GROWTH-QUALITY EVALUATION OF BLACK WALNUT WOOD. PART III—AN ANATOMICAL STUDY OF COLOR CHARACTERISTICS OF BLACK WALNUT VENEER

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(Received 10 June 1982)

ABSTRACT

Because wood color is an important quality characteristic in black walnut (*Juglans nigra* L.), a study was devised to examine some cellular features of heartwood coloration using microspectrophotometric techniques. Five commercially prepared veneer samples exhibiting low macro-luminance (darker) and five exhibiting high macro-luminance (lighter) were chosen for microscopic color analysis. Statistically significant differences were found between the high and low macro-luminance sample groups in the micro-luminance color value in some cellular features (axial parenchyma walls, ray parenchyma walls, fiber walls, and ray parenchyma inclusions). Also observed were large differences in the dominant wavelength of ray parenchyma inclusions between the sample groups. These results suggest that quality of these coloration pigments (phenolics) is more important than quantity for overall color variations.

Keywords: Microspectrophotometry, wood anatomy, wood color, *Juglans nigra* L., veneer.

INTRODUCTION

Wood color is an important wood quality characteristic in black walnut (*Juglans nigra* L.) and some other fine hardwood species. Often, the color of walnut heartwood will vary between trees of the same species and within individual trees. This color variation is not desirable because manufacturers prefer heartwood of a uniform, light brown color.

It is therefore necessary to establish some fundamental knowledge about heartwood color and about the processes (physiological and/or ecological) that influence heartwood coloration. Nelson (1978) has reported on some physiological changes that occurred during heartwood formation in black walnut and black cherry. Frey-

[1] This work was conducted while the senior author was a Research Associate at the University of Missouri and is a part of his Ph.D. dissertation. The authors would like to extend their appreciation to the North Central Forest Experiment Station for contributing some of the sample material for this study and to Mr. Jim Burroughs for his assistance in computer and statistical matters. Use of trade names in this manuscript does not constitute endorsement by the USDA Forest Service or the University of Missouri. Current address of John Phelps is University of Missouri.

Wood and Fiber Science, 15(3), 1983, pp. 212–218

Wyssling and Bosshard (1959) gave an account of some cytological changes that occurred in parenchyma during the transition from recently formed sapwood to heartwood in several European tree species. Hillis (1975) has suggested a relationship between stress conditions and the occurrence of compounds linked to heartwood coloration in some *Eucalyptus* species. Unfortunately, there is little information on the actual coloration phenomenon itself. To supply some information on this topic, we chose to examine differences in color at the cellular level in black walnut veneer samples that had visually different color characteristics and to quantify the extent to which the color differs within cell walls and cellular inclusions.

Microspectrophotometric methods have been used to measure the amounts of chromatin material in plant cells (Dhillon et al. 1978; McLeish and Sunderland 1961), the amounts of free space in plant cell walls (Berlyn 1969), and the amounts and locations of chemical compounds in plant cell walls (Lange 1954; Parameswaren and Bauch 1975). These methods have used specific stains to characterize the substance under study. Because of the highly selective nature of the stain for a particular wavelength of light, the absorbance of monochromatic light focused on the substance can be measured. This absorbance can be related to the amounts of material present under carefully defined conditions (Berlyn 1969).

Similar methods can be used to determine the "color" of cell walls and inclusions in black walnut veneer. Veneer samples were originally selected on the basis of their macroscopic color, using standard color characterization methods (Moslemi 1967). Moslemi (1967) suggested that much of the within-species variation in wood color can be characterized by the luminance (lightness) color parameter. The other color parameters, dominant wavelength (hue) and percentage purity (saturation), are less indicative of visual color differences. These observations were further substantiated by preliminary macro-color analysis (Phelps et al. 1983) on the heartwood samples used in the present study. We selected samples of veneer that exhibited extremes in the luminance parameter so that the color of cell walls and inclusions could be described and compared between samples of different macro-color properties.

As a further expansion of these microscopic analyses, cellular amounts and sample orientation were examined to better describe the anatomical nature of the samples.

Such microscopic evaluations hold promise for describing the variations in heartwood coloration that occur in economically important trees. When such anatomical studies are considered with information derived from ecological and physiological studies of black walnut, a better understanding of these interactions should result.

MATERIALS AND METHODS

The sample material

Five veneer samples that exhibited high luminance (visually lighter coloration) and five that exhibited low luminance (visually darker coloration) were used in this study (Table 1). A spectrophotometer with a diffuse reflectance attachment was used to obtain reflectance readings of each sample. Measurements were taken at 10-nanometer (nm) intervals within the visible spectrum (400 to 700 nm). The

TABLE 1. *Color parameters of the samples used in this study ranked according to percentage luminance using an averaged day light ("C") illuminant.*

High luminance group					
Sample number	Luminance (percent)	Chromaticity values		Dominant wavelength (nm)	Purity (percent)
		x	y		
1	28.129	0.356	0.347	583	20.5
2	27.988	0.350	0.342	583	17.5
3	26.658	0.356	0.345	584	20.0
4	26.637	0.348	0.340	584	16.5
5	26.388	0.358	0.347	583	21.0
Low luminance group					
Sample number	Luminance (percent)	Chromaticity values		Dominant wavelength (nm)	Purity (percent)
		x	y		
6	16.894	0.349	0.339	585	16.5
7	16.832	0.354	0.346	583	19.7
8	16.307	0.355	0.343	584	19.2
9	16.257	0.351	0.342	584	17.8
10	16.205	0.356	0.342	585	19.2

standard was a barium sulfate disc. Reflectance readings, taken at ambient relative humidity and temperature, were transformed into color parameters using previously reported methods (Moslemi 1967). Phelps et al. (1983) describe the macrocolor of the veneer in greater detail.

For the microscopic color analyses, a small square (approximately 1 cm^2) was removed from the central portion of each veneer sample. Each square approximated the area from which the original macro-reflectance data were obtained. It was mounted onto an air-dried walnut heartwood block by using cyanoacrylate glue. After the glue dried, the blocks were placed into a beaker containing boiling distilled water. After 1 to 2 hours, the material was removed and placed into cool water. A sledge microtome was used to obtain several 20-μm-thick sections from each sample square that had the same longitudinal orientation as the original face of the veneer sheets from which the samples were taken.

The sections were dehydrated in a graded ethanol-xylene series and permanently mounted on microscope slides under a coverslip. No histological stains were used. Obviously, consistency in the color of the heartwood blocks and in the dehydration schedule was important to make the comparisons reliable. No noticeable extractions were observed during the dehydrating process.

Cell distribution

One factor that might influence wood color is the relative distribution of cell types on the surface of the veneer. A cut-out method (Smith 1967) was used to evaluate the quantity of ray parenchyma, axial parenchyma, vessels, and fiber-tracheids within those samples selected for microscopic analysis. Four low power photomicrographs of a section from each veneer sample were taken at random. The above cell types were cut out and weighed. The weights were then expressed as a percentage of the total photograph weight. These photomicrographs were also used to examine the longitudinal orientation of the samples with respect to radial or tangential planes of reference.

TABLE 2. *Percent of cell type in each sample.*

Cell type	High luminance group						Low luminance group					
	Sample numbers						Sample numbers					
	1	2	3	4	5	Ave.	6	7	8	9	10	Ave.
Ray parenchyma	14.0	18.6	14.4	24.3	26.4	19.5	19.3	18.7	22.7	14.3	17.6	18.5
Axial parenchyma	19.0	9.4	12.4	8.3	8.8	11.6	11.0	15.8	10.9	15.2	18.4	14.3
Vessels	11.0	18.7	10.2	8.5	12.5	12.2	12.6	13.7	10.4	14.9	14.5	13.2
Fiber-tracheids	56.0	53.3	63.0	58.9	52.3	56.7	57.1	51.8	56.0	55.6	49.5	54.0
Sample orientation*	t	vot	t	vot	vot		sot	vot	sot	t	t	

* The symbols t, vot, and sot indicate tangential, very oblique tangential and slightly oblique tangential, respectively. The terms very oblique and slightly oblique tangential refer to the relative departure of the sample orientation from the tangential.

Cell color quality evaluations

The equipment used in this phase of the study was described previously (McGinnes and Melcarek 1976). This equipment consisted of a 150-w Xenon light source, a mirror monochromator (Littrow arrangement), and a Leitz Ortholux research microscope. A photovoltaic sensor (HAV-1000) was used as the detector. The sensing unit was connected to a digital voltmeter that was connected to a paper tape punch.

Transmittance was measured through axial and ray parenchyma walls, fiber-tracheid walls, vessel walls, and axial and ray parenchyma inclusions. The raw transmittance data from each of these cellular features were standardized, wavelength by wavelength, to transmittance values through an adjacent vessel lumen. Care was taken to obtain standard (lumen) values within the same field of focus because changes in focus were observed to influence the light intensity that was sensed. Eleven readings of each parameter were obtained for each veneer sample. These represented data from various parts of a section as well as from various sections of the same veneer sample. Percent transmittance data were obtained at 10-nm intervals throughout the visible spectrum (400 to 700 nm).

RESULTS AND DISCUSSION

Cell distribution

Wood color was evaluated in terms of relative numbers of cells, orientation of veneer samples, or location and amount of inclusions on wood color. This was done to determine if, for example, a surface with a larger amount of fibrous tissue was more reflective (lighter) than a surface with more vessels.

Table 2 presents data obtained from microscopic observations of cell distribution within the samples. Average values are given as well as individual sample values because variations were observed within the high luminance and low luminance sample groups and the averaged values suggest little variation between sample groups. These observations indicate, for example, that the high luminance group had no more fibrous area than the low luminance group. In addition, the orientation of the veneer samples apparently contributed little to the color of the wood. Samples with tangential orientation were found in the high and low sample groups as were samples more radially aligned. These observations suggest that black walnut wood color depends more on coloring matter within the cells than on the relative amounts of the cells or the orientation of the samples. Nelson and Heather (1970) observed similar interactions when they suggested that wood with

TABLE 3. *Color parameters derived from microspectrophotometric analyses of cellular properties.*

	High luminance samples			Low luminance samples		
	Luminance (percent)	Dominant wavelength (nm)	Purity (percent)	Luminance (percent)	Dominant wavelength (nm)	Purity (percent)
Fiber-tracheid walls	94.064*	581.5	3.06	87.904*	582.9	5.28
Vessel walls	86.045	584.6	4.82	77.680	586.9	7.61
Axial parenchyma walls	90.299*	582.5	4.02	85.691*	583.3	5.92
Ray parenchyma walls	87.454*	582.2	5.71	82.363*	582.5	7.67
Axial parenchyma inclusions	71.454	595.3	5.36	63.897	600.9	5.79
Ray parenchyma inclusions	64.463*	596.1	7.10	53.202*	686.0	4.76

* Indicates that the values in the same horizontal row are statistically different at the 0.10 level based on an unpaired *t*-test (n = 5). Values were weighted using source "C" illuminant conversion factors.

a darker color may be influenced more by extractives, while wood with a lighter color may be influenced more by textural properties.

Cellular inclusions can contribute to the color of wood by either being absent from the cells, by occurring as small units lining the cell walls, by partially filling the cell, or by completely filling the cell. In all sections studied, inclusions occurred as small globular units that lined the lumen of both ray and axial parenchyma cells. Some vessel walls and associated pits appeared to have a dark reddish brown color similar to the color of the inclusions in the nearby parenchyma cells. This coloration should have been expected because vessels are in intimate contact with parenchyma and thus accumulation in one could affect accumulation in the other. The color data describe this phenomenon in greater detail.

Color descriptions of the cellular parameters

Most reports on wood color have described gross (macro) reflectance properties of the material. Judd (1933) indicated that either reflectance or transmittance values can be used to evaluate color because both are functions of the absorbance characteristics of a material for a particular wavelength. Transmittance values give some indication of internal and external reflections in the material being studied. In microscopic studies, reflections (both external and internal) must be considered in the section, in the glass used for the coverslip and slide, in the mounting media, and in the immersion oil. Because each of these may influence the total transmittance through an object, the transmittance values were standardized to transmittance values through a portion of the microscope slide unobstructed by the sample (an adjacent vessel lumen). This should give data representing the transmittance properties of the cell material only.

The transmittance data were transformed into the color parameters percentage luminance, dominant wavelength, and percentage purity (Table 3) using standard techniques (Wright 1969; Judd 1933; Wyszecki and Stiles 1967, respectively). For the statistical analyses, eleven measurements within each veneer sample section were used to derive an average for that sample. This average was used to test for statistically significant differences between sample groups. Significant differences (alpha = 0.10) in micro-luminance were observed in most features except vessel walls and axial parenchyma inclusions. Furthermore, luminance of the inclusion material was less than the wall material. These luminance values indicated the departure of the sample color from pure white, i.e. the lighter the color the higher

the luminance value. The amount of purity is also an indication of the "whiteness" of the sample. In all cases, purity was low, indicating that there was little hue in the colors of the cellular material.

Even though purity was low, slight differences in dominant wavelength in the cell walls of the samples were observed between light and dark samples (maximum difference of 2.3 nm). In addition, the axial parenchyma inclusions were slightly redder (5.6 nm) in the darker samples; the ray parenchyma inclusions had a much redder hue (89.9 nm difference) in those samples with lower luminance than in those samples with a higher luminance. This finding suggests that there may be major differences in the quality of the inclusions.

Statistical analysis of the micro-data was limited to the luminance color values. Conversions of many of the individual sample dominant wavelengths fell outside the scales of the computational table. This computational problem also influenced the statistical analysis of the purity value.

These results should have some relation to the amounts of heartwood phenolics within the inclusions. Inclusions were observed to be discrete globular units, approximately 10 μm^2, in all samples lining the lumen walls. An attempt to quantitize the amounts of inclusions was futile because of the variations in sample orientation.

Previous reports suggest that the precursors to phenolic compounds are formed *in situ* in the ray parenchyma cells of the transition zone between sapwood and heartwood (Hillis 1972). These, in turn, diffuse into the cell walls where they form insoluble high polymer pigments (Frey-Wyssling 1976; Parameswaren and Bauch 1975). Therefore, a relationship may exist between the physiological conditions that occur in the transition zone and the rate and duration of phenolic synthesis (Nelson 1975). Stress and increased polyphenol production may also be related (Hillis 1975). Increased stress may concurrently affect the types of phenolic compounds formed rather than the amounts. Therefore, physiological conditions affecting metabolic processes in the tree may also influence the formation of these heartwood forming compounds.

CONCLUSIONS

Microspectrophotometry of certain cellular features from selected samples of black walnut heartwood veneer indicated that this method is a valuable tool in describing the anatomical properties that influence wood color. These observations indicate that differences in the color of most cellular features, wall material as well as inclusions, contribute to observable differences in gross wood color. Relative cellular amounts and sample orientation had little influence on wood color.

Color characterization of these cellular features indicated large differences in luminance and in dominant wavelength of ray parenchyma inclusions. This latter observation suggests a possible relation between stress conditions that produce darker heartwood color and the quality of the pigmenting compounds in the ray parenchyma.

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