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AGRONOMY AND SOILS

In-Season Assessment of Cotton Nitrogen Status from a Handheld Smartphone and an Unmanned Aerial System

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ABSTRACT

The widespread adoption of smartphones and unmanned aerial vehicles (UAVs) has the potential to ease collection of in-season cotton nitrogen (N) status. Subsequently, in-season cotton N status could be used to drive management decisions. The utility and limitations of these new platforms must be assessed and compared to current in-season measurements. The objectives of this study were to evaluate the ability of early- and late-season ground-based measurements to provide insight into cotton N status and to evaluate the ability of aerial-based measurements to correlate to ground-based measurements. Although measurements failed to correlate strongly across seasons to leaf N, moderate relationships (R^2 = 0.453) between chlorophyll meter readings and the dark green color index (DGCI) measured from a smartphone were observed in late-season measurements. Poor relationships were found between early-season UAV-acquired vegetation indices (VIs) and leaf N. Analysis of a subset of the data indicated relationships between chlorophyll meter readings and chlorophyll concentrations predicted by DGCI were strong for ground-based measurements and moderate for UAV-based measurements. (R^2 = 0.711 and R^2 = 0.511, respectively). Although additional site-years including aerial-based data are needed, this study demonstrates the usefulness of UAV-based reflectance data and VIs in predicting in-season cotton N status. Furthermore, it appears handheld DGCI measurements have the potential to replace chlorophyll meter readings for late in-season measurements of cotton N status.

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Nitrogen (N) fertilization is a key but critical component of sustainable cotton (*Gossypium hirsutum* L.) production. Deficiencies of N in cotton can lead to yield reductions and can harm overall productivity and profitability (Gerik et al., 1998; Read et al., 2006). Excessive N applications can lead to unwanted vegetative growth, increasing the need for plant growth regulator and insecticide applications, as well as increasing the difficulty of defoliation prior to harvest (Boman and Westerman, 1994; Harris and Smith, 1980). Unused N has an economic cost, as it provides no return on the input cost, and an environmental cost, as it has the potential to move offsite where it can contribute to environmental N pollution such as eutrophication (Carpenter et al., 1998). Nitrogen management in cotton has been heavily researched since the Haber-Bosch process was invented in the early 20th century. Current land grant university recommendations throughout the U.S. include optimal N rates as well as application timing, fertilizer source, and placement (Duncan and Raper, 2018; Lemon et al., 2009). Mid-season assessment of crop N status is an important method of determining whether the crop requirement has been met and the correct N rate to be applied when the application can affect final yield (Gerik et al., 1998; Raper et al., 2013). Petiole or leaf analysis methods are typically used to determine crop N status, but these methods can be laborious, costly, and challenging to use in evaluation of spatial variability due to limits of scale (Buscaglia and Varco, 2002). One popular methods of crop N assessment is the soil plant analysis development (SPAD) meter (SPAD 502, Minolta Co., Osaka, Japan), used as an indicator of chlorophyll concentration (Read et al., 2003) which decreases with N deficiency (Gerik et al., 1998). This method gives rapid results but is an in-situ method that is time-consuming and expensive.

Remote sensing of in-season crop N status is a promising method that can be performed quickly and relatively inexpensively. For decades, research has been conducted using handheld, tractor, airplane, and satellite mounted sensors (Barnes et al., 2000;

Gitelson et al., 1996; Raper et al., 2013; Rouse et al., 1973). More recently, there has been increased interest in the use of unmanned aerial vehicles (UAVs) to collect spectral information to determine crop N status (Ballester et al., 2017). Fertilizer application algorithms have been developed based on commercially available ground-based sensors (Arnall et al., 2008; Khalilian et al., 2017), but there is little information regarding the suitability of these algorithms to UAV-collected information. Tremblay et al. (2009) noted the non-transferability of application algorithms from one ground-based sensor to another, which could be extended intuitively to sensors on different platforms.

Many commercially available ground-based sensors use the normalized difference vegetation index (NDVI) as an indicator of crop N status. Although NDVI correlates strongly to N status in corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) (Ma et al., 1996; Stone et al., 1996; Zubillaga and Urricariet, 2005), studies examining the relationship between cotton N status and NDVI have revealed much weaker correlations (Bronson et al., 2005; Raper et al., 2013). In contrast, stronger relationships between cotton N status and red-edge-based vegetation indices have been reported (VIs) (Raper and Varco, 2015; Read et al., 2002). More recently, Ballester et al. (2017) found that the simplified canopy chlorophyll content index (SCCCI), also based on reflectance in the red-edge region, was the most effective VI evaluated for predicting cotton N status when using a UAV. The dark green color index (DGCI) was developed for use in turfgrass (Karcher and Richardson, 2003) then extended to applications in corn (Rorie et al., 2011a, b) and cotton (Raper et al., 2012). This index uses hue, saturation, and brightness values from a digital image of a crop leaf against a color standard. Rorie et al. (2011a, b) found strong relationships between SPAD, DGCI, and corn leaf N. A few studies have indicated that DGCI also correlates well to cotton N status and chlorophyll concentrations (Raper et al., 2012; Wang et al., 2012). These studies each concluded that DGCI has the potential to provide an accurate assessment of N status in the crops evaluated by use of an inexpensive digital camera.

These findings necessitate further investigation into the usefulness of and similarities between reflectance data and VIs developed from sensors mounted on ground and UAV platforms in estimating cotton N status. The objectives of this study were to evaluate the ability of early- and late-season ground-based measurements to provide insight into cotton N status and to evaluate the ability of aerial-based measurements to correlate to ground-based measurements.

MATERIALS AND METHODS

Trials evaluating cotton response to fertilizer N rate and fertilizer N timing were established during the 2016, 2017, and 2018 growing seasons at the University of Tennessee Research and Education Centers located in Milan (35°56’04.7”N, 88°43’40.2”W), Jackson (35°37’23.3”N, 88°50’47.5”W), and Grand Junction (35°06’53.1”N, 89°12’56.6”W), TN (Fig. 1). Soil types at Grand Junction, Jackson, and Milan locations are Loring silt loam, Memphis silt loam, and Collins silt loam, respectively. The cotton cultivar DeltaPine 1522 B2XF (Bayer CropScience, St. Louis, MO) was planted in every site-year.

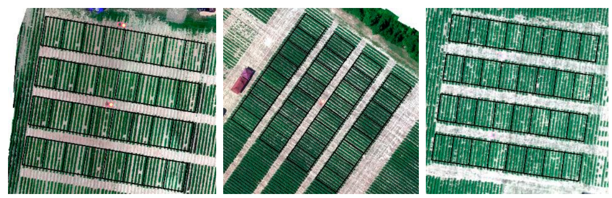

Figure 1. Cotton nitrogen (N) trial orthomosaics from (left to right): Milan 2016, Jackson 2017, and Ames 2017.

Treatments consisted of four total N rates applied at different timings (Table 1). All other agronomic management decisions were made in accordance with Univ. of Tennessee Extension Recommendations (Raper, 2016). All N was broadcast as ammonium nitrate (34-0-0) with a handheld fertilizer spreader. Application A was applied after cotton had emerged but prior to reaching the early square stage. Application B was applied during the first week of flower. Treatment application timings were selected based on management practices common to the region. Each treatment was replicated four times and arranged in a randomized complete block design. Each plot was six rows wide and 9 m in length. Row spacing at the Milan location was 1.01 m. Row spacing at Grand Junction and Jackson was 0.965 m. Planting, harvest, N application, and data collection dates are included in Table 2.

Table 1. Treatments consisted of varied rates and timings of N; application A was made after emergence but prior to early square. Application B was made during the first week of flower

Treatment #	Application A kg ha^{-1}	Application B kg ha^{-1}
1	0	0
2	45	0
3	90	0
4	135	0
5	22.5	22.5
6	45	45
7	67.5	67.5
8	0	90

Table 2. Planting, harvest, application, and data collection dates for each site-year

Location	Year	Application A	Application B	Data Collection 1	Data Collection 2	Planting	Harvest
Grand Junction	2016	25-May	8-Jul	8-Jul	9-Aug	6-May	17-Oct
	2017	16-Jun	11-Jul	11-Jul	n/a	2-May	29-Sep
	2018	15-Jun	11-Jul	11-Jul	n/a	4-May	8-Nov
Milan	2016	6-Jun	7-Jul	7-Jul	8-Aug	24-May	28-Oct
Jackson	2017	16-Jun	20-Jul	20-Jul	n/a	16-May	26-Sep
	2018	15-Jun	10-Jul	10-Jul	27-Aug	3-May	19-Oct

Ground-based Measurements. Plant measurements were collected at the first week of flower because this timing is generally assumed to be the latest timing in which an application of N could be applied and still impact seed cotton yield (Gerik et al., 1998; Raper, 2016). Furthermore, previous research has indicated the low N demand of the plant during the early growing season often prevents an accurate determination of N status before first flower (Gerik et al., 1998; Read et al., 2003). Additionally, to determine the N response at dates when N demand of each plant was substantial, a second ground-based data collection was conducted in 2016 at Grand Junction and Milan and in 2018 at the Jackson location.

Plant height, NDVI collected by a GreenSeeker handheld unit (Trimble Inc., Sunnyvale, CA), SPAD meter readings collected by a handheld SPAD 502 plus chlorophyll meter, DGCI readings from the FieldScout GreenIndex+ Nitrogen mobile application (Spectrum Technologies Inc., Aurora, IL), and leaf N content were collected at each data collection date. Plant height was manually collected from six plants within each plot. GreenSeeker NDVI was measured by walking the unit at 5 km hr^{-1} 0.75 m above row three and then row four of each plot. Five fully expanded, mainstem leaves located five nodes below the apical meristem were then removed from each plot. Three SPAD measurements were immediately collected from each leaf and the leaf was then placed on the FieldScout GreenIndex+ color board. An Apple iPhone 6 (Apple Inc, Cupertino, CA) running the FieldScout Application (Spectrum Technologies Inc., Aurora, IL) was then used to collect an image of the leaf and determine DGCI. At every data collection, collected mainstem leaves were placed on ice for transport until they could be placed within driers. After drying, samples were ground to pass a 20-mesh sieve and leaf N concentration was determined by dry combustion (ELEMENTAR Rapid N, ELEMENTAR Analysensysteme, Hanau, Germany).

Plots were harvested with either an automated weigh system outfitted on a Case 1822 or 2155 picker (CNH Industrial America, LLC, Racine, WI) or with a plot bagging system outfitted on a John Deere 9900 picker (Deere & Company, Moline, IL). Seed cotton yield was collected from the center two rows of each six-row plot.

Aerial Measurements. Aerial measurements were taken at a subset of the site-years. At early-season collection dates in 2016 in Milan (7 July) and in 2017 in Grand Junction (11 July) and Jackson (17 July), a custom quadcopter UAV equipped with a MicaSense RedEdge (MicaSense Inc., Seattle, WA) camera, which collects spectral reflectance data one image per second in five narrow bands (Table 3), was flown over the trial. Autonomous flight plans were programmed in Mission Planner software (ArduPilot, Indianapolis, IN), with 80% overlap and sidelap at 120 m altitude. In accordance with MicaSense protocol, images of a manufacturer-provided calibration panel were collected by the camera immediately prior to and after each flight. Calibration images, along with individual flight images, were uploaded into the cloud-based MicaSense Atlas (MicaSense Inc. Seattle, WA) service to create orthomosaics of the fields. Each resulting orthomosaic was downloaded in 16-bit GeoTIFF file format.

Table 3. MicaSense RedEdge camera specifications for band name, center wavelength (nm), and bandwidth (nm)

Band	Center Wavelength (nm)	Bandwidth (nm) at full width at half maximum (FWHM)
Blue	475	20
Green	560	20
Red	668	10
Red Edge	717	10
Near Infrared	840	40

Image Analysis. Image analysis of the orthomosaics was completed in ArcMap 10.5 (ESRI, Redlands, CA). Soil and shadowed areas were removed from the imagery using an unsupervised image classification technique that classified each pixel into vegetation, soil, or shadow. Pixels classified as soil or shadow were removed to minimize the influence of non-vegetative or non-illuminated areas on data intended to make crop management decisions. Plots were delineated in the images and the average and standard deviation of each in-season and yield measurement were assigned to their respective plots for further analysis.

The VIs in Table 4 were calculated using the plot-scale reflectance data collected by the UAV. Each VI was selected to capture commonly reported indices and indices that previously have been shown to correlate strongly to cotton N status (Ballester et al., 2017; Raper and Varco, 2015; Wang et al., 2012).

To evaluate correlation of UAV-calculated DGCI to cotton N status, hue, saturation, and brightness, values were calculated for each plot from UAV RGB reflectance using the equations provided by Karcher and Richardson (2003) and Rorie et al. (2011b). The DGCI was then calculated using the equation described in Table 4. A color board was fabricated using a piece of plywood with 1-m diameter disks painted to color-match the Munsell standards used by Karcher and Richardson (2003). The color board was placed in the alleys of the plot studies where it could be seen by the UAV-mounted sensor. The DGCI value of each color board was determined during each UAV flight and used in the calibration method described by Rorie et al. (2011a) to correct trial DGCI values.

Statistical analyses were conducted using JMP v14 (SAS Institute, Cary, NC). Seed cotton yield was considered to be a function of site-year, replication nested within site-year, fertilizer N rate, and fertilizer N timing. Pearson correlation coefficients were used to evaluate the association between in-season measurements and plant parameters. Yield data were subjected to analysis of variance in SAS (v9.5, SAS Institute, Cary, NC) to test for significance and means were separated using Fisher's protected least significant difference test at $p \leq 0.05$.

RESULTS

Ground-based Measurements. Observed cotton response to fertilizer N rate was weak at the early-season sampling dates (Fig. 2). Coefficients of determination for leaf N response to fertilizer N rate were greater than 0.9 at early sampling dates in half of the site-years, but less than 0.3 in half of the site-years. Relationships of NDVI, DGCI, SPAD, and height to N rate were poor with coefficients of determination never exceeding 0.4 (Fig. 2).

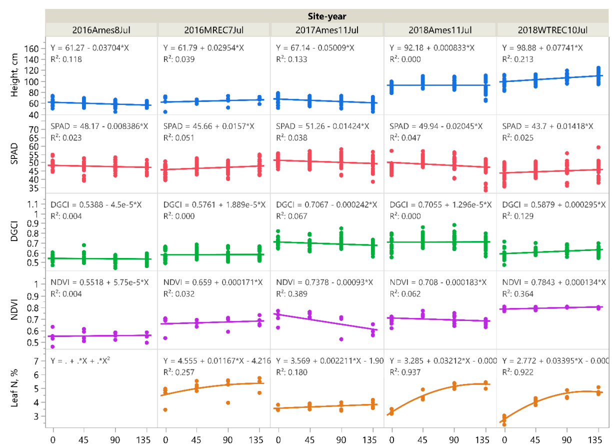

Figure 2. Early-season (prior to 15 July) response of height, soil plant analysis meter (SPAD), dark green color index (DGCI), normalized difference vegetation index (NDVI) and leaf nitrogen (N) to applied fertilizer N rate averaged across site-year. Because measurements occurred prior to the second application timing, only data from the plots receiving all N fertilizer at the early application timing were included.

Table 4. Selected vegetation indices (VIs) and corresponding references calculated within this study

Vegetation Index	Equation	Reference
Normalized Difference Vegetation Index (NDVI)	$\frac{R_{NIR,840} - R_{R,668}}{R_{NIR,840} + R_{R,668}}$	**Rouse et al., 1973**
Green Normalized Difference Vegetation Index (GNDVI)	$\frac{R_{NIR,840} - R_{G,560}}{R_{NIR,840} + R_{G,560}}$	**Gitelson et al., 1996**
Normalized Difference Red Edge (NDRE)	$\frac{R_{NIR,840} - R_{RE,717}}{R_{NIR,840} + {}_{RE,717}}$	**Gitelson and Merzlyak, 1994**
Simplified Canopy Chlorophyll Content Index (SCCCI)	$\frac{NDRE}{NDVI}$	**Barnes et al., 2000; Raper and Varco, 2015**
Dark Green Color Index (DGCI)	$\frac{\left[\frac{Hue-60}{60}+(1-Saturation)+(1-Brightness)\right]}{3}$	**Karcher and Richardson, 2003**

By the late-season sampling dates, cotton response to fertilizer N rate was stronger within some site-years (Fig. 3). A moderate response ($R^2 > 0.4$) was observed for all measured response parameters in the late sampling date from the 2016 Milan (MREC) site-year. SPAD, NDVI, and leaf N were the only parameters whose relationship with fertilizer N exceeded a coefficient of determination of 0.3 at the 2017 Jackson (WTREC) site-year, and only height and NDVI exceeded a coefficient of determination of 0.3 at the 2018 Jackson (WTREC) site-year. It is unclear why strong leaf N relationships with fertilizer N rate changed from early- to late-season sampling dates in the 2018 Jackson (WTREC) site-year, but it is suspected a rainfall event might have generated a large flush of vegetative growth immediately prior to sampling. The low leaf N observed typically would be associated with periods of rapid growth late in the year while boll development is increasing exponentially.

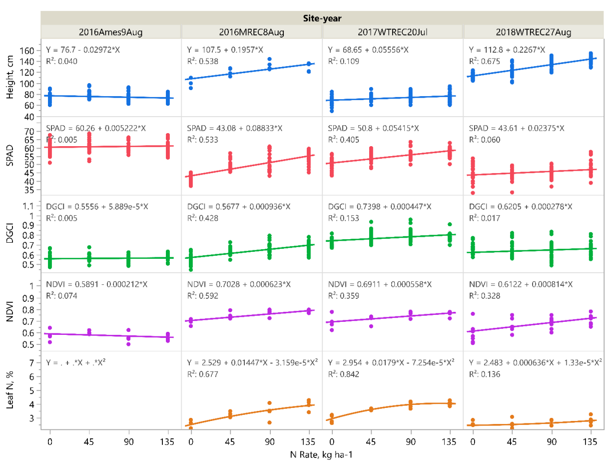

Figure 3. Late-season (after 15 July) response of height, soil plant analysis meter (SPAD), dark green color index (DGCI), normalized difference vegetation index (NDVI) and leaf nitrogen (N) to applied fertilizer N rate averaged across site-year. Plotted data consists of plots receiving all N fertilizer at the pre-squaring application timing.

The relationships of leaf N with SPAD, DGCI, and NDVI from early- and late-season measurements are shown in Fig. 4. Coefficients of determination (R^2) of leaf N with SPAD, DGCI, and NDVI were all weak ($R^2 \leq 0.13$) during early-season measurements, by the late-season measurement timings moderate to strong relationships ($R^2 > 0.4$) were observed between leaf N and SPAD as well as leaf N and DGCI. Both relationships appeared to be quadratic, with R^2 values between leaf N and SPAD, and leaf N and DGCI equaling 0.619 and 0.453, respectively. Nitrogen deficiency symptoms tend to be more visible and easier to measure later in the season as N demand is much higher as bolls are developing. However, this is generally considered to be too late to make an impact on yield through fertilizer N application.

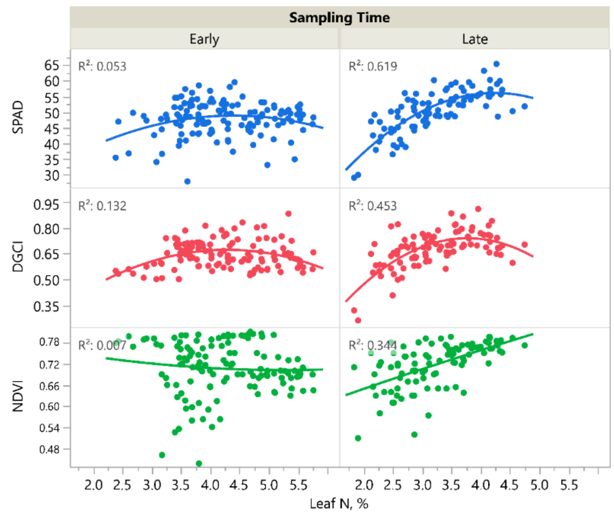

Figure 4. Relationships of leaf N with NDVI, DGCI, and SPAD graphed by sampling time, where the break between early and late the second week of flower (typically 15 July).

Across all sampling dates, a moderate relationship ($R^2 = 0.327$) was observed between SPAD and DGCI (Fig. 5). In contrast, no relationship was observed between NDVI and DGCI ($R^2 = 0.055$) and SPAD and NDVI ($R^2 = 0.002$). It is hypothesized that these poor relationships are a function of NDVI's sensitivity to biomass.

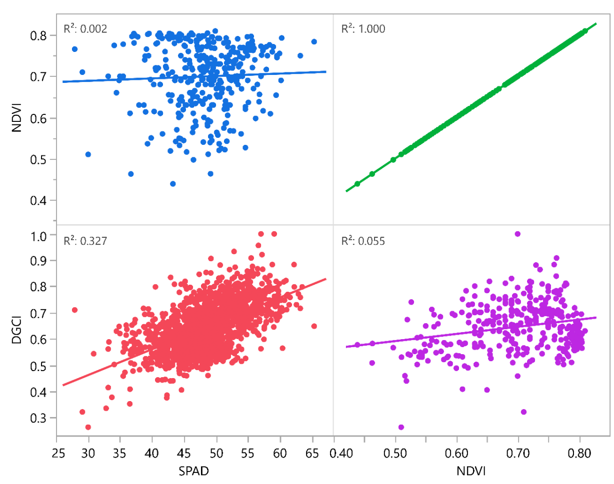

Figure 5. Relationships between SPAD, DGCI, and NDVI across early and late sampling times. Data from the late season 2016 Grand Junction site-year was excluded due to the severe drought stress present prior to that sampling date.

Aerial-based Measurements. Significant differences were observed between the three site-years evaluated; thus, correlation analysis was conducted by site-year (Table 5). The correlation between leaf N and all other measurements was not consistent for 2016 Milan and 2017 Grand Junction. It is notable that all ground-based and aerial measurements and VIs were highly correlated to leaf N at the later sampling date. This is in agreement with the ground-based analysis, where correlations of leaf N with SPAD, DGCI, and NDVI were mixed early in the season and became stronger later in the season. Of the five wavelengths evaluated, all but NIR (840 nm) were significantly correlated to leaf N at the 2017 Jackson site ($p < 0.01$) and at the 2017 Grand Junction site ($p < 0.05$). Similar trends in wavelength correlation to mid-season cotton leaf N were found in a ground-based study by Raper and Varco (2015).

Plant height was significantly positively correlated with GreenSeeker NDVI at all three site-years. Many studies have noted that NDVI is a better indicator of plant biomass than plant N status (Li et al., 2001; Raper et al., 2013). The correlation analysis for final seed cotton yield was conducted only with treatments 1 through 4 (0, 45, 90, and 135 kg N ha^{-1}) that did not receive a second N application. GreenSeeker NDVI and plant height were both positively correlated to seed cotton yield at every site-year. All reflectance readings and VIs, except NIR, were significantly correlated with final seed cotton yield at the 2017 Jackson site. Again, this is most likely due measurements being collected later as compared to the 2016 Milan and 2017 Grand Junction sites.

Both DGCI and the SPAD reading are indicative of the concentration of chlorophyll in crops such as corn (Rorie et al., 2011b) and cotton (Read et al., 2003). Wang et al. (2012) developed a model to predict cotton chlorophyll content from DGCI data with a R^2 of 0.88. This model was applied to both the ground- and UAV-based DGCI values in the current dataset and compared to SPAD measurements as the other indicator of plant chlorophyll content (Fig. 6).

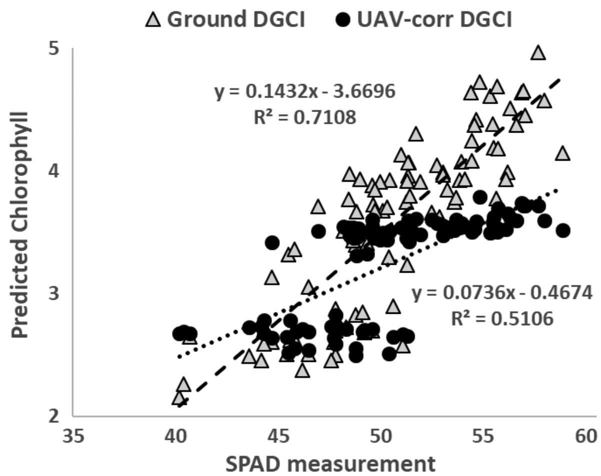

Figure 6. A regression of SPAD measurements to ground-based or UAV-based DGCI predictions of chlorophyll as modeled by Wang et al. (2012).

Chlorophyll meter measurements were moderately correlated with both methods of predicting chlorophyll content, with the Pearson correlation coefficients (r) of 0.715 and 0.843 for the UAV- and ground-based DGCI values, respectively. The ground-based chlorophyll prediction ($R^2 = 0.71$) from this dataset is stronger than the UAV-based ($R^2 = 0.51$). This can be caused by additional noise in the UAV data obtained by the wider field of view. Still, this dataset shows promise for the use of DGCI derived from early-season ground- and UAV-collected RGB images to predict chlorophyll content.

Table 5. Pearson correlation (r) values between specified plant parameters, vegetation indices, and individual wavelengths from ground-based sensors and UAV-based sensors from 2016 Milan, 2017 Grand Junction (GJ), and 2017 Jackson prior to Application B

		Leaf N						Plant height						Yield (only Trt 1-4)					
		2016 Milan		2017 GJ		2017 Jackson		2016 Milan		2017 GJ		2017 Jackson		2016 Milan		2017 GJ		2017 Jackson	
ground-based	SPAD	0.680	**	-0.224	ns	0.853	**	0.593	**	0.213	ns	0.405	*	0.526	*	-0.390	ns	0.614	*
	DGCI	0.434	*	-0.289	ns	0.578	**	0.443	*	0.112	ns	0.341	ns	0.232	ns	-0.345	ns	0.523	*
	NDVI	0.771	**	-0.095	ns	0.565	**	0.776	**	0.431	*	0.486	**	0.721	**	0.688	**	0.700	**
	Leaf N	-	-	-	-	-	-	0.845	**	-0.523	**	0.612	**	0.847	**	-0.170	ns	0.648	**
	Plant height	0.845	**	-0.523	**	0.612	**	-	-	-	-	-	-	0.733	**	0.558	*	0.558	*
UAV-based	DGCI-corr	-0.290	ns	-0.515	**	0.696	**	-0.356	*	0.468	**	0.601	**	-0.269	ns	0.339	ns	0.867	**
	NDVI	-0.394	*	-0.539	**	0.761	**	-0.413	*	0.583	**	0.700	**	-0.331	ns	0.503	*	0.820	**
	NDRE	-0.404	*	-0.395	*	0.868	**	-0.352	*	0.638	**	0.598	**	-0.331	ns	0.582	*	0.762	**
	GNDVI	-0.471	**	-0.398	*	0.842	**	-0.431	*	0.608	**	0.598	**	-0.343	ns	0.561	*	0.806	**
	SCCCI	-0.373	*	-0.303	ns	0.849	**	-0.289	ns	0.619	**	0.547	**	-0.319	ns	0.584	*	0.724	**
	B	0.208	ns	0.567	**	-0.673	**	0.325	ns	-0.599	**	-0.618	**	-0.073	ns	-0.492	ns	-0.774	**
	G	0.263	ns	0.387	*	-0.779	**	0.329	ns	-0.621	**	-0.526	**	0.011	ns	-0.517	*	-0.788	**
	R	0.261	ns	0.586	**	-0.745	**	0.382	*	-0.607	**	-0.668	**	0.003	ns	-0.466	ns	-0.842	**
	RE	0.127	ns	0.386	*	-0.779	**	0.216	ns	-0.684	**	-0.500	**	-0.110	ns	-0.518	*	-0.726	**
	NIR	-0.222	ns	-0.327	ns	0.316	ns	-0.078	ns	0.402	*	0.328	ns	-0.603	*	0.571	*	0.090	ns

[z]**Significance levels are denoted by** **$^{**}p < 0.01$ and $^{*}p < 0.05$.**

Seed Cotton Yield. Three separate models were tested to determine 1) the impact of fertilizer N rate on seed cotton yield, 2) the impact of splitting the N application versus applying all N pre-square on seed cotton yield, and 3) the impact of delaying the fertilizer N application until flower on seed cotton yield. The first four treatments of 0, 45, 90, and 135 kg N ha^{-1} applied pre-square were compared within the first model (Table 6, Fig. 7). In the second model, treatments 2, 3, and 4 (45, 90, and 135 kg N ha^{-1} applied pre-square) were compared to treatments 5, 6, and 7 (a total of 45, 90, and 135 kg N ha^{-1} split into two applications) (Table 7). In the third model, treatments 3, 6, and 8 (90 kg N ha^{-1} applied pre-square, split, and delayed until flower) were compared (Table 8, Fig. 8).

Table 6. Analysis of variance (ANOVA) table for seed cotton yield response to site-year, replication nested within site-year, and fertilizer nitrogen (N) rate WHERE N Rate equals 0, 45, 90 or 135 kg ha-1 and Timing equals early season

Source	DF	Sum of Squares	F Ratio	P > F
Model	26	45545047	7.7974	<.0001
Site-year	5	31256573	27.8263	<.0001
Replication (Site-year)	18	11630433	2.8761	0.0009
N Rate	3	2658041	3.9439	0.0117
Error	69	15501193		
Total	95	61046240		

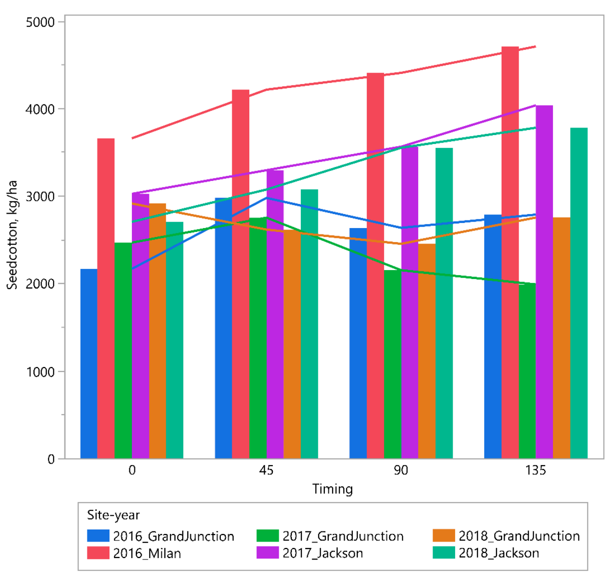

Figure 7. Response of seed cotton yield to fertilizer nitrogen (N rate) graphed by site-year. Values not sharing any letter within the N rate among all site-years are significantly different by the Fisher's protected least significant difference at the 5% level of significance.

Table 7. Analysis of variance (ANOVA) table for seed cotton yield response to site-year, replication nested within site-year, fertilizer nitrogen (N) rate, fertilizer N timing, and their interactions. Fertilizer N rate equals 45, 90 or 135 kg ha-1 and Timing equals early season or split

Source	DF	Sum of Squares	F Ratio	P > F
Model	28	63067309	12.8801	<.0001
Site-year	5	48646134	55.6353	<.0001
Replication (Site-year)	18	13600651	4.3208	<.0001
N Rate	2	324737	0.9285	0.3981
Timing	1	311759	1.7528	0.1844
N Rate x Timing	2	254017	0.7263	0.4859
Error	115	20110629		
Total	143	83177938		

Table 8. Analysis of variance (ANOVA) table for seed cotton yield response to site-year, replication nested within site-year, and fertilizer nitrogen (N) timing. Fertilizer N rate equals 90 kg ha^{-1}.

Source	DF	Sum of Squares	F Ratio	P>F
Model	25	34603307	10.7967	<.0001
Site-year	5	24581235	38.3485	<.0001
Replication (Site-year)	18	9158559	3.9689	<.0001
Timing	2	863514	3.3679	0.0432
Error	46	5897163		
Total	71	40500470		

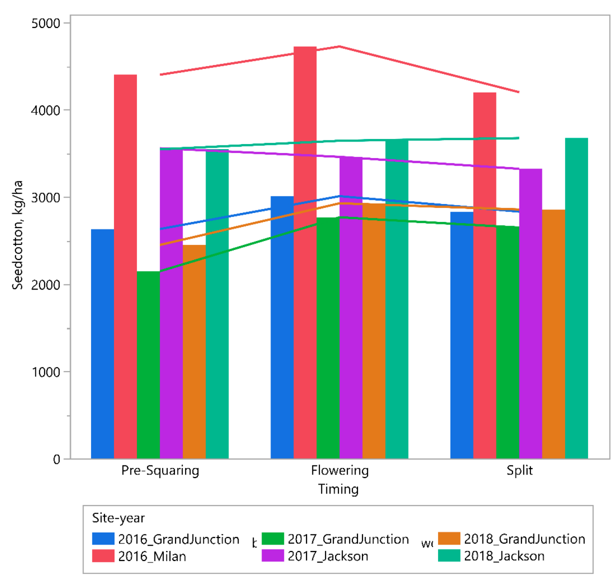

Figure 8. Response of seed cotton yield to fertilizer nitrogen (N) timing graphed by site-year. Values not sharing a letter are significantly different by the Fisher's protected least significant difference at the 5% level of significance.

Across all site years, a limited response to fertilizer N rate was observed (Table 6; Fig. 7). A significant increase in seed cotton yield was observed between the untreated and N application rates of 45 and 135 kg N ha^{-1}, but no significant differences were observed between the untreated and 90 kg N ha^{-1} treatments or the 45 and the 135 kg N ha^{-1} treatments. Limited response of seed cotton yield to N applied is suspected to be driven in some site-years potentially due to the presence and variability of soil residual N. No significant response in seed cotton yield was observed when splitting the 45, 90 or 135 kg ha^{-1} N applications (Table 7). Analysis of the 90 kg ha^{-1} treatments consisting of pre-squaring, flowering and split N timings indicated delaying the entire N application until flowering resulted in greater yields than applying the entire application pre-square (Table 8; Fig. 8). Significant differences between split application timing and a pre-square or delayed (at flower) timing were not captured (Fig. 8).

DISCUSSION

Results from this study reinforce three major issues limiting the utility of in-season cotton N status measurements to drive variable rate fertilizer N applications. First, strong cotton response to applied fertilizer N might not appear every year, especially in areas where soil nitrate levels can provide much of the N required by the crop. When Main et al. (2015) summarized cotton N response across 20 U.S. site-years, they only observed a significant response to fertilizer N in 11 of the 20 site-years. As a result, they incorporated soil nitrate data to generate strong, consistent trends across all locations (Main et al., 2015). Weak to moderate responses of cotton to fertilizer N rate also have been reported in several site-years by Oliveira et al. (2012).

Second, even when cotton response to fertilizer N is moderate to strong, cotton demand for N is low during the early season and does not increase to a substantial level until boll fill begins (Mullins and Burmester, 1990). Poor correlations between early-season measurements and applied fertilizer N are commonly reported (Oliveira et al., 2012). Most N deficiencies develop after peak bloom when fertilizer N application cannot completely alleviate the deficiency.

Finally, it is commonly recommended that fertilizer N be applied prior to bloom (Duncan and Raper, 2018; Lemon et al., 2009). Sidedress N must be moved into the effective rooting zone by an incorporating rainfall event or irrigation, and depending upon source, might not be immediately available to the plant. Although irrigation could allow producers to extend the application window, bloom typically represents the last opportunity to make a yield-impacting fertilizer N application. According to Oliveira et al. (2012), “Producers who currently apply N at the early square stage will also weigh the logistical risks of delaying N applications against the possible benefits of sensor use.” Although our results suggest these benefits could be notable, it is not clear if they will be substantial enough to warrant the added risk.

Much of the research to date has focused on the early-season assessment of cotton N status to drive variable rate fertilizer N applications within the current growing season. Because demand for N is low during the early season, soil nitrate often meets early-season demand and all fertilizer N should be applied prior to first bloom, it might be appropriate to shift the research focus towards the use of late-season assessments of cotton N status to drive variable rate fertilizer N applications for the following season. Coupled with soil nitrate sampling, it is possible that this approach could support increased N use efficiency in the cotton production system while not forcing the producer to incur unreasonable levels of risk.

CONCLUSIONS

This study provides information to researchers, producers, and advisors on the utility and current limitations of in-season measurements of cotton N status. Correlations of leaf N with both ground- and UAV-based measurements were often weak during the window of time in which a yield-impacting fertilizer N application could be made. At later season sampling dates, strong relationships were noted between leaf N and SPAD and leaf N and DGCI. Although this information will not be able to completely alleviate N deficiencies within the current season, late-season measurements could potentially provide valuable information to be used in subsequent seasons or to direct supplemental foliar N applications within the current season. Across all sampling dates and site-years, ground-based DGCI had a positive linear relationship with SPAD (R^2 = 0.327). Furthermore, early-season DGCI from ground-based (R^2 = 0.71) and aerial-based (R^2 = 0.51) images were successfully used to predict chlorophyll. These findings indicate that DGCI could potentially be used as a replacement for SPAD in determining cotton N status.

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