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The objective of this research was to (a) determine if blue stain in solid wood influenced calibration equations developed from a non-stained wood population, (b) assess the bias introduced when scanning was performed by the slave instrument without calibration transfer from the master instrument and (c) partition absorbance-based variation by instrument, stain and instrument \times stain interaction. The results helped to determine the calibration transfer needed for this case. The dependent variables assessed from clear and stained wood were lignin, extractives, modulus of elasticity (MOE), modulus of rupture (MOR) and density. When the master instrument was used for both calibration and prediction, it was found that stain-insensitive equations for the five traits could be built. However, when a slave near infrared instrument was introduced without calibration transfer, three out of five predicted traits were significantly biased by the presence of stain. Further analysis revealed an interaction between stain and instrument indicating that instrument bias was also introduced during scanning with a slave. For both multiple linear regression (MLR) and principal components regression (PCR), it was found that if a trait needed more wavelengths (or principal components) for prediction of the dependent variable, bias due to blue stain became increasingly prominent. PCR was found to perform better than MLR when stain was introduced with no calibration transfer. Such a finding alludes that PCR works better than MLR under extrapolation conditions but is not intended to support a lack of calibration transfer. Finally, the Mallows C_p diagnostic proved valuable in model selection although the well-known requirement of ($C_p - p \leq 0$) appeared conservative. For MLR and PCR, a $C_p - p \leq 5$ often yielded applicable models while $C_p - p > 7$ was about the threshold where model performance dropped.

Keywords: lignin, extractives, density, modulus, NIR, blue stain, *Leptographium*, *Ophiostoma*, Mallows, C_p

Introduction

There is currently a substantial interest to advance the internal wood quality through traditional genetic selection, DNA marker aided selection and silviculture prescription. However, to date, sampling and measurement of wood quality traits can either be expensive, time consuming, or both. Often, 1000 to 2000 trees must be measured in a traditional genetics study and further sub-sampling by age can result in 20,000 to 30,000 measurements. Such measurements may not be feasible through traditional laboratory standards but is possible with the use of near infrared (NIR) reflectance spectroscopy as a predictive tool.¹

The primary wood products subject to tree improvement are paper, lumber and engineered composites. For paper

products, density, lignin and extractives are three important variables to influence product yield and strength.^{2–9} Increased extractives and lignin can lower pulp yield while reduced extractives improves the bonding capacity between fibres.^{10,11} Solid wood density improvement will increase pulp yield but, at the same time, decreases most paper strength properties.¹²

For lumber and composite products, modulus of rupture (MOR), elasticity (MOE) and density are three important variables used by designers when choosing construction materials. As a result, manufacturers often try to non-destructively classify their products. For example, in New Zealand wood cants have been scanned and classified for MOE with acceptable error for a manufacturing environment.¹³ The use of absorbance in the NIR region to model MOE and MOR

is possible since absorbance of light by lignin and cellulose associated bonds are specific to particular wavelengths but with considerable overlap (co-linearity) between wavelengths.¹⁴⁻¹⁷

The estimation of mechanical and chemical wood properties from spectroscopic-based models can be biased if some unanticipated variable within the substrate impacts the spectra after calibration is complete. Wood blue stain is one such unanticipated source of variation that commonly occurs in lumber, chips and trees. The two most prominent staining genera are *Leptographium* and *Ophiostoma* the stain deposits of which can be detected from absorbance response between 190 to 800nm which, in turn, would bias calibration equations derived from that wavelength region.¹⁸⁻²³ On the other hand, it is unknown if the presence of stain will affect the predictability of wood mechanical and chemical regression models when wavelengths greater than 800nm are used as independent variables. Perhaps, if stain-associated wavelengths are known, one may remove those wavelengths from calibration modelling to build more robust equations, a method which has proven successful in other applications.²⁴⁻²⁶ Alternatively, if the colour change causes a shift in the full spectra range,²⁷ pre-treatments such as derivatives or multiplicative scatter correction may be useful. But perhaps most important, absorbance response in stained wood may be a function of nitrogen variation, a common component of melanin, which is the origin of the discoloration.²⁸ Identification of such variation and its covariance with spectral response is a reasonable approach to identifying which wavelengths to exclude during the model building process.

The objective of this research was to investigate if the presence of blue stain in the wood substrate would significantly bias predictive models for MOE, MOR, density, lignin and extractives for (a) when a master NIR instrument was used for calibration and scanning and (b) when a

separate instrument was used to acquire absorbance without calibration transfer from the master instrument. Also, it was of interest to measure absorbance-based variation in principal components and then partition such variation by instrument, stain and instrument \times stain interaction. It should be emphasised that this study does not support a lack of calibration transfer. Instead, it demonstrates the magnitude of error that can occur with such ill practice and that error can be inflated as the number of independent variables are increased. Such results have utility, since blue stain may not appear during calibration model development due to a low frequency of beetle infestation during that time period. Principal component regression (PCR) and multiple linear regression (MLR) procedures were chosen for model building and usually have similar predictive ability to partial least squares regression which can be harder to interpret.²⁹⁻³³ Also, MLR was attractive because, hopefully, one can directly avoid blue stain associated variation that would be captured in principal components or partial least squares regression where all wavelengths are embedded in each factor.

Methods and materials

Sample preparation and sample sets

For this study, pith wood was omitted during model building due to the low performance of models for MOE and MOR for pith wood.³⁴ Ten longleaf pine (*Pinus palustris*) trees were harvested at a stand age of 41 years for calibration and validation model development (Table 1, sample set codes NCM and NCVM). The trees were chosen to represent the lower and upper 10th percentile and mean diameter of the population. The site was managed at the Harrison Experimental Forest by the United States Forest Service in

Table 1. Sample set codes for calibration, cross-validation, and experimental data.

Sample set code	Sample size	Natural or inoculated	Population 1, 2 or calibration, validation	Master or slave	Experimental design / statistical procedure
NCM	80 to 250	N	C	M	MLR and PCR
NCVM	80 to 250	N	V	M	Leave-one-out cross-validate
N1S I1S	101 101	N I	1 1	S S	N vs I treatment a <i>t</i> -test
N2S I2S	300 300	N I	2 2	S S	N vs I treatment a <i>t</i> -test
I1M-F I1S-F N1M-F N1S-F	666 373 3328 2318	I I N N	1 1 1 1	M S M S	Unbalanced 2 \times 2 factorial design

Saucier, Mississippi, USA, at a location 30.6° north and 89.1° west. Each tree was bucked every 4.57 m in height yielding five to seven disks. The disks were used to make bending specimens 30.48 cm × random width × 1.27 cm. Spectra were acquired from the radial–longitudinal face on rings 2, 4, 8, 16, 32 and 40 from bark to pith. The strips were approximately 2 mm thick × random length. Rings with embedded knots or splits were removed from the analysis. Ring 2 was only measured if ring 1 was present to avoid pith wood.

Two populations of increment cores were used to test if blue stain variation in the wood substrate resulted in biased model predictions for the five predictive traits. For both populations, the increment cores were drilled perpendicular to the plane of the tree axis with a hydraulic drill and hollow 12 mm drill bit. The samples were taken 1.4 to 1.5 metres from the ground. Both populations of increment core were sub-sampled from the same plantation as the ten longleaf pine trees used for calibration. Also, these samples were collected one year after the ten trees were harvested.

For population 1, 20 increment cores were selected from a library of 1800 increment cores (Table 1, Sample set code N1S and I1S). These increment cores were chosen on the basis that one side had visible stain while the other side was clear of pigmentation. Since blue stain inoculation occurred naturally, population 1 represented a reasonable mixture/distribution of fungi genera to occur after bark beetle infestation within a plantation. The matched samples were next cut in half and represented the control and blue stain treatments. A sample size of 101 for the control and 101 for the blue stained side was obtained since sub-sampling at different ages within an increment core is common for tree improvement analysis.

Population 2 was chosen to corroborate or contradict any result found from testing of models developed in population 1. Table 1 summarises the treatments of population 2 (Sample code N2S and I2S). From population 2, 39 increment cores were artificially inoculated in the laboratory with *Leptographium serpens* (*L. serpens*) and *Ophiostoma minus* (*O. minus*). After sub-sampling within each increment core at ages 2, 4, 6, 8, 9, 12, 16, 32 and 40, a total of 300 data points were available for the control and stained treatment. As with the calibration samples, rings with embedded knots or splits were removed from the analysis. Ring 2 was only measured if ring 1 was present to avoid pith wood. The *L. serpens* was obtained from longleaf pine roots collected from the Palustris Experimental Forest, which is owned and maintained by the USDA Forest Service, Louisiana, USA. The *O. minus* was obtained from southern pine beetles trapped on the Bankhead National Forests, Alabama, USA. The isolates were cultured on malt extract agar [MEA (2% malt extract agar)] and representative stock cultures were maintained on MEA slants at 4°C.

Each isolate was inoculated onto 30 MEA plates and allowed to grow for seven days at 22°C ± 3 (ambient room temperature). An inoculation slurry was created for each isolate. The 30 plates of inoculum were shredded in a

blender with 500 mL dH₂O for 60 s. The cores were then briefly submerged in the inoculum and placed in separately labelled but unsealed plastic bags. At this stage, the humidity and temperature was controlled at 95% ± 2 and 22°C ± 3, respectively. The humidity chamber was a dessicator containing 1000 mL of tap water located below a ceramic plate and stirred by a magnetic stir bar. Two dessicator set-ups were used, one for each fungal treatment. After a week of fungi growth within the wood substrate, each increment core was scrubbed with sterile tissue-wipes to remove excess inoculum and then the core was placed back into the plastic bag. The plastic bag was then placed back into the moisture chamber. Sterile rubber gloves were used to handle the stained specimens and core cleaning was completed in a laminar flow hood under sterile conditions. Fungi growth was allowed to occur for a six-week sum duration and then the specimens were taken out of the plastic bags and allowed to air dry. The surface of the specimens dried within a day, as monitored by touch. The entire increment core took less than a week to dry based on weight scale measurements. The control half of the sample was air dried for two months at ambient room temperature and 60% humidity. The sample reached equilibrium with the environment when gravimetric weight became constant.

Finally, sample sets I1M-F, I1S-F, N1M-F and N1S-F were used in an unbalanced 2 × 2 factorial design where stain, instrument and stain by instrument interaction of absorbance (reduced into principal components) was tested for significance and sample sizes ranged from 373 to 3328 (Table 1). The two levels of stain were inoculated (i.e. stained) and natural (i.e. not stained) wood. The two levels of instrument were master and slave. This sample set was randomly chosen from a library of 1800 increment cores that were drilled at breast height on two plantations of longleaf pine and were from the same plantation as the calibration data set. On these increment cores, rings 3, 4, 5, 7, 9, 12, 15, 22, 30 and 40 were marked on the sample holder and identified as either inoculated or natural.

Mechanical testing

All specimens for calibration building (Table 1, sample set NCM and NCVM) were conditioned to equilibrium moisture content of 8% with a standard deviation of 1.3%. Loading was applied at a rate of 0.20 cm min⁻¹ on the tangential–longitudinal face in a three-point bending set-up. The linear slope of the stress and strain plots was used to determine MOE. MOR, moisture content and air-dry density were determined by standard test methods.^{35,36} Density was taken as the mass per volume of wood. Volumes were measured with calipers to the nearest 0.0025 cm at 8% moisture content. Weights were measured to the nearest 0.001 g at 8% moisture content.

Chemistry determination

Wood disks were ground in a Wiley mill at latewood rings 4, 8, 16, 32 and 41 from the pith and screened to between 40 and 60 mesh particle size. All samples for calibration

building were analysed for extractives and lignin by standard test methods (Table 1, sample set NCM and NCVM).^{37,38} Ground samples were then sealed in a plastic bag to minimise moisture content variation between tests. Three replicates were used and the mean reported for each extractive and lignin measurement.

NIR spectroscopy

A scanning spectrometer (Nexus 670 FT-IR; Thermo Nicolet Instruments, Madison, WI, USA) was used as a master instrument to acquire absorbances between 1000 and 2500 nm for both the calibration and for the experimental samples. Whenever the master was switched from the IR to the NIR region, due to other ongoing laboratory studies, a manufacturer's diagnostic check was run. A spot size of 8 mm was used to scan the samples and a Nexus near infra-red updrift accessory was used. Likewise, a slave instrument was used to acquire absorbance between 1000 and 2500 nm for only the experimental samples with a spot size of 5 mm (Scanning ASD Field Spec, Analytical Spectral Devices Spectrometer, Boulder, CO, USA). A fibre-optic probe was used for the slave instrument. For both instruments, a Spectralon standard (SRT-99-050, Labsphere, North Sutton, New Hampshire, USA) was used as the standard for reference and background spectra checks. The reference check was run every 10 min on the master and every 30 min on the slave. All scans were acquired at 1 nm intervals using reflectance spectroscopy. A single spectrum was acquired as the average of 40 scans from solid wood. Spectra were acquired from the radial-longitudinal face of strips, which were cut on a band saw from the increment cores, for rings 4, 8, 16, 32 and 40 from bark to pith on both the north and south side of the tree and at each disk level. The strips were approximately 2 mm thick \times random length. The origin of all experimental material was from the Harrison Experimental Forest. The north and south spectra were averaged together before model building to obtain one spectrum per sample. The north and south spectra were collected from the north and south ends of the increment core, respectively. A total of 250 samples were available for model building and cross-validation by a statistical software package.³⁹ The absorbance spectra were transferred from the spectrometer to an intermediate software package and then to SAS. The intermediate software package⁴⁰ was used to transform the original spectra to the first derivative of the spectra before exporting the data into SAS and was used to average the spectrum into 10 nm intervals.⁴⁰ The reduction to 10 nm intervals has no detrimental influence on model precision of wood properties.⁴¹

Multivariate analysis

Multiple linear regression (MLR) and principal component regression (PCR) were performed in the SAS software using PROC REG and PROC PRINCOMP macros.³⁹ Statistical diagnostic tools were applied to determine the predictive power and number of variables needed for each model. A

standard cross-validation (leave-one-out) was performed in UnScrambler to determine the root mean square error of calibration and validation (*RMSEC* and *RMSEV*).⁴⁰ The *RMSEC* from the UnScrambler software was compared with SAS *RMSEC* to ensure that the same performance was computed by both software packages since the standard cross-validation was performed in UnScrambler but model development was derived from SAS. In Table 2, the coefficient of variation (*COV%*) was computed for normal distributions (*RMSEC* or *RMSEV*/mean) while left absent for non-normal distributions due to inflated *COV*'s that occur when the distribution was skewed right resulting in the mean being lower than the median. The R^2 and adjusted R^2 were used to determine both the amount of variance accounted for by the model and the threshold where any additional variable added may result in overfit. An overfit was suspected if the adjusted R^2 was considerably lower than the standard R^2 . Additionally, the Mallows C_p was computed in conjunction with adjusted R^2 to aid in model selection. It is noteworthy to mention that the Mallows C_p is often not available in today's NIR software packages, but is a widely used tool in statistical modelling with multiple regression and has been used by the authors in another NIR study where it performed competitively to more traditional NIR model selection tools.^{34,42}

The lowest C_p tends to yield models of maximum predictive power with minimal factors.⁴² The equation is

$$C_p = \frac{SSE_p}{MSE(X_1, \dots, X_{p-1})} - (n - 2p)$$

where *SSE* is the sum of square error for the fitted subset with p parameters, *MSE* is the mean square error of the model and X is the independent variable (wavelengths or principal components). The C_p procedure is useful for identifying the sub-model with the lowest possible number of independent variables (p) and where the target C_p is less than p so that $C_p - p \leq 0$. However, for some traits, C_p was never lower than p for all possible sub-models and, thus, the lowest C_p was reported and checked through cross-validation. Wavelengths for MLR were predetermined by the most significant regression coefficients as deemed important by principal components regression loading analysis.^{43,44} For PCR, all wavelengths were reduced to principal components and then regressed by MLR in SAS using the C_p and R^2 statistic for final model determination. Likewise, the MLR procedure was chosen to see if individual wavelengths that were not influenced by stain might be used to avoid the influence of stain during predictive modelling. All independent variables were left in the model at p -values less than 0.15, a default of the SAS software package. The first derivative was also applied to the original spectra in conjunction with PCR and MLR to determine if bias due to stain was present when modelling from the original spectra. The area under the curve was not used to determine density since blue stain variation may confound the raw spectra absorbance.^{27,34}

Results and discussion

Blue stain classification

Discrimination of stained wood samples would be useful for plant pathologists interested in predicting the concentration of beetle infestation for a given plantation. In the southern United States, bark beetles can infest loblolly and longleaf pine plantations, sometimes resulting in tree mortality. One way to achieve accurate discrimination is to use absorbance at NIR wavelengths as a classifier. Since melanin in wood is the origin of stain and possesses supplementary nitrogen, it may be possible to yield good discrimination from absorbance at NIR wavelengths.

Principal components analysis is a common technique used to visually detect cluster formation. When PC1 and PC2 were cross-plotted, the potential to classify stain and unstained wood is apparent (Figure 1). The segregation of blue stain and control samples occurred along the axis of the 2nd principal component (PC), which accounted for 9% of the total variation. There was no clustering of treatments along the 1st PC axis. Clustering along the 2nd PC axis, without any discrimination along the 1st PC axis, was initially unexpected since the 1st PC accounted for 89% of the absorbance variation. However, prior work found density to primarily influence the 1st PC.³⁴ For this particular species, the density change from earlywood to latewood was extreme and often doubled in magnitude. Such a wide range in density in our study presumably dominated the matrix of absorbance values, which was embedded in the 1st PC. A different response might occur for species with less density variation and should be considered in future studies.

Eighty-eight percent of the blue stained specimens fell above zero for the 2nd PC axis while 92% of the control specimens fell below zero. A third PC was plotted against both PC1 and PC2 but no significant segregation occurred in that dimension.

The 2nd PC accounted for the blue stain variation in both the raw spectra and after the 1st derivative was applied. Applying the 2nd derivative to the raw spectra removed any segregation of treatments along the 2nd PC.

Model building

The classification of blue stain was an important 1st step because it was hypothesised that the removal of the blue stain associated wavelength before PC computation may result in more robust equations. An effort was made to this effect but, as will be discussed in further detail, was often unsuccessful due to unacceptable *RMSEC*. As a result, MLR quickly became an advantageous alternative to avoid blue stain associated wavelengths during modelling.

Table 2 demonstrates the predictive ability of PCR and MLR models for the five traits. All traits in Table 2 exhibited an $R^2 > 0.7$ except for lignin where the R^2 ranged between 0.44 and 0.55 for MLR and PCR regression, respectively. When partial least squares (PLS) regression was considered for lignin (not reported), similar R^2 values were obtained to Kelley *et al.*⁴⁵ The other four traits in this study seemed analogous to Kelley *et al.*⁴⁵ in R^2 response supporting that MLR and PCR are often an equal performing alternative to PLS.^{29–33} For lignin content, better correlations have been achieved with transmission NIR or grinding samples prior to scanning.^{2,4,17,46,47} Grinding the samples probably averages

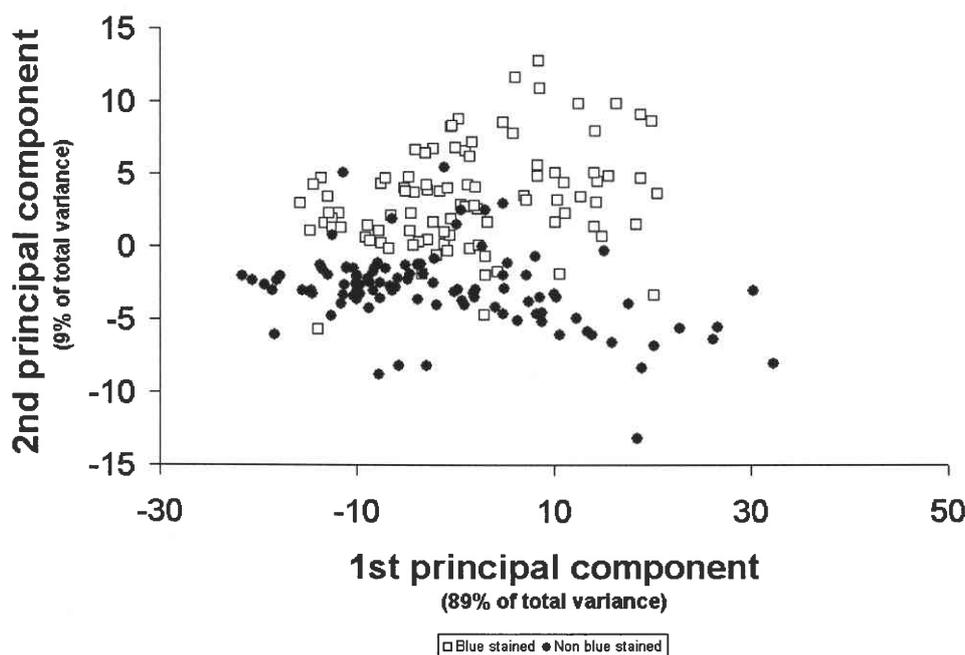


Figure 1. The segregation of blue stain and control samples when the 1st and 2nd principal components were plotted. Principal components were developed from the raw spectra between 1000 to 2500 nm. Sample set N1S and I1S (Table 1).

Table 2. PCR and MLR calibration and cross-validation correlations for lignin, extractives, modulus of elasticity, modulus of rupture and density.

Dependent variable	Model	Independent variables (p)	$N=$	R^2	Adj. R^2	$RMSEC$ (COV%)	$RMSEV$ (COV%)	C_p	C_p-p
Density	PCR	5 PCs	250	0.71	0.69	0.044 (7.3)	0.050 (8.3)	3.4	-1.6
	MLR	1495, 1885 nm	250	0.72	0.69	0.054 (9.0)	0.054 (9.0)	5.1	3.1
MOE	PCR	6 PCs	250	0.86	0.85	2048 ^b	2392	12.1	6.1
	PCR ^a	4 PCs	250	0.87	0.86	3043	3274	5.0	1.0
	MLR	1395, 1695, 1925, 1995 nm	250	0.80	0.78	2797	2861	3.6	-0.4
MOR	PCR	6 PCs	250	0.87	0.86	16.4 ^b	19.0	7.6	1.6
	PCR ^a	5 PCs	250	0.88	0.87	19.2	20.2	5.4	0.4
	MLR	1395, 1695, 1925, 1995 nm	250	0.83	0.80	21.2	21.7	3.7	-0.3
Extractives	PCR	4 PCs	107	0.72	0.70	3.06 ^b	3.48	9.7	5.7
	PCR ^a	5 PCs	107	0.64	0.61	3.30	3.04	8.0	3.0
	MLR	1405, 1685, 2185 nm	107	0.69	0.68	3.11	3.27	7.8	4.8
Lignin	PCR	4 PCs	80	0.55	0.50	1.83 (6.1)	1.99 (6.6)	3.8	-0.2
	MLR	1935, 2265 nm	80	0.44	0.40	2.01 (6.7)	2.07 (6.9)	9.5	7.5

^aA first derivative pretreatment was applied to the spectra

^bCoefficient of variations (COV) was not included for extractives, MOR, or MOE due to a non-normal distribution which resulted in inflated COVs

out any systematic variation present in solid wood. For example, when predicting extractives, one study found that the R^2 improved from 0.75 to 0.95 after grinding the solid wood samples.³ Additionally, removing extractives before scanning can improve the predictive ability of lignin, since lignin and extractives can share analogous absorption bands and, thus, may help explain our lower R^2 values for solid wood lignin.²

It was found that blue stain did not have a significant influence on the prediction of the five traits compared with predictions made from scans of clear wood. The statistical analysis is shown in Table 3 under the master column of population 1 with n.s. indicating a lack of statistical significance. As such, it was concluded that one could easily build predictive models for wood chemistry and mechanical properties by picking wavelengths not sensitive to the stain. Such

a possibility is very useful, since the presence of stain may add to the absorbance variation at some wavelengths, which in turn might bias predictions.

Next, calibration equations that were built from the master instrument were applied to the slave instrument without any calibration transfer. For some traits, the lack of calibration transfer to the slave instrument did not appear to bias predictions while for other traits gross errors occurred. While the authors do not advocate a lack of calibration transfer, it was interesting and useful to demonstrate the differing magnitudes of bias that occur depending on the trait of interest, especially since experience has shown blue stained wood often has not been included during the calibration model building stage. Furthermore, the bias was found to increase in variation and magnitude as the number of independent variables needed to predict the trait increased.

Table 3. A comparison of variance and mean difference in five traits for stain and control treatment within test population 1 and test population 2 using the same MLR model. Significant difference ($\alpha=0.05$) denoted by * while non-significant difference denoted by n.s. The symbol >, <, = denotes significantly greater, lesser, or no significant difference.

Trait	(Stain–Control) difference between means			Variance	
	Pop. 1		Pop. 2	Pop. 1	Pop. 2
	Slave	Master	Slave	Slave	Slave
Density (g cm ⁻³)	-0.03 n.s.	+0.01 n.s.	-0.05 n.s.	Stain = Control	Stain = Control
MOE (MPa)	+7166 *	+173 n.s.	+6501 *	Stain = Control	Stain = Control
MOR (MPa)	+5909 *	-0.1 n.s.	+5500 *	Stain > Control	Stain > Control
Extractives (%)	-11.3 *	+0.78 n.s.	-9.2 *	Stain < Control	Stain < Control
Lignin	+0.1 n.s.	+0.19 n.s.	+0.7 n.s.	Stain = Control	Stain = Control

During calibration modelling, it was determined by use of the C_p statistic that the number of wavelengths (or factors) needed to predict lignin was less than the number required for MOE, MOR or extractives. The number of variables needed to model density was less certain, since MLR model selection required two wavelengths while PCR model selection required five factors (Table 2). Excluding density, the more factors needed to predict a trait might imply increased complexity in the chemical structures of the polymer matrix. For example, a higher number of factors (or independent variables) needed to predict MOE and MOR was expected since density, microfibril angle, cellulose and lignin can all influence these two traits.^{12–14,16}

To determine the optimal number of factors, C_p should be equal to or less than the number of factors (p) or ($C_p - p \leq 0$).⁴² Compliance to this rule did not always occur even though the best model was chosen (Table 2). Yet these models in Table 2 still performed well during cross-validation, despite violating the rule of ($C_p - p \leq 0$). Perhaps a lack of compliance to this rule was due to the inclusion of all wavelengths during model selection. According to the literature, only important wavelengths determined *a priori* should be included in order for ($C_p - p \leq 0$) to apply.⁴² Nevertheless, for this study, performance of the sub-models in *RMSEP* were fairly stable when compared with *RMSEC* even though sometimes C_p was slightly greater than p (Table 2). This stability diminished around a $C_p - p \leq 5$ to 7 and, thus, a $C_p - p \leq 5$ seems to be an acceptable rule of thumb for model selection when absorbance at all possible wavelengths are investigated during model building. The exception to our finding was the model for lignin where the $C_p = 9.5$ while $p = 2$. As such, the finding that ($C_p - p \leq 5$) is not a hard and fast rule but perhaps a tool useful for model building in spectroscopic applications when many wavelengths must be investigated as opposed to only those suspected of influencing the dependent variable. Such a finding is important since C_p is not currently a widely used tool in the NIR field and most of the time all available wavelengths are investigated during the calibration stage.

Past research has found PCR to be more stable than MLR when slight extrapolations occur.³⁰ For our calibration samples, only ten trees were used to build these models, since the harvest of more trees was not possible. Thus, extrapolation in test increment cores for future studies is possible. Nevertheless, since within tree variation was more than between tree variation for all mechanical and chemical properties, a wide range in chemistry composition was achieved, as recommended.⁴⁴ When possible, a sampling of more trees is a worthy consideration but was not needed to meet the objectives of this study.

Influence of stain on test population 1 (observational experiment): no calibration transfer

The following experiment was performed to assess the influence of stain on absorbance across a range of NIR wavelengths when the origin of stain is unknown. In the field, various stain types may exist. In the laboratory, microscopy work could not determine the origin of the stain for population 1. It was hypothesised that stains of unknown origin can occur in the field and might differ from a controlled experiment in the lab. As a result, both scenarios were tested. Population 1 will be discussed first.

Density is perhaps the most important wood quality trait measured in tree improvement. When predicting density under stained conditions, PCR appeared more robust than MLR as indicated by the density histograms in Figure 2(a) and 2(b). The variation of predicted density for the blue stained wood was slightly larger than the variation in predicted density for the control wood. An under estimation of density occurred for specimens in the low-density region [Figure 2(a)]. Conversely, an *F*-test statistic for variance found no difference at the $\alpha=0.05$ level. When MLR was used [Figure 2(b)], the median predicted density was similar for both stain and control wood while the mean density for the control wood was significantly higher.

Of practical importance is the NIR spot size, which usually was greater than the width of the ring being scanned. For rings 30 through 40, sometimes as much as five rings

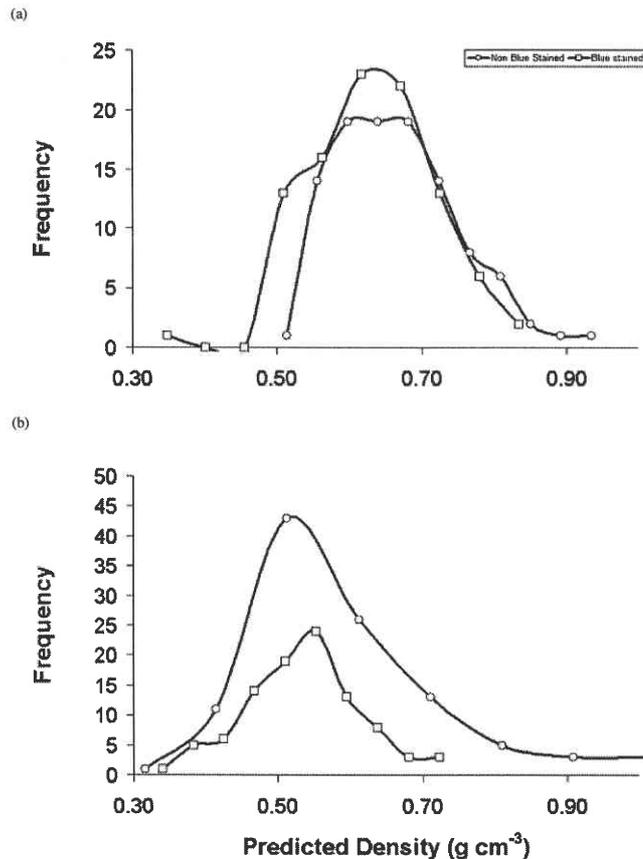


Figure 2. The prediction of density for stained ($n=101$) and unstained ($n=101$) wood using (a) PCR and (b) MLR. Sample set N1S and I1S (Table 1).

fell within the 5 mm diameter of the NIR light beam. To minimise this error, the probe was placed over the centre of each ring but earlywood would still overlap on either side and explains the wide variation in density prediction. Had the spot size been smaller than the latewood ring width for every ring, then a very narrow distribution of high-density predictions would have been expected since the variation in density within latewood is very narrow and high.⁴⁸

The influence of stain on MOE prediction was significant, regardless of which model was used (Figure 3). While histograms for MOR was not shown, MOR exhibited a similar response to MOE for all models. Even when the 1st and 2nd derivatives were applied to the spectra, a significant influence of blue stain on MOE and MOR prediction was still present. For MOE, the blue stained wood was overpredicted by 13,000 MPa (Figure 3). When the first derivative was applied and the best PCR model was determined, the mean MOE of the blue stained wood was under predicted by 10,000 MPa. Likewise, when predicting MOR, differences in mean between 6,000 to 17,000 MPa occurred, depending on model and derivative pre-treatment. As such, there was no way to avoid the systematic error introduced by blue stain when predicting MOE and MOR. MLR yielded worst models than PCR when predicting MOE and MOR under

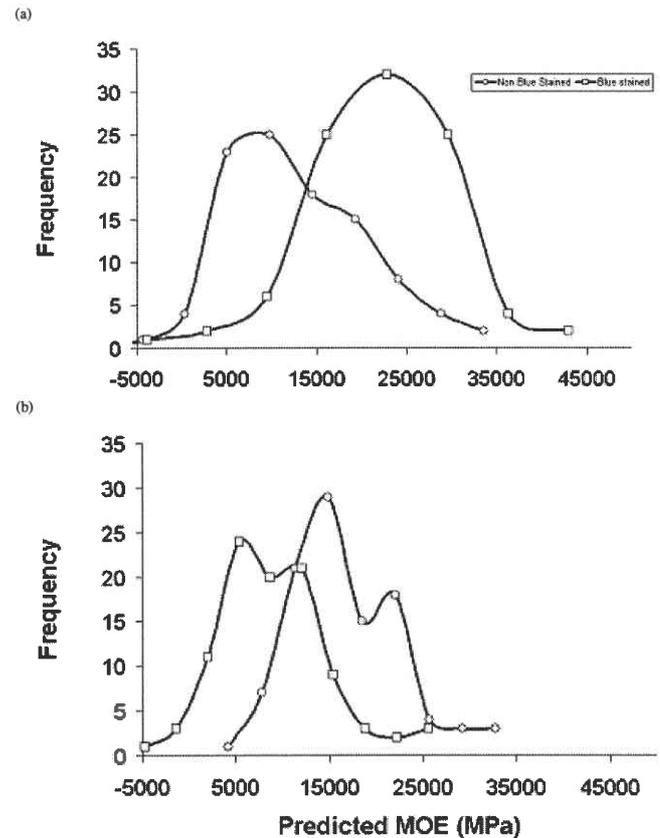


Figure 3. The prediction of MOE for stained ($n=101$) and unstained ($n=101$) wood using (a) PCR and (b) PCR from 1st derivative spectra. Sample set N1S and I1S (Table 1).

this extrapolation condition. These poor results from MLR modelling were unexpected since it was hypothesized that stain bias may be avoided by selecting specific wavelengths uninfluenced by stain.

The MLR model was no more accurate when predicting extractives content. When MLR was applied to the stained population, over 30% of the predicted extractive values was less than 0% [Figure 4(a)]. PCR modelling performed no better than MLR and, thus, a first derivative to the spectra was applied [Figure 4(b)]. Using PCR on the first derivative of the spectra still exhibited unacceptable error but was an improvement over modelling from the original spectra [Figure 4(b)]. Additionally, PCR modelling of the 1st derivative spectra removed the obvious over-prediction of extractives in Figure 4(a), which reached as high as 80%. While within the calibration set, the highest extractives content reached a maximum of 30% through wet lab chemistry. Like the mechanical properties, using PCR on the first derivative of the spectra to predict extractives content resulted in similar histogram shapes between the two treatments. Also, the extractives content was similar to the mechanical properties in that 5+ factors were commonly recommended for prediction, as determined by the C_p diagnostic.

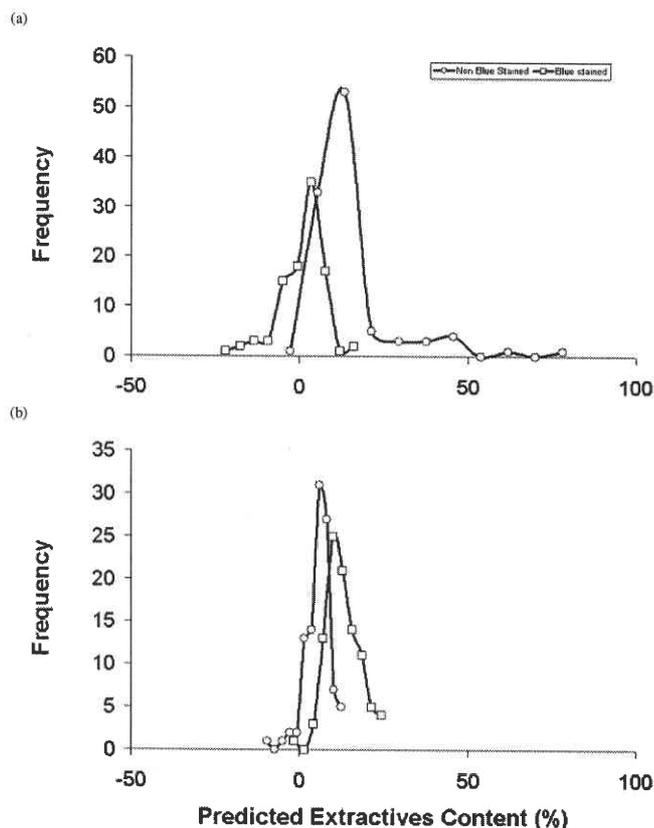


Figure 4. The prediction of extractives for stained ($n=101$) and unstained ($n=101$) wood using (a) MLR and (b) PCR from 1st derivative spectra. Sample set N1S and I1S (Table 1).

When predicting lignin content, MLR worked adequately and avoided the influence of stain with a similar mean and distribution [Figure 5(a)]. In this case, two to four independent variables were recommended by C_p , depending on the model of choice (Table 2). Perhaps the fewer number of factors needed to predict lignin made it more likely to find models not biased by the presence of stain. In contrast to other traits, MLR outperformed PCR as determined by similar distribution properties but the MLR model still yielded an offset of five percentage points when applied to the blue stained treatment [Figure 5(b)]. Also, for the PCR model, the range of the predicted lignin fell outside the range of the calibration data.

In summary, an increased number of factors yielded increased bias when stained wood was scanned and calibration transfer was not applied. For a good review of proper calibration transfer options that must be followed, the reader is referred to Reference 49 and will be discussed further in the section entitled “Instrument, stain and instrument by stain response”. With the exception of lignin content, PCR performed better than MLR in prediction of wood quality traits.

Verification of population 1 using population 2 (controlled experiment): no calibration transfer

The blue stained samples discussed so far were not control inoculated in a lab meaning that the origin and type

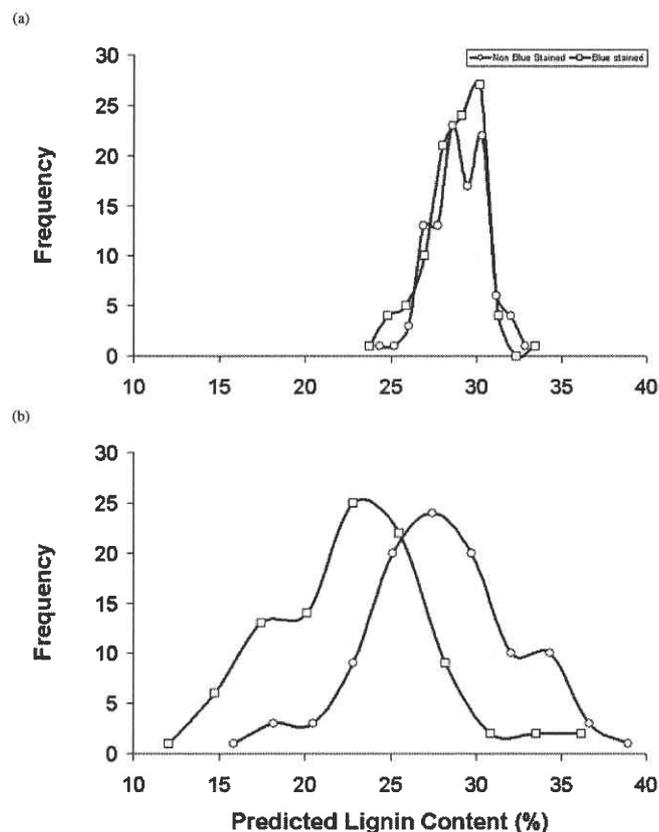


Figure 5. The prediction of lignin for stained ($n=101$) and unstained ($n=101$) wood using (a) MLR and (b) PCR. Sample set N1S and I1S (Table 1).

of stain was unknown. As such, variation in stain intensity was great suggesting that absorbance response could vary considerably within a blue stain treatment. Therefore, population 2, where the fungi growth and origin was controlled, was tested to verify the results found in population 1 (Table 1, sample set N2S and I2S). In population 2, the blue stain was naturally inoculated into the wood substrate in a controlled environment.

In Table 3, the results were strikingly similar between population 1 versus population 2. All five traits yielded similar means, variance patterns and histogram shapes. As expected, population 2 (laboratory inoculated) sometimes exhibited slightly lower variation in predictive response due to the fairly uniform stain. However, since laboratory induced stain (from known fungi genera) yielded similar results to naturally stained cores, it was concluded that predictive models were robust.

Instrument, stain and instrument by stain response

The standard measurement of lignin, MOE, MOR, extractives and density all require fairly large sample volumes. However, the rings within the increment core were, on average, 24 mm³ in volume. As a result, there was not enough material available to run traditional prediction set samples where the *RMSEV* could be computed for each trait

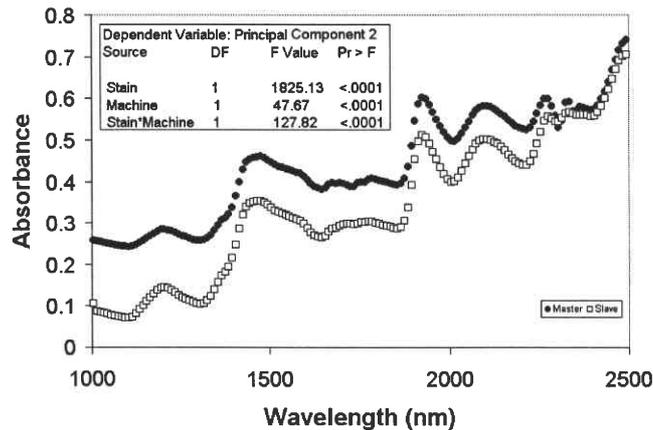


Figure 6. A plot of wavelength versus absorbance for the master and slave along with significance testing of stain, instrument, and stain by instrument along the 2nd PC.

under each instrument and staining treatment. Conversely, the absorbance at chosen wavelengths, or a PC response, can be estimated under a factorial design for each level of instrument and stain.

Figure 6 demonstrates the difference in absorbance for the master and slave instruments when an unbalanced factorial design of experiments was used. Through most of the wavelength range, the master instrument exhibited higher mean absorbance than the slave. To complicate matters, there appeared to be interaction effect between wavelength and instrument at 1394 to 1424 nm and 2284 to 2500 nm. Similarly, there was a instrument by stain interaction when the 2nd PC was used as the response variable. PC2 was chosen since blue stain significantly segregated along the 2nd PC (Figure 1).

An interaction effect between stain and instrument is an important consideration during calibration transfer. Adjustments for all levels of instrument and stain should be included during an interaction. As a result, differences between mean absorbance at each wavelength were applied.⁴⁹ However, for all five traits, this calibration transfer attempt was mostly unsuccessful due to non-homogeneous variance between treatment groups. Figure 7(a) demonstrates such non-homogenous variance for both stained and unstained wood. Furthermore, when PC2 was analysed by factorial design, the standard deviations for each group were statistically different, as determined by the *F*-Test. Unstained samples scanned by the master NIR instrument exhibited a standard deviation in PC2 response nearly twice as high as when unstained samples were scanned with the slave [Figure 7(a)]. Such a difference in absorbance and PC variance between instrument and stained treatments resulted in misleading calibration transfer and, consequently, significant errors.

Successful calibration transfer was assumed when stained and unstained histograms fell on top of one another for all five traits (as attempted in Figures 2–5 but with no calibration transfer). Histograms statistically equal in variance and

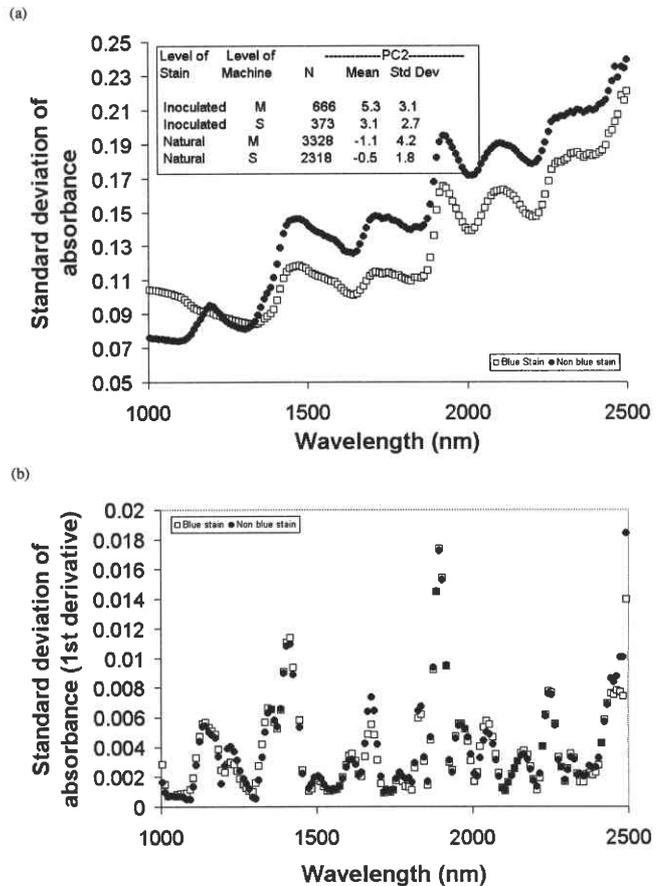


Figure 7. (a) The response in standard deviation of absorbance and PC2 for all levels of stain, instrument, and wavelength. (b) The response in standard deviation of the 1st derivative of absorbance for natural and inoculated wood for all wavelengths.

mean (not shown) were achieved for all five traits when the 1st derivative was applied before computing adjustment factors at each wavelength, as per Fearn's reported method.⁴⁹ The 1st derivative was a successful pre-treatment to the data before adjustment factor computation because of the homogenous variance that now occurred across most wavelengths for both stain and instrument treatments. Figure 7(b) demonstrates the homogenous variance in 1st derivative absorbance between stain and unstained wood for most wavelengths. Such homogenous variance across wavelengths was crucial for successful transfer of calibration equations for both PCR and MLR.

Conclusions

The introduction of blue stain did not influence prediction models for any of the five traits when both calibration and scanning were performed on the same NIR instrument. Such a finding supports the hypothesis that during model building one can pick wavelengths where absorbance is not influenced by stain. Alternatively, PCR models requiring four or five factors were insensitive to stain despite segregation of

stained wood along the 2nd PC, a factor that was required in all PCR models.

The introduction of blue stain had mixed results on predictive models when a slave instrument was used for scanning but calibration equations were not transferred from the master instrument. With the exception of lignin, PCR performance was superior to MLR, especially as the number of factors increased. PCR usually exhibited similar distribution properties with only an offset in mean while MLR more often yielded completely different distribution properties.

The performance of the Mallows' C_p diagnostic to guide which and how many variables were needed for modelling was quite useful as verified through *RMSEP*. However, the traditional rule of ($C_p - p \leq 0$) appeared quite conservative when picking models and was probably attributable to the fact that all wavelengths were investigated, even those that are not expected to play a role prior to the study.⁴² For this study, a ($C_p - p \leq 5$) seemed qualitatively to be the transition point between stable and unstable models while $C_p - p > 7$ almost always yielded inappropriate models under cross validation. Such an alternative rule of thumb is useful since, prior to calibration building, it is often unknown which wavelengths should be used in regression analysis.

Finally, the predicted mean and variance response of regression models to naturally inoculated stain was statistically equal to the predicted mean and variance response of regression models tested on a laboratory inoculated stain population. Such a finding was important because it supported the view that the models were similar regardless of stain origin. However, it should be noted that *RMSEV* were not available for the test populations since the small amount of material available in increment cores were much less than the volume of wood required by standard ASTM or TAPPI methods.^{35–38} Future work to determine the errors associated with predictions would compliment this study well.

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