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ENGINEERING AND GINNING

Cotton Leaf Reflectance Changes after Removal from the Plant

J. Alex Thomasson and Ruixiu Sui*

ABSTRACT

It is important in precision agriculture research, particularly in projects involving remote sensing, to collect a large number of field measurements on the optical properties of agricultural materials such as plant leaves. In making these measurements, maintaining a high level of accuracy and at the same time being expeditious are critical. A reasonable compromise would be to quickly collect a large number of field samples, store them appropriately during transit to the lab, and then make measurements with a laboratory-grade spectrophotometer. Concern arises, however, on the possibility that several hours of storage could significantly change the reflectance spectra of living plant material after removal from plants. Therefore, a study was conducted to quantify the change in reflectance spectra of cotton leaves over a period of time after picking. Cotton leaf samples were picked from cotton plants and then bagged in plastic zipper bags and stored in a cooler with ice underneath the samples. Reflectance spectra of cotton leaf samples were collected at 15, 180, 360, and 840 minutes after the leaves were picked with a UV/Vis/NIR (ultraviolet, visible, and near-infrared) spectrophotometer in the wavelength region from 250 to 2000 nm. Spectral data were analyzed with SAS® software to evaluate spectral changes of the leaf samples with time. It was concluded that, while certain cotton leaf reflectance spectra can be expected to change to a statistically significant degree within the first 14 h after removal from the plant, the changes are not large over that period of time. It is apparently reasonable to use a laboratory-grade spectrophotometer to measure cotton leaf spectral reflectance from 250 to 2000 nm within the first 14 h after having removed the leaves from the plant, assuming the leaves are properly stored.

Precision agriculture can be defined as optimizing inputs with respect to outputs on each unit area of a given agricultural field. Successful precision agriculture has three requirements: (1) collecting accurate data about important characteristics and conditions at various locations in the field, (2) understanding the relationships between these data and input/output interactions in the field, and (3) varying the input application rate by location.

Data collection is important to precision agriculture, and optical sensors are often appropriate for large-scale and rapid data collection. Optical data of a field can be obtained either with ground-based sensors or with sensors at a considerable distance, such as those onboard an aircraft or satellite. Deriving information about an object from a distance is known as remote sensing, which most frequently involves measuring electromagnetic energy emanating from an object of interest (Swain and Davis, 1978). Remote sensing has shown great potential for applications in agriculture, e.g., used to identify crop types and plant stresses, to measure land area and surface water resources, and to predict crop yield. Some recent applications of remote sensing and other optical sensing technologies in precision agriculture research follow.

Several studies have related spectral reflectance of crops to agricultural production applications such as yield estimation and plant growth condition assessment. Ma et al. (1996) found that the canopy reflectance of maize was strongly correlated with canopy spectral reflectance at almost all growth stages. Both canopy reflectance and field greenness measured prior to anthesis were correlated with yield at harvest. Gopalapillai et al. (1998) reported that canopy reflectance of corn 75 d after planting was correlated to nitrogen (N) application and yield. The yield could be predicted fairly accurately from the canopy reflectance of red light. Wooten et al. (1999) used multispectral satellite images to predict water stress and yield in cotton. They determined that both ground observations and yield were correlated with in-season multispectral satellite images. In subsequent work, Thomasson et al. (2000) found that average cotton yield over a 30-m square area

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J.A. Thomasson and R. Sui, Biological and Agricultural Engineering Department, Texas A&M University, College Station, Texas 77843
*Corresponding author: rsui@tamu.edu

correlated well ($R^2 = 0.88$) with the average value of Landsat picture elements (pixels) representing the same ground area. Yang et al. (2006a, 2006b) evaluated QuickBird satellite imagery for mapping yield variability in grain sorghum and cotton fields as compared with airborne multispectral imagery. Their results showed that both types of imagery had similar correlations with yield.

In addition to being an indicator of yield, reflectance measurements may provide an in-season indication of crop growth conditions such as nutrient deficiency and water stress. Thenkabail et al. (2000) used spectral data in 490 discrete narrow bands between 350 and 1050 nm to determine appropriate bands for characterizing biophysical variables of various crops including cotton. Sembiring et al. (1998) detected N and phosphorus (P) nutrient status in winter wheat with spectral reflectance. Their results indicated that the normalized difference vegetation index (NDVI) was a good predictor of the amount of biomass and N and P uptake for winter wheat. Shibayama et al. (1993) studied spectral reflectance of rice canopies in the visible, near infrared (NIR), and mid-infrared (mid- IR) ranges. Results indicated that high spectral-resolution reflectance measurements and their first derivatives in NIR and mid-IR offer promise for early detection of water stress in rice canopies. Min and Lee (2005) investigated spectral wavebands for predicting N content for citrus. They collected 1000 orange leaves from an orange grove and measured their spectral reflectance using a spectrophotometer in the lab.

Working exclusively in cotton, Lough and Varco (2000) evaluated the relationship between N treatment level and relative leaf reflectance, and found that the greatest separation between N treatments occurred at the 550-nm (green) waveband. According to Buscaglia and Varco (2002), cotton leaf N concentration had a strong linear correlation with leaf reflectance at 550, 612, 700, and 728 nm. Tarpley et al. (2000) found that reflectance ratios, calculated by dividing cotton leaf reflectance at 700 or 760 nm by a higher wavelength reflectance (755 to 920 nm) could provide accurate predictions of N concentration. Read et al. (2002) reported that remote sensing of N status in cotton was feasible with narrow-waveband reflectance ratios involving a violet-to-blue spectral band and the more commonly studied red-edge region. Wilkerson et al. (1998) found that important plant health information about cotton canopies could be found in the following spectral wavebands: 460– 490 nm, 545–565 nm, 600– 610 nm, and 740– 770 nm. Plant et al. (2000) investigated the relationships between remotely sensed reflectance data and cotton growth and yield. The results demonstrated that NDVI integrated over time showed a significant correlation with lint yield. Sui et al. (1998) and Thomasson et al. (2004) developed an optical plant health sensor for diagnosing cotton plant health. The sensor measured reflectance of the cotton canopy in four visible and NIR wavebands. The results showed that spectral reflectance of the cotton canopy was closely correlated with yield and nitrogen stress.

Objectives. For the purpose of providing ground-truth data in remote-sensing experiments and examining treatment effects, reflectance spectra of cotton leaves are often collected in the field with handheld spectroradiometers. The least accurate of these instruments use solar radiation as their energy source. The most accurate have internal energy sources. Handheld spectroradiometers are often acceptable for field data collection, but their spectral resolution and accuracy are inferior to laboratory-grade UV/Vis/NIR spectrophotometers. Because of the need to collect reflectance spectra on cotton leaves as accurately and with as much spectral resolution as possible, experiments were conducted with a laboratory-grade UV/Vis/NIR spectrophotometer. A potential problem with this data-collection method is related to the time required to collect samples, transport them from field to laboratory, and prepare them for analysis. The objective of this study was to quantify changes in the reflectance spectra of cotton leaves occurring during the time immediately after picking and the next few hours.

MATERIALS AND METHODS

Sample preparation and data collection. Five mature, randomly selected leaves from several individual cotton plants in the pinhead-square growth stage were handpicked and served as samples for spectral measurements. The plant cultivar used was ‘NuCOTN 33B’ (Delta and Pine Land Co., Scott, MS, PVP 9500109) a mid- to full-season Upland type grown at the Mississippi State University’s Plant Science Research Center, Mississippi State, MS. After picking, the leaf samples were immediately placed in plastic zipper bags and stored in a cooler with ice underneath the bags. A black plastic leaf holder (10-mm thick, 38-mm diameter) was used to present samples to the spectrophotometer. The samples were prepared for spectral measurement by cutting a round, 38-mm diameter piece from the center of the leaf. The cut leaf edge was taped onto a sample holder for measurement. After each measurement, the prepared leaf sample was placed back in the zipper bag and stored in the cooler until the next measurement. The cooler was stored at room temperature. All leaf samples were measured after picking at multiple time intervals: 15, 180, 360, and 840 min. The first measurement was made 15 min after the leaves were picked because it took about 15 min to transport leaf samples from field to laboratory. Based on the results of a brief investigation by the authors in 1999 prior to this study it was known that the spectral response of cotton leaves did not change significantly within the first 15 min after they were removed from the plant.

Diffuse reflectance spectra of the prepared leaf samples were collected with a Cary 500 UV/Vis/NIR spectrophotometer (Varian Inc., Palo Alto, CA). This spectrophotometer is equipped with a diffuse reflectance accessory that uses an integrating sphere to collect almost all the reflected radiation, remove any directional preferences, and present an integrated signal to the detector. Before collecting leaf reflectance spectra and after instrument warm up, a spectral reflectance baseline was recorded with a reference disk covering the sample port of the diffuse reflectance accessory. The reference disk was a manufacturer-provided, secondary-white-standard, polytetrafluoroethylene (PTFE) disk calibrated relative to a perfectly diffuse reflector.

Each prepared leaf sample was pressed against the sample port of the diffuse reflectance accessory, and the sphere collected the energy reflected from the leaf sample surface. The standardized spectral reflectance of a sample was calculated as the ratio of the flux reflected by the sample to that reflected by the reference disk under identical geometrical and spectral-illumination conditions. The collected leaf spectra for each sample consisted of 845 reflectance values, each with an averaging time of 0.1 sec, at wavelengths from 250 to 2000 nm. The spectral resolution selected in the 250- to 792-nm range was 1 nm, and that used in the 792- to 2000-nm range was 4 nm.

Data analysis. Data analysis was divided into two parts. In the first part, each sample spectrum was divided into 35 wavebands, each 50 nm wide. By dividing the spectrum in this manner the effect of storage time over broader spectral bands rather than those at the spectral resolution of the data could be analyzed. This is important for two reasons: (1) sensor development often employs optical filters with bandwidths in the 10- to 100-nm range, so broad-band effects could be consider; and (2) files of spectral data include samples of a magnitude that does not allow rapid computer analysis at the spectral resolution of the data (i.e., some reduction of data volume and smoothing of spectral data is often required prior to analysis). The original spectrum was normalized by the reflectance value at 450 nm as a reference value. The normalized reflectance values within each waveband were integrated over 50 nm. The integrated values at each 50-nm waveband were then analyzed with the SAS® procedure, PROC MIXED (SAS®, 1999), to determine whether the reflectance values were significantly related to the time interval after picking. Furthermore, mean normalized reflectance values were compared among sampling times with the SAS procedure, PROC GLM with Tukey's studentized range test.

In the second part of the analysis, all 845 wavebands were examined for correlation with time after picking. This analysis was conducted with the SAS® procedure, PROC MIXED. In addition, the coefficient of variation at each waveband was calculated over all sample measurements. This was done to determine whether the spectral location of correlated bands was associated with relatively high amounts of variability over all sample measurements.

RESULTS AND DISCUSSION

Spectra collected from the cotton leaf samples at increasing intervals of storage time are shown in Fig. 1A. Fig. 1B is the 1st derivative of the spectra. Each spectrum represents the average of five replicates at the given time interval. The noise evident near 800 nm was caused by a change in detectors that occurred within the spectrophotometer at roughly 800 nm. It is clear that the four spectra shown are very close to each other. No great difference could be identified visually, although it appeared that some of the reflectance curves were 1 to 2% different at certain portions of the spectrum.

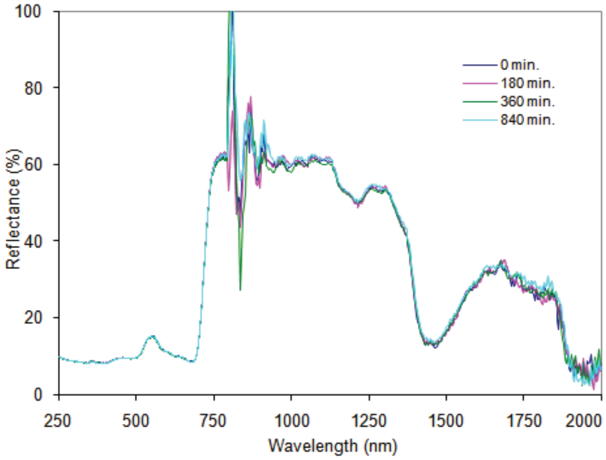

Figure 1A. Reflectance spectra of cotton leaves with varying storage time period after picking from plants.

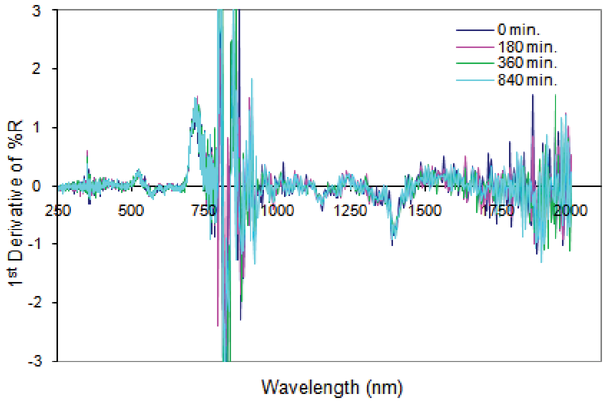

Figure 1B. First derivative of the reflectance spectra.

Results of the 50-nm bandwidth statistical analysis are given in terms of significance probabilities in Table 1, which shows that treatment effects were not statistically significant at the 5% level except for the wavebands centered at 1775 and 1825 nm. These bands are known to be strongly related with absorption of electromagnetic energy by water, so it is likely that the primary effect in these cases was related to loss of water from the leaf during the storage period (Rencz, 1999). The effects of storage time were significant at the 10% level on two other wavebands (1875 and 1925). Means at each 50-nm waveband were not significantly different between one sampling time and another except in two cases: at 925 and 1925 nm there was a significant difference between the means at the 180- and 840-min sampling times. Even at these two wavebands, 6 h of careful storage did not have a significant effect.

Table 2 includes those individual spectral bands that exhibited significant correlations at the 10%, 5%, and 1% levels. Only two wavelengths were significantly correlated with storage time at the 1% level, those centered at 1788 and 1864 nm. The ability to predict storage time based on spectral data at these two wavelengths is presented in Fig. 2. Correlation is evident, and thus reflectance change over time at these wavelengths is somewhat predictable. A number of other wavelengths exhibited significant correlations when the criterion was lowered to 5% or 10%. The coefficient of variation (CV) of percent reflectance at each spectral band is shown in Fig. 3. In relative terms, the CV was particularly low from 250 to 800 nm, high from 800 to 900 nm, low to moderate from 900 to 1850 nm, and high from 1850 to 2000 nm. It can be inferred from knowledge of the instrument's operation with regard to changing detectors that much of the cause for the high CV around 800 nm was related to noise. However, several wavelengths in the high CV range from 800 to 900 nm were significantly correlated to storage time at the 5% level. The two wavelengths that significantly correlated at the 1% level and most of the rest of those significantly correlated at the 5% level

Table 1. Significance probabilities for correlations between 50-nm spectral bands and time after picking.

Band (nm)	*P*	Band (nm)	*P*	Band (nm)	*P*	Band (nm)	*P*
275	0.9978	325	0.9922	375	0.8300	425	0.8482
475	0.9150	525	0.7729	575	0.7856	625	0.8996
675	0.9277	725	0.9968	775	0.9751	825	0.1546
875	0.9322	925	0.4440	975	0.9560	1025	0.9786
1075	0.9889	1125	0.9881	1175	0.9931	1225	0.7191
1275	0.9804	1325	0.9956	1375	0.6498	1425	0.3613
1475	0.3672	1525	0.3881	1575	0.8449	1625	0.6272
1675	0.8461	1725	0.2178	1775	0.0466	1825	0.0468
1875	0.1039	1925	0.0583	1975	0.2878		

were in the moderate CV range of 1300 to 1900 nm. Based on this analysis, a significant portion of the variability in the moderate CV range and the high CV range from 800 to 900 nm was related to the effects of storage time. Also, the high amount of variability from 1900 to 2000 nm was either related to measurement error or other factors unrelated to storage time.

Table 2. High-resolution cotton leaf reflectance spectra that are correlated with time after picking at three levels of significance probabilities.

Spectral bands (nm) significant at 10%	Spectral bands (nm) significant at 5%	Spectral bands (nm) significant at 1%
369	361	1788
377	808	1864
804	832	
812	836	
844	840	
1392	848	
1400	852	
1404	1396	
1464	1468	
1700	1696	
1720	1736	
1752	1740	
1796	1784	
1800	1828	
1816	1832	
1820	1860	
1880		
1944		
1964		

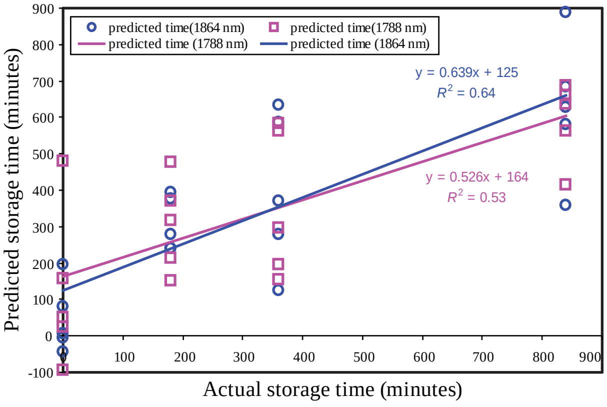

Figure 2. Predictions of storage time based on spectral data at the two wavelengths most highly correlated with storage time.

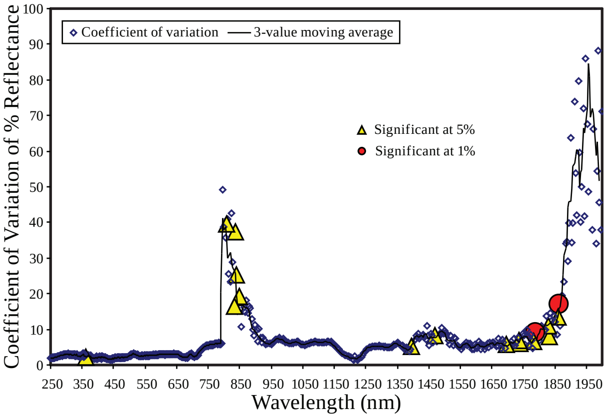

Figure 3. Coefficient of variation of percent reflectance of cotton leaves with varying storage time period after picking from plants.

CONCLUSIONS

Based on analyses of spectral reflectance data from cotton leaves stored for different periods after being removed from the plant, the following conclusions can be drawn:

- Only two 50-nm bands (1775 and 1825 nm center wavelengths) between 250 and 2000 nm were significantly correlated with storage time at the 5% level, and both of these bands are related to energy absorption by water.
- Two other 50-nm bands (1875 and 1925 nm) were significantly correlated with storage time at the 10% level.
- The mean values of two 50-nm bands (925 and 1925) were significantly different between the 180- and 840-min sampling times.
- Only two wavelengths from the original data (1788 and 1864 nm) were significantly correlated with storage time at the 1% level, whereas several other wavelengths were significantly correlated at the 5% and 10% levels.
- Areas of the spectrum where the measurement CV was moderate to high included bands that were correlated with storage time, but some high CV areas had no correlation.
- Even at the wavelengths where strong correlations existed between storage time and reflectance, the change in reflectance over the time period considered (15 to 840 min) was barely detectable upon visual interpretation of reflectance curves.

Therefore, although certain cotton leaf reflectance spectra can be expected to change to a statistically significant degree within the first 14 h after removal from the plant, the changes are not large over that period of time. The changes relate mainly to moisture loss and can be somewhat predictable at the wavelengths most affected. Further, it is apparently reasonable to use a laboratory-grade spectrophotometer to measure cotton leaf spectral reflectance from 250 to 2000 nm, within the first 14 h after having removed the leaves from the plant, if the leaves are stored appropriately. If one were taking extra care, a storage time of 6 h might be used as a maximum, as indicated by (1) differences in means at 925 and 1925 nm between the 3-h and 14-h sampling times, and (2) lack of any other differences.

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