Holzforschung, Vol. 58, pp. 375–381, 2004 • Copyright © by Walter de Gruyter • Berlin • New York

Nondestructive estimation of tracheid length from sections of radial wood strips by near infrared spectroscopy

Laurence R. Schimleck[1,*], P. David Jones[1], Gary F. Peter[2], Richard F. Daniels[1] and Alexander Clark III[3]

[1] Warnell School of Forest Resources, University of Georgia, Athens, GA, USA
[2] School of Forest Resources and Conservation, University of Florida, Gainesville, FL, USA
[3] USDA Forest Service, Southern Research Station, Athens, GA, USA

*Corresponding author.
Warnell School of Forest Resources, University of Georgia, Athens, GA 30602, USA
Fax: +1-706-542 8356
E-mail: lschimleck@smokey.forestry.uga.edu

Abstract

The use of calibrated near infrared (NIR) spectroscopy for predicting tracheid length of *Pinus taeda* L. (loblolly pine) wood samples is described. Ten-mm sections of 14 *P. taeda* radial strips were selected and NIR spectra obtained from the radial longitudinal face of each section. The fibers in these sections were characterized in terms of arithmetic and length-weighted mean tracheid length using a fiber quality analyzer, and calibrations with NIR spectra were developed for both measures of tracheid length. Relationships were good, with coefficients of determination (R^2) of 0.88 for arithmetic tracheid length and 0.96 for length-weighted tracheid length. The accuracy of NIR predicted length-weighted tracheid length was sufficient for ranking purposes.

Keywords: fiber quality analyzer; near infrared spectroscopy; NIR; *Pinus taeda*; tracheid length.

Introduction

Considerable interest presently exists in developing methods for the rapid, nondestructive determination of wood properties of plantation grown trees. Such information will be important to industry in terms of resource evaluation and will also assist tree breeders in improving the wood properties of trees. By necessity, nondestructive sampling involves removing increment cores from an easily accessible height and using the cores for analysis.

Recently, we applied near infrared spectroscopy for the estimation of wood properties of increment cores (Schimleck and Evans 2002a,b, 2003, 2004). In these studies, NIR spectra were collected from the radial longitudinal face of intact *Pinus radiata* D. Don radial wooden strips prepared for analysis on the SilviScan instruments (Evans 1994, 1997, 1999). Data provided by the SilviScan instruments was used to develop calibrations for air-dry density, microfibril angle (MFA), stiffness (determined using SilviScan-2 diffractometric data and measured density), and a number of tracheid morphological characteristics including coarseness, perimeter, radial diameter, tangential diameter, and wall thickness. Coefficients of determination (R^2) ranged from 0.65 for tracheid radial diameter to 0.97 for stiffness. The calibrations, apart from tracheid perimeter and tracheid radial diameter, performed well when applied to a separate test set of two cores that were from the same population as the calibration samples.

The SilviScan instruments provide data on a range of parameters at high spatial resolution, but an important fundamental wood property that cannot be measured is tracheid (or fiber) length. Tracheid length is important in determining the properties of both paper and solid wood products (Zobel and van Buijtenen 1989). Tracheid length is of great interest to the pulp and paper industry because it has strong relationships with a number of paper properties, including tear strength (Smook 2002). Typically, tracheid length is determined by optical methods. A dilute pulp suspension is passed through a cuvette that is illuminated by a light source. A camera detects the tracheids and image analysis is used to determine their length (Heikkurinen 1999). Several commercial instruments, such as the Kajaani FS-200 and the fiber quality analyzer (FQA), are available for the determination of tracheid length (Heikkurinen 1999). These instruments require the wood samples to first be pulped. Sample pulping is labor intensive and slow, limiting the number of samples that can be analyzed. Thus, a more rapid method for the determination of tracheid length of increment cores is desirable.

Estimation of tracheid length by spectroscopic methods has received little attention based on the paucity of existing studies. Hauksson et al. (2001) report promising results for the estimation of tracheid length in *Picea abies* (L.) Karst. (Norway spruce) using NIR spectroscopy. Ona et al. (1999, 2003) have investigated the estimation of cell length in *Eucalyptus* species by Raman spectroscopy and also reported promising results. Both of these studies used milled wood samples, and it is possible that the application of NIR spectroscopy to the solid wood of radial strips would provide improved calibration statistics. Recently, Via et al. (2004) applied NIR spectroscopy to the solid wood of *Pinus palustris* Miller (longleaf pine) and developed a number of calibrations for tracheid length. A calibration based on all available samples (from various radial positions and heights of 10 trees) provided a reasonably strong coefficient of determination ($R^2 = 0.72$). Tracheid length calibrations were also developed using age and height as constants. It was found that strong calibrations were generally obtained using

Table 1 Description of field sites.

State	County	Age	Site index	Latitude	Longitude
Delaware	Suffork	23	85	38° 70′	−75° 43′
Maryland	Nash	19	65	38° 43′	−75° 30′
Maryland	Wicomico	26	70	38° 63′	−75° 79′
Maryland	Worchester	25	70	38° 36′	−75° 41′

juvenile wood samples; however, calibrations were poor when mature wood samples of constant age were used.

The objectives of this study were to develop calibrations for arithmetic and length-weighted mean tracheid length using NIR spectra obtained in 10-mm sections from the radial longitudinal surface of *Pinus taeda* L. (loblolly pine) radial wooden strips; and to use the tracheid length calibrations to predict the tracheid lengths of sections of radial wooden strips in a separate test set based on NIR spectra obtained from the radial longitudinal face of each strip.

Materials and methods

Sample origin

Fourteen breast-height discs (approximately 40 mm thick) were collected from *Pinus taeda* L. (loblolly pine) trees growing on four sites of variable age and site index in Maryland and Delaware. A summary of the sites is given in Table 1. All samples were frozen for storage.

While the discs were still frozen, pith to bark radial strips (12.5×12.5 mm) were cut from each disc for NIR and tracheid length analysis. The radial strips were defrosted and dried overnight in an oven set at 50°C to reduce the moisture content of the samples to approximately 7%.

Near infrared spectroscopy

Four 10-mm sections were selected for each radial strip and their positions recorded on the surface of each strip. Sections were selected to be representative of wood close to the pith (juvenile wood), close to bark (mature wood), and of the transition zone between and juvenile and mature wood. NIR diffuse reflectance spectra were obtained from the radial longitudinal face of each 10-mm section using a NIRSystems Inc. Model 5000 scanning spectrophotometer (Silver Spring, MD, USA). Samples were held in a custom-made holder similar to that illustrated in Schimleck et al. (2001) with modifications to hold thicker samples. A 5×10-mm mask was used to ensure that a constant area was tested. Several samples were slightly twisted and a small gap between the spectrometer window and sample was occasionally observed, permitting stray light to interfere with the NIR measurements. To minimize stray light, the samples were tested in a light-proof environment. 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 10-mm section and the results averaged. One average spectrum was obtained per section. All measurements were made in a conditioned atmosphere maintained at 40% RH and 20°C.

Fiber length determination

At the completion of the spectroscopic work, the selected 10-mm sections were cut from each radial strip using a razor blade. Each wood section was placed in a pre-labeled 125-ml Nalgene container. To each container 100 ml of maceration solution consisting of glacial acetic acid and 30% hydrogen peroxide, mixed 1:1, was added. Samples were placed, loosely capped, in a water bath in a fume hood at 60°C for 48 h. After digestion (the wood was bleached completely white and disintegrated easily), fibers were collected by vacuum filtration using a Buchner funnel and qualitative filter paper. Waste maceration solution was collected and fibers were washed with deionized water (~2 l per sample) until the acetic acid odor was removed. Fibers were allowed to dry completely and then collected from the filter paper by peeling them off with forceps. Prior to fiber length analysis using the fiber quality analyzer (FQA), the dried fibers were soaked in deionized water overnight. Just before FQA analysis, the fibers were separated using a manual disintegrator (described in TAPPI method T-271 om-98). A small representative sample was taken from the sample and diluted, in deionized water, to the proper consistency for the FQA. Five drops of formaldehyde was added to each fiber suspension as a preservative. Data provided by the FQA included arithmetic mean tracheid length, length-weighted tracheid mean length, and weight-weighted mean tracheid length. Weight-weighted mean tracheid length was not used in this study.

The mean tracheid length is the arithmetic mean tracheid length with no correction.

$$\text{Arithmetic tracheid length} = \frac{\sum(n_i l_i)}{\sum(n_i)} \quad (1)$$

Length-weighted tracheid mean length is corrected for the natural bias present in most size distributions toward shorter fibers, where there are many more individuals. Length-weighted tracheid length is most commonly reported.

$$\text{Length-weighted tracheid length} = \frac{\sum(n_i l_i^2)}{\sum(n_i l_i)} \quad (2)$$

Where l_i is equal to the fiber length of the ith length class and n_i is equal to the number of fibers in length class i.

Calibration development

A total of 55 diffuse reflectance NIR spectra, measured from the 14 radial strips, were available for NIR analysis. One spectrum was excluded from the analysis owing to the presence of knot-influenced wood; another spectrum could not be collected from this sample owing to its short length. The spectra were split at random into calibration (39 spectra, 10 radial strips) and prediction (16 spectra, 4 radial strips each from a different site) sets. A statistical summary of the calibration and prediction sets is given in Table 2.

The Unscrambler (version 8.0) software package (Camo AS, Norway) was used to develop the tracheid length calibrations. Calibrations were developed using second derivative spectra, left and right gap widths of 4 nm were used for the conversion, limiting the wavelength range available for calibration development to 1108–2492 nm.

Calibrations were developed using partial least squares (PLS) regression with four cross validation segments. The standard error of cross validation (SECV) (determined from the residuals of each cross validation phase), the standard error of calibration

Table 2 Tracheid length range for the calibration and prediction sets.

Wood property	Calibration set (39 spectra)				Prediction set (16 spectra)			
	Minimum	Maximum	Av.	Std. dev.	Minimum	maximum	Av.	Std. dev.
A tracheid length (mm)	1.06	1.99	1.36	0.20	0.85	1.87	1.38	0.28
LW tracheid length (mm)	1.59	3.56	2.42	0.60	1.38	3.57	2.38	0.63

A = arithmetic, LW = length-weighted.

(SEC) (determined from the residuals of the final calibration), and the coefficient of determination (R^2) were used to assess calibration performance.

The standard error of prediction (SEP) was used to give a measure of how well a calibration predicts the parameter of interest for a set of unknown samples that are different from the calibration set. The predictive ability of calibrations was assessed by calculating the ratio of performance to deviation (RPD_p) (ratio of the standard deviation of the reference data to the SEP) (Williams and Sobering 1993). An RPD of greater than 2.5 is considered satisfactory for screening (Williams and Sobering 1993); however, it has been shown that calibrations with an RPD of approximately 1.5 can be useful for initial sample screening (Schimleck et al. 2003).

Results

Variation in NIR spectra

Untreated diffuse reflectance NIR spectra of three *Pinus taeda* samples having a wide range of tracheid lengths (1.61, 2.62, and 3.56 mm) are shown in Figure 1. For comparative purposes NIR spectra from three different sections of the same radial strip are shown.

The NIR spectra show differences in overall wood chemistry and physical properties and represent a gradation between juvenile and mature wood. The juvenile wood spectrum has a lower overall absorbance relative to the mature wood spectrum. The shift in baseline between the NIR spectra of the different wood samples is related to changes in wood density, with higher density wood samples having higher absorbance.

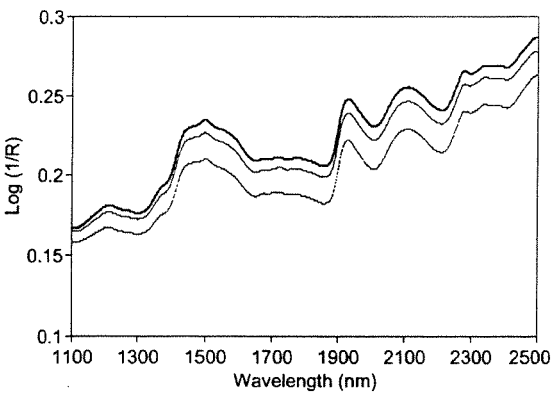

Figure 1 NIR diffuse reflectance spectra of three *Pinus taeda* samples over the range 1100–2500 nm. The dark unbroken line represents a mature wood sample having a length-weighted mean tracheid length of 3.56 mm. The light unbroken line represents a wood sample having a length-weighted mean tracheid length of 2.62 mm. The light broken line represents a juvenile wood sample having a length-weighted mean tracheid length of 1.61 mm.

Figure 2 shows the second derivative diffuse reflectance NIR spectra of the two *P. taeda* samples shown in Figure 1 that have the longest and shortest tracheid lengths. To aid interpretation, the wavelength range has been limited to 1450–2250 nm.

Considerable variation can be observed in the wavelength range shown. The most noticeable variation is in the region around 1500 nm (cellulose, O–H stretch first overtone) (Shenk et al. 1992; Osborne et al. 1993). In the region shown, many bands have been assigned to cellulose while others are aromatic (extractives, lignin) in origin (Bassett et al. 1963; Shenk et al. 1992; Michell and Schimleck 1996). The origin of bands assigned to cellulose and lignin in the region 1450–2250 nm is summarized in Table 3.

Standard linear regression was used to examine the relationship between tracheid length and second derivative absorbance at individual NIR wavelengths for all samples. Wavelengths giving moderate relationships (R^2 = 0.60 to 0.66) for length-weighted mean tracheid length included 1236, 1498, 1774, 1896, and 2146 nm. Relationships were weaker for arithmetic mean tracheid length, with the strongest relationships obtained for 1238 nm (R^2 = 0.33) and 1774 nm (R^2 = 0.36).

Tracheid length PLS calibrations

A total of 39 NIR diffuse reflectance spectra, obtained from the radial longitudinal face of *P. taeda* radial strips, were used for calibration development. Figure 3 shows the calibration plots for arithmetic and length-weighted mean tracheid length. The tracheid length calibrations both gave strong relationships (R^2 = 0.88 and 0.96, respectively). Length-weighted mean tracheid length gave the strongest relationship and also had the lowest

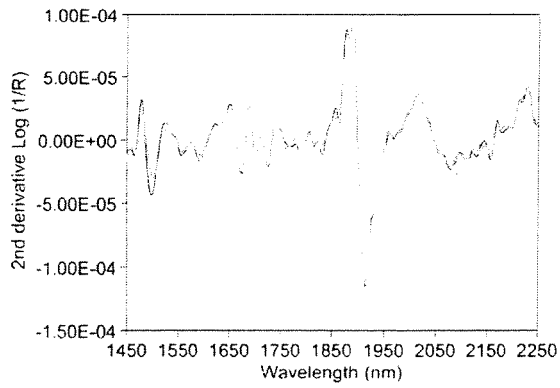

Figure 2 Second derivative NIR diffuse reflectance spectra of three *Pinus taeda* samples over the range 1450–2250 nm. To assist interpretation, only the longest (3.56 mm length-weighted) (thin, dark line) and shortest (1.61 mm length-weighted) (thick, gray line) tracheid length samples are shown.

calibration error. Examination of both plots (Figure 3) shows that the respective tracheid length calibrations fit the data very well, with very few samples having large residuals. The SECV, which is considered to be a better measure of calibration error than the SEC, was considerably larger than the SEC, indicating that the SEC was overly optimistic.

Prediction of tracheid length

The tracheid length calibrations were tested on the prediction set (16 spectra). A prediction R^2 (R_p^2) was calculated as the proportion of variation in the independent prediction set that was explained by the calibration. Relationships between measured values and NIR-predicted values for the two measures of mean tracheid length are shown in Figure 4. The length-weighted mean tracheid length calibration performed well on the prediction set; R_p^2 and SEP were similar to the R^2 and SECV. The R_p^2 (0.58) for arithmetic mean tracheid length was considerably lower than calibration R^2. The RPD_p for length-weighted mean tracheid length (2.52) was sufficiently high to indicate that the length-weighted tracheid length calibration could be successfully used to rank samples.

The relationship between PLS factors and tracheid length

When PLS calibrations are developed, factors are obtained from the NIR spectra that often explain much of the variation in the data set. Generally the first factor explains the greatest variation. For the length-weighted mean tracheid length calibration reported, the first factor explained 67% of variance. Loadings plots of factors that explain a large amount of variance in the reference data can assist in the interpretation of relationships that have made the estimation of a given parameter, by NIR spectroscopy, possible.

Figure 5 shows the first loadings plot for the length-weighted tracheid length calibration. Several wavelengths have strong loadings, indicating that these were important in the development of the length-weighted tracheid length calibration. Many strong loadings corresponded, or were close to, wavelengths that have been assigned to cellulose (broken thin lines). Weaker loadings were observed in regions of the NIR spectrum having aromatic origins (solid grey lines).

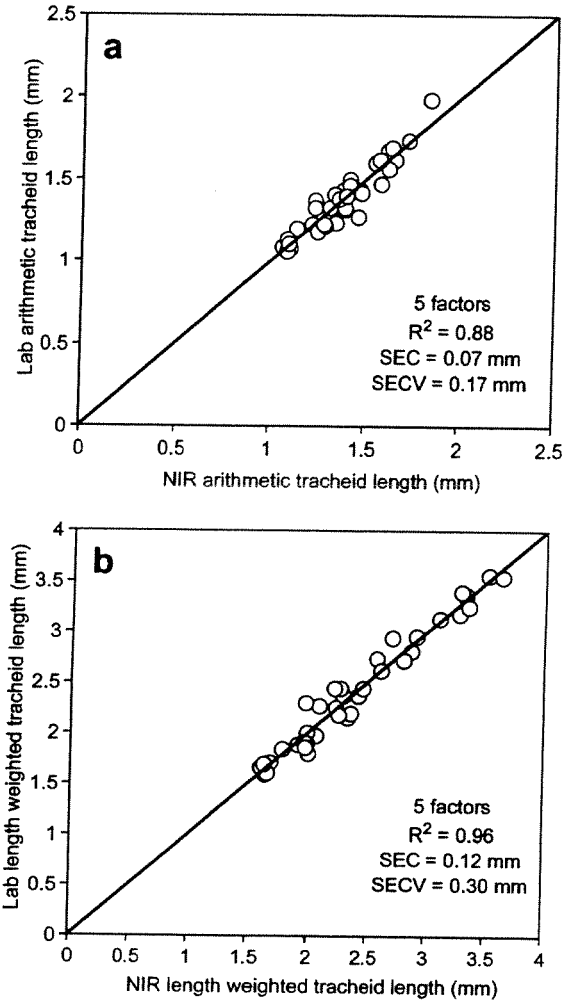

Figure 3 Relationships between measured values and NIR-estimated values for (a) arithmetic mean tracheid length and (b) length-weighted mean tracheid length. Note that the regression line has been plotted in both figures.

Discussion

Calibrations developed for arithmetic and length-weighted mean tracheid length using diffuse reflectance NIR spectra collected in 10-mm sections from the radial longitudinal surface of radial wooden strips demonstrated strong calibration statistics. When applied to a separate

Table 3 Origin of bands assigned to cellulose and lignin in the region 1450–2250 nm.

Wavelength (nm)	Bond vibration	Structure	Reference
1490	O–H str. 1st overtone	Glucose (cellulose)	Osborne et al. 1993; Shenk et al. 1992
1668	Car–H str. 1st overtone	Aromatic	Michell and Schimleck 1996
1685	C–H str. 1st overtone	Aromatic	Osborne et al. 1993; Shenk et al. 1992
1780	C–H str. 1st overtone, C–H str./HOH combn.	Cellulose	Osborne et al. 1993; Shenk et al. 1992
1820	O–H str. + C–O str. 2nd overtone	Cellulose	Osborne et al. 1993; Shenk et al. 1992
1900	O–H str. + 2 x C–O str.	Starch (cellulose)	Osborne et al. 1993
1930	O–H str./HOH combn.	Starch (cellulose)	Shenk et al. 1992
2100	O–H bend/C–O stretch combn.	Starch (cellulose)	Shenk et al. 1992
2132 (2136)	Car–H str. + C=C str.	Aromatic	Michell and Schimleck 1996

Schimleck, L.R., Doran, J.C., Rimbawanto, A. (2003) Near infrared spectroscopy for cost-effective screening of foliar oil characteristics in a *Melaleuca cajuputi* Breeding Population. J. Agric. Food Chem. 51:2433–2437.

Shenk, J. S., Workman, Jr., J.J., Westerhaus, M.O. (1992) Application of NIR spectroscopy to agricultural products. In: Handbook of Near-Infrared Analysis. Eds. Burns, D.A., Ciurczak, E.W. Marcel Dekker, New York. pp. 385–386.

Smook, G.A. – Handbook for pulp and paper technologists. Angus Wilde Publications Inc., Vancouver, 2002. pp. 17.

TAPPI method T-271 om-98. Fiber length of pulp and paper by automated optical analyzer using polarized light.

Via, B.K., Shupe, T.F., Stine, M., Groom, L., So, C. (2004) Using near infrared spectroscopy to predict tracheid length in *Pinus palustris*. Can. J. For. Res. (Submitted)

Williams, P.C., Sobering, D.C. (1993) Comparison of commercial near infrared transmittance and reflectance instruments for the analysis of whole grains and seeds. J. Near Infrared Spectrosc. 1:25–33.

Zobel, B.J., van Buijtenen, J.P. – Wood variation. Springer-Verlag, Berlin, 1989. pp. 18–19.

Received October 27, 2003