# Vessel frequency, size and arrangement in two eucalypt clones growing at sites differing in water availability

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# ABSTRACT

Vessels are the water conducting elements in *Eucalyptus* and are therefore indispensable components of the xylem. However, they cause various problems for pulp production, predominantly "vessel picking" where vessel elements lift off the paper surface during printing. This is particularly problematic with large vessels. Therefore it is of benefit to understand how variation occurs, to better understand the quality of the wood resource. This study looked at short-term variation in vessel size and frequency in two *Eucalyptus* clones at sites differing in water availability. The drier site had smaller vessels, and a higher vessel frequency than the wetter site. There was a significant change in both vessel frequency and vessel size from March to June at both sites and in both clones. It was shown that there was a significant inverse relationship between vessel size and frequency. This study was conducted as part of ongoing research to develop simulation models of vessel formation in the cambium.

# **KEYWORDS**

# Vessel formation; Cambial derivatives; Modelling xylem development; Eucalyptus

#### Introduction

Wood is a heterogenous material, which can make its use in the manufacture of various products difficult. This is particularly true for the wood of angiosperms such as Eucalyptus spp., which is an important plantation species for production of pulp in South Africa and abroad (Bernard 2003; Higgins 1984). Eucalyptus is composed of a variety of longitudinal cell types, including vessels, fibres, vasicentric tracheids and longitudinal parenchyma, compared to gymnosperms such as Pinus spp., which are composed almost entirely of tracheids (Wilson and White 1986). Vessels are particularly undesirable in wood grown for pulp as large vessels can cause problems in pulp and paper production, including "picking", where vessels lift from the paper surface during printing (Chen and Evans 2004; Colley 1975; Hicks and Clark 2001; Shallhorn and Heinze 1997). The presence of vessels in wood also affects penetrability to processing liquids (Hudson, Wilson et al. 1998) and adds to variation in basic density (Downes, Hudson et al. 1997; Hudson, Wilson et al. 1998).

In order to manage the resource effectively, it is important for forest managers to understand the properties of the material being produced, and the causes of variation in those properties, including vessel size and distribution. Hudson *et al.* (1998) showed that vessel area and distribution varied significantly between and within-rings in *Eucalyptus nitens* and *E. globulus* along a pith to bark profile, but that these properties varied in a predictable way, with increases in vessel area and decreases in vessel numbers observed from pith to bark in both species and with a clear segmentation between earlywood and latewood each year. One way to

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<sup>2</sup> School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban, 4041, South Africa predict vessel property variation in wood is to use a processbased approach. Models of xylem development have been developed by various workers (Deleuze and Houllier 1998; Fritts, Shashkin et al. 2005; Wilson and Howard 1968), however these have not tackled vessel development. In modeling the development of this cell type, it is critical to assess, at any given time, under a specific set of conditions, how many of the population of cambial initials in a tree will differentiate to become vessels vs. other cell types (Downes, Evans et al. 2000). Although the processes controlling these differentiation fates are complex and poorly understood, one hypothesis considers vessel development to be a function of auxin flows along specific paths (Aloni 2004; Lachaud 1989). Cell types in the xylem may also occur by means of the auxin-controlled mechanisms proposed to occur in phloem by Barlow (2005). It may also be possible simply to model vessel formation by introducing increases or decreases in the relative proportions of cell types as a function of various independent factors. Since vessels are essential for transport of water and soil-derived nutrients in angiosperms (fibres do not function in a conductive role), there is an important link between the properties and prevalence of vessels in wood and the water deficit/water availability at the site (Zimmerman 1983).

This paper reports on a study undertaken in 2002. The main objectives of the work were to quantify differences in vessel size and frequency between two commercially important eucalypt hybrid clones, growing at two distinctly different sites in two different seasons, largely in order to parameterize models of the process. As such, it is intended that this work will contribute to the further development of both empirical and process-based xylogenic models, but specifically for wood development in eucalypts, looking at the formation of both fibres and vessels.

# Materials and methods

Studies were undertaken at two sites in this experiment,

Table 1: Study site information.

	Palm Ridge (Dry site)	Terranera (Wet site)
Longitude (degrees E)	32.3444	32.1224
Latitude (degrees S)	28.2412	28.6484
Altitude (m AMSL)	65	74
Date of planting	9 June 1997	22 April 1997
Mean Annual Precipitation (mm)	± 1000	± 1400
Mean Annual Temperature (°C)	21.8	21.4
Soil Description	Deep yellow grey sands. Depth > 1200 mm	Deep yellow grey sands. Depth > 1200 mm

both in coastal Zululand in KwaZulu-Natal province, South Africa. The first is located north of the town of Mtubatuba (Sappi Palm Ridge estate) and the second south of the village of KwaMbonambi (Sappi Terranera estate). The sites differ substantially in terms of mean annual rainfall, with Terranera being wetter than Palm Ridge, but both sites have very similar soils and mean temperatures (Table 1). Measurements of rainfall and temperature for the two sites were obtained from three nearby weather stations, each of which was within 10 km of the relevant study site.

Three replicated plots of two *Eucalyptus* hybrid clones were studied at each site: E. grandis x camuldulensis (GC) and E. grandis x urophylla (GU). Over-bark DBH increment was measured on 28 "study trees" over the interval mid-February to mid-March (late summer), and again on the same trees ("study trees") between mid-May and mid-June 2002 (winter) to obtain a second increment, using Mitutoyo 160-128 series 0.02 mm resolution vernier callipers (Mitutoyo corporation, Japan). In March 2002, 16 samples (4 from each clone at each site) of recently formed xylem and bark were taken from trees neighbouring the study trees (these were designated "neighbouring trees") for measurements of characteristics of the growing tissue in the laboratory. This approach was taken in order to avoid damaging study trees, which needed to be re-measured. A second, final set of samples were taken in June from study trees to coincide with the measured May - June diameter growth increment. The experiment was thus set up as a 3-factor factorial experiment with two levels of "site" two levels of "clone" and two levels of "season of sampling".

Samples were taken using the technique recommended by Uggla and Sundberg (2002) using a mallet and chisel, and in each case, a depth of xylem of at least 5mm was removed to ensure that cells formed over the preceding five months were captured. The samples were reduced in size to cubes with sides about 1 cm in size and placed in formalin / acetic acid / ethanol (95%) / distilled water according to a ratio of 10/5/50/35 by volume. The samples were dehydrated in a graded acetone series, followed by penetration of Spurr's resin in a graded resin/acetone series. Samples in hardened resin were sectioned transversely using a Reichert-Jung ultramicrotome, stained with toluidine blue and mounted on glass slides using Permount mounting medium. Images were captured using the 10X objective using a Leica Leitz microcope linked to a digital camera.

The average growth increment for each plot was used as a template for assessing the xylem tissue formed during the late summer and winter study periods in each clone at each site. The total number of vessels within the growth increment were counted, and expressed relative to the area of the image. Average radial diameter of all fibres along a sub-sample of at least five radial files was also measured using Leica image analysis software. Tangential and radial diameter in each fully enlarged vessel was also measured within the relevant increment in each image. Overall vessel percentage was calculated as the total area of vessels (number of vessels \* average area per vessel) per unit area.

#### Results

The wet site received nearly three times as much rainfall as the dry site during February and March 2002 and more than four times as much rain during May and June. Both sites received less than 25% of the rainfall received in February and March during May and June. However, at both sites, the mean maximum daily temperature in March was more than 6°C hotter than in June.

Fig. 1. GC at the wet site in March 2002: Birefringence observed in the walls of vessels and fibres in which secondary wall thickening had commenced (left) and the same image without polarisation (right). Phloem and cambium are at the left of the images.



Fig. 2. GU at the wet site in June 2002. Birefringence observed in the walls of vessels and fibres in which secondary wall thickening had commenced (left) and the same image without polarisation (right). Phloem and cambium are at the bottom of the images.



Developing vessels at or near the cambial zone interface were comparatively rare in the samples taken in March compared to those taken in June. In order to ascertain if vessels were still enlarging, or at least had very recently completed enlargement, birefringence was used to test for the presence of the deposition of crystalline cellulose in the secondary cell wall (Fig. 1 and Fig. 2). In March, vessels which had recently completed expansion, or were still actively expanding were only observed in captured microscope images of about 50% of the trees sampled. In 79% of trees sampled in June at least one vessel was still enlarging, or at least had not yet begun secondary thickening. The width of the cambial zone was also significantly wider in June than in March.





ANOVA looking at the effects of site, clone and time of year found that vessel frequency was significantly higher in GC than in GU and in samples taken in March compared to samples taken in June (Fig. 3), but that there was no significant difference between sites.

It was also clear that the chance of a cambial initial becoming a vessel was significantly lower in the samples taken in June than those taken in March. Overall, in GC,

Fig. 4. The average number of vessels formed per cell file, relative to total xylem growth over the March and June 2002 measurement periods in the two clones at the dry and wet site. Error bars show mean  $\pm$  standard error x 1.96.



Fig. 5. Differences in vessel frequency between two trees of the GU clone over the same growth period in samples taken in the March (dry site left and wet site right).



vessels were found to form twice as often per mm along a given radial file of cells in the February - March period than in the May - June period. In GU, the likelihood was about 1.6 times (Fig. 4). Large variation was evident between trees in March samples (Fig. 5).

Table 2 shows the probability of vessel differentiation occurring in the two clones, under two scenarios. These data were obtained by calculating the mean average radial diameter of fibres in a sample of radial cell files over the growth increment of month t. The total number of files of cambial initials contributing to the ranks of xylem cells in each image were counted. The total number of fibres that would form in the image of known area, if no vessels formed, was then calculated (total number of files multiplied by the average radial diameter of fibres in the file). This would represent the situation, similar to what it would be in the xylem of a gymnosperm, if the rank of cells consisted of more uniformly sized cells. Since a vessel forms from a single cambial initial, the total number of vessels in the known area was divided by the total number of "hypothetical fibres". This represents, then, the relative number of vessels that formed from the total number of "differentiations" that occurred. It is also assumed, therefore, that fibres adjacent to vessels were compacted and no longer evident, but that cells did exist in those locations at the point of exit from the cambial zone.

Table 2: The percentage chance that a xylem mother cell that exits the cambial zone and begins to differentiate will become a vessel in the two clones under varying conditions

	GC Clone	GU Clone
Hot conditions with high, increasing VPD and decreasing soil water availability (high stress)	0.7 %	0.5%
Cool conditions with low, decreasing evaporative demand and increasing soil water availability	0.3%	0.3%

Flow rate through a capillary is proportional to the fourth power of the radius of the capillary (Zimmerman 1983) and this is perhaps the most meaningful expression of vessel size in terms of conductive ability. The mean of the fourth power of the vessel radius was significantly higher at the wet site than at the dry site, and in the May to June period than in the February to March period (Fig. 6), confirming the findings of other authors who have explored vessel size as a function of water stress in eucalypts (February, Stock *et al.* 1995; Leal, Pereira *et al.* 2004; Searson, Thomas *et al.* 2004).

Fig. 6. Average of the fourth power of vessel radius in the two clones growing at the dry and wet site in March and June 2002. Error bars show mean  $\pm$  standard error x 1.96.



Vessel frequency and vessel size varied significantly as a function of clone and site respectively, and in both cases between March and June samples. However, overall vessel percentage did not differ significantly as a function of site, clone or time of sampling (most likely adjusted by the trees by lowering vessel frequencies and increasing their size) (Fig. 7). There was a significant clone x season interaction for this parameter.

Fig. 7. Average vessel percentage in the two clones growing at the dry and wet site in March and June 2002. Error bars show mean  $\pm$  standard error x 1.96.



The mean fourth power of the vessel radius was significantly inversely related to the vessel frequency. The relationship was best described by a hyperbolic function (equation 1). This indicates that as the number of differentiating vessels increased, there was an initial rapid decline in vessel size (Fig. 8). However, there was eventually no change in vessel size with an increase in vessel frequency above a certain threshold.

 $[Vessel Diameter] = exp (b_0 + b_1 / [Vessel Frequency])$  1

Fig. 8. Relationship between the fourth power of the vessel radius and vessel frequency.



For both clones, the value of  $b_0$  was estimated to be 4.3, but  $b_1$  was significantly lower in GC, with  $b_1 = 4.3$ , than in GU, with  $b_1 = 6.7$ .

It is also possible to provide an estimate of the distribution of vessel sizes which can be expected to form. The Kolmogorov-Smirnov test indicated that vessel diameter was approximately normally distributed in the sample data, although the samples were small. Thus, if it we know the mean vessel size and vessel frequency, it is possible to calculate the spread of vessel diameter and calculate the total number of vessels in discrete size classes that could be expected in a section of woody tissue.

# **Discussion and conclusions**

This study found significant differences in vessel frequency between clones, but not vessel size (with GC having high vessel frequencies). In contrast, differences between sites led to significant differences in vessel size, with larger vessels forming in trees at the wetter site. There was no overall effect of site on vessel frequency. There was also a significant inverse relationship between vessel size and vessel frequency, which has been found in the same *Eucalyptus* spp. by February *et al.* (1995) and in other species by Leal et al. (2004) and Searson *et al.* (2004). Both vessel frequencies and sizes varied as a function of short-term changes in environmental conditions, with vessel frequency significantly lower, and vessel transverse

dimensions significantly higher in the June (winter) samples as compared with those taken in March (late summer).

Significant variation in vessel frequency, resulting from an increased or decreased propensity of cambial initials to become this cell type, as well as vessel size, occurs over relatively short time periods in response to changing conditions which impact on the physiology of the trees. Therefore, if an understanding of resource variation, including vessel frequency and size is important, it is important to be able to understand the short-term variations which occur, and consider these cumulatively.

The highly significant change in vessel frequencies between the drier late-summer measurement period and the wetter, cooler winter measurement period indicates that the rate at which cambial initials differentiate to become vessels was significantly higher under the higher stress conditions. Conversely, mean vessel size increased when levels of water stress at the sites decreased. The overall vessel percentage, an indication of the relative transverse area in the tissue devoted to vessels overall, did not change significantly.

Smaller vessels can be expected to pose a smaller danger of catastrophic embolism and cavitation (Tyree and Sperry 1989) and are thus desirable under conditions when water stress is high. However, once vessels reach a minimum size, presumably a minimum required for efficient conduction, size remains constant, even if frequencies increase. Possibly, trees with very high vessel frequencies have a redundancy of vessels, as they are maintaining vessels in much larger numbers than trees with vessels only slightly larger. It is possible that below a certain size, the vessel is no longer an efficient conductor, and large numbers of vessels afford the plant flexibility in loss of a number of conduits when tensions are high. Since tensions in eucalypt clones in this region are not expected to drop much below -2 MPa (Dye, Jacobs et al. 2004), this may not be an issue, however, and although it is clear that an inverse relationship exists between vessel frequencies and size, this interpretation should be considered with caution.

From these findings it is possible to propose a simple model of increased/decreased propensity for vessel formation as a basic enhancement to existing xylem developmental models, for sub-tropical eucalypt clones of the sort studied here. The varied fate of cambial derivatives can be built into such models by introducing a simple probability: the chance, under particular environmental conditions, that a cambial derivative will become a vessel or a fibre upon differentiating. It is important also to determine an approach to allocating these vessels spatially across two dimensions (tangential and radial transverse direction). Assessing the two-dimensional arrangement of vessels can conceivably be tackled by randomly selecting from a population of initials. Alternatively, it may be more accurate to model the vessel differentiation in proportions shown to occur in a spatial pattern, by incurring principles already hypothesized to cause vessel formation. The results of such models can be tested against the results in this research.

Although patterns of vessel arrangement in a stem transverse cross-section are not always obvious, certain generalizations can be made: in eucalypts, vessels typically do not occur adjacent to each other, as they do in poplar. However they do frequently occur in somewhat obliquely oriented files. In this study, in a number of cases, there was evidence of such an oblique arrangement of vessels indicating that some control of vessel differentiation is occurring along a tangential course, consistent with findings by other authors, although in the context of threedimensional studies of wood structure (Zimmerman and Milburn 1982). Preliminary consideration of the images indicated that the pattern was most obvious in the samples taken in May - June (Fig. 9). The functional significance of such an arrangement is not clear.

Fig. 9. Examples of trees where vessels formed clear oblique lines pith-ward from the cambium (CZ).



Although this study was only conducted over a short period, it is clear that smaller vessels form, but in higher frequencies, under conditions of higher water stress, compared to conditions when water stress is low, as has been shown by numerous other others in *Eucalyptus* and other hardwoods. This variation between sites, and within a site over relatively short time periods contributes to heterogeneity in the properties of the wood resource, but it is possible that this variation can be predicted, and perhaps managed. Further research exploring mechanisms of vessel growth and development is currently underway on studies looking at a number of *Eucalyptus* species, over multiple growing seasons.

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#### References

- Aloni R (2004) The induction of vascular tissues by auxin. In 'Plant hormones: biosynthesis, singal transduction, action!' (Ed. PJ Davies). (Kluwer Academic Publishers: Dordrecht)
- Barlow PW (2005) Patterned cell determination in a plant tissue: the secondary phloem of trees. *Bioessays* 27, 533-541.

Bernard M (2003) Eucalyptus: A strategic species for forests.

# feature

Revue Forestiere Francaise 55, 141-154.

- Chen F, Evans R (2004) A robust approach for vessel identification and quantification in eucalypt pulpwoods. In 'Appita Annual Conference'. Canberra, Australia
- Colley J (1975) Factors affecting the vessel picking tendency of hardwood pulps. *Appita* 28, 392 398.
- Deleuze C, Houllier F (1998) A simple process-based xylem growth model for describing wood microdensitometric profiles. *Journal of Theoretical Biology* 193, 99 - 113.
- Downes GM, Evans R, Schimleck LR, Fritts HC (2000) The commercial cambium: understanding the origin of wood property variation. In 'Cell and Molecular Biology of Wood Formation'. (Eds RA Savidge, JR Barnett and R Napier). (BIOS Scientific Publishers: Oxfordshire)
- Downes GM, Hudson I, Raymond CA, Dean GH, Michell AJ, Schimleck LR, Evans R, Muneri A (1997) 'Sampling plantation eucalypts for wood and fibre properties.' (CSIRO Publishing: Melbourne)
- Dye PJ, Jacobs SM, Drew D (2004) Verification of 3-PG growth and water-use predictions in twelve *Eucalyptus* plantation stands in Zululand, South Africa. *Forest Ecology and Management* 193, 197 218.
- February EC, Stock WD, Bond WJ, Le Roux DJ (1995) Relationships between water availability and selected vessel characteristics in *Eucalyptus grandis* and two hybrids. *IAWA Journal* 16, 269-276.
- Fritts HC, Shashkin AV, Hemming DL, Leavitt SW, Wright WE, Downes GM (2005) TREERING 2000 - Advances in process-based modelling of the softwood cambium. In 'IUFRO wood quality modelling workshop'. Auckland, New Zealand
- Hicks CC, Clark NB (2001) 'Pulpwood quality of 13 eucalypt species with potential for farm forestry.' RIRDC Publication No 01/164, RIRDC Publication No 01/164, Kingston.
- Higgins HG (1984) Pulp and paper. In 'Eucalypts for wood production'. (Eds WE Hillis and AG Brown). (Academic

Press: Sydney)

- Hudson I, Wilson L, Van Beveren K (1998) Vessel and fibre property variation in *Eucalyptus globulus* and *Eucalyptus nitens*: some preliminary results. *IAWA Journal* 19, 111 - 130.
- Lachaud S (1989) Participation of auxin and abscisic acid in the regulation of seasonal variations in cambial activity and xylogenesis. Trees 3, 125 - 137.
- Leal S, Pereira H, Grabner M, Wimmer R (2004) Tree ring structure and climatic effects in young *Eucalyptus* globulus Labill. grown at two Portuguese sites: preliminary results. *Dendrochronologia* 21, 139 - 146.
- Searson MJ, Thomas DS, Montagu KD, Conroy JP (2004) Wood density and anatomy of water-limited eucalypts. *Tree Physiology* 24, 1295 - 1302.
- Shallhorn PM, Heinze HU (1997) Hardwood vessel picking in the offset printing of uncoated fine papers. *Pulp and Paper Canada* 98, 21 - 24.
- Tyree MT, Sperry JS (1989) Vulnerablity of xylem to cavitation and embolism. Annual Review of Plant Physiology 40, 19 - 36.
- Uggla C, Sundberg B (2002) Sampling of cambial region tissues for high resolution analysis. In 'Wood formation in trees'. (Ed. N Chaffey). (Taylor and Francis: London)
- Wilson BF, Howard RA (1968) A computer model for cambial activity. *Forest Science* 14, 77 -90.
- Wilson K, White DJB (1986) 'The anatomy of wood: its diversity and variability.' (Stobart and Son: London)
- Zimmerman MH (1983) 'Xylem structure and the ascent of sap.' (Springer-Verlag: Berlin)
- Zimmerman MH, Milburn JA (1982) Transport and storage of water. In 'Physiological plant ecology II: water relations and carbon assimilation'. (Eds OL Lange, PS Nobel, CB Osmond and H Ziegler). (Springer-Verlag: Berlin)

# Big NZ Forest Service anniversary coming up in March 2007

As many NZIF members will know, on 31 March next year it will be 20 years since the NZ Forest Service since the NZFS shut its doors for the last time. A small band of people led by Bob Shaw in Rotorua are determined to ensure the occasion does not pass without notice. Instead they are starting to plan for a reunion in Rotorua next Easter (probably 7-8 April or 20 years and one week after the event) and we think it might help people with their planning if we give them plenty of warning that this is coming up. Many ex-NZFS people are also members of NZIF, while many other NZIF members will know former Forest Service people and it would be very helpful if those members could take note and also pass the news around.

The organisers are not long on details at this stage but

are planning a significant and fairly informal gathering with food and drinks and the opportunity for people to meet, mix and catch-up, and they will certainly have some memorabilia on display.

Some sponsorship is available and they are presently looking to build on that, however the focus is largely on creating an opportunity for groups of old boys and girls to come together and catch up on old times (and present times as well).

Make a note in your diary, organise your year or office to come along, and please pass the details around.

Anyone wishing for more information can get it from Bob Shaw at Bob.Shaw@FITEC.org.nz or Peter Berg at thebergs@xtra.co.nz.