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APPLYING COLORIMETRY FOR WOOD DIFFERENTIATION OF FABACEAE SPECIES GROWN IN SOUTHERN BRAZIL

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ABSTRACT

Because of the need for identification of forest species, especially for detection of illegal wood trade, the objective of this study was to evaluate the potential of colorimetry for differentiation of *Inga vera* Willd., *Muellera campestris* (Mart. ex Benth.) M.J. Silva & A.M.G. Azevedo and *Machaerium paraguariense* Hassl., species of the Fabaceae family, native to the Araucaria Forest in the state of Santa Catarina, southern Brazil. Discs at breast height were collected from three trees of each species and the colorimetric parameters (L*, a*, b*, C* and h) and visible spectra were evaluated in different radial position of the trunk (near bark, intermediate and near pith) and three different anatomical sections (transversal, radial and tangential surfaces). Mean values of hue angle (h) among the colorimetric parameters resulted in the highest potential for species discrimination. With respect to radial trunk position and anatomical section, parameters a* (green-red) and h were not statistically different, independent of the wood samples evaluated. For other parameters (L*, b* and C*), each species presented distinct results. Principal component analysis with second derivative of visible spectra discriminated all species. Colorimetry associated with chemometrics allowed to distinguish *I. vera*, *M. campestris* and *M. paraguariense*.

Keywords: Araucaria forest, colorimetric parameters, species differentiation, trade control, wood color.

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RESUMEN

Debido a la necesidad de identificar especies forestales, especialmente para detectar el comercio ilegal de madera, el objetivo de este estudio fue evaluar el potencial de la colorimetría para la diferenciación de *Inga vera* Willd., *Muellera campestris* (Mart. Ex Benth.) M.J. Silva & A.M.G. Azevedo y *Machaerium paraguariense* Hassl., especies de la familia Fabaceae, nativas del bosque de Araucaria en el estado de Santa Catarina, sur de Brasil. Se recogieron discos a la altura del pecho de tres árboles de cada especie y se evaluaron los parámetros colorimétricos (L*, a*, b*, C* y h) y los espectros visibles en diferentes posiciones radiales del tronco (cerca de la corteza, intermedia y cercana de la médula) y sección anatómica (superficie transversal, radial y tangencial). Los valores medios del ángulo de tono (h) entre los parámetros colorimétricos dieron como resultado el mayor potencial de discriminación de las especies. Con respecto a la posición radial del tronco y la sección anatómica, los parámetros a * (verde-rojo) y h no fueron estadísticamente diferentes, independientemente de la muestra evaluada. Para otros parámetros (L*, b* y C*), cada especie presentó resultados distintos. El análisis del componente principal con la segunda derivada de los espectros visibles discriminó a todas las especies. La colorimetría asociada con la quimiometría permitió distinguir *I. vera*, *M. campestris* y *M. paraguariense*.

Palabras clave: Bosque de araucaria, color de la madera, control comercial, diferenciación de especies, parámetros colorimétricos.

INTRODUCTION

The high biodiversity of native species in Brazil and different aspects of each biome in the country have been confirmed by various researchers. Andrade *et al.* (2018) commented that Atlantic Forest remnants stand out for having a high number of distinct taxonomic groups (15,179), hampering species discrimination and consequently control of wood commerce.

Among the diverse forest typologies in the Atlantic Forest biome, the Araucaria Forest stands out in Santa Catarina state, southern Brazil, in function of the presence of 925 angiosperm species. The families with the highest number of classes are Asteraceae (119 species), Myrtaceae (88 species) and Fabaceae (58 species) (Gasper *et al.* 2013). Also, in some forest fragments, the Fabaceae family has more richness (Martins *et al.* 2017). Another relevant factor is that some native trees of this family have potential for commercial logging, such as *Inga vera* Willd., *Muellera campestris* (Mart. ex Benth.) M.J. Silva & A.M.G. Azevedo and *Machaerium paraguariense* Hassl.

However, many times the trade of these wood species is illegal. Based on the list of Brazilian Environmental Ministry (MMA 2014), the genus *Machaerium* contains one species classified as vulnerable, and for genus Inga, two are classified as critically endangered, one as endangered, and nine are in vulnerable category. Nevertheless, these species are often targeted by loggers, an activity that is difficult to deter by inspections because of missing data on reproductive and vegetative material. In general, control is performed using anatomical and structural characteristics of wood, and often the time necessary for analysis and correct identification is too long. Therefore, alternative methods of wood differentiation are necessary, and one technique can be wood color. But visual classification alone is highly subjective and can be influenced by diverse factors, such as differences in luminosity (Silva *et al.* 2015), facilities available for observing anatomical structure, authentic database, practical experience and training of the technical staff involved, etc.

Thus, the use of colorimeters or spectrophotometers can be an alternative, since the evaluation is fast and needs little training. Some studies have shown the potential of this technique to evaluate wood modifications after thermal treatment (Cademartori *et al.* 2013), to characterize *Eucalyptus* wood (Mori *et al.* 2005), and to distinguish *Eucalyptus* and *Pinus* species (Nisgoski *et al.* 2017; Amorim *et al.* 2013) and Myrtaceae species (Vieira *et al.* 2019b), among others. However, for adequate species discrimination, it is necessary to have a database that includes all heterogeneous characteristics of wood, since color is influenced by various factors, like radial position in the trunk and anatomical section evaluated.

Considering the need to develop alternative techniques for species discrimination, this study evaluates the potential of colorimetry to differentiate three Fabaceae species from southern Brazil: *Inga vera, Machaerium paraguariense* and *Muellera campestris*.

MATERIAL AND METHODS

Material

The trees of the three species were cut in an area of the Araucaria Forest in Santa Catarina state, southern Brazil, set to be inundated after completion of the São Roque hydroelectric dam. Three trees of each species were analyzed (see detailed information in Table 1). Botanical material was registered with the Lages Herbarium of the Santa Catarina State University (LUSC) and access to the material is registered with Brazilian Council for Management of Genetic Heritage (CGEN/SISGEN) under number AF3EDDC.

From each tree, two discs were cut at diameter at breast height (DBH) with 50 mm thickness. Botanical material and one disc of each tree were used for species identification, registration and storage at the LUSC herbarium. So, a total of nine discs were evaluated in this study, three per species (Figure 1).

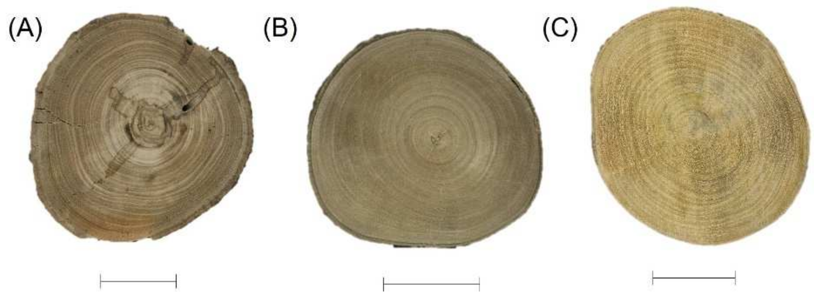

Figure 1: Example of discs used in this study: (A) *Inga vera,* (B) *Machaerium paraguariense* and (C) *Muellera campestris*. Scale bar = 50 mm.

Table 1: Scientific and popular names, LUSC registration number, diameter at breast height (DBH) and geographic coordinates of evaluated species.

Species	Popular name	Register number	DBH (cm)	Geographic coordinate
Inga vera Willd.	Ingá	LUSC 6225	26,0	lat: -27.484728 long: -50.805003 WGS84
		LUSC 6226	18,0	lat: -27.484378 long: -50.805603 WGS84
		LUSC 6227	17,5	lat: -27.484228 long: -50.805753 WGS84
Machaerium paraguariense Hassl.	Jacaranda-branco	LUSC 6243	18,3	lat: -27.489997 long: -50.805392 WGS84
		LUSC 6244	12,9	lat: -27.489997 long: -50.805417 WGS84
		LUSC 6245	11,0	lat: -27.490006 long: -50.805433 WGS84
Muellera campestris (Mart. Ex Benth.) M.J. Silva & A.M.G. Azevedo	Pau-canzil	LUSC 6237	31,2	lat: -27.496892 long: -50.810606 WGS84
		LUSC 6238	25,1	lat: -27.497081 long: -50.810536 WGS84
		LUSC 6239	15,0	lat: -27.483572 long: -50.808342 WGS84

To include most wood variation, sampling was done in three regions: near bark, intermediate and near pith. Sample size was 20 x 20 x 20 mm³ (length, width and thickness). In order and to eliminate the oxidation effects and saw marks on the wood surfaces, the samples were polished with 100 grit sandpaper. Pastore *et al*. (2004) commented that wood color is influenced by climatic characteristics, so to standardize all analyses, samples were stored, until start the analysis to maintain moisture equilibrium, in a controlled atmosphere with temperature of 25 ºC ± 2 ºC and relative humidity of 50 % ± 2 %.

Colorimetric parameters and Vis spectra

Colorimetric characterization of wood was based on the CIEL*a*b* standard, with observation angle of 10° and illuminant D65. Analysis was performed with a Konica Minolta CM-5 spectrophotometer. In each sample, two readings were carried out per anatomical section, for a total of 6 per sample and 54 per species. Data from L*= luminosity, a*= chromatic coordinate of green-red axis, and b*= chromatic coordinate of blue-yellow axis were obtained for calculation of the parameters C* = chroma or saturation (Equation 1) and h= hue angle (Equation 2). Reflectance spectra from 350 nm to 750 nm were also obtained.

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$h = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

Statistical analysis

The data were compared within each species by the Scott-Knott test in case of normal distribution and the Kruskal-Wallis test for nonparametric distribution. Factorial analysis was performed with the SISVAR software (Ferreira 2011) considering the factors anatomical section (transversal, radial and tangential) and radial position in trunk (near bark, intermediate and near pith).

Principal component analysis (PCA) was applied to verify the behavior of species regarding colorimetric parameters, with raw data and second derivative of Savitzky-Golay, with smoothing = 3, using the R Statistical software (R Core Team 2018).

RESULTS AND DISCUSSION

Colorimetric parameters of each species

Mean results for each species for the parameters L*, a*, b*, C* and h are listed in Table 2. Data on luminosity (L*), in accordance with Nishino *et al.* (1998), indicated that all studied species are classified as having high value (L*>54). In mean comparisons, *Machaerium paraguariense* presented lower luminosity (darker appearance), significantly different than the other two species (Figure 1A). The luminosity results of the three Fabaceae species are similar to Myrtaceae species from Araucaria Forest (Vieira *et al.* 2019b), which presented values from 50,01 to 69,75 for this parameter. Silva *et al.* (2015) reported values higher than 70 for *Apuleia leiocarpa*, another species from the Fabaceae family.

Table 2: Mean values of colorimetric parameters in wood of Fabaceae species.

Species	L*[2]	a*[2]	b*[2]	C*[2]	h[1]
Inga vera	68,01 A (9,66)	5,58 A (24,90)	20,49 B (10,50)	23,12 B (5,99)	75,79 C (1,57)
Machaerium paraguariense	65,27 B (7,32)	3,95 B (22,63)	20,03 B (20,44)	20,44 B (10,38)	78,70 B (3,65)
Muellera campestris	68,45 A (10,15)	4,57 B (57,94)	28,91 A (12,67)	29,38 A (12,67)	81,01 A (6,02)
For each parameter, means with the same capital letter in the column are not statistically different by the Scott-Knott ([1]) or Kruskal-Wallis ([2]) test at 95 % significance. Coefficient of variation in parentheses.					

When the analysis was performed on the chromatic coordinate of the green-red axis (a*), *Inga vera* had higher value and was considered to have the reddest pigmentation among three species (Barros *et al.* 2014). For the blue-yellow chromatic coordinate (b*) and chroma (C), *Inga vera* and *Machaerium paraguariense* were similar, and the highest parameter values were found for *Muellera campestris*, which had the lowest value of white, as also reported by Silva *et al.* (2017).

For hue angle (h), the lowest value was in *Inga vera* wood and highest in *Muellera campestris.* Camargos and Gonçalez (2001) described that even though derived from a* and b* values, hue angle can provide information about material tonality. This parameter showed the best potential to distinguish all evaluated species. Vieira *et al.* (2019b) reported the same tendency of h and b* parameters in Myrtaceae species.

Based on the color chart proposed by Camargos and Gonçalez (2001), the species where classified as having similarity with these colors: olive yellow (*I. vera*), gray rose (*M. paraguariense*) and light yellow (*M. campestris*).

Colorimetric parameters in function of anatomical section and radial position

Mean values of luminosity for Fabaceae species are shown in Figure 2. For the anatomical section factor, in general lower values were present in transversal sections, with the exception of *Inga vera* samples in the near bark region, which were similar in all anatomical sections.

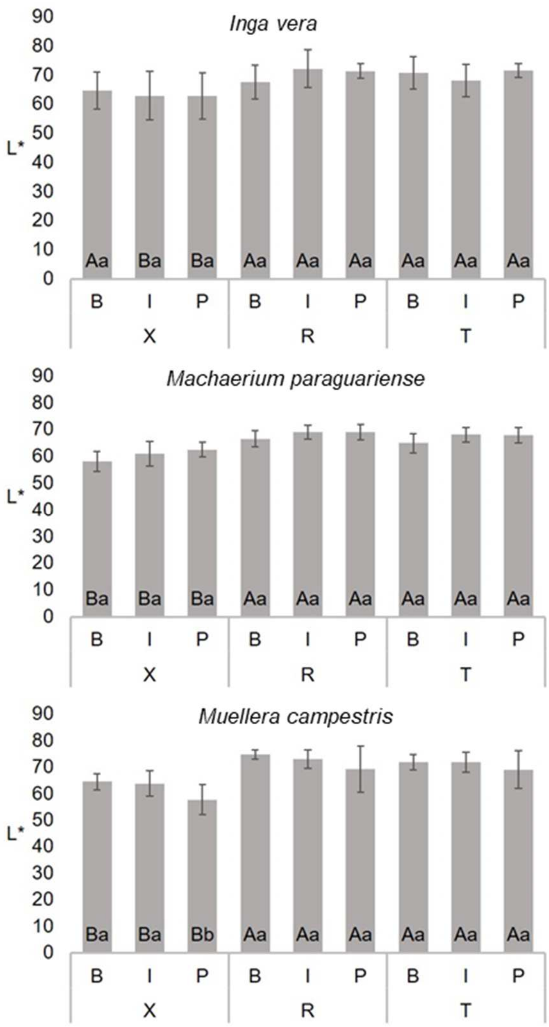

Figure 2: Mean values of luminosity (L*) of Fabaceae species. For each species, same capital letters in the same trunk position between different sections and same small letters in the same section and between trunk position do not differ significatively by the Scott-Knott test at 95 % significance. B = bark, I = intermediate, P = pith, X = transversal, R = radial and T = tangential sections.

Atayde *et al.* (2011) while evaluating differences between anatomical sections of *Brosimum* spp. wood, described statistically significant and different results for mean luminosity of samples in transversal, tangential and radial sections. Based on trunk position, in general, present results are similar, except for *Muellera campestris* in the transversal section, where mean values in the region near the pith are lower than in the other samples.

When studying forest species, statistically similar results are not common when evaluating radial variations in luminosity. For example, Moya *et al.* (2012) analyzed colorimetric parameters in *Acacia mangium* and *Vochysia guatemalensis* wood and described values with statistical differences in samples from heartwood (region near pith) and sapwood (region near bark). Vieira *et al.* (2019b) evaluated Myrtaceae species and reported a decreasing tendency of L* values from bark to pith. Considering the Fabaceae family, Cisneros *et al.* (2019) described colorimetry of *Prosopis alba* and reported different values in function of trunk position, with the highest values found in sapwood.

For chromatic coordinate a* (Figure 3 A, C, E), the results were similar for all species in all anatomical sections and trunk positions. This tendency is not standard for all wood species. Other researchers have observed variations in anatomical sections, such as lower values in transversal sections (Atayde *et al.* 2011), and different for values of radial variations between heartwood and sapwood (Moya *et al.* 2012). Another important consideration is the high standard deviation of mean values of each sample. This behavior can be explained by the species' anatomic structures, due to differences in the coloration of the fibers and axial parenchyma (predominantly aliform in *Inga vera*, and with bands in *Muellera campestris* and *Machaerium paraguariense* (Vieira *et al.* 2019a). These aspects cause high contrast with the fibers. However, according to Paula *et al.* (2016), the absence of variation of the colorimetric parameters in the different planes can be considered an advantage for wood commerce, since it means greater homogeneity of the final product.

For chromatic coordinate b* (Figure 3 B, D, F), the values were similar in all three species. In the different sections and trunk positions, *Inga vera* showed higher statistical similarity, except for mean values in transversal sections near bark, and for samples near pith in radial sections. Similar b* values for heartwood and sapwood were also found by Moya *et al.* (2012) in *Acacia mangium* and *Vochysia guatemalensis.* For *Machaerium paraguariense,* considering changes in anatomical section, smaller values were obtained in transversal sections, while for radial sections, a tendency of higher values was observed in the near-pith region. In *Muellera campestris*, when analyzing anatomical surfaces, there also was a tendency for lower values in transversal sections, while in tangential sections there was no difference between positions, but for radial and transversal sections, the near-pith position had the highest values. For parameter C* (Figure 4 A, C, E), *Inga vera* results were similar between samples, except for near-bark material in transversal and tangential sections, whose values were lower than in the radial section. In *Machaerium paraguariense,* all analyses revealed significant differences. Regarding anatomical section, lower values occurred on transversal surfaces, independent of trunk position. When considered trunk position, in radial and transversal sections, lower values were obtained in samples near the bark, while for tangential sections, higher chroma was verified near the pith. According to Ross *et al.* (2010), this behavior can be attributed to the presence of various extractives in the heartwood, or also by physiological growth characteristics and tree health. In *Muellera campestris* samples, based on anatomical section, lower chroma was found in transversal sections. Based on trunk position, no difference was observed for tangential sections, while for transversal and radial sections, higher values were obtained in the near-pith region. These results can be attributed to the presence of extractives in function of heartwood formation.

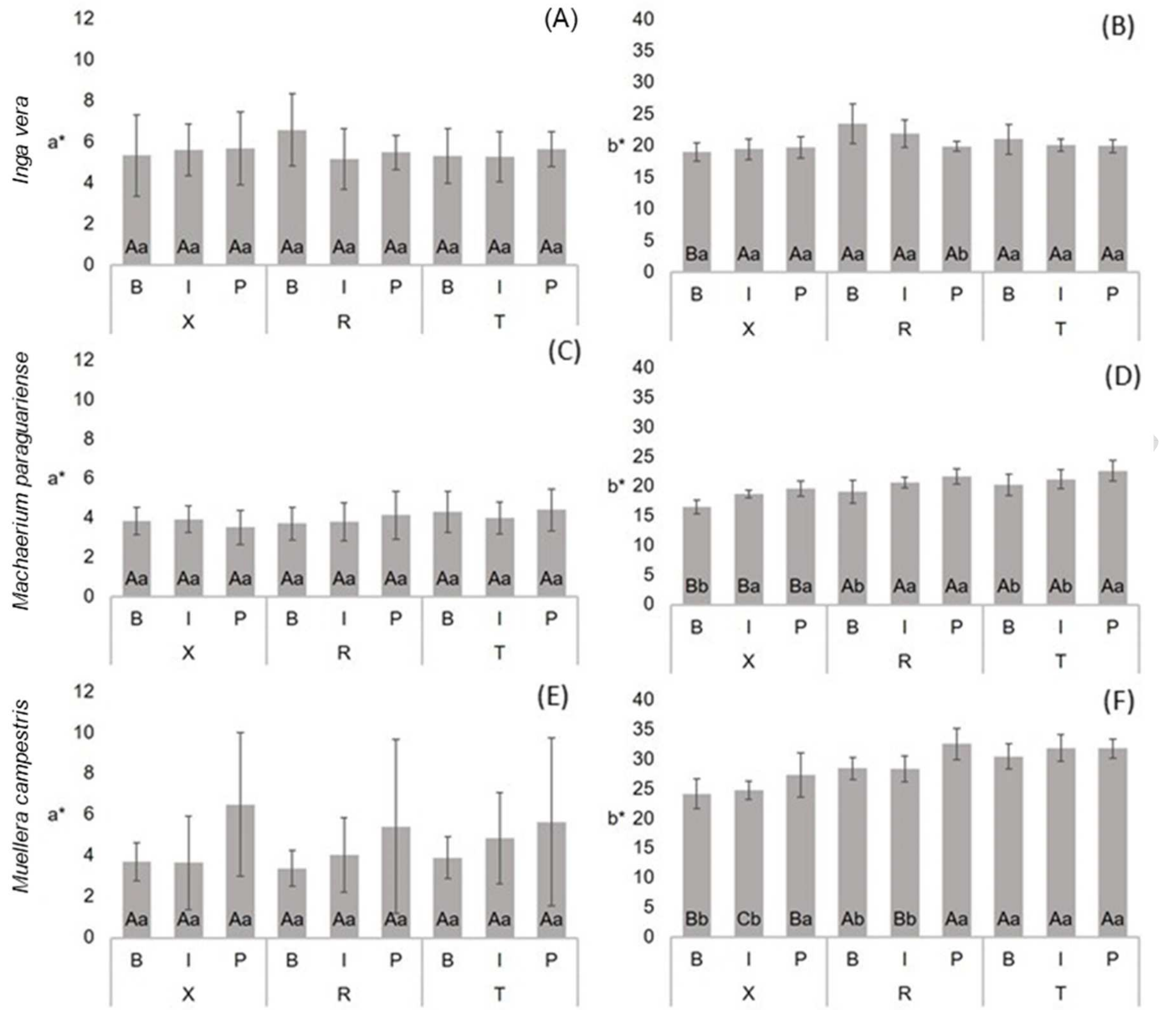

Figure 3: Mean values of chromatic coordinates a* (A, C, E) and b* (B, D, F) of Fabaceae species. For each species, the same capital letters in the same trunk position between different sections and same small letters in the same section and between trunk positions do not differ significantly by the Scott-Knott test at 95 % significance. B = bark, I = intermediate, P = pith, X = transversal, R = radial and T = tangential sections.

For hue angle (h) (Figure 4 B, D, F), no significant differences were observed in function of anatomical surface or radial position in the trunk. This was also described by Vieira *et al.* (2019b) for *Eugenia pyriformis.* This result is important because it can be a practical advantage for species discrimination, since these parameters can be used to identify all evaluated species. Studying wood from *Brosimum rubescens* and *Hevea brasiliensis*, Autran and Gonçalez (2006) described similar data for hue angle in radial and tangential sections. Describing the wood of eight native species from the state of Rio Grande do Sul (also in southern Brazil), Silva *et al.*

(2015) found similarity for the hue angle between the tangential and radial sections of the samples evaluated.

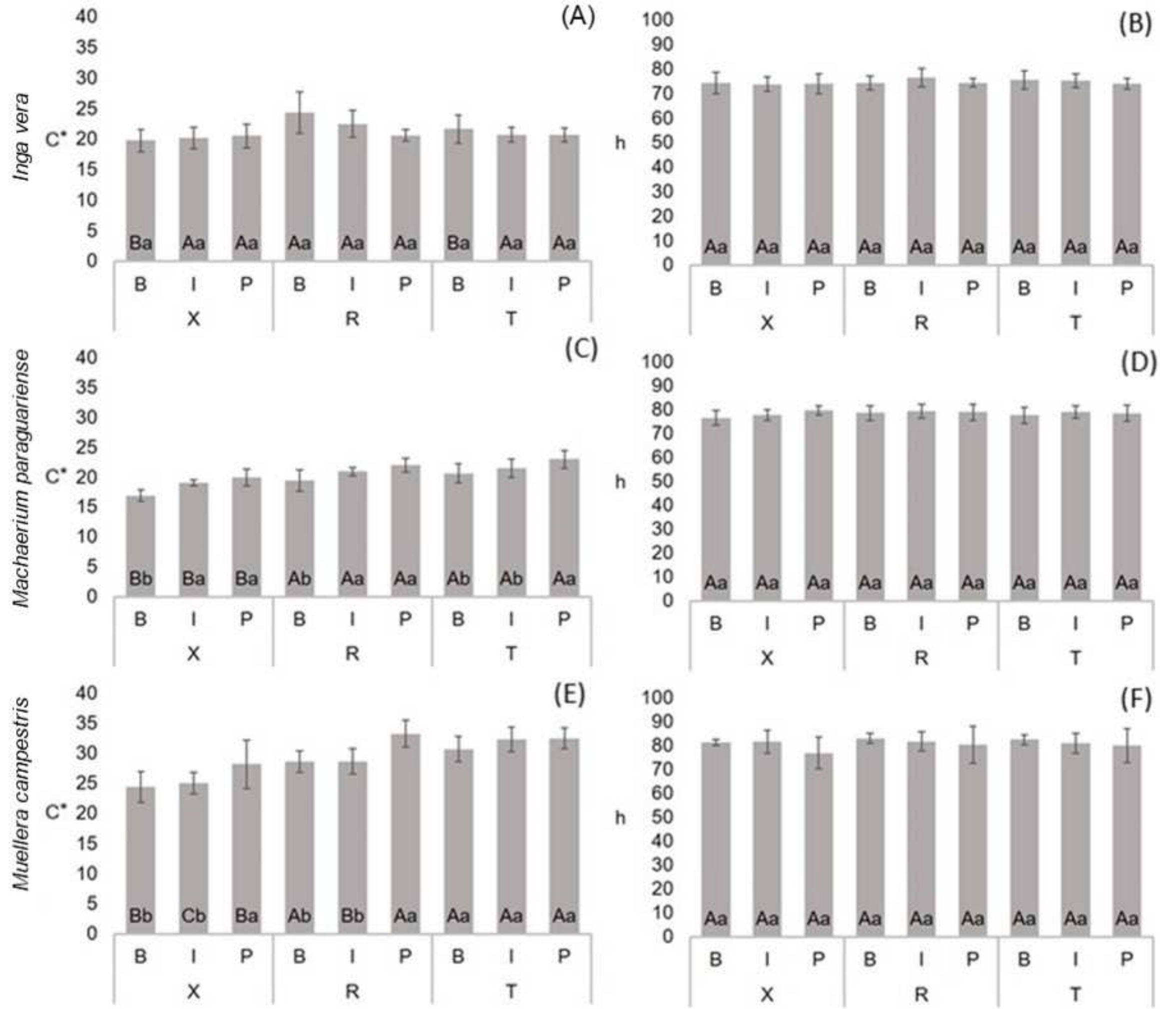

Figure 4: Mean values of parameters C*(A, C, E) and h (B, D, F) of Fabaceae species. For each species, the same capital letters in the same trunk position between different sections and the same small letters in the same section and between trunk positions do not differ significatively by the Scott-Knott test at 95 % significance. B = bark, I = intermediate, P = pith, X = transversal, R = radial and T = tangential sections.

Visible spectra

Mean spectra per species (Figure 5) indicated similar tendency in reflectance of wood, a difference in wavelength in the region from 390 nm to 500 nm (violet and blue color) and some grouping of *Inga vera* and *Muellera campestris* in bands from 500 nm to 750 nm.

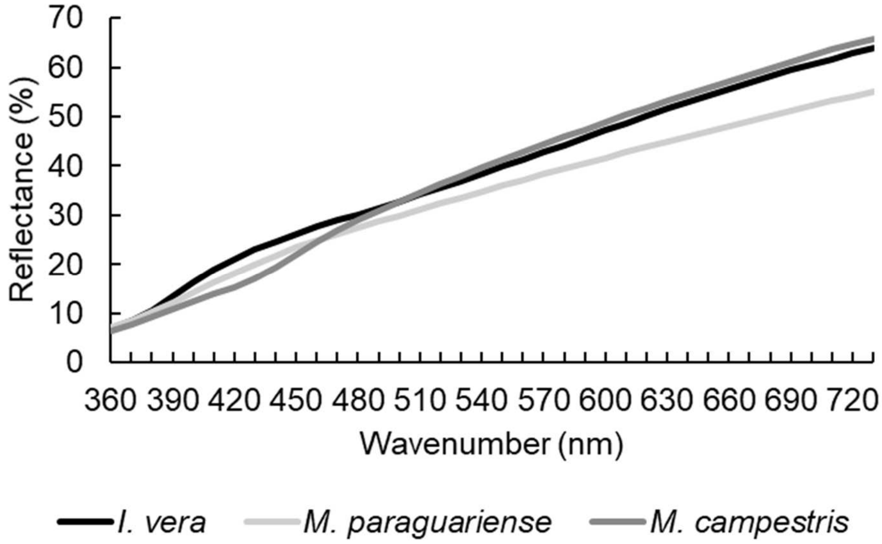

Figure 5: Visible reflectance spectra of Fabaceae species.

Principal component analysis (PCA)

Visual analysis of reflectance spectra alone cannot indicate whether species are different, so principal component analysis, which can group similar material, was performed (Figure 6). Two principal components represent 96,4 % of variation in raw reflectance spectra of species samples, the first component represented 80,4 % and second component 16,0 % of the variation. Among the species (Figure 6A), original spectra of *Muellera campestris* was separated from the others, and this distinction was confirmed by the ellipse grouping, which was the result of light-yellow color of wood. In some eucalypt species, Nisgoski *et al.* (2017) also found a distinction of samples in PCA of reflectance spectra in function of wood color.

Based on trunk position (Figure 6B), there was no distinction of species, with overlap of ellipse distribution. In analysis of anatomical sections (Figure 6C), a tendency of separation of transversal surfaces was present, along with some miscellaneous groups of radial and tangential sections. This can be explained by morphological characteristics of wood species, because individual cell arrangement and dimensions influence the formation of groups.

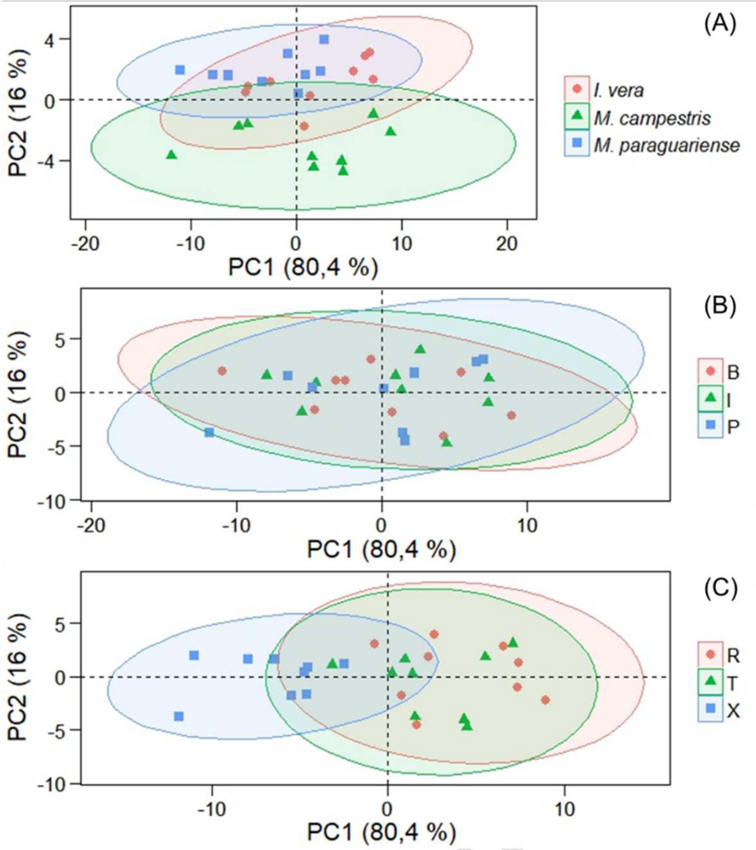

Figure 6: Score graph of PCA with mean raw spectra of Fabaceae species. (A) based on species, (B) in function of radial position in trunk and (C) for anatomical sections.

The PCA factor loading graphs with original data (Figure 7) indicated more influence on PC1 linearity after 480 nm (cyan, green, yellow, orange and red color) and for PC2 an opposite tendency, with more influence of wavelengths from 360 nm to 470 nm (violet and blue).

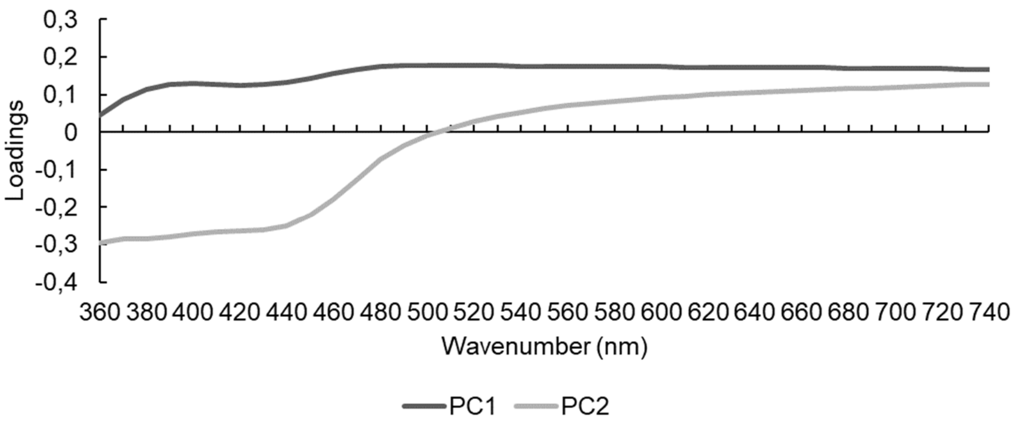

Figure 7: PCA factor loading graph with original visible spectra of Fabaceae species.

To minimize noise in spectra, mean spectral data points were transformed by taking the second derivative and PCA was performed again (Figure 8). The results indicated that PC1 represented 45,1 % and PC2 17,5 % of the variations of the samples. When considering species (Figure 8A) better discrimination occurred in comparison with raw data, and *Machaerium paraguariense* results were more similar between trees. In Myrtaceae species, Vieira *et al.* (2019b) also described better results with second derivative spectra.

When the analysis was done based on trunk position (Figure 8B) and anatomical sections (Figure 8C), the same results as found for the original data were verified. Related to position in trunk, near-bark and intermediate regions showed similarity of ellipses formed. The same was found for tangential and radial sections, while for transversal sections, the ellipses were different.

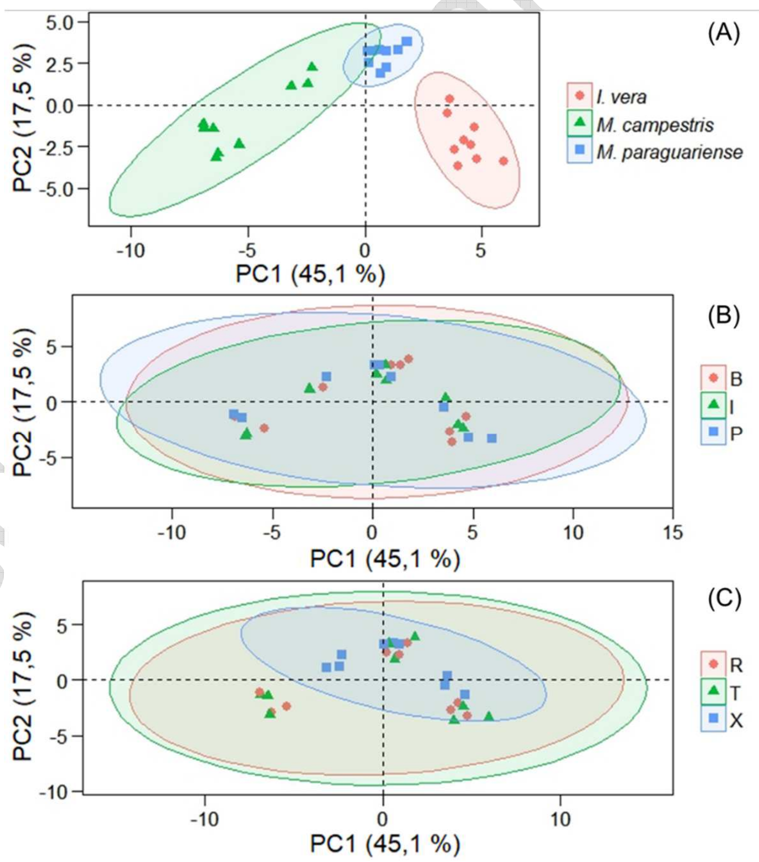

Figure 8: PCA score graph with mean second-derivative spectra of Fabaceae species. (A) based on species, (B) in function of radial position in trunk and (C) for anatomical section.

The PCA factor loading graphs with second derivative data (Figure 9) indicated an influence of wavelength in all spectral bands, i.e., it was not possible to determine with precision which region or color contributed to better discrimination of species.

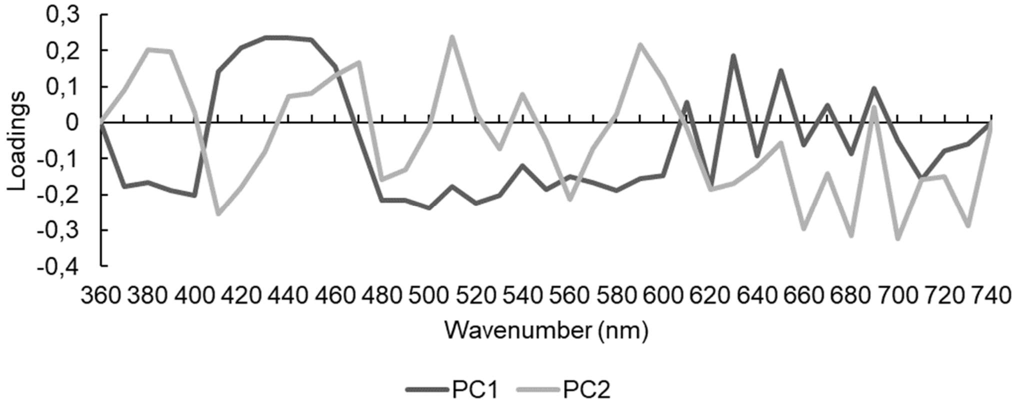

Figure 9: PCA loading graph with second-derivative visible spectra of Fabaceae species.

CONCLUSIONS

With mean data from colorimetric parameters of three species, hue angle was the color characteristic that best discriminated numerically the Fabaceae species evaluated.

The parameters L*, b* and C* were influenced by trunk position and anatomical section. Each species presented a specific tendency for each color parameter. Data from a* and h were not statistically different independent of species, trunk position or anatomical section analyzed.

In graphical analysis, similarity of species was verified. Principal component analysis with second-derivative spectra showed adequate discrimination of the three Fabaceae species.

Colorimetric parameters and visible spectra can contribute to a database for species discrimination in timber trade.

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