigan	.011	.021	.032

CONCLUSIONS

Tree breeders have made significant progress toward increasing yield of forest trees by empirical methods. It is possible that in the future productivity can be increased even faster by the use of logical methods which integrate physiology and genetics. Genetic improvement may result from studies of photosynthetic efficiency. Diminishing photorespiration and dark respiration; increasing stomatal number, size, and extent of their opening; and lowering internal diffusion resistances—all by genetic means—or by simply incorporating lines with higher than usual rates of net photosynthesis per unit leaf area into otherwise ideal plant types should provide significant increases in yield.

Results from this preliminary study indicate that there is variation in several morphological characteristics of black walnut seedlings which could provide a basis for breeding to achieve increased photosynthetic efficiency.

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