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Spectral reflectance for growth and yield assessment of irrigated cotton

Ziany N. Brandão*, Valdinei Sofiatti, José R. C. Bezerra, Gilvan B. Ferreira, José C. Medeiros[1]

Brazilian Agricultural Research Corporation, Embrapa Cotton, 1143, Oswaldo Cruz, 58428-095. Campina Grande, PB, Brazil

***Corresponding author: ziany.brandao@embrapa.br**

Abstract

The canopy reflectance using ground-based sensors has the potential to provide information on crop nitrogen content. The objective of this study was to determine relationships between canopy spectral reflectance and leaf N content, leaf area index (LAI), aboveground biomass (Biom) and yield of irrigated cotton under four nitrogen rates (0, 90, 180 and 270kg ha^{-1}). Measurements of canopy reflectance were made throughout the growing seasons (2009-2010) using a hand-held spectroradiometer. Samples for LAI and Biom were obtained three times from squaring until fruiting. The normalized difference vegetation index (NDVI), soil adjusted vegetation index (SAVI), modified soil adjusted vegetation index (MSAVI) and the modified transformed vegetation index (MTVI2) were calculated from the hyperspectral reflectance data. All vegetation indexes (VIs) and also SPAD-502 readings allowed to figure out mathematical models for N content prediction in cotton leaves with great precision (r > 0.74). It also showed good correlations (r from 0.55 to 0.96) with the LAI since the first flowering. The vegetation indexes obtained through the canopy reflectance, explained more than 64% of the variation in cotton biomass. The MTVI2 was the index that provided the best LAI prediction in advanced stages. The peak flowering stage was the best time to estimate the cotton biomass, where the MSAVI and MTVI2 showed to be excellent predictors. The results indicate that the cotton yield can be estimated through the hyperspectral reflectance since the squaring until fruiting in irrigated conditions.

Keywords: *Gossypium hirsutum*; leaf area index; nitrogen fertilization; remote sensing; vegetation indexes.
Abbreviations: *Biom*_Above ground biomass; Chl_ Chlorophyll; LAI_Leaf area index; N_Nitrogen; LAI-3100_ Leaf area meter by LI-COR; L_Soil adjustment factor; MSAVI_ Modified Soil Ajusted Vegetation Index; MTVI2_ Modified second Triangular Vegetation Index; NDVI_ Normalized Difference Vegetation Index; SAVI_ Soil Ajusted Vegetation Index; *r*_ Pearson correlation; SPAD_Minolta handheld chlorophyll meter of leaves; ρ_{800}, ρ_{670} and ρ_{550} _reflectance in the near infrared, red and green spectral bands

Introduction

Remote sensing techniques are useful to evaluate agronomic parameters. It assists to decide on nutritional supplementation and productivity assessment in many crops (Gutierrez et al., 2012). The canopy spectral reflectance has been correlated with crop growth and variables such as biomass and leaf area index (LAI), which can be determined by the shape and intensity of solar radiation interception (Pinter et al., 1994). In addition to the vegetation structural characteristics, many studies have reported that spectral indexes measured during crop development can be used to estimate crop yield, since the plant production is correlated to the amount of photosynthetic tissue. Therefore, it is correlated to growth variables of vegetation, such as plant height, chlorophyll content, vegetation density and aboveground biomass (Zarco - Tejada et al., 2005; Rossato et al., 2012). The LAI is one of the canopy biophysical variables that plays a major role in physiological processes. Many studies have been attempted to find ways to improve the relationship between LAI and spectral indices (Haboudane et al., 2004; Zhao et al., 2005). However, conventional measurements both for LAI and biomass are time consuming and costly, while measurements by spectral indices are fast, non-destructive and can be performed on a large scale (Eitel et al., 2008). The dry matter production by plants and their tolerance to stress is influenced by the amount of chlorophyll (Chl) due to the strong relationship of this pigment in photosynthetic processes (Fridgen and Varco, 2004; Brito et al., 2011). Therefore, non-destructive measurement of plants, based on the intensity of light absorbed in the red and reflected in near-infrared of electromagnetic spectrum, is useful to determine foliar thickness and biomass production (Zhao et al., 2005). In general, plants deficient in nitrogen (N) show chlorosis and reduction in the number of leaves, decreasing growth and presenting eventual necrosis (Malavolta et al., 2004; Brandão et al., 2012). Thus, the continual monitoring of N content in leaf tissues of cotton plant is important due to the fact that lack or even the excess of nitrogen negatively influence the growth and development of plant and fiber quality (Reddy et al., 2004). Application of different N rates induces wide variation in N leaf concentration and chlorophyll, considering that the crop culture is supplied with all other nutrients and water (Bagheri et al., 2013; Fridgen and Varco, 2004). So, the distinction can be detected by leaf reflectance from the visible until near-infrared spectrum. Different spectral indices can be used to estimate leaf area, biomass and yield of different crops. Despite of the use of many spectral indices, the most widely used index is the Normalized Difference Vegetation Index (NDVI), which has been successfully used to classify plant biomass (Haboudane et al., 2004; Rossato et al., 2012). Its efficiency is due to the characteristic of strong absorption of the plant in the red band and high reflectance in the near infrared band, allowing N content monitoring throughout the crop cycle (Brito et al., 2011; Gutierrez et al., 2012). Changes on leaves N content can be observed using spectral indices in different cotton growth stages. It allows assessment of crop conditions and evaluation of the canopy general state, to estimate the biomass and establish standards for the crop nutritional management leading to achieve high productivity. The objective of this study was to determine relationships between reflectance indexes and leaf N content, leaf area index (LAI), aboveground biomass (Biom) and yield of irrigated cotton across four nitrogen rates.

Results and Discussion

The leaf nitrogen accumulation is related with LAI

Plant height and LAI response to fertilizer's N rate was strong in the 2009 and 2010 growing seasons, showing a R^2 above 73% (Fig. 1). Response of plant height to changes across growth periods and years were very consistent, increasing as each growing season progressed. It showed a separation of fertilizer N rates that was often evident since squaring. A quadratic response was noted for LAI at peak flowering and fruiting in 2009, but the trend was generally linear with a low small slope early in the growing season for remaining samplings. Zhao et al. (2007) reported the direct effect of N rates at LAI, and consequently, in vegetation index measurements (Haboudane et al., 2004; Eitel et al., 2008). Leaf N response to different fertilizer N rates was strong at almost every sampling date, including those very early in the growing season. In this study, leaf N was varied from 24.5 to 47.9 g kg^{-1} in 2009 and from 22.5 to 44.9 g kg^{-1} in 2010 (Fig. 2), with the lowest values for control treatments. At peak flowering, with the exception of the control treatment, these leaf N values remnant still were within the sufficiency range for cotton on this stage, (35 to 43 g kg^{-1}) (Malavolta et al., 2004). Similar results were obtained by Motomiya et al. (2009), who found N content of 34.66 g kg^{-1} in the leaves of the cotton plants at peak flowering. According to Rosolem and Mellis (2010), the growth of the cotton plant depends on the combination of solar radiation, to create healthy leaves which produce adequate carbohydrates under optimum water supply and temperatures (Bagheri et al., 2013). The radiation is also required for a rapid and efficient plant root system development, the absorption of required nutrients to the plants basic structure and development of new plant structures. The SPAD index readings were strongly sensitive and influenced by leaf N in both years (Fig. 2), exhibited strong Pearson correlations (r) (Table 1), and good capability to predict N concentrations in cotton leaves during growing seasons. At squaring, it was observed that SPAD index was also sensitive for N tissue contents, and was confirmed as a good and fast method to evaluate changes in N availability in substitution of conventional foliar diagnosis (Brandão et al., 2009). A quick N evaluation is justified because the cotton substantially increases the daily rate of nutrient absorption after appearing the first flower buds until the opening first boll. Thus, the detection of nitrogen deficiency by SPAD allows a rapid nutrient supplementation, ensuring appropriate conditions to obtain high yields. In a nutrient solution experiment, Malavolta et al. (2004) and Brandão et al. (2009) observed a significant high correlation between SPAD index readings and leaf N levels at the second week of flowering, which is similar to the results of this study. Brito et al. (2011), studied different N rates and their availability in cotton leaves, and concluded that SPAD readings were very sensitive, significant and can be used as a foliar diagnosis method during all growing season, relating these readings with cotton yield. The higher concentrations of leaf N were founded at squaring. LAI demonstrated to be a good predictor of this nutrient for both years, during this sampling date, with linear behavior and determination coefficients of 0.90 and 0.98 to 2009 and 2010, respectively (Fig. 2). LAI increased significantly ($p < 0.001$) and rapidly as plants progressed through squaring and fruiting, especially comparing 180 and 270 kg ha^{-1} N rates with the control treatments. Response of LAI changes across growth periods and years were significantly, both to leaf N and SPAD index (Table 1). A linear trend to the LAI behavior in both years was noted, except at late stages in 2009. This index was very consistent with N leaf contents, showing very sensitive to nutrient variation (Fig. 2). Thus, LAI increased rapidly with the plants growth and chlorophyll measurements which was useful to estimate N leaf content non-destructively (Motomiya et al., 2009).

The leaf nitrogen accumulation is related with aboveground biomass

Aboveground biomass (Biom) increased with fertilizer's N rate (Fig. 1), leaf N concentrations and plant growth throughout the seasons. At squaring, the treatments of 90 and 180 kg N ha^{-1} in 2009 and 0 and 90 kg N ha^{-1} in 2010, did not differ, but in both of years after 80 DAE, all treatments showed great differences, especially when compared to the control treatment. On the final sampling date at 100 and 90 DAE (2009 and 2010, respectively), Biom of the control treatment was significantly ($p < 0.001$) less than other treatments, varying from 0.42 to 0.74 and from 0.36 to 1.36 more than treatments provided with 90 up to 270N kg ha^{-1} in 2009 and 2010, respectively. The leaf N significantly ($p < 0.001$) affected Biom and cotton yield, with correlations coefficients above 98% (Table 1). Although cotton biomass at the first sampling date did not differ statistically to the four N rates, the LAI differ significantly in both years at the same sampling dates. It was observed that the fruit weight influenced too much the total biomass, especially because malnourished plants emitted their flower buds and fruits early. According to Rochester et al. (2012), N concentration in cotton leaves decreased from the flowering to full bloom. The N levels normally decline in the leaf tissues as the crop ages, taking place early in malnourish plants and may indicate redistribution of nutrients from leaves to reproductive structures (Rosolem and Mellis, 2010). Our results are in agreement with Ducamp et al. (2012), who found that cotton biomass was significantly influenced by leaf N concentration ($p < 0.01$), providing strong evidence when occurred N dilution in plant tissues, and simultaneous decreased in cotton biomass. We confirmed that in conditions of high availability of N, cotton plants increase vegetative growth very quickly, which leads to a N dilution in biomass, reducing leaf N contents (Marschner, 2012).

Canopy reflectance related with LAI and aboveground biomass

During the seasons, NDVI, SAVI, MSAVI and MTVI2 showed significant increase with N rate rise, and noted intensification in leaf area index across growth stages. In both years, VIs showed reduced values compared to control treatment, with little variation throughout the cotton growth. However, the increase in N, promoted a large rise of VIs at different stages, and was highly correlated with the N

Table 1. Summary of Pearson correlation coefficients (*r*) between vegetation indices obtained by field spectroradiometry and SPAD index, plant height, leaf N, LAI, biomass and cotton yield during 2009 and 2010 growing seasons in Apodi, RN, Brazil.

	Year	Date	Plant Height	SPAD	Biom (t ha^{-1})	LAI	NDVI	SAVI	MSAVI	MTVI2
SPAD			0.81*	--	0.76*	0.99*	0.99**	0.91**	0.97**	0.95**
Biom (t ha^{-1})			0.25^{ns}	0.76**	--	0.70*	0.80*	0.90**	096**	0.97**
LAI		60	0.85**	0.99**	0.70*	-	0.99**	0.87**	0.96**	0.92**
N (g/kg)%		DAE	0.86*	0.92**	0.54^{ns}	0.95*	0.90**	0.67*	0.81*	0.77*
Yield (kg ha^{-1})			0.68*	0.89**	0.76*	0.86*	0.92*	0.97*	0.96*	0.98**
SPAD			0.98**	--	0.66*	0.83*	0.96**	0.97**	0.96**	0.97**
Biom (t ha^{-1})			0.79**	0.66*	--	0.82*	0.99**	0.99**	0.99**	0.96**
LAI	2009	80	0.86**	0.83**	0.82**	--	0.90**	0.89**	0.90**	0.92**
N (g/kg)%		DAE	0.98**	0.97**	0.78*	0.93*	0.98**	0.98**	0.98**	0.99**
Yield (kg ha^{-1})			0.99**	0.98**	0.79*	0.90*	0.99*	0.99*	0.99*	0.99*
SPAD			0.90**	--	0.86*	0.72*	0.97**	0.96**	0.96**	0.95**
Biom (t ha^{-1})			0.90**	0.86**	--	0.97*	0.95**	0.95**	0.95**	0.93**
LAI		100	0.82**	0.72**	0.97**	--	0.86**	0.86**	0.86**	0.85**
N (g/kg)%		DAE	0.83*	0.99**	0.80**	0.63*	0.98**	0.98**	0.98**	0.99**
Yield (kg ha^{-1})			0.94**	0.88**	0.99*	0.95*	0.97*	0.97*	0.97*	0.96*
SPAD			0.96**	--	0.85*	0.98*	0.92**	0.98**	0.96**	0.96**
Biom (t ha^{-1})			0.82**	0.85**	--	0.86*	0.79**	0.89**	0.84**	0.90**
LAI		60	0.90**	0.98**	0.86*	--	0.85**	0.94**	0.91**	0.92**
N (g/kg)%		DAE	0.95**	0.99**	0.82*	0.98*	0.97**	0.96**	0.96**	0.94**
Yield (kg ha^{-1})			0.97**	0.90**	0.69*	0.83*	0.98*	0.93*	0.97*	0.93*
SPAD			0.93**	--	0.91*	0.99*	0.98**	0.97**	0.98**	0.98**
Biom (t ha^{-1})			0.72*	0.91**	--	0.90*	0.81**	0.79**	0.81**	0.80**
LAI	2010	75	0.94**	0.99*	0.90*	--	0.98**	0.98**	0.98**	0.98**
N (g/kg)%		DAE	0.87*	0.96**	0.82*	0.94*	0.98**	0.98**	0.97**	0.98**
Yield (kg ha^{-1})			0.99**	0.89**	0.66*	0.91*	0.93*	0.94*	0.94*	0.94*
SPAD			0.95**	--	0.99**	0.99*	0.98**	0.99**	0.99**	0.99**
Biom (t ha^{-1})			0.95**	0.99**	--	0.99*	0.97**	0.99**	0.98**	0.99**
LAI		90	0.98**	0.99**	0.99**	--	0.95**	0.98**	0.96**	0.99**
N (g/kg)%		DAE	0.94**	0.98**	0.99**	0.98*	0.98**	0.99**	0.99**	0.99**
Yield (kg ha^{-1})			0.97*	0.87*	0.86*	0.92*	0.78*	0.83*	0.80*	0.87*

*and **, significant at 0.01 and 0.001 respectively. ns not significant.

Table 2. Relationships between the evaluated spectral indices NDVI, SAVI, MSAVI e MTVI2 and Leaf N Content and Aboveground Biomass measured in field experiment in Apodi, RN, Brazil, at three sampling dates during 2009 and 2010.

			Leaf N				Biomass		
Year	Index	DAE	Prediction Equations	R^2	RMSE	Leaf N	Prediction Equations	R^2	RMSE
	NDVI		$Y= 3.3837 e^{3.0423x}$	0.82***	3.77		$Y= 622.6x^2 – 960.98x + 376.06$	0.64**	2.34
	SAVI		$Y= 67.851x – 12.982$	0.45*	6.13	33.27	$Y= 335.79x^2 – 525.37x + 199.27$	0.99***	0.12
	MTVI2	60	$Y = 62.73x – 10.524$	0.60**	5.26	a	$Y= 149.55x^2 – 217.52x + 84.653$	0.95**	0.90
2009	MSAVI		$Y = 92.742x – 33.444$	0.66***	4.82	47.86	$Y= 459.57x^2 – 689.38x + 263.72$	0.92**	1.11
	NDVI		$Y = 2.1411 e^{3.5907x}$	0.97**	2.01		$Y= 642.81x^2 – 944.21x + 354.67$	0.98**	0.99
	SAVI		$Y = 1.7182e^{3.8823x}$	0.97**	2.14	25.26	$Y= 799.83x^2 – 1178.7x + 441.89$	0.99**	0.65
	MTVI2	80	$Y= 2.9863 e^{3.2566x}$	0.98**	1.41	a	$Y= 475.06x^2 – 669.22x + 243.95$	0.92**	1.06
	MSAVI		$Y= 2.1129 e^{3.6038x}$	0.97**	2.03	46.93	$Y= 647.62x^2 – 952.59x + 358.23$	0.98**	0.98
	NDVI		$Y= 750.87x^2 – 1040.5x + 385.92$	0.96**	1.70		$Y = 1.1799 e^{3.3016x}$	0.93*	1.38
	SAVI		$Y = 981.14x^2-1395x + 521.08$	0.96**	1.70	24.55	$Y= 0.9407e^{3.5643x}$	0.93**	1.37
	MTVI2	100	$Y = 728.26x^2 – 962.75x + 341.36$	0.99**	1.11	a	$Y= 2.5451e^{2.4186x}$	0.91**	0.84
	MSAVI		$Y= 857.02x^2 – 1204.1x + 448.33$	0.96**	1.69	45.34	$Y = 0.9962e^{3.5052x}$	0.92**	1.39
	NDVI		$Y= 4495.2x^2 – 7206.8x + 2912$	0.94**	3.79		$Y= 884.42x^2 – 1433.6x + 582.71$	0.89*	0.87
	SAVI		$Y= 5.6601e^{2.5618x}$	0.94**	2.99	26.07	$Y = 67.663x^2–84.065x + 28.8$	0.93**	0.65
	MTVI2	60	$Y = 6.935 e^{2.1607x}$	0.89**	3.58	a	$Y= 51.473x^2 – 66.483x + 24.128$	0.97**	0.61
2010	MSAVI		$Y = 5.8339 e^{2.3895x}$	0.92**	3.11	44.93	$Y= 88.369x^2 – 119.03x + 42.531$	0.89**	0.77
	NDVI		$Y= 0.3308 e^{5.3581x}$	0.97**	2.18		$Y= 1244.2x^2 – 2067.5x + 865.56$	0.86*	2.47
	SAVI		$Y= 0.341e^{5.2585x}$	0.97**	2.48	25.76	$Y= 1313.4x^2 - 2212x + 937.79$	0.84**	2.60
	MTVI2	75	$Y= 0.4289 e^{5.1465x}$	0.96**	2.44	a	$Y = 1335.7x^2– 2183.6x + 898.8$	0.86**	2.57
	MSAVI		$Y = 0.304 e^{5.4261x}$	0.95**	2.49	44.87	$Y= 1476.3x^2 – 2476.4x + 1044.9$	0.89*	2.47
	NDVI		$Y= 0.223 e^{5.7688x}$	0.96**	1.30		$Y= 113.9x - 84,297$	0.95**	1.08
	SAVI		$Y= 0.2551e^{5.5176x}$	0.99**	0.68	22.46	$Y= 111.76x – 84.077$	0.99**	1.01
	MTVI2	90	$Y = 0.9835 e^{4.3377x}$	0.99**	0.13	a	$Y = 85.679x – 55.025$	0.99**	0.32
	MSAVI		$Y= 0.1911 e^{5.9137x}$	0.98**	1.01	35.6	$Y = 116.82x – 87.397$	0.97**	0.84

*, ** and ** significant at 0.05, 0.01 e 0.001%, respectively.

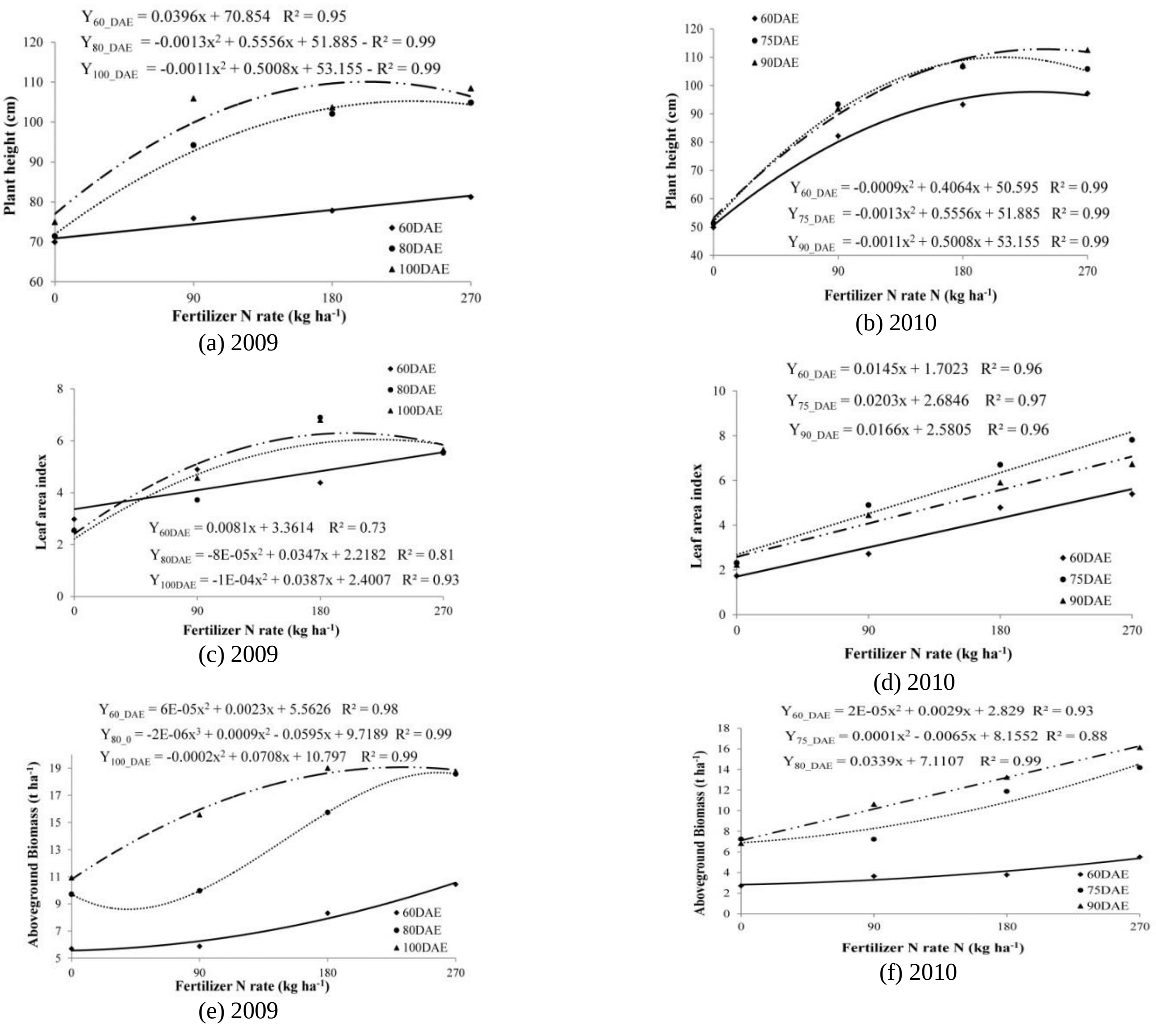

Fig 1. Cotton plant height and leaf area index (LAI) during flowering and fruiting as affected by N fertilizer treatments in 2009 (a, c) and 2010 (b, d), respectively. Each data point is the mean of four replications. Early flowering is also shown in the Figure.

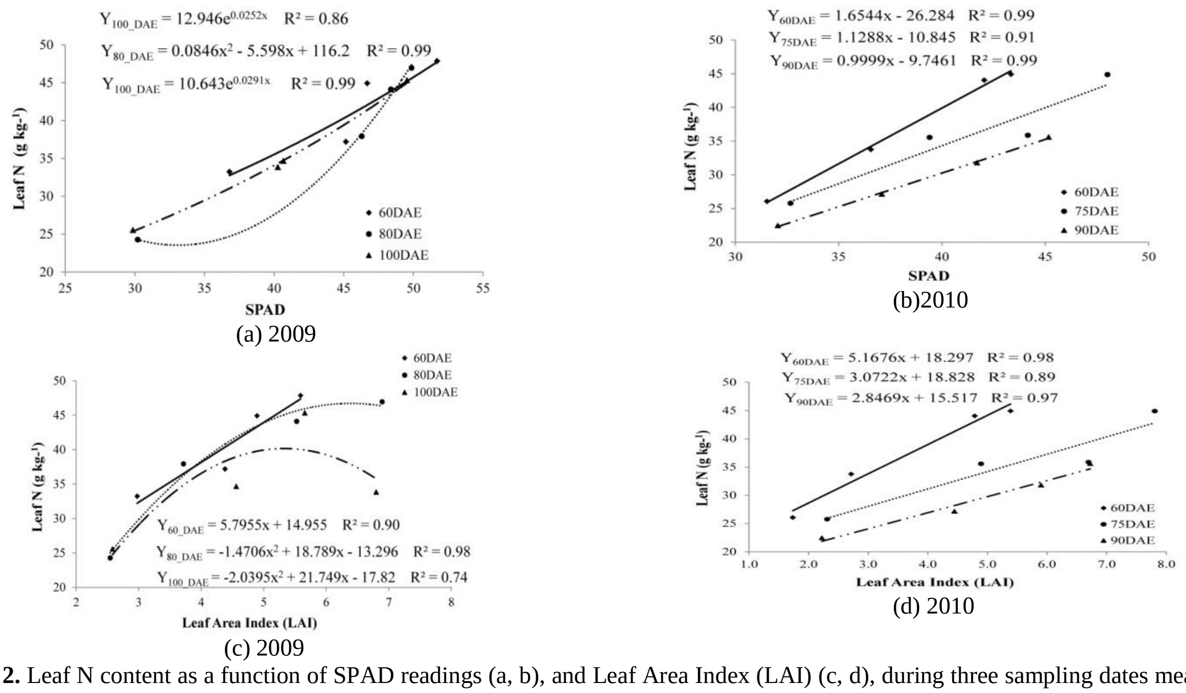

Fig 2. Leaf N content as a function of SPAD readings (a, b), and Leaf Area Index (LAI) (c, d), during three sampling dates measured in 2009 and 2010 to irrigated cotton plants in semiarid region.

concentration in leaf tissues (Table 2). The strong relationships between LAI and leaf N explained more than 74% of the leaf N content (Fig. 2) for all the sampling dates, for both years. In this regard, the spectral response of vegetation contained a valuable information about the crop effect to N availability which could be estimated quickly, non-destructively and in large-scale. This result is in agreement with many studies that reported the feasibility of using hyperspectral vegetation indices to determine LAI and aboveground biomass, as well as the chlorophyll content and nitrogen status in plants with good correlations (Zhao et al., 2005; Zarco-Tejada et al., 2005; Brito et al., 2011; Gutierrez et al., 2012). The LAI was significantly correlated with spectral indices during the entire growing season (Fig. 3). A quadratic response was noted at squaring in 2010 for NDVI and MSAVI. At the last sampling date in 2010, LAI showed a little decrease approximately when LAI exceeded 6.5, in which the relationships exhibited a weak logarithm trend. Nevertheless, the general trend in 2009 and remnant samples in 2010 presented an exponential response with an immediate rise of LAI through the N rates in cotton growing seasons. Many vegetation indexes tend to saturate at LAI levels above 4, although in some ecosystems that threshold is often surpassed (Haboudane et al., 2004; Eitel et al., 2008). Regression analysis (Fig. 3) also showed the potential sensitivity of these indices to detect small variations in LAI using predictive modeling, which is elevated due to the high slopes of the regression models relative to potential error. Relationships between some of these indices (specifically MSAVI and MTVI2) and cotton LAI estimate by direct destructive measure were strong across the seasons, with high correlation coefficients (Table 1). Both of years at squaring, the NDVI and MSAVI best represented the LAI variations (Fig. 3) with R^2 above 92% for both VIs. This result agrees with those obtained by Haboudane et al. (2004), who studied the best hyperspectral VIs for three different cultures and observed that NDVI and MSAVI and showed a good agreement to LAI variability, especially for LAI values up to 3.

They also explained that the main difference between these two indices resides in the saturation effect when LAI increases, because NDVI reaches a saturation level asymptotically when LAI exceeds 2, while MSAVI shows a better trend without a clear saturation at high LAI levels (up to 6). This explains, in part, why MSAVI has proven to be a good indicator of greenness measurement up to 60 DAE. At the peak flowering stage, density canopy was intense and MTVI2 and MSAVI provided the best estimate of the LAI, with correlation coefficients of 0.92 and 0.98 (MTVI2), and 0.90 and 0.99 (MSAVI), for 2009 and 2010, respectively (Fig. 3). Some studies have reported that hyperspectral VIs modified as MTVI2 (Haboudane et al., 2004; Zhao et al., 2007) can avoid the saturation effect observed for the NDVI, to high LAI (LAI > 3) with the advantage of being less sensitive to chlorophyll concentration changes. The best behavior across the seasons, in terms of both responsiveness to LAI changes (Fig. 3) and resistance to biomass variation (Table 2), is given by the improved index MTVI2. This VI offers the advantage of being resistant to N leaf contents changes (Table 1) and the least sensitive to the saturation phenomena. The MTVI2 during growing seasons exhibited an exponential behavior, with R^2 above 82% and 91% for 2009 and 2010, respectively (Fig. 3), without saturation to the high LAI and distinguishing N contents accurately. This index included the green band as well as a factor to reduce soil contaminations effects (Viña et al., 2011), such as those that are increased as a result of chlorophyll absorption and is influenced by the changes in leaf and canopy structures, and is insensitive to pigment level changes. Deficiencies in the supply of nitrogen causes the reduction of LAI and photosynthetic pigments content in cotton (Reddy et al., 2004; Zhao et al., 2005), resulting in reduced values of the VIs. However, for all treatments above 0 kg N ha^{-1} rate, these VIs proved to be good indicator of plant vigor, offering potential to evaluate the general condition of irrigated plants (Gutierrez et al., 2012). The spectral indexes response to aboveground biomass variability was strong for all sampling date, but similarly to LAI variability, they did not show a unique pattern of response (Table 2). Generally, the trend was a quadratic function at two early stages when leaf N was not critical. After 80 DAE, the response increased quickly with N content in 2009, represented by exponential response, and showing a linear trend at 90 DAE sampling date in 2010 (Table 2). Although, the absolute leaf N values across growth periods and years were very consistent, probably due to unchanging growing conditions (water availability, tillage systems, etc.), during 2010 the leaf N observed was more demanded in cotton plants influencing crop development. In this year, the maximum temperature was 2.5 ºC higher than 2009 (21/36 ºC and 22/38.5ºC, for 2009 and 2010, respectively).

To plants under extreme temperatures (29 to 39 ºC for day) there is a loss in the usefulness of regulators. Therefore the dose escalation was not effective in controlling the growth in these temperature regimes (Snider and Oosterhuis, 2012). According Zhao et al. (2005) cotton plants exposed to 28/36ºC, night/day growth temperature retained approximately 70% fewer bolls than plants grown under 22/30ºC grown temperature regime. Under unfavorable temperatures conditions, the real nutritional demands cannot necessarily be developed, particularly considering the importance of redistribution from vegetative to reproductive material during boll development (Rochester et al., 2012). A readily available supply of carbohydrates is essential in promoting a number of key events during plant reproductive development (Snider and Oosterhuis, 2012) and high temperature conditions, resulting in significantly lower levels of nonstructural carbohydrates in one day old cotton bolls and significantly higher abscission rates of young bolls, influencing the spectral response (Brito et al., 2011).

The MSAVI, SAVI and MTVI2 indexes showed a consistent and very similar behavior to Biom variability in many aspects. They increased rapidly with the progression of plant growth, and since peak flowering until fruiting growing seasons, they exhibited good correlation coefficients for the two years (Table 1), showing no instabilities after 70 DAE, exhibiting R^2 > 84%. The commonly used NDVI explained over 64% of variation in cotton biomass (Table 2), showing instability at early growing stages, while the remnant indexes showed better correlations with cotton biomass at this stage (Table 1). Haboudane et al. (2004) observed that NDVI saturated earlier than the other three indices, when cotton biomass increased dramatically at different N rates. Some studies have reported that relationships between canopy reflectance indexes MTVI2 and MSAVI and spatial biomass variability depend on the time of image acquisition, with the best relationships at mid and earlier growth stages (Zarco-Tejada et al., 2005; Zhao et al., 2005).

In our study, these indexes exhibited potential as good estimators of biomass since the squaring (Table 2), although MSAVI shows a better trend to predict leaf N concentration

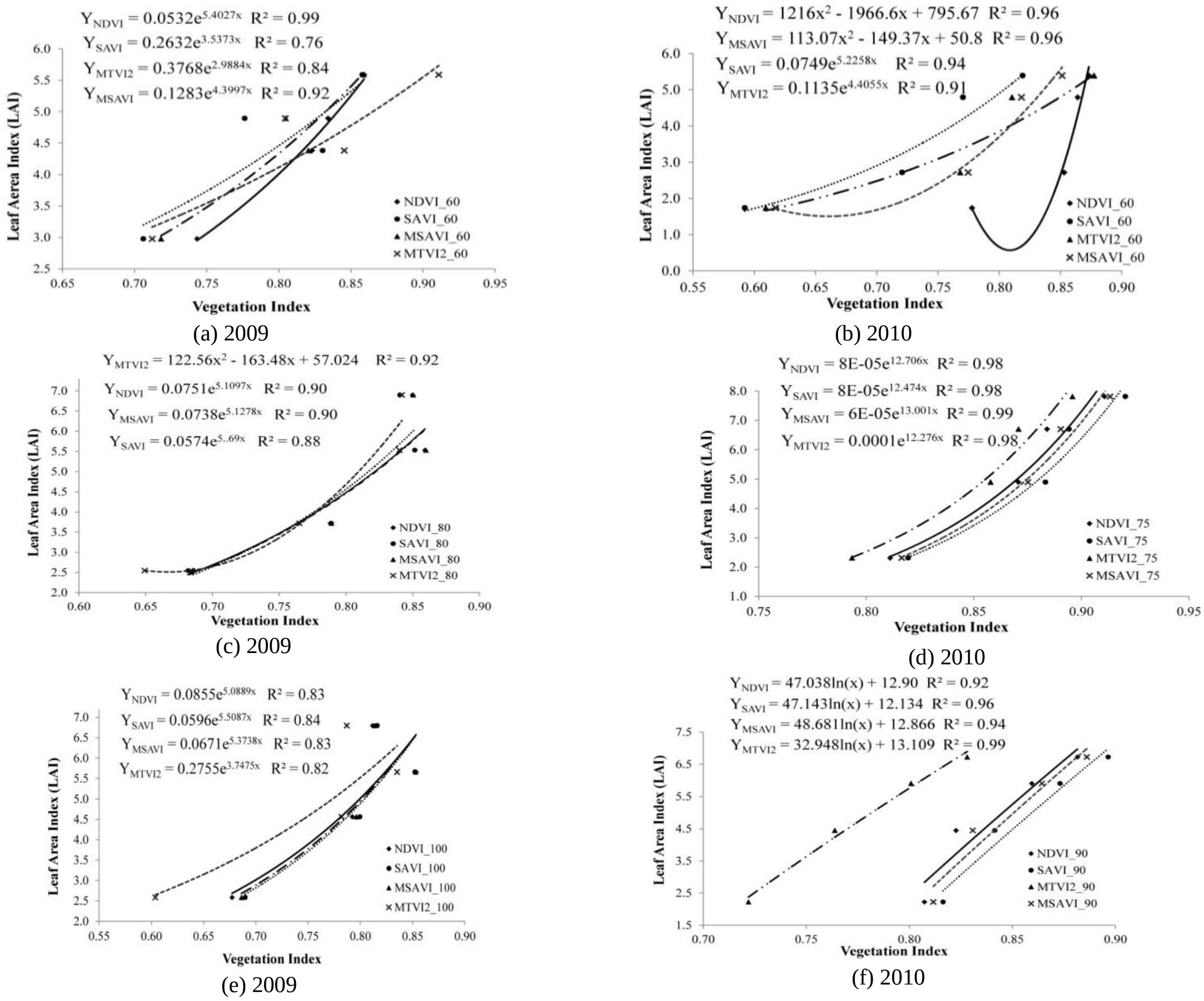

Fig 3. Relationships between the evaluated spectral indices NDVI, SAVI, MSAVI e MTVI2 and Leaf Area Index (LAI) measured at three sampling dates in Apodi, RN for all years. (a, b) at 60 DAE. (c, d) at 80 and 75 DAE, respectively. (e, f) at 100 and 90 DAE, respectively.

in earlier stages. This result is in agreement with Haboudane et al. (2004) who found that MSAVI exhibit a similar resistance to chlorophyll content variability, with clear sensitivity only at low and low-medium chlorophyll concentrations. After squaring, MTVI2 showed a similar behavior as MSAVI, but had the advantage of being less sensitive to chlorophyll concentration changes. The MTVI2 proved to be an excellent predictor of cotton biomass at fruiting stage, with determination coefficients highly significant ($p < 0.001$), 0.95 in 2009 and 0.99, in 2010, as well as root mean square errors (RMSE) less than 0.84 (Table 2). Similar results were obtained by Zhao et al. (2007) for cotton. In this study we observed that the cotton reflectance responds to both wavelength and growth stages from flowering to fruiting. According to Zhao et al. (2007), relationship between aboveground biomass and reflectance indices showed strong exponential associations after peak flowering, with R^2 = 0.64-0.75 ($p<0.001$), especially to NDVI and MTVI2. The NDVI was more stable, and MTVI2 explained more than 73% of the biomass variation, increasing rapidly as plants progressed across four N treatments (0, 90, 180 and 360 kg ha^{-1}) during two years of study.

Lint yield prediction by spectral indexes

Cotton yield was enhanced as N rate increased during the growing seasons. The vegetation indexes were sensitive to changes since early to peak flowering until reaching a maximum yield (Fig. 4). In 2009 and 2010 the cotton yield varied from 2862 to 3708 kg ha^{-1} and from 2674 to 3516 kg ha^{-1}, respectively. The maximum yield was obtained with 222 kg ha^{-1} and 195 kg ha^{-1}, to 2009 and 2010, respectively. As expected, for the two years, the lowest observed yield was for the control treatment (0kg N ha^{-1}). When the maximum potential of yield was achieved, the higher N rates caused a decrease in yield (data not shown). This yield decrease could be related to a combination of factors directly affecting some of the yield components, especially because high N levels can cause excessive vegetative growth (Rosolem and Mellis, 2010). In our study, the higher yields were observed to LAI up to 6. These results are in agreement with those obtained by Wiatrak et al. (2005), who reported a linear increase in cotton yield up to 200kg N ha^{-1}, a rate considerably high, but lower than these reported in our study.

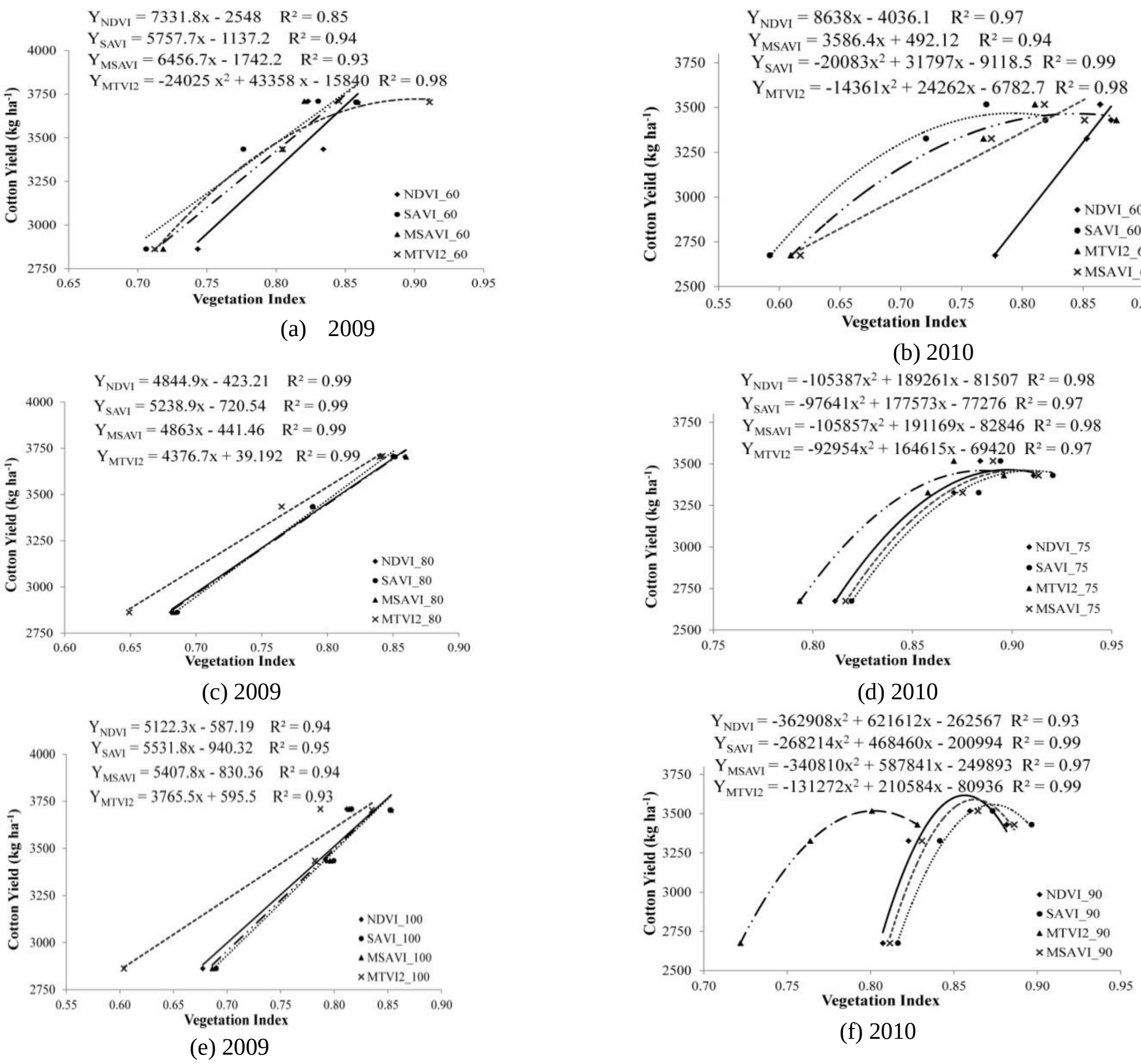

Fig 4. Relationships between the evaluated spectral indices NDVI, SAVI, MSAVI e MTVI2 and Cotton yield measured at three sampling dates in Apodi, RN for all years. (a, b) at 60 DAE. (c, d) at 80 and 75 DAE, respectively. (e, f) at 100 and 90 DAE, respectively.

Ducamp et al. (2012) also noted an optimum N rate for high N applications and reported reductions of cotton yield after reached the maximum yield. The analysis of the relationships between cotton yield and the tested reflectance indexes at different growth stages indicated that cotton yield correlated with all reflectance indexes measured after 60 DAE (Fig. 4). Relationships between VIs and cotton yield presented a linear trend in 2009, except to MTVI2 at squaring and a quadratic trend for 2010, except to NDVI, SAVI and MSAVI at the first sampling date. Even though correlations observed were above 85% since squaring, the index which best represented the yield decay was MTVI2, keeping the same behavior for two years at this sampling date. In 2010, all VIs showed the decay trend after maximum yield potential at peak flowering (Fig. 4), while in 2009, this effect was not observed. In 2009, all VIs exhibited a positive linear trend, indicating that the maximum yield potential was greater than the observed with vegetation indexes. The correlation coefficients were 0.99 (Table 1) at this sampling date in 2009, and at least 0.93 in 2010, to all VIs. As observed at last sampling date, the VIs do not follow the same pattern in the two years, and while the trend in 2009 was a linear increase, in 2010 the VIs showed a large decrease (Fig. 4). A possible cause for such a behavior is that VIs were more sensitive to N changes to cotton yield, since at this sampling date in 2010, leaf nitrogen was about 26% less than the same treatments in 2009. As reported by Reddy et al. (2004), cotton growth can respond well to nitrogen fertilization, but cotton yield not necessarily increase at the same rate of N application. Crop N demand is determined by yield potential, which varies spatially within farm fields and can be related to many factors, among which we can mention, soil, temperature, solar radiation, soil color and brightness and water availability (Pinter et al, 1994; Jiang et al., 2007; Marschner, 2012). Thus, the ability of spectral indexes can be influenced by these interactions.

Haboudane et al. (2004), stated that the best time for measuring canopy reflectance to accurately estimate lint yield is from early to peak bloom stage. They also suggested that cotton lint yield was associated with reflectance indexes (RVI, NDVI and EVI), at any growth stage from squaring through the first boll opening. The largest R^2 value was achieved around the early flower stage in both years of study (R^2 = 0.56–0.89, p < 0.001). In contrast, our study showed MTVI2 to be more sensitive and resistant to cotton yield especially compared to LAI and NDVI. Therefore we suggest that the former is a better estimator for our climate and soil conditions. This result is in agreement with Eitel et al. (2008), who studied the variations of MTVI2. They reported that this VI presented the best performance to evaluate LAI for dryland wheat. Therefore, correlations between cotton yield and canopy reflectance indexes depended both on IV and on cotton growth stage, at which measures were made. Additionally, the available N influenced the LAI and consequently, modified the VIs to yield sensing, that could be overestimated because of a greater vegetative growth, although choosing sampling dates at early stages is possible to evaluate the need of nitrogen supplementation.

Materials and Methods

Location, experimental design and plot management

The field experiment was conducted in two growing seasons, 2009 and 2010, at Apodi Plateau, located in RN State, northeastern Brazil (5°37'19 " S and 37º49'06 " W). Fields were assigned to four treatments in a randomized block design with four replications. Nitrogen fertilizer rates of 0, 90, 180 and 270 kg N ha^{-1} were used in 2009 and 2010. The N fertilizer was urea and applied in line twice. At planting, 1/3 of urea was banded with the starter fertilizer, applied during furrow opening. The remaining 2/3 of fertilizer were side-dressed into the beds 40 days after emergence (DAE) and watered immediately by overhead irrigation. To minimize the effects of growth conditions over the N treatments, (e.g. genetic varietal differences), the experiments were conducted with the same cotton cultivar for the two years. The cotton cultivar was BRS 286, which is produced by Embrapa breeding program being adapted to the Brazil northeast region conditions with short cycle. The plot size was 12 m wide by 15 m long, consisted of thirteen rows. Rows were spaced 0.9 m apart with linear plant density of 12 plants per meter. Seeds were planted in dry soil during the first week of August in both years and irrigated immediately after planting. The plots were irrigated to ensure there was no drought stress during the growing seasons. Plants were cultivated under full irrigation condition, during the winter and spring (August to December). The irrigation was scheduled using FAO-56 methodology. Soil of the experimental area is Typic Haplustox (LVA), with sand, 56.8%; clay, 33.7%; silt, 9.5% and pH, 5.7; calcium carbonate, 28 ($mmol_c$ dm^{-3}); organic matter 9%; available phosphorus, 4.4 mg dm^{-3} and extractable potassium, 4.7($mmol_c$ dm^{-3}). The crop was grown using conventional tillage practice, weed and disease control. Except for the N rates, all plots received the same field management practices, such as fertilizer applications, weed and insect control, as well as received irrigation throughout the growing seasons, scheduled according FAO-56 methodology.

Measurements

In this study, cotton crop phenology stages include the dates of squaring (flower bud until first open flower), peak flowering (first flower until first open boll), and fruiting (first open boll until last effective flower). Plant growth was assessed at 60, 80 and 100 DAE in 2009 and 60, 75 and 90 DAE in 2010. At the end of each crop cycle, which was at 125 and 115 DAE for 2009 and 2010, respectively, manual harvesting of seed cotton yield was determined for each plot and converted to kilograms per hectare.

Plant sampling was conducted to determine leaf N concentration using the leaf of the 5th main stem position and sent for determination of macronutrients. Leaf samples were oven dried for 48h at 65°C and ground through a 20 mesh sieve in a Wiley Mill. The leaf N concentrations were determined on duplicate samples of 4 to 6 mg of ground leaf material. The plant height, amount of leaves and leaf area were obtained at each sampling date in 20 plants, randomly identified in each plot. Readings were carried out with a portable chlorophyll meter SPAD-502 (Minolta Inc.) in the 5th uppermost fully expanded leaves in these plants. At each sampling date, five plants were collected for determination of aboveground cotton biomass. Previously to the biomass determination, green leaf area of these plants was measured using a LAI-3100 leaf area meter, (LI-COR Inc., Lincoln, USA). To minimize the effect of destructive sampling on subsequent perceiving date, the sampling point was moved throughout the growing seasons between external rows. Measures of reflectance, growth parameters and cotton yield were determined from the nine middle rows of each plot and cotton biomass from the four external rows. The total aboveground cotton biomass (Biom) was determined by randomly cutting four plants per plot and samples oven dried at 65ºC until constant weight. Biom was estimated using the dry weight per plant and cotton population.

Canopy reflectance and data analysis

In order to determine relationships between reflectance and plant growth, the spectral measurements were made at the same three time periods of plant measurements: 60, 80 and 100 DAE in 2009 and 60, 75, and 90 DAE in 2010. The canopy reflectance measurements were made on clear, cloudless, and sunny days above all plots using a portable PAR-NIR Apogee spectroradiometer (Apogee Instruments Inc, USA). Reflectance was measured at wavelengths ranging from 350 to 1000 nm with a 0.5 nm sampling interval. The distance between the optical head of the spectroradiometer and the plant terminal was 0.5 m. After optimization of the Apogee instrument, white panel was used to obtain reference signal prior to taking three canopy reflectance measurements from each plot. Hyperspectral reflectance indices (NDVI, MSAVI, SAVI and MTVI2) at the 550, 670 and 800 nm were calculated based on the equations provided by Haboudane et al. (2004) and Zhao et al. (2005) as follow:

$$NDVI = (\rho_{800} - \rho_{670})/(\rho_{800} + \rho_{670}) \quad (1)$$

$$SAVI = [(1+L)(\rho_{800} - \rho_{670})/(\rho_{800} + \rho_{670} + L)] \quad (2)$$

$$MSAVI = 0.5\left(2\rho_{800} + 1 - \sqrt{(2\rho_{800} + 1)^2 - 8(\rho_{800} - \rho_{670})}\right) \quad (3)$$

$$MTVI2 = 1.5[(\rho_{800} - \rho_{550}) - 2.5(\rho_{670} - \rho_{550})] \Big/ \sqrt{\left[(2\rho_{800} + 1)^2 - (6\rho_{800} - 5\sqrt{\rho_{670}}) - 0.5\right]} \quad (4)$$

Where; ρ_{800}, ρ_{670} and ρ_{550} represent reflectance in the near infrared, red and green spectral bands, respectively, and L is a soil adjustment factor and whose determination requires a prior knowledge of the amount of vegetation existing in the area (Jiang et al. 2007). The three spectral reflectance measurements, SPAD readings and growth parameters in each plot at each sampling date were obtained by averaging the data across replications to determine seasonal patterns of plant growth and reflectance indices as affected by N fertilizer rates and the mean values were used in the statistical analysis. One-way ANOVA and statistical tests of least significant differences were employed to determine effects of N fertilizer rate on reflectance indices, plant growth, and cotton yield. Values for LAI, Biom, and the reflectance indices were combined over plots, sampling dates, and years to determine relationships between reflectance indices and LAI or Biom, and regression equations were carried out. Additionally, Pearson correlation coefficients (*r*) were calculated between cotton yield and canopy reflectance at each treatment at different growth stages. Best growth stage for collecting canopy reflectance for yield estimation was determined based on the R^2 values of the regression equations.

Conclusions

Nitrogen fertilizer rate and the year significantly influenced leaf area index (LAI) and aboveground biomass (Biom). The spectral indexes allowed us to estimate leaf N content, LAI, Biom and cotton yield in both years of the study. All vegetation spectral indexes (VIs) were highly correlated with LAI, especially from squaring to peak flowering and strong similarity was observed between them on peak flowering in both years. The MTVI2 was the index that provided the best LAI prediction in advanced stages. The best period to estimate cotton biomass was after peak flowering with MSAVI and MTVI2 providing good estimates. The cotton yield can be predicted through hyperspectral VIs since the early until peak flowering in high temperature and irrigated conditions.

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