

## Anatomical characterization of *Ocotea indecora* Schott wood along a 300m altitude gradient

Caracterização anatômica da madeira de *Ocotea indecora* Schott ao longo de um gradiente de altitude de 300m

La caracterización anatómica de la madera de *Ocotea indecora* Schott a lo largo de un gradiente de altitud de 300m

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### Abstract

This study aimed to evaluate the impact of altitude on the anatomical characteristics of *Ocotea indecora* wood under various relief conditions in Cunha, São Paulo. Non-destructive stem samples of *Ocotea indecora* were collected at three altitudes within the Cunha Nucleus of the Serra do Mar State Park (1300, 1200, and 1000 m). Five non-destructive samples were taken at each altitude around the diameter at breast height (DBH) of selected trees. Trees above 1250 m were situated in plateau areas, while the remaining sampled trees were situated in escarpment areas with slopes ranging from 30 to 90%. The geolocation of sampled trees was performed using global positioning system equipment. The samples were processed following standard wood anatomy techniques. Significant differences were observed, but only in the fiber diameter of samples collected between 1000 m and 1300 m. At 1300m, fiber diameter showed the highest values, while the lowest fiber diameter was found in trees sampled at 1000 m. According to cluster dendrogram analysis, considering all analyzed variables, altitudes of 1200 m and 1300 m were grouped together, differing from the altitude of 1000m, which formed a separate cluster. Generally, anatomical elements correlate differently among themselves depending on the altitude gradient.

**Keywords:** Ecological anatomy; Environmental variation; Tropical woods.

### Resumo

Este estudo teve como objetivo avaliar o impacto da altitude nas características anatômicas da madeira de *Ocotea indecora* sob diversas condições de relevo em Cunha, São Paulo. Amostras não destrutivas do caule de *Ocotea indecora* foram coletadas em três altitudes dentro do Núcleo Cunha do Parque Estadual da Serra do Mar (1300, 1200 e 1000 m). Cinco amostras não destrutivas foram retiradas em cada altitude ao redor do diâmetro à altura do peito

(DAP) das árvores selecionadas. As árvores acima de 1250 m estavam situadas em áreas de planalto, enquanto as demais árvores amostradas estavam situadas em áreas de escarpa com inclinações variando de 30 a 90%. A geolocalização das árvores amostradas foi realizada utilizando equipamento de sistema de posicionamento global. As amostras foram processadas seguindo técnicas padrão de anatomia da madeira. Foram observadas diferenças significativas, mas apenas no diâmetro das fibras das amostras coletadas entre 1000 m e 1300 m. A 1300 m, o diâmetro das fibras apresentou os valores mais altos, enquanto o menor diâmetro das fibras foi encontrado em árvores amostradas a 1000 m. De acordo com a análise do dendrograma de agrupamento, considerando todas as variáveis analisadas, as altitudes de 1200 m e 1300 m foram agrupadas juntas, diferenciando-se da altitude de 1000 m, que formou um grupo separado. Geralmente, os elementos anatômicos se correlacionam de maneira diferente entre si, dependendo do gradiente de altitude.

**Palavras-chave:** Anatomia ecológica; Variação ambiental; Madeiras tropicais.

### Resumen

Este estudio tuvo como objetivo evaluar el impacto de la altitud en las características anatómicas de la madera de *Ocotea indecora* bajo diversas condiciones topográficas en Cunha, São Paulo. Se recolectaron muestras no destructivas del tronco de *Ocotea indecora* en tres altitudes dentro del Núcleo Cunha del Parque Estatal de la Serra do Mar (1300, 1200 y 1000 m). Se tomaron cinco muestras no destructivas en cada altitud alrededor del diámetro a la altura del pecho (DAP) de los árboles seleccionados. Los árboles por encima de los 1250 m estaban ubicados en áreas de meseta, mientras que los demás árboles muestreados estaban ubicados en áreas de escarpada con pendientes que variaban entre 30 y 90%. La geolocalización de los árboles muestreados se realizó utilizando equipos de sistema de posicionamiento global. Las muestras fueron procesadas siguiendo técnicas estándar de anatomía de la madera. Se observaron diferencias significativas, pero solo en el diámetro de las fibras de las muestras recolectadas entre 1000 m y 1300 m. A 1300 m, el diámetro de las fibras presentó los valores más altos, mientras que el diámetro más bajo de las fibras se encontró en árboles muestreados a 1000 m. Según el análisis del dendrograma de agrupamiento, considerando todas las variables analizadas, las altitudes de 1200 m y 1300 m se agruparon juntas, diferenciándose de la altitud de 1000 m, que formó un grupo separado. Por lo general, los elementos anatómicos se correlacionan de manera diferente entre sí, dependiendo del gradiente de altitud.

**Palabras clave:** Anatomía ecológica; Variación ambiental; Maderas tropicales.

## 1. Introduction

In Brazil, the Atlantic Forest covers a total area of 130,973,638 hectares with deforestation clearing around 21,642 hectares in the 2020-2021 period. This value is 66% higher than that in the 2019-2020 period (13,053 hectares) and 90% higher than that in the 2017-2018 period, which recorded the lowest deforestation value in the historical series (11,399 hectares). The loss of forests amounts to 59 hectares per day or 2.5 hectares per hour, resulting in an equivalent emission of 10.3 million tons of CO<sub>2</sub> into the atmosphere (SOS Atlantic Forest Foundation, 2022).

This calls for further and more intensive studies on species present in the Atlantic Forest biome, in particular, research related to environmental effects on secondary xylem resulting from altitudinal gradient. At higher altitudes, total atmospheric pressure decreases, along with partial pressure of oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>). Reduction in air temperature, increase in solar radiation, and higher UV-B radiation also occur at higher altitudes. Therefore, at higher elevations, plants must adapt in order to thrive (Körner, 2007).

Tropical forests are important for climate regulation and biodiversity maintenance. However, these ecosystems are threatened by climate change as temperatures rise and the frequency and duration of droughts increase. The anatomical characteristics of xylem are an essential component for understanding and predicting forest responses to changes in water availability (Lourenco et al. 2022). Therefore, environmental factors such as temperature, humidity, and pollution affect the anatomical features of plants (Dar et al. 2022). Comprehensive studies on the effects of multiple environmental factors on plant vascular tissue and water regulation should help us understand plant responses to climate change (Qaderi et al. 2019).

Research comparing species in different environments of tropical regions has shown that environmental variations are reflected in structural variations of wood, particularly in the dimensions of cellular elements, such as diameter, length, and frequency of vessels, wall thickness, fiber length, and both height and width of rays (Carlquist & Hoekman, 1985; Marcati et al., 2001).

Popularly known as “canela-broto,” *Ocotea indecora* is a species found in the (seasonal semi-deciduous forest, ombrophilous forest, and restinga forest), and its successional stage is late secondary. This species is native to Brazil with distribution in the northeast (Bahia State), southeast (Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo States), and south (Paraná, Rio Grande do Sul, and Santa Catarina States), occurring naturally in the Atlantic Forest. It is found along an altitudinal gradient of approximately 1000 m from the Serra do Mar State Park (PESM), Nuclei Cunha *Ocotea indecora* can be used for seed commercialization, and its wood can be used in civil construction (Embrapa, 2024; Flora do Brasil, 2024).

Studies related to hydraulic architecture at high altitudes have demonstrated smaller diameter cell conduits, primarily owing to the reduction in transpiration rates. This reduction is directly related to temperature, making cell differentiation and expansion more challenging. Consequently, hydraulic conductivity decreases, leading to a reduction in plant height (Körner, 1998; Petit et al., 2010). These authors examined other anatomical variations along the altitudinal gradient, e.g., xylem vessel diameter and density, as well as hydraulic architecture, and found that they all depend on tree height. Other authors have also correlated annual precipitation with the frequency and diameter of vessels. Species occurring in areas with annual precipitation ranging between 500 and 1000 mm exhibited a high density of narrow vessels, whereas species growing in very humid environments with annual precipitation exceeding 2500 mm per year showed a low density of large-diameter vessels (Lens et al., 2004).

Despite these researches, the need for expanded studies on wood anatomy in the context of environmental variations arising from the altitudinal gradient remains. Herein, we hypothesize distinct differences in the wood anatomy of *Ocotea indecora* along a 300 m altitudinal gradient. We believe that our results will promote more research to determine anatomical variations in other species growing at both high and low altitudes. Thus, this study aimed to evaluate the impact of altitude on the anatomical characteristics of *Ocotea indecora* wood under various relief conditions in Cunha, São Paulo.

## 2. Methodology

### 2.1 Experimental area

The study area is in the northern sector of the Serra do Mar State Park, Cunha nuclei (Figure 1), which is within the hydrographic basin of the Puruba River along the Cambucá Trail, connecting the plateau to the coastal plain. The vegetation is characterized as Secondary Vegetation of Dense Submontane Rainforest (from 40 to 500 m altitude) and Secondary Vegetation of Dense Montane Rainforest (from 500 to 1500 m altitude).

### 2.2 Sampling

Along a 300 m altitudinal gradient (Figure 1), samples were collected at breast height (i.e., 1.30 m above the ground) from 15 trees of *Ocotea indecora*. Samples were collected from five trees at each elevation. The crowns of all sampled trees were at the highest part of the canopy. However, local conditions made it difficult to precisely determine tree heights because of steep slopes in some areas and the variety of trees surrounding the species of interest. To identify the species, herbarium specimens (i.e., branches with leaves, flowers, or fruits) were collected and compared with specimens available at the SPSF herbarium of the Instituto de Pesquisas Ambientais in São Paulo.

To facilitate the presentation of results and, later, the discussion, altitudes were approximated as 1000, 1200 and 1300 m. The sampled trees were located at different altitudes and georeferenced using GPS equipment with precision around 7m (Garmin Global Position System, model 76CSx). We also used the UTM projected coordinate system and Datum SAD 69, a regional historical geodetic datum for South America. Subsequently, the location of each tree was entered in a digitalized IBGE 1: 50,000 chart to obtain its altimetric elevation. It is estimated that this method allowed for an accuracy of  $\pm 5$  m for altitude.

### 2.3 Climatic characterization

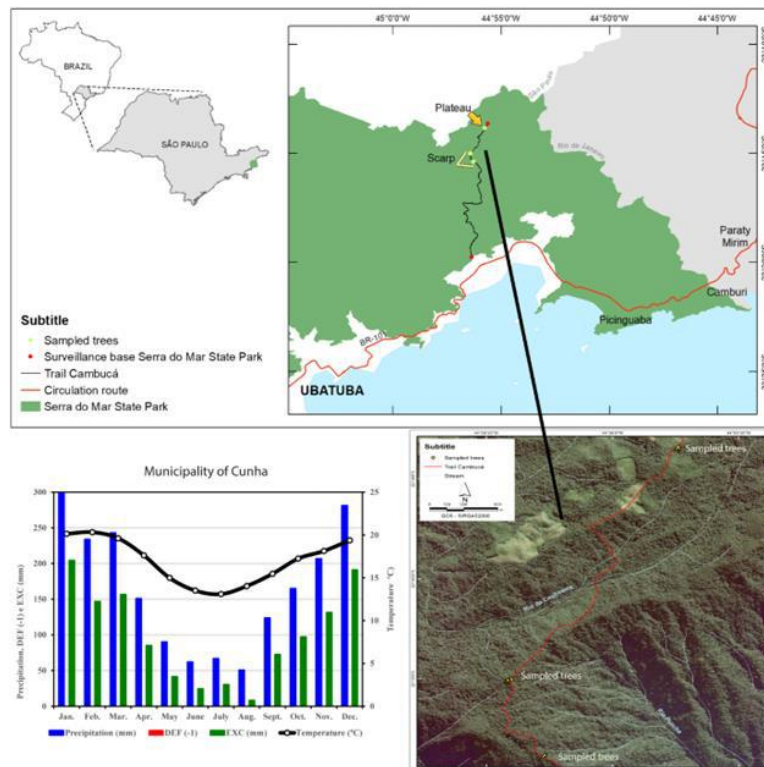
The biogeographic region of Cunha represents two extremes of altitude (Figure 1). Therefore, the meteorological data of the municipality of Cunha were obtained from Walter Emmerich Forest Hydrology Laboratory (LHFWE) since prepared meteorological data will help in understanding the anatomical variations induced in response to temperature, precipitation, and water surplus or deficit.

### 2.4 Wood anatomy

Small non-destructive pieces of wood adjacent to the cambium were cut and utilized to obtain transverse sections. These wood samples underwent boiling for approximately 60 minutes in a solution composed of water, glycerin, and alcohol (ratio of 4:1:1). Subsequently, sections with a thickness of 25  $\mu\text{m}$  were prepared using a sledge microtome. These sections were then stained with a 1% solution of safranin, followed by a rinse with distilled water. Temporary slides were prepared by mounting the sections in a solution of water and glycerin (1:1 ratio). The slides were subsequently examined using an Olympus CX 31 research microscope, and notable findings were documented using a digital camera (Olympus Evolt E330).

Measurements were performed utilizing image analysis software (Image-Pro Plus 6.3). Terminology and wood descriptions adhered to the guidelines specified in the IAWA list (IAWA, 1989). A minimum of 25 measurements were taken to determine the mean (average). The proportions of vessels, axial parenchyma, rays, and fibers in the transverse sections were assessed using a 25-point grid across ten consecutive areas.

**Figure 1** - Map showing the location of the collection area. Topography and hydrography of the entire altitudinal gradient with sampled trees indicated as yellow points. Climatic variations in the municipality of Cunha. Precipitation (blue), water deficit (brown), water surplus (green) and average temperature (red). Walter Emmerich Forest Hydrology Laboratory (LHFWE), Serra do Mar State Park (PESM) and coordinates 45°02'W and 23°25'S, data from 1980 to 2016.



Source: Authors.

## 2.5 Statistical analysis

The results were evaluated using analysis of variance (ANOVA) and Fisher's exact test at a 5% probability to detect variations among altitudinal gradients. A multivariate analysis of the data was also conducted. A grouping analysis of altitudinal gradients was also carried out, and for this purpose, a cluster dendrogram was used. To determine which properties exerted the most influence on the grouping of altitudinal gradients, a principal component analysis (PCA) was performed. The Pearson correlation test was conducted. Descriptive statistics were also used in data analysis. Statistical analyses were performed using R software (R Core Team, 2019).

## 3. Results and Discussion

In Table 1, we present the averaged results of the cellular dimensions of *Ocotea indecora* wood. Fiber length, fiber diameter, and lumen width tend to be smaller at 1000 m (Figure 4). However, only fiber diameter at 1000 m differed from that at 1300m. Fiber diameter at 1300 m presented the highest values, while fiber diameter at 1000 m presented the lowest values (Figure 4B).

The length of vessel elements, diameter, and frequency tend to be lower at 1000 m; however, these differences were not statistically significant (Figure 4E, F and G).

Nevertheless, the wood of *Eremanthus erythropappus* revealed a significant difference in the average frequency of vessels between 1000 m and 1100 m. Remarkably, trees located at 1100 m exhibited a greater quantity of conducting vessels compared to those at 1000 m. Additionally, the average vessel diameter in trees at 1000 m was significantly wider than that observed in trees at 1100 m, suggesting a tendency for larger diameters at this altitude (Mori et al., 2009).

**Table 1** - Average (mean) values and standard deviation of vessel element length (VEL), vessel diameter (VD), vessel frequency (VF), fiber length (FL), fiber diameter (FD), fiber wall thickness (FWT), fiber lumen diameter (LD), ray height (RH) and ray width (RW) of *Ocotea indecora*.

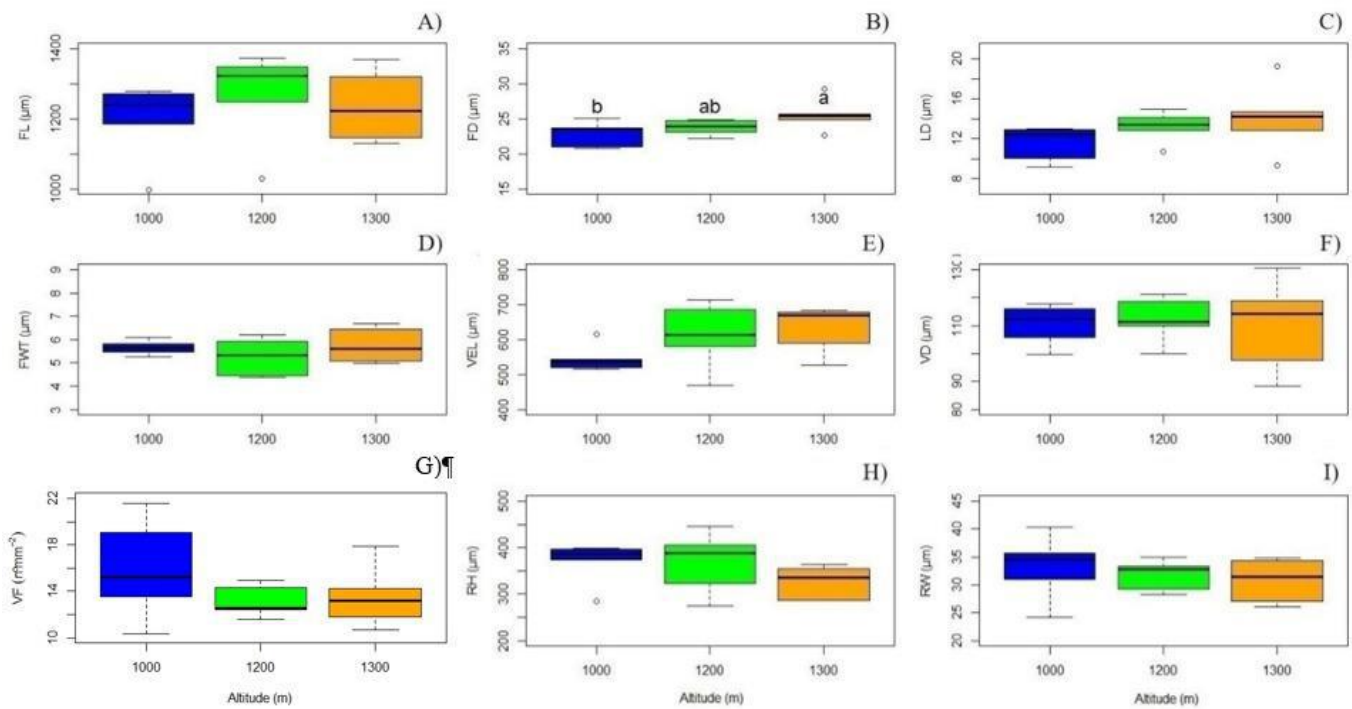
| Anatomical element       | Altitude (m)   |
|--------------------------|----------------|
|                          | mean           |
| FL (µm)                  | 1239 (116.76)  |
| FD (µm)                  | 24.07 (2.09)   |
| LD (µm)                  | 12,94 (2.56)   |
| VEL (µm)                 | 596.43 (76.65) |
| FWT (µm)                 | 5.56 (0.67)    |
| VD (µm)                  | 110.89 (10.86) |
| VF (n°mm <sup>-2</sup> ) | 14.22 (3.17)   |
| RW (µm)                  | 31.86 (4.30)   |
| RH (µm)                  | 353.90 (52.94) |

Source: Authors.

However, in a study of the secondary xylem anatomy of *Clethra scabra* branches, it was observed that modifications in vessel elements are more pronounced at higher altitudes, which could be attributed to safety and efficiency in water transport. Thus, the effects of altitude on *Clethra scabra* branches could be related to their phenotypic plasticity in response to different environmental conditions (Santos et al., 2021). According to the literature, the characteristics of wood most sensitive to environmental variations are generally vessel diameter and frequency, as well as fiber length (Marcati et al., 2001). Wood production and hydraulic conductivity are coordinated by the formation of large earlywood vessels, which may allow

for achieving higher growth rates (Camarero et al. 2021). Changes in vessel diameter and frequency occur due to the plants demand to increase their capacity for water and mineral salt conduction according to their growth and environmental adaptation (Brito et al. 2020).

**Figure 2** - Average (mean) values of the vessel element length (VEL), vessel diameter (VD), vessel frequency (VF), fiber length (FL), fiber diameter (FD), fiber lumen diameter (LD), fiber wall thickness (FWT), ray height (RH) and ray width (RW) of *Ocotea indecora* wood. Means followed by the same lowercase letter do not differ from each other, according to Fisher's exact test ( $P < 0.05$ ) probability.



Source: Authors.

For ray dimensions, both width and length showed very similar values among the different altitudes (Figure 2 H and I), indicating that this variable may be more stable in the face of altitude variations (Marcati et al., 2001).

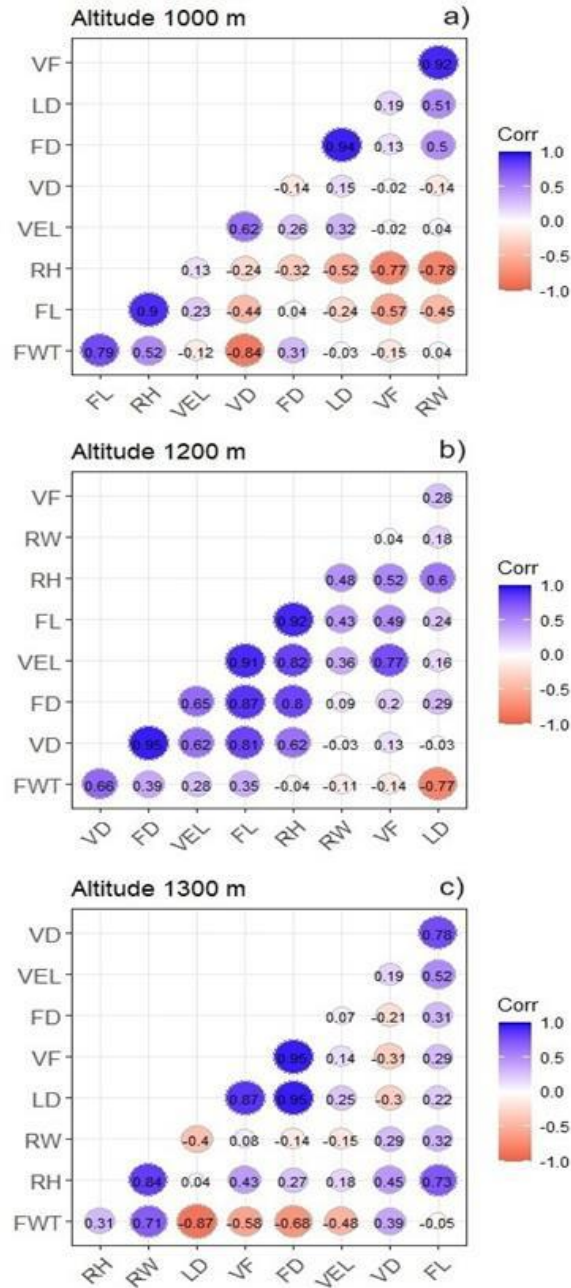
Correlations among variables were observed separately at each altitude. At 1000 m, the strongest positive correlations were between fiber diameter and lumen width, vessel frequency and ray width, and fiber length and ray height. Conversely, negative correlations were observed between fiber wall thickness and vessel diameter (Figure 3A).

At an altitude of 1200 m, we observed that the best positive correlations were between vessel element diameter and the fiber diameter, fiber length and radial height and vessel element length and fiber length. Additionally, the best negative correlation was between fiber wall thickness and vessel element diameter (Figure 3B).

At 1300 m, the best positive correlations were between vessel frequency and fiber diameter, and lumen diameter and fiber diameter, while the best negative correlation was between fiber wall thickness and lumen diameter of the fiber (Figure 3C).

Generally, therefore, depending on the altitude gradient, anatomical variables correlate differently, suggesting that there is a different dependence on each anatomical variable for each altitude gradient (Figures 3A, B, and C).

**Figure 3** - Pearson's Correlation Coefficient for vessel element length (VEL), vessel diameter (VD), vessel frequency (VF), fiber length (FL), fiber diameter (FD), fiber lumen diameter (LD), fiber wall thickness (FWT), ray height (RH) and ray width (RW) of *Ocotea indecora* by altitude separately. Negative and positive correlations are represented by red and blue, respectively. The magnitude of all correlations is represented by color intensity.



Source: Authors.

When analyzing the correlations among the variables, considering all altitudes together, it can be deduced that the best positive correlations were between fiber diameter and lumen diameter and between fiber length and radial height, while the best negative correlation was found between lumen diameter and fiber wall thickness (Figure 4).

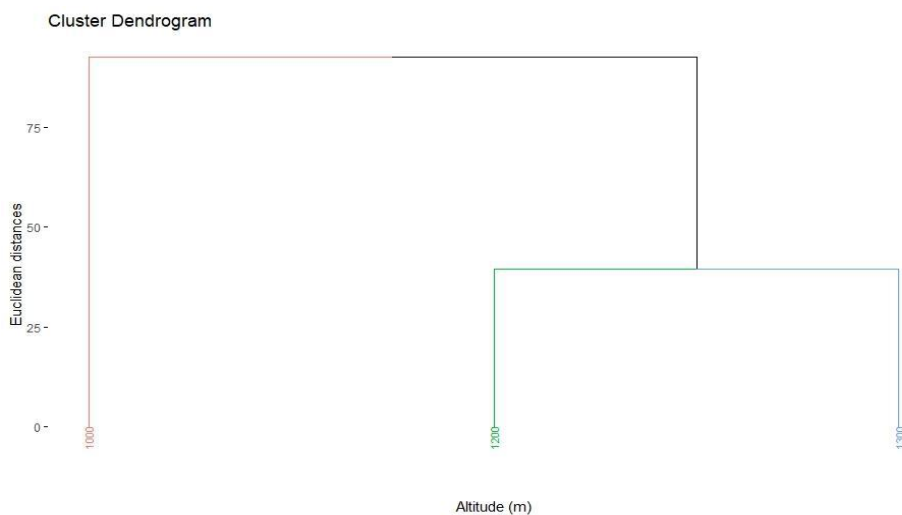
**Figure 4** - Pearson's Correlation Coefficient for vessel element length (VEL), vessel diameter (VD), vessel frequency (VF), fiber length (FL), fiber diameter (FD), fiber lumen diameter (LD), fiber wall thickness (FWT), ray height (RH) and ray width (RW) of *Ocotea indecora*. Negative and positive correlations are represented by red and blue, respectively. The magnitude of all correlations is represented by color intensity.



Source: Authors.

With the aim of attempting to group the dataset from different altitudes into homogeneous subgroups, a cluster dendrogram was created. Considering all analyzed variables, it can be seen that altitudes of 1200 m and 1300 m are grouped together, while 1000 m was considered a separate group. Additionally, 1200 m and 1300 m form a subgroup (Figure 5).

**Figure 5** - Cluster dendrogram for *Ocotea indecora*, depending on altitude.



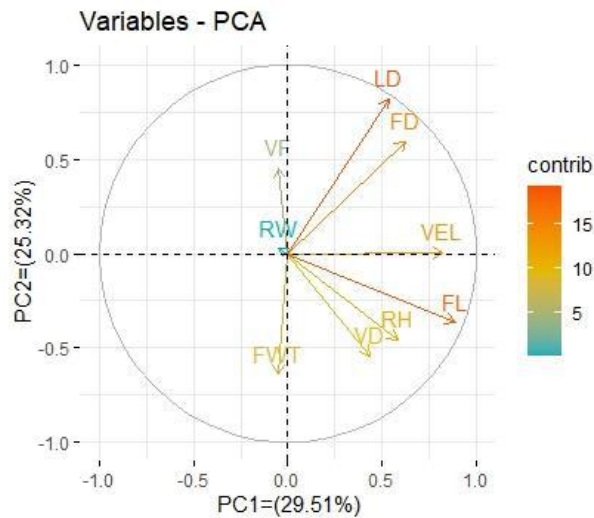
Source: Authors.

According to the principal component analysis (PCA), the first component explains 29.51% of the total variance of the data, which means that almost a third of the data contained in the 9 variables can be encapsulated in this component alone. The second component explains 25.32% of the total variance, which means that just two components explain 54.83% of the data (Figure 6).



Based on the PC1 axis, it can be observed that the variables fiber length (FL) and vessel element length (VEL) made a greater contribution to the grouping of altitude gradients, while vessel frequency (VF) and radial width (RW) made the smallest contribution (Figure 6). However, based on the PC2 axis, the variables fiber lumen diameter (LD) and fiber wall thickness (FWT) made a greater contribution to the grouping of altitude gradients, while vessel element length (VEL) and radial width (RW) made the smallest contribution (Figure 6).

**Figure 6** - Principal Components Analysis (PCA) of the anatomical elements of *Ocotea indecora* wood. The greater the intensity of the red color, the more the property contributes to the grouping. Magnitude is represented by color intensity. Additionally, the smaller the angle between the vectors, the stronger the relationship between the variables. The longer the vector and closer to the outer circle, the greater the significance of the variable represented by the vector.



Source: Authors.

#### 4. Conclusion

The anatomical characteristics of *Ocotea indecora* wood were minimally influenced by altitude. Significant differences were observed only in fiber diameter between the altitudes of 1000 m and 1300 m with fiber diameters at 1300 m showing the highest values and those at 1000 m exhibiting the lowest. Generally, anatomical elements correlate differently, depending on altitudinal gradient. According to the cluster dendrogram, considering all analyzed variables, 1200m and 1300m were grouped together, differing from 1000m, which formed another cluster. We believe that the results we obtained will foster further research to determine anatomical variations or other wood properties for species growing at different altitudes.

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