COMPARISON OF NITROGEN SAMPLING ANALYTICAL TECHNIQUES FOR ESTIMATING COTTON PLANT NITROGEN STATUS

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Abstract

Monitoring the N status of the cotton plant (*Gossypium hirsutum* L.) and understanding seasonal demands for N has been shown to be important for the implementation of sound N fertilizer management strategies that contribute to maximum profit. Conventional methods used for sampling and analysis of soil and plant N status have required considerable time and expense. Additionally, information has been unavailable about comparisons of in-field instantaneous and analytical laboratory N sampling techniques including conventional methods, chlorophyll meter, and the cardy NO_3-nitrogen meter. Eight treatment combinations of high or low soil N, high or low boll load, and with or without foliar-applied N were arranged in a split-split plot design with six replications. Tissue sampling (leaf and petiole), leaf chlorophyll content (SPAD 502), and Cardy measurements of petiole sap NO_3-N were collected weekly starting at first flower. Lint yield and yield components of all treatments were determined by hand harvesting two meters of row per plot. From first flower until five weeks after first flower, measurements of plant N status by all four methods displayed the same trend. The SPAD meter results showed showed a strong correlation with the two traditional methods of leaf blade N and petiole NO_3-N. Similar results were found between sap (Cardy meter) NO_3-N and leaf N, and between sap (Cardy meter) NO_3-N and dry petiole NO_3-N. The hand-held chlorophyll meter (SPAD-502) and the Cardy meter appear to be acceptable tools for monitoring the in-season N status of irrigated cotton in Arkansas. Further field comparisons of these methods under different environmental conditions, i.e. drought, are needed.

Introduction

Nitrogen (N) plays a crucial role in cotton plant growth and development, leaf photosynthesis, boll retention, and yield development. Excessive N fertilization can lead to rank growth and delayed maturity, while inadequate N leads to reduced yields and lower profits. Understanding the N status of the cotton plant and seasonal demands for N is important for the implementation of sound N fertilizer management strategies.

The N requirements of the cotton have been characterized using soil and tissue N analysis (Gardner and Tucker, 1967; Soundman et al., 1979; Lutrick et al., 1986; Bell et al., 1998). However, sampling and analysis for soil and plant N status require considerable time and expense. A non-destructive, instantaneous method for monitoring cotton N status would be more efficient, and possibly avoid some of the inherent problems associated with soil and tissue testing. Various new electronic field diagnostic tools have been developed for N analyses and may be of use in N management. The Minolta Chlorophyll Meter was developed in the early 1980's as a N management tool for growers (Thurow, et al., 1997). The current model (SPAD-502) was introduced in 1987. Some studies with this instrument have shown a strong correlation between leaf chlorophyll measurement and leaf N content in corn (Schepers et al., 1990), rice (Turner and Jund, 1991), and cotton (Edminsten and Wood, 1992). Furthermore, close correlation between corn leaf chlorophyll readings at the reproductive stage and final yield was reported (Thurow, et al., 1997). The higher the SPAD meter reading, the higher the yield (Thurow et al., 1997). Research in Alabama and Missouri showed that chlorophyll meter readings measured with the SPAD meter were significantly correlated with leaf-blade N concentration and petiole NO_3-N (Wood et al., 1991). The results suggest that the hand-held chlorophyll meter may be as reliable as leaf-blade N and petiole NO_3-N for predicting supplemental N fertilizer requirements of cotton. The SPAD-502 chlorophyll meter has been successfully used to determine N status of several crops (Wood et al., 1992; Peterson et al., 1993; Murdock et al., 1997; Piekielic et al., 1997). Subsequent research used the SPAD-502 meter to investigate the difference in chlorophyll content among cotton varieties and interaction effects among production practices, such as N rate, crop rotation and cover crops (Boquet et al., 1999).

The Cardy NO_3-nitrate meter was designed as a method for measuring cotton plant N status. This self-contained miniaturized digital meter uses a small amount of plant sap for diagnosis of plant N. Petiole analysis for NO3-N in cotton has been reported to be a reliable and useful indicator of plant N status (Sabbe and Zelinski, 1990). Mackown et al. (1999) reported that the level of tissue NO_3-N in tobacco might be a suitable diagnostic test of crop N sufficiency that could be used for N management decisions. They reported a close linear relationship between yield and leaf tissue NO_3-N levels of plant samples from 3 to 5 weeks after transplanting. The best fit equation was: leaf yield (y) =51.0 + 0.448 (leaf NO_3-N concentration) (r^2=0.808, p<0.001). Keisling et al. (1995) reported petiole NO_3-N was a satisfactory predictor of N nutritional status through the first three weeks of bloom for cotton grown in clay soil. Research by Oosterhuis et al. (2001) reported that the plant N status, as indicated by petiole analysis, was strongly determined by soil N level and the boll load. However, a comparison of N sampling analytical techniques including conventional methods, chlorophyll meter, cardy nitrogen meter for estimating cotton plant N status has not been reported. The objective of this study therefore was to compare conventional leaf and petiole N combustion methods with the Minolta chlorophyll meter and the Cardy meter for estimating cotton N status. The studies were conducted under both field and glasshouse conditions.

Materials and Methods

Plant Culture

Cotton (*Gossypium hirsutum* L.) cultivar 'Suregrow 215BR' was machine-planted in the field on a moderately well-drained Captina (Typical Fragiudult) silt loam at the Arkansas Agricultural Research and Extension Center in Fayetteville, on May 24, 2000. Rows, five meters in length, were spaced 1 m apart and oriented in a north-south direction. Each plot had 4 rows. Preplant fertilizer was applied at a rate of about 48 : 22 : 40 kg N: P: K per hectare. Plants were hand-thinned to a density of 10 plants m^{-1} row at the third true leaf stage. Weeds and insects were controlled during the growing season and the crop was kept well watered by furrow irrigation according to Arkansas Cotton Extension recommendations.

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The field trial was laid out as a split-split-plot design with six replications. The main plots consisted of soil N levels, high soil N (HN) and low soil N (LN), the sub plots were high (HB) and low (LB) boll load, and the sub-sub-plots consisted of foliar and no-foliar N applications. The HN plots received 72 kg N / ha prior to squaring on July 3 and the LN treatment did not receive any side-dressing N. For the LB treatment, a total of 5 quarter-sized bolls was removed by hand from each plant on Aug. 4 (3 bolls) and Aug.16 (2 bolls), whereas no bolls were removed from the HB treatment. For foliar treatments, each plot was split into two sub plots. One received foliar N application (foliar N), the other one did not (no foliar). Foliar N application was given on Aug. 30 and an additional 12 kg N / ha with 92 L of water was sprayed using a backpack CO_2-pressured sprayer.

Sample Collection and Measurements

Tissue samples were collected weekly starting one week after first flower and continued for five weeks. At each sampling date, leaf Chlorophyll content was measured on ten uppermost fully expanded main-stem leaves from each plot using a SPAD-502 chlorophyll meter between 10 AM and 12 PM. Thereafter, the leaf blades and petioles were collected immediately and taken to the laboratory. A Cardy NO_3-N meter was used to measure petioles sap N levels of half samples of the petioles. The other half of the petioles and all leaf blades were dried separately. Leaf total N and dried petiole NO_3-N were determined using traditional laboratory analytical methods.

Finally, the yield and yield components of all treatments were recorded by hand harvesting and counting the bolls from two meters of row per plot.

The ANOVA and LSD Tests were used to determine differences among treatments. Correlation coefficients were calculated to determine the relationship among the four measuring methods (Minolta SPAD meter, Cardy meter, leaf total N and petiole NO_3-N).

Greenhouse Study

A potted study was also conducted under the greenhouse conditions. Treatments for the greenhouse study consisted of high N (HN), medium N (MN) and low N (LN). When plants reached the pinhead square stage. The same methods as described above were used to SPAD meter readings, Cardy meter readings, leaf total N and petiole NO_3-N.

Result and Discussion

N Status of Cotton Plants in the Field

The ANOVA test (Table1) showed that there were no interactions among the main plot and the split plot treatments. Therefore, the main effects will be described for each treatment individually. Generally, the results from all four methods of high N, low boll load with foliar N treatment (HLF) were significantly higher than other treatments (Table 1, $p<0.05$).

During the entire period of observation (from FF+1wk to FF+5wk), the measurements of N status by all four methods displayed the same trend. The N levels of cotton plants decreased with increasing plant age. However, cotton plants with HN treatment still had higher N-levels than LN treatment (Fig. 1). There were consistent differences between HB and LB treatments, and between foliar N and no foliar N treatments (Fig. 2 and Fig. 3) although the differences were not statistically significant.

Coefficients between the SPAD reading and leaf-blade N, and between the SPAD meter reading and dry petiole NO_3-N were 0.65 ($p<0.05$) and 0.70 ($p<0.05$), respectively, which indicated that the SPAD meter had a strong correlation with the two traditional methods of leaf blade N and petiole NO_3-N. Similar observations were found between sap (Cardy meter) NO_3-N and Leaf-blade N, and between sap (Cardy meter) NO_3-N and dry petiole NO_3-N (Table 2).

Yield and Yield Components

Yield results are given in Table 3. The high boll load treatment (HB) differed significantly from low boll load treatment (LB) for boll number, seed cotton yield and lint yield ($p<0.05$). However, no differences were observed among treatments in average boll weight and lint percent. Although some numeric differences in lint yields existed, the differences were not statistically significant between the HN and LN or between foliar and no foliar N treatments. This might be due to the higher background level of N in the test soil (high soil fertility).

N-Study in the Greenhouse

In the greenhouse experiment, a comparison of the four measuring techniques was conducted to record the effects of low N (LN), medium N (N) and high N (HN). The results were similar to the field study (Fig. 5). Cotton plants from the HN treatment had significantly higher tissue N-levels than MN and LN treatments ($p<0.05$).Coefficient analysis indicated that both the SPAD reading and sap (Cardy meter) NO_3-N concentration were highly correlated with leaf-blade N, (Table 4, $p<0.001$).

Conclusion

The hand-held chlorophyll meter (SPAD-502) and the Cardy meter are potentially valuable tools to monitor the in-season N status of irrigated cotton in Arkansas. Further field comparisons of these methods under different environmental conditions, i.e. drought, are planned.

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Table 1. Significance of analysis of variances for effect of soil N level. boll load and foliar N on SPAD meter reading, sap (Cardy meter) NO_3-N, leaf blade N and dry petiole NO_3-N.

Treatment				**Cardy**	**Leaf-**	**Petiole**
Soil N-level	**Boll load**	**Foliar N**	**SPAD reading**	**Meter NO_3-N**	**blade N (%)**	**NO_3-N (ppm)**
H[Y]	H	F	45.9	169.1	3.45	321.2
H	H	N	44.2	141.7	3.44	205.2
H	L	F	46.2*	199.7*	3.46*	449.5*
H	L	N	45.4	177.7	3.42	246.8
L	H	F	41.9	133.7	3.18	153.5
L	H	N	41.4	123.8	2.97	91.4
L	L	F	43.2	121.7	3.13	291.0
L	L	N	42.3	114.0	2.93	150.0
Prob.>F						
Soil N-level (SN)			<0.0001	0.0062	<0.0001	<0.0001
Boll load (BL)			0.04	0.85	0.71	0.03
Foliar N (FN)			0.02	0.87	0.11	0.02
SN H BL			0.75	0.88	0.77	0.79
SN H FN			0.54	0.38	0.18	0.21
BL H FN			0.79	0.41	0.92	0.38
SN H BL H FN			0.50	0.21	0.92	0.29

[x] FF means the first flower;
[y] H = high level, L = low level, F = application of foliar fertilizer, N = no foliar fertilizer;
[*] P<0.05.

Table 2. Correlation coefficients (r) between each two measurement methods for field study.

	FF+3			**FF+4**			**FF+5**		
	M_2[x]	**M_3**	**M_4**	**M_2**	**M_3**	**M_4**	**M_2**	**M_3**	**M_4**
M_1	0.35	0.58*	0.73*	0.46	0.67*	0.67*	0.40	0.70*	0.69*
M_2		0.54	0.68*		0.50	0.65*		0.47	0.66*
M_3			0.54			0.51			0.59*

* P < 0.05.
[x] M_1=SPAD meter reading, M_2=Sap (Cardy meter) NO_3-N, M_3=Leaf-blade N, M_4=Dry petiole NO_3-N.

Table 3. Yield and yield components of all treatments and significance of analysis of variances.

Treatment							
N-level	Boll Load	Foliar	B.N [y] (no/m^2)	B.W (g)	L.% (%)	S.C.Y (kg/ha)	L.Y (kg/ha)
H [X]	H	F	121.3	5.15	39.2	3134	1228
H	H	N	128.5	4.69	39.0	2997	1172
H	L	F	101.0	5.06	38.2	2530	966
H	L	N	95.8	5.15	38.3	2463	943
L	H	F	124.0	4.82	38.7	2996	1159
L	H	N	116.8	4.99	39.5	2910	1152
L	L	F	105.6	5.04	38.7	2666	1032
L	L	N	95.8	5.01	38.1	2400	919
Prob.>F							
Soil N-level (SN)			0.64	0.57	0.97	0.77	0.74
Boll load (BL)			0.0001	0.17	0.10	0.0002	0.0001
Foliar N (FN)			0.88	0.54	0.68	0.78	0.73
SN H BL			0.66	0.55	0.91	0.57	0.64
SN H FN			0.58	0.37	0.79	0.86	0.81
BL H FN			0.27	0.18	0.99	0.68	0.71
SN H BL H FN			0.61	0.32	0.76	0.98	0.96

[X] H = high level; L= low level; F = application of foliar fertilizer; N = no application of foliar fertilizer.

[Y] B.N = boll number; B.W = boll weight; L.% = lint percent; S.C.Y = seed cotton yield; L.Y = lint yield.

Table 4. Correlation coefficients (r) between each two measurement methods for the greenhouse study.

	FF+1wk		FF+2 wk		FF+3 wk	
	M_2[x]	M_3	M_2	M_3	M_2	M_3
M_1	0.83*	0.97**	0.90**	0.98**	0.85*	0.86*
M_2		0.84*		0.94**		0.94**

* $P < 0.05$ and ** $P < 0.01$.[x]

M_1=SPAD meter reading; M_2=Sap(Cardy meter) NO_3-N; M_3=Leaf-blade N.

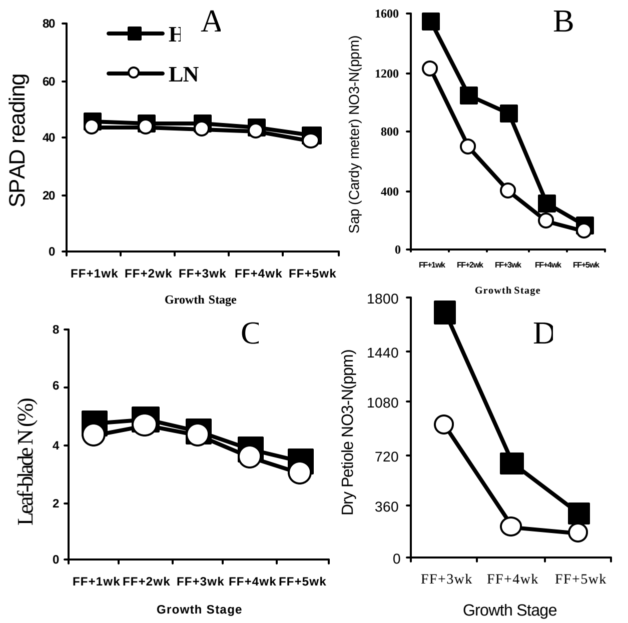

Figure 1. Effect of soil N rate on (A) SPAD reading, (B) Sap (Cardy meter) NO_3-N, (C) Leaf-blade N and (D) Dry petiole NO_3-N of field-grown cotton.

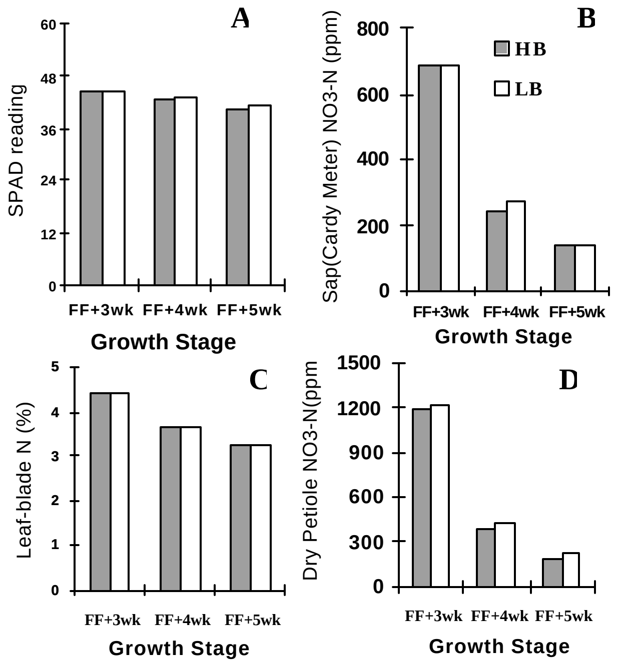

Figure 2. Effect of boll load on (A) SPAD meter reading, (B) Sap (Cardy meter) NO3-N, (C) Leaf-blade N and (D) Sap (Cardy Meter) NO_3-N of the field-grown cotton.

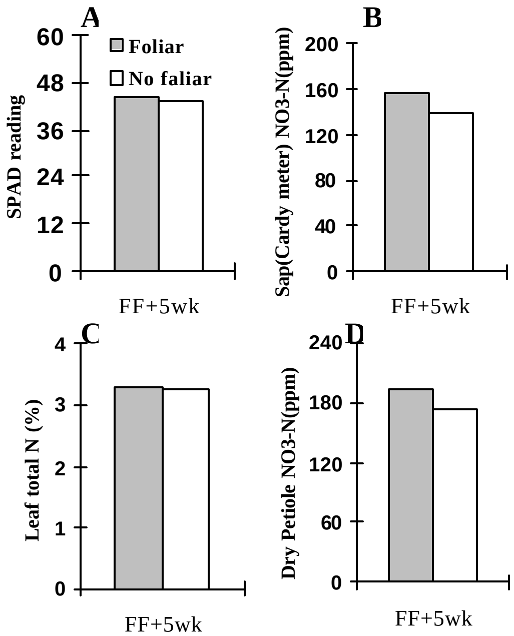

Figure 3. Effect of foliar N on (A) the SPAD reading, (B) Sap (Cardy Meter) NO_3-N, (C) Leaf-blade N and (D) Dry petiole NO_3-N.

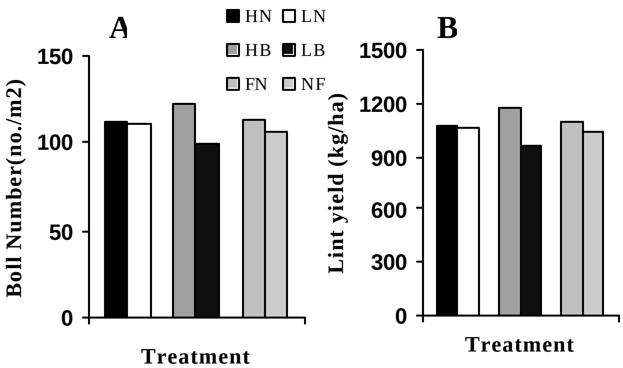

Figure 4. Effect of soil N-level, boll load and foliar N on (A) Boll Number and (B) Lint Yield

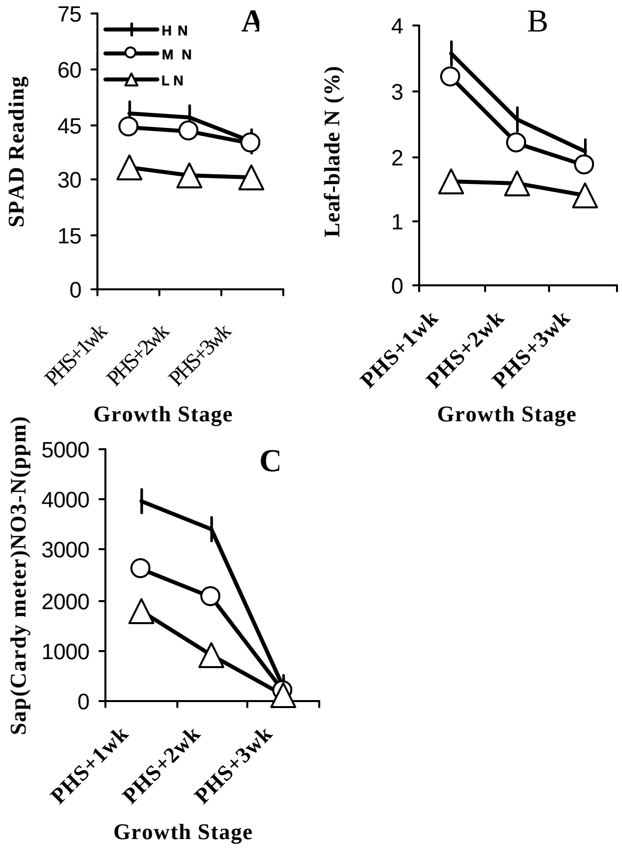

Figure 5. Effect of high N (HN), mid N (MN) and low N (LN) on (A) SPAD meter reading, (B) Leaf-blade N and (C) Sap (Cardy Meter) NO_3-N of the greenhouse-grown cotton plants.