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MULTIVARIATE ANALYSIS OF TRACHEID MORPHOLOGY IN KNOTS OF *Pinus elliottii* var. *elliottii*

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Resumo

Análise multivariada da morfologia de traqueídes em nós de Pinus elliottii var. elliottii. Durante a formação da madeira, células passam por estágios de desenvolvimento onde elementos anatômicos diferenciam-se entre regiões com nós e sem nós. O objetivo deste trabalho foi comparar, dimensionalmente, traqueídes de regiões de "nós" e de "madeira sem nós", em *Pinus elliottii* var. *elliottii*. Foram utilizadas cinco árvores extraindo-se três discos: na base da árvore (no ponto de inserção do primeiro galho), no topo (a 12 cm de diâmetro do tronco) e no meio (entre base e topo) confeccionando-se corpos de prova da região de "nó" e de "madeira sem nó". Lâminas permanentes do material foram feitas, obtendo-se fotomicrografias. Fez-se a dissociação e maceração de fragmentos de material a comparar os parâmetros comprimento total, diâmetro total, diâmetro do lume e espessura da parede e contagem e mensuração dos elementos celulares. Os dados paramétricos foram submetidos à ANOVA ($p \leq 0,05$) elevando-os ao teste Bartlett. E Não-Paramétricos ao teste Dunn. Escalonamentos Multidimensionais não Métricos (NMDS) com distância Euclidiana simples foram empregados através de Análises de Similaridades (ANOSIM) e Análises MANOVA Não-Paramétrica. Pacotes estatísticos GraphPadPrism e PAST v. 2.17c foram utilizados. Os resultados indicam que o padrão de crescimento e incremento das traqueídes estruturais mais longas e espessas da base para o tronco, não se repete nos nós. Traqueídes de nós apresentam menor incremento em tamanho e não parecem ter crescimento semelhante ao observado na madeira livre de nós.
Palavras-chave: Madeira; fibras de compressão; padrão de crescimento; fotomicrografias.

Abstract

During the formation of wood, cells go through developmental stages in which anatomical elements differentiate between knotted and knot-free regions. The objective of this study was to compare, dimensionally, tracheids of "knot" and "knot-free wood" regions in *Pinus elliottii* var. *elliottii*. Five trees were used, and three discs were extracted from each tree: at the base (point of insertion of the first branch), at the top (trunk with 12 cm diameter) and in the middle (between base and top) to make specimens from the "knot" and "knot-free wood" regions. Permanent slides of the material were made, and photomicrographs were taken. Dissociation and maceration of material fragments were performed, comparing the parameters total length, total diameter, lumen diameter and wall thickness, besides counting and measuring cellular elements. Parametric data were subjected to ANOVA ($p \leq 0.05$) and to Bartlett's test, and non-parametric data were subjected to Dunn's test. Non-Metric Multidimensional Scaling (NMDS) with simple Euclidean distance was employed through Analysis of Similarity (ANOSIM) and Non-Parametric MANOVA Analysis. GraphPadPrism and PAST v. 2.17c statistical packages were used. The results indicate that the pattern of growth and increment of the longer and thicker structural tracheids from the base to the top of the trunk is not repeated in the knots. Tracheids of knots show a smaller increment in size and do not seem to have growth similar to that observed in knot-free wood.
Keywords: Wood; compression fibers; growth pattern; photomicrographs.

INTRODUCTION

In the process of wood formation, cells go through developmental stages in which genetic, silvicultural, climatic, and positioning factors cause dimensional variations in anatomical elements (EVERT, 2013; DEUS *et al.*, 2024a). These conditions form the type of wood called reaction, commonly differentiated between traction wood in angiosperms and compression wood in gymnosperms, due to the differentiated effect of changes in the physiological state of the cambium resulting from different concentrations of growth substances (VIDAURRE *et al.*, 2013). In gymnosperms, compression wood is different from normal wood in terms of microfibril angle alignment and polymer composition (PENG *et al.*, 2020; DEUS *et al.*, 2024a).

In regions of knot, defined as the point of intersection of the branch with the trunk of the tree and surrounding areas, the cells exhibit dimensional adaptations that enable mechanical support to the branches (BUKSNOWITZ *et al.*, 2010; DONALDSON, 2013; SLATER *et al.*, 2014).

Knots have independent systems of growth rings, formed mainly by compression fibers morphologically distinct from normal wood fibers (ROCHA *et al.*, 2018).

In the knot region, moisture content is low, density is twice as high as the density of normal wood (JUNGNIKL *et al.*, 2009), and grain orientation is more or less perpendicular to the surrounding wood (JENKEL and KALISKE, 2014; SLATER *et al.*, 2014).

The combination of density and inclination angle of microfibrils from the knot along the branch confers flexibility, increases the deformation capacity of branch wood and acts as a protection against the transmission of its load towards the trunk (JUNGNIKL *et al.*, 2009), which contributes to structural damping of the movements of the branches in relation to the trunk, distributing throughout the tree the mechanical energy imposed by strong winds and not much concentrated on the trunk and roots, vital parts of the tree (SPATZ and THECKES, 2013).

However, at the same time that they are conditioned on the survival of the tree, the adaptations present in the wood cells in the knot region and in its surroundings make these regions susceptible to "defects", from the point of view of wood use (WANG *et al.*, 2013), since changes in these regions are described as the preferred points of structural failures and ruptures (JUNGNIKL *et al.*, 2009).

This is because the presence of the knot negatively affects most of the mechanical properties of the wood, especially its strength, so that the size of the area occupied by the knot has an inverse relationship with the mechanical properties of the wood (ROCHA *et al.*, 2018).

Changes in technological properties depend on the size of the knot, its state of solidity, its degree of inclination in relation to the trunk and, mainly, the stress caused by the differences between cells of the knot and cells of surrounding regions (MOHAN *et al.*, 2014; SLATER *et al.*, 2014; ROCHA *et al.*, 2018).

In this aspect, there is a gap in terms of knowledge when considering the variations along the trunk of anatomical characteristics of cells of knots and surrounding regions. Therefore, investigating these two distinct anatomical characteristics along the commercial profile of the trunk makes it possible to understand the hypothesis of how dimensional differences of anatomical elements influence technological properties of wood in different portions of the tree. In this context, the objective of this study was to compare, dimensionally, tracheids of regions of "knots" and portions of "knot-free wood" in *Pinus elliottii* var. *elliottii*.

MATERIAL AND METHODS

Material acquisition and data collection

Five 19-year-old individuals of *Pinus elliottii* var. *elliottii*, from a commercial plantation located in the municipality of Agudos, SP, Brazil (22°53'20" S; 47°04'39" W) were used in the study. The climate of the region is tropical, Cfa type, characterized as humid and hot, according to Köppen's classification. The average annual rainfall and temperature are 1367 mm and 21.8 ºC, respectively, at an average altitude of 587 m above sea level (ALVARES *et al.*, 2013).

The wood was identified and listed under the record numbers 10191, 10192, 10193, 10194 and 10195 in the Xylotheque of the Botanical Garden of Rio de Janeiro. Only samples of wood from the base region were listed and registered. Due to the limitation in the amount of knot material, no sample of this region was listed.

From each tree, three discs were extracted as follows: one disc from the point of insertion of the first branch (base), one disc from the trunk at a point with approximately 12 cm diameter (top) and one disc from the middle of the distance between the base and the top. All the discs contained at least one live knot. From the discs obtained from each section, test specimens of the "knot" and "knot-free wood" regions were made.

Permanent slides of the material were made according to Zimmermann's (2018) methodology, and photomicrographs were taken (Olympus BH2 microscope with PM-10 AB photographic equipment) to record the morphology of the cells of the knots and surrounding regions.

Fragments of the material were macerated for dissociation and measurement of the tracheids. Counts and measurements of the cellular elements followed the standards of the IAWA Committee (1989) with an increase from 25 to 30 measurements. Measurements were performed using the Image Pro Plus image processing system, version 3.0 for Windows.

Each set of 30 tracheid measurements was performed in each trunk section (base, middle and top) in each of the sampled individuals (n=5). This resulted in 180 measurements of each variable: total length (TL), total diameter (TD) and lumen diameter (LD), totaling 540 records per tree and 2700 records for each anatomical characteristic studied. Cell wall thickness was calculated according to the formula: (TD - LD)/2

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(SOUZA *et al.*, 2005). The parameters considered for the study were total length (µm), total diameter (µm), lumen diameter (µm) and wall thickness (µm) (EVERT, 2013).

Statistical analysis

All comparative data were obtained from the records of the anatomical variables of the tracheids in different segments of the trunk (base, middle and top) for the two structural conditions (“knot” and “knot-free wood”).

In all sets of measurements (single replicates) of the anatomical structures, by segment, possible outliers were identified and excluded. For the analyses performed from the means of the individuals (n=5) (true replicates), possible outliers were maintained.

Normality was assessed by the Kolmogorov-Smirnov (K-S) test and, in cases where the data sets showed parametric characteristics (normality and homoscedasticity of variances), comparisons of means were performed by ANOVA.

In the ANOVA comparisons, for significant differences ($p \leq 0.05$), Bartlett’s *a posteriori* test was applied to characterize the differences. In cases where the assumptions of normality and homoscedasticity of variances were not met, the datasets were subjected to the similar non-parametric Kruskal-Wallis test. In the K-W comparisons, Dunn’s *a posteriori* test was applied for significant differences ($p \leq 0.05$). These analyses were carried out using the GraphPadPrism v. 6 package (GRAPHPAD, 2012).

Possible differences between the three portions of the trunks (base, middle and top), in the conditions of “knots” alone, “knot-free wood” alone, and for the sets of “knots” and “knot-free wood”, from a multivariate perspective, were inferred and estimated using the mean values of the variables recorded in each individual (n=5). The PAST v. 2.17c package was used to perform the analyses (HAMMER *et al.*, 2001).

From a multivariate perspective, Non-Metric Multidimensional Scaling (NMDS) was performed. Simple Euclidean distance was used as a measure to assess the spatial proximity between the paired samples. For quantitative numerical measurement of the similarities or differences inferred in NMDS, Analysis of Similarity (ANOSIM) and Non-Parametric MANOVA Analysis (NPMANOVA or PERMANOVA) were performed (HAMMER *et al.*, 2001).

RESULTS

In the three portions of the trunk analyzed (base, middle and top), the measurements of the tracheids formed in the “knots” showed lower values and greater variation in size, compared to the segments of “knot-free wood”. When comparing the mean lengths of the tracheids of “knots”, longer structures were observed at the base and in the middle of the trunk, compared to those at the top (Figure 1a). Regarding the mean lengths of “knot-free wood”, similar values were observed between tracheids at the base and middle of the trunk. Tracheids in the top region showed values similar to those found in the middle of the trunk, but lower values than those found at the base (Figure 1b).

When comparing the mean total diameters of the tracheids of “knots”, higher values were observed in the middle of the trunk than at the top. However, no significant differences were recorded between the base and the middle, nor between the base and the top (Figure 1c). When comparing the mean total diameters of the “knot-free wood”, higher values were observed at the base, compared to those of the tracheids in the middle and at the top of the tree. Between middle and top, it was not possible to state that there are significant differences in this variable (Figure 1d).

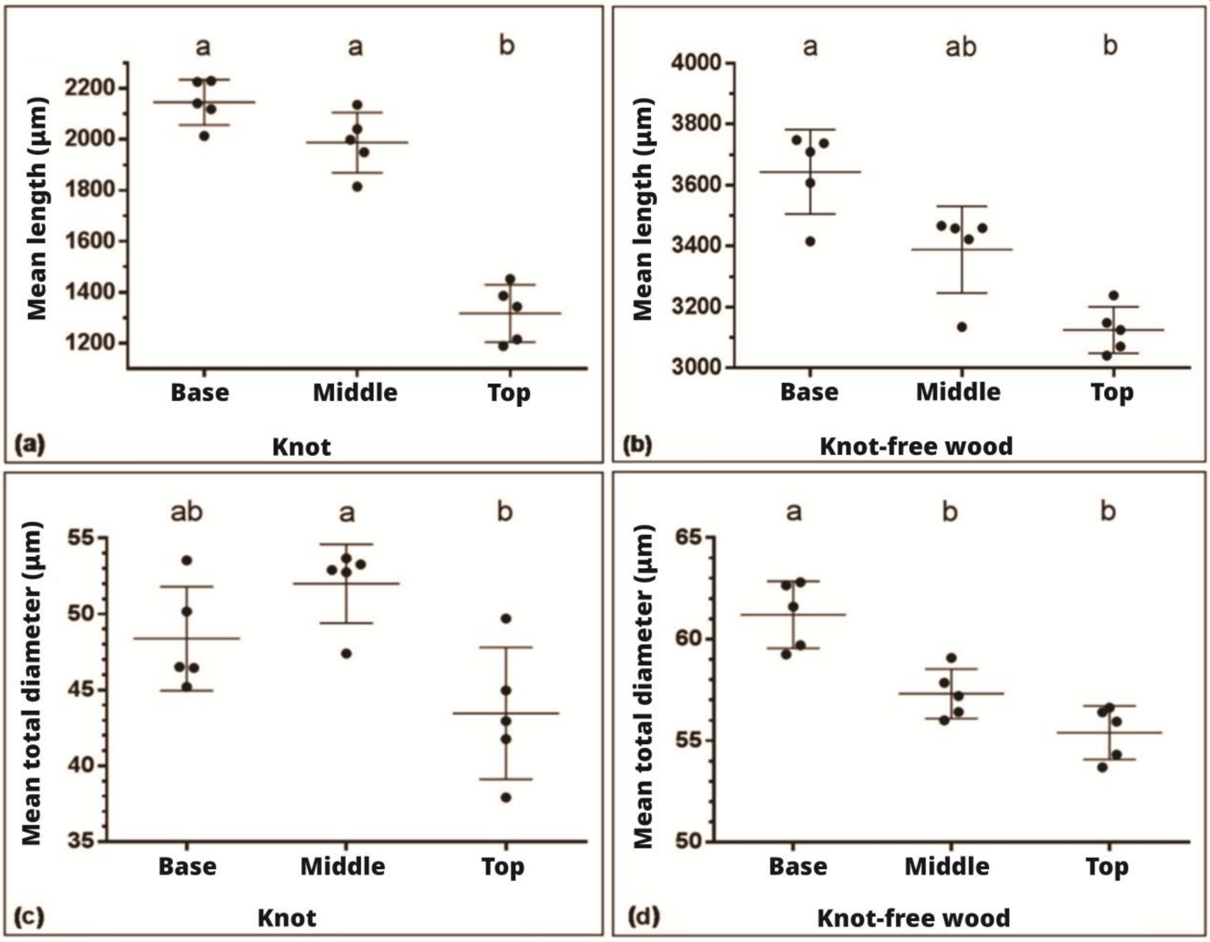

Figure 1. Comparative distribution of the mean length (μm) of tracheids in "knots" (a) and "knot-free wood" (b) of adult individuals of *Pinus elliottii* in different trunk positions (base, middle and top). Each point represents the mean of measurements (n=30) in each individual (n=5). Horizontal lines (mean ± standard deviation). Different letters indicate significant differences: (a) by ANOVA ($F [2, 12] = 84.11$; $P < 0.0001$), (b) by Kruskal-Wallis ($H = 9.74$; $P = 0.0014$). Comparative distribution of the mean total diameter (μm) of tracheids in "knots" (c) and "knot-free wood" (d) of adult individuals of *Pinus elliottii* in different trunk positions (base, middle and top). Each point represents the mean of measurements (n=30) in each individual (n=5). Horizontal lines (mean ± standard deviation). Different letters indicate significant differences: (a) by Kruskal-Wallis ($H = 8.060$; $P = 0.0092$), (b) by ANOVA ($F [2, 12] = 22.09$; $P < 0.0001$).

Figura 1. Distribuição comparativa do comprimento médio (μm) das traqueídes em "nós" (a) e "madeira sem nós" (b) de indivíduos adultos de *Pinus elliottii* em distintas posições do tronco (base, meio e topo). Cada ponto representa a média de medições (n=30) em cada indivíduo (n=5). Linhas horizontais (média ± desvio padrão). Letras diferentes indicam diferenças significativas: (a) por ANOVA $F [2, 12] = 84,11$; $P < 0,0001$, (b) por Kruskal-Wallis ($H = 9,74$; $P = 0,0014$). Distribuição comparativa do diâmetro total médio (μm) das traqueídes em "nós" (c) e "madeira sem nós" (d) de indivíduos adultos de *Pinus elliottii* em distintas posições do tronco (base, meio e topo). Cada ponto representa a média de medições (n=30) em cada indivíduo (n=5). Linhas horizontais (média ± desvio padrão). Letras diferentes indicam diferenças significativas: (a) por Kruskal-Wallis ($H = 8,060$; $P = 0,0092$), (b) por ANOVA ($F [2, 12] = 22,09$; $P < 0,0001$).

The mean lumen diameters of the tracheids in "knots" were larger in the middle of the trunk, compared to those at the top. Similar to the result observed for total diameter, no significant differences were identified between the values of the base and the middle, nor between the base and the top (Figure 2a). For the mean lumen diameters in the "knot-free wood", the values were similar between the base and middle, and between the middle and the top of the trunk. However, significant differences were observed between the base and the top (Figure 2b).

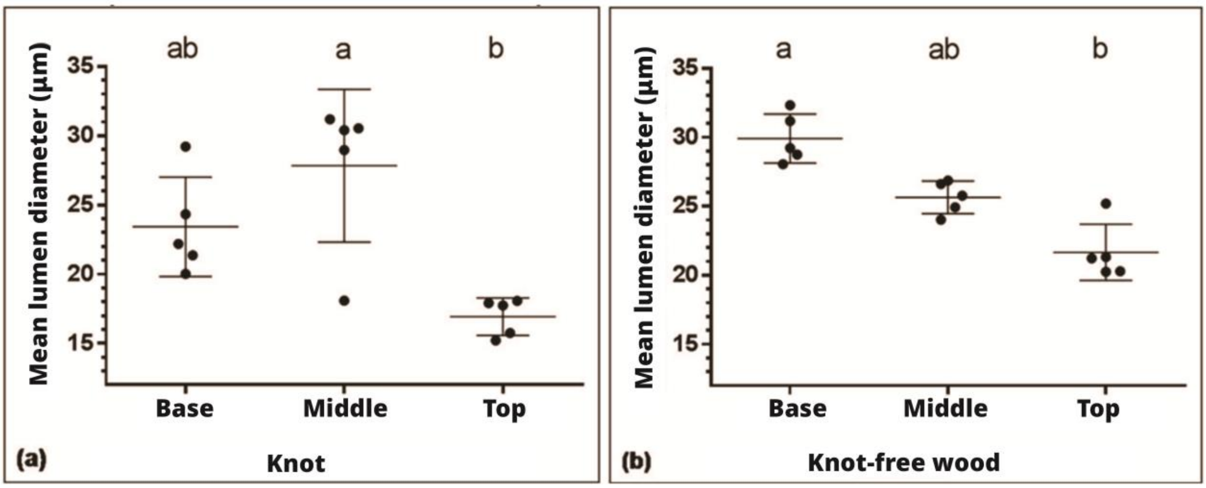

Figure 2. Comparative distribution of the mean lumen diameter (µm) of the tracheids in "knots" (a) and "knot-free wood" (b) of adult individuals of *Pinus elliottii* in different trunk positions (base, middle and top). Each point represents the mean of measurements (n=30) in each individual (n=5). Horizontal lines (mean ± standard deviation). Different letters indicate significant differences: (a) by Kruskal-Wallis (H = 10.22; P = 0.0006), (b) by Kruskal-Wallis (H = 11.58; P = 0.0001).

Figura 2. Distribuição comparativa do diâmetro médio do lume (µm) das traqueídes em "nós" (a) e "madeira sem nós" (b) de indivíduos adultos de *Pinus elliottii* em distintas posições do tronco (base, meio e topo). Cada ponto representa a média de medições (n=30) em cada indivíduo (n=5). Linhas horizontais (média ± desvio padrão). Letras diferentes indicam diferenças significativas: (a) por Kruskal-Wallis (H = 10,22; P = 0,0006), (b) por Kruskal-Wallis (H = 11,58; P = 0,0001).

Regarding the mean wall thicknesses of the tracheids in "knots" (Figure 3a) and in "knot-free wood" (Figure 3b), it was not possible to affirm that there are significant differences between the base, middle and top of the trunk.

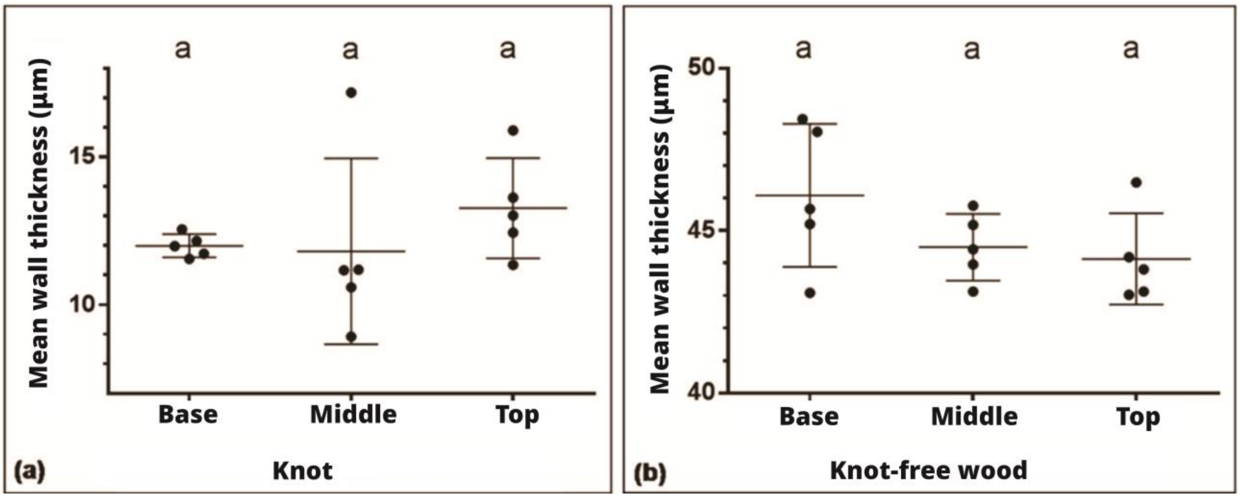

Figure 3. Comparative distribution of the mean wall thickness (µm) of the tracheids in "knots" (a) and "knot-free wood" (b) of adult individuals of *Pinus elliottii* in different trunk positions (base, middle and top). Each point represents the mean of measurements (n=30) in each individual (n=5). Horizontal lines (mean ± standard deviation). Equal letters do not indicate significant differences: (a) by Kruskal-Wallis (H = 4.22; P = 0.1226), (b) by ANOVA (F [2, 12] = 0.5952; P < 0.567).

Figura 3. Distribuição comparativa da espessura média da parede (µm) das traqueídes em "nós" (a) e "madeira sem nós" (b) de indivíduos adultos de *Pinus elliottii* em distintas posições do tronco (base, meio e topo). Cada ponto representa a média de medições (n=30) em cada indivíduo (n=5). Linhas horizontais (média ± desvio padrão). Letras iguais não indicam diferenças significativas: (a) por Kruskal-Wallis (H = 4,22; P = 0,1226), (b) por ANOVA (F [2, 12] = 0,5952; P < 0,567).

As for the multivariate analysis of “knots” *versus* “knot-free wood”, NMDS pointed to a clear distinction between the tracheid samples obtained from the “knots” located at the top of the trunks and the samples from the base and middle. It also indicated the possible difference between these last two conditions (Figure 4a). In “knot-free wood”, NMDS also pointed to the distinction between the tracheid samples and identified a gradient, considering the samples located at the top, passing through the samples from the middle, and finally reaching the samples from the base of the trees (Figure 4b).

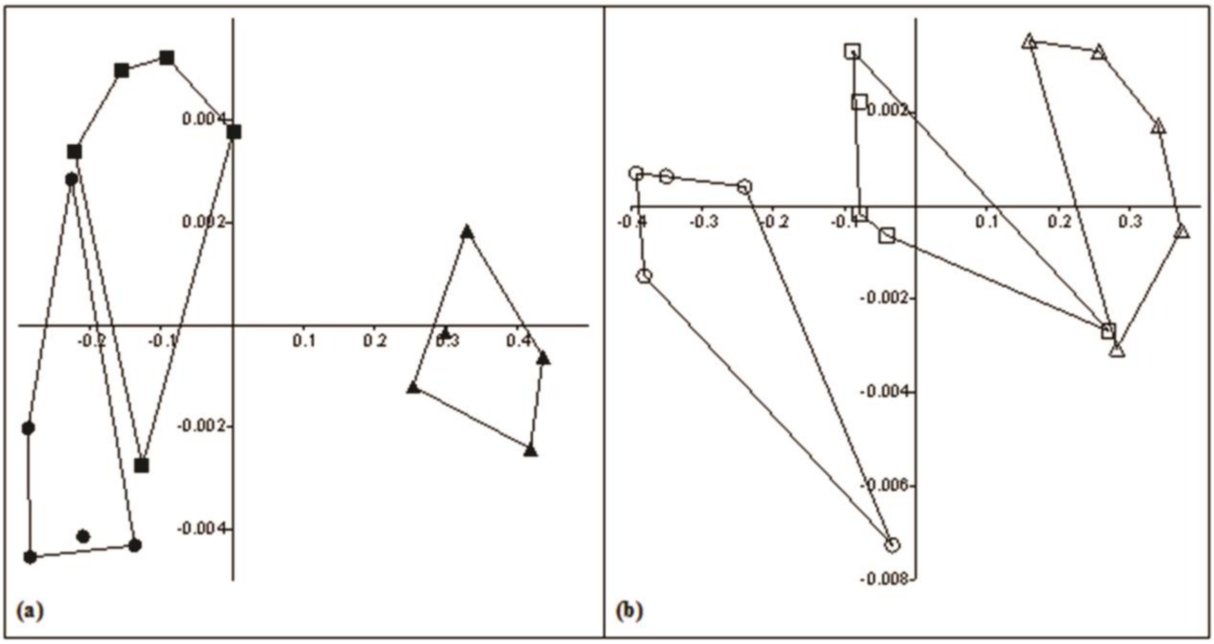

Figure 4. (a) Non-metric multidimensional scaling (NMDS) in three dimensions, from the simple Euclidean distance, in paired groups, of parts of adult individuals of *Pinus elliottii*, considering samples of tracheids obtained in “knots” at the base (circle), in the middle (square) and at the top (triangle) of the trunks. Variables considered: length (µm), total diameter (µm); lumen diameter (µm) and wall thickness (µm). Stress = 0.00226 (Abscissa axis = 1; Ordinate axis = 0). (b) Non-metric multidimensional scaling (NMDS) in two dimensions, from the simple Euclidean distance, in paired groups, of parts of adult individuals of *Pinus elliottii*, considering samples of tracheids obtained in “knot-free wood” at the base (hollow circle), in the middle (hollow square) and at the top (hollow triangle) of the trunks. Variables considered: length (µm), total diameter (µm); lumen diameter (µm) and wall thickness (µm). Stress = 0 (Abscissa axis = 1; Ordinate axis = 0).

Figura 4. (a) Escalonamento multidimensional não métrico (NMDS) em três dimensões, a partir da distância Euclidiana simples, em grupos pareados, de partes de indivíduos adultos de *Pinus elliottii*, considerando amostras das traqueídes obtidos em “nós” na base (círculo), no meio (quadrado) e no topo (triângulo) dos troncos. Variáveis consideradas: comprimento (µm), diâmetro total (µm); diâmetro do lume (µm) e espessura da parede (µm). Estresse = 0,00226 (Eixo das abscissas= 1; Eixo das ordenadas= 0). (b) Escalonamento multidimensional não métrico (NMDS) em duas dimensões, a partir da distância Euclidiana simples, em grupos pareados, de partes de indivíduos adultos de *Pinus elliottii*, considerando amostras das traqueídes obtidos na “madeira sem nós” na base (círculo vazado), no meio (quadrado vazado) e no topo (triângulo vazado) dos troncos. Variáveis consideradas: comprimento (µm), diâmetro total (µm); diâmetro do lume (µm) e espessura da parede (µm). Estresse = 0 (Eixo das abscissas = 1; Eixo das ordenadas = 0).

ANOSIM and NPMANOVA corroborated NMDS by numerically estimating similarities and differences between the “knots” of the three parts of the trunks established *a priori* (Table 1).

Table 1. Significance matrix (P) of the simple Euclidean distances between the three parts of the trunk of adult individuals of *Pinus elliottii*, considering samples of tracheids obtained in “knots” at the base, middle and top of the trunks. Variables considered: length (µm), total diameter (µm); lumen diameter (µm) and wall thickness (µm). 9,999 permutations.

Tabela 1. Matriz de significância (P) das distâncias Euclidianas simples entre as três partes do tronco de indivíduos adultos de *Pinus elliottii*, considerando amostras das traqueídes obtidos em "nós" na base, no meio e no topo dos troncos. Variáveis consideradas: comprimento (μm), diâmetro total (μm); diâmetro do lume (μm) e espessura da parede (μm). 9.999 permutações.

		ANOSIM			**NPMANOVA**	
	r=0.7253; P=0.0003			F=83.9; P=0.0001		
"Knots"		Middle	Top		Middle	Top
	Base	0.1069	0.0007	Base	0.0582	0.0083
	Middle		0.0080	Middle		0.0082
		ANOSIM			**NPMANOVA**	
	r=0.6569; P=0.0003			F=22.22; P=0.0003		
"Knot-free wood"		Middle	Top		Middle	Top
	Base	0.4666	0.0080	Base	0.0432	0.0069
	Middle		0.0249	Middle		0.0232

On the other hand, in the comparative multivariate analysis of "knots" and "knot-free wood", NMDS with the delimitation of polygons points to distinction between the "knot-free wood" and "knots" samples. This analysis also shows a clear gradient determined by the abscissa axis, from left to right, which distinguishes the groups formed by the samples obtained from the "knot-free wood" and those formed by the samples from the "knots" (Figure 5a). When the confidence ellipses of the data of each typology: "knot-free wood" and "knots" characterized *a priori* were delimited, two groups were clearly identified. Although there is some overlap between the subgroups related to the portions of the trunks from which the samples were extracted (base, middle and top), there is no probability of overlap (with a 95% confidence) between the "knot-free wood" and the "knots" (Figure 4b).

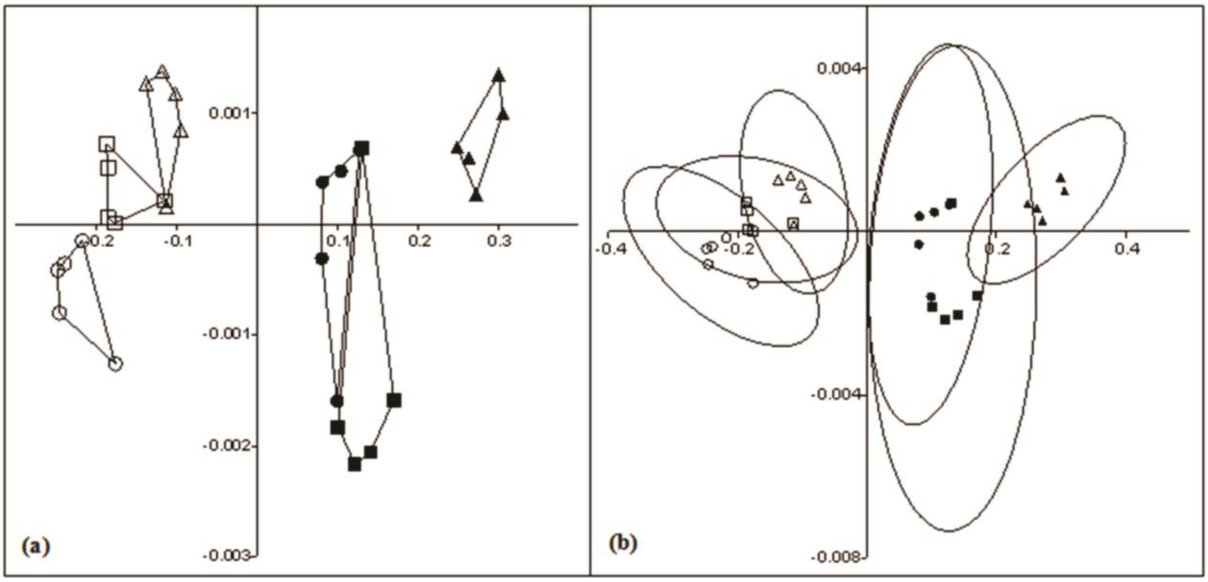

Figure 5. Non-metric multidimensional scaling (NMDS) in two dimensions from the simple Euclidean distance, in paired groups of parts of adult individuals of *Pinus elliottii*, considering samples of tracheids in the "knot-free wood", formed by the polygons on the left of the graph, representing the samples of the base (hollow circle), middle (hollow square) and top (hollow triangle) and to the right of the graph, representing the samples of tracheids in the "knots", formed by the polygons of the base (circle), middle (square) and top (triangle) samples. Stress = 0.000539 (Abscissa axis = 1; Ordinate axis = 0); (b) NMDS formed by the ellipses (95% confidence) on the left of the graph, representing the tracheids at the base (hollow circle), middle (hollow square) and top (hollow triangle) of the trunk, and on the right of the graph, representing the tracheids in the "knots", formed by the ellipses (95% confidence) of the samples at the base (circle), middle (square) and top (triangle) of the trunk. Stress = 0.000539 (Abscissa axis = 1; Ordinate axis = 0). Variables considered: length (μm), total diameter (μm); lumen diameter (μm) and wall thickness (μm).

Figura 5. Escalonamento multidimensional não métrico (NMDS) em duas dimensões a partir da distância euclidiana simples, em grupos pareados de partes de indivíduos adultos de *Pinus elliottii*, considerando amostras das traqueídes na "madeira sem nós", formadas pelos polígonos à esquerda do gráfico, representando as amostras da base (círculo vazado), meio (quadrado vazado) e topo (triângulo vazado) e à direita do gráfico, as amostras das traqueídes nos "nós",formadas pelos polígonos das amostras da base (círculo), meio (quadrado) e topo (triângulo). Estresse = 0,000539 (Eixo das abscissas = 1; Eixo das ordenadas = 0); (b) NMDS formadas pelas elipses (95% de confiabilidade) à esquerda do gráfico, representando as traqueídes da base do tronco (círculo vazado), meio (quadrado vazado) e topo (triângulo vazado); e à direita do gráfico, as traqueídes dos "nós", formadas pelas elipses (95% de confiabilidade) das amostras da base do tronco (círculo), meio (quadrado) e topo (triângulo). Estresse = 0,000539 (Eixo das abcissas = 1; Eixo das ordenadas = 0). Variáveis consideradas: comprimento (µm), diâmetro total (µm); diâmetro do lume (µm) e espessura da parede (µm).

Analysis of Similarity (ANOSIM) and Non-Parametric MANOVA (NPMANOVA or PERMANOVA) corroborated the previous analyses by numerically estimating the differences between the segments of "knot-free wood" and "knots", also considering the three portions of the trunks established *a priori*: base, middle and top (Table 2).

Table 2. Significance matrix (P) of simple Euclidean distances between the conditions of "knot-free wood" and "knots" (respectively the first letters of the acronyms, i.e., "W" and "K"), and the parts of the trunk (base, middle, and top, respectively the second letters of the acronyms, i.e., "B", "M", and "T"), in adult individuals of *Pinus elliottii*, considering tracheid samples. Variables considered: length (µm), total diameter (µm); lumen diameter (µm) and wall thickness (µm). 9,999 permutations.
Tabela 2. Matriz de significância (P) das distâncias Euclidianas simples entre as condições de "madeira sem nós" e "nós" (respectivamente as primeiras letras das siglas, ou seja, "M" e "N"); e as partes do tronco (base, meio e topo, respectivamente as segundas letras das siglas, ou seja, "B", "M" e "T"); em indivíduos adultos de *Pinus elliottii*, considerando amostras de traqueídes. Variáveis consideradas: comprimento (µm), diâmetro total (µm); diâmetro do lume (µm) e espessura da parede (µm). 9.999 permutações

	ANOSIM (r=0.8836; P=0.0001)				
	WM	WT	KB	KM	KT
WB	0.0461	0.0081	0.0056	0.0079	0.0082
WM		0.0228	0.0082	0.0082	0.0063
WT			0.0091	0.0077	0.0066
KB				0.1050	0.0082
KM					0.0086
	NPMANOVA (F=315.4; P=0.0001)				
	WM	WT	KB	KM	KT
WB	0.0386	0.0087	0.0079	0.0066	0.0057
WM		0.0248	0.0090	0.0084	0.0076
WT			0.0084	0.0082	0.0058
KB				0.0541	0.0071
KM					0.0070

DISCUSSION

The mean dimensions of the tracheids observed in an increasing manner along the positions of the trunk from base to top, both in the "knots" and in the "knot-free wood", corroborates the understanding expressed by authors such as Lazzarin *et al.* (2016), Müller *et al.* (2017) and Losso *et al.* (2018), that in gymnosperm species, especially those in the genus *Pinus*, these dimensions tend to increase in the axial direction, decreasing as they approach the crown, although with some variations of intermediate values in the middle region for the other positions.

When comparing the differences between the dimensions of "knotted" and "knot-free" wood along the trunk, Tong *et al.* (2013) suggested that the closer to the top of the tree, the greater the inclination angle of the knot cells and, consequently, the greater the dimensional differences of the anatomical elements compared to the elements of knots located in regions close to the base.

The results indicate that the pattern of longer and thicker structures from the base to the top of the trunk may not be repeated in the knots due to the different pressures exerted in evolutionary-anatomical terms.

The reduction in the lumen of tracheids of the knot, compared to the tracheids of knot-free wood, is a consequence of the increase in cellulose and lignin deposition in the cell wall, according to Aloni (2013), Vidaurre *et al.* (2013) and Deus *et al.* (2024b). This increase in deposition is a response to the need for flexion of knot cells to avoid ruptures caused by the load of the branch (JUNGNIKL *et al.*, 2009).

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The patterns found for TL, TD, LD of the tracheids in the knot-free wood portions indicate a gradient of decrease in their physical structure, considering the profile from the base to the top of the tree. This pattern is not expressed with the same clarity for the tracheids existing in the different portions of the trunk, considering the knots. The difference in growth patterns between the knot and wood tracheids can be explained by the phytohormonal factor, which interferes with plant development. The decreasing polar flow of auxin, from the apex (stem or branch), induces the differentiation of tracheary elements, promoting more cell divisions (EVERT, 2013). This gradient is also responsible for the decrease in the density of the elements, from the leaves to the root (ALONI, 2013). For being closer to the physiologically more active zones of IAA production (young leaves), the branch/trunk intercession points (knots) are more susceptible to the action of this hormone.

Souza *et al.* (2005), when analyzing anatomical parameters of the axial tracheids of wood from four provenances of *Pinus*, found that the increment in tracheid length can have an increasing linear value from the base towards the top.

In summary, considering the anatomical variables associated with the tracheids of the "knots", the multivariate analyses reaffirm some of the parameters analyzed separately in the univariate analyses, demonstrating that the base and middle of the trunk of adult individuals of *Pinus elliottii* are similar to each other, but differ from the top. In relation to the "knot-free wood", the analyses demonstrate that the base, middle and top of the trunk of adult individuals of *Pinus elliottii* have tracheids with distinct characteristics from each other.

The differences in values observed in the tracheids of the knot along the trunk may be both a response to the resistance required due to the load generated by the branch, as observed by Spatz and Theckes (2013) and Slater *et al.* (2014), and a response to greater or lesser distance from the hormone-producing zones (ALONI, 2013), or a function of different concentrations between higher lignin and lower cellulose in these regions of compression wood (VIDAURRE *et al.*, 2013).

CONCLUSION

- The results indicate that the pattern of growth and increment of the longer and thicker structural tracheids from the base to the top of the trunk is not repeated in the knots.
- The dimensions of tracheids in the knot are different from those of the surrounding wood, without knots.
- There is a gradient along the trunk in the developmental parameters of the tracheids, which decrease towards the apex of the plant.
- Tracheids of knots show a smaller increment in size and do not seem to have growth similar to that observed in knot-free wood.

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