ESTIMATION OF MICROFIBRIL ANGLE OF INCREMENT CORES
BY NEAR INFRARED SPECTROSCOPY

by

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SUMMARY

Eight *Pinus radiata* D. Don (Radiata pine) increment core samples representative of a total of thirty-two increment cores were selected. NIR spectra were obtained from the radial–longitudinal face of each core in 10-mm increments and used to develop a microfibril angle (MFA) calibration. The MFA calibration was developed using seven factors giving an excellent relationship between SilviScan-2 determined MFA and NIR fitted MFA (coefficient of determination ($R^2$) = 0.95) and a standard error of calibration (SEC) of 1.8 degrees. The MFA calibration was used to predict the MFA of NIR spectra obtained in 10-mm sections from the radial–longitudinal face of two intact *P. radiata* increment cores. NIR predicted MFA was found to be in excellent agreement with MFA determined by SilviScan-2, with $R^2$ of 0.98 (core A) and 0.96 (core B). The standard error of prediction (SEP) for core A (1.0 degree) was much lower than for core B (2.5 degrees). Both sets of predictions closely followed the patterns of MFA radial variation determined by SilviScan-2. NIR spectroscopy provides a rapid method for determining MFA variation in increment cores and is suitable for the routine analysis of large numbers of samples.

**Key words:** Near infrared spectroscopy, microfibril angle, *Pinus radiata*.

INTRODUCTION

Microfibril angle (MFA) is defined as the angle that the helical windings of cellulose chains, within the fibre wall, make with the fibre axis. MFA is recognised as an important factor in determining the mechanical properties of individual fibres (Page et al. 1972, 1977) and in conjunction with density largely determines the strength and stiffness of solid wood (Cave & Walker 1994; Megraw et al. 1999; Evans & Ilic 2001). Despite the importance of MFA its inclusion as a property in tree improvement programs has been hindered by an inability to measure it on a large scale.

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The recent development of SilviScan-2 (Evans 1997, 1999) has made the rapid scanning of increment cores a possibility for tree improvement programs and large-scale resource assessment. SilviScan-2 employs X-ray diffractometry and determines an average MFA from a composite diffraction pattern. Evans (1997) estimated that for the 0.2-mm diameter X-ray beam used on SilviScan-2, a composite diffraction pattern represents approximately 400 softwood tracheids or 2000 hardwood fibres. The diffraction patterns collected on SilviScan-2 can be integrated over specified radial sections, hence pith-to-bark profiles of MFA variation can be obtained at different spatial resolutions ranging from 0.2 to 10 mm.

It has been demonstrated (Schimleck et al. 2001, 2002b) that near infrared (NIR) spectroscopy can be used to estimate MFA, as determined by SilviScan-2. The studies by Schimleck et al. (2001, 2002b) were based on NIR spectra obtained from the radial–longitudinal face of *Eucalyptus delegatensis* R.T. Baker and *Pinus radiata* D.Don samples. The relationship between NIR estimated MFA and SilviScan-2 determined MFA was strong for both species, with coefficients of determination (R\(^2\)) of 0.83 (*E. delegatensis*) and 0.78 (*P. radiata*), respectively. In both studies the range of MFAs were limited. For *E. delegatensis* the range was 8.5 to 20.0 degrees, while for *P. radiata* it was 11.3 to 28.1 degrees.

NIR spectroscopy has also been used to investigate the radial variation in stiffness of increment cores as determined by SilviScan-2 (Schimleck et al. 2002a). In this work NIR spectra were collected from the radial–longitudinal surface of two *P. radiata* increment cores that were unrelated to the calibration samples. The stiffness calibrations used by Schimleck et al. (2002a) were developed using a number of *P. radiata* and *E. delegatensis* samples that displayed a wide range of wood properties and had been characterized in terms of E\(_{L(SS)}\) (stiffness estimated based on SilviScan-2 data and measured density (Evans & Ilic 2001)). Estimates of stiffness obtained using these calibrations agreed well with those determined using SilviScan-2 data (R\(^2\) ranged from 0.89 to 0.92).

Schimleck and Evans (2002) developed a stiffness calibration using cores that were related to the two *P. radiata* cores tested previously (Schimleck et al. 2002a) and obtained improved estimates of stiffness. R\(^2\) of 0.99 (core A) and 0.98 (core B) and standard error of predictions (SEP) of 1.6 GPa (core A) and 1.2 GPa (core B) were obtained. Both sets of predictions closely followed the patterns of E\(_{L(SS)}\) radial variation determined by SilviScan-2.

The aims of this study were:

- a) to investigate if the *P. radiata* core samples selected for development of the E\(_{L(SS)}\) calibration in Schimleck & Evans (2002) could be used to develop a calibration for MFA; and
- b) to use the MFA calibration to estimate radial changes in MFA of two *P. radiata* increment cores based on NIR spectra obtained in 10 mm sections from the radial–longitudinal face of each core.
EXPERIMENTAL

Selection of calibration samples

119 diffuse reflectance NIR spectra were collected in 10-mm sections from eight *P. radiata* increment cores. The eight cores were selected, from a total of thirty-two, for development of a calibration for MFA. All cores had been analysed by SilviScan-2 (Evans 1997, 1999) and pith to bark profiles of MFA determined. The samples were taken from breast height in 26-year-old trees, growing on a single site, that had been thinned at age fourteen. Four core samples were selected from trees that had been fertilised with nitrogen (200 kg/ha) and phosphorus (100 kg/ha) once after thinning (Nyakuengama et al. 2002). The remaining four cores were chosen from trees that had been thinned but not fertilised. The eight cores were selected from trees that demonstrated different patterns of radial variation in MFA and represented the range of tree breast-height diameters.

Core samples for the prediction of MFA

Additional two increment core samples were selected from the same set to test the MFA calibration developed using the selected core samples. The core samples were the same as those reported in Schimleck et al. (2002a) and Schimleck & Evans (2002). One sample was from a tree that had been fertilised after thinning (core A), the other was taken from a tree that had not been fertilised after thinning (core B). Core A exhibited a marked increase in ring width compared to core B.

Determination of MFA

Strips, for analysis by SilviScan-2, were cut from the selected core samples using a twin-blade saw. Strip dimensions were 2 mm tangentially and 7 mm longitudinally, radial length was determined by the pith-bark length of the selected core samples.

MFA was determined using X-ray diffractometry by SilviScan-2 (Evans 1997, 1999). All measurements were made in a conditioned atmosphere maintained at 40% RH and 20 °C. The MFA data were obtained originally over 1-mm intervals. For the purposes of this work, MFA was further averaged over 10-mm sections. Table 1 gives MFA results for the calibration set and the two cores used for the prediction of MFA.

Table 1. Microfibril angle (MFA) summary statistics for the *Pinus radiata* calibration set and *P. radiata* core A and core B.

<table>
<thead>
<tr>
<th></th>
<th>Calibration set</th>
<th>Core A</th>
<th>Core B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of spectra</td>
<td>119</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Minimum MFA (degrees)</td>
<td>10.7</td>
<td>11.9</td>
<td>11.8</td>
</tr>
<tr>
<td>Maximum MFA (degrees)</td>
<td>41.6</td>
<td>36.8</td>
<td>37.6</td>
</tr>
<tr>
<td>Average MFA (degrees)</td>
<td>23.8</td>
<td>24.0</td>
<td>22.4</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>7.3</td>
<td>6.6</td>
<td>8.5</td>
</tr>
</tbody>
</table>
Near infrared spectroscopy

NIR diffuse reflectance spectra were obtained from the radial/longitudinal face of each core sample using a NIRSystems Inc. Model 5000 scanning spectrophotometer. Samples were held in a custom-made holder (Schimleck et al. 2002b). A 5 × 10 mm mask was used to ensure a constant area was tested. The spectra were collected at 2 nm intervals over the wavelength range 1100–2500 nm. The instrument reference was a ceramic standard. Fifty scans were accumulated for each section and the results averaged. One average spectrum was obtained per section.

The spectra were converted to the second derivative mode (Shenk et al. 1992) for the development of calibrations using the instrument’s NSAS® software. A segment width of 10 nm and a gap width of 20 nm were used for the conversion.

MFA calibration development

The calibration for MFA was developed using Partial Least Squares (PLS) regression. A detailed description of PLS regression is provided by Esbensen et al. (1994). The calibration was developed using NSAS® software (version 3.52), with four cross validation segments and a maximum of ten factors. The NSAS® software recommended the final number of factors to use (Anonymous 1990).

Calibrations were developed using all spectra (119) collected from the calibration set samples.

Calibration statistics

The measure of how well a calibration fits the data is the Standard Error of Calibration (SEC). The measure of how well the calibration predicts the constituent of interest for a set of unknown samples that are different from the calibration test set is given by the Standard Error of Prediction (SEP). Formulas used to estimate the SEC and SEP are given in Schimleck et al. (2002b).

Determination of weighted cross-section averages

Weighted cross-section MFA averages were determined for the two test cores using SilviScan-2 and NIR predicted data. For the calculation of the weighted averages it was assumed that the length of the core was the radius of a disc. The disc area represented by each 10-mm section was determined and then multiplied by its respective MFA (as determined by SilviScan-2 or predicted by NIR). The sum of each radial strip was determined and then divided by the total disc area.

RESULTS

NIR calibration for MFA

A calibration for MFA was developed based on NIR spectra obtained in 10-mm increments from the radial–longitudinal surface of eight selected P. radiata increment cores (Fig. 1). The relationship between SilviScan-2 determined MFA and NIR fitted MFA was excellent. Seven factors were used for the calibration based on the recommendation of the NSAS® software, providing a coefficient of determination
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(R²) of 0.95 and a SEC of 1.8 degrees. 119 NIR spectra were used for development of the calibration and of these, eleven samples had residuals (where the residual is equal to the NIR fitted MFA minus SilviScan-2 determined MFA) greater than ± 3 degrees. The largest residual was 5.3 degrees, the same sample gave the highest residual (-2.4 GPa) for the E_L(SS) calibration reported in Schimleck et al. (2002a). The majority of samples (78.2%) had residuals less than ± 2 degrees.

Prediction of MFA by NIR spectroscopy

NIR spectra were obtained in 10-mm sections from the radial–longitudinal surface of two P. radiata increment cores (referred to in the following text as core A and core B). The current calibration was used to predict the MFA of each of these sections.

For both cores the relationship between SilviScan-2 determined MFA and NIR predicted MFA was excellent (Fig. 2). Core A, had the strongest relationship (R² = 0.98) with a very low SEP (1.0 degrees). The regression line plotted in Figure 2a is very close to the line of equivalence indicating that there is very little bias and that MFAs predicted by the selected core calibration were very close to the experimental determinations of MFA.

NIR predictions of MFA for core B were generally lower than those found by SilviScan-2 and greater bias was observed (Fig. 2b). The R² (0.96) was lower than that reported for core A and the SEP was higher (2.5 degrees). Two sections of core B had large residuals, 0–10 mm (-5.3 degrees) and 90–100 mm (-4.6 degrees) (see Fig. 3b).
Fig. 2. Relationship between SilviScan-2 determined microfibril angle (MFA) and NIR predicted MFA for *Pinus radiata* core A (a) and core B (b). Note that the regression line has been plotted and that the thin broken line represents the line of equivalence.

**Radial variation of MFA**

Plots of pith to bark (radial) variation of MFA, determined by SilviScan-2 and predicted by the selected cores MFA calibration, are shown for both cores in Figure 3. For core A, the two patterns of radial variation were in close agreement (Fig. 3a). Core A, which was from a tree that had been thinned and fertilised, showed an increase in MFA (at 110 mm) followed by a decrease that was predicted by NIR spectroscopy. The sections from 60–70 mm and 110–120 mm had the largest residual (1.7 degrees).
NIR predictions of MFA for core B also closely followed the radial pattern of MFA variation determined by SilviScan, i.e. a decrease from pith to bark (Fig. 3b). In general, the NIR predictions of MFA for each section of core B were not as close to the SilviScan-2 determined MFAs as was found for core A, with differences ranging from 0.1 to -5.3 degrees. For NIR predicted $E_{L(SS)}$ Schimleck and Evans (2002) found the opposite, i.e. NIR predictions for core B were closer to SilviScan-2 determined $E_{L(SS)}$ than were found for core A.

**Weighted cross-section averages**

For core A, a weighted cross-section average MFA of 20.7 degrees was determined based on NIR predicted MFA which compared very well with the weighted average calculated using SilviScan-2 determined MFA (20.1 degrees).

The weighted cross-section average for core B calculated using SilviScan-2 data (17.8 degrees) was 1.5 degrees higher than that determined using NIR predicted MFA (16.3 degrees).

**The relationship between PLS factors and MFA**

The correlation of MFA with the scores of the ten PLS factors used to develop the current calibration was examined. It was found that the scores of only the first factor
correlated well with MFA ($R^2 = 0.68$) indicating that considerable information relevant to MFA variation was explained by the first PLS factor. Examination of the first factor loadings plot showed that many wavelengths had large loadings, including 1320 nm, 1424 nm, 1498 nm, 1898 nm and 1932 nm. The loadings at 1498 and 1932 nm are close to wavelengths that have been assigned to cellulose (1490 nm and 1930 nm) (Shenk et al. 1992).

The relationship between MFA and individual wavelengths

The relationship between MFA and NIR spectra obtained from the radial–longitudinal surface of core samples was examined by developing MFA calibrations using individual wavelengths and standard linear regression. Several wavelengths gave good $R^2$ including 1470 nm (0.86), 1510 and 2458 nm (0.82), 2326 nm (0.74) and 2082 nm (0.62). Of these wavelengths 1470, 2082 and 2326 nm occur close to wavelengths that have been assigned to cellulose (1490, 2100 and 2335 nm) (Shenk et al. 1992).

DISCUSSION

The calibration developed for MFA using the current data set had superior statistics to those reported in Schimleck et al. (2001, 2002b). An important factor in the success of the current calibration is the wide range of MFAs present. The current data set had MFAs ranging from 10.7 to 41.6 degrees (Table 1) which is a much wider range than the limited ranges Schimleck et al. (2001, 2002b) used for *E. delegatensis* (8.5 to 20.0 degrees) and *P. radiata* (11.3 to 28.1 degrees).

The relationship between SilviScan-2 determined MFA and NIR predicted MFA was excellent for both cores. The relationship for core B was slightly weaker than for core A. The weaker relationship was caused by two sections of core B that had large residuals. These two sections were noted as having large residuals in Schimleck & Evans 2002 when an $E_{L(SS)}$ calibration was used on core B. Schimleck and Evans (2002) reported that fibre tilt and compression wood were not responsible for the large $E_{L(SS)}$ residuals observed.

An important issue that must be considered is the underlying relationships that have made the estimation of MFA by NIR spectroscopy possible. Schimleck et al. (2002b) suggested that the good calibration statistics they obtained for MFA may have been due to the systematic within-tree variation in a range of associated properties, such as cellulose content. To investigate this possibility the correlation of MFA with the scores of the ten PLS factors used to develop the current calibration was examined. Only the first factor had a strong correlation with MFA. The corresponding loadings plot had large loadings in regions of the NIR spectrum assigned to cellulose suggesting that variation in cellulose content is an important factor in the success of the MFA calibration. It was also found that several wavelengths assigned to cellulose had strong correlations with MFA providing further evidence that MFA variation is linked to variation in cellulose content.

This study found that it is possible to calibrate an NIR instrument to predict MFA (as determined by SilviScan-2). Previously (Schimleck & Evans 2002) it has been
demonstrated that NIR spectroscopy can be used to predict stiffness \( (E_L)_{\text{SS}} \)-estimated stiffness based on SilviScan-2 data and measured density). The ability of NIR spectroscopy to predict these important solid wood properties potentially makes it an important tool for wood quality assessment. Further work is required to determine if the MFA calibration reported here is applicable to cores from other sites, or to cores that have been subject to different silvicultural regimes. It will also be necessary to examine if it is possible to develop more general calibrations that include cores from trees of different ages and that include a range of sites. If an MFA calibration, based on NIR spectra obtained from increment cores, were to be used for the routine analysis of large numbers of core samples, a general and robust calibration developed using samples from several different sites and of different ages would be the preferred approach.

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REFERENCES


