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CLONAL AND SITE VARIATION OF VESSELS IN 7-YEAR-OLD *EUCALYPTUS GLOBULUS*

by

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SUMMARY

Pith to bark variation of vessel anatomy was studied in 17 clones of 7-year-old *Eucalyptus globulus* trees grown on two sites in Portugal. Vessels were measured by image analysis on transverse microsections cut from radial strips sampled at 25% tree height. The mean vessel area increased gradually from pith to bark, whereas the vessel frequency (number of vessels per unit area) decreased outwards from the innermost ring on and levelled off towards the bark. The proportion of vessels relative to other tissues remained constant across the radius. The vessel variables showed cyclic variations defined by minima (vessel area and proportion) or maxima (number of vessels). The effect of site and clone on vessel variability was significant. Clonal variation accounted for 30% and site explained 67% of the total variance of vessel proportion. At the least water stressed site, vessels appeared to be generally larger and occupied a greater proportion of total cross-sectional area.

Key words: *Eucalyptus globulus*, vessel anatomy, clone, site, radial variation.

INTRODUCTION

Structure and size of anatomical elements influence wood properties in various ways. In hardwoods, vessel proportion and vessel size affect the papermaking process (Amidon 1981). High vessel percentage facilitates penetration of the pulping liquor into wood and increases bulk, while paper surface quality is lowered since vessel elements may pick out from the paper surface during the printing process (vessel picking), leaving ink-free spots on the printed page (Colley 1973).

Despite extensive research about relationships between fibre morphology and pulping quality, only a few studies are concerned with vessel sizes and their variability. This is also true for eucalypt wood, particularly for *Eucalyptus globulus*, where vessel picking is a serious concern due to the large vessel size and to the extensive industrial utilisation of eucalypt pulp for quality printing-paper.

The wood of young *E. globulus* has a diffuse-porous vessel arrangement. Tree rings are generally difficult to recognise, with a frequent occurrence of density fluctuations

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(false rings) close to the pith. Vessels are exclusively solitary with a rounded outline and are grouped in tangential zones with no specific pattern. The number of vessels/mm^2 is low and around 4–6 vessels per square millimetre (Carvalho 1997).

Vessel size increases with cambial age, while the number of vessels per unit area rapidly decreases, levelling off gradually towards the bark (Carvalho 1962; Hudson et al. 1997, 1998). Malan (1991) reports commonly larger vessels for eucalypts at lower vessel frequency. Carvalho (1962) has observed a decrease in vessel area from pith to bark, while Hudson et al. (1997, 1998) reported a gradual increase.

Also, little is known about genetic and environmental variability of vessel characteristics and the low number of sampled trees is the most limiting factor in the existing studies. Malan (1993) has shown for several eucalypt species that site had no significant effect on vessel proportion and distribution, and only a minor effect on vessel size.

The present work reports on the pith to bark variability of cross-sectional vessel characteristics of different *Eucalyptus globulus* clones, with reference to their genetic and environmental variability.

MATERIALS AND METHODS

Samples were collected from *Eucalyptus globulus* Labil. trees grown at two clonal trials: Nogueirões (40° 44' N, 8° 26' W, 200 m) in the north-western region and Carvalhinho (37° 25' N, 8° 28' W, 150 m) in the south-western region of Portugal. The clonal trials were established in December 1992, with clones selected for growth during mass selection in Portuguese plantations, using one tree plot and four replications. Trees were planted at 3 × 3 m spacing with harrowing and initial fertilization at planting, according to the usual management practices of eucalypt forestry in Portugal. Seventeen clones were common to both sites and coded from 1 through 17. From each site two trees were randomly chosen from each clone and harvested in December 1999, at a common tree age of 7 years. The total number of investigated trees was 68. Tree heights at Carvalhinho ranged between 9.9–18.5 m, and between 13.8–23.2 m at Nogueirões. Stem diameters at breast height (DBH) were between 5.8–16.8 cm and 8.1–16.1 cm, respectively. One disk was taken from each tree stem at 25% of the total tree height in order to ensure that wood samples from different trees would have been formed by cambia with approximately the same age. Tree age at this height level was estimated using the dominant height growth model (GLOBULUS model v2.1) developed for plantation grown *E. globulus* (Tomé et al. 2001).

Although both sites show well-defined dry periods, the climate at Carvalhinho has a more Mediterranean character with 535 mm annual rainfall and a longer dry season, with almost zero precipitation during summer (Fig. 1). At Nogueirões average annual precipitation was twice the one at Carvalhinho (1108 mm), with significantly higher rainfall during the summer months. The sites had different growth indices for eucalypts. At the age of 7, the average estimated volume (Tomé & Tomé 1994) at Carvalhinho was 0.058 m^3/tree and 0.088 m^3/tree at Nogueirões.

Radial strips running from pith to bark were cut from each disk. The 2 cm wide strips were split into 2, 3 or 4 blocks, depending on its radius. The wooden blocks were sof-

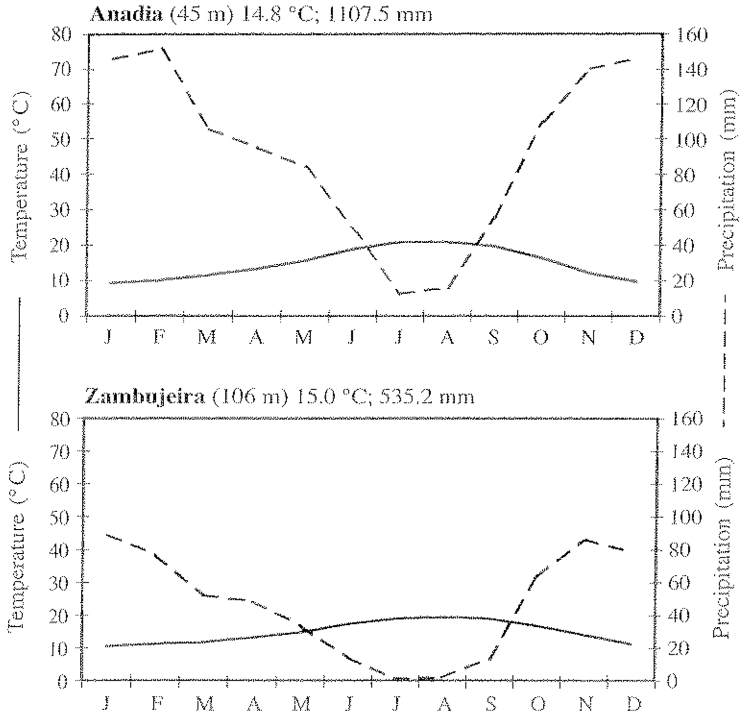

Fig. 1. Climatic diagrams of the study sites. Monthly averages over 29 years for Anadia (near Nogueirões) and 18 years for Zambujeira (near Carvalhinho).

tened for 24 hours in a mixture of water, 96% ethanol and glycerine. Transverse 20 μm thick microsections were cut from each block, using a sliding microtome. Sections were stained with methylene blue, mounted permanently in Euparal on glass slides.

Microscopic images from the wood cross sections were collected using a Zeiss Photometer-Microscope MPM800 (Zeiss Axiotron). With a total magnification of ×100, each image covered 1730 μm in the radial direction. The microscope was calibrated with a stage micrometer. Consecutive images were captured starting at the pith until the bark was reached. Images were stored in TIF graphic format using the image analysis software, PC-Image (Scion Corporation). The average number of acquired images per tree was 26.

Images were converted to binary format (black-white) and inverted to distinguish vessels as black spots, to be measured by the system, against a white background. With threshold and minimum size settings, vessels were clearly identified as separate objects (Fig. 2). In each measuring frame the number of vessels, individual vessel area, major and minor individual vessel diameter were measured. The mean vessel area (μm^2), the number of vessels per mm^2 (vessel frequency), and the percentage of the total cross-sectional area covered by vessels (vessel coverage) were calculated. To compare trees with different diameters, the relative distance from pith was recorded for each frame, calculated as a percentage of the total radius. Vessel variables have shown a very high

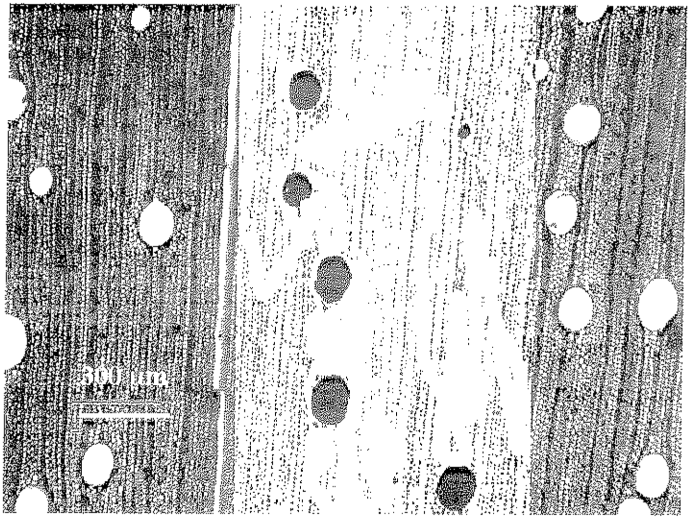

Fig. 2. Microscopic image from a transverse wood section, of clone 10, showing thresholded vessels and the frame, on the inverted part of the image, within which measurements were performed.

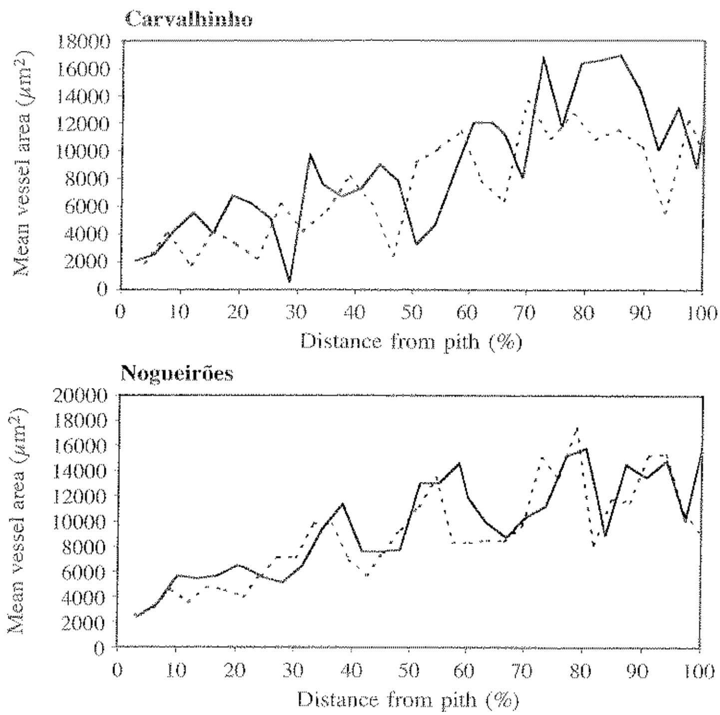

Fig. 3. Pith to bark variation in cross-sectional vessel area from two *Eucalyptus globulus* trees of clone 4 from Carvalhinho and Nogueirões.

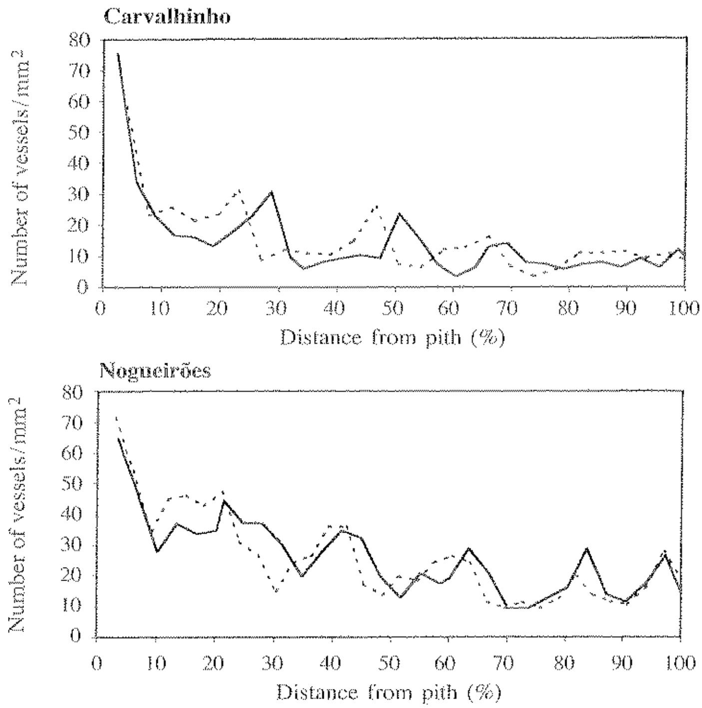

Fig. 4. Pith to bark variation in cross-sectional vessel frequency from two *Eucalyptus globulus* trees of clone 4 from Carvalhinho and Nogueirões.

between-tree variability on the innermost 20% of the radius under the influence of the near-to-the-pith growth. Therefore tree averages were obtained from the arithmetic mean of all the radial positions measured excluding this 20% of the radius. ANOVA was made, using Statistica v 6.0 software, to test the variance of the vessel variables with site (precipitation) and clone as main effects (fixed) after checking normality and equal variance assumptions.

RESULTS

Vessel anatomy

The pattern of pith-to-bark variation of vessels was similar for all trees. Variable trends of clone 4 are illustrated in Figures 3 to 5 for both sites. Mean vessel area generally increased from pith to bark starting with small and constant vessel areas (2000–4000 μm^2). Further out vessel size increased (12000–20000 μm^2) and reached its maximum close to the bark (Fig. 3). The number of vessels per unit area generally declined from pith to bark. For the between-tree range of variation, the vessel frequency started with 20–40 vessels/mm^2 around the pith, was reduced to around 10–20 vessels/mm^2 within the first half of the radius, then it levelled out with a minimum of 3–5 vessels/mm^2 close to the bark (Fig. 4). Vessel coverage varied from pith to bark between 4% and 16%, without obvious trends (Fig. 5).

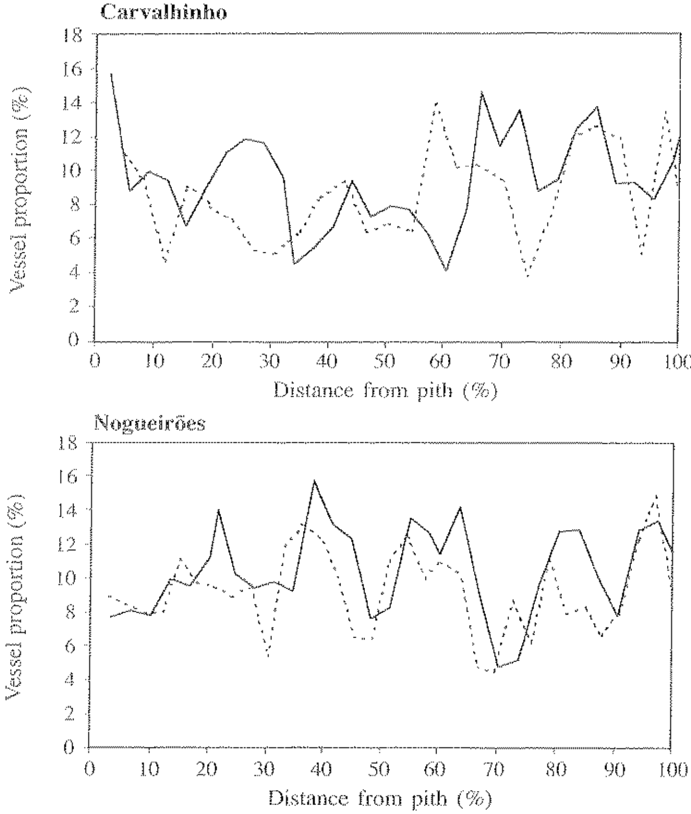

Fig. 5. Pith to bark variation in cross-sectional vessel proportion (coverage) from two *Eucalyptus globulus* trees of clone 4 from Carvalhinho and Nogueirões.

Mean vessel area and vessel proportion showed a pith-to-bark periodicity defined by sudden value changes. Mostly, five periods were identified for mean vessel area. Within sites, radial positions of these periods, i.e. maxima and minima, were similar in both trees for about half of the clones (Fig. 3–5). Figure 6 shows the pith-to-bark variation of the two trees from clone 17 from Carvalhinho, showing the least coincidence of the vessel features. Vessel shape, given by the ratio between diameter on the major and minor axis (dM/dm), changed from elliptical (dM/dm > 1), close to the pith, to more circular (dM/dm ≈ 1) with distance from pith (Fig. 7).

Site and clone effects

Between clone and site variability was evaluated by comparing mean tree values (Table 1). On each site vessel characteristics varied across clones: e.g. at Nogueirões vessel area ranged from 6677 μm^2 to 10670 μm^2, vessel frequency from 9.2 to 13.4 vessels/mm^2 and vessel coverage from 7.6% to 12.7%. For most of the 17 clones, vessels at Nogueirões were larger and less numerous than at Carvalhinho. With two exceptions (clones 6 and 7) vessel coverage was higher at Nogueirões, compared to Carvalhinho for all clones.

Significant differences were found for vessel coverage with site and clone accounting respectively for 67% and 30% of the total variation (Table 2).

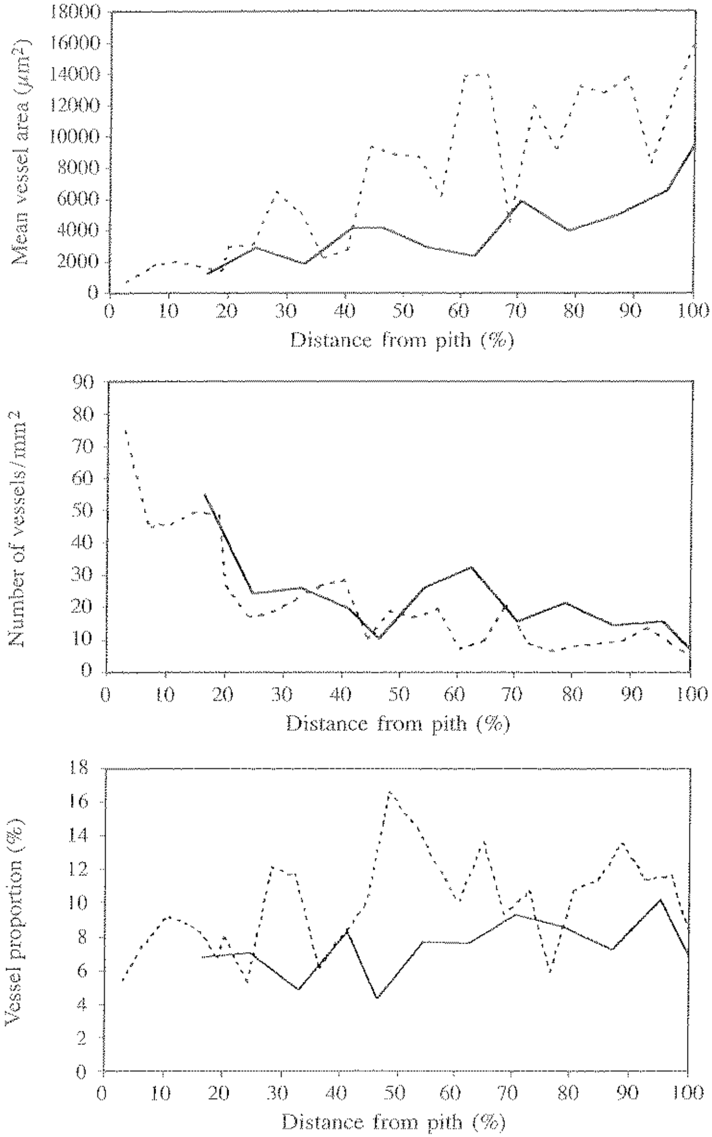

Fig. 6. Pith to bark variation in cross-sectional vessel area, vessel frequency and vessel proportion (coverage) from two *Eucalyptus globulus* trees of clone 17 from Carvalhinho.

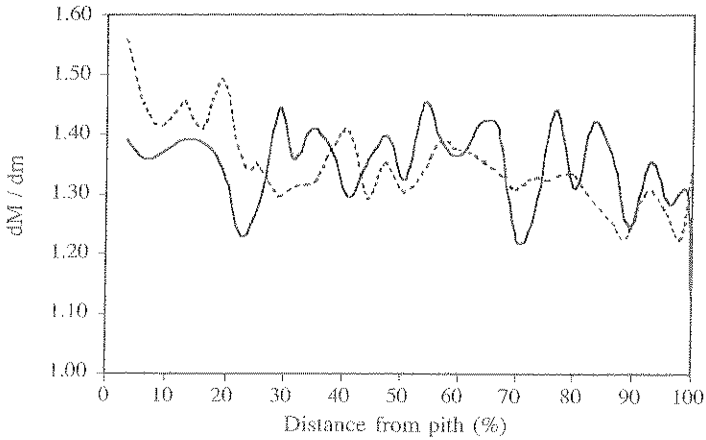

Fig. 7. Ratio between the vessel diameter on the major (dM) and minor (dm) axis from two trees of clone 15 from Nogueirões.

Table 1. Mean vessel area, number of vessels and vessel coverage of 17 *Eucalyptus globulus* clones from two sites (Nogueirões and Carvalhinho).

	Nogueirões			Carvalhinho		
Clone	Area (μm^2)	Number (n°/mm^2)	Coverage (%)	Area (μm^2)	Number (n°/mm^2)	Coverage (%)
1	10670	11.9	12.7	8331	12.4	10.3
2	6853	11.3	7.8	5775	11.6	6.7
3	8271	12.1	10.0	8708	11.1	9.6
4	9592	10.4	10.0	7802	11.5	9.0
5	8674	11.6	10.0	10354	9.2	9.6
6	6686	11.3	7.6	7843	10.6	8.2
7	9035	9.6	8.7	7837	11.5	8.9
8	8395	10.8	8.9	7201	11.3	8.0
9	9222	11.3	10.4	6645	13.5	8.9
10	6677	11.6	7.7	6098	12.3	7.5
11	9846	12.1	11.8	10468	10.1	10.6
12	9651	10.9	10.5	9009	10.5	9.5
13	10287	9.8	10.0	7872	12.2	9.6
14	9023	9.2	8.2	9479	8.3	7.8
15	9624	12.0	11.5	8090	11.8	9.5
16	9131	13.3	12.1	8264	13.4	11.1
17	7781	13.4	10.4	5588	15.4	7.8
Mean	8789	11.3	9.9	7963	11.6	9.0
St. Dev.	1212	1.1	1.6	1425	1.7	1.2

Table 2. Analysis of variance of vessel proportion in *Eucalyptus globulus* in relation to site, clone and site×clone.

Source of variation	df effect	MS error	p	% variation
Site	1	682770	0.000042	66.89
Clone	16	682770	0.000000	29.69
Site × clone	16	682770	0.405760	0.27
Error				3.18

DISCUSSION

Wood anatomy

Pith-to-bark variation found for *E. globulus* in this study confirms earlier reports by other authors on the vessel anatomy of eucalypt species. Vessel dimensions increased from pith to bark in *E. regnans* (Dadswell 1958), *E. grandis* (Taylor 1973), *E. pilularis* (Bamber & Curtin 1974), *E. nitens* (McKimm & Ilic 1987) and also for *E. globulus* (Hudson et al. 1997, 1998). A decrease with the radius for vessel frequency was seen in *E. pilularis* (Bamber & Curtin 1974), *E. nitens* (McKimm & Ilic 1987) and in *E. globulus* (Carvalho 1962; Hudson et al. 1997, 1998).

In this study, the average vessel area was less than the 35000 μm^2 reported for a 7-year-old tree by Hudson et al. (1997, 1998). Vessel frequency measured here was close to values measured by Hudson et al. (1997, 1998), who reported 17–40 vessels/mm^2 for the innermost ring and 8 vessels/mm^2 at the bark side, all measured at 20% height of 7-year-old trees.

Vessel coverage remained rather constant for all trees across clones, and was similar to that reported by Hudson et al. (1997) (8–12%).

Smaller vessels were reported to appear in association with the annual ring border while vessels in the middle of the ring were more numerous and larger (Carvalho 1962; Hudson et al. 1995a, b, 1997). In this study the periodicity of vessel area may be related to an annual ring structure but it was not recognised as such from the disks. Number of observed cycles was generally five, which could correspond to the age at 25% tree height level using estimates from the GLOBULUS model v 2.1 (Tomé et al. 2001).

Because of different growth rates, variable maxima and minima did not appear at the same radial distances from the pith. The example in Figure 6 showing the two trees of clone 17 from Carvalhinho, that differed largely in total growth, 9.9 m vs. 14.1 m of total height (data not shown), clearly demonstrates the difference in the variation patterns of vessels area, frequency and coverage.

Site and clone effects

The variation in vessel characteristics was assigned to clonal and site effects (Tables 1, 2). However Malan (1993) studied four eucalypt hybrids and found that site had only a limited effect on vessel size and distribution. Huber (1993) reported for vessel area in juvenile earlywood of *Quercus* species that within-tree variability and cambial age explain 67% of the total variation. Sass and Eckstein (1995), studying *Fagus sylvatica*, reported that the formation of vessels during the beginning of the cambial activity was controlled by internal factors (not specified), while adult wood formation was affected by external ones (e.g. rainfall).

The sites differed in climate, especially regarding rainfall. At Carvalhinho, annual precipitation in some years was low and could have caused water stress to the growing trees, while at Nogueirões water was a less limiting factor. The lower vessel coverage found at Carvalhinho may be a response to limitations in water supply. Several authors have claimed that water availability is one of the most important environmental sources of variation of the vessel anatomy. Eckstein and Frisse (1979) pointed out that a significant portion of the vessel variability in *Fagus sylvatica* was explained by climate, with precipitation being the most influential factor. Fritts (1976) stated that the cambium produces narrower cells under water stress conditions. Carlquist (1975) related number and diameter of vessels to the vulnerability caused by water stress. Knigge and Schulz (1961) have studied *Fagus sylvatica*, *F. moesiaca*, and *Quercus robur* and showed that vessel size is controlled by water supply.

CONCLUSIONS

The presented work suggests some general trends about the variability of vessel characteristics in *Eucalyptus globulus*. As reported in previous studies using a smaller number of trees, mean vessel area increases with cambial age, while vessel frequency decreases. Vessel coverage fluctuates along the radius within a given range (4–16%). Water availability had a major effect on vessel anatomy, the lower rainfall site was associated with an increase in vessel frequency and a decrease in dimensions and vessel coverage. The clonal effect on the variability of vessel characteristics was relatively small.

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