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Lima, Israel Luiz de; Longui, Eduardo Luiz; Santini Junior, Luiz; Nivaldo Garcia, José; Borges
Florsheim, Sandra Monteiro
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EFFECT OF FERTILIZATION ON CELL SIZE IN WOOD OF *Eucalyptus grandis* Hill Ex Maiden

Israel Luiz de Lima¹, Eduardo Luiz Longui², Luiz Santini Junior³,
José Nivaldo Garcia⁴, Sandra Monteiro Borges Florsheim⁵

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ABSTRACT: The use of fertilization in forest stands results in yield gains, yet little attention has been directed to its potential effects on the quality of wood produced. Information is scarce about the effect of fertilization on anatomical structures of older *Eucalyptus* wood. This work aims to study the effect of fertilization on tissue cell size of wood from a *Eucalyptus grandis* stand at age 21 years, the management system of which is based on selective thinning and fertilizer application at the start of the thinning season. Factors to consider include: presence or absence of fertilizers, two log positions and five radial (pith to bark) positions. Results led to the conclusion that fertilization significantly influenced only vessel frequency. Vessel element length was influenced by tree height. Fiber length, fiber diameter, fiber wall thickness, vessel element length, vessel diameter and vessel frequency were influenced by the radial position of the sample in relation to the log. A positive correlation was observed between fiber length, fiber diameter, fiber wall thickness, vessel element length, vessel diameter, ray width and radial position, while a negative correlation was observed between ray frequency and radial position.

Key words: Forest management, wood anatomy, wood quality.

EFEITO DA FERTILIZAÇÃO NAS DIMENSÕES CELULARES DA MADEIRA DE *Eucalyptus grandis* Hill Ex Maiden

RESUMO: A fertilização no plantio de floresta resulta em ganhos de produtividade, porém pouca atenção tem sido dada aos possíveis efeitos que ela possa ter sobre a qualidade da madeira produzida. São poucas as informações a respeito do efeito da fertilização nas características anatômicas das madeiras de *Eucalyptus* em idade mais avançada. Neste trabalho, objetivou-se estudar o efeito da fertilização nas dimensões celulares da madeira em uma população de *Eucalyptus grandis*, de 21 anos de idade, manejada pelo sistema de desbastes seletivos com aplicação de fertilizantes na época do início dos desbastes. Os fatores utilizados foram: presença ou ausência de fertilizantes, duas posições das toras e cinco posições radiais. De acordo com os resultados obtidos, pode-se concluir que a fertilização influenciou significativamente somente a frequência dos vasos. O comprimento dos elementos de vaso foi influenciado pela altura da árvore. O comprimento das fibras, diâmetro das fibras, espessura da parede das fibras, comprimento dos elementos de vaso, diâmetro de vasos e frequência dos vasos foram influenciados pela posição radial na tora. Foi observada uma relação positiva entre comprimento das fibras, diâmetro das fibras, espessura da parede das fibras, comprimento dos elementos de vaso, diâmetro dos vasos e largura dos raios com a posição radial e uma relação negativa entre a frequência dos raios com a posição radial.

Palavras-chave: Manejo florestal, anatomia da madeira, qualidade da madeira.

1 INTRODUCTION

In Brazil, although fertilization of forest stands provides yield gains, little attention has been given to its potential effects on produced wood quality. Information is yet scarce about the effect of fertilization on physical,

mechanical and chemical properties as well as on anatomical structures of woods from genus *Eucalyptus* (ANDRADE et al. 1994, BARREIROS et al. 2007, JACOB & BALLONI 1978).

Silvicultural practices intended to increase growth rates have resulted in large scale production of increasingly

¹Forest Engineer, Researcher at the Instituto Florestal do Estado de São Paulo – Divisão de Dasonomia – Rua do Horto, 931 – São Paulo, SP – 02377-000 – israeluizde.lima@yahoo.com.br

²Biologist, Researcher at the Instituto Florestal do Estado de São Paulo – Divisão de Dasonomia – Rua do Horto, 931 – São Paulo, SP – 02377-000 – elongui@if.sp.gov.br

³Student of the Biological Sciences program at Universidade Paulista/UNIP, FUNDAP Scholarship Student – Instituto Florestal do Estado de São Paulo Divisão de Dasonomia – Rua do Horto, 931 – São Paulo, SP – 02377-000 – santinijunior@yahoo.com.br

⁴Forest Engineer, Professor Dr. in Structural Engineering – Departamento de Ciência Florestais – Escola Superior de Agricultura “Luiz de Queiroz”/ESALQ – Universidade de São Paulo/USP – Cx. P. 9 – Piracicaba, SP – 13400-970 – jngarcia@esalq.usp.br

⁵Biologist, Researcher at the Forest Institute of São Paulo State – Divisão de Dasonomia – Rua do Horto, 931 – São Paulo, SP – 02377-000 – sflorsheim@if.sp.gov.br

inferior woods, making them potentially unsuitable for some applications (LATORRACA & ALBUQUERQUE 2000). According to Larson (1969), fertilization of young trees not only increases the area of juvenile wood but can also delay the transition to mature wood.

Zobel (1992) argues that fertilization of hardwoods is more common than fertilization of softwoods, yet literature is highly conflicting as to the effects of this fertilization on the quality of wood produced. Thus, no concrete assessment has been conducted of the influence of nutrients on the development and on physical, mechanical, anatomical and chemical properties of wood. While citing various authors, Vital (1990) points to conflicting results about the effect of fertilization on wood density, with values decreasing, increasing or remaining unchanged for this physical property, yet he stresses that where density decreases, the bulk wood output per hectare will increase due to greater volumetric growth. This assertion was corroborated by Andrade et al. (1994) and Barreiros et al. (2007).

Mello (1968) investigated the effect of NPK and limestone application on the quality of *Eucalyptus saligna* wood and observed that basic density, fiber length and thickness were not affected by the application of primary macronutrients, except limestone (2 t ha^{-1}), which significantly reduced fiber length. Similarly, Andrade et al. (1994) observed significant reductions in fiber length and increasing concentration of extractives in *Eucalyptus grandis* wood due to application of calcitic limestone.

Zobel (1992) argues that, overall, fertilizer application in small, continual dosages to a forest stand does not affect wood characteristics, while large, erratic dosages have stronger effect on wood quality and, additionally, that fertilizer application can cause change to the anatomical structure and chemical composition of the cell wall as well as to physical and mechanical properties. Sgarbi et al. (2000) observed that sulfur deficiency significantly reduced basic density in juvenile wood of *Eucalyptus grandis* x *Eucalyptus urophylla* while Ca deficiency produced short-fibered wood.

Silveira (2000) assessed genetic materials in an attempt to identify their efficiency in potassium absorption and also analyzed its effect on wood quality in four *Eucalyptus grandis* progenies. Progenies showed different response regarding basic density depending on potassium dosage in the nutrient solution, with higher dosages resulting in increased fiber width and length.

After investigating the effects of different boron dosages on physical and anatomical characteristics of juvenile wood in two *Eucalyptus grandis* stands at age 24 months, Bouchardet (2002) concluded that dosages did not significantly affect basic density or anatomical characteristics of wood.

This work aims to study the effect of fertilization at age five years on cell size, axially and radially, of *Eucalyptus grandis* wood as assessed at age 21 years.

2 MATERIAL AND METHODS

The material used in this study was obtained from an experimental stand of *Eucalyptus grandis* Hill Ex Maiden at age 21 years, located in the municipality of Lençóis Paulista, São Paulo, Brazil. The local soil is a medium texture red-yellow latosol, and local climate was characterized as being Cwa type, according to Köppen classification (STAPE & MARTINI 1991). The experiment consisted of two plots 900 m^2 in size, each representing one treatment. Trees were planted in 1982 with spacings of $3.0 \times 1.5 \text{ m}$, to a total of 2,222 trees per hectare. 222 kg/ha of NPK 06:30:06 was applied in this initial stage, distributed along planting furrows. In 1987, selective thinning was done at a 37% rate. After trees were thinned, fertilization was done using $1,000 \text{ kg}$ of NPK 12:06:12 and 100 kg of lime sludge per hectare, distributed in-between rows of one plot only, thus differentiating treatments with and without fertilizer.

An initial experimental inventory was compiled and, based on results of diameter distribution, an intermediate class was defined (DBH 25 to 30 cm) for the sampling procedure. Four trees were selected and duly identified in each plot. Two trunk sections 1.70 m in length were removed from each selected tree, with log 1 being cut at the base and log 2 being cut 4.70 m above ground.

Logs were sawn into diametrical planks using a bandsaw in such way as to produce representative samples of positions 0%, 25%, 50%, 75% and 100% about the tree radius, necessary to assess pith to bark variation thoroughly. The central plank was subdivided by crosswise cuts into four pieces 43 cm in length, and each piece was further cut lengthwise into two or three strips ($4 \times 4 \times 43 \text{ cm}$), as illustrated in Figure 1.

To obtain cell size in each wood strip, samples 2 cm^3 in size were taken for analysis of the following variables: vessel element length (VL), vessel diameter (VD), vessel frequency (VF), ray height (RH), ray width (RW), ray frequency (RF), fiber length (FL), fiber diameter (FD), fiber lumen diameter (FLD) and fiber wall thickness (FWT).

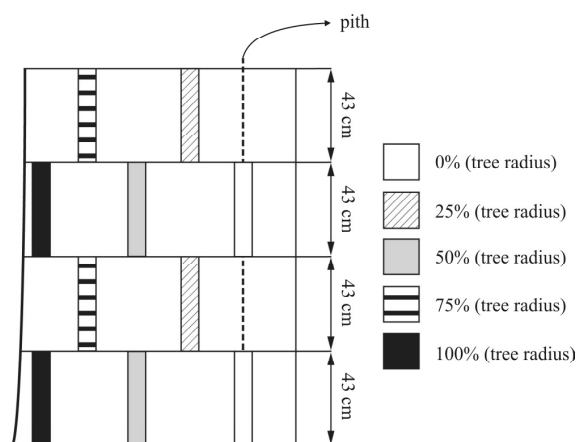


Figure 1 – Central plank representing radial positions from which samples were taken.

Figura 1 – Prancha central representando as posições radiais de retirada dos corpos-de-prova.

To obtain histological sections, specimens were softened in boiling water and glycerin (4:1) until fit for cutting. Sections 20 and 30 μm in size were obtained from the transverse and tangential planes using a 1208 Leitz microtome. Each section was bleached with sodium hypochlorite (60%), washed in water, then stained with safranin and astra blue at 1% (9:1) (JOHANSEN 1940) and mounted on water and glycerin solution. The dissociated tissue was prepared according to the Franklin method modified (BERLYN & MIKSCH 1976), then stained with alcoholic safranin and mounted on water and glycerin solution (1:1). The terminology used for anatomical analyses followed IAWA Committee recommendations (1989). All measurements were made using a trinocular optical microscope coupled to an image capturing video camera and a measurement system (Olympus BX50 with image analysis software Image – Pro Express version 6.3). Sampling was based on n=25 readings.

To calculate fiber wall thickness, the following expression was used (1):

$$FWT = \frac{FD - FL}{2} \quad (1)$$

Tested hypotheses included: a) fertilization at age five years does not influence cell size; b) there are no variations in cell size along the radius of the *Eucalyptus grandis* logs. c) there are no variations in cell size along the height of *Eucalyptus grandis* trees.

For analysis of variables, a variance homogeneity test was applied using Hartley's test. The experiment was run in a completely randomized design with a 2 x 2 x 5 factorial arrangement (fertilizer x trunk section x radial position). The Tukey test was applied, at the 5% significance level, whenever significant differences were observed between treatments in the F test. The relationship between fiber size and radial position was also analyzed using regression analysis at the 95% probability level. Data analysis was based on procedure PROC GLM and PROC REG of statistical program SAS (SAS INSTITUTE 1999).

3 RESULTS AND DISCUSSION

Table 1 provides a summary of the analysis of variance of cell element size in *Eucalyptus grandis* wood at age 21 years, as a function of each factor. It was noted that, as regards the fertilization factor, only vessel frequency was significantly influenced. As for log position along tree height axis, significant differences were observed only for vessel element length. As for radial position, significant differences occurred for: fiber length, fiber diameter, fiber wall thickness, vessel element length, vessel diameter and vessel frequency.

Vessel frequency was reduced in trees fertilized at age five in comparison to trees without fertilizer (Table 2 and Figure 2). Bamber et al. (1982) also observed a reduced vessel frequency in wood of *Eucalyptus grandis* trees at age 2.5 years that had been induced to high growth rates by fertilizer application. The same tendency was observed by Wilkes & Abbott (1983) after comparing fast growing with slow growing trees of some *Eucalyptus* species. Sette Júnior et al. (2009) concluded that application of mineral fertilizers (potassium and sodium) did not significantly influence vessel element size and frequency in wood of *Eucalyptus grandis* 24 months old. The same authors also stressed the importance of assessing vessel elements in the formation of heartwood and sapwood in *Eucalyptus* trees, at more advanced ages, key to understanding the xylem sap conducting mechanism in sapwood of trees with differing volumes of tissue and leaf area that are subjected to fertilization, with effects of fertilization being dependent on several factors, including tree age, fertilizer type and application season. In a study of *Eucalyptus grandis* trees at age 7 years, Rocha et al. (2004) observed that the wood from the dominated class (smaller trees) showed greater vessel frequency than tissue from the dominant class (larger trees).

Overall, in this study, fertilization did not influence

Table 1 – Analysis of variance of anatomical characteristics: fiber length (FL), fiber diameter (FD), fiber lumen (FLD), fiber wall thickness (FWT), vessel elements length (VL), vessel diameter (VD), vessel frequency (VF), ray width (RW), ray height (RH) and ray frequency (RF) of *Eucalyptus grandis* at age 21 years.

Tabela 1 – Resumo da análise de variância para as características anatômicas: comprimento das fibras (FL), diâmetro das fibras (FD), lume das fibras (FLD); espessura da parede das fibras (FWT), comprimentos dos elementos de vaso (VL), diâmetro dos vasos (VD), frequência dos vasos (VF), largura dos raios (RW), altura dos raios (RH) e frequência dos raios (RF) de *Eucalyptus grandis* de 21 anos.

Source	DF	Mean square									
		FL (mm)	FD (μm)	FLD (μm)	FWT (μm)	VL (μm)	VD (μm)	VF (n°mm ⁻²)	RW (μm)	RH (μm)	RF (n°mm ⁻¹)
Fertilization (A)	1	0.013 n.s.	0.362 n.s.	2.976 n.s.	0.316 n.s.	7880 n.s.	226 n.s.	107 **	0.98 n.s.	253 n.s.	0.492 n.s.
Trunk section (T)	1	0.008 n.s.	2.606 n.s.	0.017 n.s.	0.762 n.s.	42897 **	194 n.s.	10.62 n.s.	10.90 n.s.	250 n.s.	0.699 n.s.
(A) x (T)	1	0.005 n.s.	0.001 n.s.	2.899 n.s.	0.751 n.s.	345 n.s.	2.076 n.s.	4.21 n.s.	0.72 n.s.	924 n.s.	0.012 n.s.
Radial position (P)	4	0.345 **	45.420 **	6.477 n.s.	5.378 **	91909 **	6486 **	182 **	9.43 n.s.	425 n.s.	0.302 n.s.
(A) x (P)	4	0.007 n.s.	2.724 n.s.	2.457 n.s.	0.077 n.s.	11940 n.s.	215 n.s.	14.96 n.s.	2.62 n.s.	823 n.s.	0.275 n.s.
(T) x (P)	4	0.005 n.s.	3.767 n.s.	5.151 n.s.	3.767 n.s.	3985 n.s.	726 n.s.	13.42 n.s.	9.68 n.s.	410 n.s.	0.842 n.s.
(A) x (T) x (P)	4	0.003 n.s.	5.556 n.s.	5.173 n.s.	0.139 n.s.	1360 n.s.	294 n.s.	8.94 n.s.	4.42 n.s.	951 n.s.	0.517 n.s.
Residual	60	0.006	4.497	3.380	0.292	4795	396	7.32	6.65	790	0.682
Mean		0.99	22.75	13.36	4.295	630.20	130.95	13.82	13.53	206.68	9.59
SD		0.15	2.52	1.90	0.73	99.08	26.40	4.44	2.65	28.53	0.79
CV _e (%)		7.75	9.32	13.77	11.51	10.99	15.21	19.57	19.07	13.60	8.61

Where: ** significant at the 1% significance level; n.s. = nonsignificant; SD = standard deviation and CV_e = coefficient of experimental variation.

Table 2 – Means of anatomical characteristics: fiber length (FL), fiber diameter (FD), fiber lumen (FLD), fiber wall thickness (FWT), vessel elements length (VL), vessel diameter (VD), vessel frequency (VF), ray width (RW), ray height (RH) and ray frequency (RF) in *Eucalyptus grandis* at age 21 years.

Tabela 2 – Médias das características anatômicas: comprimento das fibras (FL), diâmetro das fibras (FD), lume das fibras (FLD); espessura da parede das fibras (FWT), comprimentos dos elementos de vaso (VL), diâmetro dos vasos (VD), frequência dos vasos (VF), largura dos raios (RW), altura dos raios (RH) e frequência dos raios (RF) de *Eucalyptus grandis* de 21 anos.

Treatment	FL (mm)	FD (μm)	FLD (μm)	FWT (μm)	VL (μm)	VD (μm)	VF (n°mm ⁻²)	RW (μm)	RH (μm)	RF (n°mm ⁻¹)
Without fertilizer	0.9 ^a	22.6 ^a	13.1 ^a	4.7 ^a	620.2 ^a	129.2 ^a	14.9 ^a	13.4 ^a	208.4 ^a	9.6 ^a
With fertilizer	1.0 ^a	22.8 ^a	13.5 ^a	4.6 ^a	640.1 ^a	132.6 ^a	12.6 ^b	13.6 ^a	204.9 ^a	9.5 ^a
Log 1	0.9 ^a	22.9 ^a	13.3 ^a	4.8 ^a	607.0 ^b	129.3 ^a	13.4 ^a	13.1 ^a	208.4 ^a	9.5 ^a
Log 2	1.0 ^a	22.5 ^a	13.4 ^a	4.6 ^a	653.3 ^a	132.5 ^a	14.1 ^a	13.9 ^a	204.9 ^a	9.7 ^a
Radial position (0%)	0.7 ^d	20.3 ^c	12.4 ^a	3.9 ^d	512.7 ^c	101.0 ^c	19.6 ^a	12.5 ^a	200.8 ^a	9.8 ^a
Radial position (25%)	0.9 ^c	21.6 ^{bc}	12.9 ^a	4.3 ^{cd}	600.9 ^b	121.2 ^b	13.7 ^b	13.2 ^a	201.5 ^a	9.6 ^a
Radial position (50%)	1.0 ^b	23.3 ^{ab}	13.9 ^a	4.7 ^{cd}	659.7 ^{ab}	136.1 ^{ab}	12.5 ^b	13.7 ^a	210.1 ^a	9.6 ^a
Radial position (75%)	1.1 ^{ab}	24.2 ^a	14.0 ^a	5.1 ^{ab}	705.8 ^a	145.3 ^a	11.5 ^b	13.5 ^a	208.6 ^a	9.5 ^a
Radial position (100%)	1.1 ^a	24.1 ^a	13.4 ^a	5.4 ^a	671.7 ^a	150.9 ^a	11.7 ^b	14.6 ^a	212.1 ^a	9.4 ^a

Please note: Means followed by different letters in the same column differ from each other (at the 5% significance level)

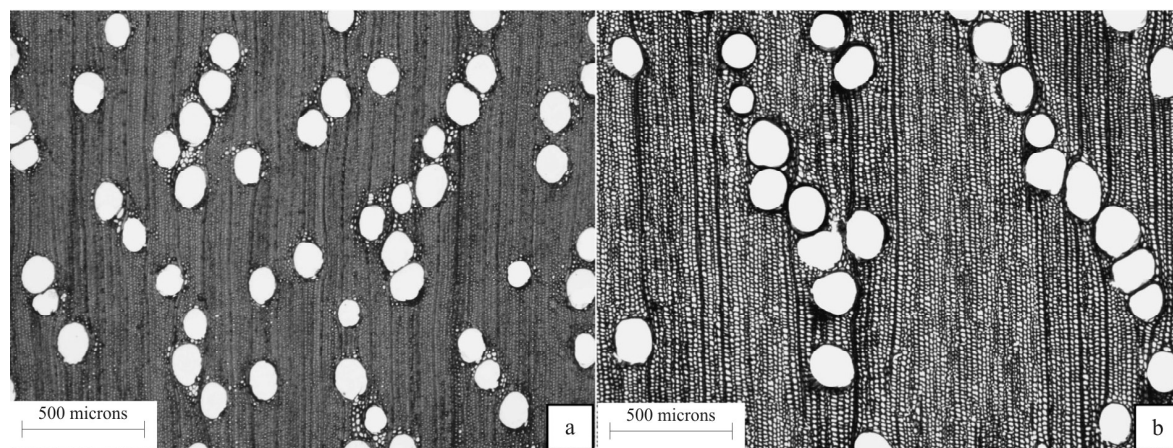


Figure 2 – Photomicrographs of *Eucalyptus grandis* wood at age 21 years. a) Transverse section, fertilized wood b) Transverse section, unfertilized wood. Bar scale = 500 μ m.

Figura 2 – Fotomicrografias do lenho de *Eucalyptus grandis* de 21 anos. a) Secção transversal madeira adubada b) Secção transversal madeira não adubada. Barra = 500 μ m.

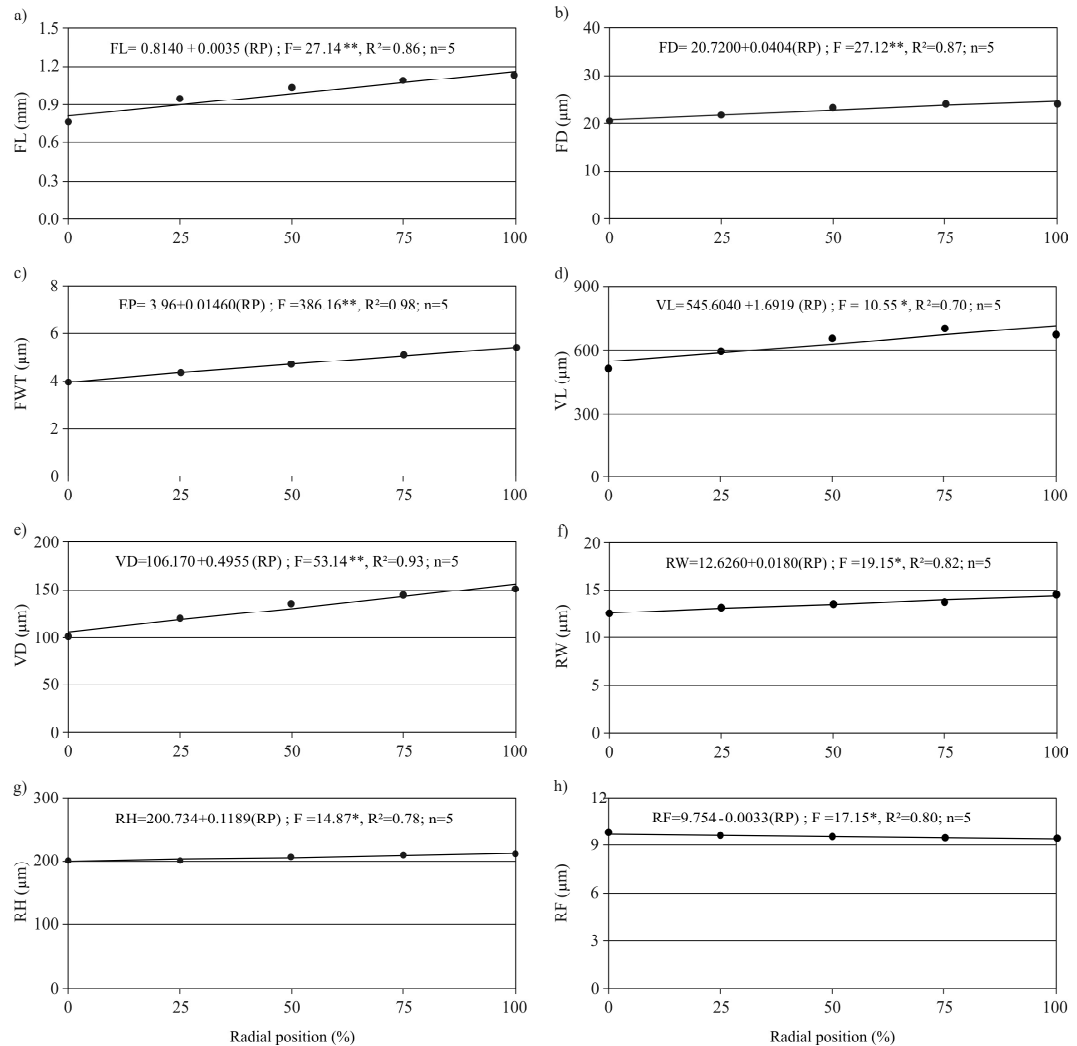
anatomical characteristics, and this outcome may be a result of wood formation at the time of fertilization or decreasing influence of fertilization with the passing of years (Table 2). These results agree Malan (1991), Malan & Hoon (1992) and Melo (1968), who observed that cell size in *Eucalyptus grandis* is hardly influenced by growth rate. Sette Júnior et al. (2009) observed that fertilizer application (potassium and sodium) induced change in fiber size of *Eucalyptus grandis*.

As regards log position in relation to tree height, this factor only influenced vessel element length (Table 2). Log section 2 showed longer length than log section 1. This result may be related to cambium maturity, considering log taper, with log 2 showing a smaller proportion of position 0% (pith) in comparison to log 1, and as this portion shows lower values of vessel element length, it is assumed that the smaller proportion of pith in log 2 has contributed toward a higher average value of vessel element length. Rocha et al. (2004) also observed vessel element length to be smaller in logs close to the base, in *Eucalyptus grandis* trees at age 7 years. Obtained results for all variables are in agreement with Rocha et al. (2004), who observed hardly any significant influence of tree height on tissue cell size. Quilhó et al. (2006) found little variation in fiber size as a function of tree height in a stand of *Eucalyptus grandis* x *Eucalyptus urophylla*.

As regards pith to bark variation, significant differences were noted, between positions 0% and 100%

of the log radius in particular, for: fiber length, fiber diameter, fiber wall thickness, vessel element length, vessel diameter and vessel frequency (Table 2). In position 100% (close to bark), higher values were found for fiber length, fiber diameter, fiber wall thickness, vessel element length and vessel diameter. And higher values of vessel frequency were found in position 0% (close to pith) (Table 2).

These variations in wood cell size result from the gradual increase in adult wood proportion in relation to juvenile wood in the pith to bark direction, and also the pattern of variation in the base to top direction. In order to better explain these variations an analysis was carried out of relationships between cell size and radial positions. Figure 3 illustrates regression models providing best fit to these variations. According to the obtained linear regression model, an increasing tendency is noted in the pith to bark direction for fiber length, fiber diameter, fiber wall thickness, vessel element length, vessel diameter and ray width, while for ray height a decreasing tendency is noted (Figure 3). These variations in cell size result from the gradual increase in proportion of adult wood to juvenile wood in the pith to bark direction. Silva et al. (2007) noted an increase in fiber length in the pith to bark direction for *Eucalyptus grandis*. With *Eucalyptus grandis*, *Eucalyptus saligna* and *Eucalyptus grandis* x *urophylla*, Alzate (2009) noted an increase in length and in width of pith fibers up until the 75% portion of the radius, followed by a tendency toward stabilization, and also an increase in wall



Where: ** significant at the 1% significance level and * significant at the 5% significance level

Figure 3 – Relationship between radial position (RP) and fiber length (FL) [a], fiber diameter (FD) [b], fiber wall thickness (FWT) [c], vessel element length (VL) [d], vessel diameter (VD) [e], ray width (RW) [f], ray height (RH) [g] and ray frequency (RF) [h] for *Eucalyptus grandis* at age 21 years.

Figura 3 – Relações entre posição radial (RP) e comprimento das fibras (FL) [a], diâmetro das fibras (FD) [b], espessura da parede das fibras (FWT) [c], comprimentos dos elementos de vaso (VL) [d], diâmetro dos vasos (VD) [e], largura dos raios (RW) [f], altura dos raios (RH) [g] e frequência dos raios (RF) [h] para *Eucalyptus grandis* de 21 anos de idade.

thickness and a reduction in fiber lumen up until the 50%-75% portion of the radius. With *Eucalyptus grandis*, Rocha et al. (2004) noted that in the pith to bark direction all fiber sizes showed an increasing tendency; vessels showed an increasing tendency concerning tangential diameter and length

and a decreasing tendency concerning frequency, while ray frequency showed a decreasing tendency. Quilhó et al. (2006) observed that fiber size in clones of *Eucalyptus grandis* x *Eucalyptus urophylla* showed no defined variation tendency in the pith to bark direction.

4 CONCLUSIONS

Vessel frequency is significantly influenced by fertilization at age five years, with lower values being found for fertilized trees. Other anatomical characteristics were found not to be significantly influenced by fertilization.

Vessel element length was influenced by log position along the trunk, with vessel element in log 1 (base log) being significantly smaller than vessel elements in log 2 (higher in the trunk). Other anatomical characteristics were found not to be significantly influenced by tree height.

Fiber wall thickness, diameter and length, and also vessel frequency, diameter and length, were influenced by radial position. In positions closer to the bark, values of fiber wall thickness, diameter and length, and also vessel diameter and length, were higher than in positions close to the pith, with inverse values found for vessel frequency (close to the pith). Ray height, ray width, ray frequency and fiber lumen were found not to be significantly influenced by radial position.

An increasing tendency was observed in the pith to bark direction, according to the linear regression model, for: fiber wall thickness, diameter and length, vessel diameter and length, and ray width, while for ray frequency in the pith to bark direction a decreasing tendency was observed.

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