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Short article

Tree-ring structure and climatic effects in young *Eucalyptus globulus* Labill. grown at two Portuguese sites: preliminary results

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Summary
Dendrochronological analyses of *Eucalyptus* are generally rare and mostly restricted to Australia. This work reports an attempt to identify annual ring patterns in young blue gum trees (*Eucalyptus globulus* Labil) grown in Europe through studying the radial variation of vessel characteristics. Common radial trends were seen such as increasing vessel areas, decreasing vessel abundances and a cyclic variability of vessel characteristics associated to the seasons. Low water availability was related to reduced ring widths in combination with more and smaller vessels. Even with the given juvenility of the investigated eucalypt trees the pith to bark trends of vessel parameters might be useful in dendroecological studies, particularly with respect to drought scenarios. The employed method of using vessel parameters to identify rings seem to be the only accurate way of identifying annual rings in *E. globulus*.

Keywords: Wood anatomy, precipitation effects, annual rings, *Eucalyptus globulus* Labill

Introduction

Tree-ring research has evolved during the past decades to a powerful tool and is today one of the most versatile methods that have gained recognition in fields ranging from archaeology, history, geomorphology, glaciology, ecology, and hydrology to climatology. Formation of tree-rings is governed by genetic components as well as by environmental conditions prevalent during tree growth. Abrupt growth rate changes might bear ecological significance (Schweingruber 1993) and event years may indicate that trees exposed to identical climatic and other ecological conditions may lead to similar growth responses (Oberhuber et al. 1997). The surrounding environment also impacts certain wood characteristics: cambia in softwoods produce narrower cells when submitted to a shortening water supply (Fritts 1976), and hardwoods also respond with smaller vessels and a larger vessel abundance, which reduces vulnerability to water stress (Carlquist 1975).

The concept of annual rings in European tree species became widely accepted around mid 19^{th} century (e.g. Dutrochet 1837) and later Wiesner (1898) claimed that all growth rings of trees grown in Germany are sharp, distinct, and always recognizable.

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This was an important base to apply the principle of "cross-dating", saying that the comparison of identifiable characteristics of rings in two or more trees should result in an assignment of dates (years) to specific rings formed by trees. While this principle has been proven for species like pines, spruces or oaks, in most of the studied situations, other species are still lacking basic information as to whether recognizable rings are annual. This is the case with *Eucalyptus globulus* where annual rings are not well defined, as lighter and darker band formations visible on the cross-section are in poor correspondence with the growing seasons. Very few studies deal with the seasonality of growth of blue gum in Portugal. However seasonal variations in the rate of height growth were evident with a pronounced decrease during the period of November to March (Pereira et al. 1989).

Overall, dendrochronological analyses of *Eucalyptus* are rare and restricted almost entirely to Australia. From the few dendrochronologically examined species in Australia, two eucalypt species are *E. pauciflora* and *E. delegatensis* (Morrow, LaMarche 1978; LaMarche et al. 1979; Banks 1991). Argent (1995) has shown dendroclimatological relationships with *E. camaldulensis* utilizing x-ray densitometry and wood anatomical features such as vessel frequency and size. Warren (1961) has utilized tree-rings of *E. viminalis* and other species to investigate erosion problems along the Tumut river in New South Wales. All these studies cover mainland southern Australia with an expressed seasonality, although highly variable. Mucha (1979) has studied tree-rings of eucalypts in the tropic northern Australia, including *E. miniata*, *E. tetradonta* and *E. nesophila*.

The present work reports results from an attempt to identify annual ring patterns in young *E. globulus* trees grown in Europe through an analysis of the radial variation of vessel characteristics. Compared with other eucalypt species *E. globulus* is known to exhibit *ceteris paribus* a rather insensitive ring structure. The ring structure was observed purely basing on wood anatomical features, and the obtained trends were interpreted with precipitation patterns prevalent at the growing sites.

Materials and methods

On two sites in Portugal, clonal trials of *Eucalyptus globulus* Labill. were planted in December 1992 as 1-tree plots, with 4 replications. The growth sites differed in their levels of annual rainfall and trees were planted with 3×3 m spacing, after harrowing and fertiliser treatment, following the common practice of eucalypt forest management in Portugal. The sites were Nogueirões (40°44′ N, 8°26′ W, 200 m), located in north-western Portugal, and Carvalhinho (37°25′ N, 8°28′ W, 150 m), situated in South-West. The climatic diagrams (Fig. 1) with data collected from the closest meteorological stations classify both sites as Mediterranean with a well-marked dry season. However, average annual rainfall was considerably higher at Nogueirões due to summer rainfalls (1108 mm), compared to Carvalhinho (535 mm). The latter site was characterized by a more extended dry season with little to no precipitation during July and August.

For this study, seventeen clones common to both sites were chosen, with two trees randomly selected from each clone. Trees were 7 year-old when felled in December 1999. Tree height and stem diameter at breast height (DBH) ranged at Carvalhinho between 9.9–18.5 m and 5.8–16.8 cm, respectively, and at

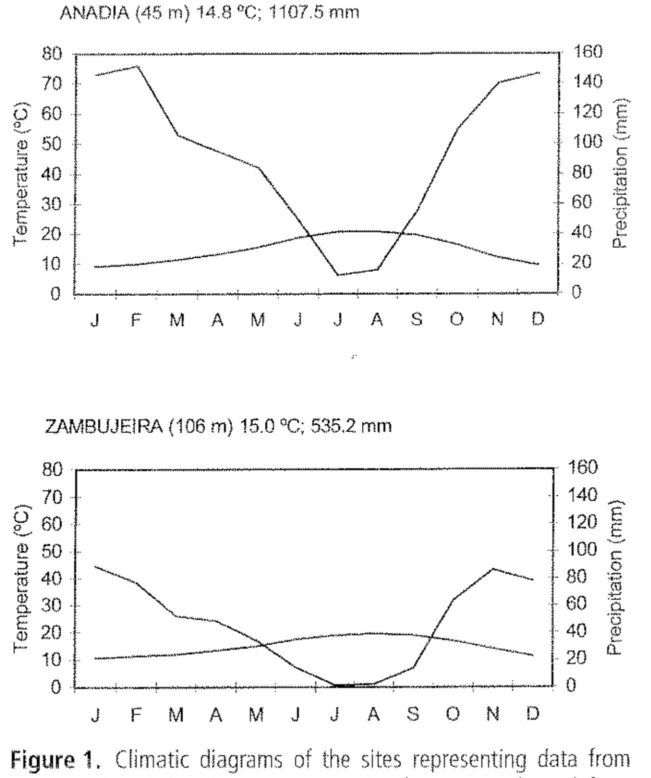

Figure 1. Climatic diagrams of the sites representing data from Anadia (above) for the Nogueirões site (1959–1988); and from Zambujeira (below) for the Carvalhinho site (1967–1985).

Nogueirões between 13.8–23.2 m and 8.1–16.1 cm, respectively. The stem volume was calculated with the formula suggested by Tomé, Tomé (1994) for *E. globulus*, with V as the total stem under-bark volume (m^3), D the diameter at breast height (cm), H the total height (m), and b_1 the empirical value 0.00003104, b_2 = 1.73130059, and b_3 = 1.22417653:

$$V = b_1 D^{b_2} H^{b_3}$$

The average estimated under-bark volume at this age was 0.088 m^3/tree at Nogueirões, and 0.058 m^3/tree at Carvalhinho. From each tree one disk was taken at 25 % relative tree height. Disks were sequentially numbered (1 through 17) to indicate the clone; the letters *a* or *b* used to represent the clone replicate. The height-growth model GLOBULUS programmed by Tomé et al. (2001) was employed to estimate a stem age of 5 years at sampling height. An approximately 2 cm wide strip that covered the complete radius from pith to bark was cut at random positions of each disk. The strips were fractionated in 2, 3 or 4 pieces, depending on the radius. Preparation for microscopic observation involved a softening treatment using a water / 96 % ethanol / glycerine mixture. Transversal sections 20 µm in thickness were cut on a sliding microtome, with subsequent staining in methylene blue before the sections were embedded in Euparal to permanently mount them on glass-slides.

The specimen were observed with a fully equipped Zeiss microphotometer – microscope MPM800 at a magnification of 100×, with sequences of images captured along the radius, which were further stored in TIF graphic format. On average 26 images were obtained from each tree. The microscope was calibrated at the used magnification and each image covered 1730 µm in radial direction. A rectangular measurement window was defined with a tangential width of 1000 µm. Images were digitally processed with the NIH-Image analysis system (Wayne Rasband, National Institutes of Health, USA, available from http://rsb.info.nih.gov/nih-image/).

The image processing allowed an automatic determination of the cross-sectional area of each vessel, and a count of the total number of vessels within the measuring frame. The following parameters were derived: mean individual vessel area (μm^2), vessel abundance (number of vessels / mm^2) and vessel coverage (total vessel area / window area × 100). The absolute radial distances were converted to relative measures to plot the variables to a common scale.

Results

Radial variability of vessel characteristics followed a similar pattern in all trees. Mean vessel area increased from 2000–4000 μm^2 near the pith, to a maximum of 12 000–20 000 μm^2 close at the bark. This trend was paralleled by decreasing vessel abundance, which varied from 20–40 vessels/mm^2 in the innermost wood, down to 3–5 vessels/mm^2 measured in the outermost wood of the stem. Vessel coverage oscillated along the radius between 13–20 % and 2–7 %, without showing a particular trend (Fig. 2). For all parameters variability was high for the inner 20 % of the radius and further out vessel area and

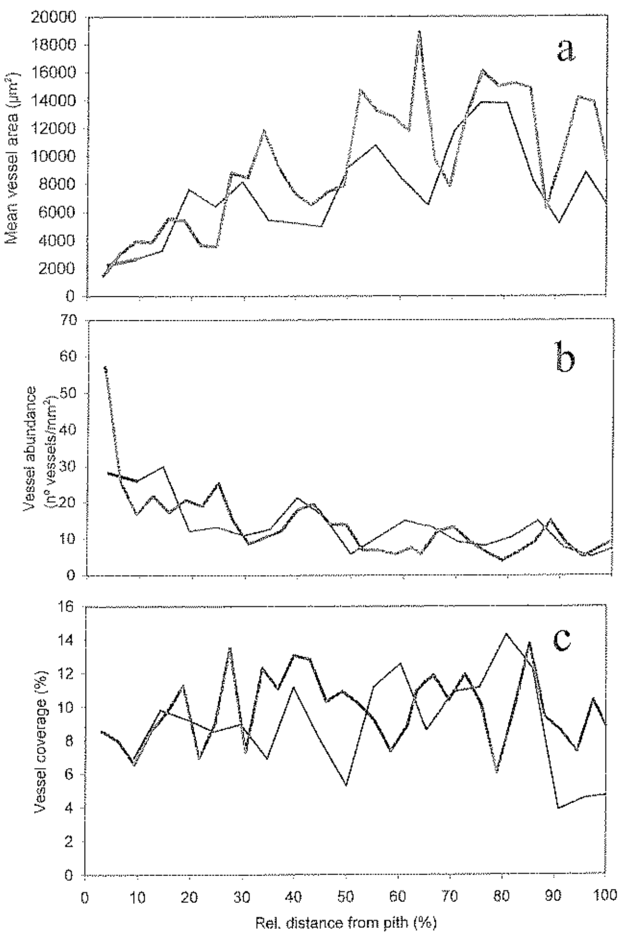

Figure 2. Radial profile of mean vessel area (a), vessel abundance (b) and vessel coverage (c) of two trees belonging to a randomly selected *E. globulus* clone, at Carvalhinho.

abundance showed a cyclic pattern. These cycles can be observed best with mean vessel area, which varied between expressed minima, followed by immediate upswings. We identified five cycles in most of the trees (Fig. 3). Fig. 4 shows a sequence of four images taken from a radial section close to the bark of one tree to demonstrate the variability in vessel sizes and distribution.

The observed cycles defined by the abrupt changes in vessel area are interpreted as annual rings. Three, sometimes four ring boundaries could be unambiguously identified, ring widths were calculated as the radial difference of subsequent vessel area minima. Ring widths at Nogueirões remained relatively constant (Fig. 5) with an average of 7.5 mm per ring.

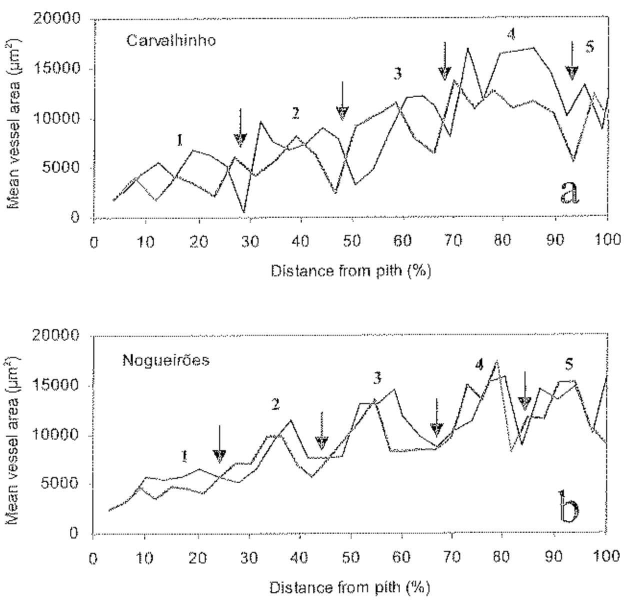

Figure 3. Radial profile of mean vessel area of two trees belonging to a randomly selected *E. globulus* clone, at Carvalhinho (a) and Nogueirões (b), with marked cyclic variation.

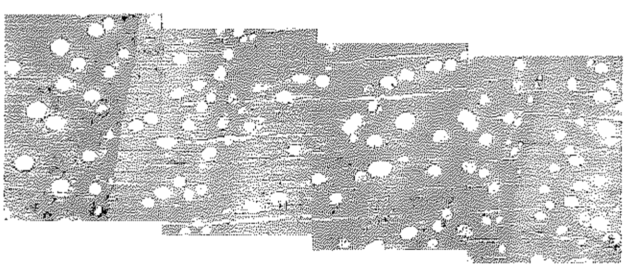

Figure 4. Series of four sequential images from one microcut in the transverse section of one *E. globulus* tree, at Carvalhinho, showing the distribution pattern of vessels.

At Carvalhinho, ring widths of the individual trees showed greater synchronicity than at Nogueirões with the 1999-ring having only 4.7 mm.

Changes in vessel characteristics (vessel area, abundance and coverage) across rings are shown in Figs. 6, 7 and 8 for all trees of the two sites. Site averages are listed in Tab. 1. A decreasing radial trend is seen for vessel abundance at the Nogueirões site. A similar pattern is present at Carvalhinho, although most trees exhibited an upswing for the outmost ring (Fig. 6). Tendency for mean vessel area was opposite to vessel abundance, with an increase from the innermost rings towards the bark; again with exception of the final rings grown at Carvalhinho (Fig. 7). Vessel coverage remained more or less constant across radius and sites (Fig. 8), with slightly higher levels at Nogueirões (10.3 % vs. 9.3 %). A two-way ANOVA of vessel characteristics identified significant factors ($p < 0.01$): for the vessel area, the fixed effects "year" and "site", respectively, for vessel abundance only "year", and for vessel coverage "site" as well as the "site × year" interaction.

The influence of water supply on ring formation was investigated on the basis of monthly precipitation data from preceding November until current year

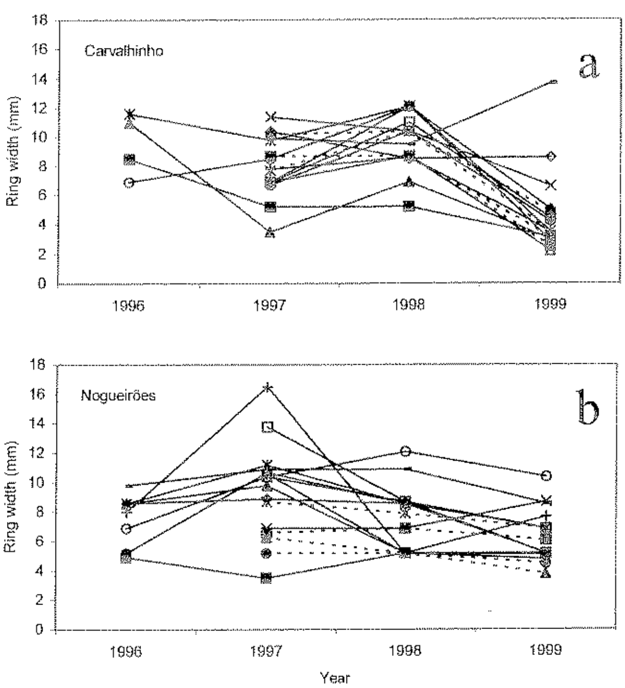

Figure 5. Ring widths of *E. globulus* trees, at Carvalhinho (a) and Nogueirões (b), for 1996 to 1999.

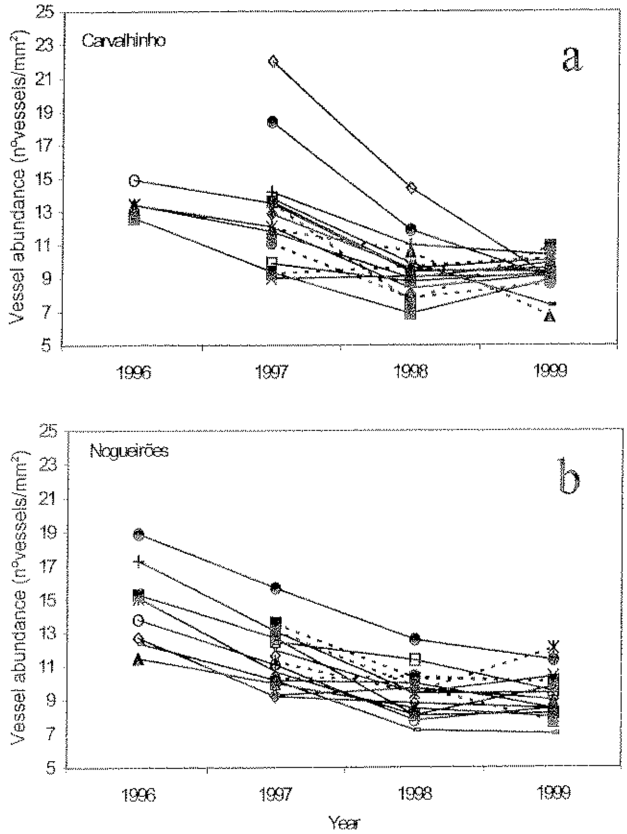

Figure 6. Ring-to-ring average vessel abundance of *E. globulus* trees, at Carvalhinho (a) and Nogueirões (b), for 1996 to 1999.

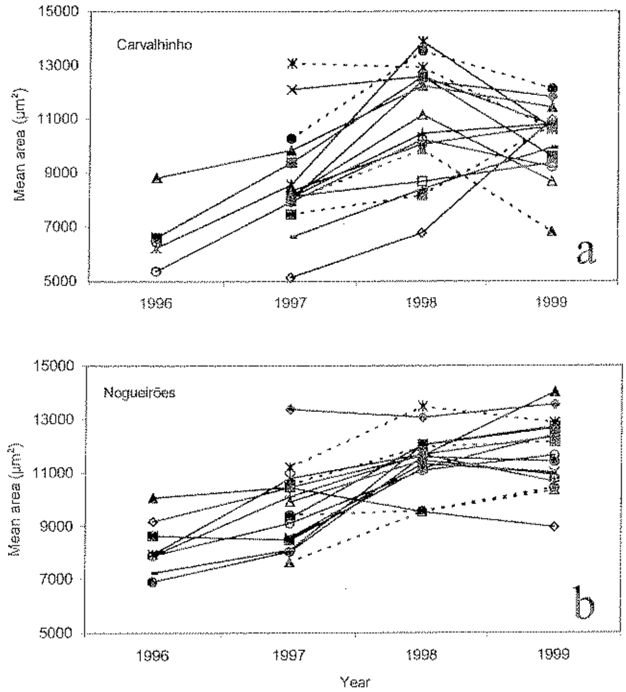

Figure 7. Ring-to-ring variability of mean vessel area of *E. globulus* trees, at Carvalhinho (a) and Nogueirões (b), for 1996 to 1999.

Table 1. Ring width and mean vessel properties (abundance, area and coverage) for the years of 1996 to 1999 corresponding to a cambial age of 2, 3, 4 and 5 years for *E. globulus* in the two sites of Nogueirões and Carvalhinho (means of 8 clones and standard deviations).

	1996	1997	1998	1999
Ring Width (mm)				
Nogueirões	7.6 ± 1.8*	9.4 ± 3.2	7.4 ± 2.2	6.3 ± 1.8
Carvalhinho	9.5 ± 2.2**	8.0 ± 2.1	9.6 ± 1.9	4.7 ± 2.9
Vessel Abundance (vessels/mm²)				
Nogueirões	14.6 ± 2.5*	11.6 ± 1.8	9.4 ± 1.4	9.3 ± 1.4
Carvalhinho	13.6 ± 1.0**	12.9 ± 3.4	9.6 ± 1.8	9.2 ± 1.1
Mean Vessel Area (µm²)				
Nogueirões	8216 ± 1035*	9728 ± 1463	11 444 ± 1128	11 769 ± 1326
Carvalhinho	6757 ± 1462**	8691 ± 1937	10 871 ± 2128	10 265 ± 1315
Vessel Coverage (%)				
Nogueirões	8.2 ± 1.0*	9.7 ± 1.5	11.4 ± 1.1	11.8 ± 1.3
Carvalhinho	8.3 ± 1.6**	9.8 ± 1.2	9.8 ± 1.5	9.3 ± 1.9

* mean of 4 clones (2 trees/clone)
** mean of 2 clones (2 trees/clone)

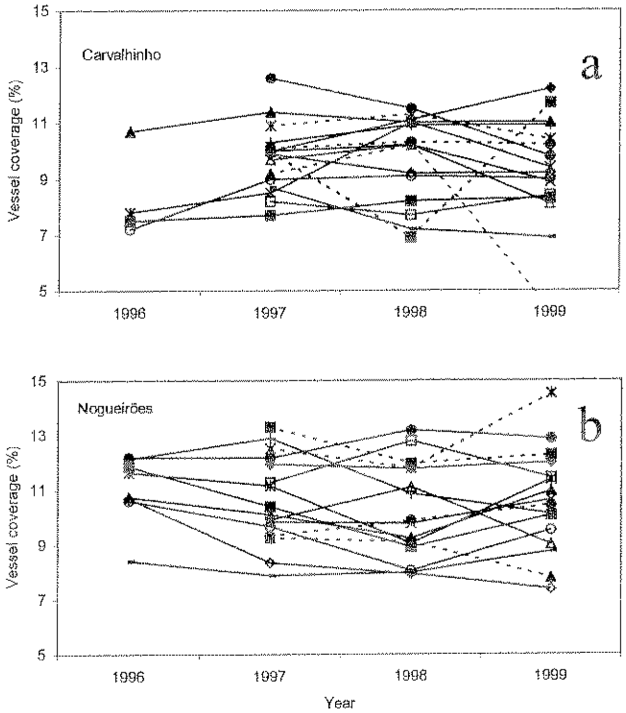

Figure 8. Ring-to-ring variability of average vessel coverage of *E. globulus* trees, at Carvalhinho (a) and Nogueirões (b), for 1996 to 1999.

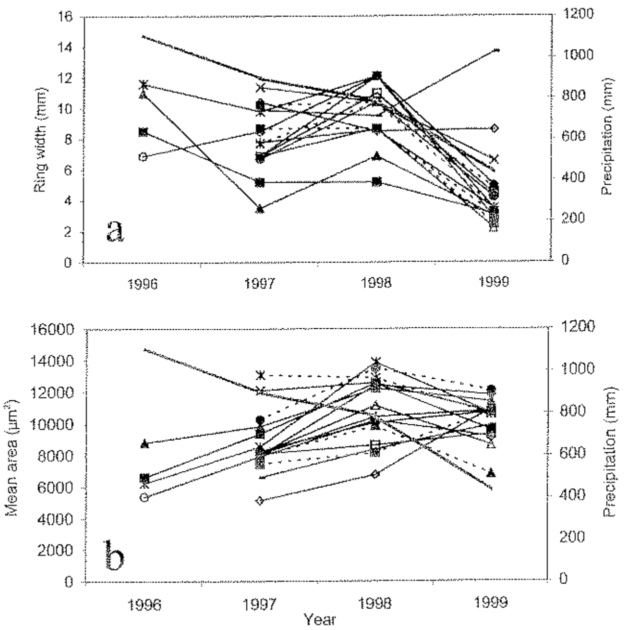

Figure 9. Ring-to-ring variability of width (a) and mean vessel area (b) of *E. globulus* trees at Carvalhinho for 1996 to 1999.

Table 2. Cumulative rainfall (mm) from November of the preceding year to October of the current year at both sites during the study period.

	1993	1994	1995	1996	1997	1998	1999
Carvalhinho	572	386	275	1107	898	788	516
Nogueirões	1146	932	689	1369	901	1365	798

October (Tab. 2). During the years 1992 to 1999 annual precipitation sums at Nogueirões were constantly higher than at Carvalhinho. Even with the short ring series conclusions on precipitation effects can be drawn. The low rainfall in 1999 (starting with November of the previous year 1998) at Carvalhinho was associated with the smallest ring width. In addition, vessel areas did not follow the generally juvenile increase and declined in this ring compared to the previous one (Fig. 9).

Discussion

The parameter vessel area increased along the radius profiles of *E. globulus*, while vessel abundance decreased. Vessel coverage lacked such a trend. High-frequency variation of vessel area and abundance followed a cyclic pattern interpreted as annual rings. Ring widths corresponded with differences in rainfall at the two sites and across years, respectively. Early studies by Dadswell (1958) and Carvalho (1962) have shown vessel sizes increasing radially with cambial age, while vessel abundance decreased sharply. Hudson et al. (1997, 1998) looked at the radial variation of vessel sizes and distribution in *E. globulus* grown in Australia and these authors identified cyclic patterns of vessel areas that were associated with the tree-ring boundaries. In a recent study of vessel features radial variation in *E. globulus* grown in Portugal, Leal et al. (2003) also noticed a cyclic pattern.

Although the results obtained in the present study are premature, they are unique in a sense that they show for the first time the existence of growth rings in European grown *E. globulus*.

Since precipitation was not limiting tree growth at Nogueirões ring widths were more or less constant across trees and years. In contrast, the low annual rainfall in 1999 at Carvalhinho has caused a considerable ring width reduction. Pereira et al. (1989, 1991) reported that water stress is the major limiting factor in *E. globulus* growth, and even under moderate climatic conditions monthly diameter increments of young *E. globulus* trees were limited by water availability during the summer months. According to Goes, Ferreirinha (1962), the minimum annual rainfall to sustain regular tree growth of *E. globulus* should be above 500–600 mm. Annual rainfall sums at both sites were well above this threshold with the exception of the year 1999 at Carvalhinho (516 mm). Araújo et al. (1990) also mentioned water availability in summer being a strong factor influencing stem growth and the efficiency of *E. globulus* wood production. However it should be mentioned that there is very little information on the seasonal variation of radial growth in *E. globulus* in Portugal (Pereira et al. 1989) and the duration and extent of growth decrease in winter months should be investigated.

In addition to ring width reduction the vessels formed in the 1999 ring at Carvalhinho were considerably smaller than in the preceding years. Knigge, Schulz (1961) described effects of the extremely warm and dry year 1959 on anatomical features of oak, beech, spruce and pine. The low rainfall was associated with smaller rings, higher proportion of earlywood vessels, a reduced latewood proportion, smaller vessel diameters, and shorter fibres. Eckstein, Frisse (1979) reported that a major portion of vessel size variability in *Fagus sylvatica* can be explained by climate, with precipitation being most influential. Knigge, Schulz (1961) stated that vessel sizes are under strong control of water supply and Carlquist (1966) has emphasized that the number of vessels increases with aridity while their individual diameter decrease. In opposition to the frequently demonstrated climate – growth relationships, links between climate and wood anatomy are scarcely documented and rarely used in dendroecological studies (Wimmer 2002). Eckstein, Frisse (1982) concluded that vessel diameters in *Fagus sylvatica* and in some *Quercus* species might be more sensitive to climate than ring widths, which should be utilized in climate reconstruction. Woodcock (1989) has documented radial patterns for *Quercus macrocarpa*, *Quercus rubra* and *Fraxinus pennsylvanica*, with ring widths being negatively related to vessel density and positively related to vessel diameter. A conclusion was that direct relationships existed between precipitation and latewood vessel diameter. Finally, Sass, Eckstein (1995) have measured vessel areas in alpine grown *Fagus sylvatica* L. and correlated them with monthly climatic data. Vessel formation at the end of the cambial activity was strongly influenced by the summer rainfall and thus determined greatly by external factors.

Conclusions

The present study has added new knowledge to the question of annual rings in *Eucalyptus globulus* grown in Portugal, through an analysis of its vessel characteristics. It was possible to show that varying growth conditions lead to different ring widths as well as vessel parameters. Even with the given juvenility of the investigated young trees it was possible to interpret the pith to bark trends of vessel parameters in terms of water supply and drought. The analysis of wood anatomy to identify rings seems to be an accurate way of identifying annual rings in *E. globulus*, a species with poorer expressed structural variability when compared to other eucalypt species. Nevertheless the seasonal variation of radial growth in *E. globulus* in Portugal and the duration and extent of growth decrease in winter months should be investigated. The results obtained with young trees need to be supplemented with studies on more mature trees, to understand better environmental and genetic effects on tree growth of European grown eucalypts, including within and between-tree variability.

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