THE ASSOCIATION BETWEEN CELLULOSE CRYSTALLITE WIDTH AND TENSION WOOD OCCURRENCE IN EUCALYPTUS GLOBULUS

by

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SUMMARY

The association between cellulose crystallite width and the occurrence of tension wood was assessed for *Eucalyptus globulus* Labill., a commercially important plantation hardwood species. Crystallite width (uncorrected for instrumental broadening) was determined from X-ray diffraction patterns collected on SilviScan-2, an instrument developed for the rapid assessment of wood microstructure. Comparisons of crystallite widths were made using 66 samples of tension wood and normal wood selected randomly from one tree known to have abundant tension wood. Tension wood was found to have significantly wider crystallites than normal wood. The mean crystallite widths were 3.6 nm for tension wood and 3.2 nm for normal wood. The normal wood crystallite widths were consistent with those reported in previous studies, allowing for an experimental broadening equivalent to about 0.5 nm in this work. This study demonstrates that SilviScan-2 is useful for the detection of tension wood in solid wood samples such as increment cores.

Key words: Tension wood, cellulose crystallite width, X-ray diffraction, SilviScan-2, *Eucalyptus globulus* Labill.

INTRODUCTION

Tension wood is a reaction wood in hardwoods that can lead to drying problems in solid wood during processing. Recent studies have shown that tension wood may be commonly encountered in young plantation grown *Eucalyptus globulus* Labill. (Washusen & Ilic 2001) which may lead to degrade in solid wood during processing (Northway & Blakemore 1996; Washusen et al. 2000). In order to understand why tension wood forms and to determine which trees are likely to cause problems during processing, rapid non-destructive detection methods may be useful.

SilviScan-2, a system developed at CSIRO for rapid analysis of wood microstructure (Evans 1994; Evans et al. 1995), had been used to show that microfibril angle (MFA) and density are insufficient predictors of the presence of tension wood in wood of cambial age of 8–10 years (Washusen et al. 2001). MFA is determined from the width of the cellulose-I 002 reflection measured in the azimuthal (φ) direction (Evans 1999). In this study crystallite width is assessed as a possible predictor of the presence of tension wood in *Eucalyptus globulus*. Crystallite width is inversely propor-
tional to the width of the 002 reflection at half-maximum intensity in the 2θ direction (perpendicular to the azimuthal direction) (Cutter & Murphey 1972). In past studies differences in crystallite width have been found between reaction wood (both tension wood in hardwoods and compression wood in softwoods) and normal wood. Goto et al. (1975) found that crystallite width observed by electron microscopy was in the range of 2.0–4.0 nm in tension wood in poplar (*Populus euramerica*). From these results Nishimura et al. (1981) suggested that crystallite width may be smaller in normal wood of this species given that crystallite dimensions in normal wood are usually less than 2.8 nm. From the width of the 002 reflection in the 2θ direction, Blaho et al. (1994) found that the crystallite width was greater in isolated cellulose from tension wood in beech (*Fagus sylvatica* L.) and Tanaka et al. (1981) found crystallite width was less in compression wood than in opposite wood of akamatsu of Japanese red pine (*Pinus densiflora* Sieb. & Zucc.). To date there has been no study conducted in *Eucalyptus globulus* that has examined differences in crystallite width between tension wood and normal wood. As SilviScan-2 was designed to measure crystallinity and crystallite width, it was applied in this study to assess its potential for detecting tension wood in *Eucalyptus globulus*.

**MATERIALS AND METHODS**

The samples used to investigate the possible differences in cellulose crystallite width were taken from a single 11-year-old tree with abundant tension wood. Microscopic observation of 18 μm thin transverse sections stained with safranin and alcian blue (Fig. 1) of randomly selected wood samples showed that the tension wood occurred in discrete bands surrounded by normal wood (Washusen 2000).

A total of 66 samples measuring approximately 8 × 8 mm in the transverse and longitudinal directions, and 10 mm in the radial direction were selected for assessment of wood anatomy. The discrete bands of tension wood varied in width in the

![Fig. 1. Photomicrographs of transverse sections of tension wood from *Eucalyptus globulus* stained with safranin and alcian blue showing the range in thickness of the gelatinous layer. – a: Tension wood with thick gelatinous layers (stained blue and appearing dark); b: tension wood with thin gelatinous layers.](image-url)
radial direction from approximately 5 mm to 10 mm. The samples were randomly selected from all parts of the tree. Each sample was identified by a number and had points of interest marked at the centre of the radial longitudinal face where transverse shrinkage to 12% moisture content had been measured. The methods employed during this process of sample selection are described fully in a second paper that details a comparison of crystallite width and shrinkage (Washusen & Evans 2001).

Determination of tension wood severity

The wood samples were re-saturated over a period of 24 days, by alternate boiling and vacuum treatment in water. Upon re-saturation, 18 μm transverse sections were cut using a microtome and stained with safranin and alcian blue. Safranin stains normal fibre walls red while alcian blue stains the un lignified gelatinous layers of fibre walls in tension wood blue. Tension wood severity was determined at the point of shrinkage measurement by assessing the proportion of gelatinous wall area from the transverse sections using Image Pro® Plus Version 3.0 software (Image Pro® 1994). The method assessed fibre wall areas tangentially across the section in sequential images at a magnification of ×25, the width of the area assessed was approximately 700 μm. Within each field of view an area of interest was established to exclude rays, parenchyma and vessels and sample as many fibres as possible. For each field of view approximately 25% of all fibre walls were assessed. Some unavoidable error was found to occur with this method as the area of blue included some extraneous dye that accumulated in the lumens and could not be differentiated from gelatinous walls in the image analysis. However, this error was found to add less than 1% to the percent-

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**Fig. 2.** Examples of wood tissue. – a: Normal wood with large lumens and thin cell walls (normal wood 1) – b: Normal wood with thick-walled fibres (normal wood 2) – c: Tension wood with isolated gelatinous fibres and thick-walled normal fibres (tension wood 1) – d: Tension wood with several gelatinous fibres (tension wood 2) – e: Tension wood with a high proportion of gelatinous fibres (tension wood 3).
age of gelatinous wall area. The percentage of gelatinous wall area was used to class each sample as either tension wood or normal wood. Samples with greater than 1.1% gelatinous wall area (the maximum was 31.0%) were classed as tension wood and samples with 0–1.0% gelatinous wall area normal wood. In the case of the normal wood group the error in determining gelatinous wall area that occurred during the image analysis meant that these sections had little or no gelatinous wall.

Figure 2 shows the variation in fibre wall thickness and tension wood severity that was observed in different regions. These images were taken at a higher magnification than used in the assessment of gelatinous wall area. Figure 3 shows the relative proportions of normal wall and gelatinous layer for these images. In these examples the gelatinous wall area determined by image analysis ranged from 0% to approximately 55% of the image.

Sample preparation for SilviScan-2

All samples were saturated in water for sectioning for the anatomical work. Following sectioning, the water was replaced with ethanol in three steps, each taking approximately one week. The samples were dried to a nominal 8% moisture content and mounted on wooden sample holders with PVA glue. Two mm strips running parallel to the grain were cut from the centre of each sample on a twin blade saw to produce strips that were approximately $2 \times 8 \times 10$ mm in the tangential, longitudinal and radial directions respectively. The strips were renumbered on one freshly cut face with the sample number, and they were also marked on the opposite face with the location where shrinkage measurements had been taken. To ensure that the strips were oriented correctly each of the samples were examined with an Olympus SZH10 stereo microscope to match the anatomical sections with the wood strips by matching vessels and the tension wood bands where they were present.
X-ray diffraction and X-ray densitometry

SilviScan-2 is a wood microstructure analysis system which incorporates a scanning X-ray diffractometer. Diffraction patterns are obtained in 7–30 seconds on a CCD area detector. These patterns contain information on MFA, crystallinity and crystallite width. At the time this work was done, the software for automatically extracting crystallite width had not been activated. All widths reported here were obtained manually from the diffraction profiles.

The samples were run in three batches of 22 on SilviScan-2. The system was set up with a rotating Cu K\(_{\alpha}\) anode operating at 45 kV and 15 mA and a capillary giving a focussed spot size of 200 \(\mu\)m. Data were acquired on the area detector for 30 seconds. Density variation was also measured at intervals of 0.01 mm and used to accurately locate where the anatomical data had been collected. The diffraction patterns were automatically collected at 200 \(\mu\)m intervals, mapped onto spherical co-ordinates and saved to disk. Mapping onto spherical co-ordinates results in images where the \(\phi\) co-ordinate and the 2\(\theta\) co-ordinate are straight and orthogonal (see Figure 4). These are here designated as phi/theta images.

![Fig. 4. A phi/theta image from a sample of tension wood.](image)

Determining the width of the 002 peak

Image-Pro 4® was used to obtain profiles of 002 peak intensity in the 2\(\theta\) direction. The azimuthal width of ± 9° (the azimuthal direction is shown in Figure 4) was chosen to cover all profiles measured in this study. Figure 5a shows the full profile obtained for the two examples from 5° to 30° (2\(\theta\)) before adjustment to normalise the height of the 002 peak. Both of these samples had low MFA but one was normal wood (Image 1042 with gelatinous wall area of 0\%) and the other tension wood (Image 319 with gelatinous wall area of 20.6\%). Figure 5b shows the profiles for the 18° to 25° (2\(\theta\)) range with the 002 peaks normalised. The height of the peak was taken from the trough between the peak at the 101 and the 002 reflection to the maximum height of the 002 before normalising. The width of the 002 peak at 50% peak height was determined by interpolation.

Following the initial development the mean 002 width for the three measurements centred on the point of shrinkage measurement was calculated for all of the 66 sample
sections. The microdensity profile was used to ensure precise alignment with the site where the anatomy was assessed (at the shrinkage measuring point). The width of the cellulose crystallites were determined by the Sherrer formula (Cutter & Murphey 1972) which is given in equation (1) below:

\[ D = \frac{0.9 \lambda}{B \cos \theta} \]

Where:
- \( D \) = is the average diameter of the cellulose crystallites (nm)
- \( \lambda \) = wave length of the radiation (0.1542 nm)
- \( B \) = width of the 002 plane reflection at half maximum intensity (in radians)
- \( \theta \) = the Bragg angle (angle of reflection)

Fig. 5. Profiles of image intensity from images 319 and 1042. – a: before standardising to 100% 002 peak height. – b: standardised to 100% 002 peak height showing differences in width of the peak at half peak height.
No correction for instrumental broadening or finite sample thickness was applied, therefore the numbers given in this paper are consistently overestimated by approximately 0.5 nm. Such a correction was not considered necessary for this initial assessment of the relationship between crystallite width and tension wood occurrence.

A one-way ANOVA and post hoc analysis with a Scheffé test were performed to compare the crystallite width between the two sample groups.

RESULTS AND DISCUSSION

The results of a one-way ANOVA were significant ($F_{(1,64)} = 38.16; p < 0.001$) and the Scheffé test was also significant. The means were 3.6 nm and 3.2 nm for tension wood and normal wood, respectively, showing that tension wood had significantly wider crystallites. The difference between the tension wood group and the normal wood group is also shown by the distribution of crystallite width data in the histogram shown in Figure 6. This figure also shows that the crystallite width distributions for normal wood and tension wood overlap around 3.2 nm. One contributor to the overlap could have been uncertainty in assignment of samples to normal wood and tension wood groups. This may be because the histochemical staining was not consistent, particularly the alcian blue stain may have failed to stain some gelatinous layers. The difference in the width of the cellulose crystallites between tension wood and normal wood samples may be explained by an increase in the extent of cellulose

![Fig. 6. Distribution of crystallite width for tension wood and normal wood samples. Mean uncorrected crystallite widths are 3.6 nm (tension wood) and 3.2 nm (normal wood).](image)
crystallisation in tension wood after cell elongation has been completed (Bamber 1978). Bamber suggests that this relative increase in crystallite size is triggered by the loss of turgor pressure in the newly formed cell where there is an absence of lignin.

Even given that crystallite width varied in both normal wood and tension wood the results show that the crystallite width in this tree was closely associated with tension wood and suggests that this ultrastructural characteristic of the fibre wall in tension wood zones may be a good indicator of the presence of tension wood. Furthermore, this would suggest that determination of crystallite width using X-ray diffraction may be used to replace the difficult and (often) tedious anatomical methods that employ histochemical staining. The results suggest that the study should be expanded to see if the relationship is consistent between a number of trees from a range of sites.

CONCLUSIONS

Differences in cellulose crystallite width between tension wood and normal wood can be detected using the scanning X-ray diffractometer on SilviScan-2. The crystallite widths found for normal wood are consistent with those reported by others, given the broadening due to instrumental effects and sample thickness. The uncorrected widths were 3.2 nm and 3.6 nm for normal wood and tension wood respectively. Further research is warranted to examine the relationship in other trees to see if SilviScan-2 can consistently detect tension wood.

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REFERENCES


