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Near Infrared Model Development for Pond-cypress Subjected to Chronic Water Stress and *Botryosphaeria rhodina*

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Visible and near infrared (NIR) reflectance correlate with chemical and physical characteristics of forest canopies. Laboratory analysis of spectral reflectance in the NIR region has advantages over wet-chemical analysis. Spectral analysis of pond-cypress (*Taxodium ascendens* Brong.) under controlled water stress and *Botryosphaeria rhodina* (Cooke) Aux. inoculation has not been reported previously. Eight models were selected for preliminary multivariate evaluation of the relationship between reflectance (NIR 1100–2500 nm) and soluble carbohydrate constituents identified in a previous study. "Best fit models" for half of the constituents were first derivative, modified partial least squares (Mod PLS) models. The remainder were second derivative, Mod PLS models. The R^2 values from the best fit models for branch-tip carbohydrates were (from greatest to least): arabinose (0.93), xylose (0.93), rhamnose (0.92), pentose:hexose ratio (0.91), total pentoses (0.87), total carbohydrates (0.83), galacturonic acid (0.81), total hexoses (0.81), mannose (0.78), glucose (0.67), galactose (0.47). Evaluation of dry, milled branch tips from natural stands of pond-cypress trees may provide better understanding of physiological impacts on trees associated with anthropogenic hydroperiod perturbations (e.g., from unsustainable aquifer yield, aquifer storage and recovery, and mining solid materials).

Keywords: arabinose, branch-tip carbohydrates, first derivative modified partial least squares models, non-wound infection, pentose:hexose ratio, rhamnose, xylose.

Water stress can occur from abnormal deficits or excesses of water, although water stress erroneously is considered to be synonymous with water deficits. Almost every aspect of plant physiology and morphology can be affected by water stress. Responses include premature and extended closure of stomata leading to reduced transpiration, premature senescence and shedding of leaves, and all forms of reduced growth (e.g., reduced leaf expansion and leaf area). All stages of reproduction (e.g., flower bud initiation/development, flowering, pollination/fruit setting, and fruit/seed maturation) are susceptible to water-deficit injury. Water stress reportedly reduces viability of some seeds and kills cypress seeds (Kozlowski et al., 1991).

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This paper addresses water stress associated with water deficits. Water stress due to water deficits commonly, but erroneously, is referenced as "drought" stress. Drought is a meteorological phenomenon involving prolonged below-average rainfall. Many native plant species have evolved effective mechanisms to cope with natural cycles of low rainfall (May and Milthorpe, 1962; McWilliam, 1986; Kozlowski et al., 1991; Salisbury and Ross, 1992), and do not experience severe stress during periods of drought. Agricultural, silvicultural and other commercially-grown plants also may have adaptations for protection against natural periods of low rainfall. In cases where irrigation is used to increase plant growth or yield artificially, however, the plants can become severely stressed (e.g., susceptible to abnormal/non-wound infection by pathogens) when water availability is reduced (see Brown and Britton, 1986 for one example). Therefore, in those cases, water stress occurred from water deficits unrelated to drought, but induced by irrigation practices. Groundwater mining resulting in induced recharge from the surficial aquifer is another example of anthropogenic activities that can result in water deficits leading to plant stress that is not due to drought directly, although groundwater withdrawals may increase significantly during periods of low rainfall.

Pond-cypress (*Taxodium ascendens* Brong.) is a species that colonized the southeastern (SE) Coastal Plain, USA during the Pleistocene epoch, and has evolved water-stress avoidance mechanisms to conserve water during natural periods of drought in that region. One of the water-conserving mechanisms exhibited by pond-cypress during periods of reduced water availability is to dehisce (drop) its leafy branchlets to halt loss of water via transpiration. A full canopy is re-established when sufficient water again becomes available. Evidence of the robustness of pond-cypress to repeated, cyclical periods of natural drought (e.g., cycles of 7–15 years) is found in thriving trees that have survived for more than 600 years (despite repeated cycles of drought). Recent investigations and hearings in Florida have linked the premature decline and death of hundreds of hectares of pond-cypress to excessive groundwater withdrawals, rather than low rainfall (drought), as was widely claimed (summarized by Bacchus, 2000).

Pond-cypress in areas where groundwater mining is occurring or has occurred, may exhibit reduced leaf area, reduced viability of pollen, and premature senescence and shedding of leaves, in addition to other symptoms attributed to water-deficit stress (Bacchus, unpublished data). Since leaves are responsible for the production of "energy", deciduous trees such as pond-cypress cannot afford to remain leafless for prolonged periods of time during the growing season. Therefore, mechanisms that plants may have evolved over thousands of years as adaptations to natural phenomena (e.g., drought) may provide no protection against rapidly-introduced and abnormally-prolonged anthropogenic perturbations, such as groundwater mining. Bacchus (2000) provides a more detailed discussion regarding groundwater mining impacts to wetlands, the aquifer matrix, and other natural resources throughout the SE Coastal Plain, on both private and public property.

Changes in carbohydrate composition in response to water stress have been reported for monocots (Pressman et al., 1989; De Ruiter et al., 1992; Richardson et al., 1992; Volaire, 1995), dicots (Barta, 1988; Miller et al., 1989), angiosperms (Parker, 1970; Parker and Patton, 1975; Parker, 1979; Smalley et al., 1991; Wang and Stutte, 1992), and

evergreen gymnosperms (Koppenaal et al., 1991; Zwiazek, 1991). Other researchers have provided information on soluble sugars present in woody plants under typical conditions (Wallbäcks et al., 1991; Ashworth et al., 1993; Stewart et al., 1994).

Chronic water-deficit stress increased the susceptibility of young pond-cypress to invasion by the facultative (opportunistic) fungal pathogen *Botryosphaeria rhodina* (Cooke) Aux. in a controlled study, which also evaluated changes in individual carbohydrates and ratios using wet-chemical analysis (Bacchus et al., 2000). Prior to that study, changes in the levels of individual carbohydrates and ratios due to chronic water-deficit stress had not been investigated for pond-cypress or related taxa, such as bald-cypress (*T. distichum* (L.) L. C. Rich.). Furthermore, other water-deficit studies reported in the literature have not subjected the test plants to chronic water-deficit stress, as would be associated with anthropogenic groundwater alteration in the SE Coastal Plain (e.g., groundwater mining, aquifer storage and recovery, and mining solid materials such is rock, sand and shell). Therefore, results from the literature cannot be used to predict what changes might occur in this unique, deciduous conifer, after exposure to chronic water deficits. The precise function of the individual non-structural carbohydrates identified in pond-cypress is not known, but was not the focus of this study.

Various approaches have been used to detect physical and chemical changes in forest canopies. Those approaches, however, are not suitable for monitoring pond-cypress wetlands in areas of anthropogenic groundwater alterations of natural hydroperiods for numerous reasons. The actual canopy of pond-cypress trees declines dramatically in response to chronic water stress associated with anthropogenic groundwater alterations of natural hydroperiods. Growth of Spanish moss and lichens on canopy branches proliferate dramatically during the advanced stages of premature decline. The canopy also may become obscured by shrub and subcanopy vegetation of opportunistic plant species (including upland species) that become established or increase in density in response to the continuing anthropogenic groundwater perturbations and subsequent thinning of the tree canopy (Bacchus, unpublished data). Windthrow (leaning/falling of the trees due to basal decay and root rot) occurs after many years of chronic water stress associated with anthropogenic groundwater alterations, thus further decreasing canopy closure.

The responses described above, in addition to the increasing influence of conditions below the canopy (as canopy closure decreases with chronic water stress); the relatively small size of pond-cypress wetlands; and the differing responses in the outer, mid, and central zones of these depressional pond-cypress wetlands (due to underlying relict sinkholes), severely hinder attempts to monitor canopy conditions via aerial photography and satellite sensor imagery. A more detailed discussion regarding the myriad difficulties associated with using aerial/satellite sensor imagery (including Landsat Thematic Mapper images) for evaluating the canopy of depressional pond-cypress wetlands subjected to stress from anthropogenic groundwater alterations is provided by Bacchus et al. (2003). Wargo (1988) describes more fully the inadequacies of current approaches to determine general tree vigor.

Spectral analysis of pond-cypress under controlled conditions, including chronic water-deficit stress, has not been reported previously. Laboratory analysis of spectral reflectance in the near infrared (NIR) region is not constrained by the problems associated

with aerial/satellite sensor imagery described above, nor with the atmospheric absorption of specific NIR bands. It also is more rapid and precise than conventional wet-chemical analysis, produces no toxic waste, and is not destructive to the sample, thus allowing for multiple analyses of the same sample. The unique advantages of using the NIR region of the electromagnetic spectrum for sample analysis is described in more detail by Barton (1989).

Although NIR reflectance can be a function of cell wall rigidity, various chemical and physical characteristics, including those not associated with cell wall structure, have been correlated with visible and NIR reflectance (summarized by Bacchus et al., 2003). The majority of those studies, including recent spectroscopic evaluations of forest stands to provide estimates of forest productivity, focus on analysis of foliage. Foliage, however, exhibits great temporal and spatial variation. Our study investigated the relationship between reflectance and soluble carbohydrate constituents in dry, milled pond-cypress branch tips from a previous study of chronic water deficit and nonwounding fungal inoculation by Bacchus et al. (2000).

Materials and Methods

Source and preparation of samples

Branch-tip samples for spectral analysis were obtained from two age classes of pond-cypress trees from central Florida that were included in a separate water stress experiment simulating annual *in situ* conditions of temperature, humidity, and day length in a growth chamber. Details of the experimental and wet-chemical methods are provided by Bacchus et al. (2000), and are summarized as follows. At the initiation of the experiment, 24 one-year old and 12 two-year old containerized pond-cypress trees were assigned randomly and in equal numbers to one of the following treatments: (1) control, (2) nonwounding fungal inoculation, (3) chronic water stress, and (4) non-wounding fungal inoculation plus chronic water stress. At the conclusion of the experiment, the distal 5 cm of each branch was removed, the tips were pooled (by tree for the older age class, and by three trees per treatment for the younger age class), dried at 45 °C for 48 h, and milled with a Wiley mill using a 20 mesh screen.

Wet-chemical analysis

Subsamples of approximately 100 mg each of the milled tissue (combined bark and sapwood) were selected as a representative pooled sample from the younger and older trees to determine composition of non-structural, soluble carbohydrates. Results from the preparation and gas chromatography-mass spectrometry (GC-MS) analysis using the trimethylsilyl (TMS) methylglycosides method (Bacchus et al., 2000), with a precision of approximately 5%, were used for spectral comparison in this study. The total non-structural, soluble carbohydrate residue recovered and individual constituents were reported as percent of the dry sample mass. Individual constituents included three pentoses (arabinose, rhamnose, and xylose) four hexoses (fucose, galactose, glucose and mannose) and galacturonic acid.

Spectral data collection

A NIRSystems 6500 monochromator (NIRSystems Inc., Silver Spring, Md.) with a spinning cup module was used to collect visible and near infrared diffuse reflectance spectral data (wavelengths 400 to 2498 nm, 10 nm spectral bandwidth), as described in general by Shenk and Westerhaus (1919). This scanner uses a silicon detector for the 400–1100 nm region and a lead sulfide detector for the 1100–2500 nm region. The visible region of the spectrum (400–700 nm) is within the range of the silicon detector. The lead sulfide-detected region commonly is referenced as the NIR region. Reference to the NIR region with respect to the research conducted for this paper is synonymous with the 1100–2500 nm, lead sulfide-detected region.

The spectral data were digitized at 2-nm intervals for each sub-sample of the same milled branch-tip samples used for wet-chemical analysis in the growth chamber experiment. The software used for both collection and analysis of spectral data was NIRS, version 3.00 (Infrasoft International, 1992). Because of the limited sample material available for analysis, a black metal ring insert with an opening 1 cm in diameter was used inside the spinning cup to reduce the size of the cavity to be filled. The dried, milled sample was packed tightly in the cavity and a white cardboard-lined foam backing was secured behind the ring to hold the sample in place. The sample was rotated and scanned automatically 32 times in 60 s, with the resulting sample spectrum recorded as the average of the 32 scans. After the initial scan, the sample was removed from the cup, the cup was cleaned with a dry, soft-bristle brush, and the sample was repacked for a second scan to minimize error due to packing. The results of the two scans were not averaged prior to analysis, so that any gross aberrations (e.g., human packing errors) that may have occurred in association with the scanning process could be detected.

The reflectance values are relative to a ceramic standard, which is scanned 16 times before and after the sample scans to adjust for any possible drift that may have occurred during scanning. A response linearization pretreatment for reflectance data to pseudoabsorbance, log(1/R), where R = reflectance, is performed automatically prior to data storage, because the spectrophotometric signals are not linearly related to the chemical composition of a sample.

Model development

Fucose was not included for model development because it was not detectable in the older age class of trees from the experiment. The wet-chemical values for the remaining six sugars, total pentoses, total hexoses, the pentose:hexose ratio, galacturonic acid, and total carbohydrates were the "actual" values for the regression analyses. Regression equation (1), was used to calculate the "predicted" constituent values, where "Y" is the carbohydrate value predicted by NIR analysis of the dry-milled tissue, "b" is the vector of regression coefficients, and "X" are wavelength responses of "unknown" samples (Infrasoft International, 1992). The b-vector was derived from the training data (both X and Y) by partial least-squares (PLS) regression, a multivariate calibration algorithm that extracts a subset of linear factors from X that are most important for estimation of the Y values (Martens and Naes, 1987).

[1] $$Y = b_0 + b_1X_1 + ... + b_nX_n$$

The performance of multivariate calibration equations generated by PLS was assessed by the statistics described immediately below, and related statistics produced by cross-validation and described later. In the equations below, SD_{range} = the standard deviation of all the reference values of the constituent in the calibration data set; SS_{total} = total sum of the squares; $SS_{residuals}$ = sum of the squares for the residuals; N = the total number of samples used for a computation (34); and K = the number of wavelengths used in an equation (692); V_E = the explained variance; and V_T = the total variance.

[2] $$R^2 = 1 - \frac{SEC}{SD_{range}}$$

[3] $$SEC = SS_{total} - SS_{residuals}$$

[4] $$F = \frac{R^2(N - K - 1)}{(1 - R^2)\,K}$$

[5] $$VR = \frac{V_E}{V_T}$$

For each carbohydrate constituent, eight types of calibration models were evaluated. Both standard PLS and InfraSoft International's (ISI) modified PLS (Mod PLS) regression algorithms were tested with four variations on spectral preprocessing. Spectral preprocessing of NIR reflectance data can remove interfering spectral variation and often enhances the performance of multivariate calibration models. Two variations were on the method of spectral normalization, which also is known as scatter correction. They included standard normal variate (SNV) alone, and SNV plus detrend (SNV+Det). Each of the normalization methods was combined with two math treatments of the spectral vectors. The first math treatment (1, 4, 4, 1) incorporated a first difference (a digital derivative) with a gap setting of 4, a first smoothing setting of 4, and a second smoothing setting of 1. The second math treatment (2, 4, 4, 1) substituted a second difference, without changes in the remaining settings. The parameter settings for the ISI software are provided in *Table 1.*

To determine the optimal number of PLS regression factors for each calibration model, the statistics were evaluated for each factor added, beginning with the results for the single factor model, and progressing through results for each additional factor (Savitzky and Golay, 1964). If an F value less than 8 was encountered, the model with the preceding number of factors was selected as the best model, and models with additional factors were presumed to be overfit. Several extreme values, which may have been outliers based on T and H values provided by the software, were identified during evaluation of the various model types. These samples, however, were not eliminated. All available samples were used (n = 17 × 2 spectral replicates = 34) in this preliminary evaluation.

Table 1

Settings for the InfraSoft International (ISI) evaluation of the relationship between NIR reflectance spectra (1100–2500 nm) predictions and wet chemistry measurements of soluble carbohydrates in dry, milled branch tips from young pond-cypress trees subjected to prolonged water stress and fungal inoculation under controlled conditions

Parameter	Setting
Derivative	1 (for 1st) 2 (for 2nd)
Gap setting	4
First smoothing	4
Second smoothing	1
Missing data value	–99
Variables	1
Equation independent	none
Maximum number of terms (factors or principal components)	6
Number of wavelengths (for 1st derivative models, 1108–2492 nm)	692
Number of wavelengths (for 2nd derivative models)	689
Minimum F value	8
Number of cross validation groups	7
Downweight outliers	no
Critical T outlier value	2.5
Critical H outlier value	3.0
Critical x outlier value	999.99
Number of outlier elimination passes	0
Selected samples to delete	0
Pause to examine results	yes

Note: The minimum F value of 8 is more conservative than the value of 7 recommended by ISI.

Results and Discussion

The final equation statistics for the pond-cypress samples from the controlled study are an average of the seventeen cross-validation groups, or internal subsets of calibration samples. During the regression calculations these subgroups are excluded alternately from the calibration to be used for internal prediction, and then are included in the regression calculations. This process is based on the conclusion of Martens and Naes (1987) that the best internal validation criterion is cross-validation, which is repeated until all calibration samples have been treated as unknowns. Seventeen groups were chosen so that each pair of spectral replicates formed a separate cross-validation group.

As predicted, results were poor for the 400–1100 nm region, and are not reported. Eight models were selected for preliminary multivariate evaluation of the relationship between NIR reflectance and soluble carbohydrate constituents in the dry, milled pond-cypress branch tips determined by wet-chemical methods referenced previously. *Table 2* provides the results of the preliminary multivariate models evaluating the relationship between NIR spectral predictions and wet-chemical measurements of the soluble carbohydrates in the dry, milled pond-cypress branch tips. For discussion purposes, first deriv-

ative models SNV/ModPLS, SNV/PLS, SNV+DA/ModPLS, SNV+DA/PLS are referenced as Models 1 through 4, respectively. Second derivative models with the same regression methods and scatter corrections are referenced as Models 5 through 8, respectively. Statistics compared for the selected models were R^2 (coefficient of multiple determination), SEC (standard error of calibration), F (test statistic for the regression), SECV (standard error of cross validation), and 1–VR (variance ratio). Mark and Workman (1991) provide a more detailed discussion of the statistical procedures.

The SECV always is larger than the SEC (Infrasoft International, 1992), and is used in the computation of several different equations for PLS, as well as for multiple linear regression (MLR), and principal components analysis (PCA). The equation with the lowest SECV usually is selected as the "best" calibration (Mark and Workman 1991), minimizing overfitting (Infrasoft International, 1992). In addition to being used to select the number of factors for the equation, cross validation results can be used to compare models. As an example, for total carbohydrates, Models 1 and 3 had the same R^2 (0.83),

Table 2

Results of multivariate models evaluating the relationship between NIR reflectance spectra (1100–2500 nm) predictions and wet chemistry measurements of soluble carbohydrates in dry, milled branch tips from young pond-cypress trees subjected to prolonged water stress and fungal inoculation under controlled conditions

Solutes		1st Derivative Models				2nd Derivative Models			
		SNV	SNV	SNV+DA	SNV+DA	SNV	SNV	SNV+DA	SNV+DA
Pentoses:		Mod PLS	PLS	Mod PLS	PLS	Mod PLS	PLS	Mod PLS	PLS
Arabinose	R^2	0.88	0.77	0.88	0.77	0.93	0.17	0.93	0.17
	SEC	0.81	1.11	0.8	1.12	0.61	2.1	0.61	2.1
	F	8.12	8.94	10.26	8.58	26.49	7.75	26.48	7.75
	SECV	1.6	1.63	1.63	1.61	2	2.29	2	2.29
	1-VR	0.53	0.55	0.51	0.52	0.26	0.03	0.26	0.28
	factors	6	5	6	5	5	1	5	1
Rhamnose	R^2	0.9	0.81	0.9	0.81	0.92	0.86	0.92	0.86
	SEC	0.23	0.32	0.23	0.32	0.21	0.27	0.21	0.27
	F	8.36	11.99	10.21	12.01	19.7	16.77	16.69	16.77
	SECV	0.55	0.53	0.55	0.53	0.44	0.43	0.44	0.43
	1-VR	0.44	0.49	0.45	0.49	0.65	0.66	0.65	0.66
	factors	6	4	6	4	4	4	4	4
Xylose	R^2	0.93	0.52	0.86	0.52	0.84	0.64	0.84	0.64
	SEC	0.71	1.32	0.72	1.33	0.77	1.14	0.77	1.14
	F	11.02	36.65	10.12	36.18	15.86	21.81	15.89	21.82
	SECV	1.26	1.67	1.19	1.66	1.4	1.55	1.4	1.55
	1-VR	0.6	0.22	0.6	0.23	0.45	0.33	0.45	0.33
	factors	5	1	5	1	3	2	3	2
Total Pentoses:	R^2	0.87	0.81	0.71	0.82	0.78	0.72	0.78	0.72
	SEC	1.4	1.69	2.12	1.68	1.83	2.08	0.82	2.08
	F	14.88	27.62	38.73	28.27	49.15	12.18	49.29	12.17
	SECV	2.36	2.23	3.11	2.19	2.98	2.92	2.98	2.92
	1-VR	0.63	0.67	0.06	0.68	0.41	0.44	0.41	0.44
	factors	4	4	2	4	2	2	2	4

Table 2 ***(cont.)***

Solutes		1st Derivative Models				2nd Derivative Models			
		SNV	SNV	SNV+DA	SNV+DA	SNV	SNV	SNV+DA	SNV+DA
Hexoses:		Mod PLS	PLS	Mod PLS	PLS	Mod PLS	PLS	Mod PLS	PLS
Galactose	R^2	0.35	0.31	0.35	0.3	0.42	0.47	0.42	0.47
	SEC	2.21	2.26	2.21	0.29	2.08	1.98	2.08	1.98
	F	18.35	16.12	18.39	14.92	24.4	9.74	24.4	9.74
	SECV	2.46	2.65	2.47	2.33	2.08	2.33	2.44	2.33
	1-VR	0.18	0.05	0.18	0.05	0.2	0.27	0.2	0.27
	factors	1	1	1	1	1	2	1	2
Glucose	R^2	0.56	0.6	0.56	0.6	0.67	0.61	0.67	0.61
	SEC	1.38	1.33	1.37	1.32	1.2	1.3	1.2	1.3
	F	14.87	9.58	17.24	10.39	26.49	11.64	26.59	11.65
	SECV	1.79	1.73	1.79	1.75	1.8	1.74	1.8	1.74
	1-VR	0.32	0.37	0.32	0.36	0.3	0.35	0.3	0.35
	factors	2	3	2	3	2	3	2	3
Mannose	R^2	0.78	0.55	0.65	0.55	0.78	0.64	0.78	0.64
	SEC	0.43	0.61	0.54	0.61	0.43	0.55	0.43	0.55
	F	9.98	13.84	31.59	14.21	8.46	12.75	8.49	12.25
	SECV	0.71	0.77	0.85	0.77	0.76	0.84	0.76	0.84
	1-VR	0.41	0.3	0.14	2.92	0.32	0.17	0.32	0.17
	factors	4	2	2	2	3	3	3	3
Total Hexoses:	R^2	0.59	0.47	0.81	0.48	0.34	0.18	0.34	0.18
	SEC	2.51	2.85	1.7	2.84	3.19	3.54	3.19	3.54
	F	12.04	12.32	8.02	12.55	17.97	8.44	17.95	8.44
	SECV	3.73	3.9	3.61	3.88	3.98	3.94	3.98	3.94
	1-VR	0.11	0.03	0.17	0.04	-0.01	0.01	-0.01	0.01
	factors	3	3	6	3	1	1	1	1
Pentose: Hexose (ratio)	R^2	0.68	0.26	0.53	0.26	0.85	0.67	0.91	0.67
	SEC	0.12	0.19	0.15	0.19	0.08	0.12	0.06	0.12
	F	12.73	12.41	17.6	12.41	9.85	16.58	9.85	16.58
	SECV	1.97	0.23	0.19	0.23	0.13	0.19	0.16	0.19
	1-VR	0.17	-0.11	0.25	0.11	0.31	0.26	0.44	0.26
	factors	3	1	2	1	3	4	4	4
Galacturonic acid	R^2	0.77	0.73	0.73	0.67	0.81	0.69	0.81	0.69
	SEC	0.64	0.68	0.68	0.76	0.57	0.74	0.57	0.74
	F	12.76	8.58	9.17	13.47	8.29	23.18	8.32	23.18
	SECV	0.86	0.91	0.87	0.94	0.87	0.01	0.87	1.01
	1-VR	0.58	0.54	0.57	0.5	0.57	0.42	0.57	0.42
	factors	3	4	3	3	3	3	3	3
Total Carbohydrates	R^2	0.83	0.8	0.83	0.81	0.72	0.69	0.72	0.69
	SEC	3.24	3.51	3.32	3.49	4.18	4.38	4.18	4.38
	F	17.44	23.32	16.68	23.55	35.22	10.48	35.15	10.48
	SECV	5.1	4.74	5.08	4.61	6.83	6.35	6.83	6.35
	1-VR	0.58	0.64	0.58	0.6	0.25	0.35	0.25	0.35
	factors	4	4	4	4	2	4	2	4

Note: SNV = standard normal variate; DA = detrend analysis; Mod PLS = modified partial least squares; PLS = partial least squares; R^2 = coefficient of multiple determination; SEC = standard error of calibration; F = test statistic for the regression (= t^2); SECV = standard error of cross validation; VR = variance ratio; Factors = PLS regression factors.

although the SECV was slightly greater for Model 1 (5.10) than for Model 3 (5.08). This suggests that Model 3 may be slightly better than Model 1 for this constituent. The advantage of SEC, SECV, and SEP (which was not used in this study), over R^2 is that these errors are in the same units as the constituent. When cross-validation is used to generate the estimate of model performance, this software uses "1–VR" instead of R^2. This statistic is similar to R^2, but very conservative in small data sets (Infrasoft International, 1992).

Models 2 and 4 failed to provide the best results for any of the solutes or combination of solutes, suggesting that first derivative, unmodified PLS regression is not well-suited for identification of soluble carbohydrates in pond-cypress branch tips *(Table 2).* Model 1 alone provided the best results for xylose ($R^2 = 0.93$; 5 factors) and total pentoses ($R^2 = 0.87$; 4 factors). Model 3 alone provided the best results for total soluble hexoses ($R^2 = 0.76$; 5 factors). Models 1 and 3 provided comparable results for total soluble carbohydrates ($R^2 = 0.83$; 4 factors). For the remaining solutes, the best results were obtained with second derivative models, except for mannose, where Model 1 ($R^2 = 0.78$; 4 factors) was comparable to second derivative Models 5 and 7 ($R^2 = 0.78$; 3 factors). Models 5 and 7 jointly provided the best results for pentoses arabinose ($R^2 = 0.93$; 5 factors) and rhamnose ($R^2 = 0.92$; 4 factors), in addition to glucose ($R^2 = 0.67$; 2 factors) and galacturonic acid ($R^2 = 0.81$; 3 factors). Model 7 alone provided the best results for the pentose:hexose ratio ($R^2 = 0.91$; 4 factors). Models 6 and 8 provided the best results for galactose ($R^2 = 0.47$; 2 factors), although this sugar produced the lowest R^2 value for the solutes evaluated.

A summary of the wet-chemical values for the constituents and results of the "best fit" models is provided in *Table 3.* The solutes can be grouped into general categories of high, medium and low based on R^2 values > 0.85, 0.71 to 0.85, and < 0.70, respectively, for correlations with spectral reflectance in the 1100 to 2500 nm region. Ranking of the

Table 3

Means, minimum values, maximum values and ranges of wet chemistry data, and summary of best fit models for non-structural, soluble carbohydrates in pond-cypress branch tips after one year of treatments

Solutes	Values (% dry wt.)			Summary of Models				
Pentoses:	Mean	Min–Max	Range	R^2	SEC	Factors	Derivative	Type
Arabinose	3.56	0.82–8.51	7.69	0.93	0.61	5	2nd	Mod PLS
Rhamnose	0.92	0.35–3.17	2.82	0.92	0.21	4	2nd	Mod PLS
Xylose	4.62	1.36–7.8	6.44	0.93	0.71	5	1st	Mod PLS
Total Pentoses:	9.09	4.22–17.69	13.47	0.87	1.4	4	1st	Mod PLS
Hexoses:								
Galactose	5.3	1.55–10.92	9.37	0.47	1.98	1	2nd	PLS
Glucose	6.34	3.63–11.16	7.53	0.67	1.2	2	2nd	Mod PLS
Mannose	2.72	1.29–4.68	3.39	0.78	0.43	4	1st and 2nd	Mod PLS
Total Hexoses:	14.41	9.33–22.94	13.61	0.81	1.7	3	1st	Mod PLS
Pentose: Hexose (ratio)	0.63	0.33–1.26	0.93	0.91	0.06	4	2nd	Mod PLS
Galacturonic acid	2.72	0.48–5.88	5.4	0.81	0.57	3	2nd	Mod PLS
Total Carbohydrates	26.36	18.5–39.3	20.8	0.83	3.32	4	1st	Mod PLS

Note: n = 17 for chemical values, n = 34 for spectral replicates; R^2 = coefficient of multiple determination; SEC = standard error of calibration; Factors = PLS regression factors; PLS = partial least squares regression analysis; Mod PLS = modified partial least squares regression analysis.

constituents from the highest to lowest categories, respectively (based on the greatest to least R^2 value), was as follows: arabinose = xylose > rhamnose > pentose:hexose ratio > total pentoses; total carbohydrates > galacturonic acid = total hexoses > mannose; glucose >> galactose. The plots of actual values from wet-chemical analysis vs. values predicted by best fit models for each constituent are shown in *Figure 1,* with confidence limits and 1:1 lines shown for reference.

The results of our study should not be inferred to be applicable to any subsequent studies on pond-cypress using different sample preparation or analysis methods (e.g., freeze-drying, drying at high temperatures). Likewise, the results of this study are not meant to be applicable to other plant tissues or other plant species, including other trees. For example, an unpublished report by Smith (1969) evaluated non-structural carbohydrates in forage species. Forage species generally are rapidly-growing herbaceous (non-woody) plants used commercially as crops for grazing animals. One of the conclusions of that report was that a higher-drying temperature (100 °C for 90 minutes, then 70 °C for the remaining time) provided more scientifically-valid results than samples dried at a lower temperature (60 °C during the entire time). The tissues evaluated in that study were alfalfa leaves and roots. Sample sizes for the various treatments were unclear (data tabulated in Appendix 3 infer there were two samples, A and B, and no statistical analysis of the results appeared to have been conducted. Within-treatment differences in that study appeared to be greater than among-treatment differences, making it difficult to draw scientifically-valid conclusions regarding any influence that drying temperatures may have had on the total non-structural carbohydrates in the alfalfa leaves and roots. Additionally, it would be more difficult to draw scientifically-valid conclusions about woody branch tips from trees subjected to controlled conditions, using data from the leaves and roots of field-grown alfalfa. Therefore, the results from our study should not be considered applicable beyond the tissue, species, and analysis methods used in our study and, conversely, results from other studies using different species, tissues, and methods are not applicable to this study.

Following the controlled, chronic water stress and nonwounding fungal inoculation experiment that generated the branch-tip samples used to develop the models in this paper, an extensive number of branch-tip samples were collected from mature *in situ* pond-cypress trees at selected sites throughout Florida and the Coastal Plain of Georgia. Collections were made during multiple winter seasons (under different rainfall and temperature regimes), immediately preceding bud-break and during full leafout in summer. Multivariate analysis of NIR spectra of branch tips in that study clearly distinguished winter samples from trees in areas of known groundwater mining impacts from those in non-groundwater mining areas. Summer samples from the same trees, however, failed to show similar clear distinctions (Bacchus et al., 2003). Since carbohydrates in branch tips of cypress are presumed to play a significant role in successful spring leafout, the results from that study provide additional support to suggest that the spectral data may be linked to the specific chemical conditions in the branch tips of pond-cypress.

In conclusion, carbohydrate contents determined by gas chromatographic analysis of trimethylsilyl glycosides prepared from dried, milled pond-cypress branch tips were correlated with spectral reflectance of the same dried material in the NIR region (1100 to

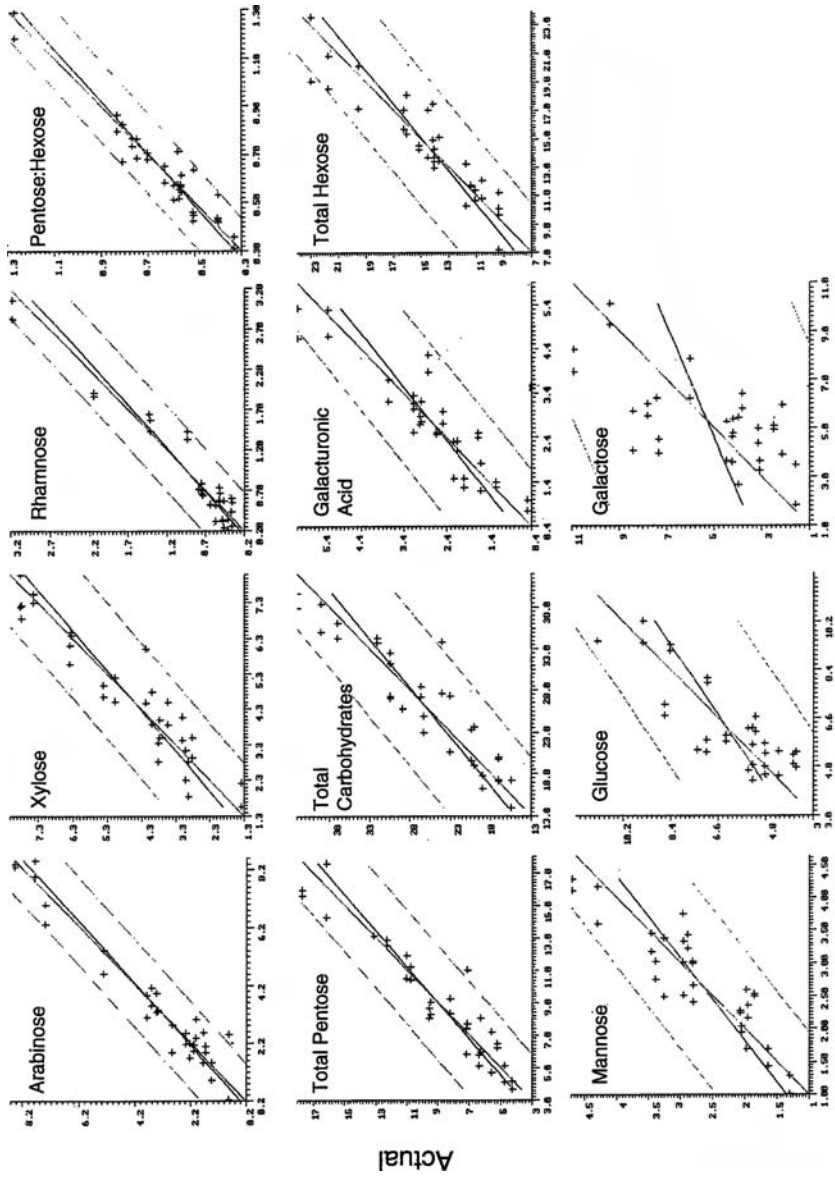

Fig. 1. Actual values from wet-chemical analysis vs. values predicted by best fit models from milled pond-cypress branch tips (ordered from greatest to least R^2 values): arabinose, xylose, rhamnose, pentose:hexose ratios, total pentoses, total carbohydrates, galacturonic acid, total hexoses, mannose, glucose, and galactose (n = 17 for wet chemical values, n = 34 for spectral replicates). Confidence limits and l: l lines are shown for reference

2500 nm). Benchtop NIR instruments appear capable of estimating the content of individual soluble pentoses (arabinose (R^2 = 0.93), rhamnose (R^2 = 0.92), and xylose (R^2 = 0.93)), the total soluble pentoses (R^2 = 0.87), and the pentose:hexose ratio (R^2 = 0.91). Moderately high correlations also were obtained for the total soluble carbohydrates (R^2 = 0.83), total soluble hexoses (R^2 = 0.81), and galacturonic acid (R^2 = 0.81). The "best fit models" for half of these constituents were first derivative, Mod PLS models, with the remaining half being second derivative, Mod PLS models.

The branch tips from the small-scale experiment that were used for model development represent values of soluble carbohydrates for young pond-cypress trees under conditions of adequate water, chronic water deficits, and exposure to the facultative (opportunistic) fungal pathogen *B. rhodina*. The data set evaluated for model development is too small to be used in a predictive capacity and is not applicable to other tissue types or other species. These results should be useful, however, in identifying key components to evaluate in larger-scale research on samples from mature trees *in situ*, to determine if similar results occur, and to evaluate the influence of seasonal changes. If results are transferable to field conditions, NIR spectral reflectance may provide a means of identifying early signs of stress in pond-cypress.

Additional work should be conducted to evaluate the primary wavelengths involved in the correlations and any correlation between the wet-chemical values of the individual constituents. Consideration also must be given to the fact that although the actual values of several constituents were highly-correlated with spectra in the 1100 to 2500 nm region, this correlation may be coincidental with other, potentially more dominant factors (e.g., cellulose, lignin, or polypeptide content). Evaluation of branch tips from mature pond-cypress trees in various natural stands may provide a better understanding of the physiological impacts on trees associated with anthropogenic hydroperiod perturbations (e.g., from groundwater mining, unsustainable aquifer yield, aquifer storage and recovery and mining solid materials).

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