The *Journal of Cotton Science 20:1–7 (2016)*
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AGRONOMY AND SOILS

Influence of Nitrogen and Mepiquat Chloride on Cotton Canopy Reflectance Measurements

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ABSTRACT

Crop reflectance sensors have been used to assess nitrogen (N) status in many crops. The SPAD chlorophyll meter and the GreenSeeker® sensor have been studied extensively to determine their ability to detect crop N status and predict crop N requirement. Mepiquat chloride (MC) is routinely used in cotton to manage vegetative growth. Pigment concentration often increases following application of MC and could confound results from GreenSeeker readings used to adjust N application. A field experiment was conducted in Georgia and North Carolina to determine the effect of MC on SPAD meter and GreenSeeker sensor readings. Treatments consisted of a factorial arrangement of multiple N and MC rates. Nitrogen was applied at the initial appearance of squares followed by MC application 14 to 21 d later. SPAD meter and GreenSeeker sensor readings were taken for 10 consecutive weeks beginning immediately before N application. The SPAD meter proved to be sensitive to MC and N, but was not associated with lint yield. The GreenSeeker sensor was sensitive to N, but not sensitive to MC. Furthermore, normalized difference vegetative index and lint yield responded similarly to N, suggesting that GreenSeeker might be a useful tool in assessing plant N status and predicting crop N requirement.

Crop canopy reflectance sensors can be used to monitor growth and development of crops. Crop reflectance sensors have been developed over the past 20 years that are active (integrated light sources), portable, and relatively inexpensive. These sensors provide canopy spectral data instantly to estimate physiological or morphological characteristic of crops, such as chlorophyll content or plant height, at the ground level (Samborski et al., 2009). Crop reflectance sensors emit light in specific wavelengths that are absorbed, transmitted, or reflected depending on the photosensitivity of the biological compounds under investigation. The reflected portion of the emitted light is measured and is dependent on the concentration of the photosensitive compounds. Red (600-700 nm) and near-infrared (750-900 nm) wavebands are used commonly because they are associated with high absorptivity of specific plant photoreceptive pigments (e.g., chlorophyll and carotenoids) and high reflectivity of leaf surfaces, respectively (Knipling, 1970). Reflectance in the red waveband is negatively correlated to green leaf area; however reflectance in the near-infrared waveband is positively correlated with leaf area (Knipling, 1970).

The SPAD (SPAD-502, Konica Minolta, Tokyo, Japan) chlorophyll meter and the GreenSeeker® sensor (Model 505, NTech Industries Inc., Ukiah, CA) are commonly used instuments that emit red and near-infrared light and sense the transmitted or reflected portion of the source light to calculate a vegetative index. The SPAD meter is a hand-held instrument that clamps on a leaf and measures the transmission of light through a leaf in the 650 nm and 940 nm wavelengths to calculate a vegetative index in SPAD units (Kim et al., 2012). These units provide an indication of relative chlorophyll density in the sampled leaf and have been highly correlated to leaf nitrogen (N) status in corn (*Zea Mays* L.), cotton (*Gossypium hirsutum* L.), rice (*Oryza sativa* L.), and sweet corn (*Zea mays* L.) (LiHong et al., 2004; Ma et al., 2007; Samborski et al., 2009; Solari et al., 2010; Wood et al., 1992). The GreenSeeker sensor can be hand held or attached to agricultural equipment, and it contains a light source that emits red light at 660 nm and near-infrared light at 770 nm and measures the reflected portion. The reflected

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light is used to calculate another vegetative index called the normalized difference vegetative index (NDVI). It is calculated as:

$$NDVI = (NIR - R)/(NIR + R) \quad (1)$$

where NIR and R are the spectral reflectance of the emitted near-infrared and red wavebands, respectively (Kim et al., 2012). The NDVI has been correlated to many plant growth and development parameters. Also, NDVI has been used to monitor leaf N status in rice and sugarcane (*Saccharum officinarum* L.) growth to optimize N applications, predict wheat (*Triticum aestivum* L.) biomass and grain yield, predict wheat N concentration, and estimate canola (*Brassica napus* L.) and corn yield potential (Holzapfel et al., 2009; LiHong et al., 2004; Osborne, 2007; Singh et al., 2006; Solari et al., 2010). The NDVI has been used in cotton as a possible indicator of crop maturity, leaf area, biomass, plant height, height-to-node ratio, nodes above cracked boll, nodes above white flower, water stress, plant N status, and lint yield (Gutierrez et al., 2012; Gwathmey et al., 2010; Plant et al., 2001; Stamatiadis et al., 2010).

Perhaps the most important role of these portable ground-based sensors is to provide real-time assessment of leaf N status. Leaf N estimates, when referenced against well-fertilized strips, can be used to predict actual crop N need, calculate an N rate, and adjust N application rates in the field. Growers can apply optimal levels of N when combining these sensors with other precision agriculture equipment, such as global positioning systems and variable rate technology. Notably, Kim et al. (2012) reported that GreenSeeker sensor NDVI readings might not be reliable when leaf coverage is less than 30%, indicating that timing of the use of the sensors is critical to their performance. Gutierrez et al. (2012) found that the best time to use remote optical sensors in cotton is during bloom because the correlation between estimated lint yield and NDVI is the highest. Khalilian et al. (2008) demonstrated that precise applications of N in cotton during bloom can maximize crop yield, while reducing N use and improving N-use efficiency.

Mepiquat chloride (MC) is commonly used in the southeastern U.S. to control plant height by reducing cell elongation in cotton (Edmisten, 2012; Ramachandra Reddy et al., 1996). Mepiquat chloride has been shown to increase leaf chlorophyll concentration, decrease ribulose bisphosphate carboxylase/oxygenase (RuBP) activity, reduce leaf area, and reduce net photosynthetic rates for as much as 20 d after application (Pettigrew, 2010; Ramachandra Reddy et al., 1996). Field experiments also indicate that MC-treated plants are consistently shorter and lint yields are affected inconsistently (Pettigrew, 2010; Wilson et al., 2007; York, 1983a, b). Mepiquat chloride can be applied as early as pre-bloom; therefore, mid-bloom applications of N relying on sensor-based N rates might be unjustifiably altered by MC. The SPAD chlorophyll meter and the GreenSeeker sensor can respond to an MC application due to increased chlorophyll concentration (green pigmentation) and reduced leaf surface area. Mepiquat chloride is most likely to reduce the R and NIR values in equation (1), but the ensuing effect on NDVI is unknown. Mepiquat chloride might confound the GreenSeeker sensor by causing a shift in NDVI without causing an actual change in plant N status.

Potential confounding of NDVI by MC has not been documented; therefore research was conducted in Georgia and North Carolina to determine the effect of MC on the SPAD meter and GreenSeeker sensor vegetative indices. Different levels of N and MC were tested to: 1) evaluate the response of SPAD meter and GreenSeeker sensor over time in response to N and 2) determine MC effects on SPAD meter and GreenSeeker sensor data.

MATERIALS AND METHODS

An experiment was conducted in Georgia during 2010 on a Tifton loamy sand soil (fine-loamy, kaolinitic, thermic, Plinthic Kandiudults) near Tifton. Experiments were also conducted in North Carolina during 2010 on a Whickham loamy sand soil (fine-loamy, mixed, thermic, Typic Hapludults) near Goldsboro and during 2011 on a Norfolk loamy sand soil (fine-loamy, siliceous, thermic, Typic Paleadults) near Clayton. Plot size was 12.2 m long and 3.7 m wide, containing four rows of cotton spaced at 0.91 m apart. Strip tillage was used within 7 d of planting and consisted of 33- to 40-cm-wide tillage zones prepared using a KMC strip tillage implement (Kelley Manufacturing Co., Tifton, GA), with in-row subsoiler followed by two sets of coulters and two rolling baskets to smooth the tillage zone. Cotton cultivars DP0949 BGRF (Monsanto, St. Louis, MO), PHY 375 WRF (Dow Agrosciences, Indianapolis, IN), and PHY 375 WRF (Dow Agrosciences, Indianapolis, IN) were planted on 19 May 2010, 6 May 2010, and 11 May 2011 in Tifton, Goldsboro, and Clayton, respectively. Cotton was planted at 13 seeds m^{-1}. These cultivars were popular in each growing region where the experiments were conducted.

Broadcast N applications of urea-NH_4NO_3 liquid N (UAN) were applied at 0, 45, and 90 kg N ha^{-1} when squares were first visible on cotton plants at approximately eight to nine true leaves. Nitrogen was applied 16 June 2010, 23 June 2010, and 22 June 2011 in Tifton, Goldsboro, and Clayton, respectively using a CO_2-pressurized backpack mounted with a single nozzle (XR8004 nozzle, Spraying Systems Co., Wheaton, IL) to deliver N evenly in row middles. The plant growth regulator MC (Mepiquat Chloride 4.2% Liquid, Makhteshim Agan of North America Inc., Raleigh, NC) was applied at 0, 0.5, and 0.10 kg a.i. ha^{-1} using a CO_2-pressurized backpack sprayer with XR8002 nozzles (Teejet Co., Wheaton, IL). Mepiquat chloride was applied 14 d after the N application in North Carolina and 21 d after N application in Georgia, based on recommendations by Edmisten (2012). All other production and pest management practices throughout the season, except N fertilization and MC application, were held constant over the experiment and were conducted in accordance with cooperative extension service recommendations for the region to optimize yield. Cotton was not irrigated. The previous crop in Georgia and North Carolina was cotton.

Cotton plant stands were counted from two, 3-m sections of the middle two rows within 3 wk of planting. A hand-held SPAD chlorophyll meter was used to determine relative chlorophyll content of the uppermost fully expanded leaf. One leaf from 10 randomly selected plants was sampled from within the center two rows of each plot and averaged to obtain a plot SPAD value. Sampling was conducted for 10 consecutive weeks beginning immediately prior to N application. NDVI values for each plot were obtained using a GreenSeeker sensor connected to a hand-held computer (Nomad®, Trimble, Sunnyvale, CA) by scanning the center two rows. The GreenSeeker sensor and Nomad computer were mounted on a pole and carried at 4.8 km h^{-1} over the row. The GreenSeeker sensor height above the crop canopy was maintained at 76 cm above the center of the row. The computer sampling rate was set at 10 samples sec^{-1}. An average NDVI value was calculated by the Nomad computer. No SPAD readings or GreenSeeker sensor scans were taken within 1 m of the end of rows. GreenSeeker scans were taken within 5 min of recording SPAD readings. The center two rows of each plot were machine harvested with a two-row spindle picker modified for small-plot research.

The experimental design was a randomized complete-block design consisting of a factorial arrangement of 3 (N rates) by 3 (MC rates). Treatments were replicated four times. Data for SPAD and NDVI readings at 7 and 14 d after N application (no MC applied at this time) were subjected to analysis of variance (ANOVA) using the general linear model in SAS (Version 9.2, SAS Institute, Cary, NC) for a 3 (sites) by 3 (N rates) treatment structure. Data for SPAD and NDVI readings at 1, 2, and 3 wk following MC application were subjected to ANOVA using the general linear model in SAS for a 3 (sites) by 3 (weeks) by 3 (N rates) by 3 (MC rates) treatment structure. Data for lint yield were subjected to ANOVA using the general linear model in SAS for a 3 (sites) by 3 (N rates) by 3 (MC rates) treatment structure. Means of significant main effects and interactions were performed using Fisher's Protected LSD at $p \leq 0.05$.

RESULTS AND DISCUSSION

A difference in SPAD meter and GreenSeeker values relative to N rates did not occur until 14 d after N was applied (Table 1). This delay was most likely caused by the time required for cotton to absorb soil N and result in a physiological response. One limitation to this research and its application is that N content in leaves was not recorded. However, differences in estimates of morphological and physiological processes were observed relative to N rate and MC treatment.

Normalized difference vegetative index was affected by N, the interaction of site and N, and by evaluation period (weeks); but NDVI was not affected by MC (Table 2). Normalized difference vegetative index was lowest when no N was added across all locations indicating that cotton plants were less green and/or smaller as expected. However, NDVI did not respond to all N rates consistently across locations. Normalized difference vegetative index values were not different when comparing sensor response at the 45 and 90 kg N ha^{-1} rates at Tifton and Goldsboro, although differences were noted at Clayton when these rates of N were applied (Table 3). Although NDVI was affected by week, there was no interaction of week with the other treatment factors. Variability of NDVI across weeks is expected because plant biomass and pigment concentration change over time (Gwathmey et al., 2010). These results suggest that NDVI is sensitive to cotton response to N but not MC application, and mid-bloom is a suitable time to scan cotton with the GreenSeeker. The lack of an interaction between week and N or week and MC indicates that NDVI responded consistently across the three consecutive weeks scanned during bloom.

Table 1. Analysis of variance for normalized difference vegetative index (NDVI) and SPAD readings 7 and 14 d after nitrogen application as influenced by nitrogen, data pooled over site

Treatment factor	Days after N application							
	7				14			
	NDVI		SPAD		NDVI		SPAD	
	F-Value	*p* > F	F-Value	*p* > F	F-Value	*p* > F	F-Value	*p* > F
Location (Loc)	141.4	< 0.0001	277.9	< 0.0001	195.7	< 0.0001	184.0	< 0.0001
Nitrogen (N)	1.4	0.2434	0.4	0.6706	7.6	0.0011	26.0	< 0.0001
Loc x N	1.4	0.2356	1.1	0.3609	1.0	0.4078	2.9	0.0245
Coefficient of variation (%)	---------- 6.2 ----------		---------- 3.5 ----------		---------- 3.8 ----------		---------- 3.2 ----------	

Table 2. Analysis of variance for normalized difference vegetative index (NDVI) and SPAD readings as influenced by location, nitrogen, MC, and evaluation period (week)

Treatment factor	NDVI		SPAD	
	F-Value	*p* > F	F-Value	*p* > F
Location (Loc)	95.8	< 0.0001	388.8	< 0.0001
Nitrogen (N)	33.4	< 0.0001	73.8	< 0.0001
Mepiquat chloride (MC)	0.7	0.5096	36.7	< 0.0001
Nitrogen x MC	1.7	0.1445	1.1	0.3306
Loc x nitrogen	5.8	0.0007	5.3	0.0010
Loc x MC	1.6	0.1921	13.1	< 0.0001
Loc x nitrogen x MC	1.8	0.0902	0.4	0.9126
Evaluation period (Week)	52.2	< 0.0001	32.5	< 0.0001
Loc x Week	2.5	0.0922	44.7	< 0.0001
Week x N	0.7	0.4990	0.3	0.7592
Week x MC	1.4	0.2650	18.9	< 0.0001
Week x nitrogen x MC	0.6	0.6564	1.2	0.3033
Loc x Week x N	0.8	0.5267	1.9	0.1135
Loc x Week x MC	0.5	0.7631	24.5	< 0.0001
Loc x Week x nitrogen x MC	0.1	0.9996	1.2	0.2992
Coefficient of variation (%)	------------------ 4.4 ------------------		------------------ 3.0 ------------------	

Table 3. Normalized difference vegetative index (NDVI) as influenced by nitrogen rate and location[z]

Nitrogen rate	Normalized Difference Vegetative Index (NDVI)		
	Tifton, GA, 2010	Goldsboro, NC, 2010	Clayton, NC, 2011
kg ha^{-1}			
0	0.861 b	0.772 b	0.621 c
45	0.884 a	0.805 a	0.718 a
90	0.884 a	0.811 a	0.699 b

[z] Means within a site followed by the same letter are not different according to Fishers Protected LSD at $p \leq 0.05$. Data pooled over weeks (1, 2, and 3wk after mepiquat chloride application).

SPAD meter data were influenced by N, MC, interaction of location by N, interaction of site by MC, week, interaction of site by week, interaction of week by MC, and interaction of location by week by MC (Table 2). Leaf SPAD meter readings were related to N rates in Tifton and Goldsboro, but not at Clayton (Table 4). The SPAD meter registered a higher SPAD value at each increase in N except in Clayton where SPAD values were the same at 45 and 90 kg N ha^{-1}. SPAD meter readings also were sensitive to MC, but responses were inconsistent across locations and weeks (Table 5). Lower SPAD values were noted in most locations and weeks when no MC was applied. SPAD values at 0.05 and 0.10 kg a.i. ha^{-1} were the same in all locations and weeks except in Goldsboro at 2 wk after MC application. These results indicate that the SPAD meter is sensitive to cotton response to N and MC applications during early to mid-bloom. However, SPAD meter response to MC is expected, because MC can increase chlorophyll concentration in leaves (Ramachandra Reddy et al., 1996).

Cotton yield was affected by N and the interaction of location and N, but not by MC (Table 6). Lint yield response to N was variable at each location (Table 7). Overall yield at Goldsboro was low due to limited rainfall during much of the season. The 45 kg N ha^{-1} rate produced at least the same amount of lint as the 90 kg N ha^{-1} rate. A review of Tables 7 and 3 reveals similar trends in lint yield and NDVI values as influenced by N rate and location. The highest NDVI values corresponded to the highest lint yields at all three locations regardless of the N rate, suggesting that the GreenSeeker might be useful in assessing plant N status and predicting N need to produce optimal lint yield.

Table 4. SPAD reading as influenced by nitrogen rate and location[z]

Nitrogen rate	SPAD Units		
	Tifton, GA, 2010	Goldsboro, NC, 2010	Clayton, NC, 2011
kg ha^{-1}			
0	41.9 c	38.7 c	44.2 b
45	45.3 b	41.9 b	46.5 a
90	47.5 a	43.1 a	47.4 a

[z] Means within a location followed by the same letter are not different according to Fishers Protected LSD at $p < 0.05$. Data pooled over weeks (1, 2, and 3wk after mepiquat chloride application).

Table 5. SPAD readings as influenced by mepiquat chloride (MC) rate, week, and location[z]

MC rate	Weeks after MC application						
	1	2			3		
		Tifton, GA	Goldsboro, NC	Clayton, NC	Tifton, GA	Goldsboro, NC	Clayton, NC
kg ha^{-1}	SPAD units						
0	41.5 b	46.3 a	33.0 c	45.5 b	43.7 b	39.8 b	45.1 b
0.05	43.0 a	46.9 a	41.4 b	46.5 ab	45.4 a	47.4 a	46.0 ab
0.10	42.8 a	47.0 a	42.8 a	47.6 a	44.1 ab	47.6 a	47.0 a

[z] Means within a location followed by the same letter are not different according to Fishers Protected LSD at $p \leq 0.05$. Data pooled over nitrogen rates and data pooled over sites at 1 wk after MC application only.

Table 6. Analysis of variance of cotton yield as influenced by nitrogen, mepiquat chloride, and location

Treatment factor	Cotton yield	
	F-Value	$p > F$
Location (Loc)	9.9	0.0089
Nitrogen (N)	19.8	< 0.0001
Mepiquat chloride (MC)	2.5	0.0905
N x MC	0.9	0.4751
Loc x N	2.9	0.0293
Loc x MC	2.4	0.0623
Loc x N x MC	0.4	0.8998
Coefficient of variation (%)	10.3	

Table 7. Cotton yield as influenced by nitrogen rate and location[z]

Nitrogen rate	Cotton yield		
	Tifton, GA, 2010	Goldsboro, NC, 2010	Clayton, NC, 2011
kg ha^{-1}	kg lint ha^{-1}		
0	1,060 b	520 b	1,070 c
45	1,240 a	580 ab	1,320 a
90	1,230 a	590 a	1,210 b

[z] Means within a location followed by the same letter are not different according to Fishers Protected LSD at $p \leq 0.05$.

SUMMARY

Our results indicate that the SPAD meter appears to be sensitive to MC, which might limit use in cotton as an indicator of leaf N status. The SPAD meter also gave little indication that it would be a useful tool to predict plant N requirement because results were not consistent with plant response to N application. GreenSeeker was the more robust sensor because it was insensitive to MC and the study confirmed that NDVI has some potential to assess N status given response to N application for NDVI and cotton yield was similar. Further conclusions from our data are limited because N status of cotton leaves was not determined. Raper et al. (2013) reported that analyzed NDVI sensors, including those used here, did not predict consistently N status prior to flowering. They also reported that analyzed NDVI sensors were sensitive to changes in cotton height. Bronson et al. (2003, 2005) also indicated that NDVI readings can be insensitive to changes in N status of cotton leaves. More research is recommended to determine if the GreenSeeker sensor can be used before bloom and across more soil types. Earlier N and MC applications plus additional N rates should be tested to obtain a more comprehensive data set to better understand the NDVI to plant N status relationship.

ACKNOWLEDGMENTS

This research was supported in part by funds administered through the North Carolina Cotton Producers Association.

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