

Spatial variation of eucalyptus fibres considering the structure of annual growth rings

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TECHNOLOGY OF FOREST PRODUCTS

ABSTRACT

Background: The fibres dimensions vary along a tree trunk, and these variations are related to the apical meristem and vascular cambium activities; understanding these variations would guide the best use of wood. There have been studies on the pattern of fibres dimensions' variation along the tree trunk in the radial and longitudinal directions; however, the vast majority do not take into account the structure of the annual growth rings. Hence, this study aimed to obtain the pattern of fibres dimensions' variation and their quality indexes along the tree trunk take into account the structure of the annual growth rings. Two *Eucalyptus urophylla* x *Eucalyptus grandis* hybrid clones were used in the study. Ten-year-old trees were selected and discs were removed from 13 longitudinal positions. The disks were prepared and the growth rings were subsequently identified and the samples used for maceration were collected. Twenty fibres were measured in the annual growth rings present in each longitudinal position.

Results: In the longitudinal direction, within the annual growth rings, the fibre dimensions and their quality indices did not show a well-defined pattern of variation. In the radial direction, the length, cell wall thickness, wall fraction, and Runkel ratio of the fibres tended to increase following the pith-bark direction, while the lumen diameter and flexibility coefficient decreased.

Conclusion: Nevertheless, the fibre width and fibre slenderness index did not present a well-defined pattern of variation in the radial direction.

Keywords: Anatomy of wood, Biometry of fibres, Clone, Fibre characteristics, Quality index.

HIGHLIGHTS

Variation maps allow a better understanding of the spatial distribution of fiber characteristics.

The mapping showed that there was no longitudinally defined variation pattern for fiber biometry.

Among the annual growth rings, the fibers showed a pattern of variation with a tendency to increase in the pith-bark direction.

The interpolation method facilitates the monitoring of variations that occur radially and longitudinally in the tree.

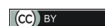
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INTRODUCTION

Approximately 210.3 million m³ of planted wood in Brazil, of which 73.3% (154.2 million m³) were eucalyptus, were destined for industrial use in 2019. Pulp and paper, wood panel, laminate flooring and charcoal industries are the main industrial sectors supplied by these planted forests. In fact, Brazil is one of the world's leading producers of pulp, paper and wood panel (Ibá, 2020). Nevertheless, to meet the industrial demand, a better understanding of wood characteristics is still necessary, as it allows for a better allocation of this raw material for the different applications.

Among the various wood characteristics, the anatomical indices are indicative of quality. These indices show the way in which the fibres intertwining occurs in the papermaking process, which influences the properties of the final product. As such, they are important indicators of wood suitability for the pulp and paper industry (Nisgoski *et al.*, 2012).

Variations in wood characteristics are related to environmental and genetic factors and genotype x environment interactions. These variations can be observed between different species, or between individuals of the same species and even within a single tree. In the tree trunk, wood characteristics can vary in the radial and longitudinal directions, between early wood and late wood, juvenile wood and adult wood, and heartwood and sapwood (Trugilho *et al.*, 1996). Knowing the variations pattern within the tree can help to better understand the wood behaviour during the production process, as well as its influence on the quality of the final product. Furthermore, it allows to determine the desired characteristic in a simpler, less expensive sampling, which will require less sample transport costs and less work at the laboratory level.

Although the biometry of the anatomical elements of the species has been widely studied, there have been few studies that investigate the radial and longitudinal variations in the cell dimensions while taking into account the structure of the annual tree growth rings, which is important during wood formation.

The annual growth rings are made up of early and late woods and indicate the growth of wood over the years in a tree. The initial wood, composed of fibres with thinner walls, is formed when the environmental conditions are favourable. Under unfavourable conditions, late wood is formed, consisting of fibres with thicker walls. Thus, environmental events such as rains, droughts, low temperature and even attacks by pathogens influence the formation and dimensions of the anatomical elements that compose the rings (Raven *et al.*, 2014). Among hardwood elements, fibres have a structural function, and their morphology influences the properties of the wood (Slater & Ennos, 2013). Hence, in the growth rings, present both radially and longitudinally in the

tree, wood anatomical variations and consequently the variations in its properties are recorded.

Studies that consider the structure of annual growth rings to evaluate the spatial variation of fibre dimensions within the tree trunk in the longitudinal and radial directions are necessary. This allows a better understanding of the pattern of these variations and consequently the application of the wood for the most appropriate purpose. Therefore, this study aimed to determine the pattern of dimensional variation in fibres and their quality indices along the tree trunk considering the structure of the annual growth rings.

MATERIAL AND METHODS

Climatological characteristics of the collection site and biological material

We conducted an experiment using trees collected in a clonal plantation located in the municipality of Luminárias-MG, in the southern region of Minas Gerais. The municipality has an altitude ranging from 1144 metres at the highest point to 943 metres in the central region. The average annual temperature is 19.6°C and the monthly averages range from 21.8°C in the summer (January) and 16.0°C in the winter (June). The average annual rainfall is 1517 mm, and according to the Köppen-Geiger climate classification, Luminárias has a humid subtropical climate (Cwb), with humid summers and dry winters (Carvalho *et al.*, 2007; Rodrigues *et al.*, 2007).

Two *Eucalyptus urophylla* x *Eucalyptus grandis* hybrid clones, namely clone A and clone B, were analysed. The trees were 10 years old (2007 – 2017) and the initial plant spacing was 3m x 2m.

Sampling

For each clone, two trees were randomly selected in the central region of the plot, approximately 10 meters away from each other, avoiding the trees positioned on the edge. The selected trees had a straight trunk, without branches, knots, bifurcation and with good phytosanitary status.

Three-cm-thick discs were removed along the tree trunk from the following longitudinal positions (base-top direction): 0.30 m, 1.30 m (diameter at breast height (DBH)) and from the DBH, every two meters up to a height of 23.30 m, as shown in figure 1. Thus, a total of 13 disks were collected from each tree.

Radial sampling (pith to bark) was performed considering each ring present in each of the 13 removed discs. The disks closer to the base had 10 annual growth rings, while in the disks closer to the top some of the rings were not present, due to the natural formation of the wood, thus a total of 119 samples per tree was used for the analysis (figure 1).

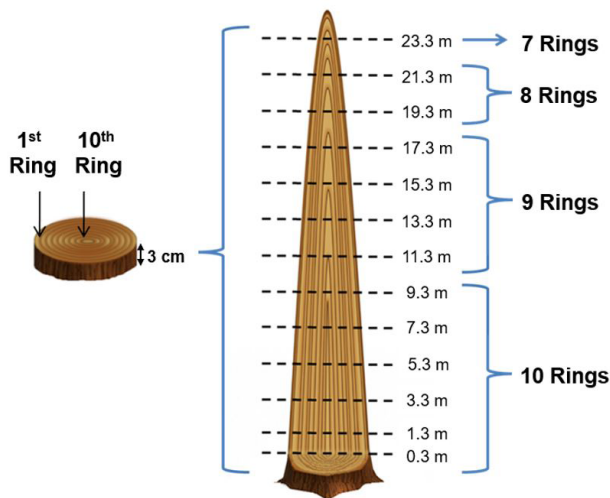


Figure 1. Sampling scheme of the trunk.

Sample preparation

The collected discs were peeled, frozen and kept in a conventional freezer for two months at the Biomaterials and Biomass Energy Multiuser Laboratory of Federal University of Lavras (Universidade Federal de Lavras-UFLA). This procedure was performed to improve the visualization of the annual growth rings, as it helps to maintain the physical and sanitary integrity of the material. To identify the annual growth rings, the disks were thawed in the open air, flattened and then sanded with 120, 220 and 320-grit sandpaper until obtaining a smooth surface that facilitated the visualization of the rings, as recommended by the standard method used in the Laboratory of Forest Ecology of the Federal University of Lavras (Universidade Federal de Lavras-UFLA). Identification of ring boundaries was performed using a stereomicroscopic magnifying glass and Sketchup Software was used to verify their contours. The rings were numbered following the outside to inside (bark-pith) direction, as recommended by Downes *et al.* (1997).

After identifying and numbering the growth rings, a radial sample was removed, passing through the pith, to facilitate the rings removal. The growth rings were separated in the radial sample with the aid of a surgical scalpel and the samples of each growth ring were submitted to the process of individualization of the anatomical elements.

Fibre measurements

The preparation of the macerate for measurements of the anatomical elements was carried out according to the method proposed by Franklin (1945) and adapted by Nicholls and Dadswell (1962), using a solution of glacial acetic acid and 30% hydrogen peroxide (1:1). The macerate was left in the oven at 60°C for three days. After this process, the samples were washed with deionized water and stained with safranin in 1% aqueous solution.

The fibres dimensions were measured with a Nikon E200 light microscope, made by Nikon instruments (Shanghai Co. China) equipped with a camera and with the aid of the software Dinocapture 2.0, according to the List of microscopic features for hardwood identification of the International Association of Wood Anatomists (IAWA, 1989). In the annual growth rings present in each longitudinal position, the lumen diameter, length, and width of twenty fibres were measured, totalling 2380 fibres measured per tree. Therefore, a total of 4760 fibres per clone were analysed.

The cell wall thickness (CWT) of the fibre, fibre slenderness index (FS), flexibility coefficient (FC), wall fraction (WF), and the Runkel ratio (RR) were obtained through the relationships between the fibres dimensions calculated according to the equations in Table 1.

Table 1. Equations for obtaining the cell wall thickness and fibre quality indices.

Parameter	Equation
Cell wall thickness (CWT)	$CWT = (FW - LW) / 2$
Fibre slenderness index (FS)	$FS = (FL / FW) \times 1000$
Flexibility coefficient (FC)	$FC = (LW / FW) \times 100$
Wall fraction (WF)	$WF = ((2 \times CWT) / FW) \times 100$
Runkel ratio (RR)	$RR = (2 \times CWT) / LW$

Legend: FW = fibre width (µm), LW = lumen diameter (µm), FL = fibre length (mm).

Mapping the fibre variation

After the fibres biometry, variation maps of fibres dimensions and their quality indices in the longitudinal and radial directions of the trunk were made using the SURFER 20 Software. Maps were prepared based on the interpolation of the biometric data and the fibre quality indices with the spatial position in the trunk using the interpolation minimum curvature method.

RESULTS

Variations Map of the fibre characteristics

Clone A had a mean fibre length (1.08 mm) greater than that of clone B (1.03 mm). However, in both clones, the fibres removed from a height of 9.3 m in the rings closest to the pith showed a length below 1 mm, but with a tendency to increase in the pith-bark direction. In clone B, the 10th ring was the fibres were less than 1 mm in all longitudinal positions where it was present, while in rings 1 to 9, the fibres were longer than 1 mm. Moreover, in both clones, in the largest portion of the trunk, the fibres length variations were not very significant (figure 2).

In clone A, all annual growth rings, except the 9th ring, present in the longitudinal positions from 7.3 m to 17.3 m, presented fibers with a width greater than 22 μm . At heights from 0.3 m to 9.3 m, the 7th, 8th, 9th and 10th annual growth ring showed fibers with a width greater than 22 μm , except some isolated points that did not follow any variation pattern. In clone B, in the 9th and 10th annual growth rings, the fibres had a width above 22 μm ,

in addition to some isolated regions, such as the region between the 2nd and 7th annual growth rings, at heights of 1.3 m to approximately 4.3 m. The width did not show a well-defined variation pattern in the longitudinal and radial directions in both clones, and in general, clone A had fibres with higher width (21.63 μm) than those of clone B (20.99 μm) (figure 3).

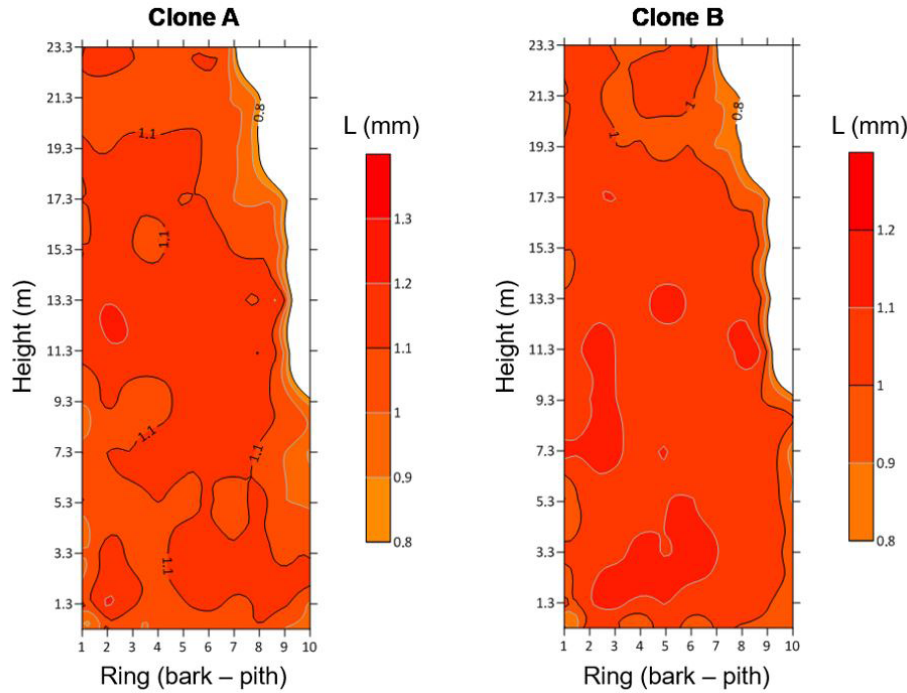


Figure 2. Variation maps of the mean fibres length (L).

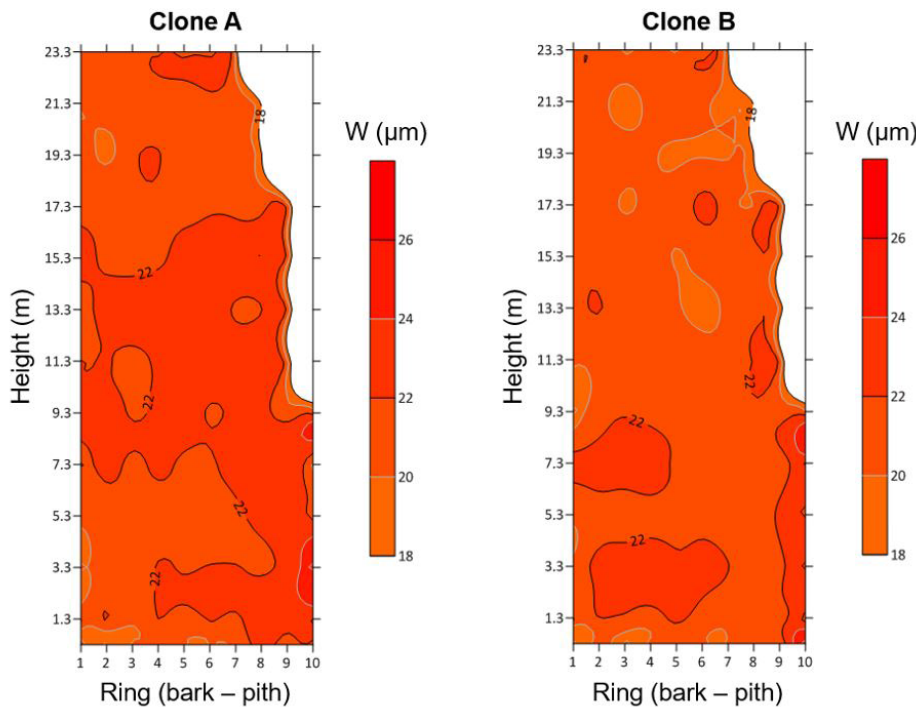


Figure 3. Variation maps of the mean fibres width (W).

The fibres with the largest lumen diameter were present in the rings located closer to the pith (9th and 10th rings) up to a height of 9.3 m. In these rings, the lumen diameter had a tendency to reduce following the pith-bark direction in both clones. In general, the fibres had a larger lumen diameter in the region closer to the pith, regardless of the height. Although the two clones showed a similar pattern of variation, clone A had fibres with a mean lumen diameter (11.75 μm) larger than those of clone B (9.96 μm) (figure 4).

The fibres cell wall thickness did not show a significant variation in the longitudinal direction within the annual growth rings, but radially, there was a tendency to increase in the pith-bark direction. In clone B, the 1st, 2nd,

3rd, 4th, 5th, and 6th annual growth rings, presented fibres with thicker cell walls (CWT > 5.5 μm) in all longitudinal positions of the trunk. In clone A, the fibres in the 6th to the 10th annual growth ring showed wall thickness below 5 μm in almost all longitudinal positions, except for some small regions. However, in the 1st and 2nd rings, the cell wall thicknesses were less than 5 μm only at a height of 0.3 m. In the 3rd and 4th annual growth rings, at heights of 0.3 m and from 15.3 m to 23.3 m, the fibres presented walls with thicknesses below 5 μm . Clone B showed fibres with thicker walls (5.51 μm) than those of clone A (4.97 μm), and both showed a pattern of increased cell wall thickness radially in the pith-bark direction (figure 5).

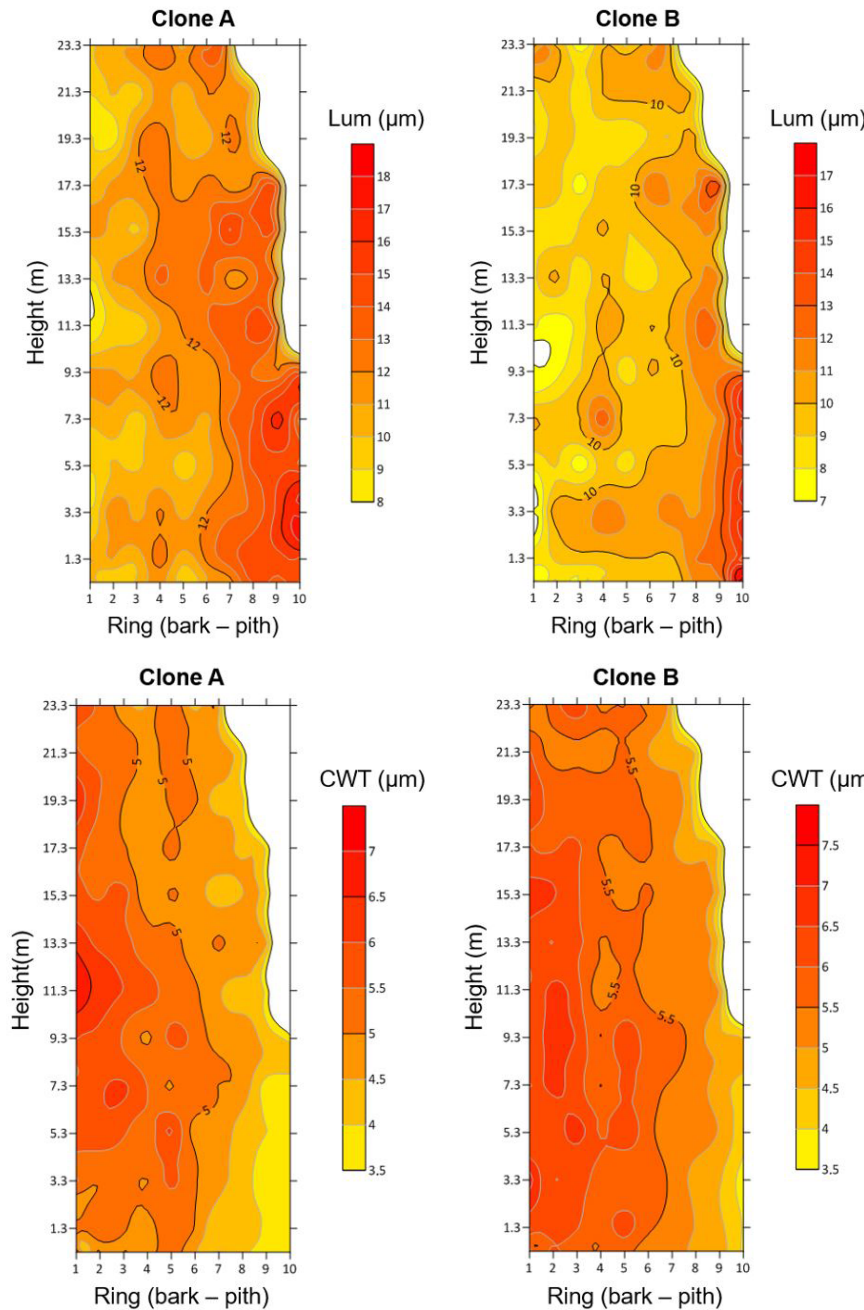


Figure 4. Variation maps of the mean fibres lumen diameter (Lum).

Figure 5. Variation maps of the mean fibres cell wall thickness (CWT).

The mean fibre slenderness (FS) of clones A and B were 49.88% and 49.03%, respectively. In the two clones, the fibres with the highest FS values (above 48%) were concentrated in the region between the 2nd and 7th annual growth rings at all sampled heights, except for some small discontinuities that appeared in the wood. Lower values occurred in the annual growth rings closer to the pith in the most basal positions and in the upper portions, especially in clone B. The 1st and 2nd rings in clone B also showed fibres with lower FS values in the most basal positions of the tree. In general, the fibres FS did not show a well-defined pattern of variation in the longitudinal or radial directions in both clones (figure 6).

The fibres with the highest flexibility coefficient (FC) were in the rings closest to the pith (9th and 10th ring) up to a height of 9.3 m in both clones. In these rings, there was a reduction in the FC in the pith-bark direction. In clone A, the fibres FC was less than 55% from the 1st to the 6th ring at all heights, except for some discontinuities existing in the trunk, while in clone B, the 1st, 2nd, 3rd, 4th, 5th, and 6th annual growth rings showed fibres with FC below 48%. Longitudinally, it was not possible to observe a significant variation pattern in the fibres FC in clones A and B, but radially, both clones showed a variation pattern with a trend of reduction in the pith-bark direction. Clone B generally presented fibres with a lower mean FC (47.39%) than clone A (54.19%) (figure 7).

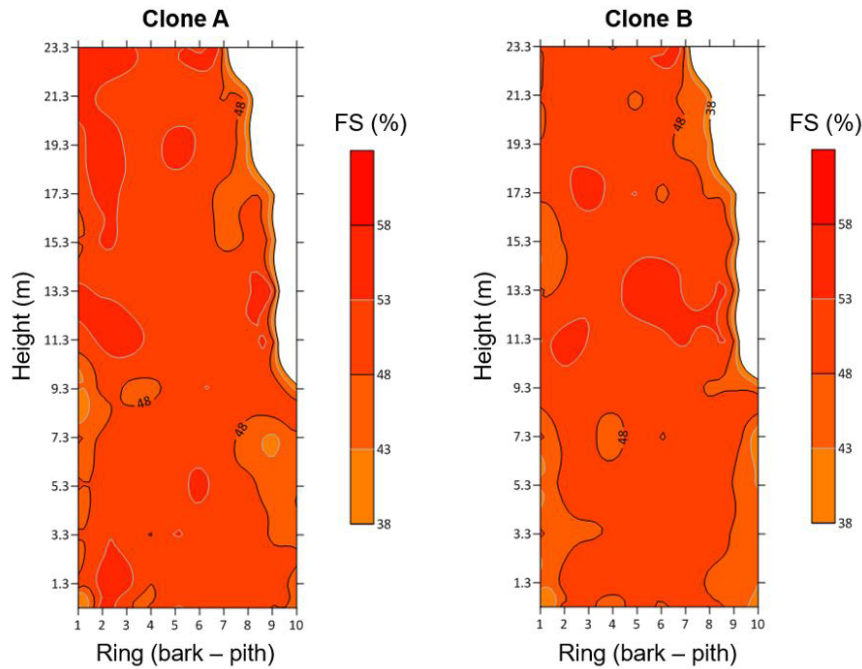


Figure 6. Variation maps of the mean fibre slenderness index (FS).

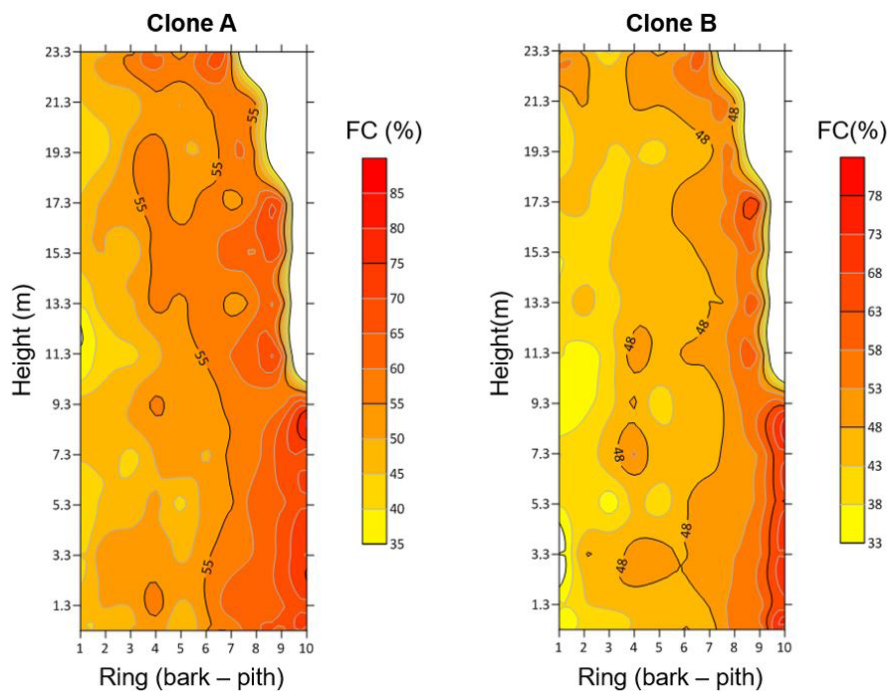


Figure 7. Variation maps of the mean fibres flexibility coefficient (FC).

The fibres wall fraction (WF) did not show a pattern of variation in the longitudinal direction within the rings, but radially, there was an increasing trend of the fibre wall fraction and pith-bark direction in the two clones. In clone B, from the 1st to the 3rd annual growth rings, the highest WF values (above 54%) were observed in all longitudinal positions of the trunk, while in the 4th, 5th, and 6th growth rings, there was discontinuity in some longitudinal positions, in which certain longitudinal positions showed fibres with WF less than 54%. In clone A, the annual growth rings closer to the bark (1st, 2nd, and 3rd) have fibres with higher WF values (above 46%) in all longitudinal positions of the trunk, whereas in the 4th, 5th, and 6th annual rings, this growth occurred with some regions presenting discontinuity, especially at heights of 13.3 m to 21.3 m. Clone B showed a mean fibre WF higher (52.61%) than clone A (45.81%) (figure 8).

In clone A, the 1st, 2nd, and 3rd annual growth rings had the highest Runkel ratio (RR) values at heights of approximately 11.3 m and 19.3 m. In clone B, the 1st, 2nd, and 3rd rings the same occurred, but at heights of 3.3 m, 5.3 m, 9.3 m, 11.3 m, and 23.3 m. In the 4th annual growth ring, the fibres showed low RR values (<0.9) up to a height of 3.3 m and started at 13.3 m to the top in clone A. In the 5th ring, the same occurred only between 11.3 m and 17.3 m. In clone B, the 5th and 6th annual growth rings, presented fibres RR values higher than 1.2 in the longitudinal positions 5.3 m to 9.3 m, 13.3 m and from 17.3 m to 19.3 m. In the 4th ring, the RR was greater than 1.2 only at the 5.3 m and 19.3 m.

In both clones, it was not possible to observe a pattern of longitudinal variation in the fibres RR, but it was possible to verify that there was radial variation, with a tendency to increase in the pith-bark direction. Clone A showed fibres with the lowest mean RR value (0.88 clone A and 1.16 clone B). Lower RR values were more frequent in the annual growth rings closer to the pith, in clone A from the 6th to the 10th ring (RR <0.9) and in clone B from the 8th

to the 10th ring (RR <1.2) at all longitudinal positions of the tree trunk (figure 9). The fibres RR showed a variation very similar to that observed in the fibre WF.

DISCUSSION

Fibre dimensions

The overall mean fibre length of clone A was 5% greater than that of clone B. At the DBH (1.30 m), which is the most commonly used for sampling, the fibres with lengths closer to the mean were in the 5th and 7th annual rings of growth in clone A, while in clone B, the value of the overall mean of the clone was found in the 8th ring. In both clones, there was a tendency for the fibre length to increase in the pith-bark direction, especially in the 1st and last rings in each longitudinal position, but this trend wasn't very obvious in the intermediate rings due to the low magnitude of the length values, which is in agreement with the results found by Tomazello Filho (1987), who evaluated the fibres length by radial portions of the trunk but did not consider the annual growth rings. Liu *et al.* (2020) considered the growth rings when analysing the anatomy of the hardwood fibres and observed fibre growth radially in the pith-bark direction, with less variation in the fibre growth in the rings closer to the bark. The authors attribute this trend of stabilization of the characteristic in the rings closer to the bark with the formation of adult wood.

Clone A had a mean fibre width 3% higher than that of clone B, and the mean value of the fibre width in the DBH was found in the 10th ring in clone A and in the 6th growth ring in clone B. However, it was not possible to observe a defined pattern in the radial and longitudinal variation in the fibre width in both clones, which is in line with the results found in studies on 4 years old (Govina *et*

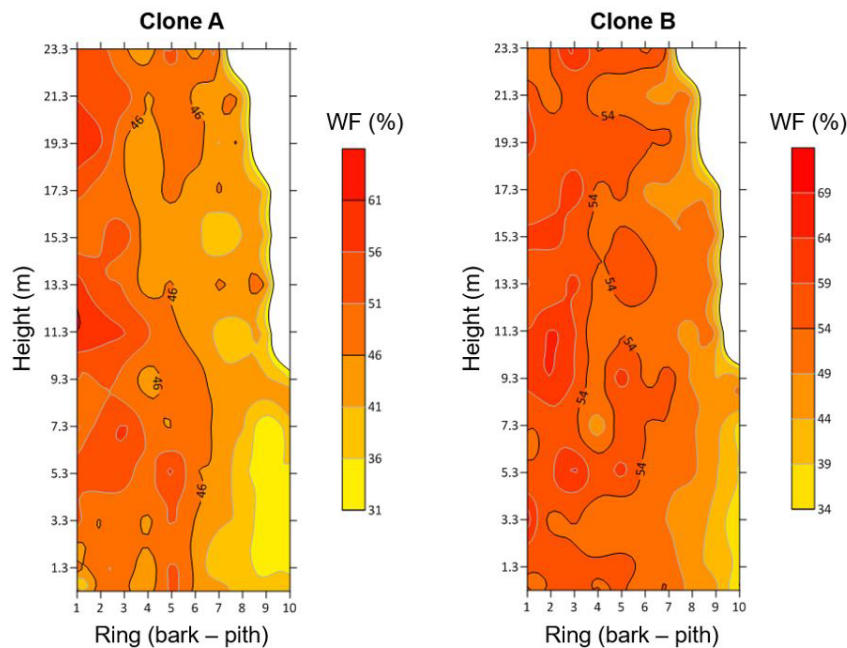


Figure 8. Variation maps of the mean fibre wall fraction (WF).

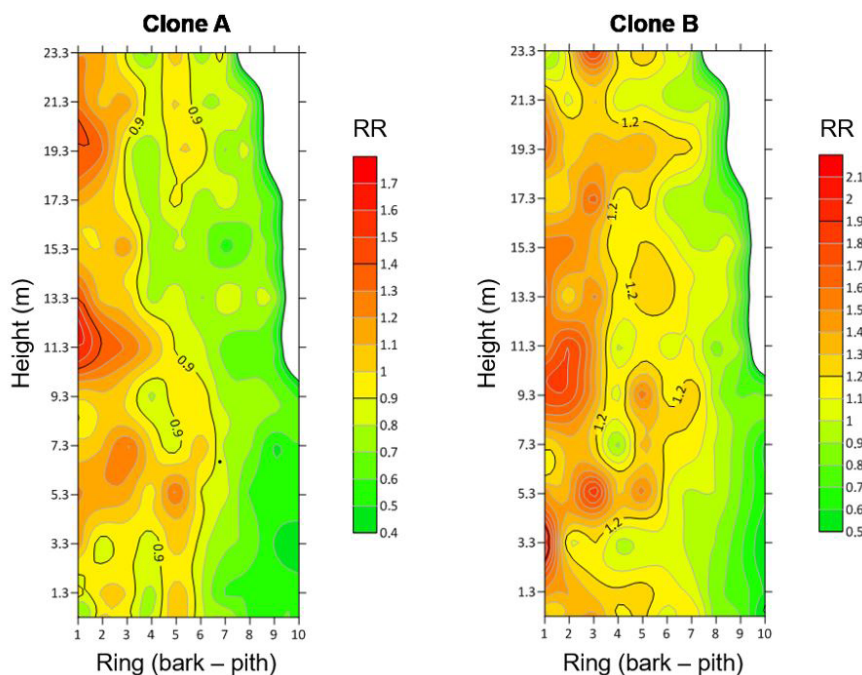


Figure 9. Variation maps of the mean fibres Runkel ratio (RR).

al. 2021) and 8 years old (Gonçalez et al., 2014) *E. urophylla* x *E. grandis* hybrids. The fibres did not show a pattern of longitudinal and radial variation with regards to the width when evaluated by portions in the trunk of the trees. The longitudinal and radial growth of the tree did not exert much influence on the width of the fibres; however, the radial growth had an influence in the lumen diameter, reducing it in the pith-bark direction, and the opposite was observed in the thickness of the fibre cell wall.

Clone A had a mean fibres lumen diameter 15% greater than that of clone B. In clone A, the mean lumen diameter value closest to the overall mean of the clone was observed in the 1st ring, and in clone B, the 5th and 6th rings presented values closer to the overall mean of the clone. A trend of reduction in the fibres lumen diameter was observed in the pith-bark direction in all longitudinal positions in both clones. These results corroborate the study by Ramos et al. (2011), who observed a reduction in the fibres lumen diameter in the pith-bark direction when studying the radial variation in the fibre characteristics of *E. grandis*.

The cell wall thickness of clone B was 10% greater than that of clone A. The values closest to the overall mean cell wall thickness could be observed in the 6th annual growth ring in both clones considering radial sampling in the DBH. In both clones, there was a tendency for the thickness of the fibre cell walls to increase in the pith-bark direction along the entire tree trunk. These results are considered to be the common variation pattern for fibre cell wall thickening according to Firmino et al. (2019). However, according to Baldin et al. (2017), it is not possible to define a general trend of radial fibre wall thickness variation since although there is an increase between heartwood and peripheral heartwood, there is a reduction in the thickness of the cell wall in the sapwood. However, the radial sampling performed by these authors

was from three portions of the disc (heartwood, peripheral heartwood, and sapwood) and only in a longitudinal position, i.e., in the DBH of the trees, differing greatly from the sampling strategy followed in this study.

Fibre quality Indices

In clone A, the mean FS was only 2% higher than that in clone B. Both clones did not show a well-defined variation pattern in the FS in the radial and longitudinal directions. Only the fibres in the annual growth rings closer to the pith, in each longitudinal position, presented lower FS values, which indicates that the clones can be used as raw materials for the cellulose industry. Fibres with FS above 50% are recommended for the production of paper with greater resistance to tearing and breaking (BALDI, 2001). In the DBH, values closer to the overall mean can be observed in the 7th annual growth ring in clone A and, in clone B, in the 5th ring.

Clone A showed a mean fibres flexibility coefficient (FC) 12% higher than that of clone B, and a tendency for the fibres FC to decrease in the pith-bark direction was observed at all heights in both clones. Fibres with higher FC values allow the production of denser paper with better strength, while low FC values indicate that the fibres have high rigidity, thus producing more voluminous and low-strength papers (Menegazzo, 2012). Considering the sampling in the DBH, the values closest to the overall mean FC of the fibres were observed in the 6th annual growth rings in both clones.

The inverse of the FC was observed for the fibres WF, i.e., a trend of the fibres WF increasing in the pith-bark direction was observed in both clones in all longitudinal positions. This is expected because with an increasing thickness of the fibre cell walls, the wall fraction increases and consequently the fibres flexibility decreases. Clone A presented fibres with a mean WF 15% lower than that in clone B. At the DBH position, in clones A and B, the values

closest to the overall mean fibres WF were found in the 6th annual growth ring. According to Talgatti *et al.* (2020), fibres with WF greater than 40% are not very flexible, i.e., are more rigid and consequently have difficulties in interconnections, which reduces the quality of the paper. However, Foelkel (1978) reports that fibres with up to 60% WF can also be used in paper production.

Clone B presented fibres with a mean RR 24% higher than those of clone A. In clone A and clone B in the 6th annual growth ring, the RR values were closer to the overall mean of each clone. The fibres RR showed an increasing trend in the pith-bark direction at all heights of the trunk in both clones. The same was observed for WF and the inverse for FC, which is in agreement with the results found by Vivian *et al.* (2021) who correlated the anatomical characteristics of hardwoods and observed that the increase in wall thickness increases its proportion in the fiber (higher WF), reduces its flexibility (lower FC) and consequently increases the value of the Runkel index. According to Nisgoski (2005), RR > 1 indicates that the fibres are more rigid and have low interconnection capacity, which is not indicated for papermaking.

CONCLUSIONS

The variation maps obtained by annual growth rings allow a broad visualization of the variations within and along the tree trunk for each characteristic, enabling a better understanding of its spatial distribution. The maps showed that in the longitudinal direction, there was no well-defined variation pattern for the studied characteristics. Radially, there was a tendency, in the pith-bark direction, for the length, cell wall thickness, wall fraction and Runkel ratio to increase, while the lumen diameter and flexibility coefficient showed the opposite effect. The fibre width and the fibre slenderness index did not show a well-defined longitudinal or radial variation in the tree trunk. For both clones, the 6th growth ring, at the DBH, best represented the overall mean of most of the tree characteristics.

The results of the research suggest that studies must be carried out through annual monitoring of growth, evaluation of environmental conditions, silvicultural interventions, and wood characteristics to better understand the dimensional variations of the fibres that occur within the tree trunk.

AUTHORSHIP CONTRIBUTION

Project Idea: PFT

Funding: PFT

Database: FMM

Processing: FMM

Analysis: PFT, FMM, JM

Writing: FMM, PFT

Review: PFT, JM, FMM, ACOC, FAP, PRGH

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