14 Evaluation of a Hand-held Radiometer for Field Determination of Nitrogen Status in Cotton

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ABSTRACT

Because the timing and amount of N fertilizer applications are important in producing high yield in cotton (*Gossypium hirsutum* L.), determining changes in plant N status is a valuable management tool. This study evaluated the Spectrum Technologies CM-1000 chlorophyll meter as compared with the GER model 1500 spectroradiometer for remote estimation of leaf chlorophyll concentration (Chl) under N-deficit conditions. In both instruments, measurements of leaf reflectance (R) at 700 and 840 nm (± 5.0 nm) were used to compute R_{840}/R_{700}, a measure associated with general plant stress. Plants were grown outdoors in large pots using half-strength nutrient solution (control) via a drip-irrigation system until some three-row plots received a restricted supply of N. Treatments comprised control N from emergence to maturity, 20% of control N at first floral bud (square) onward, and 0 and 20% of control N at first flower onward. Following 27 July 2000, treatment rankings for Chl and N were control > 20% N first flower > 0%N first flower ≥ 20% N first square. On most sampling dates, significant linear correlation was obtained between Chl and R_{840}/R_{700} across N treatments, particularly in August (r =0.55–0.93), when stress-induced changes in Chl were

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closely related to N. Treatment means for R_{840}/R_{700} were similar for the CM-1000 and GER 1500; however, SPAD readings were often more closely associated with Chl. This illustrates the difficulty of measuring leaf reflectance under ambient light levels, as compared with measuring transmitted light at two wavelengths in an enclosed chamber. We also determined the effect of a black background (R<<1%) on R_{840}/R_{700} in field-grown plants fertilized with 0 and 112 kg N ha^{-1}. Placing an absorptive background behind the measured leaf decreased R_{840}/R_{700} due to proportionately less reflectance at R_{840}, but did not change treatment rankings for R_{840}/R_{700}. In providing a rapid and reliable assessment of Chl, and hence plant N status, the CM-1000 should be useful for nutrient management in cotton.

Multispectral and hyperspectral radiometry are powerful tools for assessing plant reflectance at various wavelengths of light energy, where the amount of light not reflected is either absorbed by the plant pigments or transmitted downward to the soil surface (Jackson et al., 1980). It is known that leaf reflectance in the visible region of the spectrum (300–700 nm) varies as a function of chlorophyll (*a* + *b*) concentration (Chl). Because the majority of leaf N is contained in protein, N deficiency in cotton causes decreases in plant growth, photosynthesis, and chlorophyll production (Longstreth & Nobel, 1980; Gerik et al., 1989). When Chl begins to decline, the amount of reflected radiance from within the leaf interior increases, providing an optical (and early) indicator of stress at specific wavebands of visible light (Carter, 1993). Increased reflectance in the near infrared (NIR) region of the spectrum (740–900 nm), where light is not absorbed by chlorophyll, is caused by multiple scattering between the water-filled cells and air spaces in the leaf (Jensen, 2000). The actual amount of NIR reflected depends on the anatomical structure of leaves, which is difficult to measure. Knowledge of these leaf optical properties has been used for relative assessment of Chl, and hence N stress in cotton (Tarpley et al., 2000; Read et al., 2002). Similarly, critical N levels established by spectral radiometry were in good agreement with those established by traditional measurements of growth and plant mineral composition in corn (*Zea mays* L.) (Blackmer et al., 1996).

Remote sensing of Chl appears to have potential for field assessment of cotton N status, and hence crop productivity (Gerik et al., 1998; Tenkabail et al., 2000; Barnes et al., 2000; Read et al., 2002). Hand-held radiometers have the advantage of being rapid, nondestructive, and relatively inexpensive, as compared to the collection and analysis of dried, ground plant tissues for N concentration. Moreover, reflectance samples can be taken frequently, and quickly repeated if results are questionable (Jackson et al., 1980). Carter (1993) found several multispectral indices changed in response to altered physiological functions resulting from environmental fluctuations or plant stresses. For instance, the red-edge of leaf reflectance, which is the increase in reflectance between 680 and 740 nm, shifts toward shorter wavelengths in response to N deficiency, leaf senescence, dehydration, flooding, freezing, ozone, herbicides, competition, disease, insects, and deficiencies in ectomycorrhizal development (Carter & Knapp, 2001). The red-edge inflection point is strongly dependent on Chl *a* concentration, and several studies have related either the reflectance red-edge or reflectance indices involving the red-edge, to changes in leaf Chl concentration (Chappelle et al., 1992; Gitelson et. al., 1996) or general plant health (Carter, 1993; Trenholm et al., 2000). In cotton, single-waveband ratios that combined a low reflectance, red-edge measure with a waveband of high reflectance in the NIR region (755–920 and 1000 nm) were sensitive indicators of leaf N deficiency (Tarpley et al., 2000; Read et al., 2002). These studies suggest multispectral sensors tuned to reflectance ratios computed within narrow bandwidths (2–10 nm) offer the greatest potential in remote sensing of changes in leaf Chl, and hence stress physiology, in cotton.

The SPAD-502[1] (Specialty Products Agricultural Division, Minolta Osaka Co., Ltd., Japan) is a hand-held meter that correlates linearly with extractable Chl concentrations for a wide variety of crops, including rice (*Oryza sativa* L.) (Takebe et al., 1990), soybean [*Glycine max* (L.) Merr.] (Ma et al., 1995) and corn (Blackmer & Schepers, 1995); however, plant species and environmental environmental conditions may affect the relationship (Wood et al., 1992). The SPAD meter uses two light-emitting diodes to determine, for a 2 mm × 3 mm leaf section, the amount of light transmitted at 650 nm, which is affected by Chl content, in ratio to the amount of light transmitted at 940 nm, which is not sensitive to Chl and serves as a reference. The measurement chamber must be clamped onto a leaf in order to work. Recently, the National Aeronautics and Space Administration (NASA) patented a hand-held radiometer that uses ambient and reflected light at 700 and 840 nm to estimate Chl content (Carter & Spiering, 1999). Spectrum Technologies (Plainfield, IL) obtained the rights to develop and commercialize this instrument as a problem-solving tool, which is now marketed as the CM-1000 Chlorophyll Meter. Laser beams define an open, conical field of view (FOV) as the trigger is pressed. The meter does not contact the leaf, but is assumed to be equally accurate at distances ranging from 28 to 183 cm (1.10 to 18.8 cm diameter FOV, respectively) from the target. Canopy-level measurements with the CM-1000 are only recommended for fine-textured turf grasses, because the underlying soil contributes little to the reflectance of such canopies under most conditions.

The algorithm used to generate an index for leaf Chl is [(R_{840} sample/R_{840} ambient)/(R_{700} sample/R_{700} ambient)]. The ambient light level is obtained from two upward-looking sensors attached by gimbals to the top of the CM-1000 housing.

Reflectance-based methodologies for assessing plant N status are important to enhancing site-specific (or precision) crop management in cotton. Early detection of nutrient or Chl deficiency might assist in scheduling fertilizer application, thus reducing the amount of chemical used. Rapid delineation of field areas experiencing low N status, along with relevant soil data, also could assist in building and confirming maps for site-specific fertilizer inputs. Map accuracy is a concern for hand-held radiometers, due to error involved in extrapolating single-plant results to larger areas. The SPAD meter user manual recommends taking 30 or more readings from representative plants, including reference areas (where soil N is presumably not limiting), which can be time consuming for large fields with spatial variability in N status. Users of the SPAD meter must further be aware of the limited FOV and the relative effects of leaf veins and surface imperfections. Especially with broad-leaf (dicot) plants, the larger FOV available with the CM-1000 should allow the user to choose the best place on the leaf to sample and what area will be included in the sample. The purpose of this research is to compare the precision of

[1] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

the CM-1000 to the SPAD 502 and to a research-quality spectroradiometer (GER Model 1500) in estimating leaf Chl. We also determined whether precision of R_{840}/R_{700} can be enhanced either by subsampling individual leaves or placing a fully-absorptive black background behind the measured leaf. If our knowledge of leaf optical properties and assumptions regarding sensitivity of the CM-1000 chlorophyll meter are correct, the hand-held radiometer should be a useful tool for N management in cotton.

MATERIALS AND METHODS

Field studies were conducted at the R.R. Foil Plant Science Research Center at Mississippi State University (Lat. 33.416 N; Long. 88.782 W). The cultivar was NuCOTN 33B, a moderately tall, mid- to late-maturing plant, containing a delta endotoxin gene from *Bacillus thuringensis* that confers genetic resistance to Heliothine pests.

Outdoor Pot Experiment

The outdoor pot-culture facility was made up of 8.5-m long wooden racks (or rows) spaced 1 m apart and arranged in east–west direction. Each treatment comprised three rows with pots spaced 15 cm on center, giving 20 plants per row and 60 plants per treatment. This present study was part of a larger study comprising a total of 40 rows. Seed was sown 15 May 2000 in large (15 cm diam. by 65 cm depth), free-draining polyvinyl chloride (PVC) pots filled with a medium-fine sand. Plants were thinned to one per pot on 6 June.

Plants were fed a favorable supply of water and nutrients using half-strength nutrient solution (Hewitt, 1952), containing 85.7 mg N L^{-1}, via one Netafim dripper per pot rated at 1 L of liquid per hour. Plants were irrigated three times per day. If most plants were visibly wilted at midday, the duration of subsequent daily irrigations was increased to maintain soil water above the wilting point. Data from a weather station adjacent to the field site aided in determining plant water requirements. The different N solutions for each treatment were contained in large mixing tanks and pumped under pressure by chemical metering pumps through plastic lines to the three-row treatment plots. All timing and duration of flows to individual plots was under computer-controlled switches and solenoid valves. At various phenological growth stages (listed below), the plants in some three-row plots were watered with an osmotically-balanced nutrient solution (Hewitt, 1952) that contained either 0 or 20% of control N. The four N treatments were: (1) control, a half-strength nutrient solution supplied from plant emergence to maturity, (2) 20% N at first square (a floral bud) stage, a moderate stress imposed from first square stage 31 d after emergence (31 DAE) to maturity, (3) 20% N at first flower stage, a moderate stress imposed from first flower stage (52 DAE) to maturity, and (4) 0% N at first flower, a severe stress imposed by completely withholding N from first flower (52 DAE) to maturity.

Single-leaf reflectance, Chl and N concentration were measured approximately twice weekly between 6 July and 17 Aug. 2000. On each sampling date, five uppermost, fully expanded leaves were randomly selected in each treatment (and also in each plot row) and identified with a string tie wrapped around the petiole; this label was subsequently used to identify the corresponding SPAD and Chl samples (see below). Hyperspectral reflectance was measured from 350 to 950 nm with a resolution of about 1.5 nm using a GER model 1500 spectroradiometer (Geophysical and Environmental Research Corporation, Millbrook, NY). Each of the five leaves was measured using a 4° FOV foreoptic from a height of 0.2 m, in order to detect spectral radiance from approximately 4.2 cm^2 area (2.30 cm long × 1.83 cm wide) of the middle lobe. Immediately prior to each set of measurements, a white reference panel (Spectralon, Labsphere, North Sutton, NH) was used to collect the spectrum of incident radiation flux values. Percentage of reflectance of subsequent target leaves was computed by dividing the reflected radiance by the incident radiation flux and multiplying by 100.

Although not the primary purpose of this study, canopy reflectance also was measured using a 23° FOV foreoptic from a height of 1 m above the central portion of a canopy (40-cm width) following methods of Read et al. (2002). Thus, the reflectance measurement typically involved fully-developed plant crowns arising from three consecutive pots, and virtually no soil background. A fiber optic cable connected the foreoptic to the radiometer and was run along an extensible boom attached to the top of a tripod that also had a platform for mounting the spectroradiometer. Three (1999) or five (2000) separate rows were measured in each treatment by quickly positioning the boom as needed above each row.

Immediately following leaf reflectance measurements, the CM-1000 Chlorophyll Meter (Field Scout CM-1000, Spectrum Technologies, Plainfield, IL) was positioned nadir to a leaf and between the target and sun in order to record incident and reflected light at 700 and 840 nm (± 5 nm). Incident readings for computing R_{840}/R_{700} are obtained from two upward-looking sensors attached by gimbals to the top of the instrument. The 840 nm sensor also records a single brightness scale of 0 to 9 to estimate the amount of light incident on the leaf surface and to provide confidence that a reading was taken under adequate illumination conditions. Measurements in the present study were typically obtained at a brightness level of 5 to 6. Two laser beams define points on the perimeter of the target area when a trigger on the handle is pressed, and reflectance data of the target are recorded when the trigger is released. At a distance of 38 cm, the FOV is about 1.5 cm in diameter, giving an area of 1.77 cm^2. Typically, a single measurement was obtained from the middle lobe of each leaf, but sometimes five measurements (subsamples) were obtained in fairly rapid succession from the same middle lobe. At nearly the same time as CM-1000 measurements, five SPAD readings were recorded from five different lobes of a leaf, avoiding major veins, and the five values averaged for each leaf.

All leaf reflectance measurements were made during midday (1100–1300 h) under incident solar radiation from an angle perpendicular to target of the upper (adaxial) leaf surface according to Read et al. (2002). We minimized certain limitations to measuring reflectance under natural illumination conditions, such as specular reflectance (e.g., glare) and variability in irradiance angle and intensity by (i) positioning the radiometer between the sun and the target of the upper leaf (adaxial) surface, (ii) selecting the best angle with minimal specular reflectance, (iii) avoiding shadows, and (iv) completing measurements in <1 h. In order to avoid shadows falling on the leaf surface, the relative position of each instrument to the target was adjusted by the operator as needed.

Immediately following leaf reflectance measurements, the sampled leaves were excised from the petiole, put into plastic bags, chilled over ice, and transferred to the laboratory for determination of Chl and N. For Chl analysis, a cork borer was used to remove five discs from each leaf, avoiding areas with large veins. The discs were placed into a vial containing 4.0 mL dimethyl sulfoxide (DMSO), and kept at room temperature overnight in the dark. Absorbances of the extract at 648 and 664 nm were recorded and chlorophyll *a* and *b* concentrations were computed following the formulae of Chappelle et al. (1992). Chlorophyll concentration of the extract and the total disk surface area of 1.19 cm^2 were used to compute Chl per unit projected leaf area. For N analysis, the five leaves used for Chl analysis were pooled and dried at 70°C for 72 h. Total leaf N concentration was subsequently determined on dried, ground samples according to standard micro-Kjeldahl methods (Nelson & Sommers, 1972), and expressed as g kg^{-1} dry weight. Because leaves were pooled prior to N analysis, the number of observations for leaf N on each sampling date is equivalent to the number of treatments.

A total of 255 observations (~5 leaves/observation) of reflectance and leaf Chl, and 49 observations of leaf N (one observation/treatment), were obtained from uppermost leaves sampled at approximately biweekly intervals. Complete analysis of variance was conducted for leaf reflectance and Chl values within each sampling date, with replication being represented by five plants selected at random within each N treatment (SAS Institute, 1999). Leaf reflectance and Chl data also were analyzed to determine treatment effects across sampling dates using proc MIXED procedure in SAS. Data are presented and discussed only at the highest significant level, either $P < 0.05$ or $P < 0.01$. To evaluate interrelationships among measured traits, Pearson's correlation coefficient (r) was calculated across N-stress treatments within each sampling date for each pair of variables, namely R_{840}/R_{700} reflectance ratio, SPAD value, and the concentrations of total Chl and N in leaves.

Pearson's correlation coefficient was calculated across all treatments and sampling dates in order to evaluate the sensitivity of R_{840}/R_{700} to leaf Chl. This analysis involved determining associations between selected reflectance ratios (that is R_{700}/λ and R_{840}/λ) measured in narrow wavebands and the concentration of Chl in leaves on a plot mean basis. Because spectral reflectance from the GER 1500 radiometer is sampled in approximately 1.5-nm intervals, values for leaf reflectance were averaged across nonoverlapping, 10-nm intervals and resulting values for R_{700} and R_{840} retained for subsequent analysis and comparison with CM-1000 readings. Values for r^2 were plotted against individual wavebands to indicate the fraction (0.0–1.0) of variability in leaf Chl that could be accounted for by reflectance.

Field Experiment

Because a fully-absorptive (black) background was not placed behind a leaf during the reflectance measurements in Outdoor Pot Experiment (above), we expect R_{840} is artificially enhanced compared with measurements obtained using an integrating sphere. This additive reflectance is due to the fact that healthy green leaves in the vicinity of our field of view may reflect approximately 80% of the incident NIR energy at 900 nm (Jensen, 2000). The main reasons for increased NIR reflectance are (i) the leaf already reflects 40 to 60% of the incident NIR energy from spongy mesophyll due to internal scattering at the cell wall-air interfaces, and (ii) the remaining 40 to 50% of the energy is transmitted directly through the leaf and can be reflected once again by leaves below the measured leaf (i.e., adding to the scene reflectance). Therefore, an experiment was conducted in 2001 to determine the influence of additive reflectance on leaf optical responses to N stress at 840 nm, as well as R_{840}/R_{700} reflectance ratio. This experiment was part of a larger study to determine narrow-waveband spectral reflectance measures sensitive to N stress in field-grown cotton.

Seed was planted on 14 May, 2001 into a Leaper silty-clay loam (fine-loamy, silicious, thermic, vertic, haplaquept). Planting and pest control practices were those typically recommended for cotton in this area. Nitrogen rates of 0 (control), 56, 112, and 168 kg ha^{-1} were split-applied as urea ammonium nitrate solution (UAN, 32% N) on 29 May and on 19 June using 56 kg ha^{-1} increments. This present study measured leaves from plants in only the 0 and 112 kg N ha^{-1} treatments. Experimental design was a randomized complete block with five replications. Each plot was 8 rows wide (0.97 m centers) and 15.25 m long. Five plants selected at random from two central rows in each plot were marked on 26 June, the first sampling date, and routinely sampled on subsequent sampling dates. As in Outdoor Pot Experiment, we routinely selected an uppermost fully-expanded leaf, 4 to 5 nodes from the top of the plant for physiological measurements.

The ratio of single-leaf reflectance at 840 and 700 nm (R_{840}/R_{700}) was determined using a CM-1000 and a GER 1500 radiometer as described above. On some sampling dates, a cardboard placard painted with black fully-absorptive paint (reflectance <<1%) was placed directly behind the leaf to minimize the additive NIR background reflectance in the scene. An absorptive background should produce a more accurate measure of R_{840}/R_{700} in leaves, as R_{840} will indicate the amount of reflectance due to leaf physical structure and R_{700} will indicate the amount of reflectance due to leaf chlorophyll *a* content.

RESULTS AND DISCUSSION

Outdoor Pot Experiment

Restricted N supply decreased Chl in uppermost fully-expanded leaves on each sampling date, as compared with control plants supplied half-strength nutrient solution from emergence to maturity (Fig. 14–1). Analysis of variance across sampling dates indicated significant ($P < 0.01$) difference in Chl between control and N-deficient plants, and between 20% N at first square and 0% N at first flower onward treatments. Averaged across sampling dates, values for Chl were 57.5, 51.3, 48.5, and 44.5 μg cm^{-2} in controls, 20% N at first flower onward, 0% N at first flower onward, and 20% N at first square onward, respectively. Similarly, restriction of N at first square decreased leaf N concentration by about 40%, as compared with controls. Treatment difference in Chl increased as plants matured (Fig. 14–1), and is supported from relatively large correlation obtained in August for the relationships

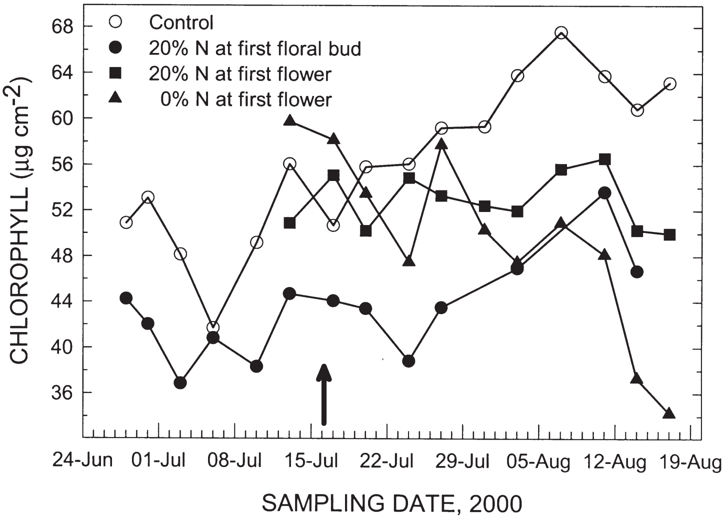

Fig. 14–1. Chlorophyll (*a* + *b*) concentration in uppermost fully-expanded leaves of cotton grown outdoors in large pots under N-limited conditions in 2000. Values ($n = 51$) represent means of five replicate leaves on each sampling date. Leaf reflectance and chlorophyll were obtained from the same leaves. The 20% N at first floral bud (square) onward treatment was imposed on 22 June. The 0 and 20% N at first flower onward treatments were imposed on 12 July (arrow).

between Chl values and either leaf N, SPAD, or R_{700} (Table 14–1). Growth of cotton has been associated with variation in N availability, due in large part to the effect of N on chlorophyll production and thus plant light interception and use (Gerik et al., 1998). Nitrogen stress also has been shown to alter the spectra of reflected radiance (Pan et al., 1997; Barnes et al., 2000; Power et al., 2000).

Hyperspectral reflectance data obtained with GER 1500 radiometer were analyzed to determine if R_{840}/R_{700} is an appropriate reflectance ratio for remote estimation of leaf Chl in cotton. Results indicated the waveband centers used in the CM-1000 appear to be properly tuned for estimation of Chl (Fig. 14–2). The best waveband ratio was R_{840}/R_{720}, which explained about 75% of the variation in Chl across treatments and samplings dates (Fig. 14–2b). The utility of the red-edge measure at 700 nm is evident when R_{700} is expressed in ratio to either strong chlorophyll *a* absorption at 675 nm or weak chlorophyll absorption at 510 nm (near the green reflectance peak) (Fig. 14–2a). Chappelle at al. (1992) similarly found maximum absorbance by chlorophyll *a* at 675 nm was useful in discovering stress reflectance indices in soybean leaves. Other Chl sensitive measures identified in the present study were R_{700}/R_{765} (r^2= 0.66) and R_{840}/R_{550} (r^2= 0.65) (Fig. 14–2a and 14–2b). Reflectance at 550 nm or its ratio with a waveband in the near-infrared was associated with corn (*Zea mays* L.) grain yield, an estimator of N status (Blackmer et al., 1996), and with chlorophyll loss during leaf senescence in horse chestnut (*Aesculus hippocastanum* L.), and Norway maple (*Acer platanoides* L.) (Gitelson et al., 1996).

The reflectance wavelength with maximum sensitivity to Chl has variously been identified at about 690 to 715 nm (Carter & Knapp, 2001; Carter & Spiering,

Table 14–1. Correlation coefficient (r) for the relationship between total chlorophyll ($a + b$) concentration and selected traits measured in uppermost leaves of cotton grown outdoors in large pots under different N-limiting treatments in 2000. The traits measured were leaf N concentration, SPAD reading, CM-1000 index, GER 1500 reflectance ratio (R_{840}/R_{700}), and GER 1500 red-edge measure (R_{700}). Data from the GER 1500 radiometer were parsed to derive 10 nm-wide waveband intervals, comparable to the CM-1000 index.

Sampling date	Treatments[a]	Leaf N[b]	SPAD	CM-1000	R_{840}/R_{700}	R_{700}
6 July	1,2	na[c]	na	0.13	0.15	–0.24
11 July	1,2	na	0.78**	0.80**	0.67*	–0.44
13 July	1,2,3,4	0.70	0.86**	0.67**	0.62**	–0.25
17 July	1,2,3,4	0.67	0.35	0.49*	0.58**	–0.43
20 July	1,2,3,4	0.48	0.79**	0.55*	0.57*	–0.38
24 July	1,2,3,4	0.93	0.75**	0.50*	0.22	–0.35
27 July	1,2,3,4	0.37	0.97**	0.66**	0.76**	–0.80**
31 July	1,3,4	0.97	0.44	0.64**	0.46	–0.42
3 August	1,2,3,4	0.95*	0.84**	0.78**	0.67**	–0.57**
7 August	1,3,4	0.88	0.93**	0.82**	0.61*	–0.72**
11 August	1,2,3,4	0.96*	0.46*	0.55*	0.15	–0.12
14 August	1,2,3,4	0.83	0.94**	0.77**	0.86**	–0.80**
17 August	1,3,4	0.99*	0.68**	0.84**	0.93**	–0.93**

**, * Significant at the 0.01 and 0.05% levels of probability, respectively; otherwise, not significant.

† Correlation analyses typically involved four N treatments ($n = 20$), initiated at various stages of cotton development, namely control at plant emergence onward (1), 20% of control N at first floral bud (square) onward (2), 20% of control N at first flower onward (3), and 0% of control N at first flower onward (4). Omission of Treatment 2 on three sampling dates was due to time constraints from the remaining experimental units in the larger experiment.

‡ Five leaves per treatment were pooled for N analysis. Consequently, the number of observations was equivalent to the number of treatments on each sampling date, and a significant linear correlation required very large r value.

§ na, complete set of data or subsamples not available for statistical analysis.

2002), depending upon species and whether a linear or a quadratic regression function was used. In cotton, a simple reflectance ratio (or Chl index) that involves a chlorophyll-sensitive wavelength and a NIR wavelength unaffected by Chl increased the value of the coefficient of determination (r^2) (Read et al., 2002), as compared with reflectance alone. Similarly, Barnes et al. (2000) used linear regression functions and found a maximally chlorophyll-sensitive waveband ratio of R_{790}/R_{720} in cotton. Using a NIR reference wavelength and different species-dependent polynomial regression functions, Carter and Knapp (2001) found that r^2 values may be higher in the green-orange spectrum from 519 to 572 nm than that obtained using a wavelength at the red edge. Read et al. (2002) found ratios sensitive to N-induced loss of Chl in cotton also may involve a violet-blue (≈420 nm) and a yellow region (≈ 610 nm) of the visible spectrum. Regardless of whether a NIR reference wavelength is incorporated, leaf reflectance wavebands most universally affected by Chl are near 700 nm (Gitelson et al., 1996; Trenholm et al., 2000; Carter & Knapp, 2001; Carter & Spiering, 2002).

Read et al. (2002) measured canopy reflectance in cotton and found single waveband reflectance ratios, R_{360}/R_{710}, R_{415}/R_{710}, R_{415}/R_{695}, R_{415}/R_{585}, and R_{840}/R_{700} were closely associated with changes in Chl. Most of these include a red-edge measure. Interestingly, among the various simple waveband ratios investigated in the present study, analysis of variance across 1999 and 2000 detected a significant ($P < 0.01$)

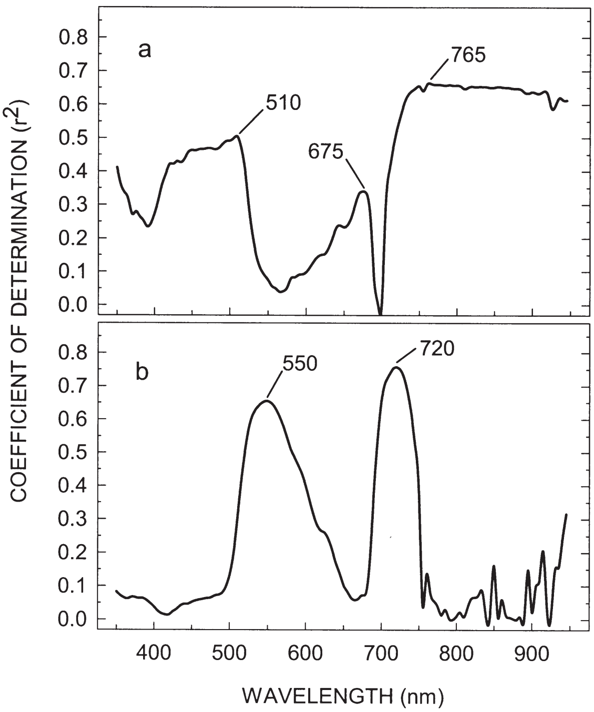

Fig. 14–2. Coefficient of determination (r^2) for the linear relationship between total chlorophyll concentration and leaf reflectance ratio in when either R_{700} (a) or R_{840} (b) is divided by reflectance at the waveband indicated by the x-axis. Correlation analysis involved all plot means of chlorophyll and hyperspectral reflectance data (n = 46), which was resampled to 10-nm wide wavebands prior to statistical analysis. The $r^2 = 0$ values at 700 nm (a) or 840 nm (b) resulted when numerator and denominator λ were identical.

difference in R_{840}/R_{700} between N-deficit treatments; whereas, R_{360}/R_{710} and R_{415}/R_{710} did not differ between N treatments (data not presented). Similar to data for Chl (Fig. 14–1), the difference in R_{840}/R_{700} between N treatments was most apparent between controls and plants supplied with 20% of control N at first square stage onward (Fig. 14–3). These results agree with those of Trenholm et al. (2000) who found strong and consistent correlation between N application and R_{813}/R_{716} for turfgrass canopies. Such results illustrate the sensitivity of a NIR/red-edge reflectance index to changes in Chl, and hence plant N status.

Cotton leaves are usually considered N-deficient if they contain <25 g N kg^{-1} dry weight (Gerik et al., 1998). Despite N values ranging from 21 to 52 g kg^{-1} across all treatments and sampling dates, the association between leaf N and Chl was relatively weak ($r^2 = 0.48$, df = 48) (Fig. 14–4a). Uncoupling of this relationship was most evident for plants with N levels considered excessive for cotton (>45 g kg^{-1}), due perhaps to excess N being stored in vacuoles or incorporated into enzymes of photosynthesis (Gerik et al., 1998). Nevertheless, N stress resulted in treatment ranking for Chl and N concentrations following the 27 July sampling date of control >20% N at first flower onward >0% N at first flower onward ≥20% N at first square

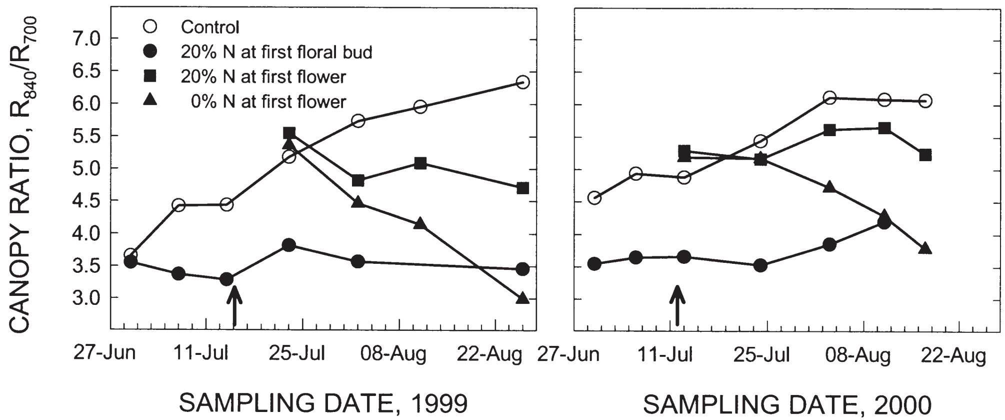

Fig. 14–3. Single waveband canopy reflectance ratio, R_{840}/R_{700}, in cotton grown outdoors in large pots under N-limited conditions in 1999 (a, n = 21) and in 2000 (b, n = 23). Data are presented from sampling dates that coincide closely between the two years. Values represent means of three replicate canopies in 1999 and five replicate canopies in 2000. The 0 and 20% N at first flower onward treatments were imposed on 15 July 1999 and 12 July 2000 (arrows).

onward. A quadratic function best described the relationship between CM-1000 values for R_{840}/R_{700} and Chl across all N-stress treatments and sampling dates ($r^2 = 0.61$, df = 44) (Fig. 14–4b). By comparison, only 46% of the treatment and seasonal variation in leaf N was explained by CM-1000 index, R_{840}/R_{700} (data not presented), which is probably due to the weak association between leaf N and Chl in the present study. The best-fit polynomial equation in Fig. 14–4 describes both the seasonal and environmental influences of N stress on R_{840}/R_{700} in individual leaves; how-

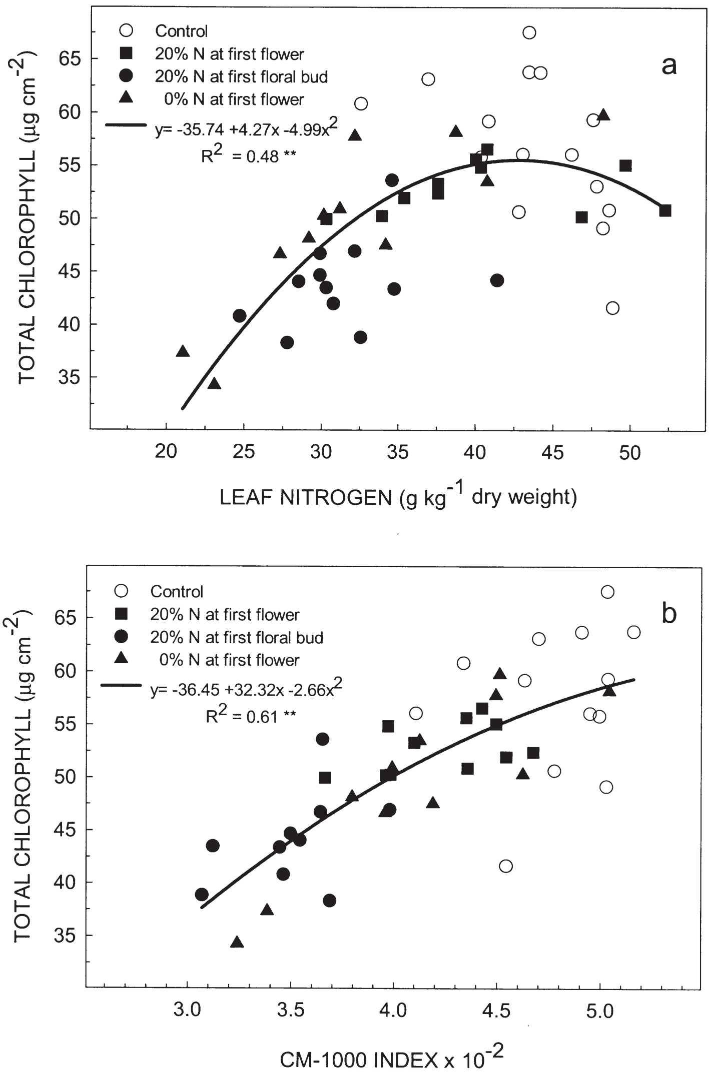

Fig. 14–4. Relationship between chlorophyll ($a + b$) concentration and either tissue N concentration (a) or CM-1000 index of R_{840}/R_{700} (b) measured in uppermost leaves of cotton grown under N-limiting conditions in 2000. Values represent means of five replicate leaves on each sampling date ($n = 51$, see also Fig 14–1. for chlorophyll data).

ever, use of the CM-1000 to fine tune N management of cotton to field conditions will require accurate assessments of N status at critical growth stages.

On most sampling dates, significant linear correlation was obtained between Chl and leaf reflectance indices across the different N treatments (Table 14–1). Values for R_{840}/R_{700} from both radiometers and R_{700} from the GER 1500 were particularly sensitive to Chl in August, when stress-induced changes in Chl were significantly related to leaf N. Because the R_{840}/R_{700} reflectance measure from either a CM-1000 or a GER 1500 radiometer, was positively associated with N-induced changes in leaf Chl concentration (r = 0.49–0.93), the CM-1000 appears to have potential in comparative studies of leaf optical responses to stress that are indicative of general plant health in cotton. Correlation analysis assumes reflectance indices measured in individual leaves are linearly related to changes in Chl, which is not strictly true (Monje & Bugbee, 1992; Carter & Spiering, 2002). Interpretation of results in Table 14–1 both within and across sampling dates indicates a strong dependency (or autocorrelation) between the measured traits. That is, strong (or weak) correlation between two variables in one sampling date was associated with similarly strong correlation between other sets of measured variables. This may be due to timing of the N fertilizer treatments or to difference in irradiance between sampling dates. Because N is a highly mobile mineral nutrient and cotton is known to use reserve N to satisfy plant requirements, cotton N status changes gradually when soil-N supplies are limited (Gerik et al., 1998). Cloud cover also may decrease N uptake, but probably has no short-term effects on leaf N and total Chl concentrations. Because the correlation between reflectance and leaf N status was not consistent from date to date (Table 14–1), it may be difficult to pinpoint the optimum time for plant sampling using the CM-1000. Moreover, unlike SPAD measurements or obtaining plant tissue samples for N analysis, users of the CM-1000 must be careful to avoid variation in irradiance incident to the leaf (e.g., shading) during a measurement; otherwise, values for R_{840}/R_{700} should be about the same regardless of the total ambient irradiance (Jackson et al., 1980; Carter & Knapp, 2001).

We found treatment means for R_{840}/R_{700} agreed closely between the CM-1000 and GER 1500 radiometers when plants were grown under N stress in large pots (Table 14–2); however, this correspondence was not evident in field-grown plants (Fig. 14–5, discussed below). Nevertheless, the consistent and strong correlation between SPAD, Chl, and R_{840}/R_{700} observed in the present study (Tables 14–1 and 14–2), support the sensitivity of a NIR/red-edge reflectance ratio for detecting unfavorable growth conditions in cotton (Barnes et al., 2000; Tarpley et al., 2000; Whaley, 2001). While the two radiometers compared favorably, SPAD readings were often most sensitive to loss of Chl. These results illustrate the difficulty of using leaf optical properties to detect N stress pre-visually in cotton. The SPAD meter transmits 650 and 940 nm light through the leaf tissue to estimate Chl content. A similar optical property is featured in R_{840}/R_{700}, as changes in R_{700} are due to strong Chl *a* absorption at 660 to 680 nm; whereas R_{840} is not affected by Chl and would only change if leaf anatomy or water content changed in response to stress (Carter & Knapp, 2001; Whaley, 2001). The use of simple reflectance ratios such as R_{840}/R_{700} may help correct for variations in irradiance, leaf orientation, irradiance angles and shading (Carter, 1993), that are commonly encountered under field conditions.

Table 14–2. Leaf optical properties or indices based on measurements with a SPAD meter, a CM-1000 Chlorophyll Meter, and a GER 1500 radiometer on selected sampling dates. Measurements were obtained from uppermost leaves of five randomly selected cotton plants grown outdoors in large pots under different N treatments in 2000. The SPAD index involved the average of five readings per leaf and the different reflectance measures involved a single measurement from the central lobe of each leaf.

Sampling date/ treatments	SPAD Index	GER 1500 CM-1000 Index × 10^{-2}	GER 1500 Reflectance Ratio R_{840}/R_{700}	Red-edge R_{700}
6 July				
Control	nd†	4.54a*	4.98a	12.73b
20% N flowering	nd	nd	nd	nd
0% N flowering	nd	nd	nd	nd
20% N squaring	nd	3.46b	3.40b	18.27a
13 July				
Control	45.66ab	4.95a	4.63b	18.25a
20% N flowering	44.90b	4.36b	4.55bc	15.46ab
0% N flowering	47.48a	4.51ab	5.56a	12.58b
20% N squaring	38.32c	3.49c	3.87c	16.91a
20 July				
Control	48.68a	4.99a	5.42a	13.22a
20% N flowering	45.76a	3.96bc	5.14a	13.45a
0% N flowering	47.44a	4.13b	4.89ab	13.93a
20% N squaring	39.32b	3.44c	4.38b	15.57a
27 July				
Control	49.76ab	4.63a	5.20a	13.59bc
20% N flowering	46.62b	4.10a	4.43a	15.33b
0% N flowering	52.68a	4.49a	5.36a	11.36c
20% N squaring	40.14c	3.12b	3.21b	19.43a
11 August				
Control	52.74a	4.91a	4.96ab	11.22b
20% N flowering	48.38b	4.43a	5.81a	11.37b
0% N flowering	43.06c	3.79b	4.16b	16.00a
20% N squaring	45.78bc	3.65b	4.53b	14.34ab

* Means within a column and sampling date followed by a different letter are significantly different according to Fishers protected LSD test at the 0.05% level of probability.
† nd, no data.

Based on *r* values across different N treatments, multiple observations (n = 5) from individual leaves did not consistently enhance the precision of CM-1000 readings to estimate total Chl and other leaf traits (compare Tables 14–1 and 14–3). With respect to improving the association between Chl and CM-1000 reflectance ratio by subsampling leaf reflectance, a numerically larger *r* value was obtained in only three of the five comparisons; on 3, 14, and 17 August. Similar to results for one CM-1000 observation per leaf (Table 14–1), *r* values between selected leaf traits increased as the plants matured (Table 14–3).

Field Experiment

Single-species comparisons using a Chl index that incorporates a NIR wavelength may need to account for environmental differences among sampled plants (Jensen et al., 2000). For instance, leaves of a given species are thicker in certain

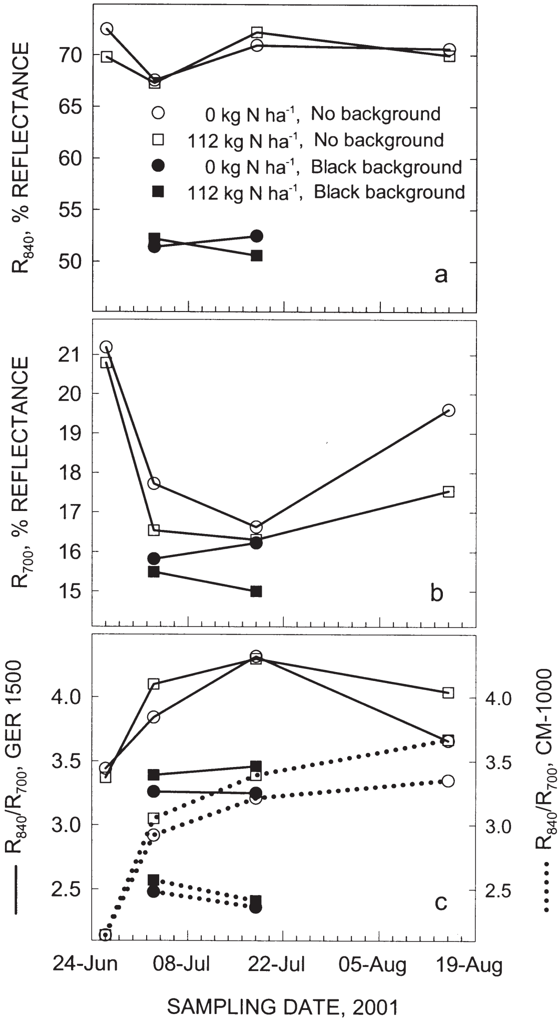

Fig. 14–5. Effects of a black absorptive background on narrow-waveband (±5 nm) leaf reflectance values of R_{840} (a), R_{700} (b), and R_{840}/R_{700} (c) measured using a GER model 1500 spectroradiometer and CM-1000 index of R_{840}/R_{700}(c). Values represent means of five plants in each of five blocks with different rates of applied N fertilizer (n = 25). Difference between N treatments was significant at $P <$ 0.05 for R_{840}/R_{700} measures obtained on 3 July using a GER 1500 and a black absorptive background; otherwise, N fertility did not affect leaf reflectance measures.

environments (e.g., dryer habitats with higher irradiance), and leaf thickness is known to affect NIR reflectance (Pearcy & Sims, 1994; Jensen, 2000). For example, Knapp and Carter (1998) found reflectance at 750 nm increased as leaf thickness increased in 26 species of plants (r^2 = 0.67). If a Chl index based on leaf reflectance is calculated using a linear mathematical function (e.g., reflected NIR/reflected red edge), a greater percentage of NIR light being reflected from the sam-

Table 14–3. Correlation coefficients (r) for the relationship between the average of five CM-1000 readings from each measured leaf and selected traits measured in uppermost fully-expanded leaves of cotton grown under different N-limiting treatments in 2000. The traits measured were total chlorophyll ($a + b$) concentration (Chl), leaf N concentration, SPAD reading, one CM-1000 reading from each leaf, and R_{840}/R_{700} with 10 nm-wide interval derived from a GER 1500 radiometer.

Sampling date	Treatments†	Total chlorophyll	Leaf N	SPAD	CM-1000	R_{840}/R_{700}
17 July	1,2,3,4	0.44	0.76	0.77**	0.67**	0.50*
3 August	1,2,3,4	0.82**	0.92	0.77**	0.85**	0.80**
7 August	1,3,4	0.70**	0.99**	0.61*	0.78**	0.62**
14 August	1,2,3,4	0.93**	0.73	0.91**	0.84**	0.84**
17 August	1,3,4	0.89**	0.98	0.63*	0.94**	0.95**

**, * Significant at the 0.01% and 0.05% levels of probability, respectively; otherwise, not significant.

† Correlation analyses typically involved four N treatments, initiated at various stages of cotton development, namely control at plant emergence onward (1), 20% of control N at first floral bud (square) onward (2), 20% of control N at first flower onward (3), and 0% of control N at first flower onward (4). Omission of Treatment 2 on three sampling dates was due to time constraints from the remaining experimental units in the larger experiment.

ple affects the index value as would a smaller percentage of red-edge light being reflected by the sample. In this example, both result in a higher Chl index. Therefore, accurate estimates of Chl may require a black background behind the measured leaf to minimize contamination from background NIR light.

Values for R_{840}/R_{700} obtained using a GER 1500 and a black absorptive panel on 3 July were significantly less in plants fertilized with 0 kg N than 112 kg N ha^{-1} (Fig. 14–5c); otherwise leaf reflectance measures did not differ significantly ($P >$ 0.05) between N fertility treatments; however, low N fertility led to numerically higher values for R_{700} throughout the study period. This red-edge feature is commonly associated with leaf Chl concentration, and is consistent with evidence from 3 July that Chl was somewhat less in N-stressed leaves than controls (58.1 vs. 61.5 μg cm^{-2}).

A black absorptive background placed behind the leaf decreased R_{840} from about 70 to 50% (Fig. 14–5), due to minimizing additive reflectance from NIR light in the scene. Because a black background produced proportionately larger decrease in R_{840} than R_{700}, values for R_{840}/R_{700} decreased in both radiometers. The GER 1500 or CM-1000 reflectance indices responded similarly to treatment difference due to N restriction, and to the apparent dampening of the R_{840} measure in leaves on 3 and 18 July. Consequently, greater accuracy in estimating Chl might be achieved by using a black absorptive material behind the leaf, but this step should not be required for relative assessment (or ranking) of plant N status. Certainly, any protocol should be supplemented with soil or tissue samples that verify the location and magnitude of the suspected nutrient deficiency.

CONCLUSION

Single-leaf reflectance measurements were used to follow changes in Chl resulting from different N stress treatments. The Field Scout CM-1000 compared favorably with two industry standards, the Minolta SPAD 502 and the GER 1500 spectroradiometer (Tables 14–2 and 14–3). Strong linear associations were obtained between Chl, SPAD values, and reflectance ratios measured using either the CM-1000 or GER 1500 spectroradiometer; however, SPAD was often a better indicator of changes in Chl than the narrow band reflectance ratio, R_{840}/R_{700} (Table 14–1). This is probably due to greater control over measurement conditions with SPAD, which encloses the area of leaf sampled in a darkened chamber, as compared to remote sampling by use of laser sighting in the two spectroradiometers tested. Nevertheless, analysis of hyperspectral reflectance data indicated R_{840}/R_{700} is an appropriate simple reflectance ratio for remote estimation of leaf Chl in field-grown cotton (Fig. 14–2).

Subsampling to minimize within-leaf variability in CM-1000 index did not enhance instrument sensitivity to Chl (Table 14–3). But the rapidity and apparent reliability of the reflectance measurements should certainly allow some form of plant or leaf subsampling in the field without compromising labor and time. Although R_{840}/R_{700} may not accurately estimate leaf N in cotton, monitoring plant nutrient status should be less costly than periodic estimates based on tissue analysis. For instance, evaluation of leaves or petioles for total N concentration using Kjedahl digestion is precise and accurate, but may be too expensive or time consuming for site-specific management. With further testing and refinements, the CM-1000 should be a very useful and practical tool for nutrient management. As with any chlorophyll meter, the CM-1000 is a tool to complement, not replace, other aspects of sound crop management.

Managed N applications are necessary to maximize cotton yields while protecting ground water from contamination (Pan et al., 1997; Boquet & Breitenbeck, 2000; Power et al., 2000). Besides direct soil testing, plant response is often used to assess site-specific N availability in cotton production (Gerik et al., 1998). Aside from decreased biomass, which can be affected by many environmental stresses, leaf Chl content is the plant response to limiting N availability with the strongest applicability to remote sensing methods. As Chl content decreases, the absorption band between the green and far-red part of the spectrum becomes more narrow, causing a sharp increase in reflectance in the red-edge region (Carter & Knapp, 2001). Measuring this change in red-edge reflectance, normalized to the near-infrared reflectance, becomes a good indicator of the Chl content in full canopies of cotton (Fig. 14–3; see also Barnes et al., 2000; Whaley, 2001; Read et al., 2002); however, application decisions must often be made early in the season, prior to full canopy closure (Gerik et al., 1998). The CM-1000 used on individual leaves appears to be a reliable means of discerning the canopy signal from the confounding (or contaminating) soil signal.

Specific information regarding the Field Scout CM-1000 Chlorophyll Meter can be obtained from the manufacturer, Spectrum Technologies, 23839 W. Andrew Rd., Plainfield, IL 60544 (Fax 815/436-4460, email specmeters@aol.com).

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