# EFFECTIVENESS OF THE DARK GREEN COLOR INDEX IN DETERMINING COTTON NITROGEN STATUS FROM MULTIPLE CAMERA ANGLES

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## Abstract

Timely in-season N determination in cotton (Gossypium hirsutum L.) can help producers combat the negative effects of inadequate or excessive applications of fertilizer N; however, current methods are often time consuming and/or expensive. More instantaneous, accurate methods of determining N status which utilize equipment already in the possession of the producer are needed. The objectives of this research were to examine the effectiveness of dark green color index (DGCI) measurements derived from standard digital photographs taken by an inexpensive digital camera and analyzed with commercially available image-analysis software to determine cotton N status and to compare sensitivities of calculated DGCI from laboratory, field nadir, and field off-nadir photographs to changes in leaf N and chlorophyll meter measurements. Results suggest DGCI measurements calculated from field off-nadir photographs are a practical and inexpensive method of cotton N determination which could possibly replace measurements using chlorophyll meters.

### 1. INTRODUCTION

Applications of fertilizer N which fail to match cotton demands are financially and environmentally costly. Although plant tissue and soil samples can be good indicators of in-season crop N status, their use as a management tool is somewhat limited due to the expense of implementing a fine sampling grid and field-laboratory-field turnaround time. The instantaneous readings and sensitivity to chlorophyll content of chlorophyll meters resulted in their emergence as a method to determine in-season N status; still, these instruments are costly and readings can be subjective and variable due to the small sampling area (Schepers et al., 1998). Cotton N deficiencies have long been associated with a shift in greenness in the visible spectral reflectance region (Nelson, 1949). These spectral characteristics and others outside of the visible spectrum have resulted in the development of many different reflectance instruments utilizing numerous indices (Samborski et al., 2009). In contrast to chlorophyll meters, canopy reflectance measurements consist of a large sampling area and therefore less readings for accurate measurements and lower labor costs (Blackmer et al., 1994). Furthermore, research in cotton has suggested that canopy reflectance measurements can provide instantaneous and accurate crop N status measurements (Zhao et al., 2005). However, many instruments measuring canopy reflectance are costly and can be complicated to integrate into management systems.

Currently, inexpensive digital cameras are being used to measure canopy reflectance for many different purposes. Previous research in cotton has described processed digital photography from a nadir angle to correlate strongly with percent ground cover and leaf area index (Stewart et al., 2007; Gonias et al., 2011). Strong relationships between processed digital photographs and N concentration in wheat (*Triticum aestivum*, L.) have also been noted (Li et al., 2010; Jia et al., 2004). Research in turf has established processed, digital-image-derived DGCI values as a greenness rating tool to replace more subjective human ratings (Karcher et al., 2009). More recent research examining this index by Rorie et al. (2011) concluded DGCI values derived from digital images in corn (*Zea mays*, L.) has potential as an in-season N status measurement tool. Calculation of DGCI from digital images in cotton could allow producers to take in-season, instantaneous N concentration measurements with lower labor and instrument costs than chlorophyll meter readings or canopy reflectance sensors.

The objective of this research was to examine the effectiveness of DGCI derived from standard digital images taken by an inexpensive digital camera and commercially available image-analysis software to determine cotton N status and to compare sensitivities of DGCI from laboratory, field nadir, and field off-nadir photographs to changes in leaf N concentration and chlorophyll meter measurements.

### 2. MATERIALS AND METHODS

A field trial was conducted at the Lon Mann Cotton Research Station near Marianna, AR. Stoneville cultivar 4288 B2FR was planted on 27 May 2011. Fertilizer N rates of 0, 34, 67, 101, 135, and 168 kg N ha<sup>-1</sup> were applied as urea in a single pre-plant application to create a wide range of plant N status. Potassium and phosphorus rates of 67 kg  $K_2O$  ha<sup>-1</sup> and 56 kg  $P_2O_5$  ha<sup>-1</sup> were blanket applied to assure N level to be the limiting growth factor. Treatments were replicated four times. Leaf sampling, chlorophyll meter readings and digital pictures were taken 1 August 2011 at the third week of flowering. Field nadir and field off-nadir (approximately 60° from nadir) pictures were taken of the canopy with an inexpensive digital camera (Canon PowerShot SD450, Lake Success, NY) against a neutral pink color board that included yellow and green disks which served as interval color standards (Figs. 1-3).

Two most recently matured, fully expanded main stem leaves 4-6 nodes from the terminal were sampled and placed on ice. Chlorophyll meter (Minolta SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan) measurements and pictures of the leaf samples were taken indoors under fluorescent lighting against a standardized color board (referred to as laboratory DGCI) within 2 hours of sampling (Fig. 2). Leaf samples were dried and ground to pass a 20-mesh sieve and leaf N concentration of the ground sample was determined by dry combustion (ELEMENTAR Rapid N, ELEMENTAR Analysensysteme, Hanau, Germany) by the Agricultural Diagnostic Laboratory at the University of Arkansas in Fayetteville, AR. Images were processed using SigmaScan Pro (v. 5.0, Chicago, IL). This software normalized each image using internal color standards prior to the calculation of DGCI. The DGCI calculation is fully described by Rorie et al. (2011). Images were manually cropped to remove bordering plots and excess amounts of bare soil (Figs. 2 and 3). Linear regressions of the replicate data examining the relationships between DGCI measurements (field nadir, field off-nadir, and laboratory), SPAD readings, and leaf N concentrations were performed in JMP 9 (SAS Institute Inc., Cary, NC).

### 3. RESULTS AND DISCUSSION

Visible differences in N status due to treatment were noted at sampling; cotton receiving 0 kg N ha<sup>-1</sup> appeared stunted and yellow in color, while cotton receiving 168 kg N ha<sup>-1</sup> appeared much larger and dark green in color (Fig. 1). Response of leaf N concentration to fertilizer N applied was significant, positive, and linear ( $r^2$ =0.55) and measured leaf N values reached and exceeded published critical values of 3.0 % leaf N (Sabbe et al., 1972; Mills and Jones, 1996). Field nadir and off-nadir DGCI readings did not correlate as strongly to leaf N as laboratory DGCI readings (Fig. 4). The laboratory DGCI readings were also slightly more sensitive to leaf N ( $r^2$ =0.603) than SPAD readings were to leaf N ( $r^2$ =0.561, data not shown). Coefficients of determination with leaf N ranged from 0.440 for the field nadir DGCI readings to 0.603 for the laboratory DGCI readings. Stronger relationships between laboratory DGCI readings and leaf N than between all other methods may be due to laboratory method's inclusion of all plant material used to determine leaf N concentration; SPAD meter only measured a portion of each of the leaves used to determine leaf N and the field nadir and off-nadir methods included upper canopy plant material which was not in the leaf N measurement.

The relationship between nadir laboratory DGCI readings and SPAD readings was very strong (Fig. 4,  $r^2$ =0.914). This strong relationship is logical, as both measurements are conducted on the same tissue. Failure of the field nadir and off-nadir DGCI readings to correlate as strongly with SPAD readings is again most likely due to the inclusion of tissue in the field images which was not actually sampled by the SPAD meter. However, the relationship between SPAD readings and field off-nadir DGCI readings was quite strong ( $r^2$ =0.818). These results suggest that field off-nadir images may be the most practical method for in-field determination of cotton N status since the relationship between laboratory DGCI readings and SPAD readings was only slightly higher but consisted of leaf sampling, storing, transportation, and more required time than other methods.

### 4. CONCLUSIONS

Results indicate that measurements of DGCI from inexpensive digital camera images are a practical and inexpensive method sensitive to cotton N status and therefore provide useful in-season N status measurements to producers.

Although laboratory images were the most sensitive to changes in leaf N and SPAD readings, field off-nadir images seem to be the most practical method of cotton N status determination for the producer since field off-nadir DGCI requires no destructive sampling and much less time. Further research across years and sites is necessary to establish critical DGCI values for cotton and streamline the image processing. However, an effective program to serve farmers could be easily set up to allow producers to email or picture message off-nadir images of the crop with a standardized color board for instantaneous determination of cotton N status.

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Figure 1: Field off-nadir images of plots recieving 168 kg N ha<sup>-1</sup> (LEFT) and 0 kg N ha<sup>-1</sup> (RIGHT) with standardized color board in the background. High N rate treatment was taller and visibly darker green than 0 kg N ha<sup>-1</sup> treatment.



Figure 2: Laboratory image of two recently matured, fully expanded cotton leaves from plot receiving 34 kg N ha<sup>-1</sup> with a standardized color board in background. LEFT: Raw Image. RIGHT: Cleaned image with excess cropped. The round standard color regions and leaves have been recognized as areas of interest by the image analysis software.



Figure 3: Field nadir image of a plot receiving 101 kg N ha<sup>-1</sup>. LEFT: Raw image. RIGHT: Cleaned image with excess rows cropped ready for image analysis.



Figure 4: Simple linear regression and coefficients of determination between laboratory DGCI, field nadir DGCI, field off-nadir DGCI, leaf N and SPAD readings at 2011 third week of flower near Marianna, AR.