Influence of toposequence position of *Stryphnodendron adstringens* trees on discrimination of samples based on spectroscopy

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ABSTRACT *Stryphnodendron adstringens* is a native species from Caatinga and Cerrado biomes. The chemical composition of species is variable and depends on the part of the tree, as well as on genetic and soil characteristics related to the origin of the population. This paper describes the influence of toposequence position of trees on the discrimination of wood and bark samples based on visible (VIS), near-infrared (NIR) and mid-infrared (MID) spectroscopy. A total of 28 trees were collected in the northern part of Cerrado State Park in the municipality of Jaguariaíva, Paraná state, Brazil, in different types of soil, at seven positions (0, 100, 200, 300, 400, 500 and 600 m) of the toposequence. The results showed the influence of the position of trees. The bark was classified as dark brown and the wood as rose-grey. VIS spectra was not efficient in sample discrimination. The Principal Component Analysis (PCA) of NIR spectra presented two groups that can be related to the the type of soil: (i) 0-300 m - Red-Yellow Latosol and Haplic Cambisol; (ii) 400-600 m - Red Latosol. MID infrared spectra of bark also showed the distinction of two previous groups; and analysis based on wood spectra of trees collected on 0-100m presented discrimination from other positions.

Keywords: infrared; colorimetry; multivariate analysis; wood and bark distinction.

Introduction

Stryphnodendron adstringens (Mart.) Coville (Fabaceae Lindl), commonly known as *barbatimão*, is a native species from Caatinga and Cerrado biomes; it might be found in the states of Tocantins, Bahia, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, São Paulo and Paraná, as well in the Federal District (SOUZA; LIMA, 2017). The species present its principal applications for medicinal purpose (FERREIRA et al., 2013). A review by Lima et al. (2016) described many composites from secondary metabolism of *Stryphnodendron adstringens*, such as alkaloids, terpenes, flavonoids, steroids and tannins, the last being the predominant constituent, adding therapeutic value to the species. The application of tannin extract in addition to bionanocomposites with natural antimicrobial properties (COSTA et al., 2013) against infections was also evaluated (LUIZ et al., 2015). In wood industry, tannin extracts from bark have been tested as adhesives in particleboards and OSB production (CARVALHO et al., 2014a, b).

The chemical composition of species is variable and depends on the part of the tree, as well as genetic and population origin. Intra-population genetic variability of *Stryphnodendron adstringens* from populations found in Cerrado regions, Goiás, Minas Gerais and São Paulo was found to be higher than inter-population diversity; and high tannin concentration in bark was related to lower percentage of polymorphic genes (MENDONÇA et al., 2012). Also, in samples from Minas Gerais, the total extractives content varied among branches (19.8%), stems (20.66%) and roots (26.8%) (GOULART et al., 2012) and total phenolic content

S. Nisgoski et al. (2018)

Influence of toposequence position of *Stryphnodendron adstringens* trees on discrimination of samples based on spectroscopy

was 13.96% in leaves, 11.47% in bark and 2.35% in stems (MACEDO et al., 2007). Soil characteristics and season also have an influence on phenol and tannin yields. Jacobson et al. (2005) observed that the highest total phenols and tannins contents in *Stryphnodendron* spp. were associated with soils having low chemical fertility and were observed in the rainy season.

Correct knowledge of species is important for adequate final use in industry and for forest conservation. Therefore, fast analysis of variations is important, for which the use of nondestructive techniques such as visible an infrared spectroscopy can be an alternative.

Wood is produced by the vascular cambium by a complex sequence of interactions between gene–protein expression and the local environment (climate and nutritional factors, growth regulators and physical stresses), which varies continuously over time (DOWNES; DREW, 2008). Wood color differs among species and within a tree, e.g., heartwood and sapwood (DÜNISH et al., 2010; KILIC; NIEMZ, 2012) and is an important factor for final applications and cost. Also, it is influenced by soil, climate, age and silviculture management (LIU et al., 2005). Extractives vary between and within trees and are related to age, environmental conditions and soil, and some studies have found that the largest variations in wood color are associated with extractives content (GIERLINGER et al., 2004; AGUILAR et al., 2009; MOYA; BERROCAL, 2010).

Near-infrared (NIR) studies with wood samples for origin discrimination were described by Sandak et al. (2011), who found relations between the spectra and the origin of trees grown in different locations in Europe; Nisgoski et al. (2016b), who observed the sensitivity of near-infrared spectra in discrimination of six provenances of *Criptomeria japonica* growing in the same region; and Hwang et al. (2016), who studied pine species from different countries. The application

of mid-infrared (MID) spectroscopy enabled the distinction of beech trees grown at different sites (RANA et al., 2008), types of wood-plastic composites (LEE et al., 2010), separation between species of the orders Fagales and Malpighiales (CARBALLO-MEILAN et al., 2014), suitability of wood species to make artistic objects (BUOSO et al., 2016), and characteristics of archeological wood (TRAORÉ et al., 2016), among others.

The above-mentioned information shows that environmental conditions have influence on the chemical composition of species, and knowledge about it is important for final industrial uses. The question is whether spectroscopy is accurate enough to discriminate trees growing in the same region but in different position of toposequence. So, in this paper it is proposed that techniques based on colorimetry/VIS, NIR and MID spectroscopy present sensibility to variations in seven toposequence positions and should be applicable to rapid discrimination of wood and bark samples from *Stryphnodendron adstringens*.

Material and Methods

Material

Trees were collected without identification of age in a natural strand, in the northern portion of Cerrado State Park in the municipality of Jaguariaíva (24º09'S; 50º18'W), Paraná state, Brazil (Figure 1), with a mixed geomorphic pattern (convex-divergent/concave-convergent). Soil characteristics were Red Latosol in the upper part of the slope, Red-Yellow Latosol in the middle part and Haplic Cambisol in the lower part (UHLMANN et al., 1997). Red Latosol and Red-Yellow Latosol are defined as distrofic, with low base saturation (<50%) in most of the first 100 cm of B horizon (including BA); Haplic Cambisol present clay of low activity and the same low base saturation of the previous soil (EMBRAPA,

S. Nisgoski et al. (2018)

Influence of toposequence position of *Stryphnodendron adstringens* trees on discrimination of samples based on spectroscopy

2014). The climate in the region, based on Köppen classification, is subtropical temperate (Cfb), with precipitation of 1400-1600 mm and mean temperature of 17-19 °C (ALVARES et al., 2013).
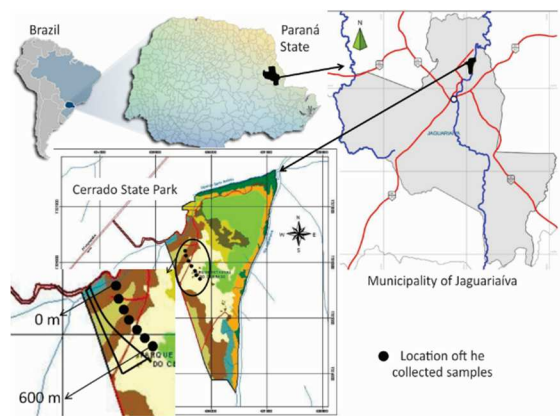

Figure 1. Location of the samples.
Figura 1. Localização das amostras.

For the selection of trees, a straight with 600 m was drawn in toposequence from downstream to upstream and seven points were marked (0, 100, 200, 300, 400, 500 and 600 m). In each point a transect line was drawn and four trees were collected at each of seven points of the toposequence (Figure 1 and Table 1). Wood and bark were obtained at breast height of each tree. Twenty-eight disks were air dried, transformed manually in chips and ground in a Wiley mill type. To avoid heating and changes in the material, an interval of 30 minutes between the grinding of each sample was established. The material was classified and the portion that passed through 60 meshes was analyzed.

Methods

The colorimetric evaluation was performed with a CM-5 spectrophotometer, with a spectral range from 350-750 nm, D65 light source and 10º observation angle (CIE-*L*a*b*** standard). Data on lightness (L*), green-red chromatic coordinate (a*) and blue-yellow chromatic coordinate (b*)

were obtained. The data were analyzed using descriptive statistics and regression analysis. The Tukey test was also performed to verify groups in function of toposequence position based on color parameters at 95% probability.

Table 1. Data from collected trees of *Stryphnodendron adstringens*.
Tabela 1. Dados das árvores coletadas de *Stryphnodendron adstringens*.

Toposequence (m)	H (cm)	DBH (cm)	Altitude (m)
0	3.5	9.5	807
0	4.5	16.6	807
0	5.0	11.8	807
0	4.5	18.8	807
100	3.6	9.2	822
100	3.0	5.7	822
100	3.4	9.2	822
100	3.2	8.3	822
200	4.7	10.5	835
200	3.8	8.6	835
200	3.7	9.5	835
200	4.4	11.8	835
300	4.0	8.6	837
300	3.9	8.6	837
300	3.6	9.2	837
300	4.0	11.1	837
400	4.1	10.5	854
400	4.3	8.9	854
400	4.1	8.9	854
400	4.6	8.0	854
500	4.7	9.5	860
500	4.8	9.5	860
500	3.8	8.0	860
500	4.3	8.3	860
600	4.5	9.9	879
600	3.9	12.7	879
600	3.7	11.8	879
600	5.5	14.6	879

Infrared analyzes were performed with a Bruker Tensor 37 spectrometer equipped with an integrating sphere and operating in reflectance mode; 64 scans were averaged with resolution of 4 cm^{-1} and a spectral range of 10,000–400 cm^{-1}. In a room with temperature of 23 ± 2 °C and relative humidity

S. Nisgoski et al. (2018)

Influence of toposequence position of *Stryphnodendron adstringens* trees on discrimination of samples based on spectroscopy

of 60%, the wood and bark samples were placed on top of the integrating sphere in a cuvette with diameter of 1 cm.

The Unscrambler X chemometric program (version 10.1, from CAMO Software AS) was used to analyze the data. Exploratory modeling was done by analyzing the score and loading graphs obtained by principal component analysis (PCA), based on the NIPALS algorithm and cross validation, to check for possible differences in samples based on toposequence position. To eliminated or minimized additive and multiplicative scattering, pretreatment of first derivative (polynomial order = 1, smoothing point = 2) and second derivative of Savitzy-Golay (polynomial order = 2, smoothing point = 3) were applied to raw data. Spectral analysis was based on American Society for Testing and Materials - ASTM E1655-05 (ASTM, 2000).

Results and Discussion

Colorimetry

The colorimetric parameters of the samples collected from the seven positions in toposequence (Table 2) showed some differences. In bark, some distinction was observed in luminosity (L*) at 500 m, but not a tendency for changes in function of position in toposequence. In the green-red chromatic coordinate (a*), two groups were observed, one from 0-200 m and other from 300-600 m. For intensity of the blue-yellow chromatic coordinate (b*), there is no increase or decrease in function of position. For wood, colorimetric parameters were more homogeneous and did not present a tendency of grouping in function of geographic coordinates.

Based on a timber color chart (CAMARGOS; GONÇALEZ 2001), bark from *Stryphnodendron adstringens* was classified as dark brown and wood as rose-grey. The results agree with Amorim et al. (2013) who comment that the a* coordinate is mainly responsible for the red color, whereas the b* coordinate influences the final color. The

application of colorimetry for species identification must be done carefully (GARCIA et al., 2014) because color variation within a species is frequent and can be related to genetic and environmental characteristics (BRADBURY et al., 2011).

Significant differences in colorimetric parameters between species, provenances and sites were described by Gierlinger et al. (2004). Sotelo Montes et al. (2013) evaluating color among natural populations of five species in Mali, observed that all species presented variation with geographical coordinates, but the relationships differed among species, and that longitude and elevation generally were better explanations for the variation than latitude. Chemical characteristics of soil and its physical properties, rainfall and water availability can also affect wood color (BRADBURY et al., 2011; MOYA; CALVO-ALVARADO, 2012).

In a study with teak wood, Moya; Berrocal (2010) observed that the variation of extractives or chemical composition of lignin produced from diverse soils explained the variation of heartwood color in young teak, and wood color was the result of the combination of plantation characteristics. In another study, Moya; Calvo-Alvarado (2012) concluded that climatic variables should be considered as the first-order causal variables to explain wood color variation, and darker b* coordinate was associated with dry climates as well as with deeper and more fertile sites. The influence of high growth rate on the standard deviation of color parameters of *Robinia* varieties was detailed by Csordós et al. (2014), but mean values were not influenced in comparison with control samples that were not fast growing.

Phenolic content was associated to differences in origins and sites, and related to correlation coefficients between color coordinates, extractives and relative decay resistance (GIERLINGER et al., 2004). In *Acacia mangium* and *Vochysia guatemalensis,* color parameters and extractives and phenolic

S. Nisgoski et al. (2018)

Influence of toposequence position of *Stryphnodendron adstringens* trees on discrimination of samples based on spectroscopy

content (in hot water and ethanol-toluene extracts) for both species varied widely, especially the parameters a* and b*, and the authors commented that one of the possible causes was the origin of the trees, which came from 30 different plantations located in two regions of Costa Rica (MOYA et al., 2012).

Reflectance curve of samples showed the similarity among all of them and principal component analysis (PCA) of visible spectra with different pretreatments was not able to distinguish bark samples based on position in toposequence. In wood, a tendency of grouping samples from 0-200 m was observed. Other samples did not present a similar trend.

Table 2. Mean and (standard deviation) of colorimetric parameters from *Stryphnodendron adstringens* bark and wood samples in seven toposequence positions.
Tabela 2. Média e (desvio padrão) dos parâmetros colorimétricos da madeira e casca de *Stryphnodendron adstringens* em sete posições na toposequência.

Position	Bark			Wood		
(m)	L*	a*	b*	L*	a*	b*
0	37.92 a (2.23)	11.15 b (1.01)	12.79 bc (1.54)	61.77 a (2.70)	9.59 cd (1.19)	19.81 a (1.80)
100	38.35 a (2.67)	10.72 b (1.32)	12.36 c (1.20)	62.26 a (1.82)	9.02 d (1.09)	19.62 a (1.00)
200	36.26 ab (1.67)	10.90 b (0.99)	11.98 c (1.22)	62.01 a (2.01)	11.08 b (0.78)	17.13 c (0.58)
300	36.62 ab (1.78)	11.01 b (0.93)	13.13 bc (1.28)	61.01 ab (8.84)	10.27 bc (1.66)	18.02 bc (1.50)
400	37.15 ab (1.94)	12.97 a (0.93)	14.16 ab (1.40)	63.40 a (2.40)	10.80 b (0.73)	17.19 c (0.75)
500	35.31 b (1.77)	12.60 a (0.85)	13.75 ab (1.15)	62.44 a (3.72)	10.44 bc (1.04)	18.12 bc (1.17)
600	37.09 ab (1.89)	12.38 a (0.55)	14.81 a (1.13)	58.34 b (1.35)	13.47 a (1.22)	18.71 ab (0.71)

Mean values followed by the same letter in the same column do not differ statistically by the Tukey test at 95% probability; L* = lightness, a* = green-red chromatic coordinate and b* = blue-yellow chromatic coordinate

NIR spectroscopy

The NIR spectra of all samples were similar, and to eliminate noise and remove additive and multiplicative effects in the spectra, as well as to improve analysis, the first and second derivatives were applied. PCA was carried out to verify the distribution of wood samples. PCA analysis (Figure 2) with derivatives showed two groups: 0-300 m and 400-600 m. The grouping is probably related to soil characteristics and distance from water-bearing strata. The soil in the 0-300 m range is classified as Red-Yellow Latosol and Haplic Cambisol, whereas that from 400-600 m is Red Latosol.

Environmental conditions can affect tree growth and wood chemical composition, which are reflected in spectra (SANDAK et al., 2011; HWANG et al., 2016; NISGOSKI et al., 2016a). Soil characteristics, such as nutrient availability, physical properties and carbon content, can also influence

tree growth and wood properties (BALIGAR et al., 2007; FAGERIA, 2012). In a study with six provenances of *Criptomeria japonica* planted in the same region, near-infrared analysis was able to discriminate them (NISGOSKI et al., 2016b), whereas in an experiment of different fertilization in pines species, the influence of individual tree was more evident than that of the treatment (NISGOSKI et al., 2016a).

In the NIR spectra, some bands presented difference between group 1 (100-300 m) and group 2 (400-600 m): bands at 6339, 6270, 6287, 6476, 7460, 7481, 7547, 7562, 8156, 8411, 8446, 8450, 8463, 8469, 8496 and 8750-8760 cm^{-1}. These regions are related to cell wall composition and some phenolic compounds (SCHWANNINGER et al., 2011). In some regions, there is a peak dislocation and in other regions the difference is related to the presence of peaks. Sandak et al. (2011) observed the influence of the environmental factors of

S. Nisgoski et al. (2018)

Influence of toposequence position of *Stryphnodendron adstringens*
trees on discrimination of samples based on spectroscopy

the origin of samples and the influence of altitude in sample discrimination. Different exposure at the same altitude can also influence NIR spectra. Hwâng et al. (2016) showed the distinction of *Pinus sylvestris* growing in two countries and *P. densiflora* from South Korea.

MID spectroscopy

MID infrared spectra from bark (Figure 3) and wood (Figure 4) showed little distinction based on position in toposequence. In bark, bands at 669, 898, 900, 952, 990, 1014, 1097, 1104, 1122, 1146, 1158, 1246, 1375, 1425, 1462 and 1650 cm^{-1} are related to variations in the components pectin, cellulose, hemicelluloses and lignin (RANA et al., 2008; LARGO-GOSENS et al., 2014). They presented variations but
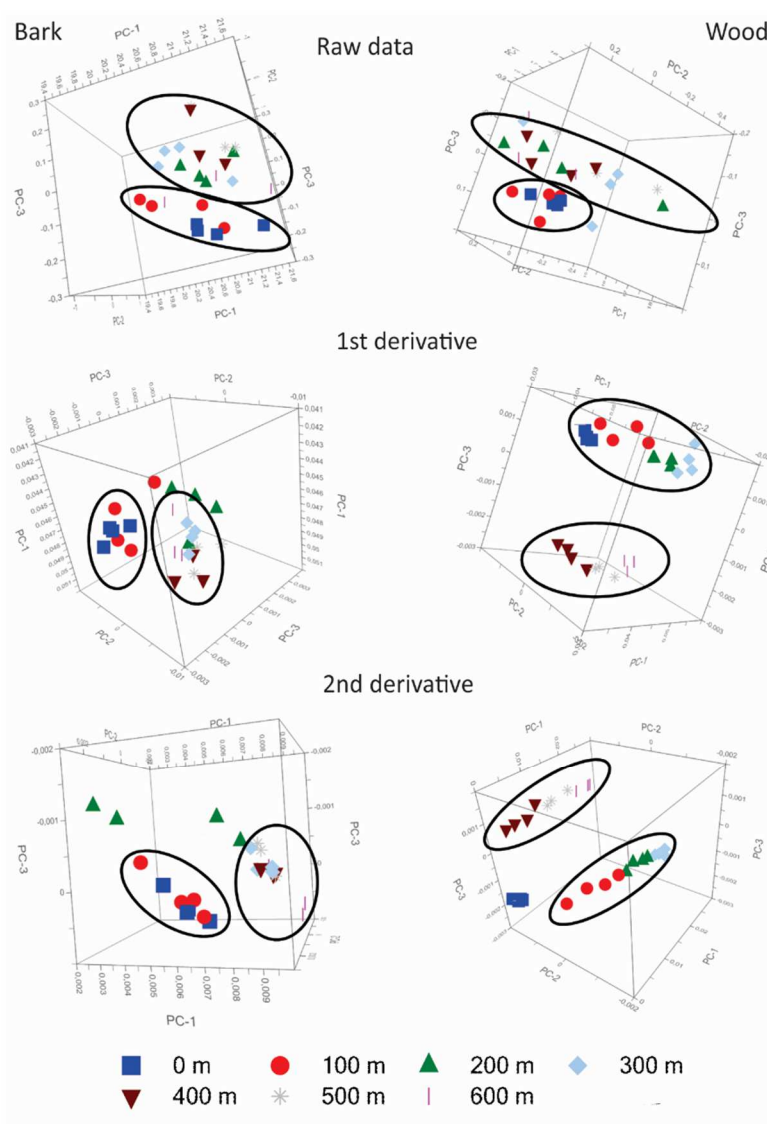

Figure 2. PCA from NIR spectra of *Stryphnodendron adstringens* bark and wood samples in seven positions in toposequence with different pretreatments.
Figura 2. PCA dos espectros NIR da madeira e casca de *Stryphnodendron adstringens* em sete posições na toposequência com diferentes pré-tratamentos.

S. Nisgoski et al. (2018)

Influence of toposequence position of *Stryphnodendron adstringens* trees on discrimination of samples based on spectroscopy

not a pattern related to toposequence position. Bands at 1550 and 1650 cm^{-1} are related to protein content in the cell wall, and the bands in the regions near 1510 cm^{-1} and 1530-1540 cm^{-1} are attributed to lignin (LARGO-GOSENS et al., 2014), showing groups from 0-200 m and 300-600 m. The region from 1400-1442 cm^{-1} was related to phenolic contents, resulting in two groups based on position: with 0-200 m and 400-500m; other samples from 300 and 600 m presented no pattern. Regions at 1040, 1041 and 1043 cm^{-1} were related to cellulose, xyloglucan and arabinogalactan, respectively (LARGO-GOSENS et al., 2014) and showed other conformation: 100 to 300 m and 400 to 600 m. This result was also observed at 1030 cm^{-1} attributed to aromatic C-H plane deformation (RANA et al., 2008).

In wood, some differences were observed in bands at 669, 898, 1030, 1158, 1330, 1375, 1525-1579, 1597-1676 cm^{-1}, related to variations in wood components pectin, cellulose, hemicelluloses and lignin (RANA et al., 2008; LARGO-GOSENS et al., 2014), but there was no linear pattern related to toposequence position.

For species discrimination, Buoso et al. (2016) related diferent peaks attributed to each species in a study of *Populus* spp. (771 and 1607 cm^{-1}), *Tilia* spp. and *Betula* spp. (1690-1800 cm^{-1} and 1200-1300 cm^{−1}), and bands attributed to chemical composition of cell wall. The influence of growing at an inclined angle on wood chemical composition was evaluated by Shi et al. (2012), who observed an increase in relative intensity of bands at 1232, 1267, 1457 and 1510 cm^{-1} and a decrease in bands at 1160, 1371 and 1739 cm^{-1}.

In bark and wood of *Stryphnodendron adstringens*, the region from 2258 to 2387 cm^{-1} showed variation between toposequence positions. These bands are attributed to CO_2 and are influenced by atmospheric conditions on spectra acquisition.
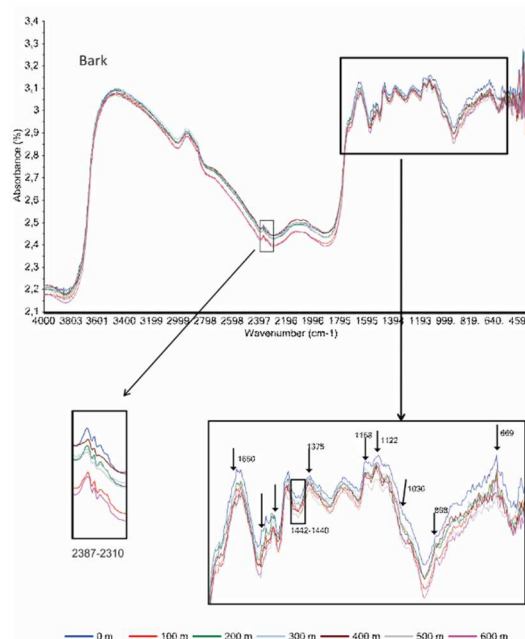

Figure 3. MID infrared spectra of bark samples of *Stryphnodendron adstringens* in seven positions in toposequence.
Figura 3. Espectros em infravermelho médio da casca de *Stryphnodendron adstringens* em sete posições na toposequência.
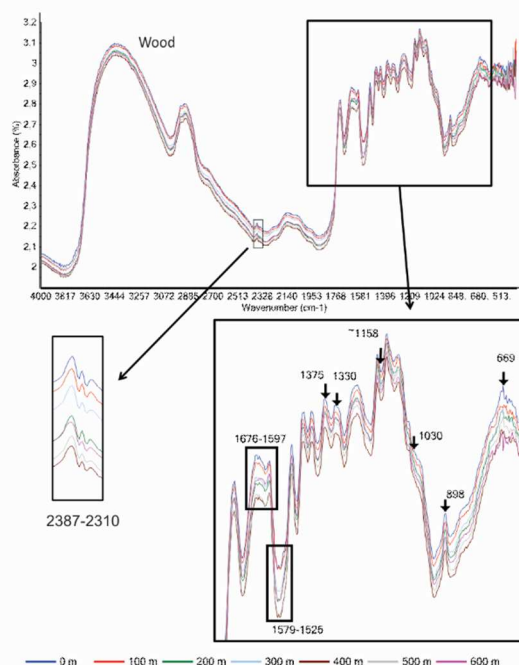

Figure 4. MID infrared spectra of wood samples of *Stryphnodendron adstringens* in seven positions in toposequence.
Figura 4. Espectros em infravermelho médio da madeira de *Stryphnodendron adstringens* em sete posições na toposequência.

S. Nisgoski et al. (2018)

Influence of toposequence position of *Stryphnodendron adstringens* trees on discrimination of samples based on spectroscopy

PCA with total MID spectra was not able to distinguish different positions of trees in toposequence. So, the analysis was performed with different regions with more contrast in wood (1550-1750 cm^{-1}) and bark (600-1700 cm^{-1}) (Figure 5). In bark, PCA with original data grouped trees from 0-100 m and from 200-600 m; in case of derivatives, the groups were 0-200 m and 300-500 m. For wood, samples from 0-100 m and 200-600 m showed similar grouping in raw data and with derivatives. The chemical composition must be evaluated to verify what is influencing in this grouping; it might also be the results of difference in soil characteristics or the proximity of water, which must be confirmed.

For samples of *Stryphnodendron adstringens* wood and bark, different regions presented the best information for discrimination of trees collected at different positions in toposequence. Rana et al. (2008), evaluating *Fagus silvatica* trees growing at five different sites, observed the potential of FTIR to distinguish wood in combination of multivariate statistical methods. The authors commented that the formations of groups were probably related to a combination of climatic factors and soil properties, and the most important bands to discriminate sites were assigned to ring vibration of carbohydrates and aromatics.
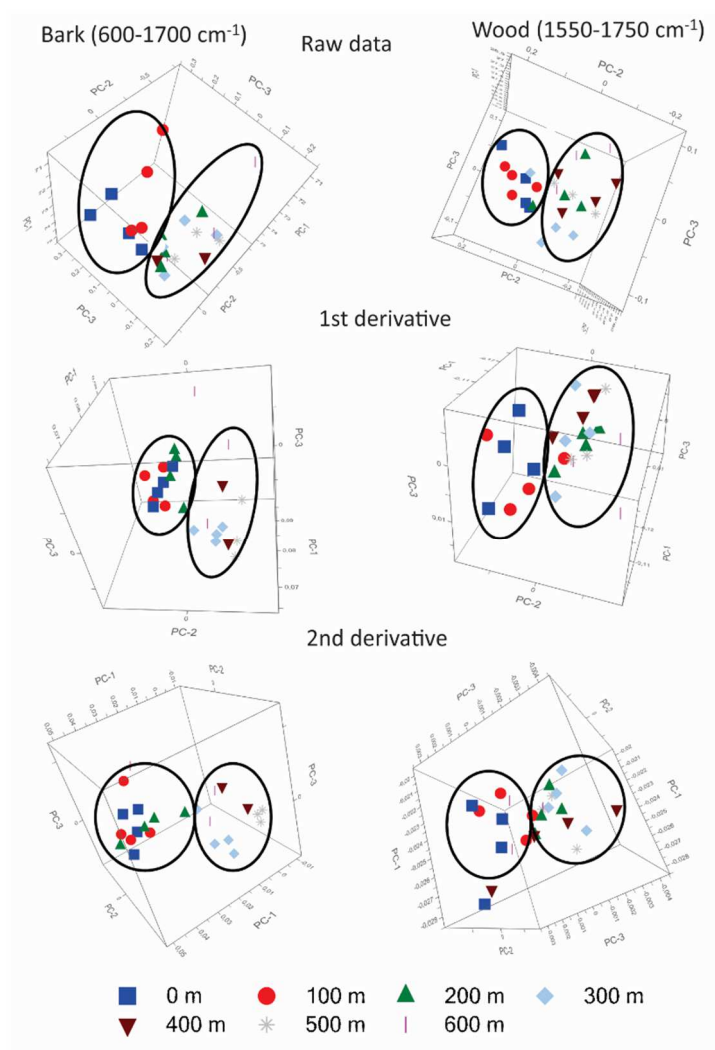

Figure 5. PCA in MID spectra of *Stryphnodendron adstringens* bark and wood samples in seven toposequence positions with different pretreatments.

Figura 5. PCA dos espectros MID da madeira e casca de *Stryphnodendron adstringens* em sete posições na toposequência com diferentes pré-tratamentos.

S. Nisgoski et al. (2018)

Influence of toposequence position of *Stryphnodendron adstringens*
trees on discrimination of samples based on spectroscopy

Acquah et al. (2016) also applied FTIR spectroscopy with PCA and LDA to identify plant part composition of forest logging residue from loblolly pine and observed that the preliminary separation was performed in function of lignin and polysaccharide concentration. Largo-Gosens et al. (2014) presented a review of FTIR applications in studies with cell wall and described its application for discrimination of wood species and determination of chemical wood composition. The authors stated that although the technique presents many advantages over traditional analysis, it is not usually employed alone to analyze cell walls, but to confirm the results obtained with other chemical methods because the information from MID spectra is complex and there are overlapping peaks.

Santoni et al. (2015) discriminated *Picea abies* from three origins and commented that chemical and physical properties of samples were different, and the success of MID spectroscopy was related to differences in the molecular configuration of lignin with some contribution of cellulose and hemicelluloses. Carballo-Meilan et al. (2016) also attributed the differences between five gymnosperm trees and 16 angiosperm trees (from the Rosids and Asterids class) to their lignin and hemicelluloses content and observed some influence of amide.

Conclusions

The position of tree in a toposequence presented influence in VIS, NIR and MID spectra from wood and bark. VIS spectra was not efficient in sample discrimination. NIR spectroscopy presented the best potential to discriminate wood and bark from *Stryphnodendron adstringens* in samples obtained from different positions. MID infrared spectra of bark showed the distinction of two groups (0-300 m and 400-600 m) and wood presented discrimination of trees collected on 0-100 m from other positions.

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S. Nisgoski et al. (2018)

Influence of toposequence position of *Stryphnodendron adstringens* trees on discrimination of samples based on spectroscopy

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S. Nisgoski et al. (2018)

Influence of toposequence position of *Stryphnodendron adstringens* trees on discrimination of samples based on spectroscopy

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