Liight Reflectance Compared with Other Nitrogen Stress Measurements in Corn Leaves

Tracy M. Blackmer
University of Nebraska-Lincoln

James S. Schepers
University of Nebraska-Lincoln, james.schepers@gmail.com

Gary E. Varvel
University of Nebraska-Lincoln, gevarvel@windstream.net

Light Reflectance Compared with Other Nitrogen Stress Measurements in Corn Leaves

Tracy M. Blackmer,* James S. Schepers, and Gary E. Varvel

ABSTRACT

New tools that can rapidly quantify the N status of corn could be valuable in N fertilizer management practices. This study was conducted to compare light reflectance from corn leaves with other parameters used to detect N deficiencies. Light reflectance (400–700 nm) as measured from corn leaves in the laboratory with a Hunter tristimulus colorimeter was compared with Minolta SPAD 502 chlorophyll meter readings (light transmittance at 650 and 940 nm), leaf N concentrations, and specific leaf N (N content per unit area). Measurements were made on individual ear leaves collected from an irrigated corn N response trial with four hybrids and five N treatments. Light reflectance near 550 nm was the best wavelength to separate N treatment differences. Reflectance at 550 nm provided a stronger relationship with both leaf N concentration and chlorophyll meter readings than between chlorophyll meter readings and leaf N concentration. The measurement of light reflectance near 550 nm has promise as a technique to detect N deficiencies in corn leaves.

INCREASING GROUNDWATER NITRATE CONCENTRATIONS have led to the need for improved N fertilizer management, especially in corn production areas. Several methods have been used over the years to improve N management in corn. These include various soil and plant analytical procedures designed to determine the amount of N available in soil and/or the concentration of N in corn tissue. These techniques all tend to be time consuming and have had varying degrees of success.

More recently, procedures have been developed to determine leaf N status, using a tool that monitors leaf chlorophyll content. This tool, the Minolta SPAD 502 chlorophyll meter,[1] works by measuring light transmittance through a leaf at ≈650 nm, which lies between the two primary wavelengths (i.e., 645 and 663 nm) associated with chlorophyll activity. Chlorophyll is responsible for leaf greenness, which has important N management implications, because chlorophyll content has been shown to be highly correlated with leaf N concentration, especially when N is deficient (Wolfe et al., 1988; Lohry, 1989; Schepers et al., 1992a). The meter provides immediate results without any time-consuming laboratory analysis. This method requires only that the user walk through the field and clamp the meter on a number of representative leaves (Peterson et al., 1993). Previous research has documented that chlorophyll meters can provide an accurate way of identifying N deficiencies when using an internal field reference (i.e., an adequately fertilized reference area) (Schepers et al., 1992a,b; Blackmer and Schepers, 1995).

[1] Mention of trade name or proprietary products does not indicate endorsement of USDA and does not imply its approval to the exclusion of other products that may also be suitable.

T.M. Blackmer, Dep. of Agronomy, Univ. of Nebraska; and J.S. Schepers and G.E. Varvel, USDA-Agricultural Research Service and Dep. of Agronomy, Univ. of Nebraska, Lincoln, NE 68583. Joint contribution of USDA-ARS and the Nebraska Agric. Res. Div., Journal Series no. 10593. Received 8 Oct. 1994. *Corresponding author.

Published in Agron. J. 86:934–938 (1994).

Labor and time might be saved if a system could use reflected instead of transmitted light, because light reflectance measurements could be made without attaching a meter or probe to a specific leaf. In addition, light reflectance measurements could substantially increase the number of plants being monitored and therefore potentially reduce variability. Also, by remotely sensing reflected light (such as from a center-pivot irrigation system or a high-clearance vehicle), the fertilization process could be automated, permitting differential fertilization rates for different parts of the field. The green color reflected from plants and seen by the human eye has a peak wavelength of ≈550 nm. This greenness is generally recognized as an indication of N status for many agronomic crops.

Thomas and Oerther (1972) showed that leaf N content could be quickly estimated by measuring leaf reflectance at 550 nm. This wavelength absorbs less energy than the red and blue region, where chlorophyll absorbs light (i.e., ≈450 and 650 nm). Decreases in chlorophyll content resulting from nutrient stresses in corn have been detected by comparing leaf reflectance at different wavelengths (Al-Abbas et al., 1974). They showed the relative reflectance at 640 and 530 nm could be used to identify some nutrient deficiencies. Later, Thomas and Gausman (1977) showed that the 550-nm wavelength is superior to the 450- and 750-nm wavelengths in relating reflectance to chlorophyll and carotenoid concentrations for eight different crops. More recently, Maas and Dunlap (1989) used normal, albino, and etiolated corn selections to identify regions of the light spectrum (380–2500 nm) that are sensitive to different pigmentations. They also found that the greatest differences in reflectance are in the 380- to 750-nm range. Their albino plants reflected more light than the normal selection, which was attributed to greater absorption by pigments of normal plants. Other research with soybean showed that leaves from plants grown at three N rates had the largest treatment separation near the 550-nm part of the spectrum (Chappelle et al., 1992).

Both light absorption and reflectance are affected by leaf thickness (Woolley, 1971). Therefore, anything that affects leaf thickness (such as water stress or nutrient deficiencies) could influence chlorophyll meter readings. Given that fertilizer N management practices may affect leaf thickness, if the impact of N fertilizer on crop growth could be expressed in terms of reflectance (i.e., greenness), then the effect of leaf thickness on reflectance would be integrated into the measurement.

Our objective was to compare a method measuring light reflectance in the visible part of the spectrum with several other techniques currently used to test for N deficiencies in corn.

MATERIALS AND METHODS

Corn leaves used in this study were collected from continuous corn research plots at the Nebraska Management Systems Evaluation Area (MSEA) project near Shelton, NE (specifically, from existing sprinkler irrigated plots established in 1991). Corn was planted in 8-row (91-cm spacing) by 15.2-m long plots and fertilized at planting with NH_4NO_3 at rates of 0, 40, 80, 120, and 160 kg N ha^{-1}. Four commercially available Pioneer brand hybrids were planted at ≈65 000 plants ha^{-1} in four replications.

In early August of 1992, N deficiencies were observed visually and detected with a SPAD 502 chlorophyll meter. At this time, the ear leaf was removed from 10 representative plants from each plot, packed in a dark cooler, and transported to the laboratory for reflectance measurements.

Reflectance measurements were made using a Hunter tristimulus colorimeter (Hunter Lab Model Labscan 6000, Hunter Associates Laboratory, Reston, VA), which measures light reflectance from 400 to 700 nm in 10-nm band widths. Illuminant A with a 2° standard observer was used as a light source. A port size with a 6-mm diameter was selected for reflectance determinations. Three measurements per leaf were taken, midlength and midway between the midrib and leaf edge for each leaf.

Immediately after reflectance measurements were completed, three chlorophyll meter readings per leaf (sensor size of 2 by 3 mm) were collected from approximately the same area on each leaf. In addition, a leaf punch (Precision Machine Co., Lincoln, NE) was used to collect six disks (1.0 cm diam.) from the same area of each leaf for total N analysis and specific leaf weight determination. Leaf disks were placed into paper coin envelopes, oven dried, weighed, and ground for 3 min using a Spex model 8000 mixer-mill (Spex Industries, Edison, NJ). Leaf N concentration was determined using a Carlo Erba model NA 1500 CHN analyzer (Schepers et al., 1989). Specific leaf weights were calculated from the above weight–surface area data. Grain yields were determined with a plot combine by harvesting three of the center rows for the entire length of each plot. Yield data were adjusted to 155 g kg^{-1} moisture.

RESULTS AND DISCUSSION

Nitrogen fertilizer application rates provided a wide range of plant nutrient conditions and significantly influenced yields for each of the four hybrids. Fertilizer applied at the 120 kg N ha^{-1} was adequate to attain maximum yields for all hybrids except Pioneer 3379, which required at least 160 kg N ha^{-1} (Fig. 1). The response of all hybrids to N fertilizer was also evaluated with chlorophyll meter readings, leaf N content, and light reflectance for the leaves. Because of differences in grain yield for each hybrid, relative grain yields for each hybrid were calculated as a ratio of the grain yield for the highest N treatment.

Reflectance Measurements

Analysis of the light spectra from the leaves for each hybrid revealed greater reflectance and more distinct separation by N rate near the 550-nm wavelength (Figures 2a–d). In this region of the spectrum, the greatest reflectance consistently occurred with the lowest N rate. This is because N deficiencies traditionally result in decreased amounts of leaf chlorophyll, which absorbs less light and therefore results in greater reflectance.

Because chlorophyll absorbs light at ≈650 nm, one might assume that this is the appropriate wavelength to monitor chlorophyll content; however, measurements of reflectance at wavelengths showing relatively little absorption (i.e., ≈550 nm) provided the most sensitive assessment of N status (Fig. 2). Zscheile and Comar (1941) also observed that chlorophyll absorbance of light was much weaker in the 550-nm region than at either 450 or 650 nm.

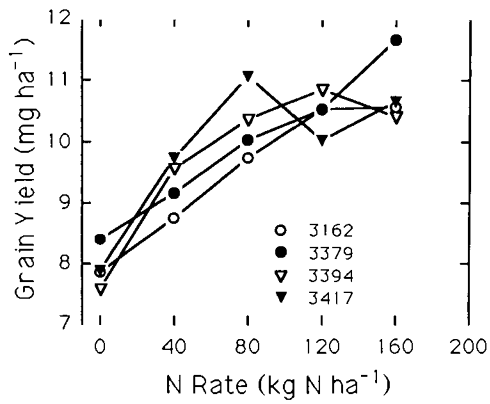

Fig. 1. Grain yield of four corn hybrids (Pioneer 3162, 3379, 3394, and 3417) at five N rates.

Nitrogen deficiencies showed little or no effect on reflectance from any of the hybrids at 450 and 650 nm (Fig. 2), which suggests that either the light at these wavelengths was equally absorbed, even though N was deficient in some cases, or that some of the light was transmitted through the leaf. The apparent inverse relationship between light absorbance and reflectance is consistent with conclusions of Thomas and Gausman (1977), who reported that the 550-nm wavelength seemed to be superior to the 450- or 670-nm wavelengths for relating leaf reflectance to either total chlorophyll or carotenoid concentrations.

Chlorophyll Meters

The positive correlation between chlorophyll meter readings for selected leaves and relative grain yield confirms that the fertilizer rates resulted in a satisfactory range of leaf N and that the 10 leaves collected from each plot provided a representative sample (Fig. 3a). Better relationships with yield would be expected had the chlorophyll meter readings been taken from 30 leaves as recommended (Peterson et al., 1993), instead of collecting 30 readings from only 10 leaves as dictated by the procedures used (i.e., time constraint) to collect the reflectance data.

Leaf Nitrogen

Leaf N concentration is commonly used to quantify crop N status, which was positively correlated with relative grain yield in this study (Fig. 3b). Converting leaf N concentration on a weight basis to an area basis (i.e., specific leaf weight) did not improve the relationship with grain yield (Fig. 3c).

Specific Leaf Weight

Specific leaf weight is defined as the mass of tissue per unit surface area on one side of the leaf. Although specific leaf weight is not a direct measurement of thickness, it can be used to estimate leaf thickness if one assumes a universal leaf tissue density across treatments. Specific leaf weight was not affected by hybrid, but increased from 44.1 to 45.7 g m^{-2} as fertilizer N rate increased from 0 to 160 kg ha^{-1}. This implies that corn leaf thickness increased ≈4% as crop N status went from deficient to excessive. By comparison, leaf N concentration, reflectance

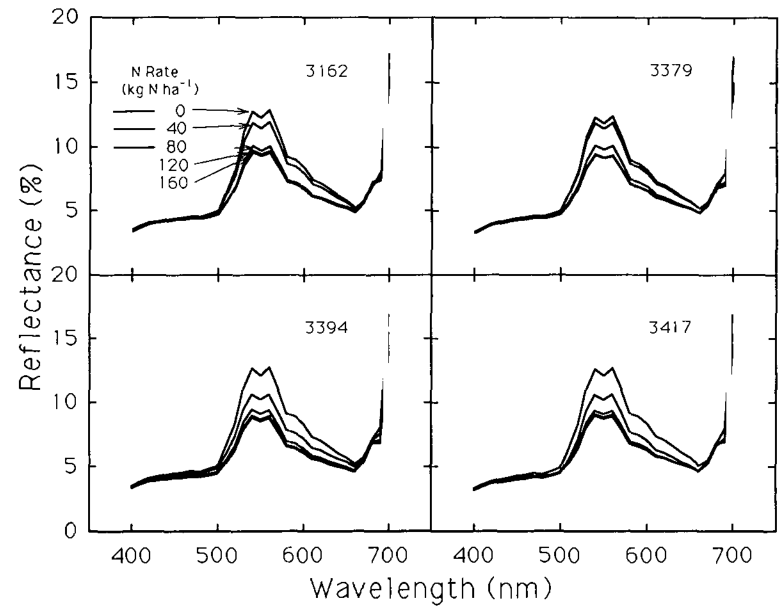

Fig. 2. Light reflectance from corn leaves of four hybrids (Pioneer 3162, 3379, 3394, and 3417) for five N rates.

at 550 nm, chlorophyll meter readings, and grain yield changed at least 20% across these N rates. Therefore, factors other than specific leaf weight or leaf thickness must be contributing to apparent metabolic activity and yield.

Correlations of Reflectance, Chlorophyll Meter Readings and Leaf Nitrogen

Leaf N, specific leaf N and chlorophyll meter readings are three of the more conventional methods of detecting and expressing N stresses. A correlation of chlorophyll meter readings to leaf N and specific leaf N provides an indication of how well the techniques relate to each other. The correlation between chlorophyll meter readings and specific leaf N was slightly better than with leaf N concentration (Fig. 4) This may be a result of changes in specific leaf weight (probably leaf thickness) affecting the transmittance measurements made by the chlorophyll meter. If this is the case, reflectance measurements may have an advantage over chlorophyll meters, because reflectance

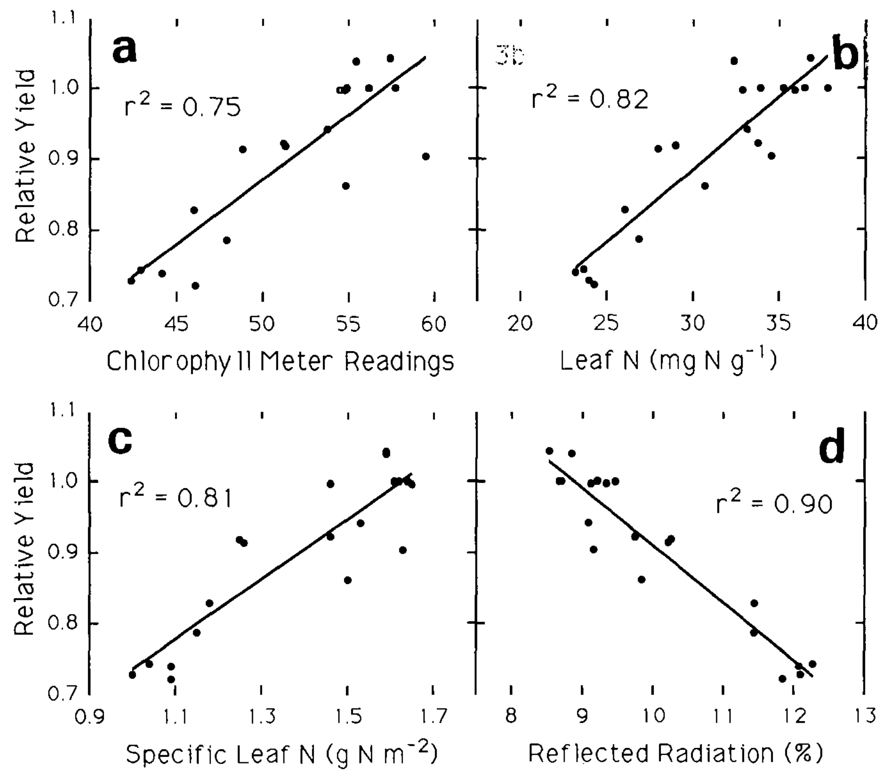

Fig. 3. Relationships between relative grain yield and chlorophyll meter readings at (a) the R3 growth stage, (b) leaf N concentration, (c) specific leaf N concentration, and (d) light reflectance at 550 nm across four corn hybrids and five N rates.

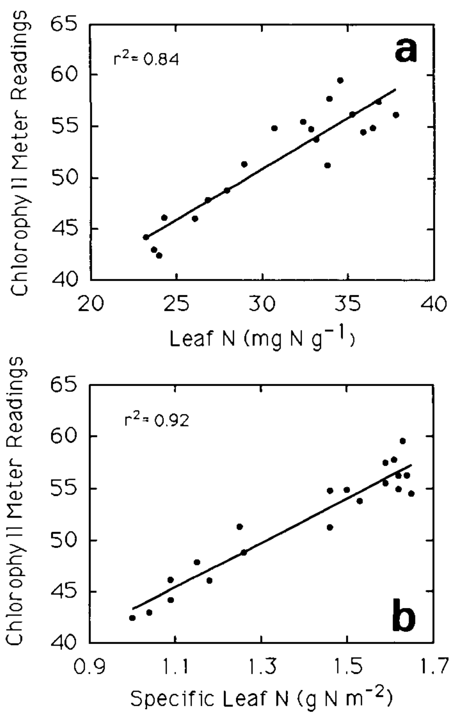

Fig. 4. Relationship between (a) leaf N content or (b) specific leaf N and chlorophyll meter readings across four corn hybrids grown over five N rates.

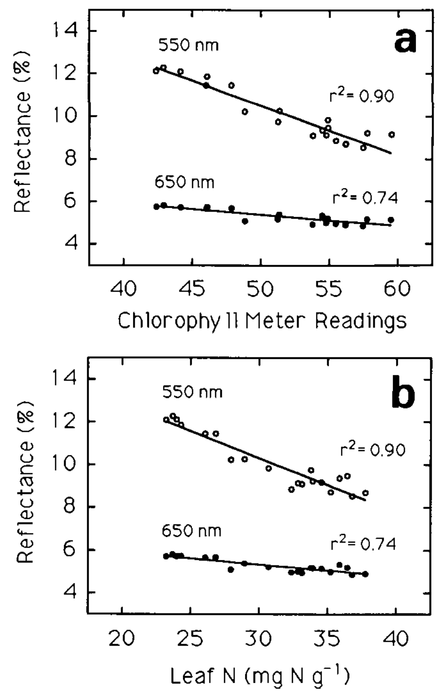

Fig. 5. Light reflectance at 550 nm and 650 nm vs. (a) chlorophyll meter readings and (b) leaf N concentration from corn leaves of four hybrids grown over five N rates.

measurements may not be as sensitive to specific leaf weight.

Correlations between chlorophyll meter readings and light reflectance data were made across all four hybrids and five N rates at 550 nm and 650 nm, because the hybrid effect was not significant. The inverse relationship between transmittance and reflectance should be expected, because chlorophyll increases light absorption, which decreases reflectance and transmittance (Fig. 5a). At 550 nm, chlorophyll absorbs less light, which increases reflectance. Similar relationships are expected between leaf N and light reflectance, because of the strong correlation between leaf N concentration and chlorophyll meter readings (Fig. 5b). Higher levels of light reflection translate into lower relative grain yields (Fig. 3d).

Both leaf N and chlorophyll meter readings had similar coefficients of determination with relative grain yield (Fig. 3a and 3b), suggesting that either type of measurement provided a similar indication of crop N status. The somewhat lower coefficient of determination for the relationship between relative grain yield and leaf N concentration than with reflectance is attributed to luxury consumption of N at the highest fertilizer rates (Fig. 3b vs. 3d). This conclusion is supported by research showing that chlorophyll meters are basically insensitive to luxury consumption (Schepers et al., 1992b).

Futuristic Applications

Efforts to address spatial variability of N availability in soil could conceivably involve a sensor to measure light reflectance that is mounted on a center-pivot irrigation system or a high-clearance vehicle. Such a system would have the advantage of automatically varying fertilizer rate across the field as plant N stress occurs. Although this work compared chlorophyll meter readings and light reflectance only from individual leaves, the use of reflected light from a crop canopy to evaluate crop N status might also prove to be an effective N management tool. Canopy reflectance measurements would have the advantage of integrating a large sampling area and would involve less labor than the use of chlorophyll meters. Additional research will be required to develop techniques to interpret canopy data collected by a moving sensor. Such research could lead to wavelength-specific sensors that are inexpensive and practical for use by consultants and producers.

CONCLUSIONS

The relatively strong correlations between light reflectance readings and relative grain yield, chlorophyll meter readings, and leaf N indicate that the reflectance procedure has promise for assessing crop N status. Light reflectance measurements near 550 nm provided the best wavelength to separate N treatment differences. Reflectance at 550 nm provided a relationship with both leaf N concentration and chlorophyll meter readings that was stronger than the relationship between chlorophyll meter readings and leaf N concentration. Both leaf N and chlorophyll meter readings were similarly correlated with relative grain yield, suggesting that either measurement is a good indicator of crop N status. Specific leaf N provided inferior indication of relative grain yield compared with leaf N concentration.

REFERENCES

Al-Abbas, A.H., R. Barr, J.D. Hall, F.L. Crane, and M.F. Baumagardner. 1974. Spectra of normal and nutrient-deficient maize leaves. Agron. J. 66:16-20.

Blackmer, T.M., and J.S. Schepers. 1995. Use of a chlorophyll meter to monitor N status and schedule fertigation of corn. J. Prod. Agric. 8 (in press).

Chappelle, E.W., M.S. Kim, and J.E. McMurtrey. 1992. Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentrations of chlorophyll a, chlorophyll b, and carotenoids in soybean leaves. Remote Sens. Environ. 39:239-247.

Lohry, R.D. 1989. Effect on N fertilizer rate and nitrapyrin on leaf chlorophyll, leaf N concentration, and yield of three irrigated maize hybrids in Nebraska. Ph.D. diss. Univ. of Nebraska, Lincoln.

Maas, S.J., and J.R. Dunlap. 1989. Reflectance, transmittance, and absorptance of light by normal, etiolated, and albino corn leaves. Agron. J. 81:105-110.

Peterson, T.A., T.M. Blackmer, D.D. Francis, and J.S. Schepers. 1993. Using a chlorophyll meter to improve N management. Nebguide G93-1171A. Coop. Ext. Serv., Univ. of Nebraska, Lincoln.

Schepers, J.S., D.D. Francis, and M.T. Thompson. 1989. Simultaneous determination of total C, total N, and ^{15}N on soil and plant material. Commun. Soil Sci. Plant Anal. 20:949-960.

Schepers, J.S., D.D. Francis, M.F. Vigil, and F.E. Below. 1992a. Comparisons of corn leaf nitrogen concentration and chlorophyll meter readings. Commun. Soil Sci. Plant Anal. 23:2173-2187.

Schepers, J.S., T.M. Blackmer, and D.D. Francis. 1992b. Predicting N fertilizer needs for corn in humid regions: Using chlorophyll meters. p. 105-114. *In* B.R. Bock and K.R. Kelley (ed.) Predicting fertilizer needs for corn in humid regions. Bull. Y-226. Natl. Fert. and Environ. Res. Ctr., Tennessee Valley Authority, Muscle Shoals, AL.

Thomas, J.R., and H.W. Gausman. 1977. Leaf reflectance vs. leaf chlorophyll and carotenoid concentration for eight crops. Agron. J. 69: 799-802.

Thomas, J.R., and G.F. Oerther. 1972. Estimating nitrogen content of sweet pepper leaves by reflectance measurements. Agron. J. 64:11-13.

Wolfe, D.W., D.W. Henderson, T.C. Hsiao, and A. Alvino. 1988. Interactive water and nitrogen effects on senescence of maize: II. Photosynthetic decline and longevity of individual leaves. Agron. J. 80: 865-870.

Woolley, J.T. 1971. Reflectance and transmittance of light by leaves. Plant Physiol. 47:656-662.

Zscheile, F. P., and C. L. Comar. 1941. Influence of preparative procedure on the purity of chlorophyll components as shown by absorption spectra. Bot. Gaz. (Chicago) 102:463-481.