



# Growth differential related to wood structure and function of *Eucalyptus* spp. clones adapted to seasonal drought stress

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## Abstract

**Aim of study:** To evaluate the growth performance, wood density and anatomical features of four drought-tolerant *Eucalyptus* spp. clones, at 4 years, and to examine the relationships between these characteristics and some functional parameters.

**Area of study:** The analyzed trees were from a clonal test installed in a region characterized by seasonal drought stress in central-western Brazil.

**Materials and methods:** Trees were felled, followed by obtaining dendrometric parameters and wood disk sampling to determine wood bulk density by x-ray densitometry, and morphometric parameters of fibers and vessels in order to evaluate the xylem hydraulic architecture. Lumen fraction (F), vessel composition (S) and hydraulic conductivity (Ks) were estimated.

**Main results:** Clone D (*E. urophylla* x *E. grandis*) presented the highest growth rates, which was related to anatomical characteristics such as low relative frequency of wide vessels. High theoretical Ks does not necessarily imply higher growth rates and were related to lower wood densities. It is possible to infer that the better xylem adjustability of *Eucalyptus* trees in response to drought stress conditions is associated with increased vessel composition to the detriment of higher hydraulic conductivity.

**Research highlights:** Vessel composition showed a greater variation among *Eucalyptus* genotypes and was positively associated with growth performance.

**Keywords:** wood anatomy; dendrometry; X-ray densitometry; water stress; hydraulic conductivity.

**Abbreviations used:** F: lumen fraction; S: vessel composition; Ks: theoretical xylem-specific hydraulic conductivity.

**Authors' contributions:** Conceived and designed the experiments: DRSS, MPC. Performed the experiments: DRSS, RFS, JLRA, ASS. Analyzed the data: DRSS, CMS. Wrote the paper: DRSS. Obtained funding: EN, MPC. Contributed reagents/materials/analysis tools: EN, CRS, MT. Contributed to the critical revision of the manuscript: CMS, EN, CRS, MT, MPC. Supervised the work: MPC.

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## Introduction

Water deficit is one of the most limiting growth factors in *Eucalyptus* plantations (Martins *et al.*, 2018). Drought stress conditions promote stomata closure to avoid water losses, and although this mechanism prevents desiccation and xylem cavitation, it also restricts CO<sub>2</sub> supply for photosynthesis, leading to a decrease in the tree productivity (Chaves *et al.*, 2009; Sperry & Love, 2015). For instance, Almeida *et al.* (2010) stated that drought may reduce the stand volume production by as much as one third over a six-seven year rotation for *Eucalyptus* trees grown near

the Atlantic Coast of Brazil. In the Brazilian scenario, the extension of *Eucalyptus* cultivation to new regions with drier climate conditions has therefore demanded superior drought-tolerant genotypes to compose commercial plantations (Pita-Barbosa *et al.*, 2016). In a wider perspective, this demand is also related to the growing concern with respect to climate change and the risk for more intense and frequent droughts all over the world (Lagergren & Jönsson, 2017) which is expected to affect forestry even in regions which are currently productive forests.

There is a coordination between stem and leaf features in the direction of high hydraulic efficiency (Tyree &

Zimmermann, 2002), and ultimately toward improving tree productivity (Brodrigg & Feild, 2000; Poorter *et al.*, 2010). Thus, the cellular structure of xylem in tree stems has important implications in acclimatization and adaptation mechanisms to drought (Crous *et al.*, 2018; Lundqvist *et al.*, 2017; Pfautsch *et al.*, 2016; Saadaoui *et al.*, 2017). This means that trees are under insufficient water supply seek to develop survival strategies which include very large modifications in their stem hydraulic system. These structural adaptations can reflect both coordinated evolutionary changes and adaptive functional responses in a given environment (Bourne *et al.*, 2017; Villar-Salvador *et al.*, 1997).

In angiosperm trees, the water transport process efficiency is directly linked to the vessel element features and indirectly to the set of adjacent cells (Barotto *et al.*, 2017). Xylem hydraulic conductivity is a measure of the vascular system morphology (Tyree & Ewers, 1991), and therefore it is a function of the vessel size and frequency. According to Zanne *et al.* (2010), trees can alter wood properties in different ways in order to adjust water provision rates through the stem. These changes may be more associated with the total sapwood area supplying a given leaf unit and the sapwood fraction occupied by vessel lumens or vessel composition with distinction to few wide or many narrow vessels. Although a positive correlation between vessel size and growth rate is expected (Brodrigg *et al.*, 2002; Meinzer *et al.*, 2010), wider vessels can be associated to higher vulnerability to cavitation. In this sense, the manner in which the vessel network is formed in order to maximize the water transport efficiency and minimize risks of loss of conductivity determines the “fitness” of tree species (Pfautsch *et al.*, 2016).

Furthermore, a close relationship between density, hydraulic safety and vessel anatomy (Barotto *et al.*, 2017; Hacke *et al.*, 2001) has been observed for most angiosperm trees. A denser wood usually has greater capacity to support tension in vessel elements (Hacke *et al.*, 2001), and this condition is mostly correlated with a high relative frequency of narrow vessels (Poorter *et al.*, 2010; Sperry *et al.*, 2008). Non-conductive elements (fiber matrix) might have an indirect effect on water transport by reinforcing vessel resistance under high tension and greater hydraulic security is generally associated with increased transverse fiber wall area and decreased fiber lumen area (Jacobsen *et al.*, 2005).

Species such as *Eucalyptus grandis*, *Eucalyptus urophylla*, *Eucalyptus camaldulensis*, *Eucalyptus pellita* and *Eucalyptus tereticornis* have been utilized for intra or interspecific hybrids in searching for genetic materials with combined drought tolerance and wood quality traits (Bassa *et al.*, 2011; Gadgil *et al.*, 2000; Paludzyszyn Filho & Santos 2011). Thus, the present study evaluated the growth performance and wood features of four *Eucalyptus* spp. elite-clones (*E. pellita* x *E. grandis*; *E. camal-*

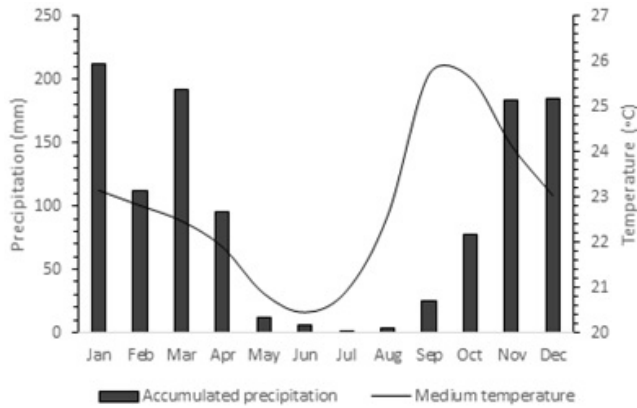
*dulensis* x *E. tereticornis*; *E. grandis* x *E. urophylla* x *E. tereticornis*; *E. urophylla* x *E. grandis*) at four years old from a breeding program located in a region of seasonal drought stress in central-western Brazil. The objective of the study was to assess dendrometric parameters and stem wood properties (bulk density and morphometric characteristics of vessels and fibers), establishing relationships with functional anatomy and hydraulic efficiency. This approach is interesting to understand how the wood traits may be related to the tree development of the distinct genotypes, contributing to expand knowledge on the functional-adaptive responses of drought-tolerant *Eucalyptus* clones. Our hypotheses were: (i) significant variations exist in wood properties at inter-specific level, which may explain the different growth responses across genotypes; (ii) increased wood density would be correlated with decreased theoretical Ks, as well as with thicker fiber wall and narrow vessels; (iii) considering that the potential of vulnerability to cavitation is linked to the spatial pattern of xylem vessels, and therefore to the hydraulic safety, trade-offs between drought tolerance strategies and growth rates are expected.

## Material and methods

### Site and wood sampling

Four-year-old trees of four elite *Eucalyptus* spp. clones were selected from a clonal trial located in Goiás state, central-western Brazil (16° 16' 37" S; 47° 44' 02" W; 930 m above sea level). The clonal trial was established in December 2012 using single tree plots and 29 replications. Trees were planted at 3 m × 3 m spacing and were managed in accordance with management practices for *Eucalyptus* plantations in Brazil (*i.e.* with limestone and NPK fertilization, and control of weeds and ants). The soil is Plinthosol characterized as gravelly with acidic pH, low base saturation and high saturation by aluminum. The region's climate is subtropical humid according to the Köppen-Geiger classification, with drier winters and wetter summers (Cardoso *et al.*, 2014). The mean annual rainfall is close to 1300 mm, with five dry months per year, and the annual average temperature is 22.0°C (NASA power climate data, 1989-2019). Figure 1 shows average monthly temperatures and rainfall for the specific growing period.

Next, the productivity performance as assessed through the mean annual increment (MAI) of all trees of the various genotypes at 2 years old and also the survival rates were considered in order to select the elite clones. The selected clones are hybrids from a diversity of species and were among the most productive of the experiment. The selection of elite-clones constitutes a strategy to indicate the adaptability to specific climatological and environmental conditions, meaning high and stable yields. The details of the selected



**Figure 1.** Monthly temperature average (°C) and monthly precipitation (mm) for the period of 2012-2016 in the municipality of Luziânia, Goiás, Brazil.

clones are provided in Table 1. Five selected trees were systematically felled for each genotype. The number of sample trees per clone was based on the sample size sufficiency calculation. The sampling error was calculated from growth data from previous inventories, being below 5% (Andrade Bueno *et al.*, 2020; Tonini *et al.*, 2019). The dendrometric parameters of the selected felled trees were subsequently verified, including total height (TH), commercial height (CH, height to minimum commercial diameter of 6 cm), and diameter at breast height (DBH, 1.3 m above the ground). Based on this data, the individual log volume was estimated using the Smalian method and MAI was predicted. Wood disks were obtained at DBH and used for determining wood density and anatomical features.

### Wood anatomy and function

First, three blocks of approximately 1 cm<sup>3</sup> were obtained from a diametrical segment of each disc representing the inner, middle and outer radial positions. Block samples were macerated with acetic acid and hydrogen peroxide, stained with Safranin and assembled in microscope slides. Images were obtained using a Zeiss Axio Scope.A1 microscope and a digital camera (Canon A640). The length, diameter, wall thickness, and lumen width of fibers were determined.

Vessel analysis was performed by macroscopic analysis from pith to bark. The surface of the samples was

sanded (increasing grain size, 120-1000 grains mm<sup>-2</sup>), followed by applying compressed air. Area, tangential diameter and frequency of vessels were obtained from digital images collected in the inner, middle, and outer positions of the total radius.

All of these morphological parameters were measured using Image Pro-Plus Software (version 5.0) in compliance with IAWA standards (IAWA, 1989). The following functional parameters were calculated from the vessel dimensions:

- Vessel lumen fraction (F, unitless): measure of the relative xylem area available for water transport. This was given by the product of the mean vessel lumen area (individual vessel) and vessel frequency for each area unit (vessels.mm<sup>-2</sup>).
- Vessel composition (S, mm<sup>4</sup>): measure of vessel size to number ratio or composition. This was calculated as the ratio between mean vessel lumen area (mm<sup>2</sup>) and vessel frequency (mm<sup>-2</sup>).
- Theoretical xylem-specific hydraulic conductivity (Ks, kg s<sup>-1</sup> m<sup>-1</sup> MPa<sup>-1</sup>): measure of xylem hydraulic efficiency, which was estimated by a modification of the Hagen-Poiseuille law (Tyree and Ewers, 1991), expressed by equation 1:

$$Ks = \left( \frac{Dh^4 \pi}{128 \eta} \right) \times VF \times \rho \quad (1)$$

Where Dh represents the weighted vessel diameter (Equation 2), η is the viscosity of water at 20 °C (1.002 x10<sup>-9</sup>, MPa.s), VF is vessel frequency (m<sup>-2</sup>) and ρ is the water density (998.2 kg.m<sup>-2</sup>).

$$Dh = \left( \frac{\sum D^4}{N} \right)^{\frac{1}{4}} \quad (2)$$

### Wood bulk density

Wood bulk density was determined by the X-ray densitometry technique (Polge, 1963) using a QTRS-01X Tree Ringer Analyzer equipment. Samples from the cross-section of the disks were sectioned (2 mm thickness). Following samples were stored in a conditioning chamber at 20°C and 50% RH until reaching 12% moisture content (Tomazello *et al.*, 2008). Complete scans of the samples (pith-to-bark direction) was performed every 0.08 mm.

**Table 1.** Species and wood productivity of the four selected elite clones (A, B, C, D) at 2 years old, being MAI = mean annual increment

Clone	Genotype/Hybrid	MAI (m <sup>3</sup> ha <sup>-1</sup> year <sup>-1</sup> )
A	<i>E. pellita</i> x <i>E. grandis</i>	55.3
B	<i>E. camaldulensis</i> x <i>E. tereticornis</i>	48.4
C	<i>E. grandis</i> x ( <i>E. urophylla</i> x <i>E. tereticornis</i> )	46.5
D	<i>E. urophylla</i> x <i>E. grandis</i>	63.1

## Data analysis

The data were submitted to a normality test (Shapiro & Wilk 1965) and homogeneity of variances (Levene, 1960). The effect of differences between clones was evaluated for all the quantified variables of wood by univariate analysis of variance (ANOVA). When the effect of clones was significant, the averages were compared by the Tukey test ( $\alpha=0.05$ ). Linear regressions were used to examine expected (or lack) relationships between parameters as hypothesized. The relative dispersion of F and S was calculated for each genotype and at the general level (Equation 3). Additionally, the relative contributions of S and F over Ks was estimated according to the model described by Zanne *et al.* (2010). The model was adapted from fitting curves to data using nonlinear regression, being  $\alpha$ ,  $\beta$ ,  $\delta$  the estimated parameters (Equation 4).

$$M_m = \frac{\max(X_i)}{\min(X_i)} \quad (3)$$

$$KS = \alpha \cdot F^\beta \cdot S^\delta \quad (4)$$

## Results

### Wood characterization of the *Eucalyptus* spp. clones

The dendrometric parameters and wood properties of the four *Eucalyptus* spp. clones at four years old (Table 2) show significant differences among genotypes ( $p<0.05$ ). Clone B showed the lowest value for total and commercial heights

(20.2 and 14.5 m, respectively), differing significantly from the other clones. Clones A and D were significantly higher regarding wood yield parameters, while clone B displayed lower performance. Moreover, fiber diameter and lumen width were the largest for clone A concerning the anatomical characteristics, with values of 22.6 and 11.0  $\mu\text{m}$  respectively, showing significant differences as regards clone C. The other fiber parameters did not have significant differences among genotypes: fiber length ranged from 848.8  $\mu\text{m}$  (clone B) to 971.7  $\mu\text{m}$  (clone A), and fiber wall thickness from 5.2  $\mu\text{m}$  (clone C) to 5.8  $\mu\text{m}$  (clones A). The largest vessel tangential diameter was found for clone D (93.3  $\mu\text{m}$ ), which differed significantly from clones A and C (84.0 and 79.7  $\mu\text{m}$ , respectively). Clone B presented the highest mean value for vessel area percentage (14.4%), and clone D had the lowest mean value for vessel frequency (10.5 vessels  $\text{mm}^{-2}$ ). Wood bulk density values ranged from 549 to 642  $\text{kg}\cdot\text{m}^{-3}$ , where clones B and D presented a lower wood density than clone C.

There were significant differences along the radial position only for diameter and frequency of vessels (Table 3). The radial variation of vessel elements and microdensity profile corresponding to each *Eucalyptus* genotype is shown in Fig. 2. It can be seen that the vessel size considerably increases for all genotypes, while the frequency decreases in the pith-bark direction. Clone A showed the greatest variation for both tangential diameter and frequency of vessels (CV: 24.1% and 44.1%, respectively). Clone C presented the lowest CV for vessel frequency (35.9%), and clone D for tangential diameter (17.1%). There was great similarity in the change patterns among genotypes regarding wood density, which generally presented lower values occurring in the central portion and a slight increase towards the bark.

**Table 2.** Dendrometric parameters and wood properties of the four *Eucalyptus* spp. clones (A, B, C, D, Table 1) at 4 years old.

Variable	Clone			
	A	B	C	D
Total Height (m)	23.4 <sup>a</sup> (1.7)	20.2 <sup>b</sup> (2.4)	22.6 <sup>a</sup> (3.6)	23.9 <sup>a</sup> (3.6)
Commercial height (m)	18.6 <sup>a</sup> (3.4)	14.5 <sup>b</sup> (2.7)	17.0 <sup>a</sup> (4.1)	18.2 <sup>a</sup> (4.9)
Diameter at breast height (cm)	16.7 <sup>ab</sup> (7.3)	14.1 <sup>c</sup> (9.7)	14.9 <sup>bc</sup> (4.1)	18.1 <sup>a</sup> (7.1)
Volume (m <sup>3</sup> )	0.21 <sup>ab</sup> (16.4)	0.12 <sup>c</sup> (20.8)	0.18 <sup>bc</sup> (11.2)	0.25 <sup>a</sup> (16.6)
Mean annual increment (m <sup>3</sup> h <sup>-1</sup> y <sup>-1</sup> )	57.5 <sup>ab</sup> (16.4)	34.6 <sup>c</sup> (20.8)	48.7 <sup>bc</sup> (11.2)	68.3 <sup>a</sup> (16.6)
Fiber length ( $\mu\text{m}$ )	971.7 <sup>a</sup> (6.4)	848.8 <sup>a</sup> (7.7)	920.0 <sup>a</sup> (2.5)	906.7 <sup>a</sup> (3.8)
Fiber diameter ( $\mu\text{m}$ )	22.6 <sup>a</sup> (12.8)	19.4 <sup>ab</sup> (4.1)	17.1 <sup>b</sup> (11.9)	19.1 <sup>ab</sup> (1.4)
Fiber lumen width ( $\mu\text{m}$ )	11.0 <sup>a</sup> (7.7)	8.5 <sup>ab</sup> (13.5)	6.7 <sup>b</sup> (18.6)	8.2 <sup>ab</sup> (2.6)
Fiber wall thickness ( $\mu\text{m}$ )	5.8 <sup>a</sup> (8.0)	5.4 <sup>a</sup> (10.6)	5.2 <sup>a</sup> (6.3)	5.4 <sup>a</sup> (4.3)
Vessel tangential diameter ( $\mu\text{m}$ )	84.1 <sup>b</sup> (4.0)	86.0 <sup>ab</sup> (7.7)	79.7 <sup>b</sup> (3.4)	93.3 <sup>a</sup> (4.2)
Vessel area (%)	12.6 <sup>b</sup> (4.4)	14.4 <sup>a</sup> (9.5)	11.2 <sup>b</sup> (11.6)	12.2 <sup>b</sup> (10.0)
Vessel frequency (n $\text{mm}^{-2}$ )	14.5 <sup>a</sup> (6.0)	15.4 <sup>a</sup> (4.5)	13.7 <sup>a</sup> (10.1)	10.5 <sup>b</sup> (7.4)
Bulk density ( $\text{kg}\cdot\text{m}^{-3}$ )	600 <sup>ab</sup> (2.68)	578 <sup>b</sup> (6.3)	642 <sup>a</sup> (5.1)	578 <sup>b</sup> (3.8)

Means and coefficients of variation (%). Different letters indicate significant differences between clones (Tukey test,  $p<0.05$ ).

**Table 3.** ANOVA test F for fibers and vessels in the different radial positions. Statistically significant differences at a confidence level of 0.05 and are indicated by “\*”.

Variable	Radial Position (within trees)	Clone x Radial Position (interaction)
	Prov>F	
Fiber length	0.711	0.987
Fiber diameter	0.283	0.465
Fiber lumen width	0.160	0.206
Fiber wall thickness	0.690	0.772
Vessel tangential diameter	<0.0001*	0.081
Vessel area	0.053	0.549
Vessel frequency	<0.0001*	0.093

### Relationship between wood characteristics and functional measures

The values for the distribution of the average range of vessel lumen fraction (F), vessel composition (S) and theoretical xylem-specific hydraulic conductivity (Ks) of elite *Eucalyptus* spp. clones are presented in Fig. 3. Clone B shows the highest mean value for F (0.011). In contrast, clones C and D have the lowest average ranges for this feature (0.061-0.070 and 0.056-0.073, respectively). The highest mean value for S is presented by clone D (0.00067 mm<sup>4</sup>). Clone B and D present the highest average ranges for Ks (17-33 and 16-28 kg s<sup>-1</sup> m<sup>-1</sup> MPa<sup>-1</sup>, respectively). When assessing the radial variation across the stem radius (Fig. 4), it can also be noted that for clone D, S presents a significant quantitative leap (p= 0.003). For F parameter, clones followed similar trends.

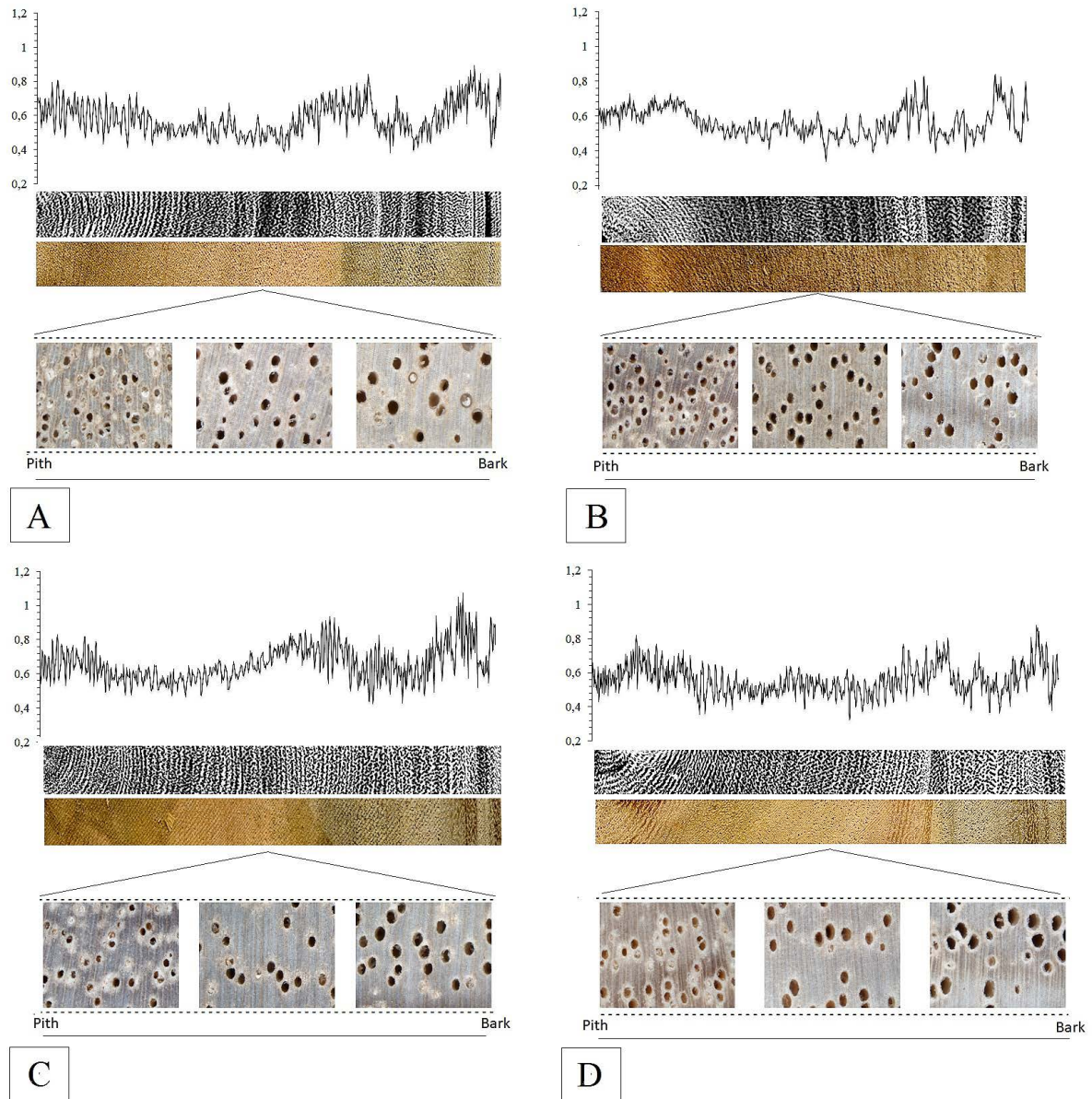
The model which relates theoretical Ks to F and S (Equation 4) showed differences between *Eucalyptus* genotypes (Table 4). The  $\beta$  values are relatively close between clones A and D (1.46 and 1.50, respectively), while it is considerably lower for clone B (1.29). The  $\delta$  values found are greater for the clones C and D (0.57 and 0.52, respectively). In analyzing the relative dispersion of F and S, from the maximum-minimum ratios of each variable, it is observed that clone B distinguishes itself from the other genotypes with a 2.2-fold and 12.5-fold variation in F and S, respectively. At the overall level, F presented a 3-fold variation, while S varied 16-fold.

The significant linear regressions between growth, xylem properties and functionality are presented in Fig. 5. There is a negative association between: vessel diameter and wood density (R<sup>2</sup>= 0.70); vessel frequency and wood volume (R<sup>2</sup>= 0.62); and hydraulic conductivity and wood density (R<sup>2</sup>= 0.38). In contrast, a positive association is found between vessel diameter and diameter at breast height (R<sup>2</sup>=0.38); S and wood volume (R<sup>2</sup>= 0.58), and hydraulic conductivity and F (R<sup>2</sup>= 0.78).

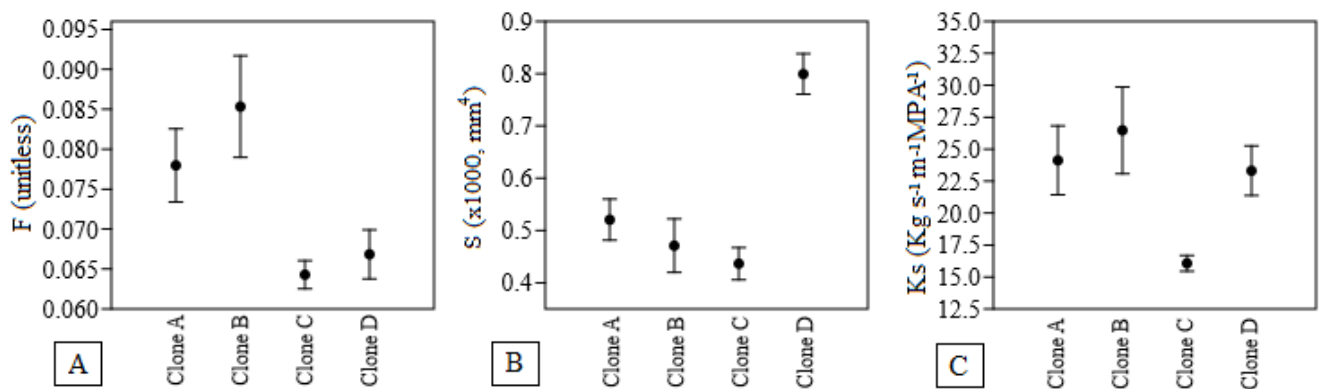
### Discussion

Despite the large variation observed in dendrometric performance among genotypes, it is possible to affirm that the wood yield values found were generally high considering the average estimate in Brazil for *Eucalyptus* industrial plantations, approximately 35 m<sup>3</sup> ha<sup>-1</sup> year<sup>-1</sup> (IBÁ, 2019). According to Stape *et al.* (2018), commercial MAI range from 20 to 70 m<sup>3</sup> ha<sup>-1</sup> year<sup>-1</sup> over 5 to 8 year rotations for many different *Eucalyptus* species and hybrids in Brazil. The good growth performance revealed by the trees in this study against climatic conditions characterized by seasonal rainfall and drought indicates that genetic materials were selected for drought tolerance. Clone D was the most prominent and is a hybrid with *E. urophylla*, which is a species with high growth potential and drought tolerance (Boland *et al.*, 2006). *E. grandis* (present in most clones) stands out for its high productivity and wood quality (da Silva *et al.*, 2019), but it has to be hybridized with other -more tolerant species- to be grown in environments where it is not well adapted as a pure species (Campos *et al.*, 2016). *E. pellita*, which is a species with broad adaptation to a range of environmental conditions (Clarke *et al.*, 2009), is combined with *E. grandis* in clone A, resulting in good performance. *E. tereticornis*, a common species in the lower performing clones (B and C), has a relative high drought tolerance (Rao *et al.*, 2002) and medium adaptability to the climatic conditions of the studied region (Flores *et al.*, 2016). Although the interspecific hybrid of *E. camaldulensis* and *E. tereticornis* (clone B) configures wider adaptability traits (Arumugasundaram *et al.*, 2011), it has relatively low productivity as compared to other *Eucalyptus* clones (Fernandes *et al.*, 2018).

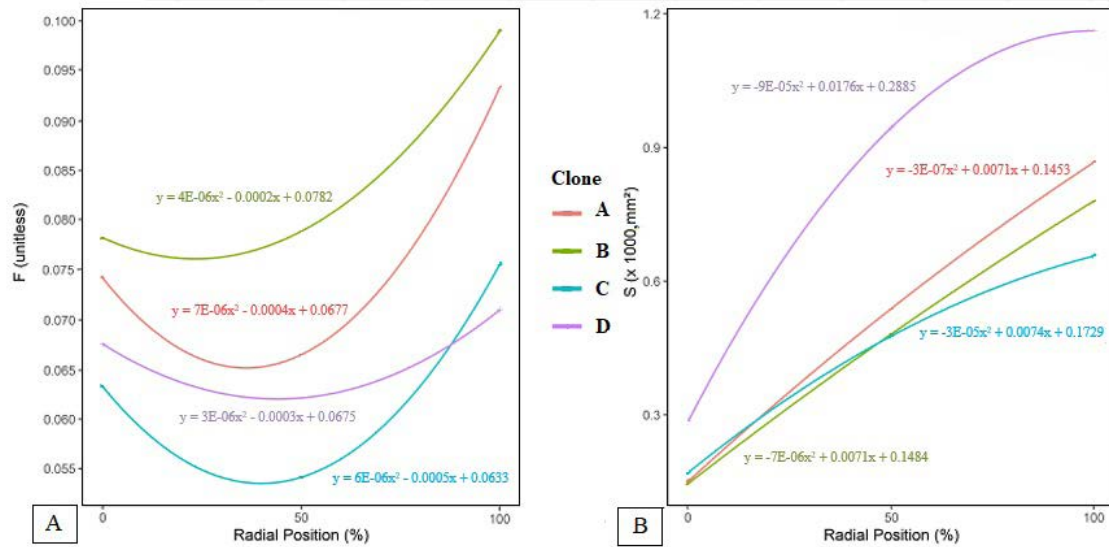
The values presented for wood bulk density are within the range of values reported by literature for various *Eucalyptus* species of the same age, which ranged between 530 and 840 kg.m<sup>-3</sup> (Knapic *et al.*, 2014). Because of its very young age, the trees showed a little variance



**Figure 2.** Illustration of radial variation of bulk density and vessel dimensions of four *Eucalyptus* clones. *E. pellita* x *E. grandis* (A), *E. camaldulensis* x *E. tereticornis* (B), *E. grandis* x (*E. urophylla* x *E. tereticornis*) (C), *E. urophylla* x *E. grandis* (D).



**Figure 3.** Distribution of average values of A) vessel lumen fraction (F); B) vessel composition (S); and C) theoretical xylem-specific hydraulic conductivity (Ks) of four *Eucalyptus* spp. clones. 95% confidence limits.



**Figure 4.** A) Changes in lumen fraction F along the 0% (inner), 50% (middle) and 100% (outer) total radius positions of wood B) Changes in vessel composition S along the 0% (inner), 50% middle) and 100% (outer) total radius positions of wood. Fitted curve for four *Eucalyptus* spp. Clones (A, B, C, D, Table 1) are shown.

of density along with the radial profile, which indicates a stem that is substantially formed by sapwood and juvenile wood (Oliveira *et al.*, 2012; Tomazello *et al.*, 2008). Since sapwood is the physiologically active portion, the relative sapwood area development profoundly influences the capacity of trees to grow during drought (White *et al.*, 1998), and is significantly related to the tree growth rate (Bamber, 1976; Cherelli *et al.*, 2018).

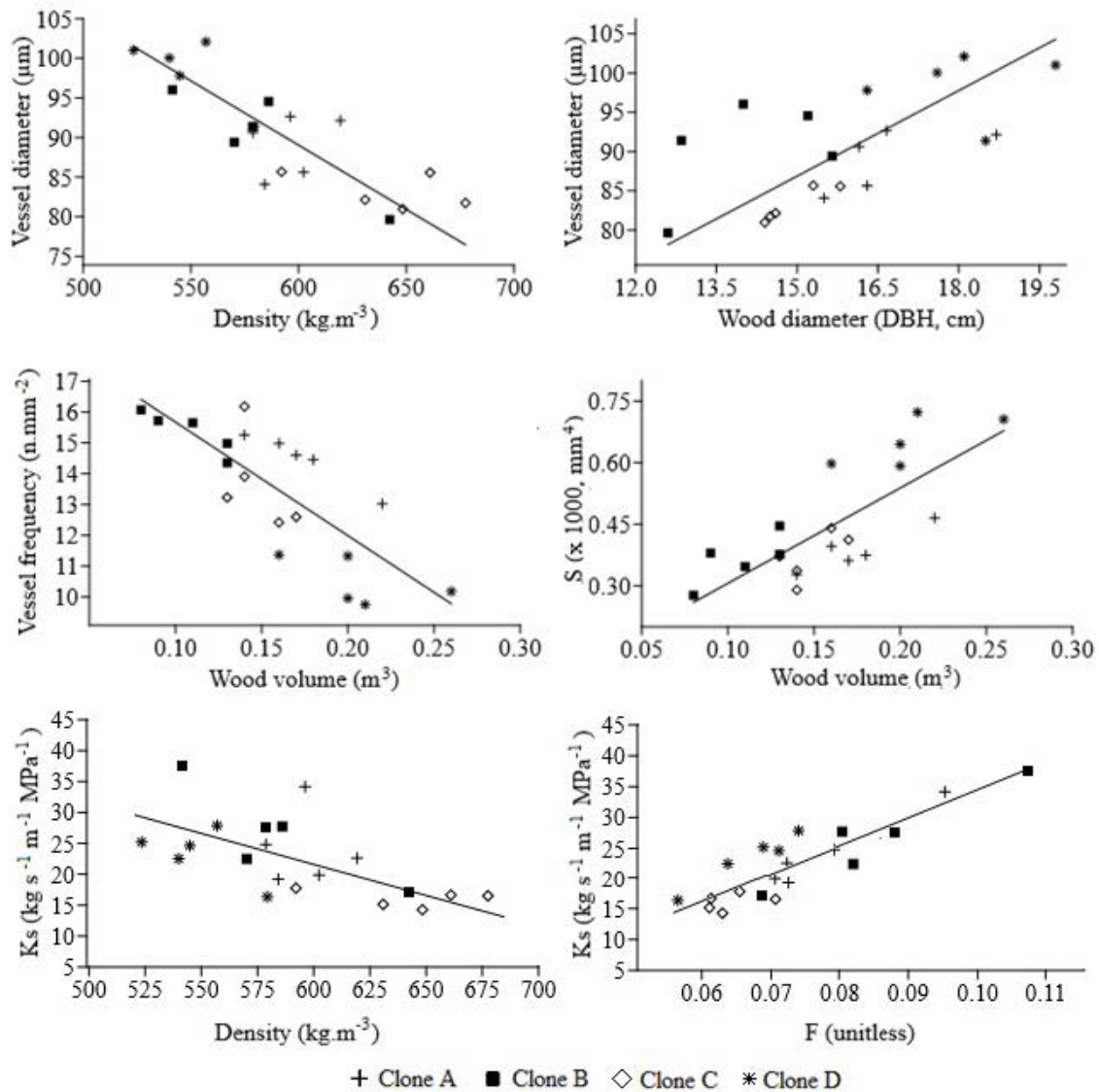
Other authors have reported similar results for anatomical dimensions for *Eucalyptus* species at similar ages (Pirralho *et al.*, 2014; Sette *et al.*, 2012). In evaluating *E. grandis* x *E. urophylla* hybrids under rainfed conditions at 5-6 years of age, Sharma *et al.* (2015) mention much higher vessel diameter compared to values reported here, from 155 to 167  $\mu\text{m}$ . In a study of drought-tolerant *Eucalypt* genotypes, Bueno *et al.* (2020) report vessel diameter and vessel frequency values of between 94-135  $\mu\text{m}$  and 7-18  $\text{n.mm}^{-2}$ , respectively. Fiber dimensions and wood density show relatively weak ecological trends (Baas, 1986; Denis *et al.*, 2013; Kube *et al.*, 2001; Sturion, 2008) and low correlation with growth mainly for fibers (Apiolaza *et al.*, 2005; de Lima *et al.*, 2019). On the other hand, it is common to observe significant correla-

tions between climate and wood vessels (Pfautsch *et al.*, 2016) due to the vessels being the hydraulic component of the angiosperm stem, directly acting on functionality and structural adaptation. However, it is important to point out that despite this strong association, species climatic range also has a large influence on molding their responses to the environmental conditions, supporting the theory that genetic factors influence the expression of xylem anatomy and stem hydraulics (Bourne *et al.*, 2017). This aspect justifies the emphasis which has been put on the importance of selecting more specific genetic materials for adverse climatic conditions.

As expected, significant relationships of wood density with vessel diameter and theoretical hydraulic conductivity were verified in this study. Clone C, which presented the lowest Ks, has anatomy characterized by relatively small-sized fibers and narrow vessels. According to Zie-mińska *et al.* (2013), wood density can be considered a measure of internal wood structure and results from the combination of features and relative proportions of the cellular elements which compose wood, including the structure of fiber and vessels. Thus, correlations of wood density and anatomy are frequently reported in the litera-

**Table 4.** Estimated parameter values  $\alpha$ ,  $\beta$ ,  $\delta$  for the model described by Zanne *et al.* (2010) (Equation 4) and maximum-minimum ratios for F and S.

Genotype	Model parameters $K_s = \alpha \cdot F\beta \cdot S\delta$				$F_{M/m}$	$S_{M/m}$
	$\alpha$	$\beta$	$\delta$	RMSE		
Clone A	22907.34	1.46	0.42	0.099	2.04	7.65
Clone B	22347.53	1.29	0.47	0.791	2.23	12.5
Clone C	58527.47	1.39	0.57	0.807	1.59	6.31
Clone D	56219.65	1.50	0.52	0.450	1.91	6.05



**Figure 5.** Relationships between growth, wood characteristics and functional indices. Values for different clones (A, B, C, D, Table 1) are shown.

ture (Pfausch *et al.*, 2016; Dadzie & Amoah, 2015; Salvo *et al.*, 2017), and are in line with our results.

The results in this work show that the wood with low relative frequency of wide vessels presented by clone D is associated with higher wood increment. The relationship between these measures can be better explored through the S ratio, where the increase in vessel composition (S) has a positive influence on wood increment, and should therefore be linked to greater hydraulic efficiency of trees. Some authors mention that trees with a high vessel composition may be more vulnerable to drought-induced embolisms (Hacke *et al.*, 2001; Tyree & Zimmermann, 2002) and therefore the relationship between the increase in S and the improvement in hydraulic efficiency is limited. However, that does not necessarily apply to the *Eucalyptus* species, where the presence of solitary vessels make unlikely the imbalance of forces between adjacent cell

as required to avoid wall collapse (Hacke *et al.*, 2001) and, therefore, there would be no obvious compromise between xylem efficiency and hydraulic safety (Barroto *et al.*, 2017). Moreover, some authors have found that large vessels of *Eucalyptus* have indeed smaller pits, so it is expected to have a lower vulnerability to cavitation (Fernández *et al.*, 2019). In this sense, higher S values do not imply that the xylem is really more prone to losing conductivity by embolism.

Contrary to what is expected, the highest average Ks (presented by clone B) contrasts its inferior growth performance. The high Ks for clone B was the consequence of a large area associated to high vessel frequency, which together occasioned F to be the highest of the four genotypes. The relationship verified between wood volume and vessel frequency suggests that, for specific site conditions, having a few large vessels per unit xylem area is



better to achieve high growth rates than having high Ks, if that is the consequence of many vessels per unit area. In this sense, the higher number of vessels per unit area presented by clone B may indicate a greater vulnerability to drought, in that the higher conduit-network connectivity would mean a higher probability of embolisms spread (Johnson *et al.*, 2020).

Based on the model that relates theoretical hydraulic conductivity (Ks) to F and S, the values estimated for coefficients  $\beta$  and  $\delta$  are similar to those obtained by Zanne *et al.* (2010) for several angiosperm species, of 1.5 and 0.5, respectively. In a study with older trees of three *Eucalyptus* species, Barroto *et al.* (2017) reported  $\beta$  values ranging from 1.32-1.61 and  $\delta$  from 0.42-0.44. Therefore, in the same way as literature, Ks more sensitively responds to changes in lumen fraction (F) than in size to number ratio (S). In contrast, S had a greater influence on differences within genotype and between genotypes, varying in a wider range than F. According to Barroto *et al.* (2017), S has a more plastic nature and its larger variability indicates that the plant can modify the conductive structure to attain greater hydraulic efficiency without substantially altering the corresponding xylem lumen fraction, which is a more stable feature. This corroborates the more remarkable influence of vessel composition on the differential growth between *Eucalyptus* trees. It should also be pointed out that the wood structural adaptations to adjust water transport are not necessarily mutually exclusive (Zanne *et al.*, 2010) and the relative contribution of each to genotype-level differences in hydraulic conductivity is variable. For example, the relative participation of S on Ks ( $\delta$  coefficient) was considerably lower in clone A, suggesting that the vessel composition for this genotype has a lower contribution to conductive efficiency. In this sense, Ks values are close for clones A and D, although clone A has relatively low S.

Forest development is a complex process resulting from the dynamic interaction of morphological and physiological traits and environmental signals in plant growth (Gonçalves *et al.*, 2017), as well as the interactions among individuals. In this sense, while the study reports variant behavior of different genotypes regarding anatomical architecture and functional response to hydraulic efficiency, water availability as a limiting growth factor and its influence on trees development, as well as adaptation mechanisms to genotype specific- level, were not previously well known for the studied genotypes. This requires further studies including a research about physiological variables in response to climatic conditions and evaluating growth based on different stem size classes.

## Conclusions

The four elite *Eucalyptus* clones presented high edaphic-climatic suitability growing under seasonal

drought-stress, highlighting the *E. pellita* x *E. grandis* (clone A) and *E. urophylla* x *E. grandis* (clone D) hybrids. There was generally considerable heterogeneity in the wood characteristics of studied *Eucalyptus* spp. clones, and therefore the comparative approach revealed important distinctions between functional-adaptative responses.

Vessel frequency was inversely associated with wood increment and increased Ks was related to decreased wood density. *E. urophylla* x *E. grandis* was the hybrid with the highest growth rates and presented the highest S, which has been linked with anatomical characteristics such as wide vessels and low vessel frequency. In contrast, *E. camaldulensis* x *E. tereticornis* (clone B), the genotype with lower growth performance, displayed high Ks as a result of the higher F. The total area occupied by vessel lumens (F) had a greater relative influence on Ks than the vessel size number ratio (S), although the latter showed greater variation among genotypes. Considering the studied *Eucalyptus* genotypes dataset, this study indicates that high theoretical Ks does not necessarily imply higher growth rates, which may be associated with greater xylem vulnerability. The results also point out the important role of vessel composition in the growth performance of young trees.

Finally, further studies are required to investigate physiological variables in response to climatic conditions in combination with growth measures and anatomical features of trees with different size classes within the stand to obtain broader knowledge of the systematic functions of wood from *Eucalyptus* spp. clones.

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