2002

FT-NIR Spectroscopic Analysis of Nitrogen in Cotton Leaves

Mark R. Riley
University of Nebraska-Lincoln, mriley3@unl.edu

Loreto C. Canaves
University of Arizona

FT-NIR Spectroscopic Analysis of Nitrogen in Cotton Leaves

MARK R. RILEY* and LORETO C. CÁNAVES

The University of Arizona, Department of Agricultural and Biosystems Engineering, Room 403, Shantz Building, Tucson, Arizona 85721

Near-infrared spectroscopy was evaluated as a means to quantify the nitrogen content in fresh cotton leaves (*Gossypium hirsutum* L. var. Delta Pine 90) subjected to a factorial design experiment of varying nitrogen and water applications. Absorbance spectra were collected in the 10 000–4000 cm^{-1} (1000–2500 nm) region from fresh cotton leaves over a two month portion of the growing season. Total nitrogen content was quantified by a wet chemistry Kjeldahl method for validation purposes. Partial least-squares regression analysis, using an automated grid search method, selected the spectral region 6041 to 5651 cm^{-1} (1650–1770 nm) for analysis based on having the lowest standard error of prediction of total nitrogen content. This region includes protein spectral features. Nitrogen predictions resulted in a correlation coefficient of 0.83, and a standard error of prediction of 0.29% for nitrogen levels ranging from 3.1 to 5.2% total nitrogen. This approach has promise for providing rapid plant chemical analyses for cotton crop fertilization management purposes.

Index Headings: **Near-infrared spectroscopy; Nitrogen determination; Leaf spectroscopy; Partial least-squares regression.**

INTRODUCTION

Nitrogen is an essential element for plants and is required in relatively large amounts by most agricultural crops. Nitrogen (N) plays a major role in plant nutrition as a component of chlorophyll, amino acids, enzymes, hormones, and vitamins.[1] In arid areas such as Arizona, the soil N availability is restricted due to the very low soil organic matter content resulting in negligible free N. As a result, N must be made available to crops through multiple fertilizations applied throughout the growing season.[2] Cotton yield has been reported to be linearly correlated with leaf N concentration.[3] Most techniques currently used for quantifying N content of crops are based on removal of plant material followed by wet chemical analyses of plant tissue composition. These techniques are tedious, time-consuming, destructive, and are difficult to repeat enough times throughout the growing season to obtain a representative evaluation of the plant's N status over time or to provide predictive capabilities so that fertilizations may be applied proactively.

Management of proper fertilizer application to crops requires frequent measurement of the nitrogen status of plants. The ideal frequency may be as often as once a day.[3] Saranga and co-workers[3] studied changes in cotton leaf nitrogen content and reported that leaf nitrogen can decline substantially from day to day and even declined substantially within several individual days. In one case, leaf nitrogen dropped by 18% over a 24-hour period. The cause or pool of N responsible for this decline is not known. To reduce the decline in leaf nitrogen, this group applied a fertilization scheme based on daily measurements of cotton leaf nitrogen. These measurements permitted application of nitrogen on an as-needed basis, resulting in 73% lower total nitrogen application required to generate similar cotton yields as in unmonitored cotton management practice.

An alternative to the laborious wet chemical methods is potentially provided by spectroscopic analyses. Spectral features within the near-infrared (NIR) region have been shown to correlate with the chemical composition of dried and ground plant material.[4–9] Only a few groups have evaluated the use of spectroscopy on fresh plant material. Lacaze and Joffre[10] compared laboratory reflectance spectra of dried and fresh leaves of several woody plants to estimate concentrations of nitrogen, lignin, and lignocellulose. Ourcival and co-workers[11] collected reflectance spectra of helm oak leaves to assess anatomical parameters such as the leaf area index and tissue thickness along with leaf nitrogen content. Saranga and co-workers[3] used near-infrared spectroscopy (NIRS) of cotton leaves to guide N fertilization by correlating spectral features to fertilization requirements rather than quantifying the N content. Borjesson and co-workers[12] determined the amount of N taken up by plants through measurement of changes in the soil N as quantified by mineralization. Their data suggest that soil components influencing mineralization are spectrally active and impact N measurement.

Much of the previous research on plant spectroscopy employs reflectance-type measurements with plant samples that have been dried to ensure low-water content. Practical application of such an approach is hindered by the time required to dry the samples and clearly is not an ideal noninvasive scheme. Measurements of plant composition preferably should be performed *in situ* without removing plant material from the field. This could be accomplished either through reflectance-type measurements which primarily sample the plant surface or with absorbance measurements which sample the entire depth of the material. Baret and co-workers[13] showed that absorbance measurements can be more sensitive than reflectance measurements in the estimation of protein content of fresh leaves. Ning and co-workers[14,15] have also evaluated absorbance-type measurements on hydrated plant material.

Many of the previous spectroscopic methods employed to determine leaf N content have focused on a few specific light wavelengths. Most often reported to correlate to plant N are absorbance features at 6329, 6211, 5931, 5599, 5500, 4878, 4608, and 4600 cm^{-1}.[4,16–17] Nearly all

Received 19 November 2001; accepted 3 July 2002.
* Author to whom correspondence should be sent.

0003-7028/02/5611-1484$2.00/0

of these studies focused on dried plant matter. Frequencies of 6329, 5760, 5931, 5500, 4878, 4608, and 4600 cm^{-1} include protein absorbance features[16,19] and may correlate to plant N.

This paper presents an analysis of the relationship between the NIR absorbance [log(1/transmission)] of fresh cotton leaves and their N content. The cotton plants were grown under a Latin square design of high and low treatments of both fertilizer and water and were followed for two months. The work evaluates the potential use of NIR spectroscopy for analyzing N concentration of fresh cotton leaves as an alternative analysis tool to traditional wet chemical methods.

MATERIALS AND METHODS

Cotton plants were grown at the University of Arizona's Maricopa Agricultural Center, Maricopa, Arizona, during the 1999 cotton season. A one-hectare field was divided into sixteen plots and planted with cotton (*Gossypium hirsutum* L. var. Delta Pine 90). Each of the plots was approximately 22 × 22 m and conformed to a Latin square design. The 16 plots accommodated four treatments with four replications. The treatments consisted of two N levels (222 Kg/Ha (high "N") and 112 Kg/Ha (low "n")) combined with two water levels (an optimal irrigation scheme based on plant requirements (high "W") and 50% of the management-allowed depletion (low "w")). Nitrogen fertilizations were distributed on 5 applications on days of the year 97, 148, 162, 176, and 197. The field was irrigated and fertilized through use of a linear-move irrigation system that was designed to apply water and fertilizer at rates specific to the treatment types. More information on the field work can be found elsewhere.[19]

Leaf Nitrogen Analysis. Leaf samples from each plot were collected once a week during the normal fertilization window for this area, July 07 (DOY 188) to September 2 (DOY 245) of 1999, totaling 6 sets of samples. To sample the leaves, the criteria recommended by Pennington and Tucker[20] was followed, collecting the youngest, most fully expanded leaves. Forty leaves from each plot were collected and immediately placed in polyethylene bags, stored in a chilled insulated box, and carried to the laboratory (requiring a 2-h drive) for collection of absorbance spectra. Samples collected on each day were divided into two sets (1) leaf blades to be dried and ground for total N analysis (30 leaves) and, (2) leaf blades for spectroscopic measurements (10 leaves). Total N content was determined by the Kjeldahl method at IAS Laboratories (Phoenix, AZ). These N measurements were used as the actual or true N content of the leaves for partial least-squares (PLS) analyses.

Near-Infrared Spectroscopy (NIRS) Measurements. Near-infrared absorbance spectra of the leaves were collected for 6 of the leaf sampling dates using a Nicolet Magna 560 Fourier Transform Infrared (FT-IR) spectrometer (Thermo Nicolet Instrument Co., Madison, WI), equipped with a 50 W tungsten source, calcium fluoride (CaF_2) beamsplitter, and liquid nitrogen cooled indium antimony (InSb) detector. Absorbance spectra of two leaves per plot were collected over the NIR region from 10 000 to 4000 cm^{-1} (1000 to 2500 nm), with 128 coadded scans per sample and a spectral resolution of 1 cm^{-1}. Triplicate spectra were collected consecutively for each leaf.

After initial analyses on the 10 000 to 4000 cm^{-1} region, measurements focused on approximately the 6500 to 5500 cm^{-1} range and only this region was collected. An interference filter (K filter from Barr and Associates, Westford, MA) was used to isolate this spectral region specifically from 6410 to 5618 cm^{-1} (1560–1780 nm). A "B" neutral density screen (Thermo Nicolet Instrument Co., Madison, WI) was used to avoid detector saturation during collection of reference spectra. This screen transmits approximately 50% of the incident light and was removed prior to collection of leaf spectra.

A total of 564 spectra were collected over a period of 2 months. All spectra were collected at ambient temperature (~27 °C), with dry air purged into the sample compartment. To perform the absorbance-type measurements, an aluminum sample holder was designed to maintain samples in an upright position. A circular hole 1 cm in diameter was drilled in the holder to permit light transmission, and leaves were held in place over this hole by use of two elastic bands. A background spectrum of the empty leaf holder was collected every 90 min. No attempts were made to standardize leaf sample thickness.

Chemometric Analyses. The collected spectra were transferred to a Silicon Graphics O_2 workstation and analyzed using partial least-squares (PLS-1) regression analysis software developed and provided by Professor Gary Small from the Center for Intelligent Chemical Instrumentation in the Department of Chemistry at Ohio University. Spectra were randomly divided into calibration and prediction data sets with triplicate spectra always placed together. A large number of calibration models were developed by varying spectral range and number of PLS factors. Prediction errors for these data sets were computed as standard error of calibration (SEC) and standard error of prediction (SEP). Models were evaluated based on minimizing the prediction error obtained. Further details on the data analysis may be found elsewhere.[21,22]

To investigate the many possible combinations of calibration parameters, a C-shell computer script was used to systematically develop calibration models with varying numbers of PLS factors from 1 to 20 and with varying spectral ranges within the 6500–5500 cm^{-1} region. The script follows a modified grid search that permits the unbiased evaluation of many calibration data sets. For each total number of PLS factors, the script searches for spectral ranges that contain significant analyte information. SEP values are calculated initially for 100 cm^{-1} wide regions at 100 cm^{-1} intervals beginning with 5600–5500 cm^{-1}. The region with the lowest SEP is assumed to contain significant analyte information and becomes the focus of further evaluation. The upper and lower values of this range are increased and subsequently decreased by a predetermined amount and the corresponding SEP values calculated. The process of modifying the maximum and minimum frequencies and evaluating ranges is repeated four times; each ensuing iteration has a more narrow step change in the spectral range. The number of PLS factors is incremented and another spectral range search is implemented. The combination of spectral range and num-

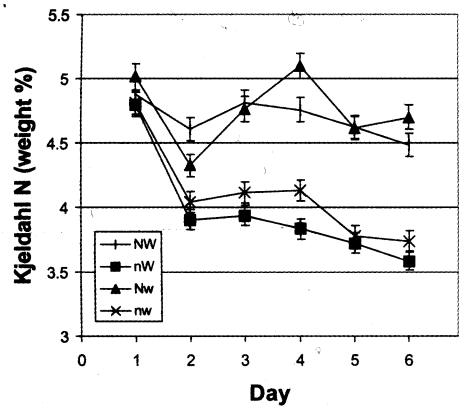

FIG. 1. Kjeldahl nitrogen analysis for different treatments by dates. **NW** (optimum N, optimum water), **Nw** (optimum N, low water), **nW** (low N, optimum water), and **nw** (low N, low water). Error bars correspond to ± one standard deviation in measurements.

ber of PLS factors that yields the minimum SEP is used to predict analyte concentrations in the prediction samples. The best calibration model for N was determined as the one with the lowest standard error of prediction as long as the model was not deemed overfitted due to use of too many PLS factors.

Samples were divided into calibration and prediction sets in several ways. One approach divided all the samples into calibration (approximately ¾ of samples) and prediction (the remaining ¼) sets. The calibration set of 140 samples consists of 420 spectra and the validation set has 48 samples, consisting of 144 spectra. Spectra were randomly assigned to the calibration and prediction sets; however, replicate spectra were always placed together. Samples were also independently divided by treatment and then into individual calibration and prediction sets. Separated by treatment, these calibration sets contained 35 samples (105 spectra) and prediction sets contained 16 samples (48 spectra). Calibrations employing different numbers of calibration samples can only be compared indirectly due to differing statistical power.

Three rounds of data processing were performed for each analysis set. For each round, sample spectra were randomly selected to be placed into calibration or prediction sets. Subsequent rounds selected alternative sample placements with the same number of samples in each set. Data presented here represent averages obtained from these analyses.

RESULTS AND DISCUSSION

Over the course of the cotton season, the impact of each treatment (high and low N, high and low water) on the N content and health of the cotton varied substantially. Figure 1 displays the average Kjeldahl N values of cotton leaves for each treatment for the six days analyzed. These "days" represent the sequence of our sampling days, each separated by approximately two weeks. A fertilization treatment was applied to all plants prior to Day 1 (DOY 188), producing similar N levels for this sampling date. Nitrogen levels in the N treatments were significantly different from Day 2 onward, while water treatments showed significant differences only once during the study period, on Day 4, due to an uncontrolled water stress that impacted all treatments. Beyond the first measurement day, low N treatments yield lower plant N content. As N content is related to cotton yield, it is desirable to increase such levels.

Near-infrared spectra of leaves collected over the 10 000 to 4000 cm^{-1} (1.0 to 2.5 μm) range showed small visual differences in absorbance features (not shown), with primary variations due to the water OH vibration located around 6900 and 5200 cm^{-1}. As light absorption by water was to be avoided for measurement of leaf N, quantitative studies focused in the region 6500–5500 cm^{-1}. N containing compounds have spectral signatures in this region including proteins features centered at 5495, 5760, 5931, and 6329 cm^{-1}.[18]

Calibration models were initially developed using the entire set of samples collected. Five hundred and sixty-four total spectra were randomly divided into calibration and prediction sets with replicate spectra placed in the same sets. Kjeldahl N concentration values were taken as true plant N content for comparison to NIRS measurements. A substantial number of calibration models were generated and evaluated for each model type using an automated grid search method to optimize spectral range and number of PLS factors. Three rounds of data processing with different distributions of samples into calibration and prediction sets were applied. Table I presents a summary of the average measurement results obtained.

For N contents varying from 3.22 to 5.22% by weight, reasonably accurate measurements are obtained using the full calibration set (see "Full set" in Table I). The models with the lowest SEPs required the spectral range from 6331–5791 cm^{-1} and 7 PLS factors. This spectral range encompasses the protein absorbance features at 5931 and 6329 cm^{-1}. These calibration models yielded an SEP val-

TABLE I. Measurement results using calibration models developed for each treatment in all days. ¾ of the spectra were used for calibration and ¼ for prediction. Correlation coefficients are calculated with an intercept through the origin (no bias).

Treatment	# of calibration samples	Range (cm^{-1})		PLS[a]	Calibration R^2	Prediction MPE[b]	Prediction SEP[c]	Prediction R^2	Nitrogen range (% of N)
Full set	140	6331	5791	7	67.8	5.41	0.287	78.8	3.22–5.22
(NW)	35	6240	5640	7	53.5	3.04	0.204	67.1	4.18–5.12
(nW)	35	6161	5951	5	37.1	9.37	0.489	29.5	3.44–5.22
(Nw)	35	6160	5950	4	45.8	4.52	0.254	27.8	4.13–5.21
(nw)	35	6010	5800	7	31.1	6.66	0.346	60.3	3.22–5.00

[a] Number of partial least-squares factors.
[b] Mean percent error.
[c] Standard error of prediction.

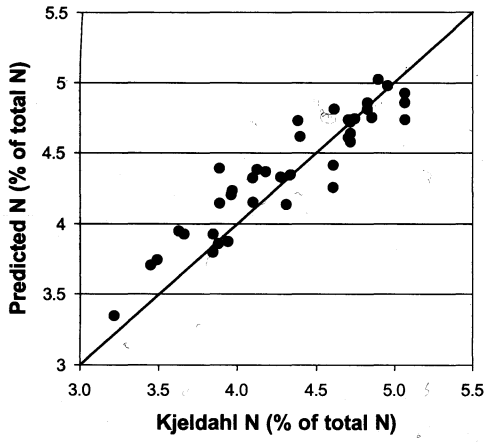

FIG. 2. Concentration correlation plot for the full set of samples. Calibration model parameters presented in Table I.

ue of 0.29% of N content. The prediction R^2 was calculated assuming no measurement bias and thus with the intercept through the origin. The mean percent error was only 5.41% of the measurement, representing fairly accurate measurements throughout the entire concentration range. Figure 2 presents a concentration correlation plot for the prediction samples. Fairly good agreement is obtained between the NIRS and Kjeldahl measurements, with close concurrence with the 45 degree line.

While fertilization management schemes are likely to focus on multiple plants within a field, a more appropriate means to evaluate these measurements would take into account the variation in N content from plant to plant within a treatment field. Figure 3 displays the average N content predictions from NIRS (with 95% confidence intervals) along with the average Kjeldahl values taken as averages for a specific treatment and sampling day. The NIRS technique performs well for most days and yields few significant differences between average NIRS measurements and average Kjeldahl measurements. High N treatments generally have a smaller variation in leaf N content throughout the season compared with the low N treatments. In the case of the NW and nw treatments, all days but one are satisfactorily evaluated by NIRS. For treatments nW and Nw, the NIRS measurements perform almost as well.

For most treatments, Day 4 presents a sizable deviation between measurement schemes. This difference is likely due to a severe water stress that occurred just prior to this day and affected all treatments, even ones that were to be given sufficient levels of water. NIRS poorly predicts the N content for the second day in treatment nW for no clear reason.

Of most interest with these measurements is the ability to predict N at low levels, thus necessitating addition of a fertilizer. This situation occurs with the low N treatments, particularly towards the end of the growing season. For the low N treatments, NIRS measurements for Days 5 and 6 are in very good agreement with the av-

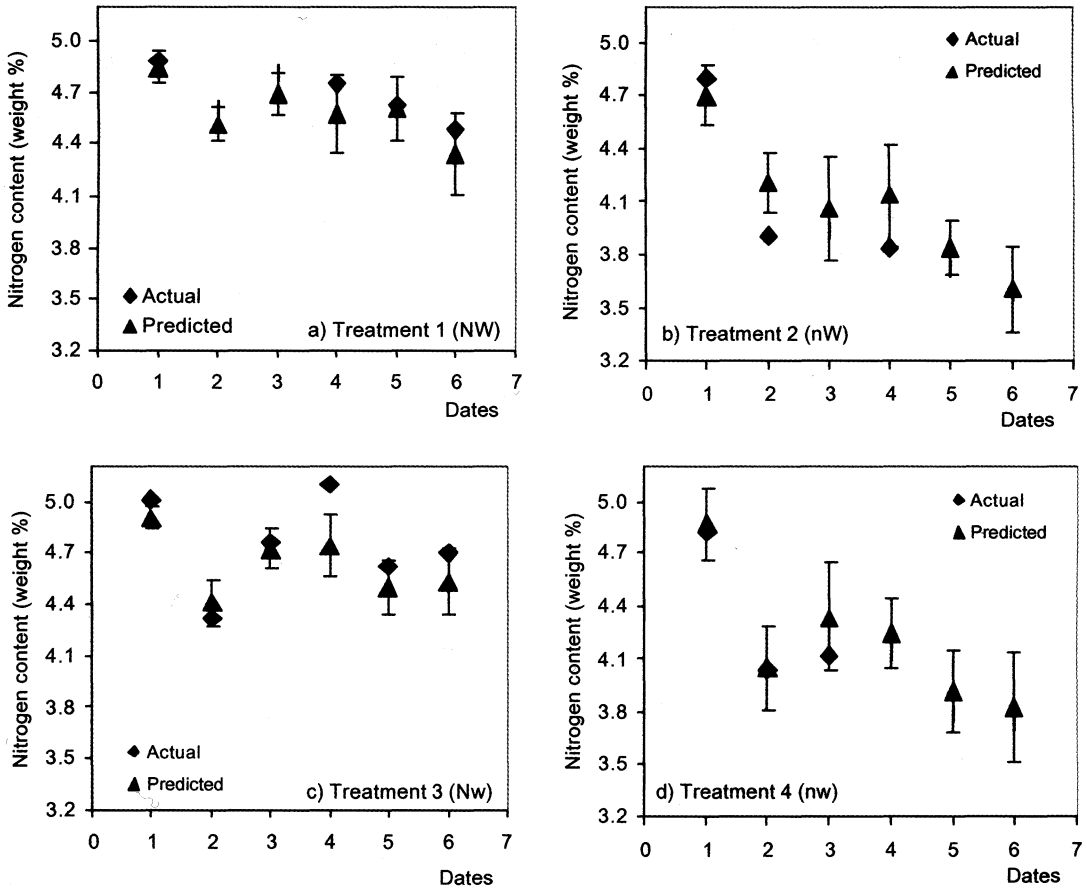

FIG. 3. Comparison of average spectroscopic measurements with average Kjeldahl measurements separated by treatments: (**a**) high N, high water; (**b**) low N, high water; (**c**) high N, low water; and (**d**) low N, low water. Error bars correspond to ± one standard deviation in measurements.

erage Kjeldahl measurements. NIRS is able to recognize substantial changes in N levels over the course of the cotton growing season.

Analyses were also performed on samples segregated by treatment types by subdividing leaf samples based on their treatment plot. In this case, only high N, high water samples were used as a calibration for other similar samples. The same approach was used for the other three treatments. Segregating samples by treatment type led to PLS models that were less successful than those composed of the entire set of samples (summarized in Table I). The less accurate measurements are likely due to the reduced number of samples present in the treatment types, resulting in poorer accounting for uncontrolled variations. The most accurate measurements based on SEP values were made with the high N level treatments. Fairly low values of SEP are obtained with 0.20 and 0.25 for the high N with high water and low water treatments, respectively. For these high N treatments, the range of N levels was narrow, which leads to low mean percent errors of only 3–4% for each treatment. The high N, low water treatment (Nw) yields a very low prediction R^2, and this is the result of measurement bias leading to overpredictions of the N content, particularly at the lower N levels within this treatment.

For the low N treatments, measurements are less accurate than those obtained for the high N treatments based on the SEP. Much of this increase in error can be attributed to inaccuracies in measurements at the lower end of the N concentration range. This results in low correlation coefficients for both the calibration and prediction sets, while having fairly low mean percent errors. Apparently, the large number of calibration samples as used in the full calibration set is required to accurately model the plant variations with low N levels. The full calibration set does not have the same measurement bias for low N measurements as do calibrations segregated by treatment type.

The automated grid search used to select wavelengths and numbers of PLS factors attempts to minimize prediction error by evaluating many spectral ranges for each number of PLS factors. The spectral regions selected typically include the 5931 cm^{-1} protein feature while some calibration sets also include the 5760 or 6329 cm^{-1} features. The two analyses segregated by treatment (nW and Nw) that do not include these features yield the poorest R^2 for prediction. The protein absorbance feature centered at 5931 cm^{-1} is fairly broad, extending from approximately 5880 to 5980 cm^{-1},[18] so some of this analytical information may be incorporated into these models. The spectral range centered at 6211 cm^{-1} has been used by other researchers to correlate NIR spectra to plant N content; however, this region has little corresponding protein spectral information. Other protein features, such as at 4878 cm^{-1}, suffer from strong absorbance due to water,[18] thus limiting its utility for plant monitoring. The spectral ranges and number of factors presented here are average values obtained from three rounds of independent sets of calibration and prediction samples.

The time of plant sampling for our NIRS measurements was selected based on the time in the growing season critical for management of N applications. Gerik[23] reported that cotton plants take up most of their N during a period of about 30 days, starting around flowering initiation (DOY 216, corresponding to our Day 2). According to this, sampling for the NIR technique beginning after Day 2 in our analysis could provide timely analysis of the plant N status. An ideal measurement scheme would likely provide measurements with a frequency of roughly once a day.

A potential limitation of the approach described here lies in the use of transmittance measurements without accounting for variation in leaf thickness, which is likely to change with treatment type and day of sampling. We have evaluated the impact of leaf thickness by comparing N predictions segregated by day of sampling. Similar measurement accuracies are obtained across the growing season, with the exception of Day 4, suggesting that variations in leaf thickness do not negatively impact our measurements. An analysis of the relationship between N content and single beam intensity for our transmission spectra reveals essentially no correlation ($R^2 = 0.04$). For comparison, the R^2 for N content from Kjeldahl analysis and N content from NIRS measurements is 0.86. Accounting for such differences presents an experimental challenge due to the variability between plants, but could also be addressed through data processing methods. Digital Fourier filtering of the spectra prior to PLS analysis has been shown to eliminate similar variations in sample thickness.[24] Digital Fourier filtering was applied to cotton leaf samples using the full calibration set in an attempt to remove such thickness variations; however, this method did not improve measurements or reduce error. This area of data analysis will be the subject of further study.

CONCLUSION

Near-infrared absorbance spectroscopy has been applied to determine the N content of fresh cotton leaves, presenting an alternative to traditional chemical analysis. The NIRS approach was reasonably robust in quantifying N content under several N and water treatments over 100 days of the cotton growing season. Nitrogen content could be quantified with mean percent errors of approximately 5.4%. Multiple spectral regions containing absorbance features of proteins provide the most accurate N concentration information in fresh plant tissue. Further studies are needed to identify the utility of other spectral regions.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Peter Waller, Dr. Christopher Choi, and Dr. Edward Barnes for their assistance on this project. We also would like to thank Dr. Julio Haberland, Emily Riley-Saxton, Rachel Wegge, and Eric Hein for collecting fresh leaves and processing samples. Partial financial support was provided by the Idaho National Environmental and Engineering Laboratory.

1. F. J. Stevenson, *Cycles of Soil. Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients* (John Wiley and Sons, New York, 1986), p. 100.
2. J. C. Silvertooth and T. A. Doerge, *Nitrogen Management in Arizona Cotton Production* (The University of Arizona, College of Agriculture, Tucson, AZ, 1990), Report 9024, p. 53.
3. Y. Saranga, A. Landa, Y. Shekel, A. Bosak, and U. Kafkafi, Agron. J. **90,** 16 (1998).
4. T. M. McLellan, M. E. Martin, and J. D. Aber, Can. J. For. Res. **21,** 1689 (1991).

5. L. Velasco and C. Möllers, Commun. Soil Sci. Plant Anal. **31,** 2987 (2000).
6. D. Gillon, C. Houssard, and R. Joffre, Oecologia **118,** 173 (1999).
7. C. A. Wessman, J. D. Aber, D. L. Peterson, and J. M. Melillo, Can. J. For. Res. **18,** 6 (1988).
8. J. A. Hattey, W. E. Sabbe, G. D. Baten, and A. B. Blakeney, Commun. Soil Sci. Plant Anal. **25,** 1855 (1994).
9. R. A. Isaac and W. C. Johnson, J.—Assoc. Off. Anal. Chem. **67,** 506 (1984).
10. B. Lacaze and R. Joffre, J. Plant Physiol. **144,** 277 (1994).
11. J. M. Ourcival, R. Joffre, and S. Rambal, New Phytologist **142,** 351 (1999).
12. T. Borjesson, B. Stenberg, B. Linden, and A. Jonsson, Plant Soil **214,** 75 (1999).
13. F. Baret and Th. Fourty, "Radiometric Estimates of Nitrogen Status of Leaves and Canopies," in *Diagnosis of the Nitrogen Status in Crops,* G. Lemaire, Ed. (Springer Verlag, Berlin, Germany, 1997), p. 201.
14. L. Ning, L. S. Daley, W. J. Bowyer, E. H. Piepmeier, G. A. Strobel, and J. B. Callis, Spectroscopy **11,** 34 (1996).
15. L. Ning, L. S. Daley, W. J. Bowyer, E. H. Piepmeier, G. A. Strobel, and J. B. Callis, Spectroscopy **11,** 68 (1996).
16. R. F. Kokaly and R. N. Clark, Remote Sens. Env. **67,** 267 (1999).
17. L. F. Johnson and C. R. Billow, Int. J. Rem. Sens. **17,** 489 (1996).
18. K. H. Norris (ASAE Annual International Meeting, St. Joseph, MI, 2000).
19. L. C. Canaves, M.S. Thesis, The University of Arizona, Tucson, AZ (2001).
20. D. A. Pennington and T. C. Tucker (The University of Arizona, College of Agriculture, Tucson, AZ, 1984), Report 8373, p. 20.
21. M. R. Riley, H. M. Crider, M. E. Nite, R. A. Garcia, J. Woo, and R. M. Wegge, Biotechnol. Prog. **17,** 376 (2001).
22. M. R. Riley, M. A. Arnold, and D. W. Murhammer, Appl. Spectrosc. **54,** 255 (2000).
23. T. J. Gerik, B. S. Jackson, C. O. Stockle, and W. D. Rosenthal, Agron. J. **86,** 514 (1994).
24. G. Lu, X. J. Zhou, M. A. Arnold, and G. W. Small, Appl. Spectrosc. **51,** 1330 (1997).