Application of near-infrared spectroscopy to predict microfibril angle of 14-year-old *Pinus patula*

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Paper presented in IUFRO symposium "Wood structure and Properties '06", 3-6 Sept. 2006, Sielnica, Slovakia

Abstract
An investigation was conducted to test the feasibility of near infrared spectroscopy (NIR) as a tool for predicting the microfibril angle (MFA) of solid wood samples of *Pinus patula*. Thirty 14 year-old trees were selected from three compartments located in KwaZulu-Natal, South Africa with three different site indices. Radial strips obtained at breast height were scanned at 5 and 10 mm intervals in a radial-longitudinal surface using a NIR spectrophotometer. The same strip samples were scanned using Silviscan-2 to determine their corresponding MFA profiles. Calibration models were developed to predict MFA from NIR spectra using partial least squares (PLS) regression.
The results showed that good correlations between NIR spectra and MFA values were achieved when data from all three sites were combined as well when considered individually. All correlation coefficients were greater than 0.9, highlighting the reliability of NIR technique to predict MFA for *Pinus patula*. When calibration equations developed using data from individual sites were tested on the other data sets, predictions ranged from 65 to 80% and standard errors of prediction from 3.4 to 3.9 degree. Calibration statistics and prediction ability improved slightly using data from scans at 10 mm resolution compared to 5 mm resolution.

Keywords: microfibril angle, *Pinus patula*, NIR spectroscopy, calibration

Introduction

Pinus patula represents approximately half of all softwoods planted in the region of South Africa (Malan, Retief and Male, 1997). The species was introduced into South Africa in 1907 and has become firmly established as the principal pine species used for timber production and an important source of fibre for pulp industry.

The microfibril angle (MFA) of the S2 layer in the fibres cell wall is known to be one of the main determinants of the mechanical properties of solid wood (Cave and Walker 1994; Evans and Ilic 2001) and shrinkage anisotropy (Megraw et al. 1998). Extensive work has been done to evaluate the relationship between MFA and strength properties of individual fibres (Watson and Dadswell 1964; Page et al. 1977). These studies have shown that fibres with smaller MFA were characterized by higher tensile and tear properties, as well as a high modulus of elasticity.

Several methods for determining the MFA have been developed over the years (Huang et al. 1997). Mostly all of them are time-consuming and not suitable for large scale utilisation. However, recently an X-ray diffraction technique developed for SilviScan-2 (Evans 1997) has successfully simplified the assessment of MFA. Although this technique enables rapid measurement of MFA, it is not always the preferred option since x-ray diffraction requires expensive capital equipment making the measurement very costly. Therefore there is a need to look for an alternative more cost-effective method of MFA evaluation.

Near infrared (NIR) spectroscopy has shown great potential within the forest products industry as a technology enabling the rapid assessment of various physical, and chemical properties. NIR analysis involves measuring the spectrum of a wood sample in the near-infrared region (700-2500 nm). Spectra within this region consist of

overtone and combination bands of vibrations of functional groups – CH, OH and NH. A NIR instrument requires the development of calibration models that relate the NIRA spectra of the wood sample to known reference data. The models are then used to predict the investigated trait of new samples from their spectra. Relative to other traditional methods of the properties evaluation, the advantages of NIR spectroscopy include minimal sample preparation, rapid acquisition time, and non-destructive spectral acquisition.

Several researchers have examined the ability of NIR to analyze wood chemical properties (Garbut et al. 1992; Shimleck et al. 1997; Poke et al. 2004), as well as pulp properties (Wright et al. 1990; Sefara et al. 2000, Shimleck et al. 2005b). More recently wood physical properties have become to the focus of assessment using NIR. It has been shown that wood density (Hoffmeyer and Pedersen 1995), mechanical properties (Kelly et al. 2004), wood anatomy (Schimleck et al. 2005a), microfibril angle (Schimleck and Evans 2002; Jones et al. 2005) can be predicted using NIR.

The aim of this work is to evaluate the potential of NIR technology for the assessment of MFA in *Pinus patula* and to investigate the influence of changing scanning resolution on the calibration statistics.

Material and methods

Sample origin

Wood samples of 14-year-old *Pinus patula* plantation trees were obtained from three compartments with different site indices located within KwaZulu-Natal, South Africa. Ten trees from each compartment were randomly selected, providing a total 30 trees for this study. The description and location of sites, mean heights and diameters are presented in Table 1.
Two discs of 30 mm were taken from different heights along the stem of each tree (at 1.3 m height, and from 15%, 35% and 65% of the total height). In this particular study, only material taken at breast height was used. In total, 30 knot-free discs were labelled, debarked, stored in plastic bags in a refrigerated environment for further analysis.

Table 1 Description and characteristics of sampled sites

Parameter	Poor	Medium	Good
Site	De Rust	Clairmont	Hodgsons
Region	Greytown	Bulwer	Umvoti
GPS co-ordinates	29°1'S 30°6'E	29°48'S 29°46'E	29°14'S 30°37'E
Altitude (m)	1041	1484	1065
MAP (mm)	810.10	959.70	910.90
MAT (ºC)	17.02	13.60	16.70
Measured Site Index	23.62	26.52	35.53
Mean DBH (cm)	19.82	23.41	21.83
Mean Total Height (m)	15.20	17.13	22.59

MAP – mean annual precipitation
MAT – mean annual temperature

Sample preparation

A 2-cm radial block was obtained from each disc and subsequently stored at 23ºC and 50% relative humidity to achieve an equilibrium moisture content of about 10%. Thin strips of uniform thickness were cut along the radius using a twin-blade saw. The sample dimensions were 12 mm in the longitudinal direction, 2.5 mm in the tangential direction and length was determined by the radius of the disc.

Near infrared spectroscopy scanning

NIR diffuse reflectance spectra were acquired using a fibre optic probe of FOSS XDS™ Smart Probe Analyzer instrument. The strips were scanned in a light-proof and conditioned atmosphere. The fibre optic probe was orientated at right angle to the radial-longitudinal face of the strip sample and the NIR spectrum was obtained over at two different scan resolutions: 5-mm and 10-mm intervals continuously starting from the bark end of the strip. During scanning the strip was held in a custom-made holder.
All spectra fell in near infrared region of the electromagnetic spectra (1100 to 2350 nm). The internal reflectance reference was 99% reflectance Spectralon. Thirty two scans were accumulated for each section which were then averaged to provide a single spectrum.

Determination of microfibril angle

MFA measurements were conducted by X-ray diffractometry using Silviscan-2 in 5 mm intervals (Evans et al. 1999). Measurements were taken along the radial – longitudinal face of the strip from bark to pith. Average of MFA values were also determined over 10-mm sections for correlations with the NIR spectra. For each sample the weighted mean value of MFA representing the disc value was also calculated.

NIR data analysis

Data analysis was conducted using Vision™ software supplied by Foss. Prior to analysis, spectra were transformed into the second derivative mode to remove baseline shift. The calibration for MFA was developed using Partial Least Squares regression. PLS is a commonly used linear regression method for highly collinear data which extracts variation in NIR spectra. A cross-validation method with four cross validation segments was used to verify the prediction power of the PLS model. It involves splitting the calibration set randomly into 4 segments with the same number of samples. One segment is excluded and remaining 3 segments are used to develop a PLS model with different numbers of factors. This is validated against the samples in the fourth segment. The process is repeated until each segment is used and validated (Williams and Norris 1987).
The coefficient of determination R^2, the standard error of calibration (SEC – determined from the residual of the final calibration) and standard error of cross-validation (SECV – determined from the residual of each cross-validation phase) generated from the regression were used to test the calibration abilities of the NIR model developed. The optimum number of components (factors) used for a model were selected by observing the response of the cross-validation residual with the added factors. Standard error of prediction (SEP) provided an assessment of how well the calibration model predicted the parameter of interest of unknown samples not contained in the calibration data set.

Calibration and prediction

Overall, 30 samples were analysed by NIR spectroscopy, producing 490 spectra from radial-longitudinal surfaces collected over 5-mm sections and 242 spectra from radial-longitudinal surfaces collected over 10-mm sections. From the 10 radial strips per compartment, 7 were randomly selected for the calibration set and the remaining 3 were included in the validation dataset to test the developed models. One strip sampled from the low quality site (De Rust) was identified as an outlier. This strip had a noticeable amount of compression wood and was therefore excluded from the subsequent analyses.
Initially, the calibration model for MFA was developed by combining samples from three sites. Table 2 gives the summary of data used for the calibration and validation datasets. From these results, it was observed that the NIR model performed better when scanned over 10-mm sections. Consequently, this resolution was used to develop individual models for each site for the prediction of MFA on other sites.

Table 2 Statistical summary of MFA values for multi-site dataset scanned over 5 and 10mm section

All sites	***Calibration set***				***Validation set***			
Section	N	Mean	Range	STD	N	Mean	Range	STD
5-mm	317	22.4	5.9-35.7	5.2	153	23.8	9.6-34.1	5.1
10-mm	155	22.3	8.4-33.8	5.2	75	23.4	6.6-34.7	5.2

Results

Comparison of MFA across different sites

Microfibril angle was measured using Silviscan-2 from bark-to-pith at 5-mm resolution on 30 samples across different sites. The profile of MFA was area-weighted for each strip and the mean value for the compartment was calculated using 10 samples. The results showed that the MFA varied significantly ($p<0.01$) with changes in site quality; the MFA being smallest for the high quality site - Hodgsons (17.9 deg) and largest for the low quality site - De Rust (23.2 deg). These results are comparable to those reported in a previous study for pine species (Donaldson 1992).

Calibrations using spectra taken from single face and from both faces

When a radial strip is scanned using a NIR spectrophotometer, the spectra are usually obtained from only one side of the strip sample. A preliminary experiment has been carried out to examine whether the averaged spectra over the scanned sections taken from two opposite sides of a strip would improve the calibration statistics compared to that of a single spectrum taken from a single surface of the strip. Spectra of 10 strip samples from the Hodgsons site for both radial faces were obtained over 10-mm sections. These spectra were also averaged to provide a single spectrum per section.

Results are presented in Table 3. Calibration based on NIR spectra taken from single surfaces (surface A and opposite surface B) gave comparable results to calibrations where average spectra of section from both sides were used. The coefficient of determination was slightly higher for the averaged surface spectra (0.88) with smaller SECV (2.3 degrees) and less PLS factors were used. In terms of predictive performance, there was a better correlation with MFA when spectra were taking only from a single surface (surface A) with R^2_p = 0.9 compared to 0.81 for averaged spectra taken from both surfaces. These results indicate that spectra taken from one random surface along the radius are sufficient to characterise NIR variation in sample and was the rationale behind the use of the method for the duration of the study.

Table 3 Summary of calibration models developed for MFA using spectra from single side of strip and averaged spectra from both sides of strip (the scan resolution of 10mm)

	Calibration set				**Validation set**	
Option	PLS factors	R^2	SECV	SEC	R^2_p	SEP
Surface A	5	0.87	2.4	1.9	0.90	2.3
Surface B	4	0.85	2.5	2.2	0.80	2.8
Averaged	3	0.88	2.3	2	0.81	2.5

Effect of scan resolution

NIR spectra were collected along the radius from the longitudinal-radial surface of the strips and subsequently correlated with the corresponding MFA values that were measured using SilviScan-2. Twenty strips combining three sites were used in the calibration set to develop the PLS models for MFA at two scan resolutions: over 5-mm and 10-mm sections. The results of calibration using NIR spectra in the range 1100 to 2300 nm are shown in Figure 1 and summarized in Table 4.

From Figure 1, it can be seen that there is a good correlation between the MFA values measured using SilviScan-2 and NIR predicted MFA values, the coefficient of determinations being 0.84 for scans recorded at 5-mm intervals and 0.89 for scans recorded at 10-mm intervals. Spectra taken over 10-mm sections performed better in terms of SECV than the spectra taken over 5-mm sections, with SECV values of 2.38 degrees and 2.9 degrees respectively. This finding was surprising since the range in MFA was narrower for the 10-mm section dataset (Table 2).

Table 4 Summary of calibration statistics and predictive power of multi-site models obtained at two different scan resolutions

	Calibration set				**Validation set**	
Scan interval	PLS Factors	R^2	SECV	SEC	R^2_p	SEP
5-mm	8	0.84	2.9	2.3	0.77	2.7
10-mm	7	0.89	2.4	1.8	0.79	2.6

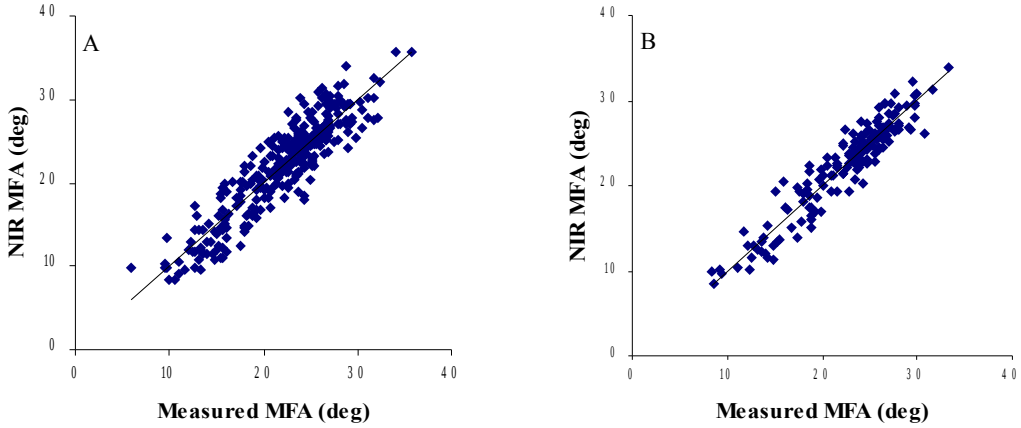

Figure 1 The calibration models for MFA using 20 strips combining all sites; NIR spectra collected over 5-mm (A) and 10-mm (B) sections

Calibration models developed for MFA were validated on a separate dataset consisting of 9 strips from combined sites which had not been used to construct the model. As shown in Figure 2 and Table 4, there were slight differences between the two datasets. The spectra collected over 10-mm sections gave better results for R^2 (0.79) and SEP (2.6 degree) compared to those collected over 5-mm sections (R^2=0.78, SEP=2.7 degree).

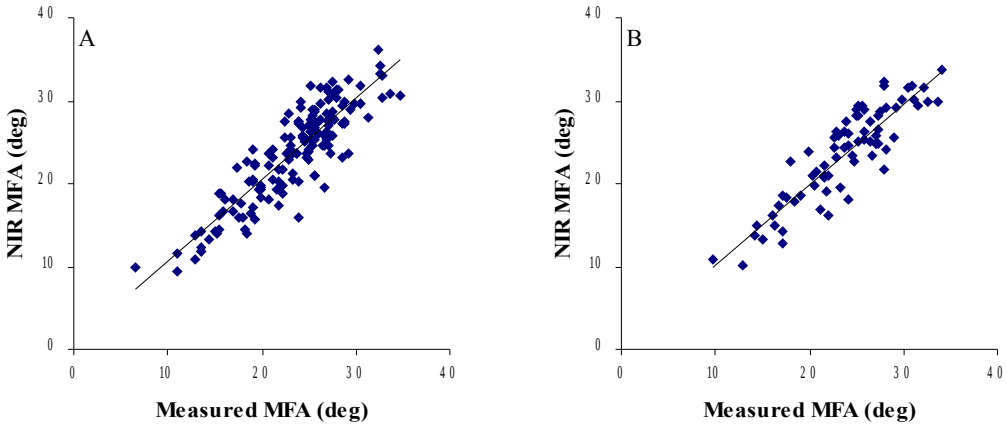

Figure 2 The prediction of MFA using 9 strips combining all sites; NIR spectra collected over 5-mm (A) and 10-mm (B) sections

Prediction at sample level

Weighted cross-sectional MFA averages were determined for the 9 strips from the validation set using data from Silviscan-2 and NIR predicted data. As illustrated in Figure 3, there is excellent agreement between two methods suggesting that NIR spectroscopy can reliably predict the MFA value of an individual sample. A slight bias was observed for the scans collected at 5-mm intervals, where an underestimation of the MFA value occurred.

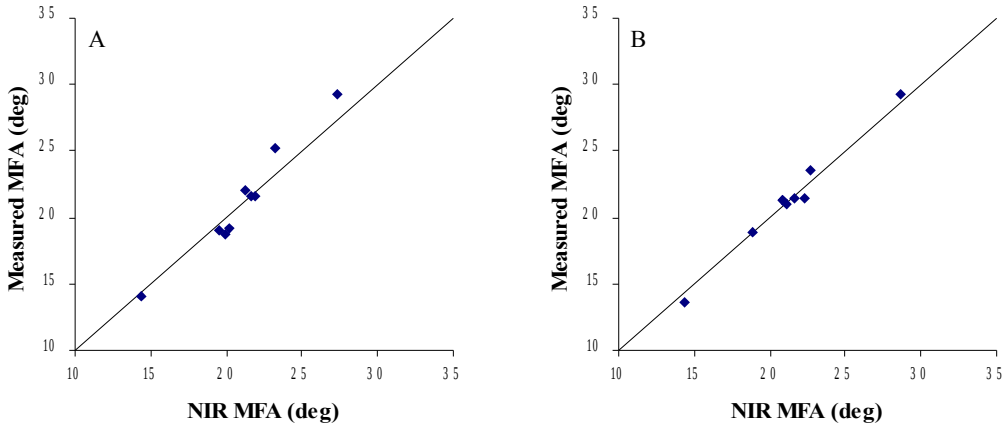

Figure 3 Relationship between weighted cross-sections averages of MFA measured by SilviScan and predicted by NIR model; NIR spectra collected over 5-mm (A) and 10-mm (B) sections. Note that the regression line has been plotted.

Calibrations for each site

It has been found out that the NIR model performed better when scanned over 10-mm sections, so at this resolution the MFA models were also developed separately for each site and then used to predict MFA on other sites. Summary of results are provided in Table 5.

All sites had very similar calibration statistics with R^2 ranging from 0.89 for the Hodgsons site to 0.91 for the De Rust site. All sites required 7 principal components to achieve the lowest SECV. SECV was lowest for Hodgsons site (2.39 degree) and highest for the Clairmond site (3.05 degree). When comparing multi-site calibration at the same scan resolution, there was a very similar correlation between the SilviScan measured MFA and the corresponding NIR spectra (R^2=0.89, SECV=2.4 degree).

Table 5 Calibration statistics and predictive power of models developed at each site and for multi-sites dataset; scan resolution of 10mm

Site	*Calibration set (all samples within the site)*				*Prediction set with R^2 and SEP in parentheses*		
	PLS	**R^2**	**SECV**	**SEC**	**De Rust**	**Clairmond**	**Hodgsons**
De Rust	7	0.91	2.64	1.58	-	0.67 (3.7)	0.8 (3.5)
Clairmond	7	0.91	3.05	2.06	0.65 (3.4)	-	0.65 (3.7)
Hodgsons	7	0.89	2.39	1.96	0.67 (3.9)	0.69 (3.9)	-
Multi-site	7	0.89	2.4	1.8	0.92 (1.9)	0.79 (2.4)	0.86 (2.5)

The local site calibration models were used to predict the MFA of other sites. As presented in Table 5, R^2 ranged from 0.65 to 0.8. For example, the calibration model constructed with all the samples from De Rust site was applied to predict the MFA for the Hodgsons site. The NIR model explains 80% variation in Hodgson's samples with an SEP of 3.5 degrees. The predictive power of the calibration model was rapidly increased when the multi-site model was used to predict the MFA value on the remaining samples from each site which had not been included in calibration set.

Discussion

When a radial strip is scanned using NIR spectrophotometer, the spectra can be obtained either from a single side of the strip or alternatively an average spectrum over the scanned section taken from both sides of strip can be used. As indicated in this study, the calibration based on NIR spectra from a single surface gave comparable results to the calibration developed using average spectra. This finding indicates that it is not necessary to scan both sides of a strip during the acquisition of the NIR spectra which reduces the data acquisition time substantially. The quality of the surface is the important factor influencing the calibration statistics (Schimleck et al. 2005a), with preference given to the side with the smoother surface.

It has been demonstrated that NIR spectroscopy can be used to estimate wood properties on different pine species, however no reports are available for *Pinus patula*. In earlier work, Schimleck and Evans (2002) have predicted MFA for *Pinus radiata* from a single site. They found excellent correlations between NIR spectra and MFA, recording an R^2 of 0.95 using 7 factors. Jones et al. (2005) estimated MFA, stiffness and wood density for *Pinus taeda* L. using 729 spectra from 9 various sites in Georgia, USA. Their ability to predict MFA was 90%, which compares favourably with the results reported in this study, where 3 sites have been included in the calibration. Previous works have considered a scanning resolution of 10 mm. We have also investigated the performance of calibration models using scanning resolutions of 5 and 10 mm. Despite the narrower range in MFA, slightly stronger calibration statistics were observed when spectra were collected over 10-mm sections. The prediction power of the model also improved at this scanning resolution.

The calibrations developed in this study were based on *Pinus patula* samples taken from contrasting sites within Kwa-Zulu Natal. When a local site model was used to predict the MFA on the other sites, the predictions were still high (more than 65% with SEP of 3.9 degree). Previous studies (Jones et al. 2005; Shimleck et al. 2005b) have shown that the addition of a few samples from new sites improves the prediction power of the model. Indeed, when the multi-site calibration model was used to predict the MFA of samples from individual sites not included in the calibration set, the predictive power improved rapidly by reducing the SEP value and increasing the R^2 values (Table 5).

The use of the NIR spectra of strip samples to predict MFA has important implications in tree breeding programs. The core samples can be obtained non-destructively from the standing trees, strips extracted and scanned by NIR spectrometer to provide the variation in MFA along the radius. A very important aspect of this study is that weighted mean values representing the whole strip can then be used to distinguish between individual trees samples within a breeding population. As shown in this study, the NIR predicted whole-disk samples correlated very well with the SilviScan measured values (Figure 3). Furthermore, several studies have shown (Evans and Ilic 2001) that MFA is strongly related to the mechanical properties of solid wood. If one can predict MFA using the NIR, an indirect prediction of mechanical properties from MFA values should be possible; further highlighting the usefulness of NIR technique as an important tool for wood quality assessment. Further work will be required to include more sites with different site qualities, ages and altitudes to widen the variation in MFA. This will allow the development of a robust calibration model for the determination of MFA for routine assess of this characteristic on a large scale and for exploitation within breeding programs.

Conclusions

Radial strips of 14-year-old *Pinus patula* obtained at breast height were scanned at 5 and 10 mm intervals along a radial-longitudinal surface using a NIR spectrophotometer. The same strip samples were scanned using Silviscan-2 to determine their corresponding MFA profiles. Calibration models to predict MFA from NIR spectra were developed using partial least squares (PLS) regression. The results showed that good correlations between NIR spectra and MFA values were achieved when data from all three sites were combined as well when considered individually. All correlation coefficients were greater than 0.9, highlighting the reliability of the NIR technique to predict MFA for *Pinus patula*. When calibration equations developed using data from any individual sites were tested on the other data sets, predictions ranged from 65 to 75% and standard errors of prediction from 3.1 to 3.9 degree. The calibration statistics and predictive power improved slightly using data from scans acquired at 10 mm resolution compared to 5 mm resolution.

Acknowledgements

We would like to thank to Dr. Rob Evans and Sharee Harper from CSIRO, Melbourne for the evaluation of MFA using SilviScan-2. This project has been funded through a cooperative between Mondi, Sappi and CSIR.

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