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WITHIN–TREE VARIATION IN PHLOEM CELL DIMENSIONS AND PROPORTIONS IN EUCALYPTUS GLOBULUS

by

Teresa Quilhó[1], **Helena Pereira**[2] **& Hans Georg Richter**[3]

SUMMARY

The axial variation of bark thickness and quantitative anatomical features of *Eucalyptus globulus* bark were analysed for one site based on individual measurements of ten 15-year-old trees at six height levels (DBH, 5%, 15%, 35%, 55% and 75% of total tree height). The parameters studied were: length, tangential diameter and percentage of sieve tubes; length, width, cell wall thickness and percentage of fibres; height and percentage of rays; percentage of sclereids in the secondary phloem. Bark thickness decreases from base to top of the tree. Fibre width and wall thickness decrease from base upwards. No distinct axial patterns of variation were observed for the other biometric variables studied. Parenchyma is the main cell type of the bark (50%) followed by fibres (27.9%), rays (12.1%), sieve tubes (2.7%), and sclereids (7.3%). The cell type proportions vary significantly within the tree, i.e., parenchyma, ray and sclereid proportions decrease, fibre and sieve tube proportions increase towards the top of the tree.

Key words: *Eucalyptus globulus*, bark, cell biometry, cell type proportions, bark anatomy, structural variability.

INTRODUCTION

Biomass utilisation of intensively grown short-rotation forest species has increased interest in bark, which represents a significant part of young tree stems, both as a potential raw-material, as well as in relation to its role in sustainability.

Eucalyptus globulus Labill. is one such species, managed in short rotation cycles of 6 to 10 years (in Brazil and Europe, respectively), and at present an important pulpwood worldwide. Its bark is fibrous and other uses, apart from combustion, might be possible. More knowledge on quantification and about variation in cell types may contribute to developing these uses.

1) Centro de Estudos de Tecnologia Florestal, Instituto de Investigação Científica Tropical, 1399 Lisboa Codex, Portugal.
2) Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, 1399 Lisboa Codex, Portugal.
3) Institut fur Holzbiologie und Holzschutz, Bundesforschungsanstalt für Forst- und Holzwirtschaft, Leuschnerstr. 91, 21031 Hamburg, Germany.

Variability of cell dimensions within tree bark has received less attention than within wood variations; few studies deal with quantitative changes during bark development (Parameswaran & Liese 1974; Yunus & Yunus 1979; Trockenbrodt 1994). Important changes occur along the plant axis and it is accepted that morphological variability is determined by the dimensions of the cambial initials and developmental changes that occur during cell differentiation (Ridoutt & Sands 1993). Apical intrusive growth also plays an important role in explaining the variability of cell size (Ghouse & Iqbal 1979).

Bark fibre length has been the main anatomical parameter studied in relation to age and tree height (Ghouse & Siddiqui 1976; Iqbal & Ghouse 1983). For the genus *Eucalyptus*, in particular for *E. globulus,* biometric studies have been conducted by Carvalho (1970) and Pereira and Araújo (1990).

Qualitative changes during the bark development of *E. globulus* were described earlier (Quilhó et al. 1999). This paper reports on variation in cell size and cell type proportions of *E. globulus* bark along the stem axis.

MATERIAL AND METHODS

Ten 15-year-old *Eucalyptus globulus* trees were harvested at the end of the 1st rotation period from a commercial pulpwood plantation in the region of Castelo Branco, in Central Portugal (39° 30' N; 7° 46' W; altitude 300 m; annual rainfall 825 mm and mean temperature 16 °C). Five trees were randomly selected in each of two 100-tree plots (P1 and P2). A cross-sectional disc was taken from each tree at five height levels (5%, 15%, 35%, 55%, and 75% of total tree height) and at breast height (DBH), which corresponds to approximately 7% ± 2% of total tree height. The material is the same as that used in a previous study (Quilhó et al. 1999).

Bark thickness was measured at two opposite radii. Transverse and tangential microscopic sections were prepared with a Reichert sliding microtome, thickness about 17 µm, after impregnation with DP 1500 polyethylene glycol (Richter 1990) and triple stained with chrysodine/acridine red and astra blue 1% aqueous solutions, and mounted in Euparal.

Tangential sections were cut from the cambium towards the periderm. Specimens were macerated in a solution of 30% hydrogen peroxide and glacial acetic acid 1 : 1 at 60 °C for two days and stained with astra blue.

All measurements were made using a projection microscope and a semi–automatic image analyser. The parameters measured and the number of measurements for each specimen were as follows: tangential diameter of 60 sieve tubes measured in the transverse section; length of 60 sieve tubes and height of 60 rays measured in the tangential section; length, width and cell wall thickness of 40 fibres, measured in the macerated material. The number of measurements necessary was previously calculated to give an accuracy of 5% at the 0.05 probability level.

The proportion of cell types was measured using a 54-point grid on each of ten areas in the transverse sections, from cambium to periderm. The phellem and phelloderm were not taken into account because the periderm was often incomplete. Sieve tubes were only quantified in the non-collapsed phloem. 'Expanded parenchyma cells' (Quilhó et al. 1999) are quantified separately from sclereids.

The tree bark volume was calculated as a difference between total tree volume and wood volume, by sections corresponding to the different height levels of sampling as a total of 5 conical sections; the top portion above 95% height (with a diameter under 3 cm) was disregarded. The tree average of cell dimensions and cell type proportions was calculated as a volume weighted mean following:

$$\overline{P} = \frac{\sum_{I=1}^{5} v_i * p_i}{\sum_{i=1}^{5} v_i}$$

where $\bar{P}$ is the tree mean parameter, v_i is the bark volume of each section and p_i the parameter measured at each height level.

A two-way analysis of variance with interaction was used to analyse the data. Bark anatomical terms are used as suggested by Trockenbrodt (1990) and Richter et al. (1996).

RESULTS AND DISCUSSION

Bark thickness

The bark thickness ranges from 6.5 mm at the base (5% of total tree height) to 2.0 mm at the top (75% of total tree height).

Bark thickness tends to increase with age, i.e., higher values always occur in the lower part of the stem (Fig. 1). This agrees with similar observations made for other taxa (Trockenbrodt 1994). Bramhall et al. (1977) also found a highly significant effect of height on bark thickness in *Tsuga heterophylla*, with thinner bark at the top position, but with very little differences between middle and butt positions, which is not the case here.

Diameter and length of sieve tube members

Table 1 summarises the tree mean and range of values for cell dimensions, as well as the mean values at the base (5% height level) and the top (75% height level).

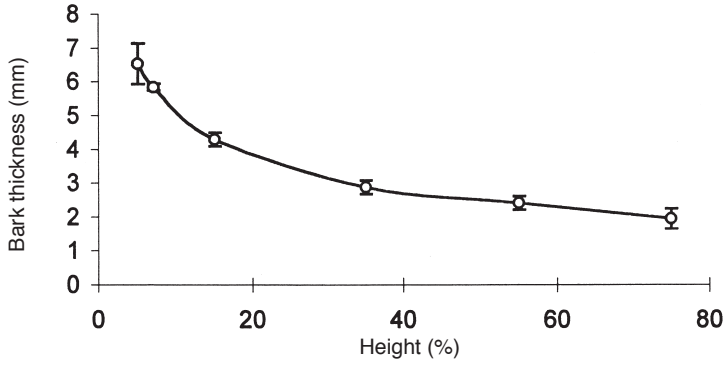

Fig. 1. *Eucalyptus globulus:* axial variation of bark thickness. Mean and standard deviation (bar) of 10 trees.

Table 1. Biometric data of the secondary phloem of *Eucalyptus globulus* for the tree weighted average (mean, minus and maximum values) and for the 5% (base) and 75% (top) height levels. Mean of 10 trees.

	Tree weighted average		Base	Top
	Mean	Min-Max	(5%)	(75%)
Sieve tube members				
Tangential diameter (μm)	39	34–42	38	37
Length (μm)	410	358–504	398	374
Fibres				
Length (mm)	1.28	1.16–1.38	1.23	1.29
Width (μm)	18	17–20	20	16
Wall thickness (μm)	7	6–7	7	5
Rays				
Height (μm)	156	133–169	148	152

Sieve tube diameter fluctuates and does not follow any regular trend within the tree. A distinct trend of development of sieve tube diameter was therefore not observed when considering individual trees. On average, there is a slight increase in trees of plot 1 and decrease in trees of plot 2 between the 15% and 35% height levels and a more or less parallel development between all the other height levels (Fig. 2). Trees from plot 1, on average, have larger sieve tube diameters than trees from plot 2, especially in the upper part of the stem (Fig. 2).

An increase of the sieve tube diameter from the upper part of the stem downwards appears to be a more common trend and was described for various taxa (Ghouse & Iqbal 1977; Iqbal & Ghouse 1983; Trockenbrodt 1994). Similarly, Vollenweider et al. (1994) observed more numerous and smaller sieve tubes in younger trees of *Fagus sylvatica* L.

Sieve tube members of *E. globulus* fit into the length categories I (< 400 μm) and II (250–400 μm) defined by Richter et al. (1996). In general, the sieve tube members

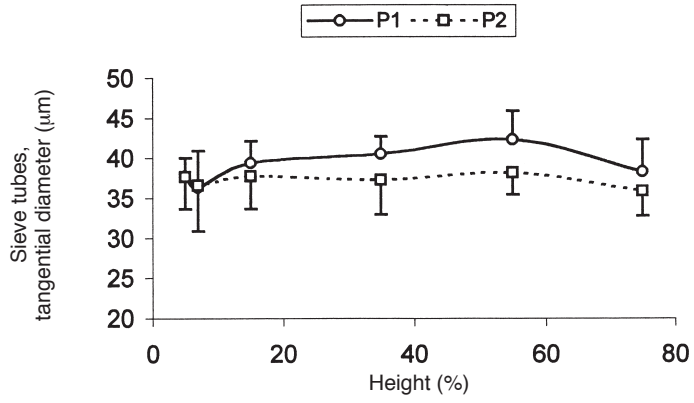

Fig. 2. *Eucalyptus globulus:* axial variation of the tangential diameter of sieve tubes. Mean and half of standard deviation (bar) of 5 trees per plot.

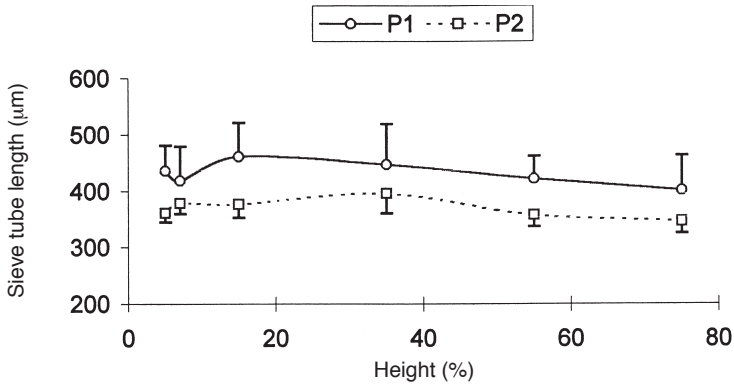

Fig. 3. *Eucalyptus globulus* bark: axial variation of sieve tube member length. Mean and half of standard deviation (bar) of 5 trees per plot.

are shorter in the younger bark of most individuals and length decreases with tree height (Fig. 3). However, this decrease does not always follow a regular gradient, e.g. in the trees of plot 1 length increases towards the 15% height level.

Iqbal and Ghouse (1983) and Trockenbrodt (1994) also observed an increase in length of sieve tube members with tree age in some individuals in various species (*Quercus robur*, *Populus tremula*, *Acacia spicigera*). This increase was confirmed by studies of radial variation where the longer elements were found near the cambium (Parameswaran & Liese 1974; Ghouse & Iqbal 1977; Iqbal & Ghouse 1983). The combined effect of cambial initials length, extent of anticlinal divisions of phloem mother cells (secondary partitioning), and apical intrusive growth determines the length of sieve tube members and may explain the observed irregular pattern of length variation (Ghouse & Iqbal 1977; Trockenbrodt 1994).

The analysis of variance for length and tangential diameter of sieve tube members shows that the height level is not a statistically significant source of variation. The plot was significant ($P < 0.05$) or highly significant ($P < 0.001$) to explain the variation of the tangential diameter and the length of sieve tube members, respectively.

Length, width and wall thickness of fibres

Fibre length varies little within the tree (Table 1) but, in general, the longer fibres occur near the top (Fig. 4). The average fibre length observed in *E. globulus* bark fits into the species range reported by Pereira (1994) for 6-year-old trees (1.00–1.04 mm).

The most common trend of fibre length variation is a decline towards the top of the tem, but Nicholls and Phillips (1970) found no apparent pattern of length variation with radial position, height or age in the bark of *E. viminalis*. Also Ghouse and Siddiqui (1976) and Iqbal and Ghouse (1983) described various trends of fibre length variation for different species. For *E. globulus* bark, longer fibres were found at the top in most of the trees (Fig. 4). Jorge et al. (1997) reported an inverse trend of fibre length variation in wood and in bark in *E. globulus*, suggesting distinct mechanisms of cell differentiation in phloem and xylem. Parameswaran and Liese (1974, in *Shorea negrosensis*) and Trockenbrodt (1994, in *Quercus robur*) also noticed an increase in

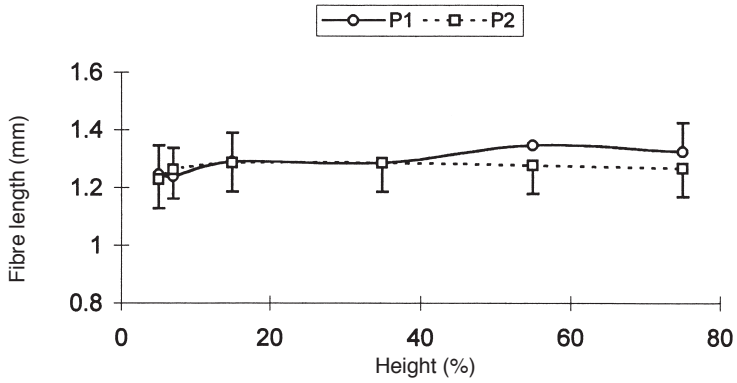

Fig. 4. *Eucalyptus globulus* bark: axial variation of fibre length. Mean and half of standard deviation (bar) of 5 trees per plot.

fibre length in the uppermost part of the stem. An increase in length of the cambial initials was responsible for the increase of length of secondary phloem fibres (Parameswaran & Liese 1974). According to Trockenbrodt (1994), the abundance of much longer primary phloem fibres in the top of the tree is also responsible for the greater lengths in this position. The variation in fusiform initial length is also an important mechanism influencing fibre length, but Ridoutt & Sands (1993, in *E. globulus*) reported that the fibre elongation during differentiation could also explain part of the longitudinal variation in fibre length.

The correspondence of shorter cambial initials at the top (Ridoutt & Sands 1993) to longer fibres in the bark of *E. globulus* trees suggests that apical elongation of fibre elements during differentiation plays a more significant role than the length of cambial initials.

Fibre width and fibre wall thickness decrease from the base to the top in most trees (Fig. 5, 6). There are no comparable studies concerning the axial variation of the width of phloem fibres. In the present study the majority of individuals showed an

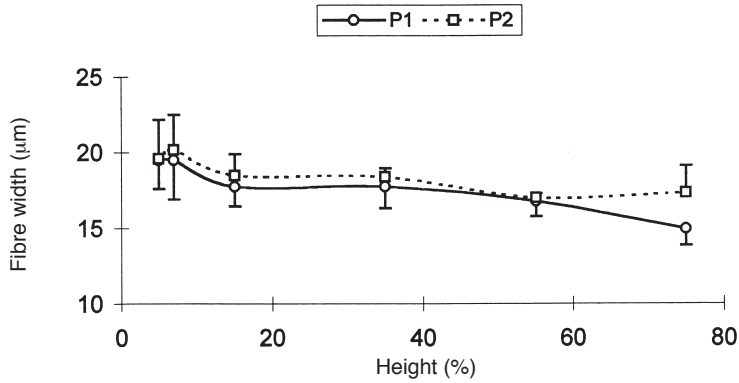

Fig. 5. *Eucalyptus globulus* bark: axial variation of fibre width. Mean and half of standard deviation (bar) of 5 trees per plot.

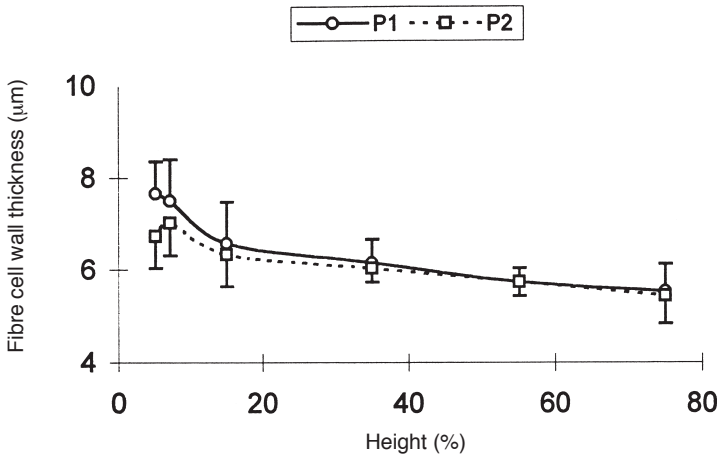

Fig. 6. *Eucalyptus globulus* bark: axial variation of fibre cell wall thickness. Mean and half of standard deviation (bar) of 5 trees per plot.

increase of phloem fibre diameter with age (Fig. 5), as occurs for xylem fibres (Jorge 1994). The changes in fibre diameter could be affected by a decreasing hormone content with increasing distance to the crown. Lev-Yadun and Aloni (1991) suggest that a longitudinal gradient of decreasing auxin concentration is responsible for the longitudinal increase in the enlargement of fibres. Additionally, basipetal variation of the duration of the wood fibre differentiation in *E. globulus* was described by Ridoutt and Sands (1994), and can also explain the well-defined pattern of axial variation found for fibre wall thickness with a steady increase with tree age (Fig. 6).

The effect of height position is highly significant ($P < 0.001$) for diameter and fibre wall thickness; no statistical differences were found for fibre length within the tree. The Tukey test shows differences for fibre diameter between 75 vs. 5, 15, 35%, and 55 vs. 5% height levels. For cell wall thickness significant differences were found between the 5 vs. 35, 55, 75% and the 15 vs. 75% height levels.

Height of phloem rays

Phloem ray height shows a large between tree variability (Table 1). However, no regular pattern of variation could be established (Fig. 7), e.g., for plot 1 there is a slight tendency for rays to be shorter at the base of the trees, but for plot 2 the top and base values were similar. This reflects in part the findings of Ridoutt and Sands (1993) who observed a decrease of ray initial width and length towards the top in 16-year-old *E. globulus* trees, possibly due to the fusion of contiguous cambial rays at the lower tree height levels. They also found fluctuations in average height of cambial rays and could not observe a consistent trend of variation. Trockenbrodt (1994) also studied ray height during development in some taxa and only observed a regular increase of ray height with age and significant differences between age groups for *Quercus robur*.

The variables plot and height level have no significant influence on the height of phloem rays.

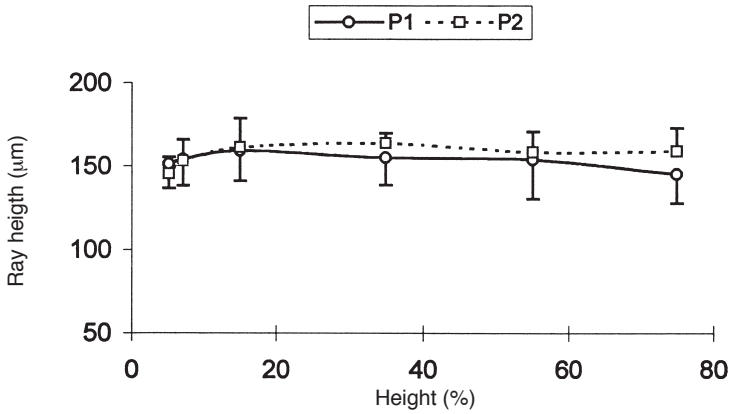

Fig. 7. *Eucalyptus globulus* bark: axial variation of ray heigth. Mean and half of standard deviation (bar) of 5 trees per plot.

Tissue proportions

The area percentage of the different tissues in the secondary phloem of *E. globulus* was determined at each height level and for all trees (Table 2).

Axial and radial parenchyma decrease towards the top of the tree after a slight initial increase at the 15% height level. Sclereid proportion decreases gradually from the base to the top. On the other hand, the proportion of fibres and sieve tubes tends to increase from the base to the top.

There is very little information on the axial variation of bark cell type proportions. They tend to differ considerably between species and between individuals (Trockenbrodt 1994). The work of Ridoutt and Sands with *E. globulus* (1993, 1994) was the first attempt to directly relate the longitudinal variation of the wood cell types with the number of fusiform and ray initials in the cambium. The results of the present study show that axial parenchyma occupies the largest area in the secondary phloem (50%), followed by the fibres (28%). These results agree with the values reported by Hillis and Udompongsanon (1990) for the bark of 4-year-old *E. globulus* and 5-year-old *E. regnans* trees. On the other hand, these authors reported proportions of 'differentiating parenchyma' (corresponding to our terminology of 'expanded parenchyma cells') and sclereids higher than in the older *E. globulus* trees studied here.

In all trees, axial and radial parenchyma proportions tend to increase towards the base. With increasing height, the area occupied by ray initials relative to fusiform initials and average ray initial width decreases (Ridoutt & Sands 1993). This may be explained by the need for efficient storage and radial transport of assimilates and plant growth regulators, as suggested by Ridoutt and Sands (1993).

The 'expanded parenchyma cells' were quantified separately from sclereids, although they have the same origin and only represent different stages of differentiation. The proportion of 'expanded parenchyma cells' shows no trend of axial variation and sometimes identical proportions occur at the base and the top of a tree. In contrast, the proportion of sclereids was always higher at the base (Table 2). The number of sclereids also increases from the cambium towards the periderm. It was

Table 2. Cell type proportions (%) in the secondary phloem of *Eucalyptus globulus*, as tree weight average and for the different height levels. Mean of 10 trees; in parentheses the standard deviation.

	Tree weighted average		Height level				
	Mean	Min-Max	5%	15%	35%	55%	75%
Sieve tubes	2.7 (0.8)	1.5–3.8	1.2 bdf	2.4 bde	3.0 bde	4.8 ace	4.5 ace
Axial parenchyma	50.0 (4.4)	42.6–58.0	51.2 a	51.3 a	50.3 a	48.2 a	45.4 b
Fibres	27.9 (3.3)	21.2–32.1	28.2 a	25.8 a	28.9 a	28.7 a	31.2 b
Rays	12.1 (1.5)	9.9–14.3	12.5 a	12.8 a	11.2 a	10.9 a	11.3 a
'Ex. parenchyma'	6.5 (1.5)	4.3–8.7	5.3 a	6.1 a	6.2 a	6.9 a	7.2 a
Sclereids	0.8 (0.4)	0.4–1.5	1.6 a	0.6 a	0.4 b	0.5 b	0.4 b

Means followed by the same letter are not significantly different at the 0.05 level, as determined by the Tukey test.

already shown that an intense lignification and sclerification of the cell walls constitutes the main structural features related to tree age in *E. globulus* bark (Quilhó et al. 1999). The increase of sclereid proportion with tree age was also observed in other species (Trockenbrodt & Parameswaran 1986; Trockenbrodt 1994).

The plot is an important source of variation in the cell type proportions, being highly significant for sieve tubes ($P < 0.001$) and significant for sclereids ($P = 0.002$). The influence of tree height is highly significant for the proportion of sieve tube members and sclereids ($P < 0.001$), and significant for the proportion of axial parenchyma ($P = 0.025$) and fibres ($P = 0.03$). Plot × height interaction is a significant ($P = 0.04$) source of variation only for the proportion of sclereids.

CONCLUSIONS

Eucalyptus globulus bark thickness, phloem fibre width and wall thickness decrease with tree height. No distinct axial patterns of variation were observed for the other biometric variables studied.

The proportions of the different cell types vary significantly within the tree, with a decrease of parenchyma, rays and sclereids and an increase of fibres and sieve cells towards the top. Parenchyma occupies the major portion of the bark (50% of total area/volume) followed by the fibres (28%).

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