Temporal variation of microfibril angle in *Eucalyptus nitens* grown in different irrigation regimes†

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Summary In 1990, a 2-ha plantation of *Eucalyptus nitens* (Deane and Maiden) Maiden was established in southeastern Tasmania and subjected to different irrigation regimes. Point dendrometers were installed in March 1995 to monitor radial stem movement every 15 min over several growing seasons. In this study, data from two growing seasons (1996–1998) were considered. From these measurements, daily increments of stem radius were determined. At the end of the second growing season, we extracted 12-mm cores and measured microfibril angles (MFA) of the wood at high resolution. Microfibril angles were rescaled on a time axis and mapped to daily and distance-based elements. Among treatments, irrigated trees in particular formed higher MFA early in the growing season (September–November) and lower MFA later in the growing season. Trees subjected to cyclic droughts showed clear relationships between MFA and soil water deficits, with MFA increasing in response to water stress release. Increases in MFA were preceded by accelerations in daily increment of stem radius. Among treatments, trees subjected to severe drought had the smallest MFA and generally low fluctuations in MFA. Irrigated trees were susceptible to changes in climate, whereas growth of the trees in the other treatments was limited by water availability. Use of path-analysis showed that temperature had an effect on stem radius increment but not on MFA; wind speed was the only factor that influenced MFA directly. Microfibril angle was correlated with stem shrinking and expansion phases; growth period length and growth rates were positively related to MFA.

Keywords: cambium, climate, dendrometer, environment, eucalypt, wood formation, wood property, wood quality.

Introduction

A primary goal in forestry has been to maximize merchantable stem volume. Accordingly, much research has focused on radial growth rate. Studies on wood quality have been limited mainly to the production of large straight stems, free of defect. However, as our knowledge of the fundamental properties of wood and fiber has increased, it has become apparent that it may be possible to improve wood properties by optimizing conditions in the cambium (Evans 1997, Downes et al. 2000).

One of the most important aspects of cell walls is the angle that cellulose microfibrils form with the long axis in the S2 wall layer of individual cells. The angle of the microfibrils (MFA) is a main determinant of the modulus of elasticity and shrinkage anisotropy (Harris and Meylan 1965, Cave 1968). Cave and Walker (1994) pointed out that, as an indicator of stiffness, MFA is at least as important as wood density. The MFA in individual tracheids of softwood has been related to the tensile strength and stretch properties of pulp fibers, with small microfibril angles resulting in a greater tensile strength and large angles resulting in greater elasticity (Watson and Dadswell 1964, Mark and Gillis 1973, Kellog et al. 1975).

The MFA in juvenile wood is large, whereas angles in mature wood are small (Bentsen and Senft 1986, Donaldson 1992). Similarly, earlywood is characterized by high MFA values, whereas low MFA values are found in latewood (Necesany 1961, McMillin 1973). Variation in MFA is also evident with changing tree height (Wang and Chiu 1988). Evans et al. (2000), who measured 15-year-old *Eucalyptus nitens* (Deane and Maiden) Maiden, found that MFA decreased with stem height to around 30–50% of tree height; thereafter, MFA increased toward the treetop. They reported that MFA was between 20 and 30° near the pith at all heights with a general decrease toward the bark.

In recent years, new technologies have allowed rapid and efficient characterization of wood, including MFA (e.g., Evans 1994, Evans et al. 1996, Downes et al. 1997). These developments have been coincident with a renewed interest in MFA as an important wood quality parameter (Butterfield 1998, Chaffey 2000).

To improve our understanding of wood properties and the factors controlling them, the spatial measurement of the targeted properties across annual rings needs to be converted from a distance scale to a timescale. However, attempts to generate time stamps in wood as it is formed in the cambium have lacked appropriate resolution. Repeated cambium wounding.

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as developed by Mariaux (1969), has been used to set time markers and the generated callus tissue remains as an artificial and datable scar in the wood. Schmitt et al. (2000) have shown that repeated wounding throughout the growing season provides information on the temporal variation of different tissues. Although cambium wounding can be used to generate time stamps for species showing indistinct growth zones or wedging rings (Worbes 1995), the method is generally destructive and operates at a very limited time resolution.

High resolution of growth processes can be obtained through the use of dendrometer bands. These bands are made of stainless steel and mounted around the tree stem to monitor changes in circumference. The band movements are measured manually through gauges or calipers, or registered electromechanically with potentiometers (Hinckley and Bruckerhoff 1975). Dendrobands have been used in a variety of studies, including investigations of tree water status (Lassoie 1973), drought effects (Hinckley and Bruckerhoff 1975), hydrologic regime changes (Keeland et al. 1997) and ozone and climate effects on tree growth (McLaughlin and Downing 1995). Alternatives to bands are point dendrometers used to monitor the radial movements of tree stems (Impens and Schalck 1965, Downes et al. 1999, Zweifel and Hässler 2000). Point dendrometers are usually mounted on stainless steel rods inserted into the wood. They provide a linear measure of stem movements, which are related to cambial processes at a particular point.

This paper reports trends in microfibril angle (MFA) in *Eucalyptus nitens* over two growing seasons. Point dendrometers were used to monitor radial stem movements at high resolution. Cores were then taken and MFA of the wood measured at high resolution and mapped on a daily time axis, rather than on a distance axis. To compare daily growth processes and MFA, trees were grown in different irrigation regimes.

### Materials and methods

#### Sites

The investigation was undertaken in a 2-ha plantation of *Eucalyptus globulus* Labill. and *E. nitens* established in 1990 in Lewisham, southeastern Tasmania (42°49′ S, 147°36′ E, elevation 30 m a.s.l.). Second generation, improved juvenile-per- sistent *E. nitens* (Pederick 1979) were used in this study. The stocking rate was 1428 stems ha⁻¹. Tree growth and water use (measured with a neutron moisture meter; see Worledge et al. 1998) have been monitored intensively since establishment in August 1990. The soil has a shallow red-brown loam A horizon and light brown, medium clay B horizon, occasionally overlaid by a light yellow–brown gritty loam from decomposing rocks. Parent material is basalt with sandstone floaters intruding in the southwestern corner of the plantation. Mean soil depth to bedrock is 0.6 m. Based on long-term meteorological measurements at Hobart airport, 9 km west of the site, mean January maximum/minimum temperature is 22.3/11.8 °C, mean July maximum/minimum temperature is 12.2/4.0 °C and mean annual rainfall is 512 ± 115 mm. Annual rainfall is below that normally considered adequate for plantation establishment. The management of the site has been described elsewhere (Honeysett et al. 1996, White et al. 1999). Six weeks after planting, phosphorus was applied as triple superphosphate at 120 kg ha⁻¹ elemental P. Nitrogen was applied as urea at 100 kg ha⁻¹ elemental N in three applications in 1990–1991 and 1991–1992 (40% in August and 30% in both December and March) and at 60 kg ha⁻¹ N each August from 1992 to 1996.

#### Experimental design

The site was divided into plots of similar area and six trees from three plots (two trees per plot) were chosen for study. The first block (A) was irrigated to avoid water stress and the other two blocks (B and C) were subjected to cycles of moderate and severe drought, respectively. Irrigation was applied through micro-sprinklers. Soil water deficit was defined as the amount of water required to bring the soil to field capacity. Soil water deficit in the irrigated treatment (Plot A) was not allowed to exceed approximately 40 mm (Honeysett et al. 1996) except on two occasions during winter to encourage root development. Irrigation was applied frequently in small amounts (10 mm) to avoid large changes in water content between measurements, which were made every 2 weeks. Plot B was subjected to a series of drying cycles interrupted either by rainfall or supplementary irrigation. These cycles created large fluctuations in soil water deficits during the 2 years of observation. Water was withheld from Plot C to allow a complete drying of the soil profile. The drought was interrupted only by rainfall, or by limited irrigation to prevent death of trees. Further irrigation details have been presented by Worledge et al. (1998).

#### Environmental data

Environmental data were taken from a station established 750 m from the plantation. Temperature and vapor pressure deficit (VPD) were measured with a screened Vaisala (Hawthorn, VIC, Australia) probe. Wind speed was measured with a cup anemometer (White et al. 1999). The instruments were mounted on a tower, 6 m above ground. Access tubes with neutron moisture meters (CPN503, Pacheco, CA) were installed within each of the plots. The tubes were placed immediately opposite the midpoint between a randomly selected pair of trees. Soil water deficit measurements were taken about every 2 weeks.

#### Monitoring tree growth

In March 1995, point dendrometers (Agricultural Electronics, Tucson, AZ) were installed on all six *E. nitens* trees at about 25% of tree height, corresponding to about 3 m actual height. Dendrometers were mounted on 4-mm stainless steel threaded rods inserted 40 mm into the wood. Each dendrometer sensing head was placed at the bark surface with loose bark removed. Dendrometers were calibrated so that a 4-µm change in stem radius corresponded to approximately 1 mV. Radial growth on the northern side of the tree was monitored at 15-min intervals throughout each growing season from August 1996 to July 1998. August represented the start of spring growth in these
trees, and radial growth accelerated around mid-August. From these measurements, hourly and daily increments of stem radius were determined.

**Wood data**

In September 1998, 12-mm cores were extracted from the trees with a powered borer approximately 300 mm below the sensing head of the dendrometer. Cores were treated as described by Downes et al. (1997) by replacing water with 100% ethanol followed by air-drying. Radial profiles of MFA were estimated from the cellulose-I 002 azimuthal X-ray diffraction peaks obtained with the rapid scanning X-ray diffractometer system SilviScan-2 (Cooperative Research Centre for Hardwood Fibre and Paper Science, University of Melbourne, Parkville, VIC, Australia), at a resolution of 200 µm (Evans et al. 2000). The equipment can perform X-ray microdensitometry at a resolution of 50 µm (Evans 1994). The densitometry data were used to identify annual growth rings.

**Relating stem growth to wood properties**

Software, written in Interactive Data Language (IDL; Research Systems, Boulder, CO), was used to process the hourly dendrometer data to obtain rates and duration of stem shrinkage and expansion for each 24-h period (Downes et al. 1999, 2000). The trees commonly experienced a shrinkage phase during the early part of the day followed by an expansion phase during the afternoon and evening. Figure 1 shows a typical diurnal cycle during 3 days in summer. From this pattern, three distinct phases were mathematically defined within a single diurnal cycle. The shrinkage phase was defined as the period during which the tree decreased in radius, usually from an early morning maximum. The recovery phase was defined as the portion of the cycle during which the radius increased until it reached the position observed previously. Finally, the increment phase was defined as the period when the stem radius continued to increase until the shrinkage phase commenced in the next diurnal phase. By processing the measured data the shrinkage, recovery and increment were resolved into rates (µm h⁻¹) and duration (h).

Software was developed to relate daily growth to radial wood increments. The measured wood properties were on a distance scale, whereas the growth data were on a timescale. If growth rates are linear and constant over the year, a direct correspondence should exist between the spatial and temporal scales of measurements. However, this was not the case in reality, so we used another approach to relate the two scales. Because dendrometers measure distance over time, a common axis exists with the measured wood properties. The axis of the dendrometer data was therefore rotated so that the radial distances of both measures could be plotted on the abscissa. Figure 2 gives an example of the association between wood properties and dendrometer data, where MFA features can be seen that correspond with dendrometer data.

Software, written in IDL, mapped the growth and weather data from a daily to a distance basis. It was assumed that phloem production was more or less constant throughout the year. The daily dendrometer data were rescaled so that the total ring width was the same for the wood and dendrometer data. The dendrometer data were then smoothed using a 7-day moving average filter with shrinkage events removed. The spatially measured wood property data was mapped onto a daily time step, using the time and distance-based arrays. A critical step in the mapping process was the identification of growth ring boundaries. The defined annual rings of *E. nitens* allowed the start and end of each year’s growth to be determined.

The hourly weather data were processed to extract the average conditions during each phase for each day. Spearman non-parametric rank correlations were used to determine relationships between MFA and stem phases mapped on equally
spaced distances. To control for interrelationships of climatic variables, path analysis was used as an extension of multiple regression analysis to assist in the interpretation of the data. The resulting standardized regression weights indicate the strength and direction of the relationships among the hypothesized variables. For each treatment, a model was used to determine how well the data fit by recomputing the correlations among variables using the $\chi^2$-test for goodness of fit. Through path analysis, the magnitudes of the linkages between MFA, stem radius increment and weather variables were estimated to provide information about the underlying causal process (Miller and Jastrow 1990). Path analysis has been successfully applied to solid wood (Zhang and Zhong 1992, Cown et al. 1999) and fiber properties (Wimmer et al. 2002). The software package EQS (Version 5.7 for Windows, Multivariate Software, Encino, CA) was used to perform the correlation and path analysis (Bentler 1998).

**Results**

**MFA trends and soil water deficit**

Mean annual ring widths were highest in irrigated trees (Group A, 10.1 mm), smallest in trees subjected to severe drought (Group C, 4.4 mm), and intermediate in trees subjected to cyclic drought (Group B, 6.9 mm). Changes in stem diameter and MFA were recorded over two growth periods commencing August 1, 1996. Figures 3a–c present seasonal changes in MFA for trees in each treatment along with soil water deficits. Microfibril angles formed during the first months of each growing season (earlywood) were larger than MFAs formed later in the season. Within a treatment, the seasonal trends in MFA of the two trees were similar, suggesting that common environmental factors were in control.

The irrigated trees (Group A, Figure 3a) showed a relatively smooth seasonal pattern with no obvious association with soil water deficits. The MFA range over the 2 years was only 9°. In trees subjected to cyclic drought (Group B, Figure 3b), variability in MFA was high, ranging from 23° in mid-November 1996 to 10° at the end of the second season. The changes in MFA were associated with changes in soil water deficits. On October 23, 1996, a soil water deficit of −88 mm was measured. This deficit was released during the following days and reached −24 mm on November 6. Between 5 and 10 days later, on November 11 to 17, 1996, the two trees formed wood with MFAs of 21 and 23°, respectively. Thus, it appears that the high MFAs were formed in response to release of soil water deficit. Further stress-release effects were seen later in the growing season, such as the MFA peak (18°) on April 7, 1997, as well as on November 13, 1997 and April 16, 1998.

For the Group B trees, the period between September 25 and December 9 was analyzed in detail. This period included the 23° peak in MFA that was recorded for mid-November 1996. Figure 4 shows that accelerated stem radius increment was associated with high MFA. Thus, temporary peaks in daily increments were recorded around October 1, 13 and 24 and were paralleled by small increases in MFA (see vertical lines in Figure 4). The release in soil water deficit starting around October 23 led to a major increase in daily stem radius increment that peaked on November 5 (Tree 1, Figure 4). Six and 12 days later, the MFAs of Trees 1 and 2, respectively, had reached their maxima. During the following weeks the daily stem radius increment of both trees steadily decreased to zero.

The trees subjected to severe drought (Group C, Figure 3c) showed low MFA variability, ranging between 10 and 15°. Although soil water deficits were high, the fluctuation in soil water deficit was less than in the cyclic drought treatment, which might explain in part the low variability in MFA in the Group C trees. In addition, growth rates of these trees were lower than for trees in the other treatments, indicating reduced cambial activity.

**Effect of weather**

In addition to soil water deficit, several weather variables were recorded during the observation period. A path-analysis model was tested for each treatment using air temperature, VPD and

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**Figure 2. Alignment of wood density (dashed line) and microfibril angle (MFA, solid black line) with dendrometer data collected hourly (gray line) for an irrigated *Eucalyptus nitens* tree over three growing seasons.**
Figure 3. Comparison of seasonal trends in microfibril angles in two *Eucalyptus nitens* trees (solid lines) and soil water deficits (dashed line) measured every 2 weeks over 2 years. Two trees are shown per treatment: (a) irrigated; (b) cyclic drought; and (c) severe drought.
wind speed as variables to predict MFA and stem radius increment. All three climate variables were assumed to act directly, but only air temperature and wind speed showed a significant correlation with MFA or stem radius increment. The $\chi^2$-test for goodness of fit was significant only for the irrigated trees (Group A) ($\chi^2 = 12.3$, $P < 0.01$), indicating that, among the treated trees, the irrigated trees were most responsive to climate.

In Figure 5, the path-analysis model shows standardized partial regression coefficients or path coefficients that indicate the degree to which the independent variable (weather) has a direct effect on the dependent variable (MFA or stem radius increment). The coefficient indicates the change expected in the dependent variable for a 1 standard deviation change in the independent variable. For example, a 1 standard deviation increase in air temperature caused a 0.65 standard deviation increase in stem radius increment. Because the coefficients are standardized, changes in the dependent variables can be directly compared. Path coefficients are influenced by correlations among predicting variables. In Figure 5, the weather variables are connected through curved two-headed arrows that symbolize simple correlations. The strongest direct effect was between temperature and stem radius increment (0.65); VPD had no effect on MFA or on increment. Wind was the only factor having a direct effect on MFA.

**MFA and stem fluctuations**

Microfibril angle correlated with the parameters derived from the stem shrinking and stem expansion phases (see Figure 1). For irrigated trees and trees subjected to cyclic droughts, stem radius increment was positively correlated with MFA (Figure 6). Longer growth periods and higher growth rates were also related to higher MFA. In trees subjected to severe drought, a high rate of stem shrinking was related to high MFAs.

**Discussion**

We used new methodology to record daily radial stem movements over a 2-year period and combined the measurements with high-resolution MFA analysis of the wood formed during the same period. Distance-based MFA measurements were transformed and mapped onto a time axis that allowed synchronous comparisons between trees across treatments. With this new approach it was possible to monitor cambial activity at the same location in the tree stem over a 2-year period without causing wound effects. Previous studies, based on repeated cambium sampling, attempted to avoid wound effects by moving the sampling locations in the tree either downward (Antonova et al. 1995) or around the circumference (Wodzicki 1971),
Because the stem radius increments determined from stem cambium is much slower than on the xylem side during mid-nor. The rate of tissue production on the phloem side of the not significant. Similarly, the frequent changes in phloemous day, it was assumed that changes in cambial width were meant by growth, because the cambial phases of cell division and expansion were not directly measured. However, we assume that most cambial activity occurred during the increment phase, when water availability to the cambium was at a maximum (Nonami and Boyer 1990, Antonova et al. 1995). Richardson (1964) reported that nighttime temperature had a stronger relationship with fiber length than mean daily temperature. Intensive growth of primary cell walls was also observed during night hours by Antonova et al. (1995). Further research is needed to combine daily dynamics of radial cell division and expansion with radial stem movements and water status of trees.

In eucalypts, cambial derivatives differentiating into xylem vessels and fiber tracheids subsequently undergo a sequence of changes including cell enlargement with continued primary wall formation, secondary wall deposition and lignification (Ridoutt and Sands 1994). The positional movement recorded by the dendrometers could be influenced by variations in cambium width during the growing season. During the mid-growing season, the width of cambium undergoing differentiation and secondary wall development can be up to 100 cells, measured at breast height in *E. globulus* (Ridoutt and Sands 1994). Because the stem radius increments determined from stem movements are the incremental changes relative to the previous day, it was assumed that changes in cambial width were not significant. Similarly, the frequent changes in phloem thickness, which occurred in the order of days, were also minor. The rate of tissue production on the phloem side of the cambium is much slower than on the xylem side during mid-season in most species (Zimmermann 1964).

We observed that higher MFAs formed during the early growing season and lower MFAs formed during the later part of the growing season, as has been observed in previous studies (e.g., Shupe et al. 1996, Megraw et al. 1998). The trees subjected to cyclic drought, in particular, showed a clear relationship between MFA and soil water deficits. Microfibril angle appeared to increase in response to release from water stress and the increase was preceded by an acceleration in daily stem radius. The development of water stress in trees influences almost every aspect of wood formation, including the duration of cambial activity (Zahner 1968). Water stress can reduce growth directly through a reduction in cell turgor and by interfering with metabolism and cell enlargement. A drought-induced reduction in growth might also occur indirectly as a result of decreased synthesis of auxin and carbohydrates and a decreased translocation to the cambium (Kramer 1964); however, it is likely that growth reduction in response to short-term changes in the severity of water stress is directly induced because the rate of polar auxin transport is not rapid enough to account for rapid reactivation of the cambium. The study trees responded to release of soil water deficits within days with accelerated radial stem increment and within 3 weeks with an increase in MFA. The rate of basipetal (polar) auxin movement is 9–10 mm h$^{-1}$ (Little 1981), implying that auxin would take over 40 days to travel 10 m from the base of the crown to the stump height. This suggests that there is another path of activation of the cambium, e.g., through hormone precursors stored in the cambium (Wareing 1951) or through a supplementary supply of hormones from adjacent tissues (Berlyn 1982).

Effects of climate were observed only in irrigated trees. Tree growth has been described as an effect of control by limiting factors (Fritts 1976). In the irrigated trees, air temperature was a limiting factor for growth. Similarly, Downes et al. (1999) reported that temperature affected the stem radius increment of irrigated *E. nitens* trees. Wind had a direct effect on MFA, probably by causing mechanical flexure stress that increased MFA—an effect described previously by Telewski (1989). The irrigated trees exhibited a larger response to wind than the trees in the other treatments, because their denser crowns and greater leaf masses made them more susceptible to wind sway.

The secondary cell walls in xylem vessels and tracheids have extremely well aligned microfibrils. The constant helical alignment within successive lamellae of the secondary wall parallels the organized arrangement of cortical microtubules found in softwoods (Abe et al. 1995) and hardwoods (Prodhan et al. 1995). In the primary wall, cortical microtubule orientation appears more or less random (Abe et al. 1995, Chaffey et al. 1997), but during cell expansion, the random orientation of microtubules and microfibrils becomes organized (Abe et al. 1995, 1997). As cambial derivatives differentiate, cell radial widths increase and the microtubules become progressively more ordered and numerous, ending up as a dense, parallel, helically aligned array.

Elongation occurs at both ends of the fiber through tip growth. At maturity, an apparent co-orientation between microtubules and microfibrils exists. Although precise time-

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**Figure 6.** Spearman rank correlations between MFA and daily stem radius increment, daily stem growth period, daily stem shrinking period, rate of stem growth and rate of stem shrinkage. Significance for correlations: $r > 0.28$, $P < 0.01$, $n = 84$. 

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scales of the changes occurring during development of cell elements have not been established, it is clear that the various microtubule arrays can co-exist and the corresponding wall elaborations can take place concurrently (Chaffey 2000). Therefore, sudden changes in stress could change MFA directly or indirectly.

In irrigated trees and trees subjected to cyclic droughts, daily stem radius increments correlated positively with MFA. McMillin (1973) reported that wood with a specific gravity of more than 490 kg m\(^{-3}\) from fast-growing *Pinus taeda* L. had a higher MFA than wood from slow-growing trees; however, a similar relationship was not observed with low specific gravity wood samples. Herman et al. (2000) found that fast-growing Norway spruce trees had a higher MFA than slow-growing trees. Erickson and Arima (1974) reported that accelerated growth was associated with an increase in MFA in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), especially in trees that had previously been growing slowly, e.g., released from suppression, and concluded that a weak relationship existed between growth rate and MFA. Similarly, Lindström et al. (1998) concluded that growth had only a minor effect on MFA. However, both Erickson and Arima (1974) and Lindström et al. (1998) investigated MFA across the radius and found that cambial age was the major factor for MFA. Compared with the age effect, growth rate was a weak factor for MFA. The major difference between these studies and ours is the resolution. We age effect, growth rate was a weak factor for MFA. The major cambial age was the major factor for MFA. Compared with the age effect, growth rate was a weak factor for MFA. The major difference between these studies and ours is the resolution. We found stem radius increment–MFA relationships across tree rings by measuring MFA every 200 µm in the radial direction in concert with measurements of stem radius fluctuations, thereby achieving a time resolution on the order of days.

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