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VARIATIONS IN WOOD ANATOMY AND FIBER BIOMETRY OF *EUCALYPTUS GLOBULUS* GENOTYPES WITH DIFFERENT WOOD DENSITY

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ABSTRACT

Six 15-year-old *Eucalyptus globulus* trees, ranging in wood density from 474 to 575 kg.m^{-3}, were sampled at breast height for anatomical study and fiber measurement. Vessel and fiber dimensions showed an increase from pith to bark, while vessel frequency decreased. Cell wall thickness, fiber length, runkel ratio and coarseness were significantly superior in the high-density genotypes (group A) in almost each section along the radius analyzed. Average cell wall thickness for samples of group A was 3.35 μm, while in group B (the low-density genotypes) was 2.86 μm. The average runkel ratio and coarseness in groups A and B were 0.73 and 7.6 mg/100 m, 0.56 and 6.7 mg/100 m, respectively. Lumen width was significantly different among the groups, being higher in group B, but no significant differences were found for vessel features. Pooled data showed significant correlations between wood density and cell wall thickness (r = 0.52), coarseness (r = 0.40) and runkel ratio (r = 0.41), while coarseness has a close relation with almost each variable evaluated, showing an effect of cambial age on anatomical variations. Also significant and positive correlations were found for coarseness vs density, wall thickness vs coarseness, fiber length vs density, density vs wall thickness, runkel ratio vs density and coarseness vs fiber length at different sections along the radius analyzed.

KEYWORDS: Cell wall thickness, fiber length, coarseness, vessel width, vessel frequency, radial variation.

INTRODUCTION

Tree selection for genetic breeding programs has been based mainly on growth rate and tree form (Raymond et al. 1998). Wood density is considered one of the most informative properties about the physical-mechanical behavior of wood for timber, pulp and paper production (Lima et al. 2000, Raymond and Muneri 2001) and has been also included as a selection parameter. However, anatomical and chemical properties of wood, which can affect wood density and the type and quality of pulp and paper produced, have not yet been included in the selection and improvement program of trees (Ona et al. 2001, Wimmer et al. 2002, Ramírez et al. 2009a).

In hardwoods, wood density is determined by its anatomical structure, cell wall thickness, fiber width, vessel width and frequency, parenchyma proportion and chemical composition, thus species with similar density can differ markedly in fiber properties and cell dimensions which affect the quality of the products obtained, such as pulp and paper (Sandercock et al. 1995, Downes et al. 1997, Butterfield 2006). In high-density hardwoods, fibers with thicker cell walls represent a larger proportion of xylem (Butterfield 2006) generating a porous paper and more compressible, giving better printability and opacity (Downes et al. 1997, Raymond et al. 1998). In low-density hardwoods, vessels occupy a major proportion of wood and fibers have thin walls, producing a denser paper, smooth and with high tensile and burst strength (Downes et al. 1997, Butterfield 2006). Fiber length usually does not directly contribute to density, but is an important descriptor factor of pulp quality, given its relationship with paper strength properties (Mansfield and Weineisen 2007, Ek et al. 2009). Density varies among trees of the same species, as well as within a tree, where it usually increases from pith to bark and with height (Downes et al. 1997, Wate et al. 1999), thus a variation of fiber and vessel dimensions is also expected among and within the trees, given their influence on basic density variation.

Eucalyptus globulus generates a great interest for forestation in different parts of the world, with extensive plantations throughout Asia, South America, Africa and Southern Europe, with at least 12 million hectares planted (Poke et al. 2005). The rapid growth rate, high pulp yield and excellent fiber quality makes *E. globulus* one of the most important commercial hardwoods for pulp and paper production (Doughty 2000, Kibblewhite et al. 2000, Patt et al. 2006). Different authors have evaluated the effect of fiber characteristics of *E. globulus* on pulp and paper properties, as well as the site effect on the variation of xylem morphology, and the variation within and between individuals (Jorge et al. 2000, Kibblewhite et al. 2000, Ona et al. 2001, Miranda and Pereira 2002, Wimmer et al. 2002, Ramírez et al. 2009a,b). The aim of this work was to determine the variations in wood anatomy and fiber biometry of two groups of *E. globulus* genotypes that presented differences in wood density (high and low density) and determine if differences found in some traits could be directly related with this property. This information could be useful for tree improvement programs to select the best genotypes for production of specified products or type of paper according to the wood density and the anatomical traits.

MATERIAL AND METHODS

Wood material

Samples were collected from six 15 year-old *E. globulus* genotypes growing in a commercial plantation established in the Bio-Bio Region, Chile (37°11’ S; 71°53’ W). Wood density of the genotypes was previously known and the trees were divided in two groups: Group A represented by three genotypes with wood density between 530 and 575 kg.m^{-3} and, Group B, represented by three genotypes with wood density between 474 and 495 kg.m^{-3}. Trees were harvested and wood disks were sampled at breast height of each tree. Diameter at breast height (DBH) for group A ranged from 15.5 to 25.2 cm and for group B ranged from 17.1 to 22.4 cm. From each disc a sub-sample was taken along the radius from pith to bark, at 10, 50 and 90 % of the total radius, labeled as section 1, 2 and 3, respectively. From each of the sub-sections, one block of 2 cm^3 and matchsticks of 0.1x0.1x0.5 cm were taken for anatomical and biometric analysis, respectively.

Transversal anatomical characterization

Wood blocks of 2 cm^3 taken at each section were macerated with distilled water and glycerin for 7 days. From each block, transversal micro-sections of 30 µm thickness were obtained using a sliding microtome (MICROM, H325). Samples were stained with Safranin and Astra blue, dehydrated with ethanol and assembled in a slide using Canada balsam. Images were obtained using a Zeiss microscope (Primo Star) connected to a personal computer and a digital camera (Canon A640) for image capture. Forty fibers were randomly selected and their cell wall thickness, fiber width and lumen width were measured with a 100x total magnification. The number of vessels was determined by counting vessels in an area of 1 mm^2, which was expressed as vessel frequency (number of vessels per square millimeter). Moreover, 20 vessel randomly selected were measured with a 40x magnification for vessel width determination. All these parameters were measured using AxioVision Software (Zeiss), which had the proper calibration for each capture lens. Similar procedure for anatomical analysis of *E. globulus* was used by Aguayo et al. (2010).

Fiber biometry characterization

Wood samples were treated according to the protocol reported by Mansfield and Weineisen (2007). Matchsticks obtained from longitudinal cuts of the sub-sample taken along the radius from pith to bark (at 10, 50 and 90 % of the total radius length) were macerated and treated using Franklin solution (30 % H_2O_2 and CH_3COOH, 1:1 v/v) for 8 hours at 70°C. The solution was decanted and the remaining fibrous material was washed with water until a neutral pH was achieved. Fiber length distribution, average fiber length and coarseness were determined in a Fiber Tester equipment (Lorentzen & Wettre, Sweden) where 200 mg of sample were previously disaggregated in 200 ml distiller water for 10 minutes. During analysis of this suspension, the equipment was setting to measure approximately 35.000 fibers of each sample.

Data analysis

Statistical analyses of anatomical characteristics from the six genotypes were performed using the software SAS system 9.0 (SAS Institute). Unpaired t-test was used to compare the properties between both groups and between each section. Correlation analysis of pooled data and data from each section analyzed was performed between anatomical features calculating Pearson coefficient.

RESULTS AND DISCUSSION

Cell wall thickness, fiber lumen width, fiber width, vessel width and vessel frequency were determined using microscopic analysis across the transversal section of *E. globulus* genotypes, and fiber length and coarseness were measured by a fiber analyzer equipment. Each one of these variables was measured at different sections along the radius from pith to bark at breast height. Section 1 belongs to the wood close to the pith, section 2 to the wood within the half of the radius, and section 3 to the wood close to the bark. The radial variation pattern of the anatomical characteristics evaluated in each group is shown in Fig. 1. Vessel frequency range of the six genotypes analyzed at the zone close to the pith was 8 – 14 vessel.mm^{-2} and decreased to a range of 5 – 9 vessel.mm^{-2} close to the bark, while vessel width increased from 75– 114 μm in the pith to a range of 115 – 142 μm in section 3. The radial variation pattern of vessel features in each group is shown in Fig. 1a. Vessel width presents no significant differences between groups across different radial sections, but vessel frequency is higher in group B at sections 2 and 3. The variation pattern of vessel width and frequency found in this study is consistent with the existing reports, which indicated an increase in the size of vessels as cambial age increases, while vessel frequency decreases (Carvalho 1997, Hudson et al. 1998, Leal et al. 2003, Ramírez et al. 2009b). There are several detailed studies about vessels features in different species of *Eucalyptus*, which show the same radial variation pattern for *E. regnans* (Dadswell 1958), *E. grandis* (Taylor 1973), *E. nitens* (McKimm and Ilic 1987) and *E. globulus* (Hudson et al. 1998; Ramírez et al. 2009b).

Regarding fiber morphology, fiber width increases from pith to bark (Fig. 1b); however, there are not clear differences between groups. The same pattern of radial variation was reported for *E. globulus* clones (Ramírez et al. 2009b) and for *E. grandis* x *E. urophylla* hybrids (Quilhó et al. 2006). The range for fiber width of the six genotypes evaluated was 12.63 – 14.35 μm in section 1 and 15.70 – 19.42 μm close to the bark. For the radial variation of fiber length, both groups showed an increase from pith to bark (Fig. 1b), a trend that has been described in the genus and specie, and has been considered as the main source of variation within a tree (Wilkes 1988, Jorge et al. 2000, Miranda and Pereira 2002, Ohshima et al. 2003). However, only in section 2 the groups differ significantly. The fiber length range of the six genotypes analyzed in the wood close to the pith was 0.65 – 0.80 mm and 0.90 – 1.02 mm for the wood close to the bark.

Coarseness, defined as fiber mass per fiber length (expressed as mg of fibers/100 m of fibers), has a close relationship with wood density and is, among the fiber dimensions, a good index for predicting pulp properties, besides being closely related to the biometric properties of wood (Via et al. 2004, Mansfield and Weineisen 2007). Coarseness shows an increase from pith to bark, being higher in the group A in each of the sections analyzed (Fig. 1c). In section 1, the range of the six trees evaluated was 4.9– 7.5 mg/100 m and increases to 7.3– 9.8 mg/100 m in the zone close to the bark. Similarly, the variation pattern of cell wall thickness is in accordance to the models of radial variation within the tree commonly described for *Eucalyptus* genus, increasing from pith to bark (Miranda and Pereira 2002, Ohshima et al. 2003, Quilhó et al. 2006). In section 1, the range of cell wall thickness was 2.37 – 2.90 μm and 3.15 – 4.54 μm in section 3. Ramírez et al. (2009b) reported no significant variation from pith to bark for cell wall thickness in *E. globulus* clones, contrary to the results found in this study, where in addition, the cell wall thickness was significantly higher in group A at each section analyzed (Fig. 1c). Runkel ratio, which is a parameter derived from cell wall thickness and lumen width (2 x cell wall thickness/ lumen width) is commonly used to predict paper properties through fiber morphology. Runkel ratio increases from pith to bark in the radial direction, at section close to the pith Runkel ratio is 0.49 –0.72 and close to the bark is 0.66 – 0.96 The same radial variation pattern was found for *E. globulus* and *E. camaldulensis* by Ohshima et al. (2005).

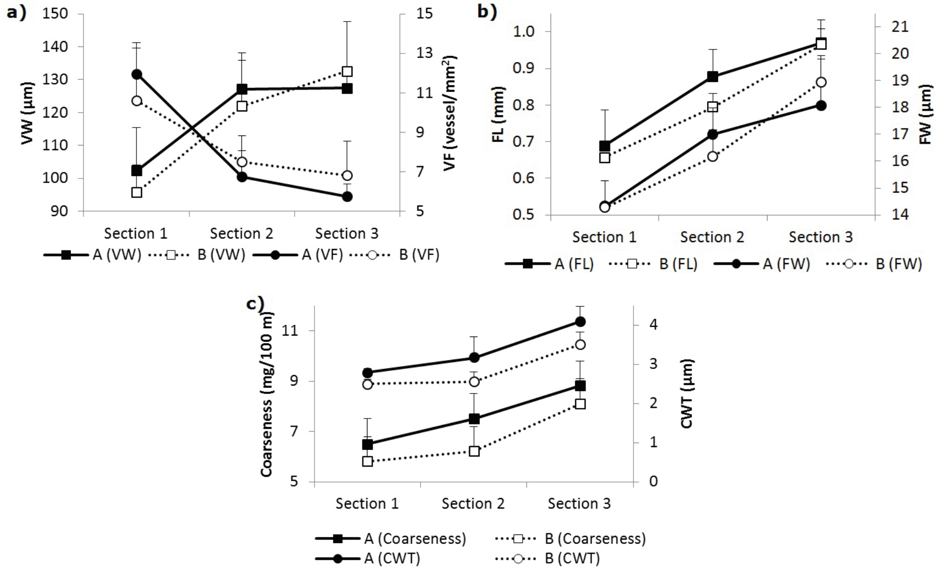

Fig. 1: Radial variation pattern of group A (high wood density) and B (low wood density) for anatomical properties measured. Mean of 3 trees per group and half standard deviation as bar. a) Vessel width and vessel frequency, b) fiber width and fiber length and c) coarseness and cell wall thickness. VW: Vessel width; VF: Vessel frequency; FL: Fiber length; FW: Fiber width; CWT: Cell wall thickness. Radial distance from pith to bark in section 1 was 10 %, in section 2 was 50 % and in section 3 was 90 %.

The average values of anatomical characteristics for trees of group A and B are summarized in Tab. 1. The average of biometric characteristics of both groups was determined considering the data from each section. The information obtained for the different variables evaluated was consistent with reports published for *Eucalyptus* genus. Specifically for *E. globulus*, the range reported for fiber length was 0.6 – 1 mm, for fiber width was 12 – 21 µm and for cell wall thickness was 2 – 4 µm, approximately (Ohshima et al. 2003, Ramírez et al. 2009b, Aguayo et al. 2010, Ona et al. 2001). No significant differences were found between group A and B for fiber width, fiber length, vessel width and vessel frequency, however, the fiber cell wall thickness was statistically higher in group A, as well as coarseness and Runkel ratio. Conversely, fiber lumen width was significantly higher in group B. High coarseness and cell wall thickness values are expected in trees with high density, due to that coarseness is directly related to cell wall thickness (Mansfield and Weineisen 2007). Within-tree variation of Runkel ratio was considered to be affected only by cell wall thickness (Ohshima et al. 2005) and also shows a significant variation between group A and B. In papermaking with hardwood fibers, Runkel ratio lower than 1.0 is desirable for good conformability and fiber-to-fiber contact in paper (Dean 1995), showing that trees from both groups are suitable for this purpose.

Tab. 1: Average values and standard deviation of biometric characteristics evaluated in E. globulus trees. Comparison between group A and B is based on unpaired t-test.

Variable		Group A	Group B	t-test
Cell wall thickness (μm)	Mean	3.35	2.86	***
		± 0.67	± 0.53	
Lumen width (μm)	Mean	9.27	10.24	***
		± 1.05	± 1.52	
Fiber width (μm)	Mean	15.96	15.95	NS
		± 1.95	± 2.18	
Fiber length (mm)	Mean	0.85	0.81	NS
		± 0.14	± 0.14	
Vessel width (μm)	Mean	119	117	NS
		± 14	± 22	
Vessel frequency (vessel/ mm^2)	Mean	8	8	NS
		± 3	± 3	
Coarseness (mg/100 m)	Mean	7.6	6.7	*
		± 1.4	± 1.2	
Runkel ratio (2CWT/ LW)	Mean	0.73	0.56	**
		± 0.14	± 0.10	

NS: non significant; * significant at p=0.05; ** significant at p=0.01; *** significant at p<0.001

The entire data set was analyzed in order to evaluate the influence of anatomical features on wood density and coarseness. Pearson coefficients for basic density and coarseness with anatomical features are presented in Tab. 2. The pooled data showed significant correlations of density with cell wall thickness, coarseness, runkel ratio, and fiber length. Coarseness had more significant correlations with anatomical characteristics than wood density, being fiber width, cell wall thickness and fiber length the properties that presented the highest coefficients. The radial variation between groups of the cell wall thickness, fiber length, runkel ratio and coarseness can explain the differences found in wood density due to the effect of cambial age on these properties. Coarseness is directly related to fiber cell wall thickness, fiber length and cell wall density, therefore, a positive correlation between coarseness and wood density is expected, considering that woods with high density often have thicker cells walls (Malan et al. 1994), which was also observed in this study. A similar situation occurs with runkel ratio, which is also influenced by fiber wall thickness (Ohshima et al. 2005).

Tab. 2: Pearson correlation coefficients for pooled data of basic density and coarseness with anatomical properties, n = 18.

	Density	p-value	Coarseness	p-value
Density	1	-	+0.40	0.09
Coarseness	+0.40	0.09	1	-
Fiber length	+0.33	NS	+0.79	<0.001
Fiber width	+0.16	NS	+0.85	<0.001
Lumen width	-0.25	NS	+0.75	<0.001
Cell wall thickness	+0.52	0.02	+0.80	<0.001
Vessels width	+0.26	NS	+0.52	0.025
Vessel frequency	-0.23	NS	-0.61	0.006
Runkel ratio	+0.41	0.08	+0.38	NS

NS: non significant.

Correlation index of the anatomical properties evaluated at different sections along the radius is shown in Fig. 2. Density vs coarseness (Fig. 2a), cell wall thickness vs coarseness (Fig. 2b) and fiber length vs density (Fig. 2c) showed positive and significant correlations at 50 % of the total radius. Density vs cell wall thickness (Fig. 2d) and runkel ratio vs density (Fig. 2e) showed positive and significant correlations at each section analyzed, while fiber length vs coarseness (Fig. 2f) presented significant correlation index at 10 and 50 % of the total radius, demonstrating the wide variability between anatomical properties at different stages of xylem development.

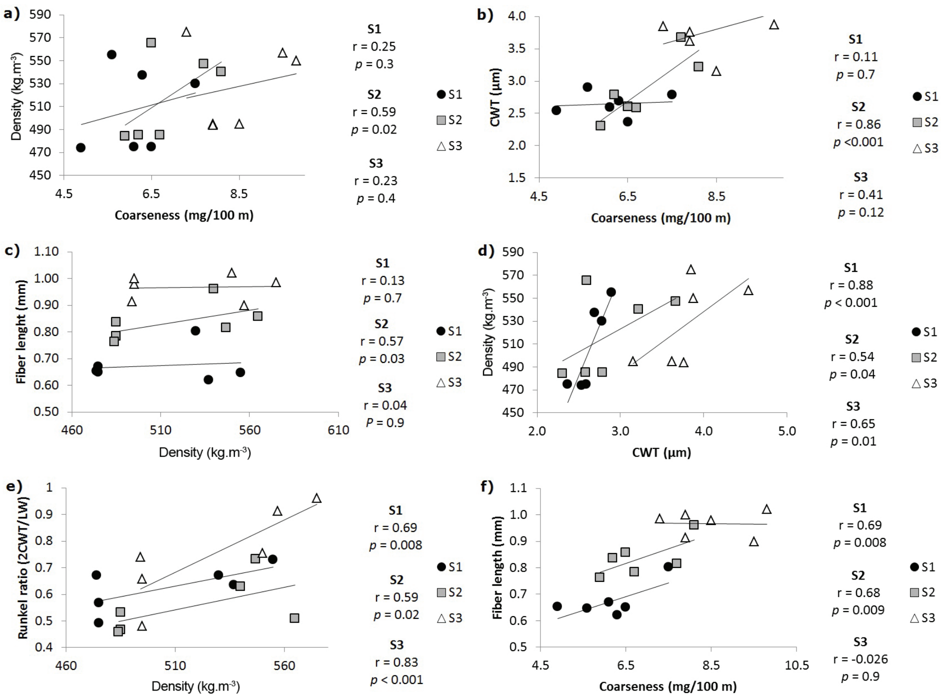

Fig. 2: Regression lines and correlation index between anatomical properties at each section analyzed are shown. a) density vs coarseness, b) cell wall thickness vs coarseness, c) fiber length vs density, d) density vs cell wall thickness, e) Runkel ratio vs density and f) fiber length vs coarseness. Radial distance from pith to bark in section 1 was 10 %, in section 2 was 50 % and in section 3 was 90 %.

Different authors report different correlation coefficients estimates for wood density and anatomical properties of *Eucalyptus*. Wimmer et al. (2002) studied 8 year-old *E. globulus* trees from different sites, estimating a negative relationship between fiber length and wood density (r=-0.44). Quilhó et al. (2006) reported for 6.8 year-old and 5-6 year-old *E. grandis* x *E. urophylla* hybrids that Pearson's correlations for fiber dimensions (length, width and cell wall thickness) and wood density was highly significant ($p<0.001$) and positive, and that cell wall thickness and fiber length would explain a 25 and 16 % of wood density variation, respectively. Raymond et al. (1998) reported for *E. regnans* weak correlation coefficients between density and fiber length (r=0.35), density and coarseness (r=0.14), and between coarseness and fiber length (r=0.11). Kube et al. (2001) reported for 12 year-old *E. nitens*, from different sites, positive and significant correlations (p=0.05) between density and fiber length (r=0.26), density and coarseness (r=0.22), and between fiber length and coarseness (r=0.25). All these works reflected the wide variability in morpho-anatomical features presented within *Eucalyptus* species. It has been suggested that given the strong genetic determinism on the structural characteristics of wood, is expected to find differences between trees, even in plantations developed on the same site and time (Wilkes 1988). In this study, the correlation coefficients among some anatomical properties and wood density were highly significant in genotypes with different density and from the same site of plantation, minimizing the effect of environment in the results obtained.

CONCLUSIONS

Eucalyptus globulus trees with high density had thicker fiber cell walls, lower fiber lumen width, higher runkel ratio and higher coarseness than low density trees. The pattern of radial variation observed in both groups is the same for all variables evaluated, but cell wall thickness, fiber length, coarseness and Runkel ratio are significantly superior in the high-density group. From these results, wood density was correlated with cell wall thickness, runkel ratio and coarseness. Vessel dimensions and vessels frequency had no influence in wood basic density variation. Therefore, considering the influence of fiber characteristics on pulp and paper quality, it would be important to consider these traits in *E. globulus* breeding programs, including a parameter that could explain fiber characteristics more precisely than basic density, as coarseness, which can be easily measured using nondestructive methods.

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