THE RESPONSE OF VISIBLE/NEAR INFRARED ABSORBANCE TO WOOD-STAINING FUNGI

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(Received May 2005)

ABSTRACT

The influence of blue-stain fungi [*Ophiostoma minus* (Hedgcock) H. and P. Sydow and *Leptographium serpens* (Goid.) Siemaszko] on absorbance at the visible and near infrared wavelengths was investigated. Forty trees were sampled at breast height from longleaf pine (*Pinus palustris* Mill.). One half of each increment core was inoculated with one of two fungi treatments while the other half served as a control.

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Wood and Fiber Science, 38(4), 2006, pp. 717–726

Visible and near infrared spectra were acquired between rings 3–40 for the stained and control-clear wood samples ($n = 304$). Absorbance was greater for the stained than the control wood at wavelengths between 464 to 1334 nm. Statistical techniques were applied to the NIR data to determine which wavelengths, and their corresponding chemical assignments, were most affected by the fungi. First and 2nd derivative pretreatments to the original spectra resulted in some blue-stain sensitive wavelengths throughout the 350 to 2500 nm range, some of which are associated with nitrogen in the melanin present in blue stain. However, for the 2nd derivative pretreatment, the stained wood exhibited a different signal to noise ratio than the control wood, and thus the pretreatment method should be used with vigilance. For the raw, 1st, and 2nd derivatives, the absorbance of *L. serpens* ($n = 164$) significantly differed from *O. minus* ($n = 140$) between 424–554 nm. The results of this study are important because the absorbance at visible and NIR wavelengths may be used to classify stained wood.

Keywords: Blue stain, fungus, *Leptographium*, melanin, modeling, NIR, *Ophiostoma*, pine, sapstain, spectra.

INTRODUCTION

With proper calibration, near infrared (NIR) spectroscopy is becoming an important tool to quantitatively assess the chemical, mechanical, and physical properties of wood (Hoffmeyer and Pedersen 1995; Schimleck et al. 1997) but can be influenced by extraneous factors. Thus, an understanding of how blue stain influences the absorbance at different wavelengths is critical when modeling wood properties. The visible range just below the NIR region also deserves consideration. Zulpa et al. (2003) found blue stain to influence the absorbance in the visible range between 190 and 700 nm with 700 nm being the most impacted (Table 1).

Melanin is a nitrogen-rich compound left by invading fungi and may impact the absorbance within the visible and NIR region. The melanin is produced by fungi, which are transported to trees by bark beetles (Wood 1982). In the tree, melanin can impede the rate of water conductance, causing tree mortality or suppression. In wood products, melanin causes an undesirable discoloration that can lead to lower product value. Fungal hyphae are located in parenchyma cells and resin canals but can also grow in tracheids (Liese 1970). The two most prominent melanin-producing genera are *Leptographium* sp. and *Ophiostoma* sp. The former is more virulent to the host, grows twice as fast in media and sapwood, and can withstand an oxygen-deficient environment (Solheim et al. 2001). Alternatively, *Ophiostoma* sp. grows at a moderate rate

TABLE 1. *Chemical or polymer assignments to visible and near infrared wavelengths.*

Chemical bond or polymer association	Wavelength (nm)	Reference
Glucose (O-H) vibration	1480, 1580	Via et al. 2003
Cellulose	1678, 2132, 2332, 2460	
Cellulose	1766, 2140	
Cellulose	1754, 1898, 2076, 2252	
Hot-water extractives	2300, 2100, 1900	
Lignin	870, 1230, 1275, 1438, 1460, 1505, 1600, 1660, 1708, 1828, 1445, 2154, 2218, 2386	
Components associated with melanin:		
Nitrogen	700–1100	Zhou and Wang 2003
Nitrogen	1650–1770	Riley and Cánaves 2002
Nitrogen	2090, 2174, 2378	McLellan et al. 1991 a,b
CH_3	740, 900, 1015, 1152, 1360, 1695, 1705, 2280	
C-H	1225, 1440	Osborne and Fearn 1986
C=O	1920, 1950, 2030, 2294	Osborne and Fearn 1986
ROH	738, 970, 1410, 1520, 2080, 2380	Osborne and Fearn 1986

and occurs more in sapwood than *Leptographium* sp.

Fungi to wood interactions are important in understanding which visible and NIR wavelengths may be impacted by stain. Wang et al. (1995) reported that for *O. piceae* and *O. ainoae*, the resin acid content decreased by up to 67% in conjunction with fungal growth. Likewise, in lodgepole pine (*Pinus contorta* Loud.), the presence of *O. piceae* for two weeks reduced the resin acid content by 66% (Gao et al. 1994). For quaking aspen (*Populus tremuloides* Michx.) logs, the total extractives content decreased by 30% after inoculation with *O. piceae* and *O. pluriannulatum* (White-McDougall et al. 1998).

The carbohydrates within wood are another site of reaction for blue-stain fungi which may in turn influence light absorbance. When both *Leptographium* sp. and *Ophiostoma* sp. interact with wood, the xylose and galactose content doubles while the mannose and glucose content decreases (Fleet et al. 2001). Since a glucose unit in cellulose is most likely to be in a crystallized state, it is probable that most glucose reduction occurs in the hemicellulose matrix. In Norway spruce [*Picea abies* (L.) Karst.], sucrose concentrations decrease immediately after inoculation of *Ceratocystis polonica* (Viiri et al. 2001a). Terpene concentration also changes sharply with respect to inoculation of *C. polonica* (Viiri et al. 2001b). Finally, for the melanin residue left by the fungi, mid infrared spectroscopy shows significant peaks for OH, C-H, C=O, C-CH_3, and C-H bonds (Zink and Fengel 1990). But perhaps most critical to this study is the percentage of nitrogen in the melanin compounds, which was found to be between 1.5 to 2.5% (Zink and Fengel 1988). Since the stem, branch, and roots of most woody species commonly fall between 0.02 and 0.12% nitrogen content (Likens and Bormann 1970), the addition of melanin to wood from blue-staining fungi may greatly increase the total nitrogen concentration in a localized portion of the wood substrate, which may in turn impact the absorbance of nitrogen-associated wavelengths.

The objective of this research was to determine which regions of the visible and NIR wavelength absorbance were significantly influenced by the inoculation of *O. minus* and *L. serpens* into longleaf pine (*Pinus palustris*). In addition to the raw spectra, the 1st and 2nd derivatives of the data were also investigated since they are commonly used pretreatments for calibration modeling. Finally, *O. minus* versus *L. serpens* stain treatments were compared to distinguish differences in absorbance. Such objectives should be useful for determining the applicability of using visible or NIR wavelengths to sort blue-stained wood from clear wood.

MATERIALS AND METHODS

Increment core sampling

Fifty increment cores were randomly chosen from longleaf pine trees in a 41-year-old plantation on the Harrison Experimental Forest, which is owned and maintained by the USDA Forest Service near Saucier, MS. The location was 30.6º north and 89.1º west and the understory of the site was free of competition due to periodic prescribed burns over the lifetime of the stand. Full increment core specimens were collected from bark to pith to bark for all samples. Tree identification numbers were recorded during tree selection and cross-referenced with a map to ensure minimal genetic relation between trees. Five increment cores were randomly chosen to determine if blue-staining fungi were present in existing trees. Microscopic examination did not find the presence of any stain fungi. Furthermore, no blue stain discoloration occurred on any of the 50 increment cores taken from the site; neither when green nor after air-drying. Finally, local forestry experts reported no major bark beetle attacks for the two years prior to sampling.

Forty of the fifty increment cores were used for the final study. Each increment core was broken in half and sawn such that the radial face was exposed. One half of the core was inoculated with the fungus treatment while the other half served as a paired control. Two stain fungi were used for this study, *L. serpens* and *O. minus*.

Inoculation and colonization of O. minus and L. serpens

The fungal species used in this experiment were isolated from longleaf pine roots and southern pine beetles from the Palustris Experimental Forest, LA, and the Bankhead National Forest, AL, for *Leptographium serpens* and *Ophiostoma minus*, respectively. The isolates were cultured on malt extract agar (MEA) (2% malt extract agar), in the dark for comparison to species described in Jacobs and Wingfield (2001), and representative stock cultures were maintained on MEA slants at 4°C.

Each isolate was inoculated onto forty MEA plates and allowed to grow for 7 days at 22 ± 3ºC. A dipping slurry was created for each isolate to inoculate the cores by blending forty plates of inoculum with 500 ml H_2O for 1 min. The cores were then dipped in the inoculum and placed in separately-labeled plastic bags. None of the plastic bags were sealed and needle pin holes were punctured into the bags to provide ventilation. The labeled bags were placed in a moisture chamber where the humidity and temperature were controlled at 95 ± 2% and 22 ± 3ºC, respectively (Wang 1994; Viitanen 1997; Jacobs et al. 2000). The moisture chamber was a desiccator containing 1000 mL of water below a ceramic plate agitated by a magnetic stir bar. Two desiccator setups were used, one for each fungal treatment. Condensation commonly occurred inside the plastic bags holding the increment core. After a week of growth, each increment core was scrubbed with separate sterile tissue-wipes to remove excess inoculum and 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. Growth was allowed to occur for a six-week duration and then the specimens were taken out of the plastic bags and allowed to air-dry (Viitanen and Bjurman 1995). The surface of the specimens dried within a day ensuring that the surface was below fiber saturation point. The entire increment core took less than a week to dry, based on weight scale measurements. The control half of the increment cores were air-dried for two months at ambient room temperature and humidity. After two months, the increment cores reached equilibrium with the environment as indicated by gravimetric weight.

Processing and NIR scanning of increment cores

The increment cores, control and inoculated halves, were sawn after drying using a fine-toothed band saw. A clean radial-longitudinal face was exposed with minimal deviation from the radial plane. Any sample that showed significant deviation from the radial-longitudinal plane was identified and removed from data analysis. Rings from bark to pith were counted with the first ring being year 41. Years: 40, 30, 22, 17, 12, 9, 7, 5, and 3 were marked on each control and inoculated core. The markings were made away from the scanning area, thus avoiding spectra contamination. A total of 164 and 140 rings/data were available for *L. serpens* and *O. minus*, respectively, while 304 paired control samples were available.

NIR and visible absorbance was obtained using a FieldSpec Pro FR (Analytical Spectral Devices Inc. Boulder, CO) at wavelengths between 350 and 2500nm at 1 nm intervals. Thirty scans were collected and averaged into a single spectrum. Spectra were obtained from the latewood region of each marked year on the control and inoculated half of the increment core. Since the inoculated (or control) broke down into two clean faces, the cleanest cut face was used for scanning. Any surface burns attributable to the band saw were not considered for NIR scanning.

Statistical analysis

Significant differences between mean absorbance at a given wavelength were determined using the 95% confidence interval (Ott 1993)

$$\hat{y} \pm t_{\alpha/2}{}^{*}\left(\frac{s}{\sqrt{n}}\right) \qquad (1)$$

with $\hat{y}$ being the mean absorbance, s being the standard deviation of absorbance for given

wavelengths, n being sample size, and t being the t-value at a given confidence ($\alpha = 0.05$). Additionally, the 95% confidence interval was an indication of the precision of the mean absorbance for a given wavelength(s) for the sample populations examined.

The spectra were pretreated by 6 different methods: a) original raw spectra of wood and stain, b) 1st derivative of wood and stain, c) 2nd derivative of wood and stain, d) original raw spectra of paired data, e) 1st derivative of paired data, and f) 2nd derivative of paired spectra. A computer software package was used to create 1st and 2nd derivative treatments to the original spectra by the Savitzky-Golay method of transformation (Unscrambler 1999). For the matched samples, the spectra from the control (clear wood variation in absorbance) were subtracted from the stained wood spectra for the original, 1st, and 2nd derivatives. Paired data were possible since half of the increment core was used as a control while the other matching half was used for stain treatment.

The F-test was used to determine if variances differed between the absorbance of solid wood and stained wood (Ott 1993)

$$F = \frac{s^2_{stain}}{s^2_{control}} \qquad (2)$$

When the F estimate exceeds a tabulated value, then the variation between the two populations was deemed significantly different ($\alpha = 0.05$). Such information was used to determine if the addition of blue stain added to the variation in absorbance already present in clear wood.

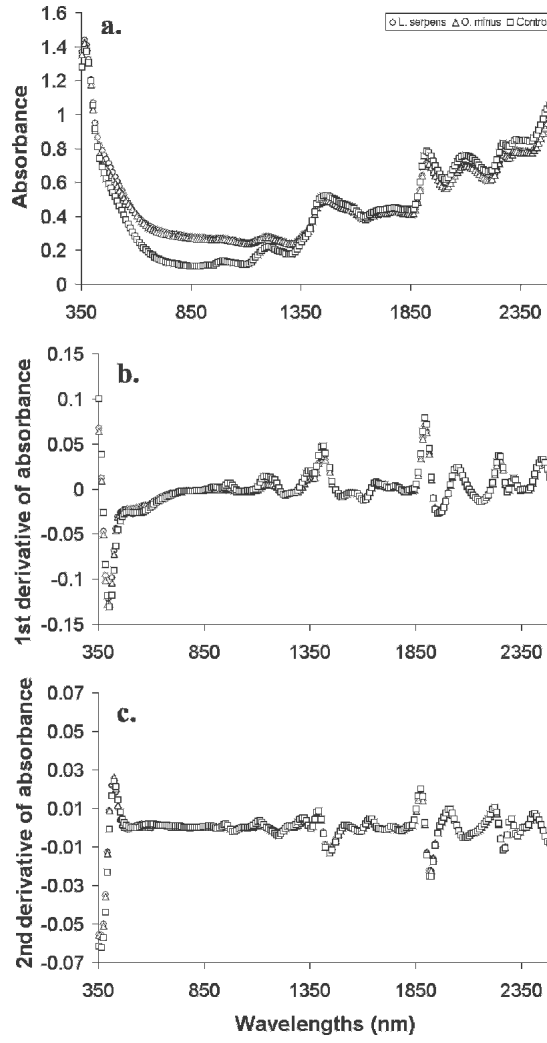

FIG. 1. a) Absorbance between wavelengths 350 through 2500 for untreated wood and wood treated with *L. serpens* and *O. minus,* b) 1st derivative of absorbance ($dA*dw^{-1}$), and c) 2nd derivative of absorbance (d^2A*dw^{2-1}).

RESULTS AND DISCUSSION

Clear wood (control) versus stained wood

Both *L. serpens* and *O. minus* treatments resulted in a similar absorbance response per wavelength for most of the wavelength range (Fig. 1a). However, the control wood differed from the stained wood for both staining fungi. A substantial difference occurred between 424–1104 nm (Fig. 1a). Other differences in mean absorbance between both stain and control treatments were detected between 354–364 nm and 1114–1354 nm. The differences were partially detectable due to the large sample size as listed in Table 2. The upper region of the spectra (1894–2500) appeared to differ in absorbance, but was not statistically different (p-value > 0.05) due to increased variance in absorbance. The variation in absorbance generally increased with increasing wavelength, which is typical for spectra collected from wood and made detection

TABLE 2. *The influence of* O. minus *(O),* L. serpens *(L), and control (C) treatment on absorbance between the wavelenghts of 350–2500 nm. Significance differences were determined at the 0.5 level (*p*-value < 0.05).*

Treatment to spectra	Comparison	Significant wavelengths	Sample size
Original	L vs. C	354–364, 424–1354, 464–1354	L = 164, C = 304
	O vs. C	464–1334	O = 140, C = 304
	L vs. O	424–504	L = 164, O = 140
1st derivative	L and O vs. C	354–384, 414–474, 554–734, 854–1014, 1064–1204, 1234–1244, 1294–1624, 1694–1714, 1854–2004, 2044–2074, 2124–2194, 2224–2264, 2294–2304	L and O = 304, C, = 304
	L vs. O	484–554	L = 164, O = 140
2nd derivative	L and O vs. C	374–754, 684–764, 834–1364, 1414–1514, 1924–1964, 2294–2304, 2454–2464	L and O = 304, C = 304
	L vs. O	444–504	L = 164, O = 140

of differences in the higher wavelength region more difficult.

The variation in absorbance between stained wood and the controls was determined to significantly differ at wavelengths between 424–1324 nm (p-value < 0.05). *O. minus* and *L. serpens* treatments exhibited lower variance in absorbance than the control treatment. For wavelengths greater than 1324 nm, there was no statistical difference in variance between the control and stain treatments.

Figure 1b shows the mean 1st derivative pretreatment of the spectra. From the graph, all three treatments fell on top of one another throughout the wavelength range. Significant differences were detected in part due to the large sample size (Table 2). However for the first derivative, when the variation in absorbance of wood was compared to that for the wood and stain, there was no significant difference in variance for wavelengths greater than 1000 nm. A lack of difference in variance suggests that wood absorbance dominates the spectra variation relative to stain.

Figure 1c shows the mean absorbance after 2nd derivative pretreatment of the original spectra. By visual assessment, no wavelengths stood out which might classify either stain treatment from the control. However, as Table 2 designates, there were significant differences at various ($d^2A\ dW^{2-1}$) of the spectra. The highest difference in absorbance occurred between 374 to 754 nm. What was not plotted on the graph was the variation in absorbance for each treatment. At a given point, for the 2nd derivative pretreatment, the confidence interval of absorption often varied wildly between stain and control treatments. These oscillations were not present in the raw data and 1st derivative pretreatments. The severe fluctuations in confidence intervals were an indication of increased noise over signal for the 2nd derivative application.

L. serpens versus O. minus treatment

To determine the *in situ* absorbance patterns of *L. serpens* and *O. minus*, the spectra of the clear wood were subtracted from the wood-stained spectra. The absorbance at 424–504 nm was significantly different for the two fungi species. The slope of *L. serpens* was steeper than *O. minus* between 414–474 nm as determined statistical tests (p-value < 0.05).

The first derivative of the paired analysis was plotted on Fig. 2b. At wavelengths between 484–554 nm, respectively. *O. minus* had a significantly greater absorbance than *L. serpens*. *L. serpens* and O. *minus* exhibited a peak and valley of 504 and 564 nm, respectively (Fig. 2b). The remainder of the wavelengths, 564–2500 nm, showed no significant difference between the treatments. Similarly, only between 444–504 nm did *L. serpens* and *O. minus* significantly differ in absorbance when the 1st derivative pretreatment was applied (Fig. 2c). When the 2nd derivative pretreatment was applied, *O. minus* and *L. serpens* differed at 474–554 nm as determined by p-values less than 0.05.

When the wood variation in absorbance was not removed from the overall spectra (Fig. 1a),

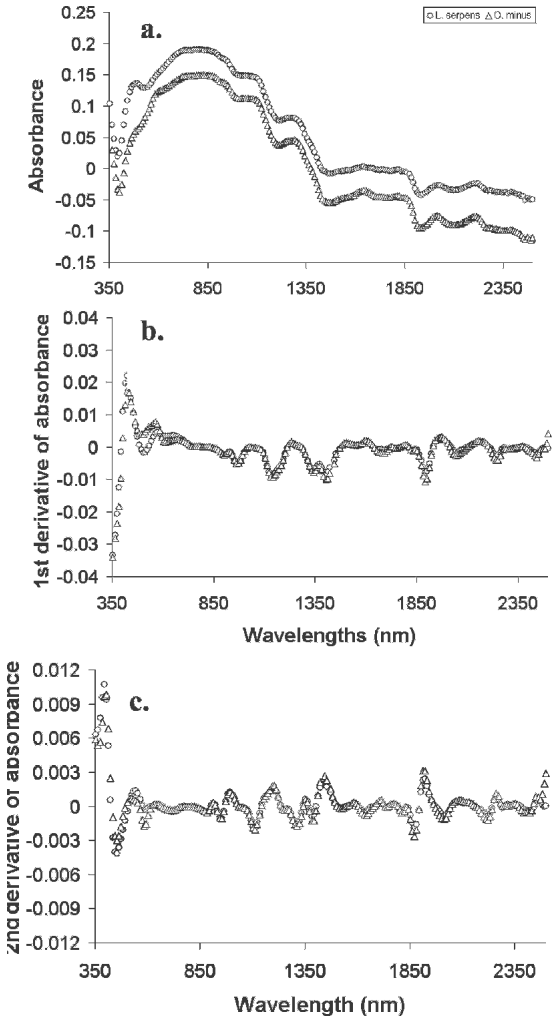

FIG. 2. a) Absorbance between wavelengths 350 through 2500 for paired data where the spectra of stained wood was subtracted from clear wood to yield the spectra of the *L. minus* and *O. minus*, b) 1st derivative of absorbance ($dA*dw^{-1}$), and c) 2nd derivative of absorbance (d^2A*dw^{2-1}).

there was no single region where *L. serpens* and *O. minus* differed. However, when the 1st derivative was computed (Fig. 1b), *L. serpens* differed from *O. minus* between 474–554 nm which agreed with the paired analysis of Figs. 2a-c.

The influence of stain on spectra

The response of absorbance in the visible and NIR range to blue-stain invasion is significant if it increases error or adds bias to prediction models. One method to build predictive models is to avoid using wavelengths associated with uncontrollable covariates (Wülfert et al. 2000). In wood, the prediction of mechanical and physical properties from visible/NIR spectra is viable. Since blue stain is a common contaminant of lumber, one needs to be careful when building predictive models. However, using wavelengths most associated with blue stain may be useful in classification of clear wood, stained wood, and perhaps even fungus genera.

As shown in Fig. 1a, wavelengths 654–1114 nm exhibited the largest difference in mean absorbance for clear wood when compared to wood stained by *L. serpens* and *O. minus*. Zhou and Wang (2003) found the wavelengths of 700 to 1100 nm to be sensitive to nitrogen (Table 1). It has been reported that blue stain is composed of melanin, which possesses much higher nitrogen concentrations than unstained wood (Zink and Fengel 1990). As the fungi compete for nutrients, the nitrogen in the host is concentrated by 20-fold when extracted from the phloem and excreted in feeding chambers (Hodges and Lorio 1969; Eckhardt et al. 2004). The close parallel between Zhou and Wang (2003) and this study suggests that increased nitrogen concentration is a potential basis for the increase in absorbance between 654–1114 nm.

Wavelengths greater than 1650 nm can be influenced by nitrogen and potentially blue stain (Table 1). However, significant differences in absorbance between clear wood and blue stain-infected wood did not occur at wavelengths greater than 1650 nm for the original spectra (Table 2). Table 1 shows that wavelengths greater than 1650 nm are commonly influenced by lignin, cellulose, extractives, and hemicelluloses. Given that the concentration of wood polymers is of many magnitudes greater than nitrogen concentration (Likens and Bormann 1970), it was reasonable that blue stain differences could not be detected at wavelengths greater than 1650 nm. However, when the 1st and 2nd derivatives were applied, differences in absorbance between the control and blue stain emerged above 1650 nm (Table 2).

For wood, an increase in density will shift the absorbance in the NIR region upward (Via et al. 2003). Such large variations in the original spectra are likely to dominate absorbance response, making smaller variations, such as nitrogen concentration in melanin, harder to detect. Taking the 1st or 2nd derivatives is a common spectroscopic technique to reduce baseline variation. Still, while small differences between the control and stain were detectable for the 1st and 2nd derivative applications, realistically, this difference at wavelengths greater than 1650 nm may have been somewhat attributable to the high sample size (Table 2 and Figs. 2b–c). Also, the success of detecting such small differences was aided by the controlled growth of *L. serpens* and *O. minus* species in a laboratory environment. The procedure used in the laboratory lasted six weeks under optimal humidity and temperature conditions. Such duration and environment allowed for complete inoculation. The uniform fungi growth resulted in maximal color change with less contrast in stain from ring to ring. When these test cores were visually compared to naturally-inoculated cores, it was clear that these cores had increased stain concentration, which should heighten differences in spectra. As a result, more verification at wavelengths greater than 1650 nm is recommended when building discriminant models for stain.

When the variation in clear wood absorbance was compared to that for the stain, the variation was found equal at wavelengths greater than 1354 nm. Equal variation is often an important assumption during model building. Additionally, equal variation in absorbance between stain and control wood suggests that absorbance variation was dominated by the wood substrate and not the blue stain. Likewise, when the wood variation was subtracted from the spectra leaving only the absorbance attributable to the *in-situ* stain, the region above 1354 nm was relatively insensitive to blue stain as indicated by the fluctuation around zero (Figs. 2a-c).

When compared to the control treatment, the confidence intervals around the mean absorbance became unstable for the stain treatment with the pre-application of the second derivative. Differences in range by an order of magnitude often occurred when the confidence intervals at the same wavelength were compared. Hiukka (1998) noted that the second derivative was very sensitive to noise, similar to that in this study. It is thus recommended that the noise be addressed when both stained and unstained woods are to be monitored by NIR or visible wavelength signal when using the 2nd derivative as a pretreatment. The increase in noise to signal was not a problem when the 1st derivative was applied or when the original spectra was analyzed.

Differences in absorbance between L. serpens versus O. minus

The detection of *L. serpens* versus *O. minus* might be possible if absorbance at a given wavelength(s) reacts differently to the two stain treatments. To achieve differentiation, the power of the paired experimental design was utilized. While a shift in the 1st derivative occurred, when compared to the original and 2nd derivative spectra, the range of wavelengths that differentiated *L. serpens* and *O. minus* was between 424–554 nm. While this fell within the range of spectra (424–1354 nm) influenced by blue stain in Fig. 1a, the region of 424–554 nm was a direct influence of the stain while the remainder of the range (564–1354 nm) could have been confounded by some stain to wood interaction.

With the exception of 424–554 nm, the mean absorbance across all wavelengths had an equal slope for *O. minus* and *L. serpens* spectra (Fig. 2a). The parallelism between absorbance trends suggests that some degree of similarity in chemical structure exists between the two genera. While not plotted, the variation in absorbance at each wavelength was considerable with *O. minus* and *L. serpens* overlapping in standard deviation below 424 nm and above 554 nm.

Within- and between-core variation

The heartwood of most cores appeared resistant to stain when visually observed. Observable stain usually began to occur at rings 9 through

40. However, when the data were partitioned by sapwood and heartwood, blue stain were detectable in the heartwood region despite the lack of visible stain. As a result, NIR may be a practical method to detect stain otherwise unobservable. Such rapid detection is needed by tree physiologists to determine the ratio of trees infected by fungi.

The range of stain variation between increment cores was large with some increment cores being only slightly stained while most were heavily stained. The occurrence of some lightly-stained cores suggests that some trees had increased resistance to stain. The lightly blue-stained cores usually possessed lower absorbance in the 564–1354 nm range than the heavily-stained cores, which was probably attributable to decreased nitrogen content. Qualitative assessment suggested this to be a possibility and merits attention in future work.

The response in NIR and visible absorbance appeared to be a promising tool for the separation of stained and clear wood. The high nitrogen content, due to the melanin from the fungi, has been found to impact the spectra from the stained wood. This was evident from the wavelengths assigned to the nitrogen components associated with melanin. Future research is needed to determine if NIR/visible region can distinguish between blue stained wood from control wood for different tree populations and species. For those wishing to monitor other traits like stiffness, density, lignin, or extractives, it may be useful to avoid wavelengths significantly impacted by stain during the calibration process.

CONCLUSIONS

The objective of this research was to determine the influence of blue-stain fungi [*Ophiostoma minus* (Hedgcock) H. and P. Sydow and *Leptographium serpens* (Goid.) Siemaszko] on absorbance at the visible and near infrared wavelengths. Absorbance was greater for the stained than the control wood at wavelengths between 464 to 1334 nm. First and 2nd derivative pretreatments to the original spectra resulted in some blue stain sensitive wavelengths throughout the 350 to 2500 nm range, some of which are associated with nitrogen in the melanin present in blue stain. However, for the 2nd derivative pretreatment, the stained wood exhibited a different signal to noise ration than the control wood. Therefore, the pretreatment method should be used with vigilance. For the raw, 1st, and 2nd derivatives, the absorbance of *L. serpens* ($n = 164$) significantly differed from *O. minus* ($n = 140$) between 424–554 nm. The results of this study are important because the absorbance at visible and NIR wavelengths may be used to classify stained wood.

ACKNOWLEDGMENT

This paper (No. 04-40-0266) is published with the approval of the Director of the Louisiana Agricultural Experiment Station. This study was funded by the USDA National Research Initiative Competitive Grants Program Agreement No. 2001-35103-10908. All data collection was performed at either Louisiana State University Agricultural Center or the USDA Forest Service, Southern Research Station in Pineville, LA.

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