REMOTE SENSING OF COTTON NITROGEN STATUS USING THE CANOPY CHLOROPHYLL CONTENT INDEX (CCCI)

D. M. El-Shikha, E. M. Barnes, T. R. Clarke, D. J. Hunsaker, J. A. Haberland, P. J. Pinter Jr., P. M. Waller, T. L. Thompson

ABSTRACT. Various remote sensing indices have been used to infer crop nitrogen (N) status for field-scale nutrient management. However, such indices may indicate erroneous N status if there is a decrease in crop canopy density influenced by other factors, such as water stress. The Canopy Chlorophyll Content Index (CCCI) is a two-dimensional remote sensing index that has been proposed for inferring cotton N status. The CCCI uses reflectances in the near-infrared (NIR) and red spectral regions to account for seasonal changes in canopy density, while reflectances in the NIR and far-red regions are used to detect relative changes in canopy chlorophyll, a surrogate for N content. The primary objective of this study was to evaluate the CCCI and several other remote sensing indices for detecting the N status for cotton during the growing season. A secondary objective was to evaluate the ability of the indices to appropriately detect N in the presence of variable water status.

Remote sensing data were collected during the 1998 (day of year [DOY] 114 to 310) and 1999 (DOY 106 to 316) cotton seasons in Arizona, in which treatments of optimal and low levels of N and water were imposed. In the 1998 season, water treatments were not imposed until late in the season (DOY 261), well after full cover. Following an early season N application in 1998 for the optimal (DOY 154) but not the low N treatment, the CCCI detected significant differences in crop N status between the N treatments starting on DOY 173, when canopy cover was about 30%. A common vegetation index, the ratio of NIR to red (RVI), also detected significant separation between N treatments, but RVI detection occurred 16 days after the CCCI response. After an equal amount of N was applied to both optimal and low N treatments on DOY 190 in 1998, the CCCI indicated comparable N status for the N treatments on DOY 198, a trend not detected by RVI. In the 1999 season, both N and water treatments were imposed early and frequently during the season. The N status was poorly described by both the CCCI and RVI under partial canopy conditions when water status differed among treatments. However, once full canopy was obtained in 1999, the CCCI provided reliable N status information regardless of water status. At full cotton cover, the CCCI was significantly correlated with measured parameters of N status, including petiole NO$_3$-N ($r = 0.74$), SPAD chlorophyll ($r = 0.65$), and total leaf N contents ($r = 0.86$). For well-watered cotton, the CCCI shows promise as a useful indicator of cotton N status after the canopy reaches about 30% cover. However, further study is needed to develop the CCCI as a robust N detection tool independent of water stress.

Keywords. Canopy reflectance, Fertility detection, Radiometers, Spectral analysis, Water stress.

The efficient application of fertilizer improves economic returns of cultivated crops. Proper management confines nutrients within the root zone of the crop, protecting the underlying groundwater.

Over-fertilization of some crops like cotton can be counterproductive by promoting vegetative rather than reproductive growth (Maples and Keogh, 1971) and reducing harvestable yields (Pennington and Tucker, 1984). Under-fertilization can also reduce quality and yield (Bondada et al., 1996). Most N fertilizer recommendations are based on tissue NO$_3$-N concentrations determined from destructive sampling of a specific part of the crop (i.e., leaf petioles in cotton, lower stalk in corn, lower stem in wheat, etc.). Samples are usually collected from a number of locations within a field, and then combined so that a single N recommendation is obtained for the entire field. However, obtaining efficient fertilizer management based on average field conditions represents a significant challenge to many growers because N requirements often have extensive spatial variability (Pan et al., 1997). A method to determine crop N status in the field involves the use of a hand-held SPAD chlorophyll meter (Minolta SPAD 502 meter, Spectrum, Inc., Plainfield, Ill.; Peterson et al., 1993). Since leaf chlorophyll content is mainly determined by N availability (Filella et al., 1995; Osborne et al., 2002), the detection of crop N stress using a chlorophyll meter could be a useful tool to maintain an adequate N supply by fertilizing as needed (Blackmer and Schepers, 1994, 1995). The meter is clamped to a plant leaf, and a relative measure of chlorophyll concentration is displayed. The SPAD readings are also taken in a reference area where the...
crop is known to have ample N. The ratio of the field measurement to the ample N measurement forms a “sufficiency index,” which is used to infer the N status of the crop. Pinter et al. (1994) found that the SPAD meter could provide estimates of chlorophyll concentration in cotton if properly calibrated. However, the number of SPAD readings needed to adequately determine variable application rates might not be feasible with a hand-held meter. Another limitation to practical application of SPAD may be that many farmers would not expend the extra time and effort to plant ample N reference strips in their fields, which is vital for this method. Thus, a definitive solution that uses an absolute measure instead of a relative comparison is more desirable and practicable.

Remote sensing provides field-scale diagnostic methods that could enable detection of variable canopy N status of plants within fields (Pinter et al., 2003) without the reference strip requirement. A number of studies have examined the use of various reflectance factors and spectral indices to infer crop N status (e.g., Bausch and Duke, 1996; Blackmer et al., 1994; Reed et al., 2002). One of the primary approaches uses vegetation indices that combine near-infrared (NIR) and red canopy reflectance factors to assess whole-canopy N status (Stone et al., 1996; Raun et al., 2001). This approach assumes that the variation in canopy density, as described by the vegetation index, is primarily related to plant-available N. There has also been considerable interest in detecting chlorophyll content at the leaf scale using the red edge position (REP) between the strong red light absorption by chlorophyll and the highly reflective NIR wavelengths in plant canopies (Gates et al., 1965; Collins, 1978; Horler et al., 1983; Jago et al., 1999). For detecting chlorophyll content, the REP has been exploited to measure the change in reflectance in the red edge (far-red) wavelengths (Tarpley et al., 2002; Blackmer et al., 1994; Reed et al., 2002). One limitation of these aforementioned leaf-scale approaches is that changes in canopy density can dominate spectral response. This makes it difficult to relate spectral variations to other crop properties, especially for incomplete canopy cover, where reflectance measurements are subject to interference from the soil background. Thus, the methodology requires full canopy cover in the sensor’s field of view, and a spectrometer with a fine enough spectral resolution to determine the REP.

Vegetation indices developed from spectral observations in the red and NIR are highly correlated with plant variables such as chlorophyll content, percent canopy cover, above-ground phytomass, and yield (Rouse et al., 1973). Vegetation indices have been used to determine nutrient status and infer fertilizer application rate (Blackmer et al., 1994; Schepers et al., 1996; Stone et al., 1996). A particular form of vegetation indices, such as ratio vegetation index (RVI), can track deviations of various plant growth parameters from normal conditions. However such indices have not yet been proven useful for distinguishing among various possible plant stress factors.

A promising approach to measure cotton nitrogen status was suggested by Barnes et al. (2000) and Clarke et al. (2001). This approach involves predicting N–sufficient and N–stressed limits as a function of crop cover and then calculating a normalized index between these limits to form what is referred to as the Canopy Chlorophyll Concentration Index (CCCI). The approach is similar to two–dimensional methods used to account for canopy cover in the determination of crop water stress (Moran et al., 1994; Clarke, 1997). Rodríguez et al. (2006) and Fitzgerald et al. (2006) demonstrated the CCCI to be an accurate indicator of spatial N status for wheat, having great independence of water status and canopy density. Kozerszwezi et al. (2003) examined the spatial resolution requirements of this index for cotton. Remote sensing data collected during two cotton experiments in Maricopa, Arizona, were used to derive CCCI and several other remote sensing indices. The CCCI was also evaluated for broccoli and reported to provide reliable broccoli N status information, regardless of water status (El–Shikha et al., 2007).

The primary objective of this article is to evaluate the ability of these remote sensing indices to detect cotton N status. A secondary objective was to evaluate the extent of the confounding effects of limited water on the ability of the indices to distinguish N status.

**Materials and Methods**

Experiments were conducted during two cotton seasons (1998 and 1999) on a 1.3 ha field site at the University of Arizona’s Maricopa Agricultural Center, located approximately 40 km south of Phoenix (33° 04’ 21” N, 111° 58’ 45” W, and 361 m above sea level). The site is in an arid area, receiving an annual average of only 185 mm of rainfall, with maximum summer temperatures ranging from 32°C to 46°C. The soil type in the field is Casa Grande (reclaimed, fine-loamy, mixed, superactive, hyperthermic Typic Natrargids). A full-season cotton (*Gossypium hirsutum*, cv. Delta Pine 90b) was planted on 24 April 1998 and on 16 April 1999 in east–west oriented beds, spaced 1.0 m apart. The cotton seed was planted in a single line on beds at a rate of 10 plants m⁻². Before each planting, multiple composite surface soil samples were collected from the field and analyzed to determine the residual nutrient contents in the top 0.3 m of soil. Based on local recommendations for cotton (Doerge et al., 1991), residual soil N content (98 kg N ha⁻¹) precluded the need for a preplant application of N prior to the 1998 season. However, the residual soil N content (22 kg N ha⁻¹) prior to the 1999 season was insufficient for early season development. Consequently, a uniform application of 34 kg N ha⁻¹ was incorporated into the field soil prior to planting in 1999 (table 1).

The field site was divided into sixteen 22 × 22 m plots, separated from one another by unplanted 2 m wide strips. The experimental design was a two–factor, water × nitrogen, Latin

<table>
<thead>
<tr>
<th>Date</th>
<th>DOY</th>
<th>Optimal N</th>
<th>Low N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998 Cotton</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 Mar.</td>
<td>89</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>3 June</td>
<td>154</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>9 July</td>
<td>190</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>188</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>1999 Cotton</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 Mar.</td>
<td>85</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>7 Apr.</td>
<td>97</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>28 May</td>
<td>148</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>11 June</td>
<td>162</td>
<td>43</td>
<td>24</td>
</tr>
<tr>
<td>25 June</td>
<td>176</td>
<td>71</td>
<td>24</td>
</tr>
<tr>
<td>16 July</td>
<td>197</td>
<td>46</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>244</td>
<td>134</td>
<td></td>
</tr>
</tbody>
</table>

[a] Pre-plant soil analysis (available residual N).
[b] Pre-plant application.
square with four treatments and four replicates. Treatments consisted of two levels of water application [optimal (W) and low (w)] and two levels of N application [optimal (N) and low (n)]. One treatment, WN, received both optimal water and N and served as the control treatment. Additional treatments consisted of optimal water and low N (Wn), low water and optimal N (wN), and both low water and low N (wn). For both cotton seasons, irrigation scheduling for the W treatment was based on daily crop evapotranspiration estimates calculated using the crop coefficient-reference evapotranspiration methodology presented in FAO-56 (Allen et al., 1998).

A linear-move irrigation system, which would eventually enable variable water applications for treatment plots, was under construction during the 1998 season and was not operable until September 1998, after irrigation was essentially complete. Consequently, plots in the 1998 season were surface-irrigated and all plots received the same amount of water application during nine surface irrigations events. When the linear-move irrigation system became operable in September 1998, three late-season water applications were given to the W treatment but not to the w treatment (table 2). For the 1999 season, only the linear-move system was used to irrigate plots. Drop hoses on the linear-move system applied water directly to furrows between the raised beds. Solenoid-controlled boom sections below the main overhead pipe controlled both water and N application amounts for individual plots. To avoid runoff, all plots were diked once the linear-move irrigation system was used for applying water and nutrients.

The objective for the w treatment in 1999 was to simulate water stress that may be commonly encountered in commercial cotton production, i.e., to create a scenario in which a timely water application is delayed for a short period due to scheduling errors or other constraints. However, severe cumulative water stress for the w treatment was avoided over the course of the season. For 1999, the w treatment was imposed by delaying the irrigations two times toward the end of the cotton development stage (i.e., vegetative, flowering, early boll development) and for three times during the midseason stage (late flowering, mid-late boll development) (table 2). However, after delayed irrigations for the w treatment in 1999, subsequent irrigation amounts for the w treatment were increased to bring the root zone soil water contents back to the W treatment level.

During the two growing seasons, the low N treatment received one-half (1998) and from about one-third to one-half (1999) of the amount of N applied to the optimal N treatment. For 1998, management of the optimal N treatment followed a common split-application practice used in the area for surface-irrigated cotton. The 1998 optimal N treatment plots were side-dressed two times with ammonium sulfate (21–0–0) at a rate of 45 kg N ha⁻¹; first about one month after crop emergence on 3 June, and then just before canopy closure on 9 July (table 1). The fertilizer was immediately incorporated during subsequent cultivations on the same days as the side-dress. The low N treatment in 1998 received only the second of the two applications. For 1999, all plots received an application of N via the linear-move system on 28 May 1999, about one month after crop emergence. Afterwards, the management of the optimal N treatment was predicated on petiole NO₃-N contents obtained from weekly plot samples, which will be described later. Based on petiole samples, three more N applications were given to the optimal N treatment in 1999 (table 1). The low N treatment in 1999 received N on the same days as the optimal treatment, but at approximately half the rate (table 1). For 1999, N applications were made by injecting 32% liquid urea ammonium nitrate into the irrigation water.

Cotton growth parameters were determined by in situ measurements and destructive plant sampling that occurred weekly in treatment plots starting on 2 June 1998 and on 9 June 1999. Measurements and sampling were made within three designated areas of each plot that were 22 m long and 2 m wide, running north to south (i.e., perpendicular to plant rows). On each sampling date, growth measurements, including canopy heights and widths, were made within the each of the three areas along the 2 m length of a pre-specified plant row, in which different plant rows were sampled on each date. Canopy height and width were recorded at 0, 0.5, 1.0, 1.5, and 2.0 m along each of the three rows. Canopy width measurements were used to approximate the fraction of canopy cover. The stem diameter for each plant within the 2 m length of row was also measured. Subsequently, one plant from each row was destructively sampled; plant selection was based on stem diameter, which had to be close to the median diameter of all plants measured in the row. The sampled plants were analyzed for various growth parameters, including leaf area index (LAI).

Measurements for plant N were not made during the 1998 season but were determined weekly for all plots during 1999. Following each weekly destructive plant harvest in 1999, 30 leaves per plot, corresponding to the most recent fully expanded leaves, were randomly collected from remaining plants within the sampling rows. After a hand-held SPAD chlorophyll meter (Minolta SPAD 502, Spectrum, Inc., Plainfield, Ill.) reading was taken on each leaf, the petiole was detached from the leaf blade and both were taken to the lab for analysis.

Planting and harvest dates, as well as dates of key growth stages for the WN treatment, are presented for each season in table 3. Final yield data were obtained by mechanically harvesting two undisturbed rows of cotton (44 m²) located in the middle of each plot.

**REMOTE SENSED DATA**

During both the 1998 and 1999 cotton seasons, canopy reflectance was measured in all plots with a hand-held radiometer (CropScan MSR, CropScan, Inc., Rochester, Minn.).

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**Table 2. Dates on which irrigation water was applied to optimal water treatments and withheld from low water treatments, and total seasonal water application amounts for water treatments for 1998 and 1999.**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Date</th>
<th>DOY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998 Cotton</td>
<td>18 Sept.</td>
<td>261</td>
</tr>
<tr>
<td></td>
<td>22 Sept.</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>30 Sept.</td>
<td>273</td>
</tr>
<tr>
<td>Total application:</td>
<td>1219 mm (optimal water treatment)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1165 mm (low water treatment)</td>
<td></td>
</tr>
<tr>
<td>1999 Cotton</td>
<td>12 July</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>27 July</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>11 Aug.</td>
<td>223</td>
</tr>
<tr>
<td></td>
<td>16 Aug.</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>30 Aug.</td>
<td>242</td>
</tr>
<tr>
<td>Total application:</td>
<td>1066 mm (optimal water treatment)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>998 mm (low water treatment)</td>
<td></td>
</tr>
</tbody>
</table>
The radiometer consisted of eight silicon detectors filtered to different reflective bands. Five of the reflective bands were relatively narrow (12 to 27 nm half-peak width): blue (460 nm), green (559 nm), red (661 nm), red-edge (710 nm), and near-infrared (NIR, 810 nm). The remaining three bands were similar to bands 3, 4, and 5 of the Landsat TM sensor with central wavelengths (and half-peak widths) of 660 nm (58 nm), 830 nm (137 nm), and 1650 nm (229 nm), respectively. During the two cotton seasons, radiometer readings were made in all plots every two to five days starting shortly after planting until the cotton was defoliated. The instrument was held 2 m above the soil surface, providing a 1 m diameter view area at the soil surface. For each set of measurements, the radiometer was centered over an undisturbed crop row and positioned to collect data over the same five locations of the row for all plots. Radiometer data were converted to reflectance by reference to a calibrated, painted BaSO4 reflectance panel following the methods of Jackson et al. (1987).

The linear-move irrigation system was used as a remote sensing platform (Agricultural Irrigation Imaging System, AgIIS, i.e., “Ag Eyes”) during the 1999 season. AgIIS used a downward-looking sensor package with 15° field of view optics positioned 4 m above ground level (1 m diameter view area). As the sensor traveled along the length of the linear move, north-south measurements were taken at 1 m intervals centered on a planted row. A differentially corrected global positioning system (GPS) receiver was located at one end of the linear move, and processing algorithms were developed that assigned a Universal Transverse Mercator (UTM) coordinate to every sensor measurement. The linear-move system was operated at a speed that allowed sensor measurements to be gathered at approximately 1 m intervals in the direction of travel. Thus, when the data were displayed spatially, the “pixel” resolution was 1 × 1 m. AgIIS data collection typically began at solar noon, and the entire field was measured in approximately 2 h. The AgIIS sensor package was composed of four silicon detectors (manufactured by Intor Inc., Socorro, N.M.) filtered to narrow wavelength intervals (~10 nm) in the green (555 nm), red (670 nm), red-edge (720 nm), and near-infrared (NIR, 790 nm) portions of the spectrum, and an infrared thermometer (Everest model 3000). Images were obtained at a minimum of weekly intervals, with as many as three images per week during the period of rapid crop development. Data were calibrated to reflectance using a painted plywood panel mounted on the center tower of the linear-move system. The plywood’s spectral reflectance characteristics with respect to solar zenith angle were determined using the CropScan radiometer. Additional details on AgIIS are presented by Kostrzewski et al. (2003).

Reflectance factors from these sensor packages were used to compute the following vegetation indices:

\[ RVI = \frac{NIR}{Red} \]  
\[ NDVI = \frac{(NIR - Red)}{(NIR + Red)} \]

where RVI is the ratio vegetation index (Jordan, 1969), and NDVI is the normalized difference vegetation index (Rouse et al., 1973).

**DESCRIPTION OF THE CCCI**

The CCCI uses normalized difference based on NIR and red-edge (NDNE) reflectance as a relative measure of chlorophyll concentration. The NDNE index is computed as:

\[ NDNE = \frac{(NIR - Edge)}{(NIR + Edge)} \]

where NIR is the NIR reflectance factor (810 nm, CropScan; 790 nm, AgIIS) and Edge is the red-edge reflectance factor (710 nm, CropScan; 720 nm, AgIIS). For comparison purposes, a normalized difference index based on NIR and green (G) reflectance (NDNG) was also computed from the AgIIS data sets by replacing the Edge reflectance factor in equation 3 with a Green reflectance factor (555 nm).

NDNE limits for high (N sufficient) and low (N stressed) chlorophyll levels were determined empirically as a function of NDVI, which describes relative canopy cover. The CCCI was then calculated as:

\[ CCCI = \frac{NDNE_{measured} - NDNE_{low}}{NDNE_{high} - NDNE_{low}} \]

where NDNE_{measured} is the NDNE measured over the crop, NDNE_{low} is the value of NDNE expected at a given crop cover (i.e., NDVI) for low canopy chlorophyll concentrations, and NDNE_{high} is the NDNE value expected at a given crop cover for high chlorophyll concentrations. A CCCI value of zero indicates low chlorophyll concentrations (N stress with NDNE_{measured} = NDNE_{low}), whereas a value of one indicates high concentrations (N sufficient).

Figure 1 illustrates the high and low limits for NDNE for an idealized situation where the measured reflectance is a simple composite of crop canopy and/or sunlit soil. Under these conditions, the composite reflectance at the sensor (ρ_λ_c) is given by:

\[ \rho_\lambda_c = F_p \rho_\lambda_p + (1 - F_p)\rho_\lambda_s \]

where \( F_p \) is the fraction of the measured area with plant cover, \( \rho_\lambda_p \) is the plant canopy reflectance at wavelength \( \lambda \), and \( \rho_\lambda_s \) is the soil reflectance at wavelength \( \lambda \). The relative magnitude of the differences in composite reflectance due to chlorophyll differences in plants increases as the crop canopy cover increases.

The component reflectance factors used to construct figure 1 are provided in table 4 and are assumed constant (no

**Table 4. Reflectance factors used in the linear mixing model for figure 1.**

<table>
<thead>
<tr>
<th>Band</th>
<th>Status</th>
<th>Crop (ρ_p)</th>
<th>Soil (ρ_s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>High [Chl]</td>
<td>0.030</td>
<td>0.300</td>
</tr>
<tr>
<td>NIR</td>
<td>High [Chl]</td>
<td>0.700</td>
<td>0.370</td>
</tr>
<tr>
<td>RE</td>
<td>High [Chl]</td>
<td>0.230</td>
<td>0.333</td>
</tr>
<tr>
<td>Red</td>
<td>Low [Chl]</td>
<td>0.040</td>
<td>0.300</td>
</tr>
<tr>
<td>NIR</td>
<td>Low [Chl]</td>
<td>0.650</td>
<td>0.370</td>
</tr>
<tr>
<td>RE</td>
<td>Low [Chl]</td>
<td>0.270</td>
<td>0.333</td>
</tr>
</tbody>
</table>
other variables) for a given canopy cover. The reflectance factors in table 1 were determined empirically using cotton data from previous experiments. The generalized conditions in figure 1 do not account for variations in soil background conditions, differential changes in band reflectances due to changes in canopy architecture at different growth stages, changes in leaf spectral properties with age, or shaded soil conditions. However, these changes occur, so high and low limits for CCCI were determined empirically using data collected throughout the season by:

- Assuming a linear relationship between NDNE and NDVI for both high and low chlorophyll concentrations.
- Determining the average values of NDNE and NDVI for bare soil conditions with different cultivations, soil moistures, etc., and anchoring the lower left ends of the two limit lines to the average bare soil levels.
- Adjusting the slope of the limit lines so that 95% of the NDNE values at a given NDVI are less than the high limit and 95% of the values are greater than the low limit. This step is a first iteration in establishing the location of the limit lines.
- Removing the data points that are higher than the high limit or lower than the low limit by 25% or more of the range.
- Redrawing (second iteration) the limit lines so that 99% of the data are less than the high limit and greater than the low limit.

Canopy reflectance levels are very different between 710 and 720 nm, so it was necessary to draw the limit lines separately for each of the two different sensors.

Utilizing the procedures described above, we found that the CCCI increased during the season as a function of canopy cover, regardless of N treatment. While the CCCI provides a relative measure of chlorophyll content at a given canopy cover, it does not account for the seasonal differences in chlorophyll contents of leaves, where younger leaves during early growth stages have less chlorophyll than mature leaves at full cover. Accordingly, we attributed the observed CCCI trend to increasing leaf chlorophyll during the season. To account for the differences in leaf chlorophyll contents due to growth stage, we normalized the CCCI, using NDVI to characterize the growth stage. Thus, normalized CCCI values were calculated by dividing the value obtained with equation 3 by NDVI.

Statistical significance of treatment effects and interactions were determined using analysis of variance. Treatment comparisons were made using the protected least significant difference test (Snedecor and Cochran, 1980). A probability level of 0.05 was used to evaluate significance differences.

RESULTS AND DISCUSSION

NDVI VERSUS CANOPY COVER

If it is assumed that NDVI has a one-to-one relationship with fraction of canopy cover, then the NDVI from AgIIS slightly overestimated cover, whereas the NDVI from CropScan slightly underestimated cover (fig. 2). For both imaging systems, NDVI reached a maximum value of 0.9 when the canopy cover was about 90% (fig. 2). To a large extent, dependence of these relationships on sensor type is caused mainly by differences in sensor field of view and to a lesser extent on the wavelength of the red and NIR filters. Despite the differences in NDVI for different sensors, the data presented in figure 2 demonstrate that the NDVI achieved a reasonable estimate of crop cover and thus can provide an estimate of relative variations in canopy cover for application of the CCCI. However, the data indicate that CCCI relationships developed for one sensor cannot be used to calculate the CCCI for the other sensor.

HIGH AND LOW LIMITS OF NDNE

Figure 3a shows the high and low NDNE limits for the CropScan data, with data points from both 1998 and 1999 cotton seasons. In general, data from both seasons fall within the same limits and overlap, with no significant differences between seasons. Figure 3b presents the limits determined with data from the 1999 AgIIS sensor. For NDVI levels below 0.6, the NDNE based on 1998 and 1999 CropScan data remained close to the low limit (fig. 3a), whereas the NDNE based on the 1999 AgIIS data remained between the high and low limit lines (fig. 3b). The NDNE values for the 1999 AgIIS data form a bimodal distribution for NDVI between 0.6 and 0.8. Most of the values close to the lower limit represent data points collected later in the season. Some of this data grouping is expected, as leaf chlorophyll concentrations decline during the season. In general, the high and low limits were

![Figure 1. Theoretical relationship between NDNE and NDVI for high and low canopy chlorophyll concentrations as fractional crop cover varies from 0 to 1.](image)

![Figure 2. Measured canopy cover versus NDVI (a measure of green cover) for NDVI data determined from the CropScan in the 1998 and 1999 cotton experiments (CropScan 98, 99) and from AgIIS in the 1999 cotton experiment.](image)
lower in magnitude for AgIIS than for CropScan. The differences might be attributed to differences in sensor field of view and band wavelength. This would suggest using different limits for different sensors.

Figure 3. High and low limits of chlorophyll contents for NDNE vs. NDVI based on (a) CropScan (CS) data collected during both the 1998 and 1999 cotton seasons and (b) AgIIS data collected only during the 1999 cotton season.

Figure 4. Seasonal treatment trends for normalized petiole NO₃-N contents for treatments during the 1999 cotton season.

Table 5. Measured petiole NO₃-N contents for treatments during the 1999 cotton season.

<table>
<thead>
<tr>
<th>DOY</th>
<th>WN</th>
<th>Wn</th>
<th>wn</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>10606</td>
<td>8740</td>
<td>11114</td>
</tr>
<tr>
<td>188</td>
<td>10114</td>
<td>5958</td>
<td>10428</td>
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<td>195</td>
<td>10941</td>
<td>10126</td>
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</tr>
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<td>202</td>
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<td>8453</td>
<td>8049</td>
</tr>
<tr>
<td>208</td>
<td>4486</td>
<td>5679</td>
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[a] Sampling dates when optimal N and low N treatments were significantly different.

Figure 5. Seasonal trends in leaf area index (LAI) during the (a) 1998 cotton, (b) 1999 cotton experiments for the control (WN), low N (Wn), low water (wN), and low water, low N (wn) treatments.
The impact of the N treatment on crop development can be seen by viewing the LAI data presented in figure 5a (1998) and figure 5b (1999). For 1998, the low N treatments had lower LAI values than the optimal N treatments starting on DOY 200, although not significantly lower until DOY 215. The LAI for Wn and wn were not significantly different until measurements on DOY 287, following the three water stress events that occurred in late September in 1998. However, in 1999, the water stress treatments had significantly lower LAI than the optimal water treatments beginning on DOY 202, following the first water stress event that occurred on DOY 193 in 1999 (fig. 5b). The LAI for the Wn treatment did not begin to separate significantly from the WN treatment until DOY 215. At the end of the season, the Wn and wN treatments had the same LAI levels during cotton boll maturity. The high N needed during that growth stage could have lowered the LAI of the Wn treatment.

Figure 6 shows the seasonal treatment variations for the CCCI (based on CropScan) in 1998 along with percent cover as averaged across all treatments. Values for the CCCI were lower than expected for the optimal N treatments until shortly after the second application of N on DOY 190. Although an application of N had been given to the optimal N treatments on DOY 154 (table 1), the application rate may have been insufficient to fully satisfy the cotton N requirements during the period of high canopy N uptake in the subsequent weeks. However, as the canopy matured to near full cover following the second N application on DOY 190, the N requirements for the cotton canopy decreased. The CCCI data for the optimal N treatment reflect this shift in canopy N needs and N status, as the CCCI increased to about 0.80 to 0.90 at full cover. On the other hand, values for the low N treatment, which did not receive the early N application, remained lower than those for the optimal N treatment, except for a short recovery period following the N application to all treatments on DOY 190.

In order to more clearly observe the CCCI differences for the 1998 treatments, the CCCI values (CropScan) for the three non-control treatments were divided by the CCCI value for the control treatment (fig. 7a). Treatment RVI data based on CropScan are also shown relative to the control treatment (fig. 7b). There were no clear treatment differences for CCCI prior to DOY 173. Before this date, crop cover was less than 20%, suggesting that the response of the CCCI was dominated by changes in soil surface conditions due to cultivation and soil surface wetness. However, after crop canopy reached about 30% on DOY 173 (fig. 6), the CCCI values for the low N treatments were indicated to be significantly lower than for the optimal N treatments. After N was applied to all treatments on DOY 190, the CCCI for the low N treatments approached the optimal N level, such that by DOY 196, CCCI differences were not significant between treatments. After DOY 198, there was a consistent decrease in the CCCI for the low N treatments, which eventually became significantly lower than that for the optimal N treatments by DOY 205. As expected, there was no interaction between the water and N treatments, since water treatments had not yet been imposed. While the RVI for the low N treatment eventually became significantly lower than that for the optimal N treatment by DOY 189, its response occurred 16 days later than for the CCCI. After N was applied on DOY 190, differences for RVI between the low and optimal N treatments remained significant, except for DOY 215. Thus, unlike the CCCI, the RVI did not indicate a sudden change in N status for the low N treatment due to the second N application.

It can be visually observed (fig. 8a) that the CCCI for 1999 (based on AgIIS), prior to the first differential irrigation on...
DOY 193, separated the low N treatments from the optimal N treatments much better than did the RVI data (fig. 8b). However, N treatment differences for CCCI during this period were not found to be statistically significant. The data for CCCI and RVI between DOY 193 and 211 correspond to times of water stress for low water treatments with incomplete canopy cover. During this particular period of water stress events, leaf wilting was observed for wN and wN, particularly on DOY 202. However, the data for wN and wN show “spikes” for both the CCCI (rapid increase in low water treatments) and RVI (rapid decrease in low water treatments), which resulted in significant interactions between water and N for the CCCI, as well as for the RVI. This suggests that neither index was able to differentiate between N treatments during this period of water stress. Furthermore, the CCCI indicated higher N status for low water treatments than for optimal water treatments, regardless of N treatment. Following DOY 202, there was an appreciable decrease in the rate of LAI development for low water treatments (wN and wN) compared to WN and Wn (fig. 5b). Eventually, however, the LAI for Wn decreased below the LAI for WN. By DOY 230, the LAI for Wn decreased to the same LAI as for wN.

The relative treatment trends for RVI (fig. 8b) were similar to the LAI trends, except that the RVI became the same for Wn and wN about DOY 220. On the other hand, while the RVI generally followed the LAI differences between the low and optimal water treatments, the CCCI was able to differentiate significantly between the N treatments starting when canopies were near 100% cover, on DOY 216 (fig. 8a). Thus, the CCCI was not affected by the interaction between w and N once full cover was reached, although low water treatments continued until DOY 242 in 1999. At this stage (after DOY 216), peak blooming occurs and there remains about 30% of total N uptake to be assimilated by plants (Crozier, 2006). Because a timely application of N during peak blooming could have a positive effect on final cotton yield, clear N status information provided by the CCCI during this period would be useful in determining final N applications for the season.

**CORRELATION BETWEEN SPECTRAL REFLECTANCE FACTORS AND INDICES WITH PLANT N STATUS**

Correlation coefficients of measured petiole NO3-N, SPAD chlorophyll, and total leaf N content data with AgIIS reflectance factors and indices during the 1999 cotton season are shown in table 6. Correlations were calculated for (a) data over all growth stages, (b) all data except dates when water stress occurred during partial canopy development (i.e., excluding data from DOY 195 to 208), and (c) all data after full canopies were attained (i.e., excluding all data except from DOY 216 to 236). Correlations using data within each separate treatment were also calculated but are not shown since none were significant.

The 1999 data based on all sampling dates indicate that most indices and all band reflectance factors were not well correlated to N status measurements across the entire season (table 6a). For the entire season, the CCCI had low correlation with both petiole NO3-N (r = 0.41) and leaf N (r = 0.41). Correlations for NDNE (r = 0.65) and for NDNG (r = 0.56) versus petiole NO3-N were significant and statistically higher than those for the CCCI. However, CCCI was the only index significantly correlated with SPAD (r = 0.54). After excluding the water stress days under partial canopy (table 6b), correlations for the CCCI with petiole NO3-N, SPAD, and total leaf N were each significant. Correlation values for NDNE were higher, although not significantly so, than the CCCI for petiole NO3-N. Both red-edge and green reflectance factors had significant but negative correlations with total leaf N. Table 6c indicates that removing all days prior to full cover further improved CCCI correlations with petiole NO3-N (r = 0.74), SPAD (r = 0.65), and leaf N (r = 0.86). NDNG and NDNE also had significant correlations with petiole NO3-N and total leaf N for full cover, but were not statistically different from those for the CCCI. All other indices and band reflectance factors had significant correlations with leaf total N, but few were significantly correlated with petiole or SPAD.

The CCCI was significantly correlated with SPAD for the entire season, while its correlations with petiole NO3-N and total leaf N were affected during times of water stress under partial canopies. The correlations of NDNE with petiole NO3-N and total leaf N were relatively stable over the season and appear less affected than the CCCI for days before full cover. For correlations of NDNE with petiole and leaf N to be statistically greater than for CCCI during partial canopy periods suggests that the CCCI was adversely affected by inaccurate canopy cover characterization by NDVI during
partial canopy periods. However, once full cover was achieved and NDVI was near maximum, slight inaccuracies in NDVI did not appear to pose a problem for the CCCI.

**SUMMARY AND CONCLUSIONS**

A hand-held radiometer and a remote sensing monitoring system, AgIIS, were used during cotton experiments in 1998 and 1999 in Arizona to evaluate and compare the ability of several remote sensing indices to discriminate cotton N status as affected by N and water treatments. The CCCI was demonstrated to be an effective indicator of N status during 1998 when the cotton was only affected by N application differences until late in the season. Thus, when cotton irrigation is well-managed, the CCCI should provide appropriate N status detection well before full cover, possibly as early as 30% canopy cover. However, prior to this canopy size, early season management of cotton needs to be guided by other non-canopy cover. However, once full cover was achieved and NDVI was near maximum, slight inaccuracies in NDVI did not appear to pose a problem for the CCCI.

While the CCCI clearly reduced ambiguity of treatment N status indicated by differential water treatments after full cover in 1999 (DOY 216), it was confounded by variable treatment water status during partial canopy conditions. Hence, confounding effects of water stress during partial canopy may seriously limit the success of the CCCI as an effective N management tool for cotton. However, when considering overall results, the CCCI provided superior discrimination of N treatments compared to common vegetation indices, such as RVI. For example, the RVI was able to adequately detect and follow growth changes for treatments, but the RVI’s ability to discriminate N status lagged behind the CCCI discernment by about 16 days during 1998. The inability of RVI to differentiate between the two primary sources of sub-optimal growth was demonstrated during the late 1999 season when the RVI indicated the same response for the low N under optimal water treatment as for the optimal N under low water treatment. Although correlations of NDNE with measured petiole NO$_3$-N and total leaf N were significantly higher than for CCCI across the entire 1999 season, correlations of CCCI with petiole NO$_3$-N and total leaf N were significantly improved and comparable to NDNE when considering only data during full cover. Thus, the ability of the CCCI to appropriately monitor N status during partial canopy periods could potentially be improved through better methods to characterize actual canopy cover.

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