

# REMOTE SENSING

## Selection of Optimum Reflectance Ratios for Estimating Leaf Nitrogen and Chlorophyll Concentrations of Field-Grown Cotton

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### ABSTRACT

Leaf N and chlorophyll (Chl) concentrations of cotton (*Gossypium hirsutum* L.) are important indicators of plant N status. Laboratory determinations of plant tissue N are time consuming and costly. Measurements of leaf reflectance may provide a rapid and accurate means of estimating leaf N and Chl. Studies were conducted to determine the relationships between leaf hyperspectral reflectance (400–2500 nm) and Chl or N concentration in field-grown cotton. One study consisted of four N rates of 0, 56, 112, and 168 kg N ha<sup>-1</sup>, and another study consisted of four mepiquat chloride (MC) rates of 0, 0.59, 1.17, and 2.34 L MC ha<sup>-1</sup>. Chlorophyll and N concentrations and reflectance of uppermost, fully expanded mainstem leaves were measured throughout the growing seasons. Reflectance at 556 and 710 nm increased significantly as N fertilizer rate decreased. Averaged across years and sampling dates, the percentage increase in reflectance at these two wavelengths was 8, 10, and 19% greater in the 112, 56, and 0 kg N ha<sup>-1</sup> treatments, respectively, compared with the 168 kg N ha<sup>-1</sup> treatment. The effect of MC on leaf reflectance was more complex than the N effect. In both the N and MC studies, a linear relationship was found between leaf N and a simple ratio of leaf reflectance at 517 and 413 nm ( $R_{517}/R_{413}$ ) ( $r^2 = 0.65\text{--}0.78^{***}$ ). Leaf Chl concentration was associated closely with reflectance ratios of either  $R_{708}/R_{915}$  or  $R_{551}/R_{915}$  ( $r^2 = 0.67\text{--}0.76^{***}$ ). Our results suggest leaf reflectance can be used for real-time monitoring of cotton plant N status and N fertilizer management in the field.

NITROGEN FERTILIZATION management is an important issue in cotton production systems. It is more difficult to balance demand and supply of cotton plant N nutrition compared with other nutrient fertilizers because of the complexity of N cycling in the soil and the indeterminate growth habit of cotton (Gerik et al., 1998). Both deficient and excessive N negatively affects lint yield and fiber quality (Gerik et al., 1998; MacKenzie and van Schaik, 1963). Insufficient N supply decreases leaf area (Fernandez et al., 1996; Reddy et al., 1997; van Delden, 2001), photosynthesis (Ciompi et al., 1996; Reddy et al., 1997; Lu et al., 2001), and biomass production (Fritschi et al., 2003), resulting in lower yields (Howard et al., 2001; Fritschi et al., 2003). On the other hand, excessive applications of N fertilizer

often result in immoderate growth, increased input costs, and potential adverse environmental impacts, especially water quality (Jaynes et al., 2001). Therefore, one of the goals of farm managers is to accurately detect plant N status and provide N fertilizer in a timely manner to improve yield, increase N use efficiency and profit, and minimize N losses to the environment. Changes in leaf N concentrations depend on not only environments, but also the stage of crop development (Oosterhuis et al., 1983). Recently, Bell et al. (2003) reported that the critical leaf-blade N concentrations associated with seed cotton yield loss were 5.4% at the pin-head square, 4.3% at early flower, and 4.1% at 3 wk after flower. Therefore, multiple determinations of leaf N concentration are required for N recommendations to optimize cotton yields.

Leaf N concentration is an important indicator for diagnosing plant N status (Gerik et al., 1994; Bell et al., 2003). Traditional methods of determining tissue nutrient concentrations in a laboratory are time consuming and costly. Furthermore, by the time the symptoms of plant nutrient deficiency become clearly visible, many physiological processes may have been severely disrupted by nutrient stress.

Recent advances in remote sensing, coupled with lower cost of acquiring images, have allowed the collection of timely information on crop growth and physiological parameters temporally and spatially as affected by environmental stresses. Such information can be used for in-season crop nutrient assessment and management (Filella et al., 1995; Daughtry et al., 2000; Zarco-Tejada et al., 2000a, 2000b; Afanasyev et al., 2001). Several studies have assessed N status and other physiological parameters of field crops using leaf or canopy spectral reflectance parameters (Gausman, 1982; Chappelle et al., 1992; Blackmer et al., 1994; Thomas and Gausman, 1977; Peñuelas and Filella, 1998; Peñuelas and Inoue, 2000; Zhao et al., 2003). Nitrogen deficiency causes a decrease in leaf Chl concentration, resulting in an increase in leaf reflectance in the visible spectral region (400–700 nm) (Buscaglia and Varco, 2002; Carter and Estep, 2002; Read et al., 2002; Zhao et al., 2003). However, several other stresses may also result in increased reflectance due to reduced amounts of Chl (Carter and Knapp, 2001). Furthermore, diagnosing a specific nutrient deficiency with remotely sensed data can be difficult when plants are subjected to deficiencies of multiple

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**Abbreviations:** Chl, chlorophyll; DAS, days after sowing; DW, dry weight; FF, first flower; FS, first square; MC, mepiquat chloride;  $R_i$ , reflectance at  $i$  nanometers; RD, reflectance difference; RS, reflectance sensitivity.

elements (Masoni et al., 1996). Recently, several studies have shown the relationships between cotton plant N status and spectral reflectance (Tarpley et al., 2000; Buscaglia and Varco, 2002; Read et al., 2002). Buscaglia and Varco (2002) documented that cotton leaf N concentration was linearly correlated with leaf reflectance at 550, 700, 612, or 728 nm, but the regression parameters (i.e., the slope and intercept) varied significantly with growth stage, and the sample size was limited ( $n = 12$ ). Tarpley et al. (2000) reported that using specific reflectance ratios (i.e., leaf reflectance values at 700 or 716 nm divided by reflectance values at 755 to 920 nm) could improve precision and accuracy in predicting cotton leaf N concentration. Read et al. (2002) also found that some specific reflectance ratios were more closely related to leaf N concentration (greater  $r^2$  values) than single reflectance measures. However, all these studies were conducted in controlled environmental conditions in growth chambers, greenhouses, or pots, and some plants faced severe N shortage or other environmental stresses during the experiments. The conclusions from these reports have not been validated under field conditions with varying N levels or with other management practices such as application of plant growth regulators.

Mepiquat chloride is a plant growth regulator that successfully controls cotton plant growth and size (Reddy et al., 1995; Zhao and Oosterhuis, 2000). Approximately 50% of the cotton grown in the USA receives MC or other plant growth regulators containing MC. In general, plants receiving MC have greener, thicker, and smaller leaves compared with plants not receiving MC application (Zhao and Oosterhuis, 2000). Previous studies have shown MC application is effective in regulating plant height and leaf area development (Reddy et al., 1995) and alters several physiological processes in cotton such as photosynthesis (Hodges et al., 1991; Zhao and Oosterhuis, 2000), respiration (Hodges et al., 1991), specific leaf weight (Xu and Taylor, 1992; Zhao and Oosterhuis, 2000), and the synthesis of leaf Chl (Stein et al., 1983; Xu and Taylor, 1992) and nonstructural carbohydrate concentrations (Zhao and Oosterhuis, 2000). So far, there have been no studies conducted on the interrelationships between MC-induced morphophysiological changes and reflectance properties of cotton leaves.

We hypothesized that some particular leaf reflectance ratios may be used to rapidly estimate leaf N and Chl concentrations of field-grown cotton over the growing season and across a wide range of N fertilization and MC applications. To test our hypothesis and to investigate responses of cotton leaf N concentration and leaf spectral properties to N supply and MC applications under field conditions, we conducted two separate field experiments in 2001 and 2002. The specific objectives were to: (i) determine the seasonal trends of leaf N and Chl concentrations as affected by N fertilizer rates and MC applications and (ii) develop functional relationships between leaf hyperspectral reflectance or simple reflectance ratios and leaf N or Chl concentrations in cotton.

## MATERIALS AND METHODS

Two separate field studies were conducted in 2001 and 2002 on a fine, smectitic, nonacid, thermic Vertic Epiaquept (Leeper silt clay loam) soil at the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi State, MS. Seeds of cotton cultivar NUCOTN 33B, a midseason upland Bt (*Bacillus thuringiensis*) variety, were sown on 14 May 2001 and 24 May 2002. Rows were spaced 1 m apart and oriented in an east–west direction. Seedlings were hand-thinned to a density of 9 plants  $m^{-2}$  at the second true-leaf stage. The stages of first square (FS) and first flower (FF) are defined as the dates when 50% of plants have first visible floral bud (square) with a 3-mm dimension and when 50% of plants have the first white flower, respectively. Dates of the FS and FF stages recorded in all treatments were on 24 June [41 d after sowing (DAS)] and 17 July (64 DAS), respectively, in 2001 and 28 June (35 DAS) and 19 July (56 DAS), respectively, in 2002.

The N-rate study included four treatments of (i) no N applied during the growing season, (ii) 56 kg N  $ha^{-1}$  applied at the second true-leaf stage, (iii) 112 kg N  $ha^{-1}$  split evenly and applied at the second true-leaf stage and at the FS stage, and (iv) 168 kg N  $ha^{-1}$  (control) split-applied as 56 kg N  $ha^{-1}$  at the second true-leaf stage and 112 kg N  $ha^{-1}$  at the FS stage. The MC study also included a control without MC and three MC treatments of 0.59, 1.17, and 2.34 L MC  $ha^{-1}$ . These MC treatments were split evenly and applied foliarly at FS and FF stages using a backpack  $CO_2$ -pressured sprayer with 94 L water  $ha^{-1}$ . All plots in the MC study received 168 kg N  $ha^{-1}$  (56 kg at the second true-leaf stage and 112 kg at the FS stage). Liquid N fertilizer of N solution and suspensions, containing 32% N (NSOL, Mississippi Chem. Corp., Yazoo City, MS), was injected beside each row.

The experimental design was a randomized complete block with three replications in both N-rate and MC studies. Plot size was 8 m wide by 15 m long. Weekly or biweekly measurements of N and Chl concentrations and hyperspectral reflectance of uppermost, fully expanded mainstem leaves were made during the growing seasons. Five uppermost, fully expanded mainstem leaves were randomly collected from plants at about 1100 h beginning from FS stage and ending about 3 wk after the first open boll. The leaves were placed in a cooler immediately and brought to the laboratory. Leaf hyperspectral reflectance measurements were made using a portable ASD FieldSpec FR spectroradiometer (Analytical Spectral Devices Inc., Boulder, CO) with a wavelength ranging from 350 to 2500 nm. The optical sensor of the spectroradiometer was mounted in the frame of a supplemental light source (ML 902, Makita Corp., Aichi, Japan) with a 50-mm distance from target leaf surface. A Spectralon reference panel (white reference) was used to optimize the instrument to 100% reflectance at all wavelengths before taking measurements. When measuring leaf reflectance, the individual leaves were placed adaxial side up on top of a nonreflecting black polyurethane background.

In 2001, leaf Chl concentration was not determined spectrophotometrically due to unavailability of instrument, but relative Chl levels in the uppermost, fully expanded leaf from 10 plants in each plot were measured using a Minolta SPAD-502 Chl meter (Minolta Corp., Osaka, Japan). In 2002, leaf Chl was extracted and quantified using a spectrophotometric method (Chappelle et al., 1992). After measuring leaf reflectance, five leaf discs (38.5  $mm^2$  each) were punched from the five leaves and placed into a vial with 5 mL of dimethyl sulphoxide and incubated in the dark at room temperature for 24 h to allow for complete extraction of Chl into the solution. Absorbance of the extract was measured using a SmartSpec 3000 spectro-

photometer (Bio-Rad Laboratories, Hercules, CA) at 470, 648, and 664 nm to calculate concentrations of Chl *a* and Chl *b* according to Chappelle et al. (1992). Chlorophyll concentration in 2002 reported in this paper is the sum of Chl *a* and Chl *b*.

Leaf area was determined using a LI-3100 leaf area meter (LI-COR Inc., Lincoln, NE) after collecting the leaf discs for Chl measurements. Then, the five leaves from each plot were together placed into a paper bag and immediately dried in a forced-air oven at 70°C for 72 h, weighed, and ground for determination of leaf N concentrations according to standard micro-Kjeldahl procedure (Nelson and Sommers, 1972). Specific leaf weight was calculated based on leaf dry weight (DW) and leaf area. When determining leaf N concentration, several standard samples of apple (*Malus domestica* Borkh.) and spinach (*Spinacia oleracea* L.) leaves (U.S. Commerce, NIST, Gaithersburg, MD) were included to eliminate systematic errors during the laboratory process. Concentrations of leaf Chl in 2002 were expressed on a leaf area basis ( $\text{g m}^{-2}$ ). Leaf N concentrations were expressed on both DW ( $\text{g kg}^{-1}$ ) and leaf area basis.

The spectral reflectance data measured on the five leaves in each plot at each sampling date were averaged. To determine the effects of N fertilizer rate and MC application on leaf hyperspectral reflectance, the mean reflectance values for each treatment of both studies were obtained by averaging the data across sampling dates and replications. The reflectance differences (RD) at each wavelength and reflectance sensitivity (RS) to N fertilizer rate or to MC application were calculated based on the following formulas:

$$\text{RD} = \text{reflectance of N or MC treatments} - \text{reflectance of the control}$$

$$\text{RS} = [\text{RD}/(\text{reflectance of the control})] \times 100$$

Data for leaf N and Chl concentrations were subjected to analysis of variance (SAS Inst., 1997) to determine N and MC treatment effects. To determine relationships between leaf N or Chl concentrations and leaf reflectance values at different wavelengths, data of leaf N and Chl and corresponding reflectance were pooled across plots, treatments, and sampling dates in each study and each year. Coefficients of determination ( $r^2$ ) were calculated and used to evaluate linear relationships of leaf N concentrations with reflectance at 1-nm intervals throughout the range of 400 to 2500 nm for the N study. Thereafter, the reflectance values at 517 and 701 nm ( $R_{517}$  and  $R_{701}$ ) with greatest  $r^2$  values with leaf N content were used as the numerators and the reflectance values at all other wave-

lengths ( $R_i$ ) as denominators to calculate reflectance ratios ( $R_{517}/R_i$  and  $R_{701}/R_i$ ), and the  $r^2$  values of the reflectance ratios with leaf N were further determined. The best reflectance ratio ( $R_{517}/R_{413}$ ), which had the greatest  $r^2$  value with leaf N concentration, was selected. Data of leaf N concentrations from the N and MC studies within each year were plotted with the corresponding reflectance ratios ( $R_{517}/R_{413}$ ), and linear regression was performed. The same methods described above were used to determine functional relationships between Chl concentration and reflectance or reflectance ratio values.

## RESULTS AND DISCUSSION

### Leaf Nitrogen Concentrations

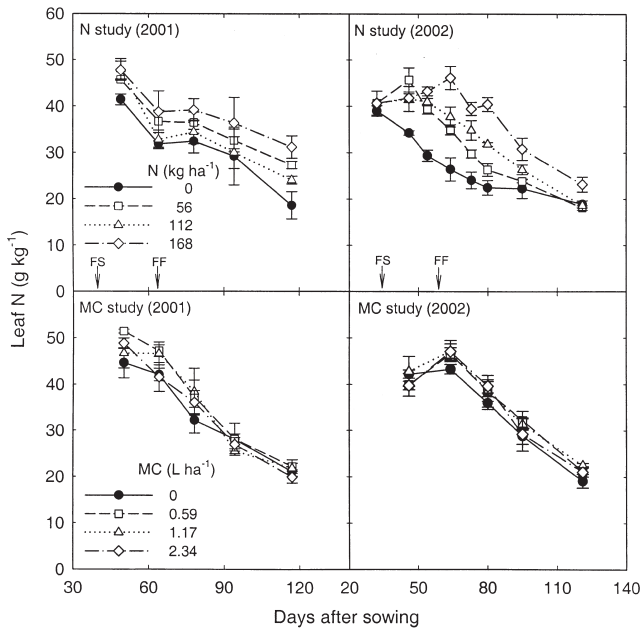
Leaf N concentration on a DW basis did not differ between years for either the N or MC study but differed significantly across sampling dates and treatment levels (Table 1). The year  $\times$  sampling date interactive effects on leaf N concentrations were significant. Overall, leaf N concentration was highest between 40 and 70 DAS (FS to FF stages) and decreased as plants aged (Fig. 1). The seasonal trends of leaf N concentration in our study are consistent with whole canopy dynamics of cotton leaf N reported by Oosterhuis et al. (1983) and Milroy et al. (2001).

In the present N study, leaf N concentration was closely related to the N fertilizer rate and consistently increased with increasing amount of N fertilizer application (Fig. 1). Averaged across sampling dates, values of leaf N of the 0, 56, 112, and 168  $\text{kg N ha}^{-1}$  treated plants were 30.4, 35.8, 33.9, and 38.7  $\text{g kg}^{-1}$  DW, respectively, in 2001 and 27.2, 32.4, 34.1, and 38.2  $\text{g kg}^{-1}$  DW, respectively, in 2002.

In the MC study, leaf N levels ( $\text{g kg}^{-1}$  DW) were not statistically different among the four MC treatments within any sampling date (Fig. 1). Averaged across sampling dates and years, leaf N concentrations of the 0, 0.59, 1.17, and 2.34 L MC  $\text{ha}^{-1}$  treatments were 33.8, 36.5, 36.2, and 35.1  $\text{g kg}^{-1}$  DW, respectively. When leaf N levels were expressed on a leaf area basis, however, the three MC treatments had 11 to 17% higher leaf N concentrations than the control ( $P < 0.05$ , data not shown). Because application of MC usually results in smaller leaves and greater specific leaf weight (Zhao

**Table 1.** Analysis-of-variance mean square (MS), *F* values, and  $P < F$  for cotton leaf N and chlorophyll concentrations.

Source	N study				Mepiquat chloride study			
	df	MS	<i>F</i> value	$P < F$	df	MS	<i>F</i> value	$P < F$
<b>Leaf N concentration</b>								
Year (Y)	1	6.2	0.37	0.5438	1	0.0	0.00	0.9885
Date (D)	4	1047.2	62.76	<0.0001	4	2578.6	179.54	<0.0001
Treatment (T)	3	579.4	34.73	<0.0001	3	47.0	3.27	0.0254
Y $\times$ D	4	55.5	3.33	0.0143	4	95.1	6.62	0.0001
Y $\times$ T	3	57.1	3.42	0.0211	3	22.8	1.58	0.1997
D $\times$ T	12	17.1	1.03	0.4325	12	4.8	0.33	0.9810
Y $\times$ D $\times$ T	12	24.1	1.44	0.1636	12	7.7	0.53	0.8861
<b>2001 Chlorophyll concentration</b>								
Date (D)	9	87.9	12.49	<0.0001	7	247.0	69.3	<0.0001
Treatment (T)	3	152.1	21.60	<0.0001	3	309.7	86.9	<0.0001
D $\times$ T	27	11.7	1.66	0.0428	21	5.02	1.41	0.1486
<b>2002 Chlorophyll concentration</b>								
Date (D)	6	317.8	19.95	<0.0001	5	449.9	10.92	<0.0001
Treatment (T)	3	1400.8	87.94	<0.0001	3	19.1	0.46	0.7095
D $\times$ T	18	142.19	8.93	<0.0001	15	50.8	1.23	0.2811



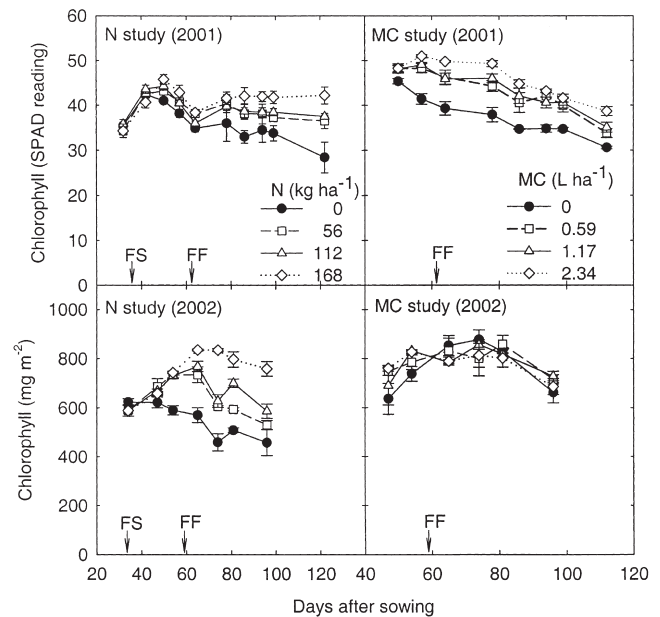
**Fig. 1.** Changes in N concentrations of cotton uppermost, fully expanded mainstem leaves over the growing seasons in 2001 and 2002 as affected by N and mepiquat chloride (MC). Data are means  $\pm$  standard error of three replications. Two arrows from left to right indicate the first square (FS) and the first flower (FF) stages, respectively.

and Oosterhuis, 2000), increased leaf area-based N concentration due to MC application was probably related to MC slowing down leaf expansion (Reddy et al., 1995). Although changes in leaf area-based N concentration ( $\text{g m}^{-2}$ ) in response to N fertilizer or MC rate were similar to those of leaf DW-based N concentration, the declines in leaf area-based N as plants aged were smaller than those in leaf DW-based N due to the increases in specific leaf weight as plants aged (data not shown).

### Leaf Chlorophyll Concentrations

Sampling date and N fertilizer rate significantly affected Chl concentration of uppermost, fully expanded leaves ( $P < 0.0001$ ) in the N study, and the interaction was significant ( $P < 0.05$  in 2001 and  $P < 0.0001$  in 2002) (Table 1). Changes in Chl concentration with plant development in our study are similar to those during individual leaf ontogeny found by Wells (2001). Starting from FF, the 56, 112, and 168 kg N ha<sup>-1</sup> treatments had consistently higher Chl concentration than the 0 kg N ha<sup>-1</sup> treatment ( $P < 0.05$  to 0.0001, Fig. 2). Application of MC significantly increased leaf relative Chl levels (SPAD readings) at most measuring dates in 2001, but no statistical differences were observed in Chl concentration among the MC treatments in 2002 (Fig. 2).

Many studies have shown that there is a close relationship between cotton leaf Chl and N concentrations (Wood et al., 1992; Wu et al., 1998). Our results indicated that although leaf Chl levels were positively correlated with leaf N concentrations ( $r^2 = 0.66\text{--}0.80^{***}$ ,  $n = 120$ ), changes in Chl with growth stages were much smaller than changes in leaf N concentration, except perhaps in MC study in 2001 (Fig. 1 and 2). Averaged



**Fig. 2.** Changes in chlorophyll concentrations of cotton uppermost, fully expanded mainstem leaves over the growing seasons in 2001 and 2002 as affected by N and mepiquat chloride (MC). Data are means  $\pm$  standard error of three replications. Vertical arrows from left to right indicate the first square (FS) and the first flower (FF) stages, respectively.

across sampling dates, leaf Chl concentrations of the 0, 56, 112, and 168 kg N ha<sup>-1</sup> treatments were 35.8, 39.0, 39.3, and 41.2 (SPAD reading), respectively, in 2001 and 548, 639, 668, and 745 mg m<sup>-2</sup>, respectively, in 2002. The results of leaf Chl concentration response to N fertilizer rates in the present study are in agreement with those of Boggs et al. (2003). The Chl concentrations of the 0, 0.59, 1.17, and 2.34 L MC ha<sup>-1</sup> treated plants were 37.4, 43.0, 43.4, and 45.8 SPAD readings, respectively, in 2001 and 766, 790, 785, and 780 mg m<sup>-2</sup>, respectively, in 2002. Our results of leaf Chl response to MC application are consistent with earlier reports of Stein et al. (1983) and Xu and Taylor (1992). The increase in leaf area-based Chl concentration in MC-treated cotton leaves might be associated with a greater specific leaf weight (Xu and Taylor, 1992; Zhao and Oosterhuis, 2000). Wells (2001) reported a significantly positive linear relationship between leaf Chl concentration and canopy photosynthesis in cotton. Boggs et al. (2003) documented that leaf Chl correlated significantly with soil NO<sub>3</sub>-N and cotton yield. Therefore, to improve crop C assimilation rate and yield, it is important to maintain appropriate leaf N and Chl concentrations by N fertilizer and other management practices.

### Leaf Hyperspectral Reflectance

Leaf hyperspectral reflectance showed similar patterns for all the N (Fig. 3A) and MC treatments (data not shown). Nitrogen fertilizer rate mainly affected leaf reflectance in the visible range (400–700 nm) and in the red edge (690–730 nm). Specifically, the leaf reflectance at 556 and 710 nm rapidly increased with the decrease in N fertilizer rate. This phenomenon could be clearly

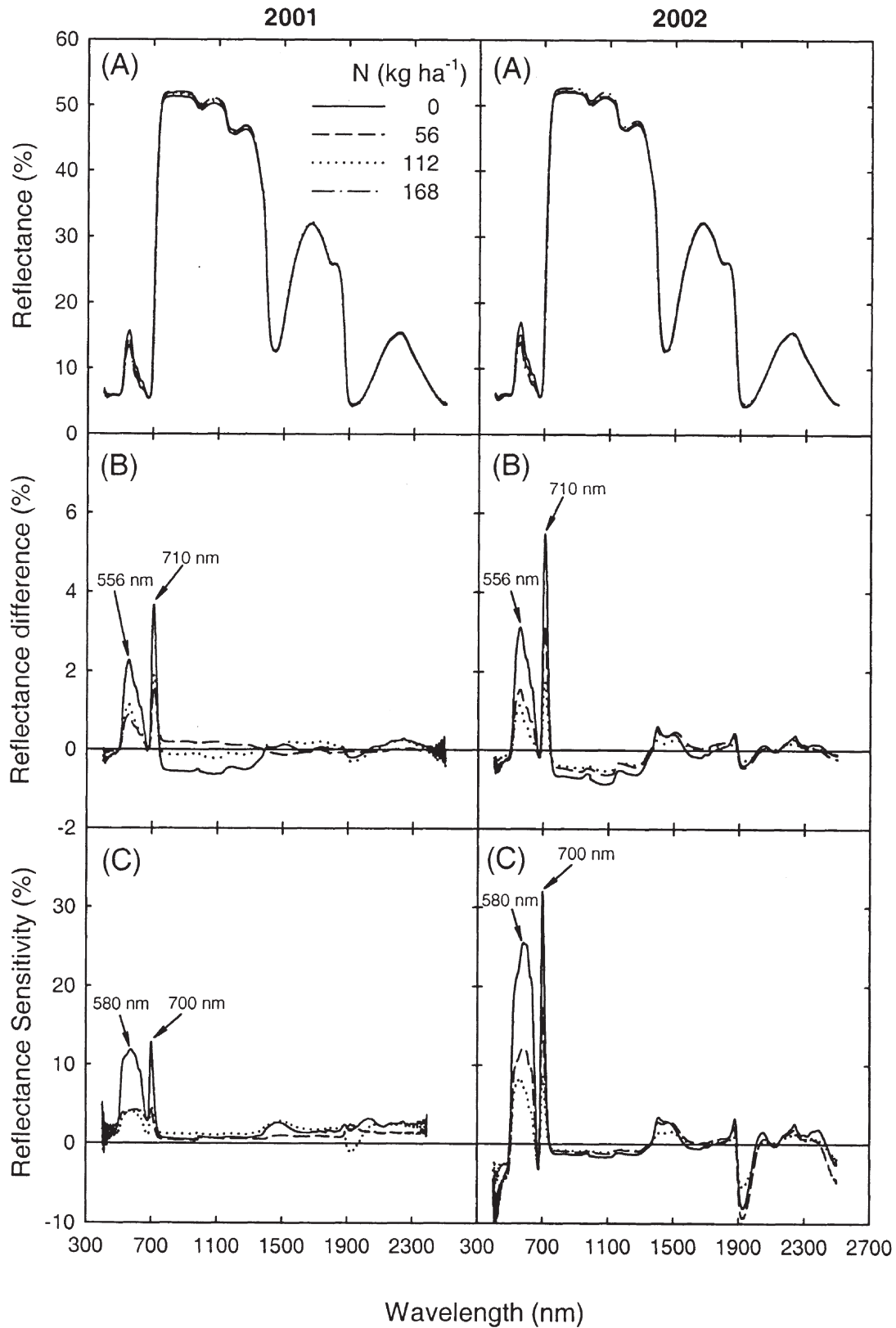


Fig. 3. Cotton leaf (A) hyperspectral reflectance of the four N treatments in the N study, (B) reflectance differences of treatments from the control (168 kg N ha<sup>-1</sup>), and (C) reflectance sensitivity to N fertilizer rate. Data are means of five (2001) or eight (2002) measuring dates. Each time, measurements were made on 15 individual leaves from three replications of each treatment.

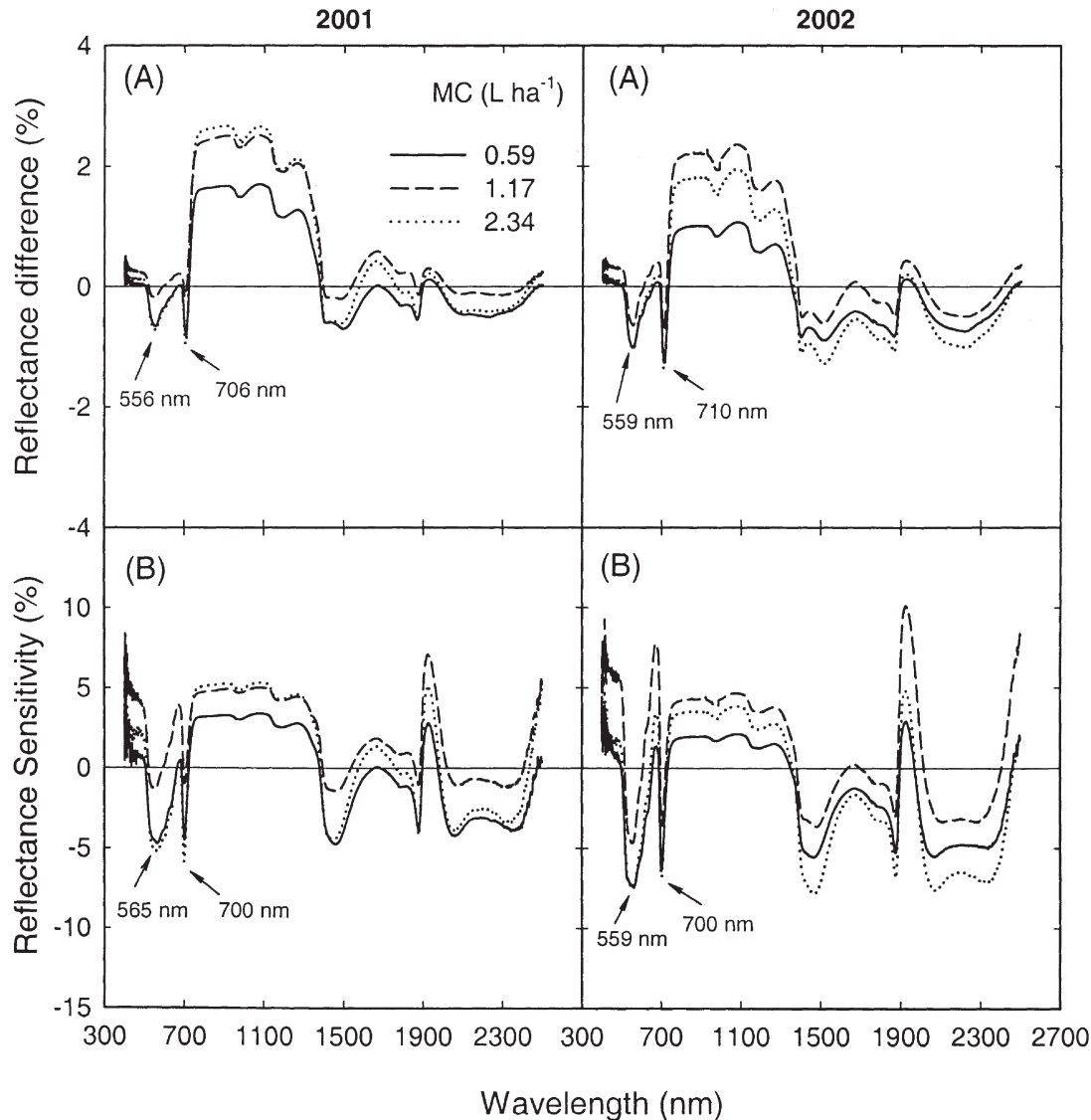


Fig. 4. Effects of the plant growth regulator mepiquat chloride (MC) on cotton (A) leaf reflectance difference and (B) reflectance sensitivity based on the control without MC application. Data are means of five measuring dates. Each time, measurements were taken on 15 individual leaves from three replications of each treatment.

seen from values for RD and RS (Fig. 3B and 3C). Averaged across the years and measuring dates in the growing seasons, the 0, 56, and 112 kg N ha<sup>-1</sup> treatments had 20, 9, and 8% higher reflectance at 556 nm, respectively, and 18, 10, and 7% higher reflectance at 710 nm, respectively, compared with the 168 kg N ha<sup>-1</sup> treatment ( $P < 0.05$ ). The two wavelengths where N fertilizer rate mostly affected cotton leaf reflectance in the present study are consistent with earlier reports in corn (*Zea mays* L.) (Blackmer et al., 1996; Carter and Estep, 2002; Zhao et al., 2003). When calculating RS by dividing RD by the reflectance of the 168-kg N treatment, we found that leaf reflectances at 580 and 700 nm were the most sensitive to N application rate (Fig. 3C). Several studies have shown that leaf reflectance values around these two wavelengths are closely associated with leaf Chl level (Jacquemoud and Baret, 1990; Daughtry et al., 2000; Carter and Spiering, 2002; Zhao et al., 2003). Therefore, N fertilizer rate mainly affected leaf reflec-

tance in the visible range and the red-edge feature by modifying leaf Chl content. Leaf reflectance around these two wavelengths could be used to detect crop plant N deficiency.

The leaf reflectance patterns of all the MC treatments in the MC study were similar to those in the N study (data not shown). Based on the leaf RD and RS to the MC, the effect of MC on leaf reflectance was complex (Fig. 4). Foliar applications of MC decreased the reflectance in visible range around 556- and 710-nm regions similar to those found in the N deficiency study where N deficiency increased reflectance (Fig. 3). In addition, plants treated with MC had increased leaf reflectance between 800- and 1400-nm wavelengths and decreased/increased reflectance in 1450 to 2500 nm. Decreased reflectance at 556 and 710 nm by MC was related to the increased leaf area-based N or Chl levels in MC-treated plants as described earlier. Evidence shows that leaf reflectance in 800- to 1400-nm region is associated

with leaf surface properties and leaf structure, whereas reflectance in 1500- to 2500-nm region may be associated with leaf water content and other chemical compositions (Peñuelas and Filella, 1998). Therefore, MC application seems to modify not only leaf photosynthetic pigment concentration, but also other leaf physiological and morphological properties (Zhao and Oosterhuis, 2000).

### Relationships between Leaf Nitrogen Concentration and Reflectance or Reflectance Ratios

When data were pooled across treatments and growing seasons, leaf N concentrations ranged from 16.4 to 52.3 g kg<sup>-1</sup> DW or 1.34 to 2.75 g m<sup>-2</sup> leaf. Coefficients of determination ( $r^2$ ) for leaf N, on both leaf DW basis and leaf area basis, with leaf reflectance at each wavelength are presented in Fig. 5A. Although N fertilizer rate mainly affected leaf reflectance at 556 and 710 nm (see Fig. 3), two specific wavelengths where reflectance provided the greatest  $r^2$  values with leaf N concentration were 517 and 701 nm. It is noted that the first wavelength of 517 nm did not match the N fertilizer sensitive wavelengths (Fig. 3B, 3C), but the second one (701 nm) was very similar to the N sensitive wavelength of 700 to 710 nm described in Fig. 3. Our finding of reflectance at 517 nm having greater  $r^2$  with leaf N content is in contrast to earlier reports by Buscaglia and Varco (2002) and Read et al. (2002), who indicated that in the green region, the reflectance around 550 or 585 nm was closely correlated with cotton leaf N concentration.

Leaf N concentration was closely related to reflectance at 517 and 701 nm among 2100 wavelengths from 400 and 2500 nm in the present study. However, using the single reflectance values at any one of these two wavelengths could only explain 62 to 65% of leaf N variations (Fig. 5A). The  $r^2$  values of leaf N concentrations, expressed in both leaf DW basis and leaf area basis, with the reflectance ratios of  $R_{517}/R_i$  and  $R_{701}/R_i$  were further calculated (Fig. 5B, 5C). Compared with single reflectance, the reflectance ratios improved  $r^2$  values at most wavelengths measured, and the reflectance ratio of  $R_{517}/R_{413}$  showed the best linear relationship ( $r^2 = 0.83^{***}$ ,  $n = 60$ ) with leaf N concentrations.

Correlations of leaf N of both the N and MC studies in the 2 yr with  $R_{517}/R_{413}$  are presented in Fig. 6. These results indicate that the reflectance ratio of  $R_{517}/R_{413}$  decreased linearly as leaf N concentrations increased ( $r^2 = 0.65\text{--}0.78^{***}$ ,  $n = 120$  in 2001 and 156 in 2002). Several studies have shown that the use of simple reflectance ratios can improve precision and accuracy of predicting cotton leaf N concentration compared with single reflectance (Tarpley et al., 2000; Read et al., 2002). Our results support their conclusions although the reflectance ratios are not exactly the same as in those studies. In a pot-culture study, Read et al. (2002) found quadratic relationships between cotton leaf N levels and several canopy reflectance ratios, but our results indicated linear relationships between leaf N and  $R_{517}/R_{413}$  for field-grown cotton. Results from several field experi-

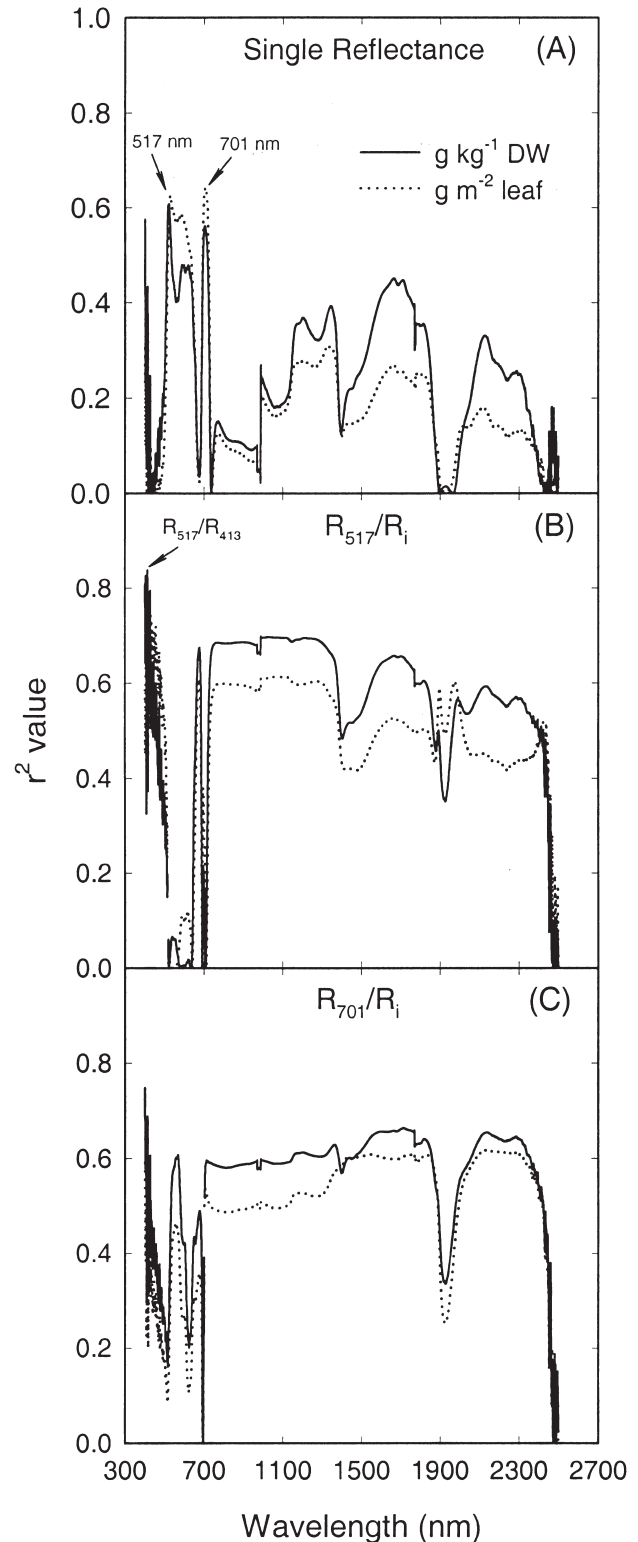
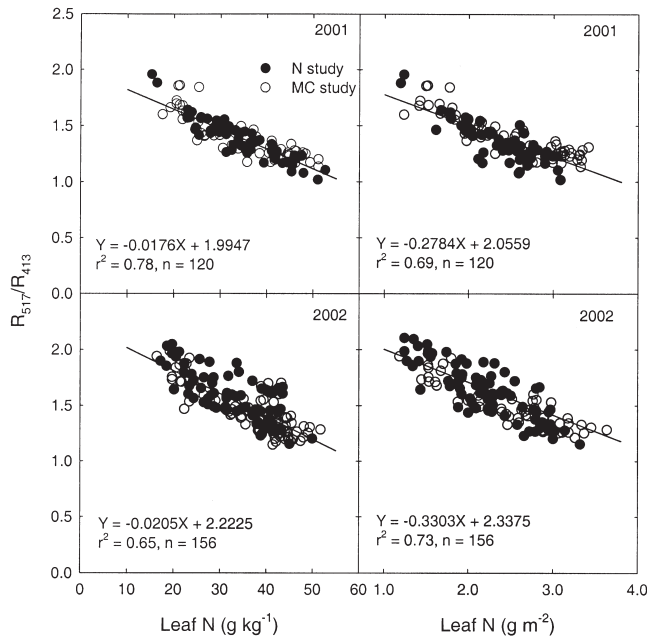


Fig. 5. Coefficients of determination ( $r^2$ ) vs. wavelengths for the relationships between cotton leaf N concentration and (A) leaf reflectance at all wavelengths (400 to 2500 nm) and leaf reflectance ratios of (B)  $R_{517}/R_i$  and (C)  $R_{701}/R_i$  for the 2001 N study. The  $r^2$  values were based on a linear model, and data were pooled across the four N treatments, five sampling dates, and three replications ( $n = 60$ ). Wavelengths with the greatest  $r^2$  values are presented in the figure. Leaf N concentrations were expressed on both dry weight (DW) and leaf area basis.

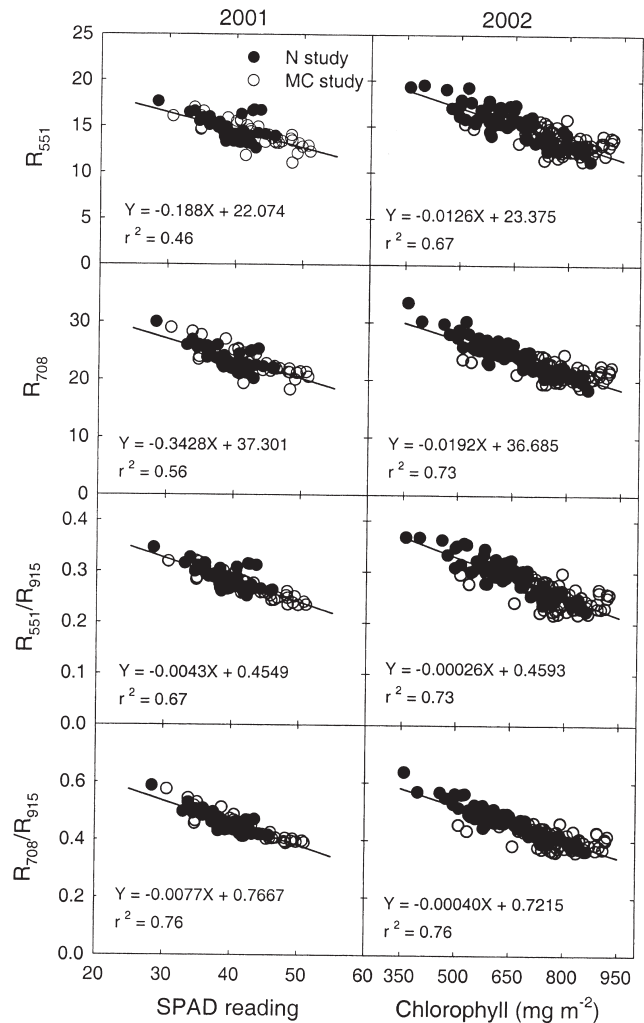


**Fig. 6.** Linear regression of cotton leaf N concentrations with the values of a specific reflectance ratio ( $R_{517}/R_{413}$ ) that had the greatest  $r^2$  value with leaf N. Leaf N concentrations were expressed on both (left) a leaf dry weight basis and (right) a leaf area basis. Data were pooled across the studies, treatments, replications, and sampling dates ( $n = 120$  in 2001 and  $n = 156$  in 2002). MC, mepiquat chloride.

ments conducted for 2 yr across in the Midsouth USA indicated that critical leaf-blade N concentration associated with seed cotton yield loss was  $54 \text{ g kg}^{-1}$  DW at FS stage,  $43 \text{ g kg}^{-1}$  at early-flower stage, and  $41 \text{ g kg}^{-1}$  at midflower stage. (Bell et al., 2003). Clearly, cotton plant N status is closely related to yield (Gerik et al., 1994; Bell et al., 2003), but traditional methods of plant tissue N quantification in a laboratory are time consuming and costly. Estimation of leaf N concentration using nondestructive leaf spectral reflectance measurements can be an alternative method for plant N diagnoses and N fertilizer recommendation. For instance, based on critical leaf-blade N values reported by Bell et al. (2003), and from our findings of linear function between leaf N and reflectance ratio (see Fig. 6), cotton critical leaf reflectance ratio ( $R_{517}/R_{413}$ ) at the three key crop developmental stages of FS, early bloom, and midbloom was 1.044, 1.238, and 1.273, respectively. These specific leaf reflectance ratios may be used for nondestructive detection of cotton plant N deficiency in a fast and reliable fashion.

### Relationships between Leaf Chlorophyll and Reflectance or Reflectance Ratios

Among 2100 wavelengths from 400 to 2500 nm, the reflectance values at 551 ( $R_{551}$ ) and 708 ( $R_{708}$ ) nm had the best linear relationships with leaf Chl ( $r^2 = 0.46$ – $0.73$ ,  $n = 76$ – $156$ ). These two Chl-specific wavelengths matched the two N fertilizer sensitive wavelengths described earlier (see Fig. 3B, 3C). The  $R_{551}/R_{915}$  and  $R_{708}/R_{915}$  had the greatest  $r^2$  values (0.67–0.76) with Chl concentration among all the reflectance ratios of  $R_{551}/R_i$



**Fig. 7.** Linear regression of cotton leaf chlorophyll with corresponding reflectance values at wavelengths of 551 ( $R_{551}$ ) and 708 ( $R_{708}$ ) nm and two reflectance ratios ( $R_{551}/R_{915}$  and  $R_{708}/R_{915}$ ) that had the greatest  $r^2$  values with chlorophyll. Data were pooled across studies, treatments, replications, and sampling dates ( $n = 76$  in 2001,  $n = 156$  in 2002). Chlorophyll in 2001 was measured using a SPAD-502 chlorophyll meter. MC, mepiquat chloride.

and  $R_{708}/R_i$  (Fig. 7). Similar to leaf N and reflectance relationships, these simple reflectance ratios slightly improved the  $r^2$  values of the linear models compared with single reflectance values.

Our results of leaf Chl most closely correlating with the reflectance at either 551 or 708 nm (Fig. 7) are in contrast to Boggs et al. (2003), who found that hyperspectral reflectance at about 808 nm had the largest correlation with cotton leaf Chl. Results showing a strong correlation between  $R_{708}/R_{915}$  and Chl in the present study are similar to those of Read et al. (2002), who reported that  $R_{705}/R_{930}$  was one of the best ratios correlated to cotton leaf Chl, but are in contrast to those in other species reported by Gitelson et al. (1996) and Lichtenthaler et al. (1996). In their studies,  $R_{700}/R_{750}$  was linearly correlated to Chl content in tobacco (*Nicotiana tabacum* L.) genotypes (Lichtenthaler et al., 1996) and in senescing leaves of two tree species (Gitelson et al., 1996). Recently, Carter and Spiering (2002) investigated



the relationships between leaf Chl concentration and leaf reflectance or reflectance ratios in several tree species and found that Chl concentration was associated with leaf reflectance at 549 and 715 nm, but the relationship followed a power function, rather than a linear function. The differences between our results and these earlier reports might be associated with experimental conditions and species. Overall, cotton leaf Chl concentrations could be estimated using leaf reflectance at  $R_{551}$  and  $R_{708}$  or reflectance ratios  $R_{551}/R_{915}$  or  $R_{708}/R_{915}$  (Fig. 7).

## SUMMARY AND CONCLUSIONS

Cotton leaf N concentration declined as plants aged from early squaring through boll opening and was correlated closely to N fertilizer rate. Leaf Chl was positively correlated with leaf N concentration. Nitrogen fertilizer rate mainly affected cotton leaf reflectance at 550 and 710 nm, and N deficiency increased leaf reflectance at these two wavelengths. Among leaf reflectance values at all wavelengths (400–2500 nm), the reflectance values at 517 and 701 nm had the highest correlation with leaf N levels. The effects of MC on leaf reflectance were more complex than the N effects. Compared with single reflectance measures, the simple reflectance ratios improved  $r^2$  values of best-fit linear regression with leaf N. Leaf N concentrations were highly and linearly correlated with spectral reflectance ratio of  $R_{517}/R_{413}$  with the greatest  $r^2$  value. Chlorophyll concentrations were highly correlated with either leaf reflectance at a single wavelength of 551 or 708 nm or the reflectance ratios of  $R_{551}/R_{915}$  and  $R_{708}/R_{915}$ . Application of MC did not affect these relationships between leaf reflectance and leaf N or Chl concentrations. Thus, the selection of these specific wavelengths and utilization of the leaf reflectance ratios appear to provide a quick, inexpensive, and reliable means to precisely estimate cotton leaf N and Chl concentrations throughout the growing season under a broad range of management practices. Our results clearly indicate that leaf reflectance measured nondestructively can be used for real-time monitoring of cotton plant N status and N fertilizer management in the field.

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