Colorimetric Analysis, Genetic Control, and Effects on Wood Properties of Green Vein in Wild Cherry

Michela Nocetti,* Michele Brunetti, and Paolo Burato

Green vein is one of the most severe defects that affect wild cherry (*Prunus avium* L.). It consists of green streaks that alter the typical color and uniformity of the cherry wood, causing considerable value losses. A colorimetric analysis was performed on wild cherry clones using the CIE L*a*b* system, and the influences of environmental and genetic factors on green vein as well as the effects of the presence of green vein on the physical properties of the wood were investigated. Discriminant analysis shows that the color parameter that best discriminated green vein were low values of *L** and *a**. The cloning effect was the most important, but the environment also played an important role in the development of green vein. Finally, the presence of green vein was found to mainly affect the longitudinal shrinkage of wood and, to a lesser extent, wood density. These same features are typical of tension wood, to which green vein was strictly linked, as confirmed by some preliminary anatomical observations.

Keywords: Prunus avium; Tension wood; Genetics; Wood quality; Physical wood properties; Wood color

*Contact information: CNR-IVALSA, Istituto per la Valorizzazione del Legno e delle Specie Arboree, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy; *Corresponding author: nocetti@ivalsa.cnr.it*

INTRODUCTION

Cherry is a valuable timber species that is mainly used in the furniture industry for both veneer and sawn timber. Hence, appearance can be considered a key factor in determining its quality (Nepveu 1992).

In this context, color and texture are the properties commonly considered to constitute the aesthetic value of wood, and their natural variability at various levels (among different species, but also within the same tree) is vast. Thus, factors affecting wood color have been studied in previous works, and some factors that have been proven to influence wood color variation include environment (Rink 1987; Mosedale *et al.* 1996; Sotelo Montes *et al.* 2008), silvicultural treatments (Wilkins and Stamp 1990), tree growth and age (Klumpers *et al.* 1993; Moya and Barrocal 2010), and genetic aspects (Mosedale *et al.* 1996; Gierlinger *et al.* 2004; Sotelo Montes *et al.* 2008). In particular, Janin (1996, in Gierlinger *et al*. 2004) found a high heritability in wild cherry (*Prunus avium* L.) at the family level for wood color, while Signorini (2006), in a study on various wild cherry clones, reported on the influence of genotype over this trait and also a high variability both within the clone and within the tree.

Given its importance, wood color changes have been investigated in black cherry (*Prunus serotina* Ehrh.) due to the wood's extractive and element content (Mayer *et al.* 2006; Mayer and Koch 2007), and in wild cherry after processing via steaming (Straže *et al*. 2008), heat treatment (Sevim Korkut *et al.* 2013), and thermo-vacuum modification (Ferrari *et al.* 2013). After processing, changes were often observed, but the

homogenization of wood color was rarely achieved, thus underlining the importance of optimizing wood color properties in living trees to obtain high-quality products (Mayer and Koch 2007; Aydemir *et al.* 2010).

Uniformity of color is generally a characteristic much appreciated by the customers of wood products and, consequently, by the industry. In this respect, one of the most widespread defects that affect wild cherry wood color is the presence of green vein. This is a chromatic anomaly consisting of green streaks, detectable only after tree felling. Its presence results in a huge change in the "typical" color of cherry wood and often causes considerable losses of material value because it is underappreciated by the market. Green vein is difficult to eliminate during wood processing and is often even emphasized by the varnishing process (Polge 1984).

Green vein, in addition to causing aesthetic depreciation, seems to be associated with more severe processing problems such as greater difficulty with planing, warping subsequent to veneer seasoning, and weaker joints in the solid wood. These features are also linked to the presence of tension wood, to which green vein seems to be related (Ferrand 1983; Langbour 1986; Nepveu 1992; Polge 1984).

The causes of the formation and development of green vein in wild cherry wood are various: the characteristics of the growth site (mainly climate and soil) can have an important role, and genetics have also been often considered crucial in determining the incidence of this anomaly (Masset 1979; Polge 1984). However, up to now evidence of this is still lacking in the scientific literature.

This paper presents a colorimetric analysis of green vein in two wild cherry clonal trials with the aim of better describing and detecting this anomaly and providing useful information on the possible environmental and genetic influences on green vein. The effects of the presence of green vein on the physical properties of wood are also analyzed and discussed.

EXPERIMENTAL

Materials and Laboratory Measurements

The raw materials were collected at two different sites, Marani and Forestello, located in the north and in the center of Italy, respectively. The characteristics of the sites, the sample preparation, and the laboratory measurements were the same as described in detail in previous papers (Nocetti *et al.* 2010, 2012), and are reported briefly below.

The two plantations are wild cherry (*Prunus avium*) clonal trials and belong to the experimental network of the CRA (Council for Research on Agriculture) - Research Centre for Silviculture of Arezzo (CRA-SEL) (Ducci 2005). The clones were micro-propagated from selected phenotypes located in the central Apennines at different altitudes (between 150 and 1000 m a.s.l.) and planted in 1986. At the Marani farm the initial spacing was 3 x 3 m and a systematic thinning was carried out in 1995; at Forestello the plantation was mixed with *Alnus cordata* and the spacing was 3 x 3 between trees and 6 x 6 m between wild cherry. A total of 71 wild cherry trees were felled. Six clones (8 ramets per clone) in Marani and four of the same clones (6 ramets per clone) in Forestello were sampled.

After felling, a 1-m-long log was collected 50 cm above ground level from each tree and immediately processed in the laboratory. Defect-free specimens, each measuring 20 x 20 mm for the transversal section and 30 mm long, were cut from the four radial

planks of the logs. Three different states of the wood were measured in this study. Firstly the green state (freshly cut), then conditioned at 65% relative humidity and at 20 °C, and finally oven-dried. For wood densities, linear shrinkages, and shape factors (the ratio of tangential to radial shrinkage) determinations, the weight, the length, and the cross section (tangential and radial dimensions) of each specimen were recorded both at green and oven-dried state. The basic wood density was calculated as the ratio of oven-dried weight to fresh volume; the longitudinal, radial, and tangential shrinkages were calculated as the percentages of the differences between green and oven-dried measurements of the respective specimen dimensions in relation to the green ones.

The heartwood or sapwood position of each specimen was recorded, and only the heartwood was included in the subsequent colorimetric and statistical analysis, because the green streaks are not visible in the sapwood. In total, 466 heartwood specimens were obtained for color measurements. In Table 1, the sample is summarized for the two sites.

Table 1. Sampling Description for the Two Sites

Site	N of clones	N of ramets per clone	N specimen per tree	Total N of specimens
Marani	6	8	6	275
Forestello	4	6	8	191

Color measurements

The color measurements were performed on specimens in the conditioned state using a Minolta Croma-Meter CR-200. For each specimen, measurements from four non-overlapping spots (8 mm in diameter) in the transversal section and five in the radial face were collected with the help of a grid, gathering an overview of the wood color of the specimen surface in a systematic way (Fig. 1). The CIE L*a*b* system was used, and the color parameters of L^* (lightness), a^* (the chromatic coordinate on the green-red axis) and b^* (the chromatic coordinate on the blue-yellow axis) were registered. The hue angle (h) was calculated as $h = \tan^{-1}(b^*/a^*)$.

Because green vein is characterized by irregularly distributed streaks, the color variation was thought to be an important feature for describing its presence. Therefore, the mean color parameter, as well as the minimum and maximum values, were calculated for each specimen and color parameter and used in further analysis.

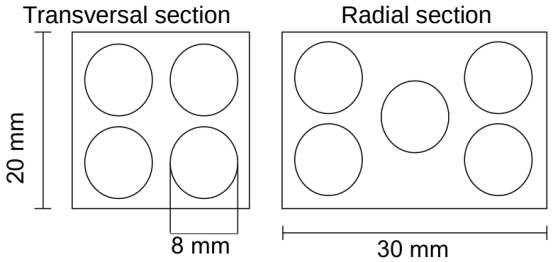

Fig. 1. Scheme of the spot position for the color measurements on each specimen

Anatomical investigation

Furthermore, some anatomical observations were carried out: samples were collected from different wood zones (green vein wood, normal heartwood, and sapwood), and sections of 20-µm thickness were prepared using a sliding microtome, stained, and

subsequently mounted on glass slides. The sections were then examined under a light microscope and compared.

Data Analysis

The first part of the analysis aimed to describe the color of cherry specimens affected by the presence of green vein using the colorimetric measurements, and therefore to translate in numerical way what is perceived by the human eye. Hence, the samples were classified according to the presence/absence of green vein. To that aim, 50 specimens were randomly selected and visually classed into three groups: n = absence of green vein; v = presence of green vein; vv = heavy presence of green vein (dark green streaks). These 50 specimens were used as a training set for a linear discriminant analysis, such as to classify the entire sample. The discriminant analysis determines the linear combination of model variables that best separated the groups (the analysis maxims the between-group variance relative to the within-group variance). In the analysis, the factorial groups were the green vein classes (n, v, and vv) and the numeric variables were the colorimetric parameters described above (mean, minimum, and maximum values of L^*, a^*, and b^*). Afterwards, the two linear combinations of numeric variables (called discriminant functions) best explaining the variance among the three groups were applied to the whole sample, and the two discriminant variables, D1 and D2, were calculated for each specimen. Subsequently, the allocation of each specimen to a green vein class was predicted according to the discriminant variables. The discriminant analysis and the allocation were performed with the *lda* function of the package MASS in R software (R Development Core Team 2010).

The discriminant variables, D1 and D2, can be considered a sort of "synthetic index" of the presence of green vein. Therefore, simple correlations (Pearson correlation coefficients) were then calculated between the colorimetric parameters and the discriminant variables to highlight the color description of green vein.

To check the significance of the differences among groups, an analysis of variance was applied wherein the green vein classes were the factors and the colorimetric parameters the variables. Tukey's test was then performed.

After the specimen allocation and the colorimetric description of green vein classes, the environmental and genetic factors were tested as sources of variation of wood color and green vein "index". Firstly, an analysis of variance was performed, incorporating *site*, *clone*, and *tree within clone* as the sources of variation for each colorimetric parameter to investigate the effects of these factors on wood color. Subsequently, the same analysis was performed for the two discriminant variables, D1 and D2, to investigate the influence of the site and clone effects on the green vein.

Finally, an analysis of variance was calculated to examine the influence of the green vein presence on wood properties with *site*, *clone*, and *green vein class* as effects and the physical characteristics of wood as the numerical variables.

RESULTS AND DISCUSSION

Colorimetric Analysis and Specimen Classification into Green Vein Classes

Figure 2 shows the results of the discriminant analysis on the 50 selected specimens. The hit ratio (percentage of correctly classified cases) was 66%. It was compared with a prediction due to chance using both the Maximum Chance Criterion

(MCC), which predicts that all cases are in the group with the largest number of cases, and the Proportional Chance Criterion (PCC), which randomly classify the cases proportionate to the number of cases in either group. With the MCC a percentage of 48% was obtained, and with the PCC a percentage of 39%. Therefore, the analysis yielded a prediction that was far better than chance.

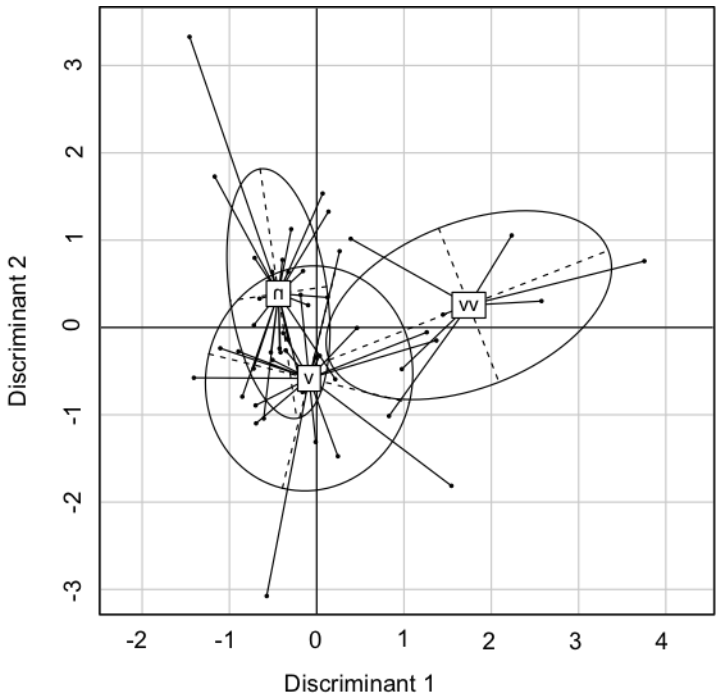

Fig. 2. Results of the discriminant analysis performed on the 50 specimens selected by visual observation (n = absence of green vein; v = presence of green vein; vv= heavy presence of green vein)

The discriminant functions reported in Table 2 were used to class the whole sample. The allocation of each specimen in the sample to one of the three green vein classes resulted in 240 specimens in class n (absence of green vein), 140 in class v, and 86 in class vv.

The color parameter that best discriminated the groups was *L**, as the minimum and mean values of lightness were highly correlated with D1, and the maximum value was highly correlated with D2 (Table 3). The green-red coordinate (*a**) was also an important predictor, while the *b** coordinate demonstrated low class predictability.

Table 2. Coefficients of Colorimetric Variables for Each Linear Discriminant Function

Function	*L** mi	*L** m	*L** ma	*a** mi	*a** m	*a** ma	*b** mi	*b** m	*b**ma
Discriminant 1	-0.000	-0.816	0.402	-0.427	-0.490	-0.103	0.780	-1.783	1.033
Discriminant 2	-0.008	-0.438	0.754	-0.489	0.336	-0.046	-0.360	1.608	-1.013

mi = minimum; m = mean value; ma = maximum

Table 3. Pearson Correlation Coefficients between Colorimetric Parameters and Discriminant Variables (D1 and D2) for Entire Sample

	*L** mi	*L** m	*L** ma	*a** mi	*a** m	*a** ma	*b** mi	*b** m	*b** ma
D1	-0.50***	-0.44***	-0.21***	-0.44***	-0.52***	-0.54***	-0.23***	ns	0.16***
D2	ns	0.37***	0.76***	-0.11*	-0.26***	-0.37***	0.21***	ns	-0.30***
* significant at 5% level; ** significant at 1% level; *** significant at 0. 1% level; *ns* not significant									

mi = minimum; m = mean value; ma = maximum

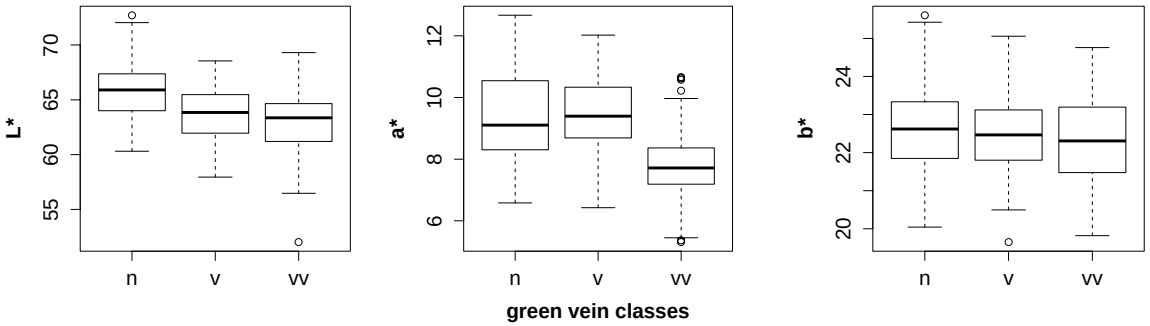

Fig. 3. Boxplots of lightness (*L**), green-red (*a**), and yellow-blue (*b**) coordinates for green vein classes. Bold line = median; box = interquartile range; dot lines = whiskers; small horizontal lines = smallest and largest non-outlier values; points = outliers

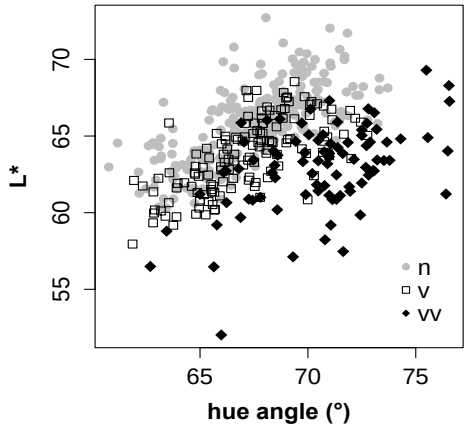

Fig. 4. Lightness *vs.* hue angle. The whole sample is displayed, highlighting the green vein class allocation.

To better describe the colorimetric characteristics of the three groups, boxplots of lightness and color coordinates were determined for the green vein classes (Fig. 3). In addition, lightness *vs.* hue angle was plotted, which highlighted the different groups (Fig. 4). The three classes differed significantly from each other in terms of *L** values (Tukey's test: n-v $p < 0.001$; n-vv $p < 0.001$; v-vv $p = 0.03$), and the vv class distinguished itself from the others in terms of *a** values (Tukey's test: n-vv and v-vv $p < 0.001$). Clearly, the green vein was characterized by a darker and greener color (lower values of *L** and *a**). No difference among the groups was detected on the yellow-blue axis (*b**). Signorini (2006) analyzed wild cherry wood color and reported an increase in the variability of *L** values as a result of the presence of green vein. A similar outcome can be observed in

Fig. 3 for these specimens. The same author also noticed lower values of *a**, as reported here, and higher values of *b** (yellowish color), which differed from what was observed in the current sample.

Genetic Influences on Wood Color and Green Vein

In Table 4, the results of the analysis of variance are reported for the color parameters and the discriminant variables. All the sources of variation included in the analysis demonstrated significant influence on wood color and green vein, but the weights of these influences varied. The greatest effect was the clone effect (except for *a**), while *tree-within-clone* had a negligible effect, indicating the presence of a strong genetic factor in green vein development. Except for the *b** coordinate, the site effect was also important, as was the *site* x *clone* interaction (mainly for *a** and D1).

Table 4. Values of *F* and Significance as results of the Analysis of Variance for Color Parameters (*L**, *a**, and *b**) and for Discriminant Variables (D1, D2)

Source of Variation	*L**	*a**	*b**	D1	D2
Site	17.0***	185.0***	4.8*	17.0***	10.4***
Clone	62.3***	123.5***	27.2***	33.9***	16.3***
Tree-within-Clone	2.0***	4.3***	1.6*	2.3***	2.6***
Site x Clone	3.4*	107.9***	24.4***	43.7***	7.2***
* significant at 5% level; *** significant at 0.1% level					

In previous studies, the wood color of wild cherry was demonstrated to be under moderate genetic control (Janin 1996, in Gierlinger *et al*. 2004; Signorini 2006). The results of the present study can confirm those conclusions, but above all they show the importance of the clone effect as well as the clear influence of growth conditions on the green vein formation. In the past, various other authors hypothesized that genetic factors were one of the possible causes of this phenomenon (Masset 1977, in Polge 1984), but they did not produce any evidence.

Presence of Green Vein and Wood Properties

Because of the supposed connection between the presence of green vein and the wood properties, an analysis of variance was performed on the physical properties with green vein class as an additional factor. The results are presented in Table 5.

Firstly, it must be noted that most of the interactions were not significant, particularly the *clone* x *green vein* interaction, which was only significant for longitudinal shrinkage, suggesting that the presence of green vein acted independently, regardless of clone type, in determining physical characteristics of wood. The site effect, the clone effect, and the *site* x *clone* interaction were highly significant for all the properties examined, in agreement with previous findings (Nocetti *et al.* 2010). In addition, green vein exhibited a very strong effect on the longitudinal shrinkage, whereas its influences on the basic density, radial shrinkage, and shape factor were significant but much lower. No effect was noticed on the tangential shrinkage.

Both basic density and longitudinal shrinkage increased significantly for the green vein specimens (Fig. 5), (Tukey's test: n-v $p > 0.05$; n-vv $p < 0.001$; v-vv $p < 0.001$). The

same characteristics are described in the literature on tension wood; higher density (Hughes 1965b; Ruelle *et al*. 2007; Washusen *et al*. 2001) and higher longitudinal shrinkage (Clair *et al*. 2003; Hughes 1965b; Ruelle *et al*. 2007) were found in tension wood compared to normal wood. These two properties best distinguish tension wood, although other properties of tension wood have been reported with conflicting results. With regards to radial and tangential shrinkages, no clear trends have been discovered: Clair *et al*. (2003) did not notice significant differences between tension and normal wood regarding radial and tangential shrinkages in chestnut, and likewise Ruelle *et al*. (2007), in a study of 10 tropical species, observed unclear differences in radial shrinkage.

The association between green vein and tension wood has been previously hypothesized by Ferrand (1983). Polge (1984) also observed a high percentage of tension wood in the green vein zones in cherry wood; the samples of green vein had higher longitudinal shrinkages, pulp yields, fiber lengths, and ultrasonic wave velocities than did the controls. The author reported an association between green vein and tension wood at the statistical level, but not at the individual level (he found many exceptions).

Langbour (1986), using the association between green vein and tension wood, tried to predict the presence of green vein in standing trees by measuring longitudinal growth stresses, the main cause of tension wood formation, but the results were not convincing.

Table 5. Values of *F* and Significance as results of the Analysis of Variance for the Physical Characteristics of Wood

Source of Variation	Basic Density	Longitudinal Shrinkage	Tangential Shrinkage	Radial Shrinkage	Shape Factor
Site	180.9***	16.8***	424.3***	43.3***	5.2*
Clone	29.2***	13.3***	49.6***	27.4***	18.9***
Green Vein	8.2***	66.5***	2.3 ns	6.1**	6.1**
Site x Clone	17.3***	19.3***	27.7***	27.3***	14.0***
Site x GV	0.5 ns	1.3 ns	15.1***	4.0*	0.2 ns
Clone x GV	0.9 ns	1.9*	1.4 ns	1.0 ns	1.3 ns
Site x Clone x GV	0.3 ns	3.1**	2.0 ns	0.8 ns	0.3 ns
* significant at 5% level; ** significant at 1% level; *** significant al 0. 1% level; *ns* not significant					

(GV = Green Vein)

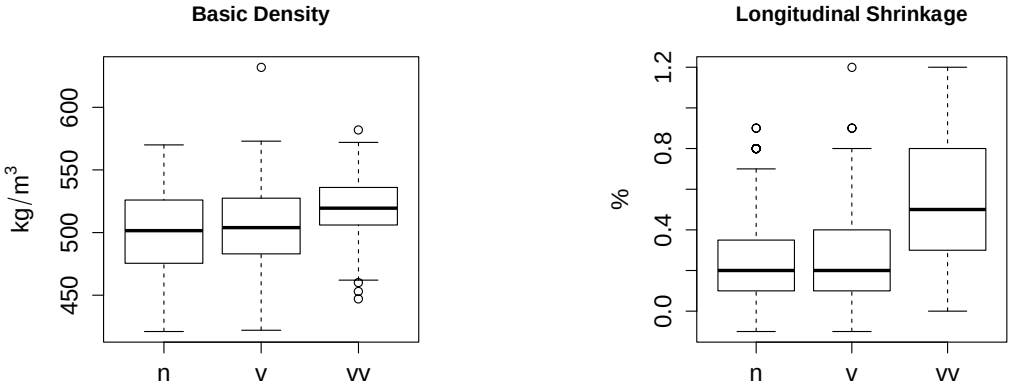

Fig. 5. Basic density and longitudinal shrinkage for the green vein classes

Anatomical Observations

To verify the presence of tension wood, anatomical investigations were carried out on the specimens analyzed here.

Firstly, samples from the same tree ring, some from the green vein zone and some from the normal wood zone, were compared. The micro-sections clearly showed tension wood cells, identified by the presence of the gelatinous layer, mainly composed of cellulose, in the cell wall and by a reduction in the size and number of vessels (Hughes 1965a; Jourez *et al.* 2001; Pilate *et al.* 2004), corresponding to green vein, as well as to unmodified cell ultra-structure in the normal-colored wood (Fig. 6, a and b). In these specimens tension cells and normal ones were homogeneously distributed in the wood tissues.

Next, sections obtained from light green wood zones (essentially the v green vein class), observed under the microscope, once again showed typical tension wood cells, but they were heterogeneously distributed (Fig. 6, c) and characterized by a thinner G layer and a much more evident cell wall lignification. Finally, some sections were prepared from the sapwood zone, where the color changes due to the heartwood formation had not yet begun. Here, tension wood was also observed, although no green vein was macroscopically visible.

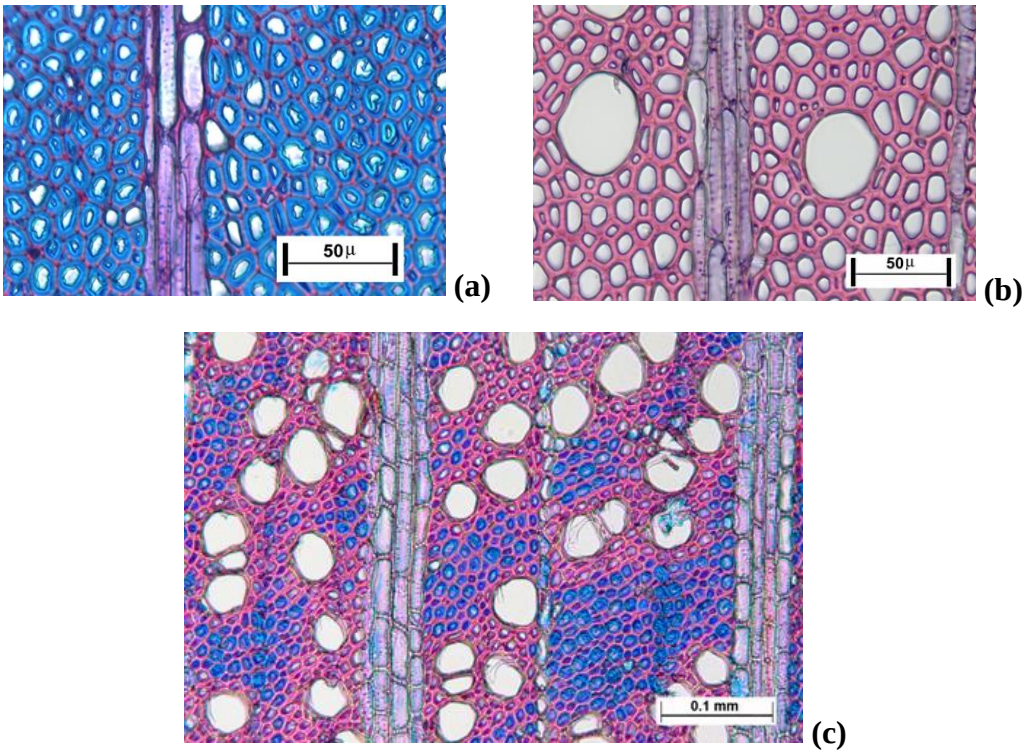

Fig. 6. Light microscope image of heartwood zone (a) with green vein, (b) without green vein, and (c) with light green vein. Typical tension wood cells are visible in (a) and (c) (the G layer with high cellulose content appears blue), but in the last image, they are heterogeneously distributed, the G layer appearing thin and the cell wall lignification (red) rather evident.

It was previously stated that green vein can be discerned only in heartwood. The coloring of wood is usually associated with heartwood formation and the synthesis of heartwood substances by parenchyma cells (Hillis 1987). From the chemical point of view, wood color is mainly due to the presence of extractives (Burtin *et al*. 1998), and in

black cherry, wood color variation has been linked to the chemical composition and the distribution of flavonoids (Mayer *et al.* 2006).

This suggests that the green coloring occurs during heartwood formation and that it can be linked somehow to the different anatomical structure of the tension wood cells: here, after heartwood formation, the quantity or the typology of the extractives might be different from those in the normal tissues. Several gradations of tension wood formation, as well as the random distribution of tension wood cells, have been described previously (Araki *et al*. 1982; Dadswell and Wardrop 1955; Hughes 1965a), but from our observations we can hypothesize that the darkness of the green color might be linked to different degrees of abnormality of the tension wood.

Signorini (2006) discovered variations in the quantitative compositions of extractives between green vein samples and normal ones, although the study examined a small number of specimens and the results were different due to the extraction methodology applied. In addition, Phelps and McGinnes (1983) suggested the importance of the quality rather than quantity of phenolics in determining significant differences in walnut veneer color.

Naturally, these last assumptions are only suppositions and need further investigation. Further studies should be carried out on the evolution of green vein during heartwood formation as well as on the relationship between green vein, tension wood tissues, and wood extractives.

CONCLUSIONS

1. The colorimetric analysis of green vein produced interesting results. The discriminant analysis was successful in detecting the green vein and classing our sample accordingly. The color parameters that best discriminate the groups were L^* (lightness) and a^* (green hue), such that the green vein could be described with lower values of L^* and a^*.

2. The presence of green vein was strongly controlled by genetic factors, and among these, the clone effect was the most important. However, the environment also played an important role in its development.

3. The presence of green vein mainly affected the longitudinal shrinkage of wood and, to a lesser extent, wood density. These same features are typical of tension wood, to which green vein was strictly linked, as confirmed by some preliminary anatomical observations.

4. It could be possible to reduce the green vein development in cherry wood by attempting to limit the tension wood formation.

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