ESTIMATION OF TRACHEID MORPHOLOGICAL CHARACTERISTICS OF GREEN *PINUS TAEDA* L. RADIAL STRIPS BY NEAR INFRARED SPECTROSCOPY

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:(Received May 2003)

ABSTRACT

The application of near infrared (NIR) spectroscopy to the green wood of radial samples (simulated increment cores) and the development of calibrations for the prediction of several tracheid morphological characteristics are described. Twenty *Pinus taeda* L. (loblolly pine) radial samples were characterized in terms of coarseness, perimeter, radial and tangential diameter, specific surface, and wall thickness. NIR spectra were obtained in 10-mm steps from the radial-longitudinal and transverse face of each sample and were used to generate calibrations for each property. NIR spectra were collected from all samples when the wood was green (moisture content ranged from approximately 100 to 154%), and when dried to approximately 7% moisture content. The relationships between measured and NIR-estimates for green wood were strong for coarseness, specific surface, and wall thickness, with coefficients of determination (R^2) ranging from 0.89 to 0.73. Differences between calibrations developed using radial-longitudinal and transverse face NIR spectra were generally small. Dry wood calibrations demonstrated strong relationships for all parameters apart from perimeter and radial diameter; R^2 ranged from 0.59 to 0.91. Calibrations were tested on an independent set; relationships for coarseness, specific surface, and wall thickness were strong. Good calibrations can be obtained for some tracheid morphological characteristics using NIR spectra collected from the surface of green *P. taeda* wood.

Keywords: Near infrared spectroscopy, SilviScan, increment cores, *Pinus taeda,* tracheid morphological characteristics.

INTRODUCTION

Recently Schimleck et al. (2003) demonstrated that near infrared (NIR) spectroscopy could be used to estimate the air-dry density, microfibril angle (MFA), and stiffness of 10-mm sections of green *Pinus taeda* L. radial strips. NIR calibrations for each property were developed using data provided by SilviScan-1 (Evans 1994) and SilviScan-2 (Evans 1997, 1999) and

Wood and Fiber Science, 36(4), 2004, pp. 527–535

NIR spectra obtained in 10-mm sections from the radial-longitudinal face and transverse face of pith to bark radial samples cut from breast height discs.

The SilviScan instruments analyze wooden strips cut from increment cores or discs and can also provide information that describes several fundamental tracheid morphological characteristics at high resolution. SilviScan uses image analysis to measure radial and tangential tracheid diameter. Properties that are very difficult to measure, such as wall thickness and coarseness, are estimated from the measured properties using long-established relationships (Evans 1994). The development of calibrations for these properties using NIR spectra collected from air-dried wooden strips used for SilviScan analysis has been investigated (Schimleck and Evans 2004). It was found that calibrations for coarseness and wall thickness were excellent, with coefficients of determination (R^2) of 0.91 and 0.89, respectively. Calibrations for tracheid perimeter and radial and tangential diameter were not as strong, with R^2 ranging from 0.65 to 0.69.

The *P. taeda* samples utilized by Schimleck et al. (2003) were also characterized in terms of their tracheid morphological characteristics. These samples provide the opportunity to investigate if calibrations can be developed for these properties using NIR spectra obtained in 10-mm sections from the radial-longitudinal and transverse face of green *P. taeda* radial samples.

METHODS

Sample origin

P. taeda wood samples examined in this study were obtained from a regeneration trial established by the North Carolina State Forest Nutrition Cooperative in 1979 on a poorly drained site in Williamsburg County, South Carolina (Latitude: 33º 59′, Longitude: –79º 48′). The study received a factorial combination of two levels of each site preparation, fertilization, and herbicide treatment at establishment. The treatments were applied in a split-plot design with the two site preparation treatments as main plots and the fertilizer × herbicide treatments as subplots in four replications (blocks). Nilsson and Allen (2003) provide a complete description of the treatments; the trees utilized in this study were from site 1101. Trees from five different treatments were selected for examination: 1. control (low site preparation), 2. intensive site preparation, 3. intensive site preparation plus fertilization, 4. intensive site preparation plus herbicide application, and 5. intensive site preparation plus fertilization and herbicide application.

From each block-treatment combination, one tree of average size was selected for sampling from the buffer zone surrounding each measurement plot. A total of 20 sample trees were felled, measured for total height, and sectioned. For each tree, ten to twelve wood discs were cut from fixed heights (1.5-m intervals). Only discs obtained at breast height (1.4 m) were utilized in this analysis. All samples were frozen for storage.

Radial samples representing pith to bark variation were cut from each of the breast height discs while they were still frozen. The dimensions of the radial samples were 12.5 mm tangentially and 12.5 mm longitudinally; radial length was determined by the pith-bark length of each sample.

Sample preparation for near infrared spectroscopy and SilviScan analysis

The frozen radial samples were defrosted overnight in sealed plastic bags to minimize moisture loss. When they were defrosted, loose sawdust on the surface of the samples was brushed off and NIR spectra were collected from the green samples. Upon completion of the NIR analysis, the green samples were dried overnight in an oven set at 50ºC. The dried samples were cooled, and NIR spectra were collected from the dried samples.

Moisture content determination

The moisture content of the dried radial samples was measured using an electrical moisture meter and was found to be approximately 7%. Based on this moisture content and the weight of

each dried sample, an oven-dried weight was estimated and then used to determine the sample's green moisture content. Green moisture contents ranged from 100 to 154%. The radial samples were not oven-dried to 0% as it would have damaged the samples for SilviScan analysis.

SilviScan analysis—the measurement of tracheid morphological characteristics

Strips for analysis by SilviScan-1 and -2 were cut from the 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 samples.

Tracheid morphological characteristics were measured in 50-micron steps. The SilviScan-1 image analysis system (Evans 1994) was used to determine radial and tangential tracheid dimensions. Tracheid coarseness, perimeter (external perimeter of rectangular tracheid cross-section), specific surface, and tracheid wall thickness were determined from relationships that have been in use in various forms for several decades (Evans 1994). All measurements were made in a conditioned atmosphere maintained at 40% RH and 20°C.

Wood property averages were determined over 10-mm sections for correlation with the dry wood spectra. To account for the radial shrinkage of the green samples when they were dried, wood property averages, for correlation with the green wood spectra, were determined over 10.5-mm sections. Haygreen and Bowyer (1996) reported that the radial shrinkage of *P. taeda* is 4.8%. SilviScan data were not available for some spectra collected near the pith owing to excessive ring angles.

Near infrared spectroscopy

NIR diffuse reflectance spectra were obtained from the radial-longitudinal face and transverse face of each sample when green and when dried to approximately 7% moisture content. All spectra were obtained using a NIRSystems Inc. Model 5000 scanning spectrophotometer. Samples were held in a custom-made holder similar to that illustrated in Schimleck et al. (2001). The holder was modified to hold larger samples. A 5-mm × 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 were averaged. One average spectrum was obtained per section.

Calibration development

Fifteen cores, three per silvicultural treatment, were selected at random for calibration development. The remaining five cores (one per silvicultural treatment) were used to test the predictive ability of the calibrations. Table 1 gives a statistical summary of each wood property for the green and dry wood calibration and prediction sets.

WinISI II (version 1.50) software package (Infrasoft International, Port Matilda, PA) was used to develop the tracheid morphological characteristic calibrations. For calibration development, the wavelength range was limited to 1108 to 2492 nm. Calibrations were developed using untreated spectra, untreated spectra plus multiplicative scatter correction (MSC), second derivative spectra (gap 4 nm, smooth 4 nm), and second derivative spectra (gap 4 nm, smooth 4 nm) plus MSC. The strongest relationships were consistently obtained using the second derivative treated spectra and only these are reported.

Calibrations were developed using Modified Partial Least Squares (MPLS) regression (Shenk and Westerhaus 1991). Calibrations were developed 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 (SEC) (determined from the residuals of the final calibration), and the coefficient of determination (R^2) were used to assess calibration performance.

TABLE 1. *Range of each parameter for the calibration and prediction sets.*

Wood Property	Calibration set				Prediction set			
	Minimum	Maximum	Av.	Std. dev.	Minimum	Maximum	Av.	Std. dev.
Green samples								
Coarseness (μg/m)	371.3	689.4	518.1	73.0	350.8	676.1	542.8	84.5
Perimeter (μm)	115.8	138.6	125.0	5.1	118.0	140.2	125.8	5.4
Radial diameter (μm)	28.6	36.7	32.8	1.9	29.0	36.0	32.8	1.8
Specific surface (m^2/kg)	199.9	335.6	261.2	28.6	204.1	348.2	252.9	31.2
Tangential diameter (μm)	27.2	34.1	29.7	1.4	26.9	35.5	30.1	1.9
Wall thickness (μm)	2.2	4.7	3.3	0.6	2.1	5.0	3.5	0.7
Dry samples								
Coarseness (μg/m)	373.7	697.2	519.4	72.3	382.6	684.7	549.4	81.3
Perimeter (μm)	114.6	139.2	125.1	5.2	118.0	139.0	126.0	4.9
Radial diameter (μm)	28.8	39.1	32.8	2.0	28.3	36.3	32.9	1.7
Specific surface (m^2/kg)	206.1	331.6	260.6	28.9	197.5	323.3	250.2	27.5
Tangential diameter (μm)	27.3	34.0	29.7	1.4	27.0	35.5	30.1	1.9
Wall thickness (μm)	2.2	4.5	3.4	0.6	2.4	5.1	3.6	0.7

The Standard Error of Prediction (SEP) gives 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 WinISI II software recommended the number of factors to use for each calibration.

RESULTS AND DISCUSSION

Development and application of MPLS calibrations—green wood

Calibrations for each tracheid morphological characteristic were developed using MPLS regression and NIR spectra obtained in 10-mm sections from the radial-longitudinal and transverse faces of green *P. taeda* radial samples. The calibrations were then applied to a separate test set of 32 samples that represented five cores (one per treatment). Table 2 provides summary statistics of each calibration.

Coarseness, specific surface, and wall thickness calibrations gave strong relationships with coefficients of determination (R^2) ranging from 0.73 to 0.89; weaker relationships ($R^2 < 0.5$) were observed for the other properties. Generally, the two sets of NIR spectra provided calibrations that were similar for each property.

TABLE 2. *Summary of calibrations obtained for each* P. taeda *tracheid morphological characteristic using spectra collected from the radial-longitudinal and transverse face of green wood samples.*

Wood Property	Calibration set				Prediction set	
	No. of factors	R^2	SECV	SEC	R_p^2	SEP
Radial-longitudinal spectra						
Coarseness (μg/m)	3	0.82	33.9	30.8	0.70	49.3
Perimeter (μm)	1	0.24	4.5	4.4	0.0	6.0
Radial diameter (μm)	1	0.24	1.7	1.6	0.01	1.9
Specific surface (m^2/kg)	3	0.77	15.3	13.6	0.58	20.6
Tangential diameter (μm)	3	0.49	1.1	1.0	0.34	1.6
Wall thickness (μm)	3	0.87	0.23	0.21	0.79	0.36
Transverse spectra						
Coarseness (μg/m)	3	0.81	36.3	31.5	0.87	40.4
Perimeter (μm)	1	0.27	4.4	4.4	0.01	6.4
Radial diameter (μm)	2	0.36	1.7	1.5	0.0	2.4
Specific surface (m^2/kg)	3	0.73	17.7	14.9	0.78	15.7
Tangential diameter (μm)	3	0.50	1.1	1.1	0.21	1.7
Wall thickness (μm)	4	0.89	0.24	0.20	0.83	0.34

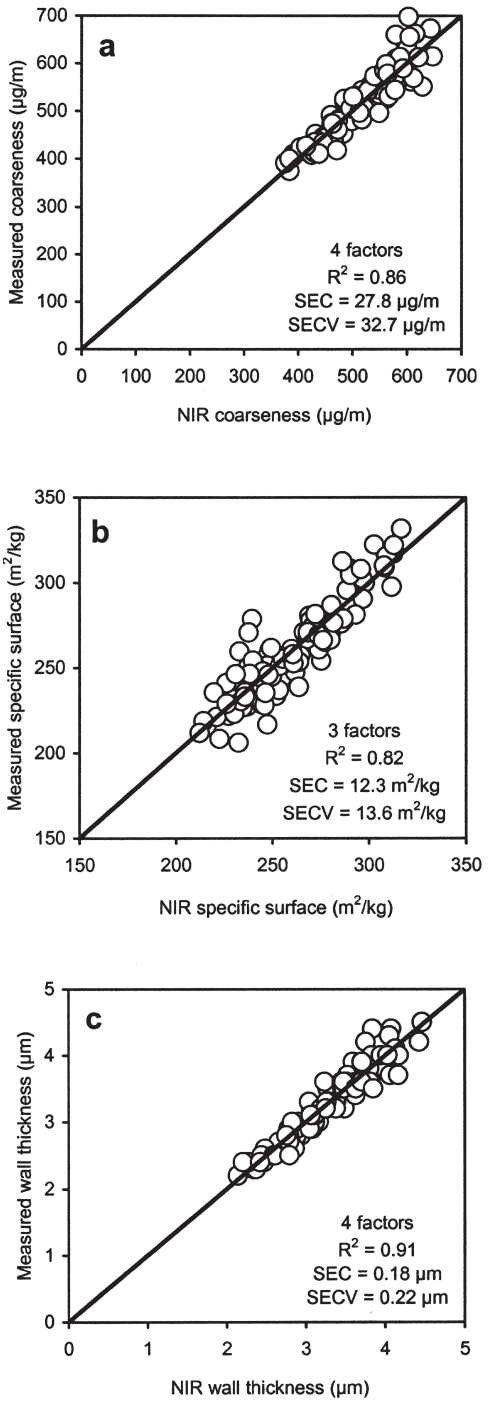


FIG. 1. Relationships between measured values and NIR-estimated values for (a) coarseness, (b) specific surface, and (c) wall thickness. Calibrations were developed using 98 green wood NIR spectra collected from the radial-longitudinal face.

Relationships between measured values and NIR-estimated values for coarseness, specific surface, and wall thickness are shown in Fig. 1 (the results shown are for NIR spectra obtained from the radial-longitudinal face).

The green wood tracheid morphological characteristic calibrations were used on a separate test set of five cores (32 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. The strongest relationships were obtained for NIR-predicted coarseness and wall thickness. For NIR-predicted coarseness, a large difference was observed between NIR spectra collected from the radial-longitudinal and transverse faces, with NIR spectra from the transverse face giving a better relationship. For the transverse spectra NIR-predicted coarseness and specific surface gave stronger R_p^2 than the respective calibrations. Predictions of tracheid perimeter and radial and tangential diameter were poor for both sets of spectra, which could be expected, considering the poor calibration statistics for these properties. Relationships between measured values and NIR-predicted values for coarseness, specific surface, and wall thickness are shown in Fig. 2 (the results shown are for NIR spectra obtained from the transverse face).

Development and application of MPLS calibrations—dry wood (7% moisture content)

NIR spectra obtained from the radial-longitudinal and transverse faces of *P. taeda* samples dried to 7% moisture content were used to develop MPLS regression calibrations for each tracheid morphological characteristic. The calibrations were then applied to the separate test. Summary statistics for each calibration are provided in Table 3.

The dry wood coarseness, specific surface, tangential diameter (radial-longitudinal face only), and wall thickness calibrations all had R^2 greater than 0.8. Perimeter, radial diameter, and tangential diameter calibrations were weaker (R^2 ranged from 0.59 to 0.70). Perimeter, radial diameter, and tangential diameter calibrations were greatly im-

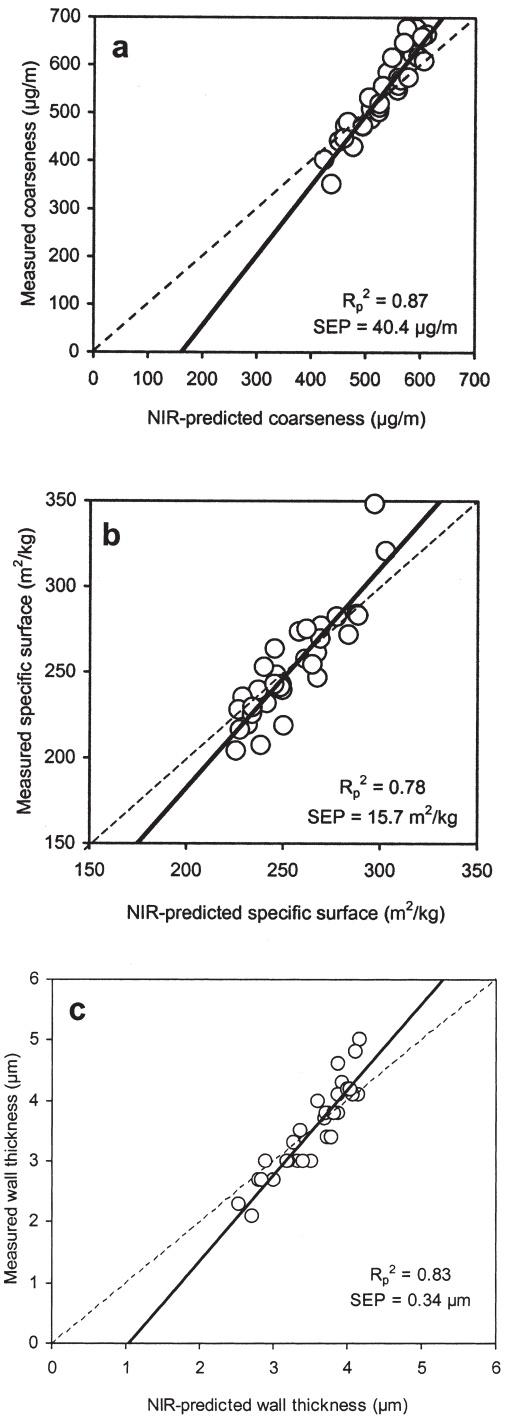


FIG. 2. Relationships between measured values and NIR-predicted values for (a) coarseness, (b) specific surface, and (c) wall thickness. Predictions were made on a set of 32 green wood NIR spectra collected from the transverse face.

proved compared to those reported for green wood spectra. NIR spectra collected from the radial-longitudinal face generally provided stronger relationships than those obtained with NIR spectra collected from the transverse face. Coarseness and wall thickness calibrations obtained using NIR spectra collected from the radial-longitudinal face had superior statistics despite one less factor being recommended. Relationships between measured values and NIR-estimated values for the coarseness, specific surface, and wall thickness are shown in Fig. 3 (the results shown are for NIR spectra obtained from the radial-longitudinal face).

Dry wood calibrations were used on the separate test set of 32 NIR spectra. Predictions of coarseness, specific surface, and wall thickness all gave strong relationships with measured values (R_p^2 ranged from 0.75 to 0.91). NIR spectra collected from the radial-longitudinal face gave the strongest prediction statistics (R_p^2 were very similar to calibration R^2). Predicted tangential diameter was reasonable (R_p^2 = 0.61, SEP = 1.3 µm) when NIR spectra from the radial-longitudinal face were used but poor for NIR spectra from the transverse face. Predictions of perimeter and radial diameter were poor for both sets of NIR spectra, particularly for NIR spectra obtained from the transverse face. Relationships between measured values and NIR-predicted values for coarseness, specific surface and wall thickness are shown in Fig. 4 (the results shown are for NIR spectra obtained from the radial-longitudinal face).

Calibrations based on NIR spectra collected in 10-mm sections from the radial-longitudinal face and transverse face of green *P. taeda* radial strips provided strong relationships between measured and NIR-estimated values for coarseness, specific surface, and wall thickness. The green wood coarseness and wall thickness calibrations were comparable to those obtained for these properties using NIR spectra collected from the same wood samples when dried to approximately 7% moisture content. The perimeter, radial diameter, and tangential diameter calibrations were poor when NIR spectra from green wood were used. Stronger calibration statistics were obtained for these properties using

TABLE 3. *Summary of calibrations obtained for* P. taeda *tracheid morphological characteristic using spectra collected from the radial-longitudinal and transverse face of dry wood samples.*

Wood	Calibration set				Prediction set	
Property	No. of factors	R^2	SECV	SEC	R_p^2	SEP
Radial-longitudinal spectra						
Coarseness (μg/m)	4	0.86	32.7	27.8	0.84	35.7
Perimeter (μm)	5	0.62	4.3	3.2	0.32	4.0
Radial diameter (μm)	6	0.70	1.6	1.1	0.36	1.5
Specific surface (m^2/kg)	3	0.82	13.6	12.3	0.83	11.6
Tangential diameter (μm)	6	0.80	1.0	0.6	0.61	1.3
Wall thickness (μm)	4	0.91	0.22	0.18	0.91	0.22
Transverse spectra						
Coarseness (μg/m)	5	0.85	38.2	28.3	0.75	44.4
Perimeter (μm)	5	0.63	4.4	3.2	0.01	5.5
Radial diameter (μm)	5	0.59	1.7	1.3	0.16	1.7
Specific surface (m^2/kg)	5	0.83	15.7	11.9	0.81	12.1
Tangential diameter (μm)	4	0.73	1.0	0.7	0.16	1.7
Wall thickness (μm)	5	0.90	0.27	0.19	0.86	0.29

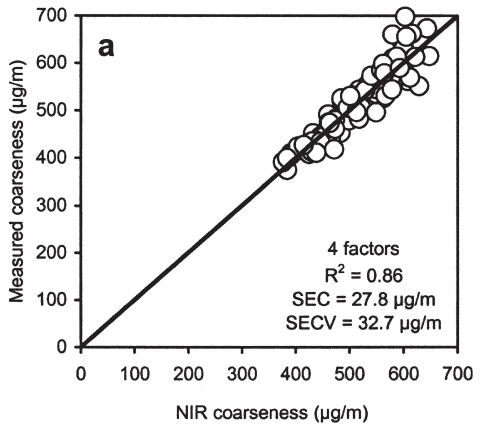
NIR spectra of dried samples, but generally the calibrations failed to perform well in prediction.

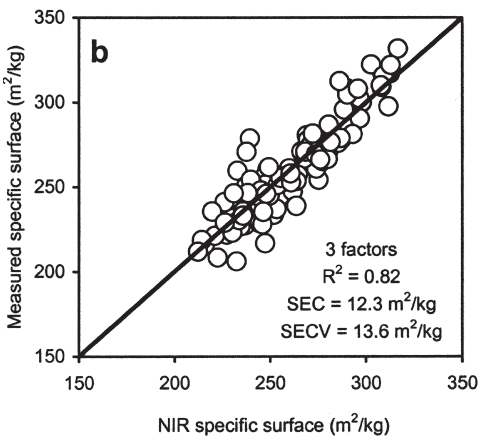
Strong calibrations for coarseness and wall thickness and the weaker relationships for perimeter, radial diameter, and tangential diameter have also been reported for *P. radiata* (Schimleck and Evans 2004), a related species. As noted by Schimleck and Evans (2004), the success of the coarseness and wall thickness calibrations relies on their strong relationships with air-dry density. For the green and dry *P. taeda* calibration samples used in this study, the R^2 for coarseness and air-dry density was 0.76, while it was 0.95 for wall thickness and air-dry density. Specific surface also had a strong relationship with air-dry density ($R^2 = 0.85$). The weaker calibration statistics observed for the remaining morphological characteristics may be attributed to the low resolution (10 mm) of the NIR measurements (Schimleck and Evans 2004). At this resolution, the relationship that exists between radial diameter and air-dry density at fine resolution is weakened. It is possible that improving the resolution of NIR measurements (to 2 mm, for example) will improve calibration statistics for these properties. Experiments are being conducted to investigate the impact of improved resolution on calibration development and will be reported in a later study.

Green wood tracheid morphological characteristic calibrations were similar regardless of which face NIR spectra were collected from. This finding is in agreement with calibrations obtained for air-dry density, MFA, and stiffness using the same sample set (Schimleck et al. 2003). When green wood calibrations were used for prediction, it was found that the calibrations based on NIR spectra collected from the transverse face provided the best results, particularly for coarseness and specific surface.

Dry wood calibrations were stronger when NIR spectra collected from the radial-longitudinal face were used. Schimleck et al. (2003) noted that the transverse surface of the radial samples was much rougher than the radial-longitudinal surface and that it was probable that surface roughness contributed to differences between calibrations. For green wood, the variation in NIR spectra was dominated by water absorption, and variation caused by surface roughness was relatively unimportant.

The green wood samples used in this study for calibration purposes had a moisture content range of approximately 50% (100% to 146%). Sample moisture content was determined for the whole radial sample, not individual 10-mm sections, so it is probable that the actual moisture content range represented in the calibrations was even greater. Despite the wide range of moisture contents, it was shown that calibrations for coarseness, specific surface, and wall thickness





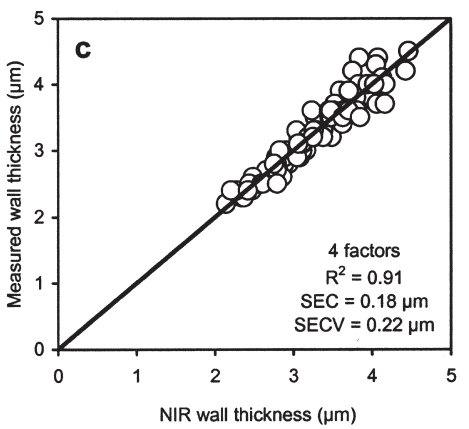
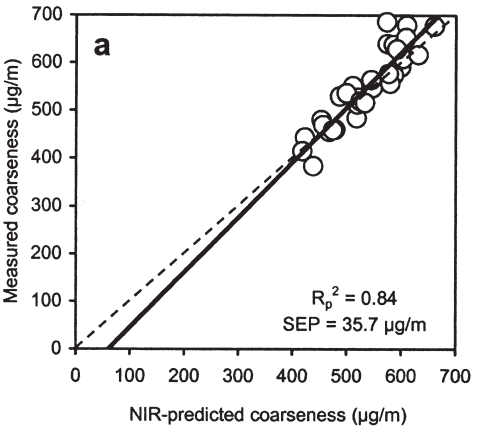
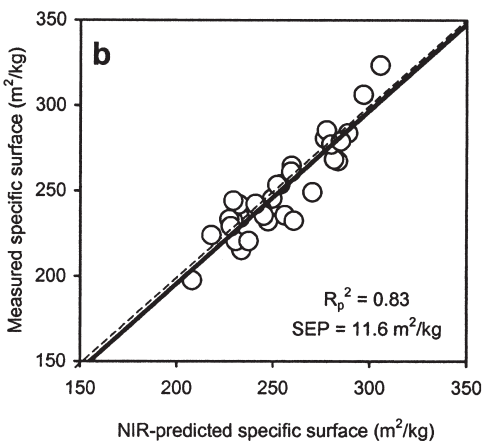


FIG. 3. Relationships between measured values and NIR-estimated values for (a) coarseness, (b) specific surface, and (c) wall thickness. Calibrations were developed using 101 dry wood NIR spectra collected from the radial-longitudinal face.





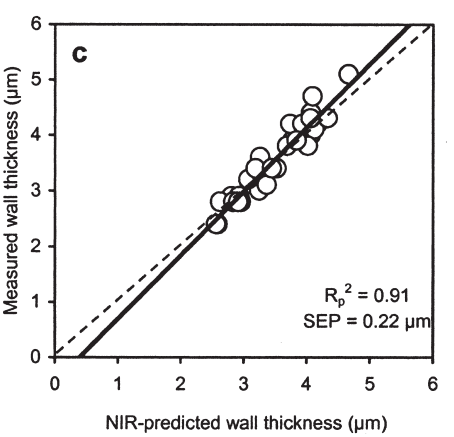


FIG. 4. Relationships between measured values and NIR-predicted values for (a) coarseness, (b) specific surface, and (c) wall thickness. Predictions were made on a set of 32 dry wood NIR spectra collected from the radial-longitudinal face.

were relatively successful when applied to samples in the prediction set that had a similar range of moisture contents (approximately 100 to 154%). These findings and those of an earlier study (Schimleck et al. 2003) demonstrate that it is possible to use calibrated NIR spectroscopy to estimate the wood properties of green radial samples. The ability to analyze green wood provides the opportunity to estimate wood properties in real-time and negates the need to dry samples prior to analysis.

CONCLUSIONS

NIR spectra obtained in 10-mm sections from the radial-longitudinal or transverse face of green *P. taeda* wood radial samples can be used to develop calibrations for coarseness, specific surface, and wall thickness.

NIR spectra obtained in 10-mm sections from the radial-longitudinal or transverse face of the same wood samples when dried to approximately 7% moisture content can be used to develop calibrations for coarseness, perimeter, radial diameter, specific surface, tangential diameter, and wall thickness. Stronger calibration statistics were obtained using NIR spectra collected from the surface of the dried samples.

When NIR spectra were collected from green wood, the face used for analysis (radial-longitudinal or transverse) was not important, but for dry wood, NIR spectra collected from the radial-longitudinal face gave stronger relationships than NIR spectra collected from the transverse face.

ACKNOWLEDGMENTS

The authors thank Mr. Alex Clark for preparing the samples for NIR analysis and Dr. Lee Allen for the provision of samples.

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