REVIEW - MICROFIBRIL ANGLE: MEASUREMENT, VARIATION AND RELATIONSHIPS

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SUMMARY

Microfibril angle (MFA) is perhaps the easiest ultrastructural variable to measure for wood cell walls, and certainly the only such variable that has been measured on a large scale. Because cellulose is crystalline, the MFA of the S2 layer can be measured by X-ray diffraction. Automated X-ray scanning devices such as SilviScan have produced large datasets for a range of timber species using increment core samples. In conifers, microfibril angles are large in the juvenile wood and small in the mature wood. MFA is larger at the base of the tree for a given ring number from the pith, and decreases with height, increasing slightly at the top tree. In hardwoods, similar patterns occur, but with much less variation and much smaller microfibril angles in juvenile wood. MFA has significant heritability, but is also influenced by environmental factors as shown by its increased values in compression wood, decreased values in tension wood, and often, increased values following nutrient or water supplementation. Adjacent individual tracheids can show moderate differences in MFA that may be related to tracheid length, but not to lumen diameter or cell wall thickness. While there has been strong interest in the MFA of the S2 layer, which dominates the axial stiffness properties of tracheids and fibres, there has been little attention given to the microfibril angles of S1 and S3 layers, which may influence collapse resistance and other lateral properties. Such investigations have been limited by the much greater difficulty of measuring angles for these wall layers. MFA, in combination with basic density, shows a strong relationship to longitudinal modulus of elasticity, and to longitudinal shrinkage, which are the main

reasons for interest in this cell wall property in conifers. In hardwoods, MFA is of more interest in relation to growth stress and shrinkage behaviour.

Key words: Microfibril angle, cellulose microfibrils, X-ray diffraction, microscopy, wood properties.

INTRODUCTION

The primary and secondary cell walls of plants contain a scaffold of cellulose microfibrils embedded in a matrix of polysaccharides such as pectin, hemicellulose, and often lignin, especially in vascular tissues (Harris 2006). In primary cell walls, the orientation of cellulose microfibrils is often random, but may show varying degrees of alignment in tissues where cell elongation is taking place (Wardrop 1958; Imamura et al. 1972; McCann and Roberts 1991; Abe et al. 1995b; 1997). In other tissues, including those containing sclereids and some types of non-xylem fibres, secondary cell walls may show many alternating layers of opposing microfibril orientation, known as a helicoidal arrangement (Reis and Vian 2004).

In the secondary cell walls of xylem cells, the cell wall typically has three layers, an outer S1 with transversely oriented microfibrils, a thick S2 layer with axially oriented microfibrils, and an inner S3 layer also with transversely oriented microfibrils, in a S-Z-S helical organisation (Fig. 1) (Wardrop and Preston 1947; Preston and Wardrop 1949; Harada et al. 1951; Preston 1952; Meylan and Butterfield 1978; Butterfield and Meylan 1980; Brändström et al. 2003, Brändström 2004a, b; Donaldson and Xu 2005) also reviewed by Barnett and Bonham (2004), and by Abe and Funada (2005). This crossed structure provides high axial stiffness while at the same time providing high collapse and burst resistance, thus allowing the plant to adopt an erect growth habit, while also allowing efficient water conduction up the stem. Mutant studies confirm that both cellulose and matrix are required to achieve these mechanical and physiological functions, as when either component is reduced, prostrate growth and collapsed xylem phenotypes result (Kajita et al., 1997; Turner et al., 1997; Jones et al 2001).

From a utilitarian viewpoint, the orientation and organisation of cellulose microfibrils contribute to the physical properties of sawn timber and processed fibre. The S2 layer is generally much thicker than the other layers and may therefore dominate the physical and chemical properties of the cell wall. It has been shown that the longitudinal stiffness (longitudinal modulus of elasticity or MOE_L) of wood is very dependent on S2 microfibril angle (Cave 1968; Cave and Walker 1994). The average MFA of the S2 layer in mature wood lies between 5º - 20º to the fibre axis, but much larger angles are found in the juvenile[1] wood of conifers, particularly at the base of the tree, contributing to the low stiffness of wood in the butt log (Donaldson 1992; Cave & Walker 1994; Walker & Butterfield 1995; Cown et al. 1999; Xu et al. 2004). In contrast, the S1 and S3 layers are relatively thin, but are nevertheless thought to have a crucial role in strengthening the cell against deformation by water tension forces, as well as contributing to the lateral hardness and crushing strength of timber (Booker, 1993, 1996; Booker and Sell 1998; Koponen 1998). The S1 layer may play an important role in determining pulp fibre properties, contributing to fines formation (Jordan and O'Neill 1994) and determining the transverse mechanical properties and surface properties of fibres (Bergander & Salmén 2000, 2002; Bardage et al. 2003; Brändström et al. 2003). Booker and Sell (1998) have suggested that the S3 layer is comparatively more effective at stiffening the wall in the transverse plane than the S2 layer, and thus contributes to collapse resistance in functional xylem.

Measurement methods

The literature on MFA is dominated by method description, often to distraction from interesting experimental results. Perhaps there are few parameters that have so many different methods for assessment and so many variations on individual methods. Measurement techniques for MFA are of two types, either measurement of individual tracheids or fibres using microscopy, or measurement of bulk wood samples using X-ray diffraction or near infrared (NIR) spectroscopy. Microscopy-

[1] Juvenile wood is used to refer to the inner 10-15 growth rings from the pith following common usage. For a detailed discussion see Burdon et al. (2003).

based techniques are divided into those that rely on the optical properties of crystalline cellulose, employing variations on polarised light techniques (Preston 1934; Manwiller 1966; Page 1969; El-Hosseiny and Page 1973; Leney 1981; Donaldson 1991; Verbelen and Stickens 1995; Batchelor et al., 1997; Ye and Sundström 1997; Jang 1998; Palvianen et al., 2004; Ye 2006 a, b), and those that directly or indirectly visualise the orientation of the microfibrils themselves. Such methods include iodine precipitation (Bailey and Vestal 1937; Senft and Bendtsen 1985) and other biological, chemical or physical treatments (Huang 1995; Anagnost et al., 2000), confocal reflectance microscopy (Donaldson and Frankland 2004), fluorescence microscopy (Marts 1955), micro-Raman spectroscopy (Pleasants et al., 1998), scanning electron microscopy (SEM) (Meylan and Butterfield 1978; Abe et al., 1991), and transmission electron microscopy (TEM) (Hodge & Wardrop 1950; Wardrop 1954, 1957; Wardrop and Preston 1947; Frei et al., 1957; Harada 1965a, b; Preston 1965; Dunning 1968; Reis and Vian 2004; Donaldson and Xu 2005). Some of these techniques are more suited to quantitative applications while others are used for simple imaging. These techniques are described in more detail below.

1. Polarisation microscopy

The earliest techniques for assessing microfibril orientation were based on various forms of polarised light microscopy. Because cellulose is partially crystalline, and the microfibrils within each secondary wall layer are highly aligned (Müller et al. 1998, 2006; Lichtenegger et al. 2003; Abe and Funada 2005), thin sections of wood are birefringent when viewed between two crossed polarising filters. In cross-sectional view, this type of microscopy can be used to identify the three secondary cell wall layers, which have different brightness at different orientations of the section. Unfortunately, this approach cannot be used to easily measure MFA in cross-sections (Crosby et al. 1972), but in longitudinal sections, where the section is thin enough to contain only a single cell wall, it is possible to measure the MFA as a weighted average of the whole secondary wall (Preston 1934; Page and El-Hosseiny 1974). The effect of the transversely oriented S1 and S3 layers on the

birefringence of the whole fibre wall is generally small, but varies with total cell wall thickness (Page and El-Hosseiny 1974). This technique simply involves rotating the tracheids or fibres relative to the fibre long axis until the bright cell wall becomes dark, the so called maximum extinction position (MEP) (Fig. 2). Usually it is necessary to determine the correct direction of rotation (clockwise or anticlockwise) to avoid measuring the complementary angle, using either a compensator, or by observing nearby pits. The difference between the fibre axis and the MEP is the average MFA, which approximates the S2 MFA because the S1 and S3 layers are relatively thin compared to the S2 layer. The constraint of a single cell wall thickness is required because in opposite walls from the front and back of a tracheid or fibre, microfibrils will be oriented in opposite directions, and hence the MEP cannot be found. The various polarisation techniques vary in their approach to achieving a single cell wall for observation. It is possible to simply cut very thin longitudinal sections, but this approach has the disadvantage that the section will always have only some regions containing a single cell wall, thus limiting the sites where measurements can be made (Preston 1934; Cousin 1972; Leney 1981). Other approaches include filling single-fibre preparations with mercury (Page 1969), which has safety considerations, and using the holes formed by bordered pit apertures, where the pit membrane has been removed by maceration, to view the single cell wall on the opposite side of the cell (Donaldson 1991).

A novel method which avoids the need for single-cell wall preparations is confocal bifluorescence microscopy (Verbelen and Stickens 1995; Jang 1998; Bergander et al. 2002; Sedighi-Gilani et al. 2005). This technique uses the natural polarisation of some fluorescent dyes such as congo red or calcofluor when bound to cellulose molecules, in combination with the optical sectioning ability of confocal microscopy, to make MEP measurements within the S2 region of the secondary wall simply by focusing on this region. A similar approach using the polarisation of reflected light, was used by Batchelor et al. (1997). The z resolution (depth-of-field) of a high numerical aperture objective lens is sufficient to exclude the S1 and S3 layers unless the cell wall

thickness is less than 1 µm. The disadvantage of this approach is the need for relatively slow electronic image acquisition over a range of orientations, where the MEP is calculated from a plot of brightness versus orientation (Batchelor et al. 1997; Jang 1998). Because confocal imaging usually requires an electronic light detector and signal averaging, this process is relatively slow, although multiple fibres can be measured simultaneously within the field of view. Some types of confocal microscope such as Nipkov disk or slit scanning devices do allow real-time confocal imaging, but this type of instrument has not been applied to the task of measuring S2 microfibril angles.

For automated measurement of pulp fibres, spectroscopic imaging ellipsometry has been used to characterise S2 MFA (Ye and Sundström 1997; Ye 2006a, b). This technique is independent of fibre orientation and measures the spectral transmission function of the fibre, which can be used to measure MFA, using an optical system based on polarisation microscopy and spectroscopy. This technique does not require single cell walls on which to make measurements, making it ideal for measurement of commercial pulp samples without any specialised sample preparation.

More recently, the single-cell wall approach has been extended to single cell wall layers by cutting much thinner sections of embedded wood using an ultramicrotome (Donaldson & Xu 2005). This is the only method that allows quantitative measurement of individual cell wall layers including the S1 and S3 layers, for single tracheids.

2. Direct visualisation using physical or chemical methods

It is relatively easy to directly image the microfibril orientation on cell wall surfaces, especially if the surface is produced by fracturing, as this reveals the “grain” of the cell wall (Donaldson and Frankland 2004). It is not necessary to be able to see individual cellulose microfibrils to determine the MFA because a fracture will produce a coarse surface texture based on microfibril clusters or lamellae that can be seen with a simple brightfield light microscope. Since the position of cell wall fracture is unpredictable, it may be necessary to search for an appropriate

region where S1 or S2 layers are revealed. Often there is a preferred fracture plane between the S1 and S2 layers (Donaldson 1995). However, fracture surfaces are much less likely to reveal the texture of S3 layers. Microscopy of the lumen surface does not always reveal a clear image of microfibril textures because of the dense matrix in the S3 layer. Marts (1955) used fluorescence microscopy of split radial surfaces to measure MFA by visualising checks on the wood surface. Using pulp fibres, Crosby and Mark (1974) used ultraviolet (UV) illumination combined with phase contrast microscopy to observe micro-checks in the fibre walls. In this case, the use of UV illumination allowed improved resolution, although the exact nature of the micro-checks was not determined. Phase contrast microscopy with white light illumination has also been used to measure MFA in pulp fibres by visualising the microfibril texture (Peter et al. 2003).

Greater measurement accuracy requires more image detail, and techniques such as confocal reflectance microscopy (Donaldson & Frankland 2004; Donaldson et al. 2004) (Fig. 3) or electron microscopy (Hodge & Wardrop 1950; Wardrop 1954, 1957; Wardrop and Preston 1947; Frei et al. 1957; Harada 1965a, b; Dunning 1968), especially low-voltage field emission scanning electron microscopy (FESEM), can all produce suitable high-contrast images (Abe et al. 1991, 1992, 1997; Kataoka et al. 1992; Brändström et al. 2003, Brändström 2004b; Abe and Funada 2005). Some investigations have examined cell wall layers during deposition, but prior to or during lignification, and in these cases microfibril textures can be clearly seen and measured (Abe et al. 1991, 1992, 1997; Kataoka et al. 1992; Fujino and Itoh 1998).

Iodine precipitation has been used to visualise microfibril orientation using either brightfield microscopy (Bailey and Vestal 1937; Senft and Bendtsen 1985) or confocal microscopy (Donaldson and Frankland 2004). This technique relies on the precipitation of iodine crystals within the cell wall, which is quite an interesting process in itself. Originally, it was thought that iodine crystals were deposited in minute checks within the cell wall that were induced by drying (Bailey and Vestal 1937; Senft and Bendtsen 1985). However, more recent studies have shown that

the iodine crystals form cavities within the cell wall by compressing the surrounding cell wall material. Such cavities may occur in regions of greater porosity within the cell wall, such as at the S1/S2 boundary (Donaldson and Frankland 2004). Although useful, some caveats must be remembered with the iodine-precipitation technique. Not all wood samples react equally well so that iodine crystals may be patchy, or present only in certain cells, or not at all in some samples. The iodine precipitation requires concentrated nitric acid, the fumes from which may damage expensive light microscope equipment. Iodine crystals sublime rapidly so the effect may disappear before measurements can be completed. In a modification of the direct visualisation of iodine crystals, it is instead possible to make images of the cavities produced by the crystals using confocal reflectance microscopy (Donaldson and Frankland 2004) (Fig 3). The crystals themselves are easily removed by washing in ethanol. This has the advantage of removing volatile/corrosive chemicals from the sample and improving the detail of microfibril orientation. Soft-rot cavities (Fig. 3) are also used in a similar way (Anagnost et al. 2000, 2002; Khalili et al. 2001; Brändström et al. 2002), but have the disadvantage of requiring a relatively long time (6-14 weeks) for the fungus to produce sufficient cavities, and the cavities are relatively coarse in size (Anagnost et al. 2000; Brändström et al. 2002).

Mechanical fibrillation using ultrasonic treatment, either alone or in combination with chemical treatments, has also been used to visualise MFA by brightfield light microscopy (Crosby and Mark 1974; Huang 1995, Huang et al. 1998; Wang et al. 2001). Congo red has been found to enhance ultrasonic fibrillation of cell walls (Huang 1995). However, such treatments may induce checking more easily in large diameter tracheids with high MFA, resulting in some bias in the measurements (Huang 1995). Wang et al. (2001) using a range of softwoods and hardwoods, found that treatment with cobalt and copper salts enhanced fibrillation by sonication and hence facilitated measurement of MFA in latewood, even in *Pseudotsuga menziesii* (Mirb.) Franco, where spiral thickenings often make measurement of MFA difficult.

The orientation of bordered and cross-field pit apertures is known to often follow the orientation of microfibrils, and has been used to measure MFA (Pillow et al. 1953; Cockrell 1974). Typically latewood tracheids are examined because the pit apertures are more elongated and hence it is easier to measure the orientation, but this may bias the results, as latewood is known to often have lower MFA values than earlywood (Wellwood 1962; McGinness 1963; Hiller 1964a; McMillin 1973; Paakkari & Serimaa 1984; Stuart & Evans 1995; Donaldson 1998; Herman et al. 1999; Anagnost et al. 2005; Deresse et al. 2003; Sarén et al. 2004; Jordan et al. 2005). Pinoid cross-field pits are easier to measure than fenestriform cross-field pits and bordered pits, because of their elongated shape (Pillow et al. 1953). Ray tracheid pit apertures can also be used, and may be more reliable than cross-field pits (Shumway et al. 1971; Huang et al. 1998; Lichtenegger et al. 2003).

3. X-ray diffraction

X-ray diffraction is currently perhaps the most popular method for measuring MFA (Cave 1966; 1997; Boyd 1977b; Evans 1999), and automated devices capable of scanning increment cores at high spatial resolution have been developed to exploit this technique (Evans et al. 1999). Several procedures are available for interpreting diffraction patterns from radial or tangential surfaces of wood, and a detailed description of each is beyond the scope of this review (Cave 1997). However, typical methods obtain the MFA by measuring characteristics of the 002 equatorial reflection (Cave 1968; Yamamoto et al. 1993; Stuart and Evans 1995; Evans 1999). The method proposed by Meylan (1967) requires calibration against other methods, while the variance method proposed by Evans (1999) is directly related to MFA but with the disadvantage that precision is less at very high angles because of the relatively weak diffraction signal from juvenile softwood. In theory it is possible to determine the MFA directly from the 040 reflection but this is confounded by overlapping reflections from other planes (Cave and Robinson 1998). The variance method proposed by Evans (1999) has been used to develop automated MFA measurements of the S2 layer by X-ray diffractometry using the SilviScan device (Evans et al. 1996, 1999; Evans 1999) (Fig. 4).

Using *Pinus sylvestris* L. and *Picea abies* (L.) H.Karst., Paakkari and Serimaa (1984) attempted to deconvolve the 002 reflection to give an estimate of MFA in the S1, S2 and S3 layers. However, their results do not agree very well with accepted MFA values for these cell wall layers, giving very low angles for the S1 and S3 layers, and this approach has not been used or modified in more recent studies.

Small-angle X-ray scattering (SAXS) has also been used to measure MFA, with the added advantages of measuring microfibril diameter and the ability to measure within-cell variations at small spatial resolutions (Kantola and Kähkönen 1963; Kantola and Seitsonen 1969; Reiterer et al. 1998, 1999; Lichtenegger et al. 1998; 1999a; 2003; Entwistle et al. 2005). Microdiffraction has been used to measure orientation on transverse sections (Lichtenegger et al. 1999b).

4. Infrared Spectroscopy

Near infrared (NIR) spectroscopy can be used to predict MFA by scanning of wood surfaces on the radial longitudinal face of increment cores using multivariate modelling techniques (Schimleck et al. 2001a, b, 2002, 2003; Schimleck and Evans 2002; Jones et al. 2005; Schimleck et al. 2005). The prediction algorithm, which uses various undefined features of the NIR spectrum to predict MFA, seems to involve compositional information such as cellulose, lignin and hemicellulose contents, although the exact factors involved in prediction are poorly understood. The importance of density in the prediction relationship has been investigated. Schimleck and Evans (2002) examined *Pinus radiata* D. Don samples where there was a strong correlation between density and MFA. However, subsequent studies using *Eucalyptus nitens* (H. Deane & Maiden) Maiden samples, where the density/MFA correlation was poor, have also successfully predicted MFA, suggesting that the correlation with density is not important (Schimleck et al. 2003). While the prediction was less accurate in these samples, this was attributed to the narrow range of MFA values.

In a later study, Schimleck et al. (2005) using *P. radiata* and *Pinus taeda* L. confirmed that accurate MFA prediction is possible even when density variation is small, with R^2 values of 0.93 using 6 predictive factors. However, this study showed that prediction was poor below 500 kg m^{-3} density, and prediction improved with increasing density (Schimleck et al. 2005). Prediction of MFA in samples with high angles and low density (juvenile wood) is problematic, at least in part because the X-ray diffraction data used for calibration are less precise for high angles due to a reduction in signal-to-noise ratio for the 002 reflection of the diffraction pattern (Schimleck et al. 2005).

5. Comparison among techniques

The different techniques discussed above all estimate the same parameter and show good relationships with physical properties. However, they may not give exactly the same result for a given sample. A number of studies have compared different techniques to gain some understanding of factors affecting accuracy.

Good correlations were found between microscopic (bordered pit aperture) and X-ray diffraction measurements of MFA in *Pinus elliottii* Engelm. (Jurbergs 1963). Meylan (1967) compared MFA measured by X-ray diffraction, iodine staining, polarisation, a method involving shadowed replicas of fibre surfaces, and the spiral checks present in compression wood samples, using *P. radiata*. There was good agreement among these techniques, with iodine and polarisation methods giving comparable results. The relationship between the iodine method and X-ray diffraction was curvilinear, probably due to the unreliable method used to measure the diffraction patterns at that time, and as a result, one of many subsequent modifications to the method was proposed (Meylan 1967). In a comparison of polarised light microscopy and X-ray diffractometry, Prud'homme and Noah (1975) found considerable differences between the two methods using *Picea mariana* (Mill.) Bruch & Schimp. The relatively higher values provided by microscopy may have been due to the effect of high angles in the S1 and S3 cell wall layers and a relatively thin S2

layer (Page and El-Hosseiny 1974). Peter et al. (2003) compared phase contrast, polarisation microscopy and X-ray diffraction and found identical results for both earlywood and latewood for *P. taeda* samples showing a wide range of average MFA (5-50^{o}).

Huang et al. (1998) compared microscopic methods with X-ray diffraction, evaluating not only accuracy, but ease of sample preparation, ease of measurement, and availability of equipment. Pit-aperture techniques worked better for latewood than earlywood, probably because pit apertures tend to be rounded in earlywood, making measurement of orientation difficult. Pit aperture was generally the least accurate method, but iodine staining and polarised light microscopy were almost always within a few degrees of X-ray diffraction measurements, bearing in mind that X-ray diffraction was calibrated using iodine staining in this experiment. In *P. abies* and *P. sylvestris*, Saranpää et al. (1998) found that polarised light measurements yielded slightly higher MFA values compared to X-ray diffraction, possibly as a result of the small effects of the transversely oriented S1 and S3 layers on polarisation measurements.

In *P. taeda*, comparisons have been made among X-ray diffraction, soft rot cavities and iodine precipitation. There was good agreement among these methods although correlations were somewhat better for latewood compared to earlywood (Anagnost et al. 2000, 2002). Pleasants et al. (1998) compared micro-Raman spectroscopy with helical checks in compression wood fibres and found good agreement, although Raman measurements were a few degrees higher. Surprisingly, these techniques did not agree with results from polarisation and pit-aperture methods. A comparison of X-ray diffraction and confocal bifluorescence microscopy using *P. abies* and *P. radiata*, found good agreement between these two techniques (Long et al. 2000). Peura et al. (2005) found disagreement between SAXS and polarisation microscopy in *P. abies*, probably again because of the effect of S1 and S3 layers in polarisation microscopy for thin-walled tracheids.

Kretschmann et al. (1998) compared X-ray diffraction and iodine staining, finding a similar correlation to Huang et al. (1998) and confirming a lack of precision at high MFA for X-ray

diffraction measurements. Lichtenegger et al. (1998) have compared (SAXS) and wide-angle X-ray diffraction, with both techniques giving the same result. SAXS has the advantage of higher spatial resolution, allowing measurement on single cells, which is useful for hardwoods to differentiate cell types, although it requires a synchrotron X-ray source.

For X-ray diffraction, the choice of analysis method may influence results. Using several hardwood and softwood species, Yamamoto et al. (1993) found that Cave's method (Cave 1968) gave accurate results only for MFA values below 25^{o} when compared to iodine staining. Yamamoto et al. (1993) provided a more accurate analysis method that gave better results, especially for reaction wood. Evans (Stuart & Evans 1995; Evans 1998, 1999) later developed methods based on curve fitting to allow automation of measurements.

Choice of technique often depends on what equipment is available. X-ray techniques offer potential automation (Evans 1998) and large sample size, while microscopic techniques offer single-cell (Donaldson 1991) or within-cell (Anagnost et al. 2002) resolution, so choice of method will also depend on the nature of the study, and the desired outcome. In some cases, for example, screening of breeding populations with the goal of selecting for improved stiffness, MFA can be measured by proxy using sonic velocity techniques to measure wood stiffness directly on logs or in standing trees (Evans and Ilic 2001; Kawamoto and Williams 2002; Huang et al. 2003).

MFA Variability

1. Within-tree variability

In conifers, MFA varies from pith to bark, with the highest angles occurring in the first five growth rings from the pith at the base of the tree (Phillips 1941; Preston 1948, 1949; Wardrop & Dadswell 1950; Pillow et al. 1953; Echols 1955; Hiller 1964a; Manwiller 1972; McMillin 1973; Erickson & Arima 1974; Bendtsen & Senft 1986; Pedini 1992; Donaldson 1992; Cave & Walker 1994; Sarén et al. 2004; Xu et al. 2004; Fukunaga et al. 2005; Jordan et al. 2005; Zhang et al. 2007). Microfibril angles are high at the base of the stem and decrease exponentially with height in the lower stem,

remaining constant beyond about 7m, but increasing again near the top of the stem (Pillow et al. 1953; Manwiller 1972; Donaldson 1992; Hirakawa and Fujisawa 1996; Downes et al. 2003; Jordan et al. 2005, 2006; Zhang et al. 2007). With increasing height, a stable MFA is achieved closer to the pith so that in *Chamaecyparis obtusa* (Siebold & Zucc.) Endl., MFA becomes stable at about ring 20 at breast height, but at ring 10 at 8m height, with little variation in the stable value (Fukunaga et al. 2005).

There have been few systematic comparisons of MFA in the stem with branches and roots. Using root wood from *P. radiata* and *Pinus nigra* J.F.Arnold pines, Matsumura and Butterfield (2001) found that high MFA values were confined to the first 2-3 rings from the pith compared to 10-15 rings in stem wood. In *C. obtusa*, Fukunaga et al. (2005) investigated the possibility of predicting mature stem wood MFA from measurements on root wood. Little variation was found along the length of the root, or with root diameter, and it was possible to predict mature wood MFA from root wood MFA. Unfortunately, the correlation was less for juvenile growth rings.

In hardwoods, there are generally fewer data on within-tree variation in MFA, most of the data being for *Eucalyptus* trees (Boyd 1980; Bendtsen et al. 1981; Yoshida et al. 1992; Baillères et al. 1995; Stuart & Evans 1995; Baba et al. 1996; Li et al. 1997; Evans et al. 2000; French et al. 2000; Kibblewhite et al. 2004, 2005; Lima et al. 2004). In *E. nitens*, MFA decreases with height, reaching a minimum at 30-50% of stem height before increasing again towards the crown (Evans et al. 2000). MFA declines from pith to bark but, unlike conifers, the angles are much lower near the pith, typically 15-20^{o}. Based on average trends for 29 trees, MFA in *E. nitens* declines from 20^{o} at the pith to 14^{o} at the bark for 15-year-old trees. In *E. nitens* and *Eucalyptus globulus* Labill, French et al. (2000) found angles of 0-13^{o} with only a 5^{o} difference between inner and outer stem regions. In *E. globulus*, MFA remains constant with height apart from higher angles at ground level (Downes et al. 2003). In *Eucalyptus grandis* (G.Forst.) Maiden x *urophylla* S.T.Blake clones, Lima

et al. (2004) found almost no change (~1^{o}) in MFA from pith to bark. However, the trees in this study were only 8 years old.

In *Betula pendula* Roth., MFA declines from 19^{o} at the pith to 12^{o} at the bark at 1m height, with slightly lower values at greater heights (Bonham and Barnett 2001). Most of this decrease occurred within the first 15 growth rings in the 40 year-old tree examined. Similar values have been measured for *Populus deltoides* Marshall (Bendtsen et al. 1981; Bendtsen and Senft 1986; Li et al. 1997), *P. deltoides* x *euramericana* (Dode) Guinier (Fang et al. 2006), *Quercus robur* L. and *Fagus sylvatica* L. (Lichtenegger et al. 1999b) confirming that MFA values are generally below 20^{o} in hardwoods.

Pith to bark variation in *Populus* clones showed MFA values ranging from 28^{o} (pith) to 8^{o} (bark) in 11-year-old trees at breast height (Fang et al. 2006). MFA was significantly correlated with growth ring number from the pith (R^2=0.83) and was reduced by up to 10^{o} beyond 5m height with pith to bark trends becoming flatter (Fang et al. 2006).

2. Among-tree variation

Significant variation in MFA among trees has been observed in a number of studies. Differences among trees are generally more apparent in the juvenile wood. In conifers, neighbouring trees will often show a broad range of juvenile wood MFA values. However, by age 15 and beyond, the trees generally have comparable low MFA values (Donaldson 1992). It is thus relatively uncommon to find trees with both high juvenile MFA and high mature wood MFA when compression wood is excluded (Donaldson 1992, 1993; Donaldson and Burdon 1995). The tendency of MFA to show less among-tree variation in the mature wood (15 + years) than in the juvenile wood is a reason for MFA being a significant predictor of stiffness only in the juvenile wood (Cown et al. 1999). MFA has a significant broad-sense heritability of 0.7 (Donaldson and Burdon 1995; Youming et al., 1998; Cown et al., 2004; Dungey et al., 2006) and not surprisingly this varies from growth ring to ring being highest in the juvenile wood and somewhat lower in the

mature wood (Youming et al., 1998; Dungey et al., 2006). This may go some way toward explaining the findings of Vainio et al., (2002) who have shown significant variation in MFA between provenances in *Picea sitchensis* (Bong.) Carrière, with trees from California and Queen Charlotte Islands provenance having higher MFA than trees of Washington and Oregon provenances.

In hardwoods there are also differences in among-tree variation in MFA. The most notable difference is that among-tree variation at the pith is only slightly greater than at the bark in 15-year-old *E. nitens* (Evans et al. 2000). In hardwoods, limited data show much lower heritabilities for MFA than in conifers (Lima et al. 2004).

3. Cell-to-cell and within-growth ring variation

MFA varies considerably between tracheids within a growth ring, typically varying over a range of 35-40^{o} about its mean value in *P. radiata* (Donaldson 1998; Donaldson & Xu 2005) and with similar results in *P. abies* (Bergander et al. 2002; Sarén et al. 2005). This variability does not change with cambial age unlike the average MFA, but the frequency distribution of MFA changes, becoming skewed toward lower angles in mature wood compared to juvenile wood (Donaldson 1998).

In conifers, the trend from earlywood to latewood is for a gradual decline in MFA towards the latewood with a steeper decline in the last few latewood tracheids, at least in some growth rings (Wellwood 1962; McGinness 1963; Hiller 1964a; El-Osta et al. 1972, 1973; McMillin 1973; Tang 1973; Bucur 1982; Paakkari & Serimaa 1984; Cave & Walker 1994; Stuart & Evans 1995; Donaldson 1998; Herman et al. 1999; Anagnost et al. 2002, 2005; Deresse et al. 2003; Sarén et al. 2004; Jordan et al. 2005). Some reports however, suggest that the decline in MFA in the latewood is more apparent with increasing distance from the pith, and may even be reversed in juvenile wood, with higher latewood MFA compared to earlywood until about ring 7 from the pith (Megraw et al. 1998; Lichtenegger et al. 1999b; Deresse et al. 2003; Myszewski et al. 2004). Both Jakob et

al. (1994) and Reiterer et al. (1998) found significantly higher MFA in latewood (20^{o}) compared to earlywood ($<5^{o}$) of *P. abies*. In contrast, Sahlberg et al. (1997) found comparable values for earlywood and latewood, also in *P. abies*. The earlywood/latewood difference may be less apparent in growth rings containing compression wood (Bergander et al. 2002; Donaldson et al. 2004). The method used to measure MFA may influence the amount of earlywood/latewood difference that can be detected. X-ray diffraction is typically less sensitive compared to microscopy methods because of the large sample of tracheids being measured by X-ray diffraction (McMillan 1973; Kyrkjeeide 1990; Sahlberg et al. 1997; Huang et al. 1998; Herman et al. 1999; Bergander et al. 2002). In hardwoods the same trend occurs, but the variation is much smaller (Stuart and Evans 1995; Anagnost et al. 2005). MFA in latewood was generally 1-5^{o} lower than in earlywood in *Populus* clones (Fang et al. 2006).

4. Variation among cell wall layers and within cells

Most measurements of MFA are carried out on radial cell walls but some studies have compared radial and tangential walls. In *P. sylvestris*, MFA measured using soft rot cavity orientation was found to be greater in radial walls compared to tangential walls (Khalili et al., 2001). Similarly, Anagnost et al., (2005) found that MFA on the radial wall was significantly larger than on the tangential wall in *Drimys winteri* (J.R.Forst & G.Forst.). In contrast, in *P. taeda*, radial and tangential walls had similar MFA (Anagnost et al., 2002). Likewise, in the hardwoods *Acer saccharum* Marsh, *Prunus serotina* Ehrh. (Anagnost et al., 2005) and *E. nitens* (Stuart and Evans, 1995), radial and tangential wall MFA values were very similar. However in *Drimys winteri* J.R.Forst & G.Forst., Anagnost et al., (2005) found that MFA on the radial wall was significantly larger than on the tangential wall.

Differences in MFA between radial and tangential walls can vary among trees within a species. Using X-ray diffraction, Kretschmann et al. (1998) found that for the same growth ring, one tree of *P. taeda* showed no difference while a second tree showed higher MFA on the

tangential walls. Donaldson and Xu (2005) found quite large differences between radial and tangential walls in *P. radiata* tracheids using polarised light microscopy, with some samples showing higher MFA values on tangential walls and other samples showing the higher values on radial walls. It seems likely that differences between radial and tangential walls are quite variable. None of the investigations comparing radial and tangential walls compare measurements on single tracheids for the different wall orientations, so this remains a challenge for future work.

The overall pattern of MFA variation among cell wall layers has been known since the 1930's from studies using polarised light microscopy (Preston 1934; Bailey & Kerr 1935; Bailey & Vestal 1937; Harada et al. 1951; Wardrop and Preston 1947; Bucher 1957; Wardrop 1964; Mark 1965; Tang 1973). In cross-sections, the S1 and S3 layers appear bright while the S2 layer is dark, indicating that the MFA of the outer and inner secondary wall layers are more or less horizontal with respect to the fibre axis.

Electron microscopy has been used to confirm this pattern (Hodge & Wardrop 1950; Wardrop 1954, 1957, 1964; Wardrop and Preston 1947; Frei et al. 1957; Harada 1965a; Dunning 1968; Meylan and Butterfield 1978; Abe et al. 1991, 1992; Kataoka et al. 1992; Brändström 2004a, b; Brändström et al. 2003; Abe and Funada 2005). However, few quantitative studies have been carried out to provide actual measurements (Wardrop & Preston 1947; Harada 1965a; Mark 1965, 1967; Manwiller 1966, 1967; Crosby et al. 1972; Tang 1973; Donaldson & Xu 2005). This is partly due to difficulty in measuring the S1 and S3 layers, and partly because the much thicker S2 layer has a more direct influence on wood properties, such as stiffness, and has thus been of greater interest.

Early studies of MFA in S1 and S3 layers used a very tedious method based on Senarmont compensation, a variation on polarisation microscopy (Wardrop and Preston 1947; Preston 1952; Manwiller 1966). This method involves making matched measurements of birefringence on serial sections at various known angles to the transverse plane (Manwiller 1966). Crosby et al. (1972),

found a general trend of decreasing MFA from juvenile to mature wood in *Pinus resinosa* Aiton in all three secondary wall layers. Using *Pinus virginiana* Mill. Tang (1973) found MFA values of 80^{o} for the S1 layer and 75^{o} for the S3 layer, with little or no difference between radial and tangential walls.

Using FESEM, Abe et al. (1992) have measured MFA on the inner surface of developing tracheids in *Larix leptolepis* Gordon, *P. abies* and *Picea jezoensis* (Siebold & Zucc.) Carrière, studying the variation from earlywood to latewood in single growth rings. Both S and Z helices were observed, although smaller Z helices seemed to occur more often in the latewood, angles ranging from 40^{o} (Z) to 160^{o} (S).

Donaldson & Xu (2005) using oblique sectioning, polarisation microscopy and transmission electron microscopy, were able to measure MFA for S1, S2 and S3 layers for a range of samples from *P. radiata*. The S1 layer was usually an S-helix with MFA ranging from 79-117^{o}, the S2 layer was a Z-helix with angles ranging from 1-59^{o}, and the S3 layer was also usually a Z-helix ranging from 50-113^{o}. Unlike MFA in the S2 layer, which shows well-defined trends of within-tree variation, the S1 and S3 layers show only random variations from pith to bark and with height (Donaldson and Xu 2005). Donaldson and Xu (2005) defined S helices as being >90^{o} while most other studies have defined the S or Z helix as an angle to the left or right of the fibre axis, leading to confusion when angles change from Z to S within or between layers. For example, as MFA changes from 80^{o} Z to 10^{o} S (100^{o}) there is actually only a rotation of 20^{o}, not 90^{o} as might be implied by the older definition. It is also worth noting that, when viewed from outside the fibre, a Z helix leans to the right (Fig. 1) but when viewed from the lumen it leans to the left.

Using transmission electron microscopy, Donaldson and Xu (2005) were able to measure the continuous variation of microfibril orientation from lumen to primary wall, showing a relatively abrupt transition zone from S2 to S3 but a more gradual transition from S1 to S2 in *P. radiata*. In *P. abies*, Müller et al. (2002) studied the S1 layer during secondary wall formation using X-ray and

electron microdiffraction, and found orientations of 70 – 90°. Similar results were also found by Brändström et al. (2003), using a variety of microscopy-based methods, including softrot cavities, ultrasonic and chemical treatments, combined with light and electron microscopy. Early studies considered the S1 layer to have a crossed structure produced by alternating S and Z helices (Wardrop 1954, 1957, 1964; Emerton and Goldsmith 1956; Frei et al. 1957; Jurbergs 1963; Harada 1965a; Preston 1965; Dunning 1969; Tang 1973; Abe et al. 1991; Kataoka et al. 1992). More recent studies have failed to find such a crossed structure within the S1 layer, suggesting that earlier investigations were mistakenly observing the outer part of the S2 layer which forms a transition zone between the S helix of the S1 layer and the Z helix of the S2 layer (Abe et al. 1997; Khalili et al. 2001; Brändström et al. 2003; Donaldson and Xu 2005).

Some studies have measured local variations in MFA at different positions along single tracheids. Lichtenegger et al. (2003), using X-ray microdiffraction, have shown that most of the tracheid wall contains parallel-aligned microfibrils, whereas Abe et al. (1991) found evidence for non-parallel alignments outside of the S2 region. Müller et al. (1998, 2006) also found a high degree of alignment; tilt angle distribution was 5.4° in tension wood of *Populus maximowiczii* Henry, and 7° in bast fibre of *Linum* sp. Local deviations in MFA occur around pits, but angles are usually consistent along the length of the tracheid when measured between bordered pits (Anagnost et al. 2002; Lichtenegger et al. 2003; Sedighi-Gilani et al. 2005, 2006). Variation in angle may be less in latewood tracheids compared with earlywood (Anagnost et al. 2002). Pit apertures are generally assumed to be oriented parallel to the local MFA, which has been confirmed in latewood but there are sometimes large discrepancies for earlywood (Fig. 2) (Lichtenegger et al. 2003).

Environmental influences

1. Reaction wood

Compression wood typically has a higher MFA than opposite wood (Wardrop and Dadswell 1950; Kantola and Seitsonen 1961; Kantola and Kähkönen 1963; El-Osta et al. 1972; Paakkari and

Serimaa 1984; Sahlberg et al. 1997; Färber et al. 2001; Donaldson et al. 2004; Yeh et al. 2006), but in mild compression wood, juvenile compression wood, and occasionally even in mature severe compression wood (Donaldson et al. 2004), the MFA may be similar to or the same as the relevant opposite wood control within individual growth rings (Nečesaný 1955; Harris 1977; Donaldson & Burdon 1995; Donaldson et al. 2004). In mild compression wood, MFA may be, on average, about 5^{o} higher than opposite wood, while in severe compression wood, MFA is on average 8^{o} higher than opposite wood in *P. radiata*, with the largest observed difference of 17^{o} (Donaldson et al. 2004). In contrast, Yeh et al. (2006) found that all compression wood samples had MFA greater than that found in juvenile wood for a single tree of *P. taeda*. However this study did not use ring-by-ring comparisons, nor opposite wood controls. Wardrop and Dadswell (1950) found that growth rings beyond the compression zone may also have increased microfibril angles, but other studies have shown the opposite effect, with lower MFA values in growth rings formed subsequent to compression wood zones (Donaldson et al. 2004). Within growth rings, the MFA pattern may be different between opposite and compression wood. Hiller (1964a, b) found that MFA decreases from earlywood to latewood in both opposite and compression wood, while Park et al. (1979) found the highest MFA values in the centre of the growth ring for compression-wood rings. In *P. radiata* compression wood, there was an increase in MFA in the latewood, compared to the gradual decline in MFA across the growth ring in opposite or normal wood, although the latewood MFA was still lower than at the beginning of the earlywood (Donaldson et al. 2004).

Limited information is available on MFA in layers other than the S2 layer for compression wood. Since the S3 layer is usually absent in compression wood, studies have examined only the S1 layer. In *P. abies*, Brändström (2004b) found that the S1 layer of compression wood tracheids is almost always perpendicular to the fibre axis (90^{o}) and shows less variation than normal wood tracheids. Donaldson et al. (2004) also found that the S1 layer was perpendicular to the fibre axis in *P. radiata* compression wood.

The general consensus for tension wood is that MFA is very small in the g-layer of gelatinous fibres (Wardrop & Dadswell 1948, 1955; Kantola and Kähkönen 1963; Baba et al. 1996; Yoshida et al. 2000; Washusen et al. 2001; Hori et al. 2003; Hillis et al. 2004; Washusen et al. 2005a; Daniel et al. 2006; Donaldson 2007; Ruelle et al. 2007) but it would be of interest to measure tension wood MFA in a wider range of species. Yoshida et al. (2000) using field emission SEM and X-ray diffraction, found that microfibrils were parallel to the fibre axis in gelatinous fibres of *Prunus spachiana* Kitamura, regardless of the angle of stem inclination. In contrast, tension wood of *Liriodendron tulipifera* L., which does not form gelatinous fibres, had microfibrils oriented at about 20^{o} compared to about 30^{o} in upright controls. Washusen et al. (2005b) found significantly higher MFA values in opposite wood of branches of *E. grandis* and *E. globulus* exceeding 40^{o}, which seems to be the highest recorded value for a hardwood. In *Laetia procera* (Poepp.) Eichl., a tropical hardwood from South America, tension wood has a distinctive polylamellate secondary wall containing layers with alternating high and low microfibril angles and associated variation in degree of lignification, low angles being associated with low levels of lignification (Ruelle et al. 2007). Interestingly, in the layers with high MFA, microfibrils showed a reduced degree of parallelism.

2. Site and Silviculture

Site and silviculture may have small effects on MFA, apparently in response to stimulated growth rate. MFA in *Cryptomeria japonica* (L.f.) D.Don clones shows variation with site, but this is generally small compared to genetic effects and does not seem to be related to growth rate (Hirakawa and Fujisawa 1995; Hirakawa et al. 1998; Nakada et al. 1998, 2003). The effect of growth rate may interact with other wood properties. For example McMillin (1973) found that MFA increases with growth rate, but only in trees with higher specific gravity. *Pseudotsuga menziesii* also shows a short-term increase in MFA in response to enhanced growth rate from fertilisation and thinning (Erickson and Arima 1974).

In *P. taeda* from 31 provenances growing in China, Youming et al. (1998) found that latitude, annual temperature, annual rainfall and length of frost-free season, had significant effects on MFA. The environmental effect on MFA increased with tree age. Myszewski et al. (2004) found significant, but unspecified, environmental influences on MFA in *P. taeda*. Jordan et al. (2006, 2007) found significant site variation, also in *P. taeda*, but could not relate this to any specific site factor other than growth rate. In *P. taeda*, significant variation in MFA was found from a range of sites in the southern US (Shupe et al. 1996; Clark et al. 2006) but these differences were thought to be related to seed provenance rather than site effects (Clark et al. 2006). Clonally replicated trials would be beneficial in distinguishing site and genotype effects.

P. radiata growing on ex-pasture sites in Australia, which are characterised by elevated soil nitrogen, was found to have significantly higher microfibril angles, and although the difference was less than 10^{o}, this amounts to a 14% increase (Raymond & Anderson 2005). In *Pinus resinosa* Ait., Deresse et al. (2003) found that increased growth rate leads to increased MFA and reduced modulus of rupture and modulus of elasticity. It is notable that in New Zealand, where there are large plantation areas growing on fertile ex-pasture sites, there have been no studies showing the effect of soil fertility, specifically nitrogen, on MFA.

Lindstrom et al. (1998) found a small effect of growth conditions (temperature, precipitation, fertilisation, initial stocking) measured as growth rate, on MFA in *P. abies*, while Herman et al. (1999) also found increased MFA when growth rate was increased by thinning treatment. Irrigation, but not fertilisation, was found to have a small but significant effect on MFA, also in *P. abies* growing in Sweden (Lundgren 2004). The effect was greater on a poor-quality site where the growth response to fertilisation and irrigation was larger. Wood from faster-growing trees consistently had a higher MFA in this study (Lundgren 2004). Sarén et al. (2004) studied the effect of growth rate on MFA in *P. abies* grown on a fertile site in southern Finland. These fast-grown trees showed a more gradual decline of MFA with cambial age compared to trees from a medium-

fertility site. In *P. sitchensis*, Cameron et al. (2005) found slightly higher MFA in faster-growing progenies in juvenile wood. Pedini (1992) also found higher MFA in faster-growing trees of *P. sitchensis* but also found higher MFA in narrow growth rings from suppressed trees.

Other studies on softwoods have failed to show significant effects of site or growth rate (Manwiller 1972; Markstrom et al. 1983; Shuler et al. 1989; Hirakawa & Fujisawa 1995; Donaldson 1996; Myszewski et al. 2004; Chiu et al. 2005). In *P. taeda*, soil moisture conditions had no apparent affect on MFA (Hiller and Brown 1967) in contrast to the significant effects of drought and irrigation in *E. nitens* trees found by Wimmer et al. (2002). Changes in MFA were not associated with severity of Swiss needle cast disease in *P. menziesii* (Johnson et al. 2005).

In *E. nitens* grown under varying irrigation schemes, MFA showed a significant relationship with water deficit (Wimmer et al. 2002). Irrigated trees formed higher MFA values early in the growing season and lower MFA values later in the growing season compared to un-irrigated trees. Trees subjected to drought cycles produced wood with increased MFA in fibres formed after release from water stress (Wimmer et al. 2002). Wind speed had an apparent direct effect on MFA, and growth rates were positively related to MFA (Wimmer et al. 2002). Lima et al. (2004) also found a significant effect of site on MFA, but did not relate this to specific site characteristics. In a similar study, Washusen et al. (2005b) found an increase in MFA with growth rate in response to thinning or fertilisation in *E. globulus*, and this was discussed in relation to tension wood formation, which they claimed was reduced by fertiliser treatment.

Propagation method may have a significant effect on MFA. *P. radiata* trees grown from physiologically aged cuttings had significantly lower juvenile wood MFA compared to trees grown from seedlings, although mature wood values were comparable in both types of tree (Donaldson 1996). Tsutsumi et al. (1982) also found differences in pith to bark trends in MFA between seedlings, cuttings and grafts.

Relationships between MFA and other wood properties

1. Cell Dimensions

MFA has long been known to have a moderate to strong correlation with tracheid length (Echols 1955; Kantola and Seitsonen 1969; Crosby et al. 1972; Erickson and Arima 1974; Megraw 1985; Shupe et al. 1996; Bonham & Barnett 2001; Chiu et al. 2005). However, it is not clear if these parameters are causally linked, or if their covariance is merely coincidental. Wellwood (1962) found a higher correlation between MFA and tracheid length in latewood (-0.67) than in earlywood (-0.35) in *P. menziesii*. Jurburgs (1963) found only a small correlation between tracheid length and MFA in *P. elliottii*. In the phytoplasma disease "rubbery wood" of apple (*Malus pumila* P.Mill), MFA and fibre length were independent, resulting in low tensile strength and high extensibility, also related to reduced lignification in this material (Nelmes & Preston 1968). Among a range of *C. japonica* cultivars, Hirakawa et al. (1998) found that MFA is not directly correlated to tracheid length among cultivars, even though the two parameters vary inversely from pith to bark within individual stems. Matsumura and Butterfield (2001) also found that MFA and tracheid length were independent in root wood of *P. radiata* and *P. nigra*.

Studies showing changes in MFA and tracheid length in compression wood (Kibblewhite et al. 2005) have the potential to suggest a more causal relationship, independent of ring number from the pith, but have not been analysed on a within-ring basis, making interpretation difficult. There is a need to study this relationship in more detail by examining the correlation orthogonally, comparing samples of fixed cambial age among trees.

There have been few studies comparing microfibril angles with cell wall thickness or lumen diameter, and more importantly, doing this comparison on individual tracheids. In *P. elliottii* and *P. taeda*, Hiller (1964a) found a curvilinear relationship between tracheid wall thickness and MFA using the pit aperture technique. In this study, cell-wall thickness accounted for 64-81% of the variation in latewood MFA. In a second study, Hiller (1964b) found that cell wall thickness was the

1 best single predictor of MFA (R^2 = 80%) among nine variables including age, distance from pith,
2 ring width, percent latewood, tracheid length, tracheid width, wall thickness, length/width, and age
3 × tracheid length. All nine variables were significant predictors, accounting jointly for 88% of the
4 variation in MFA.

5 In southern pine (*Pinus* sp.), Anagnost et al. (2002) found no relationship between MFA and
6 tracheid width along the length of individual tracheids using soft rot cavities. Clark and Daniels
7 (2004) found that specific gravity and MFA have a strong inverse correlation in *P. taeda*, attributed
8 to increased amounts of latewood, which has reduced MFA. Interestingly, Myszewski et al. (2004)
9 also working on *P. taeda*, found no such correlation. In *P. radiata* clones, Lindström et al. (2005)
10 found that clones with high MOE, and hence lower MFA compared to low-MOE clones, had longer
11 tracheids (1.8 mm cf. 1.5 mm) and larger tracheid diameters (37.5 μm cf. 34.7 μm).

12 In *E. nitens*, MFA and density show a significant correlation (Evans et al., 2000). This study
13 also claims that fibre wall thickness is the main determinant of density in *E. nitens*, and suggest that
14 as wall thickness (and hence density) increases, the contribution of the S2 layer increases relative to
15 the transition layers between S1 and S2, and S2 and S3. In *P. resinosa*, Crosby et al. (1972) found
16 no significant relationship between MFA and transverse cell dimensions. In *P. abies*, Bergander et
17 al. (2002) found no correlation between MFA and fibre length or width. As described above, MFA
18 does often vary between earlywood and latewood, as do lumen diameter and cell wall thickness, but
19 published studies investigating these relationships seem to be lacking.

20 2. Density

21 MFA shows a variable relationship with wood density. In some cases MFA and wood density
22 are correlated, while in other cases they are not (Evans et al. 2000; Bergander et al. 2002;
23 Schimleck and Evans 2002; Lin and Chiu 2007). The correlation between density and MFA may be
24 stronger over a small number of consecutive growth rings but interestingly, the relationship
25 between MFA and density does not hold among trees (Evans et al. 2000).

It seems likely that any relationship between these properties is entirely coincidental since MFA is not related to tracheid wall thickness. However, the amount of juvenile wood and latewood might be responsible for relationships in some cases since both MFA and density are related to these factors as discussed elsewhere.

3. Stiffness

MFA in the S2 layer is widely considered to be an important determinant of timber and fibre quality (Horn 1974; Armstrong et al. 1977; Bendtsen and Senft 1986; Shupe et al. 1996; Walker and Butterfield 1995; Butterfield and Pal 1998; Raymond 2002; Kijidani and Kitahara 2003; Courchene et al. 2006). The curvilinear relationship between MFA and longitudinal stiffness (MOE_L or Young's modulus) has been repeatedly demonstrated in the literature (Harris & Meylan 1965; Cave 1968; Cave & Walker 1994; Cown et al. 1999; Yamashita et al. 2000; Deresse et al. 2003; Xu et al. 2004). The longitudinal stiffness of the cell wall is determined by MFA, which in turn is related to the MOE_L of a piece of wood by the amount of cell wall per unit volume, usually measured as basic density. In other words, the properties of the cell wall material (specifically MFA) and the amount of cell wall (density) both affect the mechanical properties of the wood (MOE_L). Hence, both MFA and basic density can be related to wood stiffness, either theoretically or experimentally (Cave 1969, 1976; Tang & Hsu 1973; Armstrong et al. 1977; Cave & Walker 1994; Hirakawa et al. 1997; Cown et al. 1999; Xu et al 2004). Because MFA tends to vary within and among trees mainly in the juvenile wood, whereas density varies in the mature wood, correlation studies comparing MFA and density to MOE_L tend to show a greater effect of MFA in the juvenile wood and in the butt log (Cown et al. 1999), although in some cases MFA may be a significant factor in both juvenile and mature wood (Kijidani and Kitahara 2003). Xu et al. (2004) compared the distributions of MFA, density and MOE_L along the length of butt logs of *P. radiata* and found that MFA was the main determinant of stiffness variation with height. This result is not surprising, since density shows little variation within the butt log. Evans & Ilic (2001) showed that

MOE_L could be predicted from density and MFA in *Eucalyptus delegatensis* R.T.Baker, accounting for 96% of the variation in MOE_L in a sample of 104 clearwood specimens. MFA is also related to modulus of rupture (MOR) in small clearwood samples (Bendtsen & Senft 1986; Treacy et al. 2000; Deresse et al. 2003).

Using *P. radiata* clearwood, Booker et al. (1998) found high correlations between MOE_L, MFA and density (r = 0.69 and -0.78 respectively), but for specific modulus (MOE per unit of mass), path analysis showed that MFA was the only significant causal factor. This was interpreted to indicate that MFA was the only significant variable in the cell wall structure of the samples examined. Nakada et al. (2003) showed that clonal selection for low MFA resulted in improved stiffness of logs in *C. japonica*, even when using MFA of just the second growth ring. There was no difference in selection for improved stiffness by MFA, or directly by log stiffness.

MFA shows a good correlation with the mechanical properties of single fibres, where fibres with larger MFA also show increased extensibility (Page et al. 1972, 1977; Page and El-Hosseiny 1983; Mott et al. 2002). Short-term creep shows a positive linear relationship with MFA (El-Osta and Wellwood 1972). Using small-angle X-ray scattering, Reiterer et al. (1999) also found a relationship between MFA and extensibility of wood foils. Maximum longitudinal strain increases from 0.5 to 11% as microfibril angle increases from 5 to 50^o. Most of the increased extensibility at higher microfibril angles is due to irreversible deformation of the cell wall. Reiterer et al. (2001) also found that tangential strain increases with microfibril angle reaching a maximum at 27^o. Tensile strength decreases with increasing microfibril angle, from 220 MPa at 5^o to 35 MPa at 50^o. Using nano-indentation of cell wall regions, Gindl et al. (2004) confirmed a relationship between MFA and MOE_L, especially for large MFA values, but found that hardness is independent of MFA. Sedighi-Gilani and Navi (2007) have modelled the effect of local variations in MFA on wood cell rigidity, indicating that localised damage to the matrix and reorientation of microfibrils are responsible for the elasto-plastic response of single wood fibres.

Cown et al. (2004) studied the relative effects of MFA and basic density on MOE_L in boards of *P. radiata* clones, but found a low (non-significant) contribution of MFA compared to other factors such as spiral grain and knot area ratio. Two factors seem to have contributed to this reduced effect of MFA. First, the clones studied were physiologically aged and hence may have had a smaller range of pith to bark variation in MFA than in trees grown from seedlings (Donaldson 1996). Secondly, the clones all had approximately the same average MFA and hence the between-tree component of variation in MFA would have been small, resulting in a bias toward the contribution of basic density.

Keckes et al. (2005) studied changes in wood behaviour under conditions of cyclic loading, using wide-angle X-ray diffraction with thin wood foils prepared from *P. abies*, *Ginkgo biloba* L., and *Juniperus virginiana* L. They found that MFA decreased with time under cyclic loading and this change seemed to be relatively uniform compared to similar behaviour in individual fibres, which showed large but localised changes in MFA (Kölln et al. 2005). These experiments demonstrated the two interacting effects of MFA and matrix properties on stiffness (Keckes et al. 2005).

4. Shrinkage

Various models have been developed to deal with shrinkage behaviour of wood, and in particular, the anisotropic nature of such shrinkage (Barber & Meylan 1964; Barber 1968; Barrett et al. 1972; Cave 1972a, b; Boyd 1974, 1977a; Koponen et al. 1989, 1991; Yamamoto et al. 2001; Pang 2002; Yamamoto & Kojima 2002). The most popular of these models is the "reinforced matrix" hypothesis proposed by Barber and Meylan (1964). MFA is one of the dominant parameters that affect shrinkage and shrinkage anisotropy. For example, compression wood with increased MFA shows a corresponding increase in longitudinal shrinkage (Harris & Meylan 1965; Harris 1977). Shrinkage is assumed to occur in the cell wall matrix below fibre-saturation moisture content, and hence the rigid microfibrils are orthogonal to the shrinkage of the matrix, and their

orientation accounts in part for the anisotropic nature of the shrinkage. Cell walls with very low MFA tend to have greater tangential shrinkage, while cell walls with very high MFA tend to have greater longitudinal shrinkage. Microfibrils themselves may shrink slightly in the longitudinal direction, due to water loss from the non-crystalline regions, causing some non-linearity in the shrinkage process (Abe & Yamamoto 2005, 2006).

In *P. taeda*, Megraw et al. (1998) found that the curvilinear relationship between longitudinal shrinkage and MFA was highly dependent on ring position and height, with evidence for factors other than MFA influencing longitudinal shrinkage, since MFA accounted for only 60-70% of the variation in longitudinal shrinkage. Trees with (unevenly distributed) high longitudinal shrinkage produced boards with larger amounts of crook. Donaldson and Turner (2001) confirmed that crook in window frames was associated with uneven distribution of zones of high MFA associated with compression wood. Samples with evenly distributed compression wood did not show crook.

Nakano (2003) has demonstrated the resistance to swelling caused by the S1 and S3 layers which have microfibril angles more or less orthogonal to the fibre axis, by comparing the behaviours of intact wood with wood powder. Microfibrils have been shown to contract longitudinally using a range of softwoods, including *Abies sachalinensis (*Schmidt) Mast., *Larix kaempferi* (Lamb) Carrière, *P. jezoensis*, and also a hardwood *Betula ermanii* Cham. (Ishikura and Nakano 2007), as indicated by changes in the anisotropy of longitudinal and transverse swelling rates.

5. Pulp and Paper properties

Paper properties are a function of the network properties of the paper as well as the properties of individual fibres (Horn 1974). MFA is related to the tensile strength and elastic modulus of pulp fibres, where small MFA values lead to stronger and stiffer fibres (Wellwood 1962; Watson & Dadswell 1964; Mark 1967; Page et al. 1972, 1977; Mark & Gillis 1973; Kellogg et al. 1975; Armstrong et al. 1977; French et al. 2000; Burgert et al. 2002; Groom et al. 2002a, b; Downes et al.

2003). Using single southern pine (*Pinus* sp.) fibres, Mott et al. (2002) found that latewood fibres had 33% higher MOE_L and 73% higher ultimate tensile stress compared to average earlywood fibres, differences that were partially attributed to lower MFA in latewood fibres. In plantation-grown *E. globulus*, density and MFA account for 70% of kraft pulp variation in bulk, burst, stretch, tear index and tensile strength (Downes et al. 2003). Using unbleached kraft pulps from 10 individual loblolly pine trees with similar density, coarseness, cell wall thickness and fibre length, but differing in MFA, Courchene et al. (2006) found that MFA was a major determinant of handsheet tensile strength, stretch, modulus of elasticity, stiffness and hygroexpansivity.

6. Growth stress

Growth stresses accumulate in the stem as the tree grows, and can result in significant splitting in felled logs, as well as bow and crook when the log is sawn into boards (Yang 2005). Growth strain originates in developing wood fibres by two mechanisms (Okuyama et al. 1993; Yamamoto 1998), where cellulose crystallisation results in longitudinal shrinkage (Bamber 1979, 1987, 2001) while lignification results in transverse swelling of fibres (Boyd 1985b). Since the maturing wood fibres are attached to the fully developed wood fibres already formed, a strain develops resulting in progressive compression of the wood fibres in the centre of the stem, and the formation of tension at the periphery of the stem (Boyd 1985b).

Growth stress can also be generated in reaction wood by similar mechanisms (Bamber 2001). Bamber (2001) has proposed that cellulose is involved in both compressive and tensile stress generation in reaction wood. The reduced lignification of the g-layer in tension wood facilitates generation of tensile stress by allowing contraction of microfibrils oriented close to the fibre axis. Cellulose microfibrils have recently been confirmed to be in a state of tension by measurements of lattice spacing (Clair et al. 2006). In compression wood, the increased lignification is considered only as a mechanism to increase compression strength (Bamber 2001), in conflict with Boyd

(1985b) who regards the increased lignification as the primary method for generation of compressive stress in compression wood.

MFA is related to the directionality of growth stress, particularly in reaction wood. As discussed above, compression wood generally has a high MFA and hence can resist high compressive stress, while tension wood has a low MFA and hence can resist a high tensile stress (Boyd 1980; Yamamoto 1998). Theoretical models predicting the effect of MFA (Yamamoto 1998; Guitard et al. 1999; Alméras et al. 2005) are in good agreement with experimental measurements at the fibre level (Yamamoto 1998).

7. Other factors

MFA is known to influence Young's modulus and it has been shown that low MFA values in both earlywood and latewood result in a high Young's modulus and low-loss tangent resulting in attributes suited to violin or piano soundboards. Among a sample of 12 (mostly asian) softwood species, *P. sitchensis* showed the most desirable acoustic properties (Hori et al. 2002). Unfortunately *P. abies*, the favoured species for musical instruments, was not included in the comparison (Wegst 2006).

Using a combination of SAXS and FTIR, Hori et al. (2003) have shown that for *C. japonica*, MFA shows a significant positive correlation with lignin content and a negative correlation with cellulose content in samples containing compression wood. Since galactan content is an indicator of compression wood severity (Nanayakkara et al. 2005), MFA should also show a correlation with galactan content in compression wood. Using data from Yeh et al. (2006) yields a correlation of MFA with galactan content of 0.8 ($p<0.05$), based on 7 samples of normal and compression wood collected throughout a single tree of *P. taeda*. In *L. tulipifera*, MFA shows a positive correlation with xylan content, but no correlation with cellulose content in samples containing tension wood.

MFA influences the fracture properties of cell walls. There are many studies that have examined the effect of MFA on fracture properties indirectly through effects on stiffness and

extensibility, but relatively few reports describe direct effects on fracture morphology. The greater frequency of fractures at the S1/S2 interface compared to the S1/middle lamella may depend on MFA (Wardrop and Addo-Ashong 1963). MFA is related to the frequency of transwall fracture in *P. radiata* explaining 39% of the variation within trees, but is not related to variation among clones (Donaldson 1996). MFA affects not only extensibility in the longitudinal direction but also influences deformation perpendicular to the applied load (Reiterer et al. 2001). Wood with high MFA has a greater energy absorption capacity, showing fractures with greater tearing and deformation representing a more ductile behaviour, compared to the smooth fracture surfaces in samples with low MFA (Reiterer et al. 2001). The fraction of absorbed energy resulting from elastic deformation is only about 10% in samples with high microfibril angles (Stanzl-Tschegg 2006). Comparing the fracture properties of normal and compression wood in *Larix decidua* Mill., Gindl and Teischinger (2003) found that while both transwall and intrawall fracture predominate in normal wood, fracture is mainly by intercellular failure at the middle lamella in compression wood. The S1/S2 interface was found to be more resistant to failure in compression wood, probably due in part to the reduced difference in MFA between the two layers in compression-wood tracheids (Gindl and Teischinger 2003).

Functional significance

The possible functional reasons for the variations in microfibril orientation among cell wall layers have received little attention from researchers, with only a few studies addressing this issue. Booker (1993, 1996), and Booker and Sell (1998) have considered the various functions of the secondary wall layers and provide a discussion of possible functional roles for microfibril orientation in each layer. The S3 layer is thought to provide resistance to collapse from the compressive stresses caused by water translocation in the living tree, resistance to crack propagation in the radial and tangential directions, and protection of the S2 layer from checking (Booker 1993, 1996; Booker and Sell 1998). The S3 layer may be important in determining tangential modulus of elasticity, but it is

likely to be its thickness rather than any variation in MFA that contributes to variation in properties (Koponen 1998). The S2 layer supports the weight of the crown and resists the compressive and tension forces generated by the wind. The S1 layer limits the maximum cell expansion under load and acts as a buffer layer between the S2 and middle lamella (Booker 1996). It is also thought that the high microfibril angles in juvenile wood near the base of the tree allow the stem to bend in the wind when the tree is young thus reducing the chance of broken stems (Booker and Sell 1998). The smaller angles in mature wood are more efficient at supporting the crown. By comparing the swelling properties of intact wood and wood powder, Nakano (2003) was able to demonstrate the role of S1 and S3 layers in resisting swelling due to the flat helix of the microfibrils, resulting in a lower isotherm curve in the intact wood compared to powdered wood.

A number of studies have been carried out to understand the functional significance of reaction wood. Conifer branches have been studied with respect to their mechanical properties. Microfibril angles of 30^{o} or more are needed to generate compressive stress (Yamamoto 1998). In *P. abies* branches, Färber et al. (2001) found that MFA on the lower side decreases continuously from the trunk to the tip of the branch. In the opposite wood, especially in the outer growth rings, very small MFA values were found near the mid-length of the branch acting as a reinforcement to prevent further bending of the branch. Relatively high MFA values were found in the inner growth rings, providing high flexibility when the branch is young. MFA values throughout the branch were larger than in the stem.

In *P. abies* and *Taxus baccata* L., Burgert and Jungnikl (2004) found that in order to maintain horizontal growth, the compressive stress generated by the compression wood in the underside of the branch should not be overcompensated by formation of stiff opposite wood on the upper side, which will tend to resist the compressive force. At the base of the branch, stiffness and MFA remain relatively constant from pith to bark, thereby allowing the branch to maintain its

horizontal growth. In one-year-old stems of *Abies fraseri* (Pursh) Poir. subjected to flexing, MFA was increased, although other features of compression wood were absent (Telewski 1989).

Control of microfibril orientation

Microfibril orientation is controlled at two levels in secondary xylem. Within each cell, MFA varies systematically among wall layers, being often random in primary walls, while in secondary walls MFA is transverse in the S1 and S3 layers, and of variable longitudinal orientation in the S2 layer. Secondly, the MFA of the S2 layer varies systematically with cambial age, height, presence of reaction wood, and among taxa as discussed above. Although a detailed discussion of microfibril orientation mechanisms is beyond the scope of this review, it is worthwhile to review recent progress as it relates to secondary xylem, keeping in mind that much of the work on microfibril orientation has been done in primary tissues because of the interest in control of cell elongation, and hence plant morphogenesis.

Microfibril orientation is known to be often associated with the orientation of microtubules (MT) within the living cell protoplast during wall formation, but this is not always the case (Heath 1974; Abe et al. 1994, 1995a, b; Barnett et al. 1998; Baskin 2001; Barnett and Bonham 2004). A β-tubulin gene in *E. grandis* (*EgrTUB1*) has recently been shown to be associated with microfibril orientation in secondary fibre cell walls (Spokevicius et al. 2007). Whether or not microtubules are involved, there is still the question of an exact mechanism for controlling the orientation.

In *E. nitens* and *E. globulus*, Thumma et al. (2005) found that single nucleotide polymorphisms (SNP's) in the lignification gene cinnamoyl CoA reductase are associated with variations in MFA. Since lignification occurs after formation of the secondary cell wall the significance of this result is unclear, but it is tempting to attribute this to a reaction-wood effect.

Cellulose is known to be synthesised from protein complexes in the plasma membrane known as terminal complexes or "rosettes". Although originally seen in algal cells, these rosettes have now been observed in vascular plants (Kimura et al. 1999), and confirmed to have cellulose synthetic

activity (Itoh and Kimura 2001). Of the 10 cellulose synthase (*CesA*) proteins currently known, at least three are required for cellulose synthesis during secondary-wall formation (Tanaka et al. 2003; Taylor et al. 2003). Some *CesA* proteins are specific to cellulose synthesis in the primary wall (Samuga and Joshi 2004). The movement of rosettes (*CesA6* tagged with yellow fluorescent protein) has been shown to follow both microtubule and microfibril orientation (Paradez et al. 2006). Cellulose synthase complexes move bidirectionally and appear to have some intrinsic self-organising capability in the absence of associated microtubules (Paradez et al. 2006).

Emons and co-workers have developed a theory that involves the density of *CesA* in the plasma membrane, the distance between microfibrils, and cell geometry, which explains random, axial, helical, helicoidal, transverse and crossed-polylamellate cell wall textures (Emons et al. 1992; Emons 1994; Emons and Kieft 1994; Emons and Mulder 1998; Emons et al. 2002; Mulder et al. 2004). Reducing the level of cellulose synthetic activity, using either chemical treatment or mutants, results in a loss of parallel orientation of microfibrils (Sugimoto et al. 2001, 2003; Pagant et al. 2002). Microfibril orientation may also be related to the rate of cellulose synthesis (Sugimoto et al. 2001). The already-formed cell wall may also act as a template to maintain orientation of microfibrils when cortical microtubules are depolymerised using drug treatments or in mutants, but this is not an essential part of the control system (Sugimoto et al. 2003; Himmelspach et al. 2003).

The *fra2* mutant in *Arabidopsis thaliana* L. Heynh. shows disorganised microfibrils associated with equally disorganised cortical microtubules (Burk and Ye 2002). The katanin-like protein encoded by this gene may therefore be involved in microfibril orientation. Gibberellins have been shown to influence microtubule organisation by changing katanin levels (Bouquin et al. 2003). In a similar study, the *fra1* mutant of *Arabidopsis*, which also has abnormal microfibril organisation, encodes a kinesin-like protein that binds microtubules and may also be involved in microfibril orientation (Zhong et al. 2002).

Xylans may have a role as the twisting agents acting at the transition from one microfibril orientation to the next (Reis and Vian 2004). Xylans have been specifically localised to the transition zone between the S1 and S2 layers in *Tilia platyphyllos* Scop. (Vian et al. 1992), and are hypothesised to act as helper molecules controlling the orientation, reducing aggregation and favouring parallel alignment of microfibrils (Reis and Vian 2004).

Paradez et al. (2006) recently demonstrated that exposure of cells to blue light can result in a change in MT orientation from predominantly transverse to predominantly longitudinal but how this equates to what happens inside an intact plant is unclear. Control of microfibril orientation among wall layers may thus involve cellulose synthase, microtubules and microtubule orienting proteins, but the signals and mechanism are not understood.

The variation in S2 MFA that occurs within tree stems shows a relationship with growth strain and it seems likely that this variation in MFA is just a consequence of the inherent growth strain in the stem at the time of formation (Boyd 1980, 1985a). The influence of genetic and environmental factors is at least partly understood in relation to reaction-wood formation, but how this relates to events at the cellular level is unclear. Conceivably, growth strain can influence the self-organising ability of cellulose synthase complexes and hence influence the deposition process directly. For example, Wu et al. (2000) have described a cellulose synthase gene from aspen xylem that responds to tensile stress. This also fits well with the often observed relationship between MFA and tracheid or fibre length; the greater the tensile strain the longer the tracheid and the greater the distortion of the *CesA* complexes in the plasma membrane, resulting in smaller MFA.

The molecular analysis of genes that function in control of microfibril orientation will no doubt contribute to future progress in understanding these mechanisms (Moran et al. 2002; Pilate et al. 2004; Roudier 2005).

CONCLUSIONS

A sizable body of literature exists that explores MFA and its relationship to wood properties. Clearly, a wide range of different methods are available to characterise MFA in wood. The variation in MFA in softwoods has been extensively characterised in many species, especially in the Pinaceae. The commercial importance of MFA, as it relates to wood quality, is well established for softwoods, but is less clear for hardwoods. Relatively few hardwoods have been characterised, so there is a need to extend the range of species and ecotypes that have been investigated. Additionally, more well-designed studies relating MFA and its interaction with other wood properties to timber quality are needed. Likewise, the relationships between MFA and other cell wall properties, such as tracheid length, lumen diameter, cell wall thickness and chemistry, require further study. Finally, the means through which trees control changes in MFA in response to developmental and environmental influences are poorly understood, but the use of model plant systems, molecular biological and genetic techniques is already making a significant contribution to this aspect of plant cell biology. Further inroads are anticipated through combinations of such methods with proven physical and chemical techniques.

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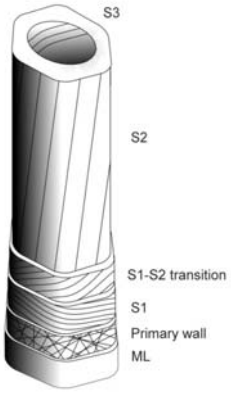

Fig. 1. A diagram showing the microfibril orientation in the cell wall layers of a typical tracheid.

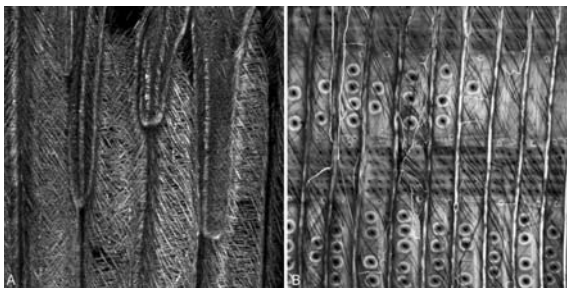

Fig. 2. Polarised light image of a *P. radiata* tracheid showing the procedure for measurement of maximum extinction position. The bordered pit aperture allows observation of the single cell wall behind (or in front of) the pit. The tracheid is rotated in the direction of tilt of the pit aperture (to the left) until the cell wall visible through the pit aperture becomes dark. In this case the MFA is 18^{o}. The angle as determined from the orientation of the pit aperture itself (as opposed to measurement of extinction position) is 36^{o}, indicating poor agreement between these two techniques in this example. Scale bar = 30 μm

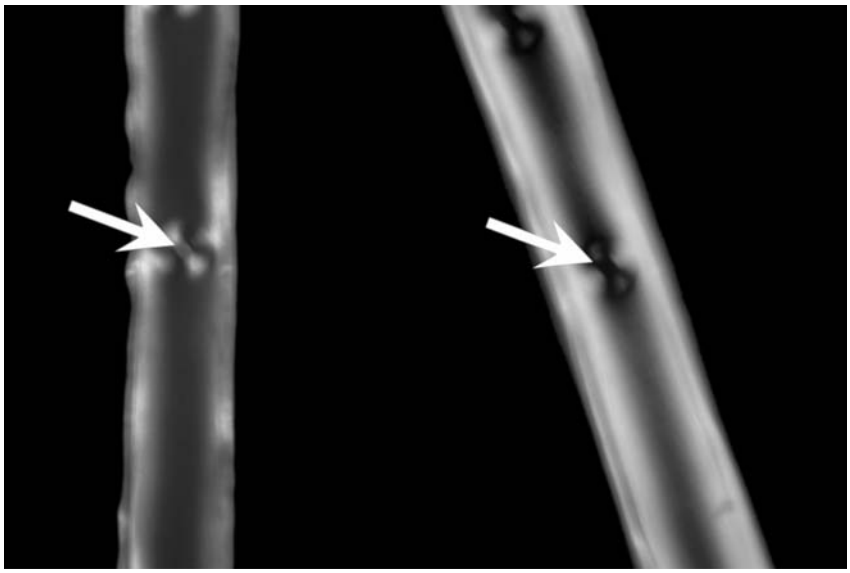

Fig. 3. A. Confocal reflectance image of iodine stained *P. radiata* wood showing the microfibril orientation in S1 and S2 layers of tracheids. Field of view 160 x 160 μm B. Soft rot decay in *Larix* sp. showing microfibril orientation. Field of view 500 x 500 μm

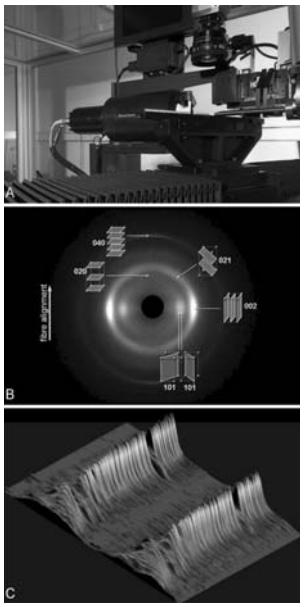

Fig. 4. Microfibril angle measured by X-ray diffraction. A. The SilviScan-3 X-ray diffraction apparatus. B. An X-ray diffraction pattern of wood showing the major diffraction peaks. C. A stacked series of 002 azimuthal X-ray diffraction profiles from pith to bark for *P. abies* showing large angles near the pith (at left) and a narrow band of compression wood (at right). The spread of each peak is approximately proportional to microfibril angle. [Rob Evans, Ensis].