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Estudo comparativo da anatomia de madeira e casca de duas espécies de eucalipto

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Resumo: Neste trabalho foram caracterizadas e comparadas, do ponto de vista da anatomia da madeira e casca, duas espécies de eucalipto: *Eucalyptus sideroxylon* e *E. viminalis*. O trabalho aqui apresentado faz parte do projecto "EucPlus-Novos processos e utilizações para madeira de eucalipto" (PTDC/AGR-CFL/119752/2010), coordenado pelo Centro de Estudos Florestais.

Os eucaliptos, com 4 anos de idade, cresceram numa área experimental localizada no campus do Instituto Superior de Agronomia da Universidade Técnica de Lisboa. A cada árvore foi retirada uma rodela ao nível do DAP que serviu de base para o estudo da variação radial dos vasos, fibras e raios.

A madeira foi estudada macroscópica e microscopicamente. Ao nível macroscópico foram determinadas a área de vasos e o número de vasos por mm^2 ao longo do raio (da medula para a casca). Fizeram-se cortes histológicos (com utilização de micrómetro), segundo três planos - tangencial (em três posições do raio 30%, 60% e 90%), transversal e radial - onde se caracterizaram as duas espécies ao nível microscópico. A caracterização biométrica das fibras da madeira foi realizada em material dissociado recolhido em três posições do raio (30%, 60% e 90%), determinando-se a largura, espessura e o comprimento das fibras. Os raios foram medidos nas três posições no plano tangencial. Utilizaram-se técnicas de microscopia de luz transmitida e análise de imagem. A casca foi caracterizada nos três planos: tangencial, transversal e radial. A anatomia da casca destas duas espécies não tinha sido ainda investigada. A caracterização e variabilidade anatómica (lenho e casca) destas duas espécies permitirão contribuir para a avaliação do seu potencial comportamento do ponto de vista físico, mecânico e químico.

Palavras-chave: Eucaliptos, vasos, caracterização biométrica, fibras, casca.

Abstract: Two eucalypt species - *Eucalyptus sideroxylon* and *E. viminalis* - were characterized and compared regarding the anatomy of wood and bark. This work is part of the project "New EucPlus processes and uses for eucalyptus wood" (PTDC/AGR-CFL/119752/2010), coordinated by the Center for Forest Studies.

The 4-year-old eucalypt trees grew up in an experimental area located on the campus of the School of Agriculture, Technical University of Lisbon. From each tree a sample was taken at 1.30 m height (DBH) and used for studying the radial variation of the vessels, fibres and rays.

The wood was studied at a macroscopic and microscopic level. Macroscopically the area and number of vessels per mm^2 along the ray (from pith to bark) was determined. Histological sections were obtained (using a micrometer) in three directions - tangential (representing 30%, 60% and 90% of the ray), transverse and radial - and the two species characterized at the microscopic level. The biometric characterization of wood fibres was done in dissociated material at 30%, 60%

and 90% of the ray, by measuring width, thickness and length. The rays were measured at the three positions in the tangential direction. Transmitted light microscopy and image analysis were used. The bark was characterized in three directions: tangential, radial and transversal. The bark anatomy of these two species has not yet been investigated. The characterization and anatomical variability (wood and bark) of these two species will contribute to assess their potential behavior regarding physical, mechanical and chemical properties.

Keywords: Eucalyptus, vessels, biometric characterization, bark.

1. INTRODUCTION

The knowledge of size and structure of anatomical elements which influence the wood and bark properties in *Eucalyptus* are a useful tool to improve the quality of the final products (CARVALHO 1997, QUILHÓ *et al*. 2000, MIRANDA and PEREIRA 2002, RAMÍREZ *et al*. 2009, PEREIRA *et al*. 2011).

Although *Eucalyptus* had their origin in Australia, they are present today in other regions, as is the case of *Eucalyptus globulus* in Portugal and Spain (PEREIRA *et al*. 2011). Only a few species are from Indonesia and Papua New Guinea (GONZALEZ *et al*. 2011). Several species are exotics in different regions i.e. *E. nitens* in Portugal and Spain as well as in Argentina and Chile*; E. grandis* in the sub-tropical and tropical zones of Argentina, China, Brazil, India, South Africa, Uruguay and Vietnam (FORRESTER *et al*. 2010). In spite of the numerous anatomical studies of wood (CARVALHO 1997, MIRANDA and PEREIRA 2002, RAMÍREZ *et al*. 2009) and bark (CHATTAWAY 1955a, 1955b, 1955c; ALFONSO 1987, QUILHÓ *et al*. 1999, 2000) of the genus *Eucalyptus*, the information on *E. viminalis* e *E. sideroxylon* is scarce.

The study of the anatomic characteristics is important because performance and potential value of products depend on a wide range of interlinked fundamental wood characteristics (HUANG *et al*. 2003). The secondary xylem includes different cell types (i.e vessels, fibres, axial and radial parenchyma in xylem or in phloem) originated by the vascular cambium; this cambium also differentiates the secondary phloem with sieve tubes, fibres, axial and radial parenchyma and sclereids (EVERT 2006). The secondary phloem, the periderm and rhytidome represent the bark (RICHTER *et al*. 1996). For trees growing in plantations, these wood and bark cells are affected by some factors such as site, ecological conditions, management, genetics and age (ZOBEL and VAN BUIJTENEN 1989).

The main objective of this study was to characterize and compare the anatomic structure of wood and bark of *E. viminalis* and *E.sideroxylon*.

2. MATERIAL & METHODS

This study was conducted in *E. viminalis* and *E. sideroxylon* trees with 4 years of age from an experimental site located in the campus fields of the School of Agriculture, Technical University of Lisbon, at Tapada da Ajuda, Lisboa, Portugal (38°42´N; 09°10´W). The region is under the influence of a mesothermal humid climate, with a dry season in the summer extending from June to August, and registering above 10°C in the coldest month and below or equal to 22°C in the hottest month. The soil is a vertisol characterized by a fine, or medium to fine, texture, derived from tuffs or basalts, frequently with limestone on the inferior horizons, or from calcareous rock (in much less extension).

In each tree samples with 10 cm thickness were collected at 1.30 of the tree height (DBH) and wood and bark were separated.

The wood was studied macroscopically: vessel area was determined from pith to bark, using the Leica software Qwin V 3.5.0, after acquisition of a sequence of images of each ray through a digital camera Leica DFC 320 coupled to Leica Magnifier MZ6. Microscopically the wood was characterized at three radial points: near pith, 30% of the radius; middle, 60%; and near the cambium, 90%. Transversal, tangential and radial sections with 17 µm thickness were obtained, using a micrometer, stained with safranin and mounted in Eukitt. Ray height and number of cells were measured in 40 uniseriate rays, in tangential sections. The length, width and wall thickness of 40 fibres were measured on dissociated material using image analysis LAS software V4.2 assisted by a digital camera EC3 coupled to a transmitted light Dialux 22 EB microscope. Descriptive terminology follows the IAWA List of Microscopic Feature for Hardwood Identification (IAWA COMMITTEE 1989).

The bark samples were impregnated with DP 1500 polyethylene glycol. Transversal, tangential and radial sections of approximately 17 µm thickness were prepared with a Leica SM 2400 microtome using Tesafilm 106/4106 adhesive for sample retrieval (QUILHÓ *et al*. 1999). The sections were stained with a triple staining of chrysodine/acridine red and astra blue and mounted on Eukitt. Light microscopic observations were made using Leica DM LA and photomicrographs were taken with a Nikon Microphot-FXA. The terminology follows RICHTER *et al*. (1996).

3. RESULTS & DISCUSSION

3.1. Anatomical characterization

Figure 1 shows the wood structure of *E. viminalis* (Fig. 1 A-C) and *E. sideroxylon* (Fig. 1D – E). The two species are diffuse-porous with solitary vessels, which are circular to oval and arranged in a diagonal pattern (Fig. 1A, D); the axial parenchyma is of different types i.e. apotracheal diffuse and diffuse in aggregates or paratracheal vasicentric uni or circunvascular (Fig. 1A, D); the rays are mostly uniseriate, occasionally biseriate (Fig. 1B, E) and homogenous with procumbent cells (Fig. 1C, F). The same type of vessels, axial and radial parenchyma are found in other *Eucalyptus* spp. (OLIVEIRA and FREITAS 1970, ALFONSO 1987, PEREIRA *et al*. 20011).

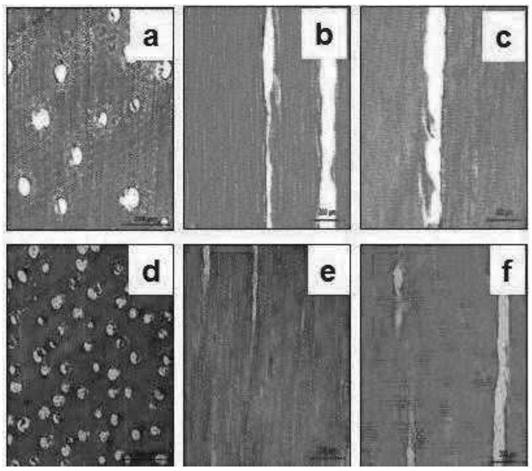

Figure1. Wood microscopic details: **a** Transverse section of *E. viminalis*; **b** Tangential section of *E. viminalis*; **c** Radial section of *E. viminalis*; **d** Transverse section of *E. sideroxylon*; **e** Tangential section of *E. sideroxylon*; **f** Radial section of *E. sideroxylon.* Scale bar = 300 µm.

Figures 2 and 3 show the bark structure of *E. viminalis* and *E. sideroxylon*. The bark of *E.viminalis* is described as almost wholly smooth, or rough grey and persistent on the lower part of the trunk, shed in long ribbons from the upper trunk and branches, leaving a smooth white or yellowish surface; in contrast, *E. sideroxylon* has an ironbark, persistent to the small branches, hard and deeply furrowed, dark brown to black (BOLAN *et al*. 1992). However the studied trees were very young and exhibited a similar structure, in both species; this is in accordance with observations of CHATTAWAY (1955a, 1955b, 1955c) and ALFONSO (1987) from young eucalypt barks.

The barks present the following features: a periderm with two types of phellem cells (suberized and lignified cells) and a poorly developed phelloderm (Fig. 2A and 3A); a thin layer of non-collapsed phloem where tangential bands of fibres alternate with thin layers of axial parenchyma cells and sieve tube elements (Fig. 2B and 3B); the collapsed phloem includes some dilatation tissue as a result of tree growth i.e expanded axial parenchyma cells (Fig. 2C and 3C); the rays are mainly uniseriate (Fig. 2D and 3D) and homogenous (Fig. 2E and 3E); clusters of expanded parenchyma cells are present in both species (Fig. 2E and 3E), more evident in *E. viminalis.* The bark structure of these species at this age resembles the bark structure of *E.globulus* (QUILHÓ *et al*.1999).

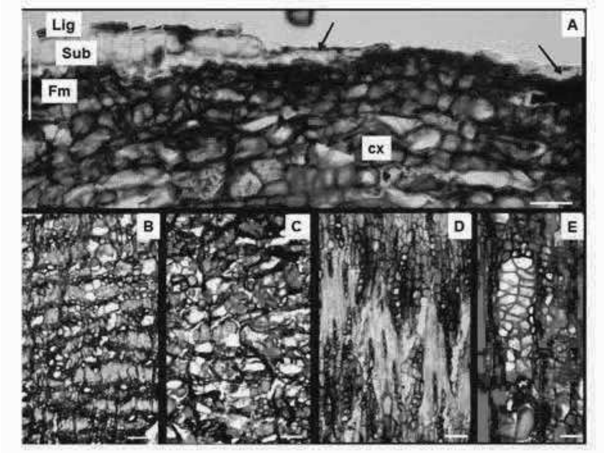

Figure 2. Microscopic structure of *E. viminalis* bark. A – Periderm including the lignified (lig) and suberized (sub) cells of the phellem and 2-3 cells of the phelloderm; deposits of suberin (arrow); cortex (cx). (transverse section) B – phloem with non collapsed phloem and collapsed phloem (transverse section); C- collapsed phloem (transverse section); D- phloem (tangential section); E- phloem (radial section). Scale bar = 50 µm.

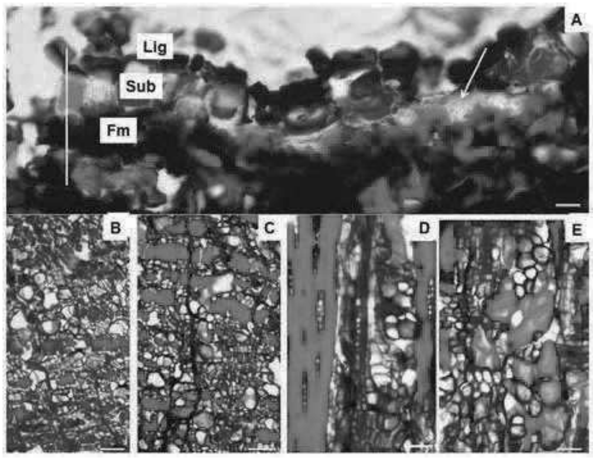

Figure 3. Microscopic structure of *E. sideroxylon* bark. A – Periderm including the lignified (lig) and suberized (sub) cells of the phellem and 1 cell of the phelloderm; deposits of suberin (arrow); cortex (cx). (transverse section) B – phloem with non collapsed phloem and collapsed phloem (transverse section); C- collapsed phloem (transverse section); D- phloem (tangential section); E- phloem (radial section). Scale bar = 50 µm

3.2. Biometry

E. sideroxylon fibre length increased from an average 740.5 µm near the pith to 859.7 µm near the cambium (Table 1). This trend was not observed for *E. viminalis*. The most frequent pattern of radial variation of fibre length and wall thickness in the *Eucalyptus* genus is an increase from pith to near cambium (PEREIRA *et al*. 2011). This pattern of radial variation was found in *E. sideroxylon*. *E.*

tereticornis showed a higher fibre length value of 850 at 4 years of age (RAO *et al*. 2002), when compared to *E. viminais* and *E. sideroxylon*.

Fibre width increased radially in *E. viminalis* (19.8 µm near pith and 20.6 µm near bark), but decreased in *E. sideroxylon*. Fibre wall thickness decreased from 3.8 µm to 3.0 µm and 2.7 µm, respectively, at the pith, middle and near cambium positions in *E. viminalis*. In *E. sideroxylon* the fibre wall thickness was not significantly different from pith to bark, with average values ranging from 3.8 µm of 4.2 µm and to 3.8 µm in the three radial positions. These values of wall thickness are within the range of 1.7 µm to 6.6 µm reported for *E.globulus* at 9 and 12 years of age (PEREIRA *et al*. 2011).

Vessel area increased from pith to bark for *E.viminalis* and *E. sideroxylon* (Table 1). Vessels were smaller near the pith (0.0078 mm^2 for *E. viminalis* and 0.0034 mm^2 for *E. sideroxylon*), increasing afterwards (0.0079 mm^2 for *E.viminalis* and 0.0035 mm^2 for *E. sideroxylon*) and reaching its maximum near the bark (0.0082 mm^2 for *E. viminalis* and for *E.sideroxylon*). The same pattern of variation was found in *E. globulus* (PEREIRA *et al*. 2011) i.e. vessels area increased from the pith to near cambium.

Table 1. Results of fibre variables (length, width and wall thickness) and vessel area measured at tree radial position (30%, 60% and 90% of radius) of *Eucalyptus viminalis* and *E. sideroxylon*

Species	*Position (%)*	*Fibre length (µm)*	*Fibre width (µm)*	*Fibre wall thickness (µm)*	*Vessel area (mm^2)*
E. viminalis	30	616.1±83.2	19.8±3.9	3.8±0.8	0.0078±0.003
	60	908.8±84.7	17.2±2.8	3.0±0.38	0.0079±0.041
	90	699.7±73.5	20.6±3.0	2.7±0.7	0.0082±0.004
E. sideroxylon	30	740.5±68.7	13.3±1.5	3.8±0.45	0.0034±0.0009
	60	816.8±97.8	20.4±3.0	4.2±0.76	0.0035±0.0009
	90	859.7±74.8	13.3±1.3	3.8±0.59	0,0035±0.001

Table 2 shows the dimensions of uniseriate rays. The ray height increased radially from pith to bark in *E. sideroxylon* (108.6 µm to 124.7 µm), and decreased in *E. viminalis* (166.5 µm to 153.3 µm). *E. sideroxylon* showed 28% of bisseriate rays near pith and 34% near the cambium. In *E. viminalis* only uniseriate rays were observed.

Regarding the variance analysis (table 3) results indicated that species and radial position and interaction between species and position had a very significant effect in all the fibre biometric variables ($p<0.001$). Interaction between species and position accounted for most of the variability (35%, 52% and 24% of the total variation for fibre length, width and wall thickness, respectively). Species had a significant effect in fibre wall thickness (28%). Between-fibre variation (error component) was high at 50%, 64% to 60% of the total variation respectively.

Table 2.Variable of uniseriate rays measured at three radial position (30%,60%,90%) of *E.viminalis* and *E.sideroxylon*

Specie	*Position (%)*	*Height (µm)*
E.viminalis	30	166.5±45.7
	60	149.0±44.2
	90	153.3±43.5
E.sideroxylon	30	108.6±25.4
	60	107.9±28.1
	90	124.7±38.9

Table 3. Summary of variance analysis for fibre length, width and wall thickness measured at tree radial position (pith, middle and bark) for *E.viminalis* and *E.sideroxylon*

		df	*F*	*P*	*Exp.Var.(%)*
Species	Fibre length	1	36.6	<0.001	8
	Fibre width	1	95.1	<0.001	19
	Fibre wall thickness	1	83.1	<0.001	28
Position	Fibre length	2	101.1	<0.001	4
	Fibre width	2	15.1	<0.001	5
	Fibre wall thickness	2	13.5	<0.001	7
Species*Position	Fibre length	2	55.1	<0.001	35
	Fibre width	2	85.8	<0.001	52
	Fibre wall thickness	2	23.9	<0.001	24
Error	Fibre length		234		26
	Fibre width		234		25
	Fibre wall thickness		234		41

4. CONCLUSIONS

The anatomic structure of wood and bark of *E. viminalis* and *E. sideroxylon* was compared. The wood and bark structure of young *E. viminalis* and *E. sideroxylon* species are similar.

Both species presented the same radial pattern of the vessels area and similar to *E .globulus* i.e. vessels area increased from pith to near cambium. Fibres of *E. sideroxylon* were higher and wider than *E. viminalis* fibers, but their wall thickness was similar. *E. viminalis* reported a higher height of unseriate rays. Interaction between species and the radial position was the most significant effect for the variation of fibre biometric variables. *E.sideroxylon* showed a similar radial pattern of fibre length to that found in the genus *Eucalyptus*.

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