# ANALYSIS OF SWITCHGRASS CHARACTERISTICS USING NEAR INFRARED SPECTROSCOPY

Nicole Labbé,[a*] Xiaofei P. Ye,[b] Jennifer A. Franklin,[a] Alvin R. Womac,[b] Donald D. Tyler,[b] and Timothy G. Rials [a]

Switchgrass varieties grown under various environments were investigated by dispersive and Fourier Transform Near-Infrared (NIR) spectrometers. The collected NIR spectra were analyzed using multivariate approaches. More specifically, principal component analysis (PCA) and projection to latent structures (PLS) regression techniques were employed to classify and predict characteristics of the switchgrass samples. The multivariate results were compared to reflectance indices that are commonly used to study the physiological performance of plants. From near infrared spectra, discrimination between the two growth locations was successfully achieved by PCA. Separation based on the ecotype and the rate of fertilizer applied to the field was also possible by the multivariable analysis of the spectral data. For the classification/ discrimination of the switchgrass samples, the near infrared spectra collected by the dispersive and the Fourier Transform spectrometers provided similar results. From the two near infrared data sets robust models were developed to predict non-structural carbohydrates content and the rate of nitrogen applied to the field. However, the spectra collected by the dispersive spectrometer resulted in more accurate models for these samples.

*Keyword: Switchgrass; Near infrared spectroscopy; Multivariate analysis; Principal component analysis; Reflectance indices*

*Contact Information: a: Department of Forestry, Wildlife and Fisheries, University of Tennessee, 2506 Jacob Drive, Knoxville, TN 37996-4570; b: Department of Biosystems Engineering and Soil Science, University of Tennessee, 2506 E. J. Chapman Drive, Knoxville, TN 37996-4531; *Corresponding author, nlabbe@utk.edu*

## INTRODUCTION

Developing renewable energy from plants is an attractive proposition for many reasons, including minimizing release of sequestered carbon in fossil fuels. Perennial energy crops such as switchgrass (*Panicum virgatum* L.) may improve agricultural sustainability through crop diversification, decreased erosion, and improved water quality compared with a traditional annual row crop system (Tolbert and Wright 1998). This warm-season (C4) grass has been proposed for biofuel production in the central, mid-western, and southeastern United States (McLaughlin 1998), and Canada. Stated traits for selecting switchgrass as a crop for energy and fiber production include high production rate, low production costs, low nutrient requirement, high water use efficiency, large range of geographic adaptation, ease of establishment by seed, adaptation to marginal

soils, and potential for carbon storage in soil (Christian and Elbersen 1998; McKendry 2002; Samson and Omielan 1992; Sanderson et al. 1996).

Two switchgrass ecotypes are generally defined based on morphological characteristics and habitat preferences. Lowland ecotypes are vigorous, tall, thick-stemmed, and adapted to wet conditions, while upland ecotypes are short, rhizomatous, thin-stemmed, and adapted to drier conditions (Moser and Vogel 1995). Lowland cytotypes are tetraploids, while the upland cytotypes can be either tetraploids or octaploids (Martínez-Reyna et al. 2001). Switchgrass varieties have been cultivated since the 1930s, and new lowland and upland varieties are now being developed specifically for biomass production for biofuel (Taliaferro and Hopkins 1997). Quality of biomass for energy purposes depends on the type of application: high lignin and cellulose content is more suitable for co-firing, while high cellulose content and non-structural carbohydrate content (sugars and starches) is desired for ethanol production. Energy density, ash and nutrient content, and other bioenergy-related chemical traits vary widely among switchgrass ecotypes and varieties (Lemusa et al. 2002). High genetic variation in these important traits should make possible the development and selection of improved varieties for bioenergy production.

In addition to differences between cultivars, growth conditions and cultural practices such as fertilization also have a significant effect on the chemical composition of switchgrass plants. Nitrogen management is an important component of any non-leguminous cropping system, especially bioenergy systems. Nitrogen has important economic cost in switchgrass production, and as a waste product of energy production, an ecological cost as a potential contributor to air and stream pollution (FAIR 5-CT97-3701, 2003). Nitrogen application rates must maximize growth rate while maintaining low foliar nitrogen and ash concentrations for biomass combustion applications. High ash concentrations could cause corrosion, slagging, and fouling of the boilers and other components of the power plant, decreasing efficiency and increasing maintenance costs (Miles et al. 1996), while high nitrogen content increases NOx emissions.

Widespread interest in the use of biomass for bioenergy and biomaterials production has increased the demand for efficient, accurate analytical methods. Current methods (Karr and Brink 1991; Karr et al. 1991; American Society for Testing and Materials 1993) for chemical characterization of biomass feedstock are not applicable in a commercial setting because they are very expensive (labor intensive) and cannot provide analytical information in a time frame useful for feedstock management and process control. The expanding application of spectroscopic methods to crop production, both remote sensing and near-range, will allow for the rapid assessment of plant characteristics. Rapid biomass compositional analysis utilizing near infrared techniques, which may instantly provide information of heating value and contents of sugars, lignin, and ash, is in high demand for biomass feedstock suppliers, bio-ethanol manufacturers, and bio-power producers (Hames et al. 2003). NIR has been successfully used to predict total nitrogen in forage (Valdes et al. 2006) and nitrogen deficiency in corn (Clay et al. 2007). However, mixed success has been reported for the use of NIR spectra in quantifying biochemical characteristics, particularly when used in conditions of high environmental or genetic variability. The use of multiple indices has been suggested as a way to improve the predictive capacity of spectra (Belanger et al. 2005; Prasad et al.

2007), while multivariate methods such as principal components analysis (PCA) are another alternative statistical technique. The quantification of sugars by mid-FTIR has been successfully used for plant extracts (Cadet 1998), and models from NIR spectra have predicted starch content of sweet potato (Lu et al. 2006), and total sugars in intact fruit (Walsh et al. 2000). For biomass, the quantity of easily extractable carbon may be important in determining pre-treatment methods.

As a step towards rapid biomass compositional analysis, the goal of this study is to conduct a comprehensive evaluation of the feasibility of NIR technology, including both dispersive (D-NIR) and Fourier Transform (FT-NIR) near infrared, in differentiating switchgrass ecotypes and varieties, and for the evaluation of environmental effects on plant composition. Specific objectives are: 1) to determine the extent of spectral variation between cultivars and ecotypes, and examine the relative abilities to distinguish between these using principal components analysis (PCA) of D-NIR and FT-NIR data; 2) to determine the extent of spectral variation resulting from nitrogen (N) fertilization, and the relative abilities to distinguish between these using principal components analysis (PCA) of D-NIR and FT-NIR data; and 3) to determine the effects of cultivar and N fertilization on non-structural carbohydrate concentrations, and test the ability to predict these parameters using Projection to Latent Structures (PLS) models constructed from D-NIR and FT-NIR data. The hypothesis tested is that NIR scan readings in combination with principal component analysis (PCA) can provide rapid classification of switchgrass samples without previous statistical calibration curves.

## EXPERIMENTAL

### Plant Materials

Tables 1 and 2 summarize the information about the twelve cultivars.

**Table 1.** Treatment Description of Switchgrass Cultivars

| Treatment # | Ecotype [1] | Variety | Nitrogen application, kg ha$^{-1}$ | Location |
|---|---|---|---|---|
| 1 | Lowland | Cave-in-Rock | 0 | Knoxville |
| 2 | Lowland | Alamo | 0 | Knoxville |
| 3 | Lowland | Kanlow | 0 | Knoxville |
| 4 | Upland | Shelter | 0 | Knoxville |
| 5 | Upland | NC 1-16 | 0 | Knoxville |
| 6 | Upland | NC 2-16 | 0 | Knoxville |
| 7 | Lowland | SL-93-3 | | Milan |
| 8 | Lowland | GA-993 | | Milan |
| 9 | Lowland | GA-992 | | Milan |
| 10 | Lowland | Alamo | 0 | Milan |
| 11 | Lowland | Alamo | 67.2 | Milan |
| 12 | Lowland | Alamo | 201.7 | Milan |

[1] Taliaferro, C.M. 2002. Breeding and selection of new switchgrass varieties for increased biomass production http://www.ornl.gov/info/reports/2002/3445605360105.pdf accessed January 8, 2007, and Bouton, J.H . Bioenergy crop breeding and production research in the southeast – final report for 1996 to 2001. http://bioenergy.ornl.gov/pdfs/TMUniv_Georgia_final_report.pdf accessed January 8, 2007

**Table 2.** Characteristics of Switchgrass Cultivars at Time of Harvest

| Treatment # | Cultivar | Nominal Height and Range (m) (Range) | Mean Stem Base Diameter (mm) (Standard Deviation) | Characteristic Colors and visual description |
|---|---|---|---|---|
| 1 | Cave-in-Rock | 1.32 (1.12 – 1.52) | 2.92 (0.31) | Dark green leaves<br>Dark pink stems<br>50% tan seeds fallen off |
| 2 | Alamo | 0.96 (0.84 – 1.12) | 2.78 (0.33) | Light green leaves<br>Pale green stems<br>Seeds fallen off |
| 3 | Kanlow | 1.73 (1.29 – 2.01) | 3.64 (0.44) | Light green leaves<br>White w/ brown patches on stems<br>Tan seeds present |
| 4 | Shelter | 1.04 (0.91 – 1.12) | 2.28 (0.38) | Dark green leaves<br>Yellow stems<br>Seeds fallen off |
| 5 | NC 1-16 | 2.01 (1.55 – 2.44) | 4.01 (0.57) | Light green leaves<br>Yellow stems<br>Brown bold seeds |
| 6 | NC 2-16 | 1.70 (1.24 – 1.98) | 4.53 (0.97) | Light green leaves<br>30% pink on stems<br>Pink seeds |
| 7 | SL-93-3 | 2.04 (1.93 – 2.16) | 3.63 (0.80) | Brown with some light green leaves<br>Light green stems<br>Tan seeds present |
| 8 | GA-993 | 2.24 (2.01 – 2.46) | 3.52 (0.89) | Light green and brown leaves<br>Light green stems<br>Tan seeds present |
| 9 | GA-992 | 2.36 (2.11 – 2.59) | 4.13 (1.14) | Light green and brown leaves<br>Light green stems<br>Tan seeds present |
| 10 | Alamo | 2.06 (1.78 – 2.29) | 3.66 (0.80) | Light green and brown leaves<br>Light green stems<br>50% tan seeds fallen off |
| 11 | Alamo | 2.17 (2.08 – 2.24) | 3.40 (0.98) | Light green and brown leaves<br>Pale green stems<br>Tan seeds present |
| 12 | Alamo | 2.24 (2.08 – 2.31) | 4.30 (1.11) | Light green and brown leaves<br>Pale green stems<br>50 % tan seeds fallen off |

Twelve switchgrass cultivars of experimental populations were included in the study. Among the twelve, six were grown in Knoxville, in eastern Tennessee, and the other six were from Milan in western Tennessee. Both lowland and upland ecotypes were

included, and three different nitrogen fertilization treatments were applied to the Alamo variety grown in Milan.

The experiment was conducted in a randomized block design with two replications and three sub-samplings (a total of 72 samples). All of the switchgrass samples at both locations were freshly harvested in the fall of 2006 and transported to the Knoxville campus of the University of Tennessee in a time window of three days. Samples were oven-dried at 105 °C for 12 hours, then ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to 5 mm particle size. The use of dried and ground samples in this study reduces sample variation and the effects of tissue water on spectral reflectance.

## Collection and Processing of Spectral Data

Near infrared spectra were collected on the dried samples using both D-NIR and FT-NIR. Thirty (for D-NIR) or thirty two (for FT-NIR) scans were collected and averaged into a single average spectrum. Three average spectra were taken for each sample. Table 3 lists the instruments and the corresponding scan parameters. The complete NIR data sets (D-NIR and FT-NIR) were transferred to Unscrambler v. 9.2 software (CAMO Software Inc., Woodbridge, NJ). The reflectance spectra were converted to absorbance spectra. The data sets were reduced by averaging the spectra (D-NIR and FT-NIR) to a spectral data set of 4 nm intervals. Averaging the spectra reduces the size of the spectra matrix and significantly minimizes the time required to compute the multivariate models without decreasing their quality. The data were then mean-normalized. Multiplicative scatter correction (MSC) was also applied to compensate for multiplicative and/or scatter effects in the data.

**Table 3.** NIR Instruments and Scan Parameters Used to Collect the Near Infrared Spectra

| Instrument | Instrument type | Scan range | Scan resolution | Co-addition | Diameter of scanned area |
|---|---|---|---|---|---|
| Advanced Spectral Devices® | D-NIR | 350-2500 nm | 1 nm | 30 | 40 mm |
| Varian Excalibur® | FT-NIR | 1000-2500 nm | 0.39 nm | 32 | 10 mm |

In near infrared spectroscopy, the influence of compounds usually reflects throughout the whole spectral region instead of only within a certain specific region. As a result, visual analysis of NIR spectra does not reveal fingerprints of chemicals in the near range (700-2500 nm) as they are in the mid range (400-4000 $cm^{-1}$). However, a NIR spectrum is a unique representation of a substance or a mixture. Signals from bonds such as C-O, C=O, O-H, and N-H, as well as the overlap of their first and second overtones are included within this region. The composition-informative spectra need to be combined with multivariate mathematical methods to extract structural information. Therefore, multivariate models are, besides being predictive, also useful in the interpretation of overlapping and wide overtone bands. All of the multivariate analyses of NIR data were performed using a commercial software Unscrambler v. 9.2. Principal component

analysis (PCA) was used to analyze the spectra of dry samples, using all samples for the analysis of cultivars, and Alamo only for analysis of nitrogen fertilization effects. PCA is a non-direct analysis of spectral data and can be employed to visualize outliers, trends, or clusters in large data sets. In order to interpret/compare the loadings obtained for the D-NIR and FT-NIR data sets, a factor rotation procedure (Varimax rotation) was performed using Matlab (Abdi 2003).

Projection to Latent Structures (PLS) was applied to build regression models between the spectral data and parameters of interest, such as amount of fertilizer applied to the field, as well as starch and sugar content. PLS models were constructed by cross-validation, and the number of PLS-components was selected as the minimum in the residual variance versus components plot. PLS is a regression technique that provides a model for the relationship between a set of predicator variables X (n objects, m variables) and a set of response variables Y (n objects, p responses). If the spectral data contain information about the property of interest, then a reliable model can be constructed. The multivariate calibration models were constructed using the full cross-validation technique. The cross-validation systematically removed a single sample from the data set, constructed the model with the remaining samples, and used that model to predict the value of the Y variable for the extracted sample (validation). In addition to a coefficient of correlation for the calibration ($R_{calibration}$) and for the validation model ($R_{validation}$), a root mean square error of calibration (RMSEC) and root mean square error of cross validation (RMSECV) were provided to assess the quality of the PLS models. Jack-knifing was employed to study the significance of the X variables in the regression coefficients plot, which expresses the link between the variation in the predictors (wavelengths) and the variation in the response (sugar and starch content, fertilization effects).

## Reflectance Indices

The data set collected on dry samples by D-NIR was used to calculate several reflectance indices that have been proposed for the assessment of crop characteristics. All are based on reflectance of plant pigments at 680 nm and 705 nm, which are sensitive to genetic and environmental factors, as compared to a reference value. Specific indices were selected to represent simple, normalized and chlorophyll: carotenoid indices (Sims and Gammon 2002). The R4 estimates chlorophyll content by a simple ratio of reflectance at 430 to reflectance at 672 (Brach et al. 1981). The Structure Independent Pigment Index (SIPI) assesses the ratio of chlorophyll to carotenoid pigments, using an infrared wavelength to account for structural changes that may be related to observed pigment changes (Penuelas et al. 1995), and is defined as:

$$SIPI = (R_{800} - R_{445}) / (R_{800} - R_{680}) \quad (1)$$

where $R_x$ = reflectance at a wavelength of x nm

The Plant Senescence Reflectance Index (PSRI) has been proposed to describe changes in the ratio of chlorophylls to carotenoids in leaves as a result of senescence (Merzlyak et al. 1995):

$$PSRI = (R_{678} - R_{500}) / R_{750} \quad (2)$$

ND680 and ND705 are normalized indices developed by Sims and Gamon (2002) based on the normalized difference vegetation index (NDVI) and on the maximum absorbance of total chlorophyll at 705 nm. These indices were calculated on the raw reflectance data.

$$ND680 = (R_{800} - R_{680}) / (R_{800} + R_{680}) \quad (3)$$

$$ND705 = (R_{750} - R_{705}) / (R_{750} + R_{705}) \quad (4)$$

ANOVA was used to test for effects of fertilization and of cultivar on the above indices, with a Duncan post-hoc used when treatment effects were significant (SPSS version 13.0, SPSS Inc. Chicago, IL).

### Non-Structural Carbohydrates

Soluble sugars were extracted twice with 85% ethanol at 90° for 10 min. The two aliquots were combined, the extract was reduced under vacuum until all ethanol was removed, the extract was rehydrated to a set volume, and then sugars were measured by HPLC. The analysis was carried out on a Waters 2695 Alliance Separations Module coupled with a Waters 2414 Refractive Index Detector. The sugar separation utilized a Biorad Aminex HPX-87P column heated to a constant temperature of 80ºC in an external column heater. Standard solutions were prepared using HPLC-grade glucose (Thermo Fisher Scientific, Waltham, MA). Sugars other than glucose were found to make up only a small fraction of soluble sugars (<5%) and were not used in analysis. The remaining residue was dried and starch was converted to sugars using the enzymatic digestion method described by Hendrix (1993), which uses α-amylase and amyloglucosidase to break glucoside bonds in starch and dextrins into glucose units, with minimal effect on cell wall polysaccharides. Soluble starch (Fisher Scientific S-516) was used as a standard. ANOVA was used to test for effects of fertilization and of cultivar, with a Duncan post-hoc used when treatment effects were significant, and PLS models were developed for sugar and starch.

## RESULTS AND DISCUSSION

Figure 1 shows some D-NIR (a) and FT-NIR (b) spectra collected on the oven-dried switchgrass samples. All absorbance spectra collected by the two methods had generally similar spectral patterns. Prominent absorbance bands were observed at the same characteristic wavelengths across all samples, such as bands at 1475, 1723, 1925, 2100, 2275, and 2330 nm. The bands at 1925, 2100, and 2275 nm were generally assigned to OH stretching vibration, the bands at 1723 and 2330 nm were attributed to C-H stretching vibration, and the peak at 1475 nm was assigned to N-H stretching vibration (Shenk et al. 2001).

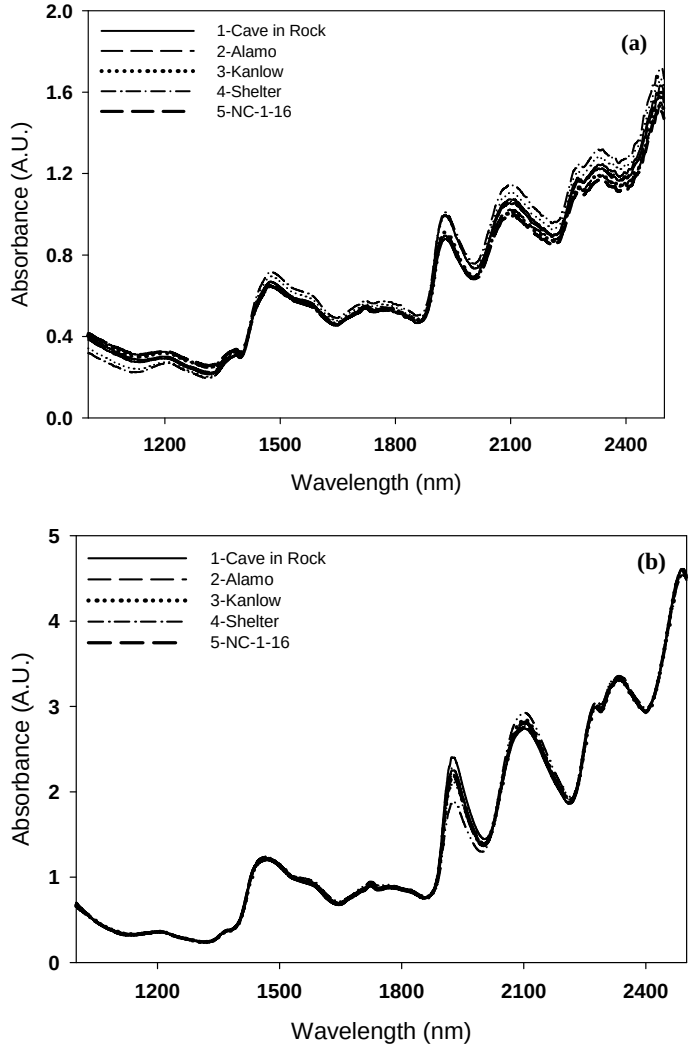


**Fig. 1.** D-NIR (a) and FT-NIR (b) spectra of switchgrass samples

Principal Component Analysis (PCA) followed by Varimax rotation was first conducted on the whole data sets (D-NIR; FT-NIR), in order to determine the variation across all samples, and between cultivars. Eigenvalue scores indicated that the first three principal components accounted for most of the total variance (97% for the D-NIR data and 98% for the FT-NIR). These patterns in the spectra indicate that the switchgrass samples could be classified by their location of growth (Knoxville versus Milan, Fig. 2). While growth location was associated strongly with PC1 in the model constructed from D-NIR data, the separation was not as clear and more closely associated with PC2 in the model constructed from FT-NIR data. Both approaches indicated measurable differences in switchgrass chemistry between growth locations, the result of differences in soils, and/or climate between the two locations.

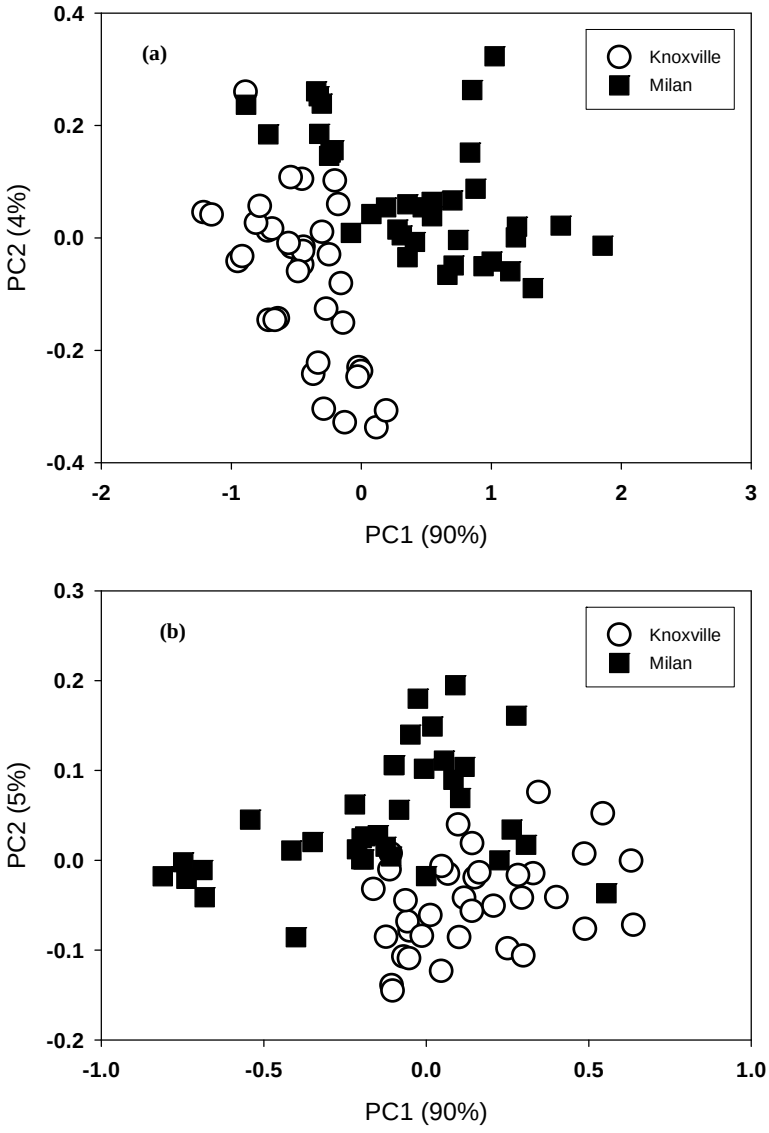


**Fig. 2.** Scores plot of the first two principal components derived from D-NIR (a) and FT-NIR (b) of absorbance spectra of dried switchgrass samples

Varimax rotation was performed in order to maximize the variance of factor loadings by making high loadings higher and low ones lower for each factor. These PCA loadings describe the data structure in terms of variable correlation. Each variable has a loading on each PC. The loadings of the first two principal components (after Varimax rotation) for the two sets of spectral data (D-NIR and FT-NIR) are shown in Fig. 3. From these plots it was possible to identify the spectral regions that contributed the most to each principal component.

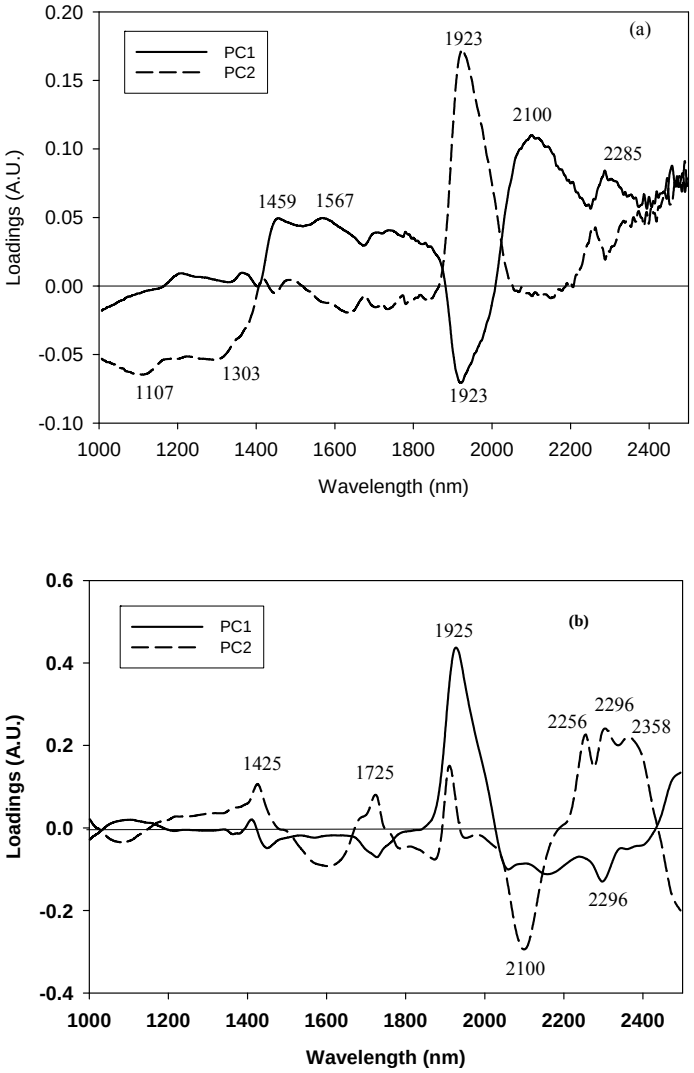


**Fig. 3.** Loadings (after Varimax rotation) of the first two principal components derived from D-NIR (a) and FT-NIR (b) of absorbance spectra of dried switchgrass samples

Several significant bands were responsible of the classification of the samples. For the D-NIR data set (Fig. 3a), the significant bands of principal component one were 1459, 1567, 1923, 2100, and 2285 nm, and for principal component two 1107, 1303, and 1923 nm. For the FT-NIR data set (Fig. 3b), the bands at 1425, 1725, 1925, 2100, 2256, 2296, and 2358 nm possessed high loadings. The significant bands in the 1925, 2100 regions suggest that these portions of the spectrum may have been the most affected by the growth environment. The bands at 1725, 2256, 2296, and 2358 nm were generally assigned to C-H functional groups present in biomass components, and the bands at 1425, 1925, and 2100 nm were assigned to the hydroxyl groups.

In order to identify any spectral patterns related to the switchgrass varieties (in the absence of environmental differences) PCA was performed on the samples collected only at the Knoxville location. Figure 4 represents the scores plots obtained for the D-NIR and FT-NIR sub-data sets.

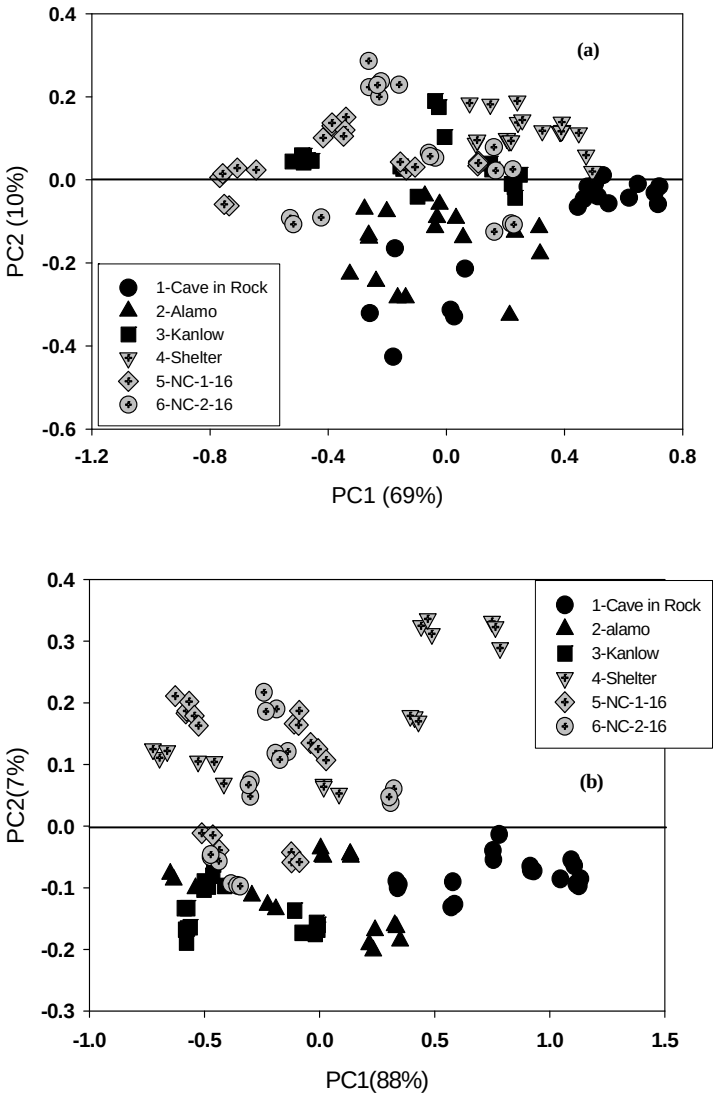


**Fig. 4.** Scores plot of the first two principal components derived from D-NIR (a) and FT-NIR (b) of absorbance spectra of switchgrass samples grown in Knoxville

Especially for the FT-NIR data (Fig. 4b), there was a clear separation of upland (Shelter, NC-1-16, NC-2-16) and lowland (Cave-in-Rock, Alamo, Kanlow) ecotypes along PC2. Figure 5 shows the most significant wavelengths responsible for this discrimination. One band dominated the PC1 loadings at 1925 nm, which is usually due to hydroxyl groups present in biomass (Tsuchikawa and Siesler 2003). More specifically, this band is related to the intramolecular hydrogen bond between water and OH of switchgrass components. Numerous bands were significant in the PC2 loadings (1425, 1722, 1907, 1953, 2116, and 2260-2380 nm). The assignment of all these bands to specific switchgrass components was not possible due to the nature of near infrared, and more than one assignment is possible to give the observed bands. The band at 1722 nm

resulted from the first overtone of CH stretching vibrations. The band occurring at 1907 nm was assigned to the combination band of free OH group stretching vibration. The absorbance in the combination region 2260-2380 nm occurred for C-H functional groups present in cellulose, hemicellulose, and proteins (Shenk et al. 2001).

In both models, Cave-in-rock could be picked out from other cultivars, and of the six cultivars compared here, only Cave-in-rock was of northern origin. This cultivar also had a significantly greater sugar and starch content at the time of harvest, as compared with other cultivars (Fig. 7a).

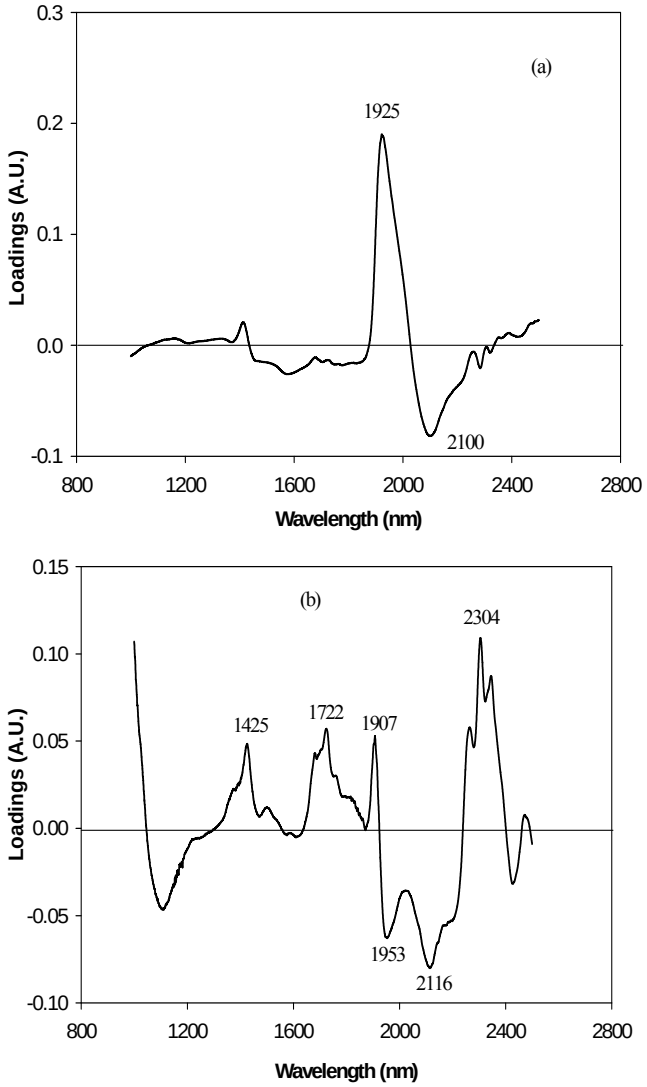


**Fig. 5.** Loadings plot of the first (a) and second (b) principal component derived from FT-NIR of absorbance spectra of dried switchgrass samples

The lack of distinct clusters indicates that switchgrass chemistry varied widely within a cultivar. Genetic analysis of cultivars and populations has found greater variation between individuals of a population than between cultivars (Casler et al. 2007), although latitude of origin also affects switchgrass development and yield (Van Esbroeck

et al. 2003; Elbersen et al. 2001). FT-NIR models showed that the two cultivars originating in the southeast, NC-1-16 and NC-2-16, appeared to have a similar chemical composition, as did the two western cultivars, Alamo and Kanlow (Fig. 4).

Reflectance indices calculated with specific wavelengths were also used to compare the different cultivars (Table 4). Of the 5 reflectance indices tested, SIPI, ND680, and ND705 were significantly different between cultivars. Cultivar groupings by these 3 indices were similar, but did not necessarily coincide with plant color recorded from visual observation (Table 2). Alamo and GA992 with light green leaves were grouped at one end of the spectrum with Shelter, which had dark green leaves at the time of harvest. These had a relatively low ratio of carotenoids:chlorophylls, indicated by a low SIPI, which may have been due to higher levels of total chlorophyll, as indicated by the ND680 and ND705 indices. At the far end of the range was SL-93-3, which showed senescence at the time of harvest, and Cave-in-Rock.

**Table 4**. Values of 5 Different Reflectance Indices by Cultivar, and by Nitrogen Application Rate.

| Treatment # | Cultivar | SIPI | PSRI | ND680 | ND705 | R4 |
|---|---|---|---|---|---|---|
| 1 | Cave-in-Rock | 1.719 cd (0.016) | 0.317 (0.005) | 0.329 a (0.004) | 0.126 a (0.003) | 8.286 (0.353) |
| 2 | Alamo | 1.644 ab (0.018) | 0.276 (0.005) | 0.346 de (0.007) | 0.132 d (0.003) | 7.371 (0.163) |
| 3 | Kanlow | 1.615 abc (0.017) | 0.218 (0.008) | 0.358 bcd (0.005) | 0.137 bcd (0.002) | 7.590 (0.121) |
| 4 | Shelter | 1.575 a (0.014) | 0.255 (0.010) | 0.377 e (0.005) | 0.136 cd (0.002) | 7.698 (0.081) |
| 5 | NC 1-16 | 1.665 cd (0.025) | 0.311 (0.014) | 0.347 de (0.008) | 0.132 ab (0.002) | 7.700 (0.180) |
| 6 | NC 2-16 | 1.700 bcd (0.009) | 0.322 (0.014) | 0.336 abc (0.003) | 0.129 abc (0.002) | 7.634 (0.211) |
| 7 | SL-93-3 | 1.731 d (0.026) | 0.308 (0.006) | 0.318 a (0.007) | 0.121 a (0.003) | 7.691 (0.238) |
| 8 | GA-993 | 1.717 bcd (0.036) | 0.298 (0.013) | 0.314 ab (0.010) | 0.119 d (0.004) | 7.019 (0.150) |
| 9 | GA-992 | 1.607 a (0.044) | 0.251 (0.015) | 0.352 cde (0.014) | 0.134 cd (0.006) | 7.449 (0.249) |
| 10 | Alamo 0 N | 1.613 (0.030) | 0.272 a (0.008) | 0.359 (0.011) | 0.138 (0.005) | 7.653 (0.244) |
| 11 | Alamo Low N | 1.678 (0.019) | 0.275 a (0.005) | 0.331 (0.006) | 0.125 (0.002) | 6.627 (0.186) |
| 12 | Alamo High N | 1.678 (0.020) | 0.287 b (0.009) | 0.332 (0.006) | 0.126 (0.002) | 7.494 (0.115) |

Standard errors are shown in parentheses. Letters indicate a significant difference between treatments at $p \geq 0.05$, with treatments 10-12 analyzed separately.

Although senescence was not visually noted in the latter cultivar at the time of harvest, phenology is known to be affected by photoperiod (Van Esbroeck et al. 2003), and like other genotypes from the north, would be at a later stage of development than local genotypes when grown in the south (Parrish and Fike 2005). However, it is possible that chlorophyll loss during post-harvest handling was different between cultivars, resulting in differences in the visible portion of the spectrum (Sestak 1971).

There was no clear relationship found between reflectance indices based on the visible portion of the spectrum and either of the first two principal components from NIR models. Both reflectance indices and NIR models may be detecting chemical differences resulting from differing maturity between cultivars, as both growth stage and genotype of winter wheat have been shown to significantly affect a variety of reflectance indices (Prasad et al. 2007). Although differences in reproductive development were noted (Table 2), neither reflectance indices nor NIR models grouped switchgrass cultivars by the presence/absence of seed, so observed differences appear to have been the result of switchgrass chemistry.

Multivariate analysis was also performed on the cultivar Alamo from different growth environments (Table 1), to examine the ability of NIR to detect fertilization-related differences between switchgrass samples. Both D-NIR and FT-NIR showed clear spectral differences between plants grown at different fertility levels (Fig. 6), demonstrating that the environment had a dramatic effect on the chemical signatures of the biomass.

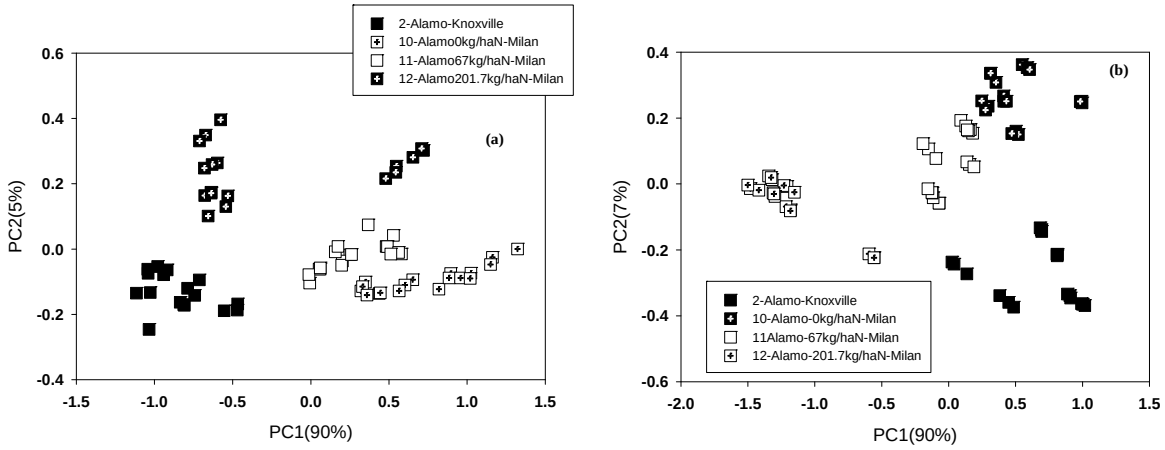


**Fig. 6.** Scores plot of the first two principal components derived from NIR (a) and FT-NIR (b) of absorbance spectra of alamo variety grown under different amount of fertilizer.

Of the five indices used to compare spectral data using in the 400-750nm range, only PSRI was sensitive to fertilization treatment of Alamo, with plants in the high fertility treatment being significantly different from the other two treatments. At an application rate of 200 Kg $ha^{-1}$, chlorophyll may be higher as N approaches the saturation point, as this rate of fertilization resulted in the greatest biomass production of Alamo (Miur et al. 2001).

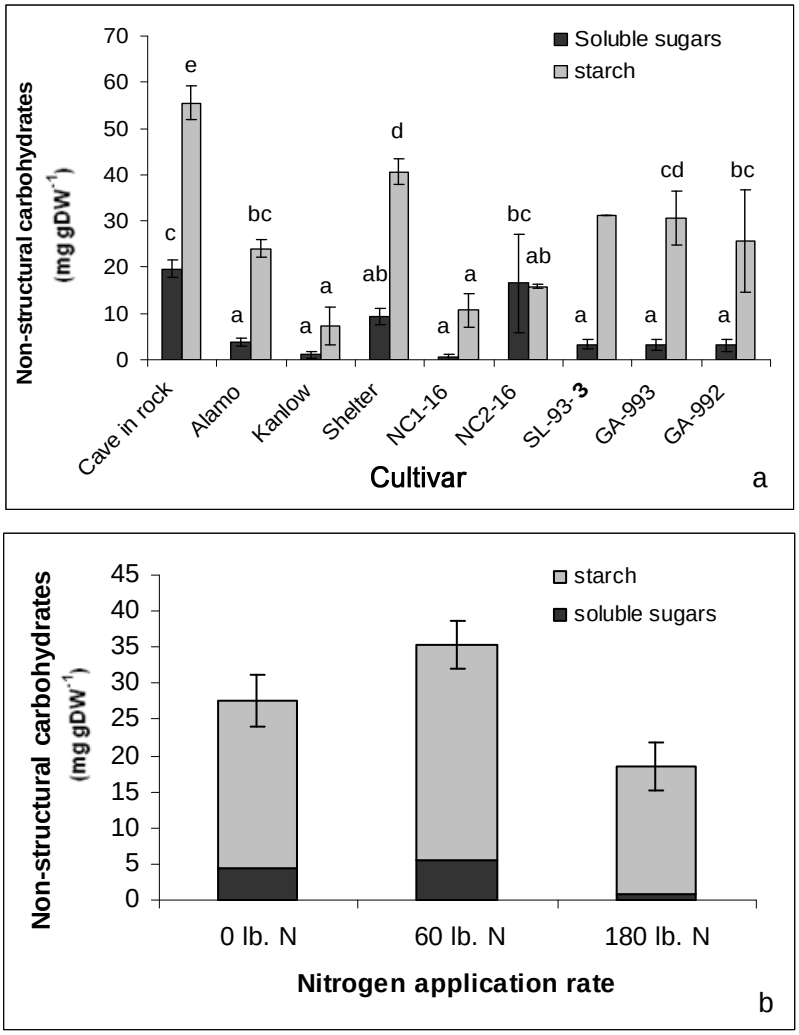


**Fig. 7**. Concentrations of soluble sugars and starch in switchgrass sample as related to a) cultivar and b) nitrogen application rate. Bars indicate standard error. Different letters indicate significant differences between cultivars at $p \leq 0.05$.

Indices based on reflectance in the 350-1050 nm range and the use of multiple indices have been used successfully to classify corn foliage by nitrogen application rate (Clay et al. 2006; Strachan et al. 2001). The multivariate approach proved to be more efficient in classifying and identifying differences in switchgrass samples related to soil fertility. A very strong model ($R_{calibration}$ = 0.97) was created to predict the amount of fertilizer that was applied to the field during the switchgrass growth. In this case, both data sets (D-NIR and FT-NIR) provided very similar models (Table 5). NIR with PCA has been used to predict with good accuracy the total nitrogen content of foliage in forage (Starks et al. 2004; Valdés et al. 2006). The specific chemical differences between plants in differing fertilization treatments is unknown, but increases in foliar total N of switchgrass have been found with increasing fertilization (Bransby et al. 1998). The

ability to determine N of switchgrass will be necessary for fertility management, and for the selection of feedstock with low nitrogen content.

Non-structural carbohydrates did not differ significantly between fertility treatments (Fig. 7b), but did differ between cultivars with a 6-fold difference between foliage of Cave-in-Rock and Kanlow. This may be due to differences in the timing of growth cessation, as Cave-in-Rock was one of the smaller cultivars, but sugar content of plant tissue is also highly sensitive to the time of day so harvesting effects cannot be ruled out. The multivariate approach was employed to develop regression models to predict the amount of starch and sugar present in the switchgrass samples by near infrared spectroscopy and to predict the amount of fertilizer applied to the field. Table 5 summarizes the results for the different models developed with both, D-NIR and FT-NIR spectra. In general, the best models (higher correlation, lower RMSEC and RMSECV) were obtained when the D-NIR spectra were utilized.

**Table 5.** PLS Models Developed with D-NIR and FT-NIR Spectra for Sugar, Starch, Total Non-Structural Carbohydrates (NSC) and Nitrogen Content

| Models D-NIR/FT-NIR | | Starch (mg/g) | Sugar (mg/g) | Total NSC (mg/g) | Nitrogen Kg/ha |
|---|---|---|---|---|---|
| D-NIR | $R_{calibration}$ | 0.96 | 0.91 | 0.96 | 0.97 |
| | $RMSEC_{Calibration}$ | 4.59 | 2.18 | 6.48 | 20.09 |
| | $R_{validation}$ | 0.91 | 0.82 | 0.92 | 0.95 |
| | $RMSECV_{validation}$ | 6.83 | 3.11 | 8.95 | 27.11 |
| | Nb PCs | 4 | 3 | 4 | 2 |
| | Most Significant Wavelenghts (nm) | 1091,1411 1563,1910 2099,2243 2371,2451 | 1411,1910 2119,2255 2371 | 1107,1415 1595,1910 2119,2260 2371 | 1083,1603 1771,1927 2107 |
| FT-NIR | $R_{calibration}$ | 0.82 | 0.92 | 0.81 | 0.97 |
| | $RMSEC_{Calibration}$ | 8.90 | 2.62 | 12.28 | 20.31 |
| | $R_{validation}$ | 0.72 | 0.80 | 0.64 | 0.95 |
| | $RMSECV_{validation}$ | 10.82 | 4.22 | 16.60 | 27.38 |
| | Nb PCs | 3 | 6 | 4 | 2 |
| | Most Significant Wavelenghts (nm) | 1513,1598 1731,1983 2045,2120 2218,2312 2350 | 1460,1704 1731,1922 2056,2144 2210,2337 2272, 2439 | 1704,1732 2005,2133 2221,2272 | 1441,1505 2043,2089 2187,2241 2350,2434 |

A model to predict total non-structural sugar in switchgrass with a coefficient of correlation of 0.96 for the calibration and 0.92 for the cross validation model was built. Four principal components were needed to establish these models, and several wavelengths (1415, 1910, 2119, 2260, and 2371 nm) were found significant by jack-knifing. The band at 1415 nm is assigned in the literature to the first overtone of hydroxyl groups, the band at 1910 nm is related to the interaction between carbohydrate hydroxyls and water, and the band in the 2260 nm region is attributed to the C-H stretch groups.

## CONCLUSIONS

The current study demonstrates the ability of using NIR methods for classifying, determining, and predicting characteristic features of switchgrass biomass that could be used as a feedstock for biofuel production. Classification of the samples by their growth location and their ecotypes were achieved, while no clear separation was obtained by varieties, demonstrating that the switchgrass chemical features were more dependent on growth conditions than varieties. NIR calibration models developed from the dispersive and Fourier Transform near infrared data were comparable with slightly better models with the D-NIR spectra. Robust models were built to predict characteristics such as starch, sugars, and total non-structural carbohydrates content, as well as the amount of fertilizer applied to the field during the growth. Results showed that NIR can be used as a tool for the rapid analysis of switchgrass, and may prove useful for the selection of cultivars or fertility regimes that will provide the most desirable qualities for use as a bioenergy feedstock.

## ACKNOWLEDGMENTS

The authors would like to thank Aamir Saleem, Shirley Liu, and Stéphane Jeckel for technical assistance.

## REFERENCES CITED

Abdi, H. (2003) "Factor rotations in factor analyses," In: *Encyclopedia of Social Sciences Research Method*s. Lewisbeck M., Bryman, A., Futing T. Eds. Thousands Oaks (CA): Sage.

American Society for Testing and Materials (1993). D-1106-84. "Standard test method for acid-insoluble lignin," In 1993 Annual Book of ASTM Standards; American Society for Testing and Materials: Philadelphia, PA.

Belanger, M. C., Viau, A. A., Samson, G., and Chamberland, M. (2005). "Determination of a multivariate indicator of nitrogen imbalance (MINI) in potato using reflectance and fluorescence spectroscopy," *Agronomy J*. 97(6), 1515-1523.

Brach, E. J., Mack, A. R., and St-Amour, G. T. (1981). "Mobile field laboratory instrumentation to measure spectral characteristics of agricultural crops," 626. Eng. and Stat. Res. Inst.–Institut de recherche technique et statistique, Ottawa, ON, Canada.

Bransby, D. I., Mclaughlin, S. B., and Parrish, D. J. (1998). "A review of carbon and nitrogen balances in switchgrass grown for energy," *Biomass and Bioenergy* 14(4), 379-384.

Cadet, F. (1998). "Measurement of sugar content by multidimensional analysis and mid-infrared spectroscopy," *Talanta* 48, 867-875.

Casler, M. D., Stendal, C. A., Kapich, L., and Vogel, K. P. (2007). "Genetic diversity, plant adaptation regions and gene pools for switchgrass," *Crop Sci*. 47(6), 2261-2273.

Christian, D. G., and Elbersen, H. W. (1998). "Switchgrass (*Panicum virgatum* L.)," In: N. El Bassam (ed.), *Energy Plant Species. Their Use and Impact on Environment and Development*, London, James and James publishers, p. 257-263.

Clay, D. E., Kim, K. I., Chang, J., Clay, S. A., and Dalsted, K. (2006). "Characterizing nitrogen and water stress in corn using remote sensing," *Agron. J*. 98, 579-587.

Elbersen, H. W., Christian, D. G., El-Bassem, N., Bacher, W., Sauerbeck, G., Alexopoulou, E., Sharma, N., Piscioneri, I., Visser P. D., and Berg, D. (2001). "Switchgrass variety choice in Europe," *Aspects Appl. Biol.* 65, 21-28.

FAIR 5-CT97-3701 (2003). "Switchgrass (*Panicum virgatum* L.) as an alternative energy crop in Europe-Initiation of a productivity network," Final Report for the period from 01-04-1998 to 30-09-2001. http://www.switchgrass.nl/pdf/Sw_FinalRep_full2.pdf

Hames, B. R., Thomas, S. R., Sluiter, A. D., Roth, C. J., and Templeton, D. W. (2003). "Rapid biomass analysis: New tools for compositional analysis of corn stover feedstocks and process intermediates from ethanol production," *Applied Biochemistry and Biotechnology*. (Spring) 105-108, 5-16.

Hendrix, D. L. (1993). "Rapid extraction and analysis of nonstructural carbohydrates in plant tissues," *Crop Sci.* 33, 1306-1311.

Karr, W. E. and Brink, D. L. (1991). "The complete analysis of wood polysaccharides using HPLC," *J. Wood Chem. Technol*. 11, 447-463.

Karr, W. E., Cool, L. G., Merriman, M. M., and Brink, D. L. (1991). "Simplified analysis of acid soluble lignin," *J. Wood Chem. Technol*. 11, 465-477.

Lemusa, R., Brummerb, E. C., Moore, K. J., Molstad, N. E., Burras, C. L., and Barker M. F. (2002). "Biomass yield and quality of 20 switchgrass populations in southern Iowa, USA," *Biomass and Bioenergy* 23, 433-442.

Lu, G., Huang, H., and Zhang, D. (2006). "Prediction of sweet potato starch physiochemical quality and pasting properties using near-infrared spectroscopy," *Food Chemistry* 94, 632-639.

Martínez-Reyna, J. M., Vogel, K. P., Caha, C., and Lee, D. J. (2001). "Meiotic stability, chloroplast DNA polymorphisms, and morphological traits of upland x lowland switchgrass reciprocal hybrids," *Crop Science* 41, 1579-1583.

McKendry, P. (2002). "Energy production from biomass (part 1): Overview of biomass," *Bioresource Technology* 83, 37–46.

McLaughlin, S. B., and Walsh, M. E. (1998). "Evaluating environmental consequences of producing herbaceous crops for bioenergy," *Biomass and Bioenergy* 14, 317–24.

Merzlyak, M. N., Gitelson, A. A., Chivkunova, O. B., and Rakitin, V. Y. (1999). "Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening," *Physiol. Plant.* 106, 135-141.

Miles, T. R., Miles, Jr., T. R., Baxter, L. L., Bryers, R. W., Jenkins, B. M., and Oden, L. L. (1996). "Alkali deposits found in biomass power plants: A preliminary investigation of their extent and nature," National Technical Information Service, Springfield, VA.

Miur, J. P., Sanderson, M. A., Ocumpaugh, W. R., Jones, R. M., and Reed. R. L. (2001). "Biomass production of 'Alamo' switchgrass in response to nitrogen, phosphorous and row spacing," *Agron. J*. 93, 896-901.

Moser, L. E., and Vogel, K. P. (1995). "Switchgrass, big bluestem, and indiangrass," In: Barnes, R. F., Miller, D. A., and Nelson, C. J. (eds.), *Forages, Vol. 1: An introduction to Grassland Agriculture*. Ames, IA: Iowa State University. p. 409–20.

Parrish, J. and Fike, J. H. (2005). "The biology and agronomy of switchgrass for biofuels," *Critical Reviews in the Plant Sciences* 24, 423-459.

Prasad, B., Carver, B. F., Stone, M. L., Babar, M. A., Raun, W. R., and Klatt, A. R. (2007). "Potential use of spectral reflectance indices as a selection tool for grain yield in winter wheat under great plain conditions," *Crop Sci*. 47, 1426-1440.

Peñuelas, J., Baret, F., and Filella, I. (1995). "Semi-empirical indices to assess carotenoids/ chlorophyll a ratio from leaf spectral reflectance," *Photosynthetica* 31, 221-230.

Samson, R. A., and Omielan, J. A. (1992). "Switchgrass: A potential biomass energy crop for ethanol production," In: The Thirteenth North American Prairie conference, Windsor, Ontario. p. 253-258.

Sanderson, M. A., Reed, R. L., McLaughlin, S. B., Wullschleger, S. D., Conger, B. V., Parrish, D. J., Wolf, D. D., Taliaferro, C., Hopkins, A. A., Ocumpaugh, W. R., Hussey, M. A., Read, J. C., and Tischler, C. A. (1996). "Switchgrass as sustainable energy crop," *Bioresource Technol*. 56, 87–93.

Shenk, J. S., Workman, J. J., Jr, and Westerhaus, M. O. (2001). "Application of NIR spectroscopy to agricultural products," In *Handbook of Near-Infrared Analysis, Second Edition*, D. A. Burns, and E.W. Ciurczak (eds.), Marcel Dekker, Inc. New York.

Sims, D. A., and Gamon, J. A. (2002). "Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages," *Remote Sensing of Environment* 81, 337-354.

Strachan, I. B., Pattey, E., and Boisvert, J. B. (2001). "Impact of nitrogen and environmental conditions on corn as detected by hyperspectral reflectance," *Remote Sensing of the Environment* 80, 213-224.

Sestak, Z. (1971). "Determination of chlorophylls a and b," In: *Plant Photosynthetic Production*, Sestak, Z., Catsky, J., and Jarvis, P. G. (eds.) Dr. W. Junk Publishers, The Hague. Pp. 672-701.

Starks, P. J., Coleman, S. W., and Phillips, W. A. (2004). "Determination of forage chemical composition using remote sensing," *J. Range Manage* 57, 635-640.

Taliaferro, C. M., and Hopkins, A. A. (1997). "Breeding and selecting of new switchgrass varieties for increased biomass production," Five year summary report. Oak Ridge National Laboratory, Oak Ridge, TN.

Tolbert, V. R., and Wright, L. L. (1998). "Environmental enhancement of US biomass crop technologies: Research results to date," *Biomass and Bioenergy* 15, 93–100.

Tsuchikawa, S., and Siesler, H. W. (2003). "Near-infrared spectroscopic monitoring of the diffusion process of deuterium-labeled molecules in wood. Part I: softwood," *Appl. Spectrosc*. 57, 667-674

Valdés, C., Andrés, S., Giráldez, F. J., García, R., and Calleja, A. (2006). "Potential use of visible and near infrared reflectance spectroscopy for the estimation of nitrogen fractions in forages harvested from permanent meadows," *J. Sci. Food Agric*. 86, 308-314.

Van Esbroeck, G. A., Hussey, M. A., and Sanderson, M. A. (2003). "Variation between Alamo and Cave-in-Rock switchgrass in response to photoperiod extension," *Crop Sci.* 43, 639-643.

Walsh, K. B., Guthrie, J.A., and Burnie, J. W. (2000). "Application of a commercially available, low cost, miniaturized NIR spectrometers to assess the sugar content of intact fruit," *Aust. J. Plant Physio*. 27, 1175-1186.

Article submitted: July 14, 2008; Peer review completed: Sept. 2, 2008; Revision accepted: Oct. 21, 2008; Published: Oct. 23, 2008.