

# RATES OF ETHYLENE PRODUCTION BY PARENCHYMA CELLS IN BLACK WALNUT SAPWOOD

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## ABSTRACT

Black walnut sapwood samples, representing high and low extremes in ethylene production rates, were anatomically analyzed to determine the relation between the amount of parenchyma in the sapwood and the rate of ethylene production. These analyses indicated that ethylene production in sapwood is controlled not by the amounts of parenchyma, but by the rate of ethylene production within each sapwood parenchyma cell.

*Keywords:* *Juglans nigra* L., ethylene, heartwood formation, wood anatomy, sapwood, parenchyma, hormones.

## INTRODUCTION

The phytohormone ethylene is thought to be associated with heartwood formation in walnut and other woody plants (Shain and Hillis 1973; Hillis 1975; Nelson 1978) and has been shown to be related to the formation of phenolic compounds in both herbaceous and woody plants (Craker and Wetherbee 1973; Rhodes and Woollorton 1973; Hillis 1975). Ethylene production within the xylem may be related to several quantitative factors that influence wood quality in black walnut (*Juglans nigra* L.). These factors include the rate of heartwood formation (Nelson 1978), the amount and composition of phenolic extractives produced during heartwood formation (Hillis 1975, 1977), and the amount and composition of phenolic extractives produced in response to stress and injury (Hillis 1975, 1977; Shigo and Hillis 1973). Thus, understanding the physiological control(s) of ethylene production may be important for interpreting how heartwood is formed in walnut and how phenolics are formed in other higher plants (Abeles 1973).

Within any specific sapwood region, ethylene emanating from excised tissue can originate from at least two sources: (1) living cells outside the specific sapwood region, with ethylene transported to the region prior to excision of the tissue by diffusion or mass flow within the liquid and gas phases of the tree, and (2) living axial and ray parenchyma cells within the region. The first source is probably unimportant in excised tissue in which *in vitro* ethylene production is

followed for several hours (Abeles 1973; Ben-Yehoshua and Aloni 1974; Nelson and Hillis 1978), as was the case in the present study. Ethylene production by the second source can be affected by two primary factors: (1) biochemical control over biosynthesis of ethylene within the parenchyma cells, and (2) the amount of parenchyma cells in the wood. This paper is concerned with the latter potential mode of control of ethylene production rates in living xylem, i.e., the relation between the amount of parenchyma and ethylene production. In addition, the study presented an opportunity to calculate ethylene production per volume of parenchyma tissues and per parenchyma cell. These data are rare in the literature and are applicable to a broad spectrum of plant physiology research on ethylene.

#### MATERIALS AND METHODS

The trees used in this study were from five families of walnut grown on two plantations (Union County, Jackson County) in southern Illinois. In a previous study, increment cores were removed in November 1978 from forty, 9-year-old walnut trees to study *in vitro* sapwood ethylene production rates (Nelson et al. 1981). As a consequence of these analyses, sample cores were selected for the present study. In the present study, one increment core from twelve trees (six from each plantation) was selected for anatomical analysis. The cores were chosen to represent three high and three low ethylene producers per plantation and were matched cores to those used to determine ethylene production rates. A more complete description of the trees and the procedures used in the ethylene analysis is given in Nelson et al. (1981).

The following criteria were used to select high and low ethylene-producing samples. The sapwood in each core was divided into thirds, and an average ethylene production rate was computed for each one-third sapwood segment within each plantation. The tree was classified as a high ethylene producer if the outer region of the sapwood had a substantially higher ethylene production rate than the average rate for the outer segment of the other trees in the plantation and if at least one of the other two segments (middle and/or inner) also had higher rates than the plantation average for the respective region. Low ethylene-producing samples were selected using the inverse of the above criteria.

All measurements of growth ring width and amounts of parenchyma were obtained from transverse microtomed sections from the selected increment cores. The sections were stained with safranin and permanently mounted on microscope slides. Percentages of axial and ray parenchyma were determined with a fixed dot grid system similar to that described by Smith (1967). The grid was attached to the projection screen of a light microscope, and ten random measurements were obtained using a 24× objective within each annual ring of each sapwood increment core. To estimate the area occupied by parenchyma in the growth ring, the number of dots that fell on the parenchyma (axial or ray) were averaged for the ten readings. This average was divided by the number of dots on the screen and was subsequently multiplied by 100 to express the value as a percentage of the area occupied by parenchyma. Percentage parenchyma by area was assumed to be a valid estimate of percentage parenchyma by volume. After determining the amount of parenchyma in each growth ring, the data were converted to one-third segments of the sapwood to correspond to the ethylene measurements. Ring widths were used as the weighting factors for these conversions.

Specific gravity (oven-dry weight/green volume basis) of each one-third segment of the sapwood was obtained using the maximum moisture content method (Smith 1954). To express parenchyma on a volume per gram basis, the axial and/or ray parenchyma value was divided by the specific gravity from that same segment. In this and all subsequent calculations, parenchyma were expressed as fractions rather than percentages. To calculate ethylene production per volume of parenchyma, the ethylene production rate was multiplied by the quotient of the specific gravity divided by the parenchyma value. To calculate ethylene production on a per cell basis, the ethylene production rate per gram of dry wood per hour was divided by the number of parenchyma cells per gram. The number of parenchyma cells per gram of wood was calculated by dividing the amount of parenchyma by the specific gravity, which was further divided by the average volume of an individual parenchyma cell. Volumes of individual axial and ray parenchyma cells were obtained from data in Appendix F of Nelson (1973) for walnut trees similar in age to those used in the present study.

Analysis of variance (ANOVA) was done using a Statistical Analysis System (SAS) program. One-way analyses were run using ethylene production rates (high versus low) as the treatments. Analyses of variance were run on several variables including axial parenchyma and ray parenchyma percentages; parenchyma expressed on density, volume, and weight basis; and ethylene production per gram of wood, per total parenchyma volume, and per parenchyma cell.

#### RESULTS AND DISCUSSION

The objective of this study was to examine possible contributions of parenchyma cells to ethylene production rates. Because parenchyma cells are the predominant living cells in the sapwood of woody plants and ethylene production has been shown to occur *in vitro* in isolated sapwood samples (Shain and Hillis 1973; Hillis 1975; Nelson 1978; Nelson and Hillis 1978; Nelson et al. 1981), this study was developed to examine the relation between amounts of parenchyma cells (on an area, volume, and number per gram of wood basis) and ethylene production rates. If no positive relation between parenchyma amounts and ethylene production rates exists, then it could be assumed that variation in ethylene rates is a biochemical phenomenon rather than one dependent on numbers of parenchyma. Our data suggest that such a biochemical control relation exists (Table 1). Significant differences in parenchyma variables occur, but these are contrary to the assumption that increases in numbers of parenchyma lead to increases in levels of ethylene production. In fact, the samples that produced low levels of ethylene actually had larger numbers of parenchyma cells (based on cells per unit volume) and more parenchyma tissue (based on a volume to volume and volume to mass relation) than those samples that had high ethylene production rates.

These observations are further strengthened when ethylene production rates per dry weight of wood, per volume of parenchyma, and per parenchyma cell are compared (Table 2). For example, note that the high ethylene-producing samples had rates approximately five times greater than the low ethylene-producing samples regardless of how the rate was expressed. This comparison indicates that the rate of ethylene production in the sapwood is controlled by the rate of ethylene production within each parenchyma cell of the sapwood and not by the amounts

TABLE 1. *Parenchyma characteristics of high and low ethylene-producing sapwood samples (n = 18).*<sup>a</sup>

Sapwood sample	Axial parenchyma			Ray parenchyma		
	(%) <sup>b</sup>	(cm <sup>3</sup> /g) <sup>c</sup>	(cells/g) <sup>d</sup>	(%) <sup>b</sup>	(cm <sup>3</sup> /g) <sup>c</sup>	(cells/g) <sup>d</sup>
High ethylene-producing	3.6*	0.059*	4.5 × 10 <sup>6</sup> **	15.3**	0.247*	3.9 × 10 <sup>7</sup> *
Low ethylene-producing	4.3*	0.068*	5.2 × 10 <sup>6</sup> **	17.3**	0.275*	4.4 × 10 <sup>7</sup> *

<sup>a</sup> Data are the means of 18 observations (6 cores × 3 regions of sapwood).

<sup>b</sup> Cross-sectional area basis.

<sup>c</sup> Green volume of parenchyma per gram of oven-dry wood.

<sup>d</sup> Number of parenchyma cells per gram of oven-dry wood.

\* Indicates that the difference between the values in the column is significant at the 5% level.

\*\* Indicates that the difference between the values in the column is significant at the 1% level.

of parenchyma. Thus, it appears that biochemical control of ethylene biosynthesis is of primary importance in determining ethylene production rates per unit weight of sapwood. Nelson (1975) reached a similar conclusion for the biosynthesis of phenolics within the inner sapwood of walnut during heartwood formation. That is, the quantity of phenolic extractives produced per unit weight of wood by axial and ray parenchyma cells during heartwood formation was not influenced by the amount of parenchyma cells present.

The data on ethylene production rates per volume of parenchyma tissue and per parenchyma cell are applicable to a range of plant physiology research on ethylene. To our knowledge, such data are not available in the literature for any plant species, with the exception of simulated calculations of endogenous ethylene per cell in Abeles (1973). Possible uses of this type of data in physiological research include calculation of the number of ethylene attachment sites in a cell (Abeles 1973), refinement of ethylene dose-response relations to a per cell basis, a better understanding of the ratio of internal to emanated ethylene in woody tissue, and improved knowledge of the stimulation of phenolic synthesis in wood by ethylene (Shain and Hillis 1973; Hillis 1975).

#### SUMMARY

Black walnut sapwood samples, representing high and low extremes in ethylene production rates, were anatomically analyzed to determine the relation between the amount of parenchyma in the sapwood and the rate of ethylene production.

TABLE 2. *Specific ethylene production rates of black walnut sapwood (n = 18).*<sup>a</sup>

Sapwood sample	Ethylene production rate		
	Per gram of wood (nl g <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>	Per total parenchyma volume (nl cm <sup>-3</sup> h <sup>-1</sup> ) <sup>c</sup>	Per parenchyma cell (nl cell <sup>-1</sup> h <sup>-1</sup> ) <sup>d</sup>
High ethylene-producing	31.73*	106.17*	7.44 × 10 <sup>-7</sup> *
Low ethylene-producing	6.66*	19.77*	1.39 × 10 <sup>-7</sup> *

\* Denotes significant differences between values in the column at the 5% level.

<sup>a</sup> Data are the means of 18 observations (6 cores × 3 regions of sapwood).

<sup>b</sup> Nanoliters of ethylene per gram of oven-dry wood per hour.

<sup>c</sup> Nanoliters of ethylene per cubic centimeter of parenchyma per hour.

<sup>d</sup> Nanoliters of ethylene per parenchyma cell per hour.

Statistical analysis revealed that low ethylene-producing samples had slightly more parenchyma than high ethylene-producing samples. When expressed as a function of oven-dry weight of total wood substance, total parenchyma volume, or individual parenchyma cells, ethylene production was approximately five times greater in high ethylene-producing samples than in low ethylene-producing samples. This indicates that ethylene production in sapwood tissue is controlled not by the amount of parenchyma, but by the rate of ethylene production within each sapwood parenchyma cell. Expression of ethylene production on an individual cell basis is a novel approach and could be applicable to other plant physiology research on ethylene. Furthermore, because ethylene production is thought to be related to heartwood formation, analyses such as these may be important selection criteria in those species in which heartwood formation is an important wood quality characteristic.

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