Relationships between chlorophyll meter readings and leaf chlorophyll concentration, N status, and crop yield: A review[1]

C. W. Wood[2], D. W. Reeves[3] and D. G. Himelrick[4]

[1] Contribution of the Alabama Agricultural Experiment Station in cooperation with the USDA-ARS National Soil Dynamics Laboratory.
[2] Department of Agronomy and Soils, 202 Funchess Hall, Auburn University, Alabama, 36849-5412, USA.
[3] National Soil Dynamics Laboratory, USDA-ARS, P.O. Box 3439, Auburn, Alabama 36831-3439, USA.
[4] Department of Horticulture, 101 Funchess Hall, Auburn University, Alabama 36849-5412, USA.

Abstract

Prediction of crop nitrogen (N) requirements is necessary for efficient fertilizer N utilization and protection of groundwater against contamination. Soil tests for available N have been successful in predicting crop N needs in arid regions, but have met with less success in humid regions. Tissue tests have shown promise for crop N requirement prediction, but time required for sampling and laboratory analyses may disallow timely producer response to crop N deficiencies. Recently, hand-held chlorophyll meters, which measure crop leaf green color intensity, have become available. Since green color intensity of crop leaves is directly related to crop N status, chlorophyll meters may have utility in prediction of crop N requirements. Research on chlorophyll meter use has been conducted for a variety of crops including rice (*Oryza sativa* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and cotton (*Gossypium hirsutum* L.), and horticultural crops. These studies have shown high correlations between chlorophyll meter readings and extractable leaf chlorophyll, as well as high correlations between chlorophyll meter readings and crop N status. Some research has identified critical chlorophyll meter reading levels that allow confirmation of N responsive sites. However, identification of appropriate N rates with chlorophyll meters at N responsive sites is more difficult at this time. Moreover, chlorophyll meter readings for a particular crop N status likely vary with crop varieties, cultural practices, and environmental factors. More research will be required before chlorophyll meters are routinely used for crop N requirement prediction.

Additional key words: *tissue testing, soil testing, water quality, extractable chlorophyll, N recommendations.*

Introduction

Producers, consultants, and researchers have long sought a quick, reliable method to determine crop N requirements. The goal has been to develop a means of tailoring N fertilization programs to specific conditions under which crops are grown. Poor N fertilizer management can result in economic losses to farmers. Too little N limits crop growth and yield, while in some situations excess N can cause crop damage. Moreover, expenditure for N fertilizer that is not taken up by crops represents an economic loss. In addition to economic consequences, excessive N fertilization can cause contamination of groundwater with NO_3-N, which constitutes a potential human and livestock health hazard (Stevenson, 1986). Past research confirms the potential for NO_3-N losses to groundwater under cropping systems (Aldrich, 1980; Bergstrom and Johansson, 1991; Chaney, 1990; Chichester and Smith, 1978). These and many other studies point to a need for developing tools to insure environmentally sound N management while maintaining economically viable crop production.

The principle associated with eliminating NO_3-N contamination of groundwater while maintaining viable crop production is simple: NO_3-N that is taken up by growing plants will not leach into groundwater. The probability is high that properly managed N applications at economically optimum levels for crops will not result in significant net loss of N via leaching from the root zone (Stanford, 1973). However, owing to the dynamic nature of N in soil systems, developing crop N requirement recommendations that do not result in economic loss or environmental contamination is often a formidable task. Current, widely used methods for determining crop N requirements include soil NO_3-N testing, tissue N tests, and calibrations from long-term field trials.

As recently as 1965, evaluating N availability via soil NO_3-N testing was considered of little value by most soil scientists (Meisinger, 1984). Since then, however, research has proved the importance of using soil NO_3-N concentrations as a tool for predicting crop N requirements (Meisinger, 1984). This soil test has met with greatest success in regions of low rainfall where minimal NO_3 leaching occurs (Stanford, 1981). Recent research, however, indicates that soil NO_3-N testing may be a viable approach for predicting crop needs in humid regions as well (Magdoff, 1991). The key to Magdoff's (1991) approach, commonly referred to as the pre-sidedress nitrate test (PSNT), is the collection and analysis of soil samples immediately prior to the rapid phase of plant growth (e.g., six- to ten-leaf stage in maize, early jointing in wheat, and first-square in cotton). At present, much research is focusing on the PSNT in humid regions such as the southeastern U.S. (Wells and Thompson, 1992).

Tissue N analyses have also been used in an attempt to develop N recommendations for crops. For wheat, methods tried include measurements of stem NO_3 (Roth *et al.*, 1989), N uptake (Alley *et al.*, 1986; Reeves *et al.*, 1993), and tissue N at various stages of growth (Roth *et al.*, 1989; Hargrove *et al.*, 1983; Reeves *et al.*, 1993; Vaughan, 1990 a,b). Of these tests, it appears that tissue N concentration and/or N uptake at various stages of growth hold the greatest promise for predicting wheat N requirements. Various tissue N tests have also been proposed for maize, and as with wheat, it appears that measurement of N concentration at defined growth stages have the greatest potential for prediction of N needs. We conducted a study in Alabama and found that tissue N concentrations at the 10-leaf stage of growth (V10; 10 leaf tips visible) were well correlated (r^2 = 0.81) with maize grain yields (Wood *et al.*, 1992). This good correlation early in the maize life cycle is of particular interest, because supplemental N applications could easily be made at V10.

Petiole NO_3-N analyses are a popular means of monitoring cotton N status in many U.S. production areas (Baker *et al.*, 1972; Lutrick *et al.*, 1986; Sunderman *et al.*, 1979; Tucker, 1963), but have been less successful and have not been adopted in some U.S. cotton producing states (Howard and Hoskinson, 1986; Jenkins *et al.*, 1982; Touchton *et al.*, 1981). Sabbe and Zelinski (1990) found that cotton leaf-blade N analyses were less affected by seasonal climatic changes than petiole NO_3-N tests, thus potentially offering a more stable measure of cotton N status.

Although soil NO_3-N and tissue N tests appear promising for predicting crop N requirement/sufficiency status, they require substantial time on the producers part, the use of sophisticated laboratory equipment, and costs associated with analyses. Time required for sample collection and analyses may disallow timely producer response to crop N deficiencies. Recently, new technology (chlorophyll meter) has become available that may allow rapid sampling and determination of crop N status, hence, allowing rapid decision-making on the producer's part with regard to the need or lack of need for supplemental N fertilizer. The purpose of this paper is to provide a review of research to-date on the use of chlorophyll meter technology for ascertaining crop greenness, N requirement, and yield.

Chlorophyll Meter Technology

Theory and operation

The theory behind using leaf greenness measurements (i.e., chlorophyll meter readings) to predict crop N status is based on the relationship between plant N concentration and plant chlorophyll concentration. The chlorophyll molecule contains four N atoms (the molecular formula for chlorophyll a is $C_{55}H_{72}N_4O_5Mg$ and for chlorophyll b is $C_{55}H_{70}N_4O_6Mg$), and extractable chlorophyll in leaves has been shown to be positively correlated to leaf N concentration (Takebe *et al.*, 1990). Increasing leaf chlorophyll concentration serves to increase chlorophyll density in chloroplasts rather than increasing the number of chloroplasts (Terashima and Saeki, 1983). It follows that leaf N concentration increases with chlorophyll density in leaf chloroplasts, and that measurement of leaf greenness provides an avenue for determining leaf N status. As previously discussed, leaf N status has been shown to be a good predictor of crop N status and N requirement. Thus, measuring greenness of crop leaves at appropriate times may offer a means of predicting crop N requirements.

Inada (1963) first devised the measurement of leaf greenness *in vivo* via light transmission using the highest peak of attenuance spectrum of chlorophyll in leaves (670 nm; red region), and the light region with constant minimum attenuance at (750 nm; infrared region). Building on this relationship, he proposed that differences in light attenuation between 670 and 750 nm in fresh leaves can be used as an index of leaf chlorophyll concentration, or greenness. Based on his ideas, Minolta Camera Co., Ltd., Japan developed a portable, dual-wavelength (645 and 790 nm) chlorophyll meter, the SPAD-501, which was subsequently widely marketed in Japan for determination of rice N status (Takebe and Yoneyama, 1989). An improved version of this meter (SPAD-502), which utilizes the light

attenuation difference between 650 and 940 nm for determination of leaf greenness, is currently being marketed worldwide.

Light sources for the SPAD-502 are light emitting diodes (LEDs), including a red (650 nm; peak chlorophyll absorbance) and an infrared LED (940 nm; nonchlorophyll absorbance), which emit light in sequence through the leaf. The meter has two silicon photodiode detectors, one sensitive to red light and the other sensitive to infrared radiation. Electrical currents converted from light received by the silicon photodiodes are received by a microprocessor, which linearlizes the signal and calculates a SPAD (Soil Plant Analysis Development; unitless) value according to equation 1:

$$SPAD = A(\log (I_{or}/I_r)-\log(I_{of}/I_f)+B \quad (1)$$

where A and B are constants, I_r and I_f are currents from red and infrared detectors with sample in place, respectively, and I_{or} and I_{of} are currents from red and infrared detectors with no sample, respectively.

The meter is lightweight (225 g), is powered by two AA alkaline batteries, has a 2-second interval between measurements, and can store up to 30 values for averaging. Operationally, measurement of leaf greenness is accomplished by inserting a leaf blade into the head of the SPAD-502 (Fig. 1).

Relationship with extractable chlorophyll

Leaf chlorophyll concentration is often well correlated with plant metabolic activity (e.g., photosynthetic capacity and RuBP carboxylase activity; Evans, 1983; Seeman *et al.*, 1987), plant stress (Eagles *et al.*, 1983; Fanizza *et al.*, 1991), as well as leaf N concentration. Standard *in vitro* colorimetric methods for measuring chlorophyll (e.g., Arnon, 1949; Moran, 1982) are accurate (±5%), but are destructive, time consuming, and require sophisticated laboratory equipment (Monje and Bugbee, 1992). Measuring leaf chlorophyll *in vivo* spectroscopically via chlorophyll meter could provide a quick, reliable alternative to *in vitro* techniques.

Extractable leaf chlorophyll concentrations have been compared with chlorophyll meter readings for a wide variety of field and horticultural crops [e.g., apple (*Malus* x *domestica* Borkh.) (Campbell *et al.*, 1990); grape (*Vitis vinifera* L.) (Fanizza *et al.*, 1991); wheat, rice, and

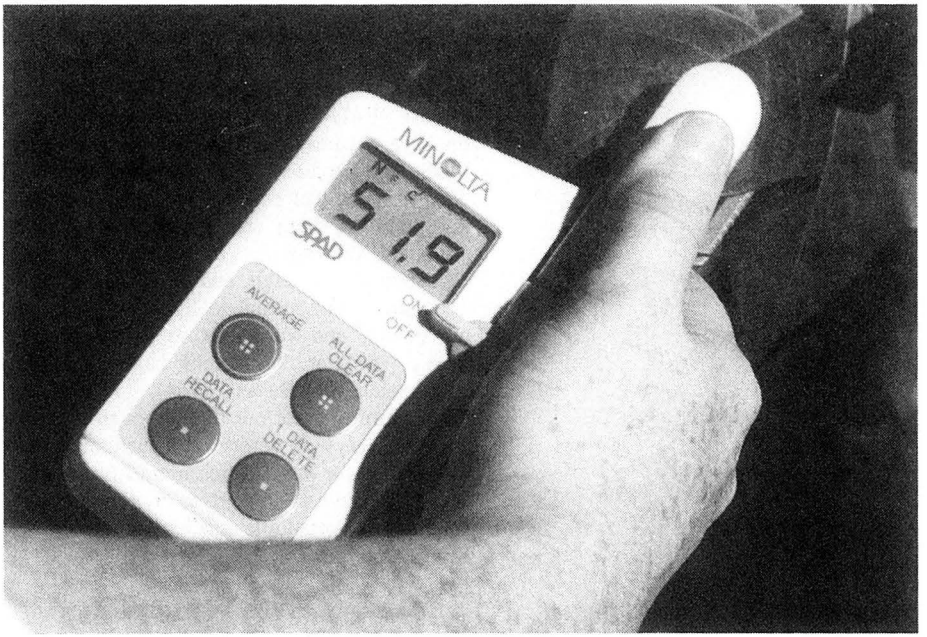

Figure 1. Measurement of leaf greenness with the SPAD-502 chlorophyll meter.

soybean (*Glycine max* [L.] Merrill) (Monje and Bugbee, 1992); and several species of tropical fruit trees (Schaper and Chacko, 1992)]. In most studies, the relationship between extractable chlorophyll and chlorophyll meter readings has been linear. This relationship is illustrated by the strawberry (*Fragaria* x *Ananassa*) data of Himelrick *et al.* (1992) (Fig. 2).

A single equation relating *in vivo* and *in vitro* chlorophyll measurements across species would be useful for an array of research applications. Yadava (1986) compared extractable chlorophyll from leaves of 22 species under diverse growing conditions with SPAD-501 readings; pooled regression (all 22 species included) showed a linear relationship between extractable chlorophyll and SPAD-501 readings (r^2 = 0.48). The poor relationship between extractable chlorophyll and SPAD-501 readings in Yadava's (1986) study suggest that either plant species differences and/or environmental conditions greatly affected the relationship. In another study, regression of SPAD-501 readings against leaf chlorophyll extracted from 12 species with N,N-dimethylformamide produced a highly significant linear relationship (r^2 = 0.89) (Marquard and Tipton, 1987). Although the pooled regression conducted by Marquard and Tipton (1987) was good, regressions for individual species showed significant differences among species. The environment under which plants are grown (e.g., soil fertility, water status, pest damage, etc.) has also been shown to affect the relationship between *in vivo* and *in vitro* chlorophyll measurements (Campbell *et al.*, 1990; Kaakeh *et al.*, 1992; Fanizza *et al.*, 1991). It is well known that water stress causes greater thickness of outer walls of the leaf epidermis and cuticle (Parker, 1968), and leaf thickness has been shown to affect leaf greenness as measured by the chlorophyll meter (Peng *et al.*, 1992). These studies suggest that separate regression models relating *in vivo* and *in vitro* chlorophyll measurements are required for different plant species and growth conditions.

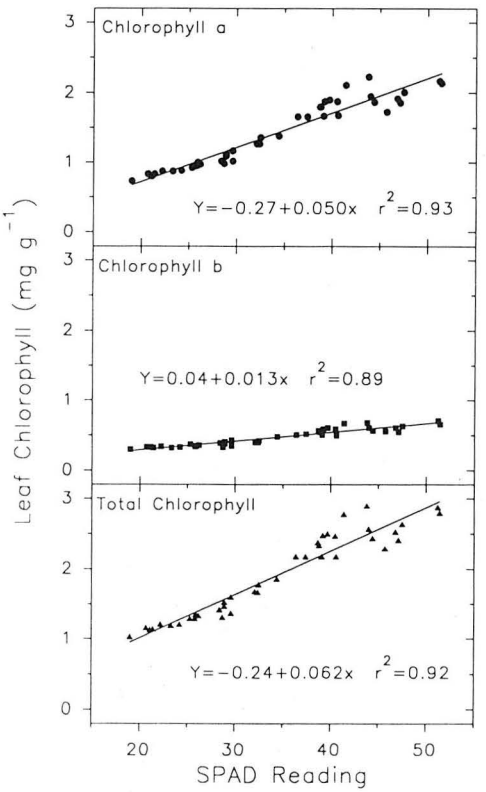

Figure 2. Relationship between SPAD-502 meter readings and extractable chlorophyll (fresh weight basis) in strawberry leaves (adapted from Himelrick *et al.*, 1992).

If light absorbance in leaves is solely dependent on pigment concentration per unit area not per unit tissue volume, the relationship between *in vivo* and *in vitro* chlorophyll measurements should follow the Beer-Lambert Law and be linear. However, curvilinear regression (r^2 = 0.97) of pooled data for wheat, rice, and soybean samples extracted with dimethyl sulfoxide against SPAD-502 readings was an improvement over linear regression (r^2 = 0.93) (Monje and Bugbee, 1992). Monje and Bugbee (1992) observed that the SPAD-502 overestimated leaf chlorophyll concentrations with very low and very high chlorophyll concentrations. They attributed the meter's overestimation of chlorophyll concentration in high and low ranges, and thus the curvilinear relationship, to light scattering, leaf surface reflectivity, and variations in pigment distribution in leaves. Similar conclusions have been purported by other scientists (Butler, 1964). Monje and Bugbee (1992) further point out that although limitations to the use of chlorophyll meters exist, such meters are valuable instruments for *in vivo* measurement of leaf chlorophyll if: 1) properly calibrated to a given set of growing conditions, 2) multiple readings are taken, and 3) leaf veins are avoided.

Relationship with crop N status and yield

Rice: The application of chlorophyll meter readings to determination of crop N status first began with rice in Japan. Takebe and Yoneyama (1989) studied the

relationship between SPAD-501 readings and N concentrations of second uppermost leaves of four rice varieties during 1984 to 1987. They found highly significant linear relationships between chlorophyll meter readings and leaf N concentration. Data collected for rice at panicle formation in four successive years had correlation coefficients ranging from 0.82 to 0.94, with the pooled data set having a correlation coefficient of 0.82. The relationship between SPAD-501 readings and rice N concentration did, however, vary with variety, study site, and growth stage. Takebe and Yoneyama (1989) concluded that to diagnose the N requirement of rice that knowledge of the relationship between chlorophyll meter readings and leaf N concentration would be required for specific varieties, sites, and growth stages.

Turner and Jund (1991), working at several sites in Texas over three years, evaluated the SPAD-502's potential for determining the need for N fertilizer by semi-dwarf 'Lemont' rice. They applied 56 to 280 kg N ha^{-1} to paired plots before planting to establish a range of leaf chlorophyll levels. At one of four growth stages (tillering, pre-panicle initiation, panicle differentiation, and heading), one of the paired plots was topdressed with 50 kg N ha^{-1} subsequent to measuring greenness on the most recent fully developed leaf with the SPAD-502. Turner and Jund (1991) found no significant correlation between SPAD-502 readings on the uppermost fully developed leaf and the yield increase owing to topdressing at tillering or heading. They attributed the failure of the SPAD-502 to predict the need for topdress N at tillering and heading to a narrow range of SPAD-502 readings at those growth stages; preplant N applications were apparently adequate at tillering, while the potential for topdressing to increase rice yields at heading was low. During pre-panicle initiation and panicle differentiation, the range in SPAD-502 values reached maximum, and a good relationship between SPAD-502 values and the yield increase due to topdressed N was evident. Turner and Jund (1991) determined the SPAD-502 critical value (the reading below which a producer would expect to get an economic yield increase) at pre-panicle initiation and panicle differentiation to be 40 SPAD units for semidwarf 'Lemont' rice.

Maize: Takebe and Yoneyama (1989), after examining the efficacy of the SPAD-501 in predicting the N status of maize, suggested that the N status of upland crops cannot be as easily determined with chlorophyll meters as it can for rice. They attributed poor performance of the SPAD-501 in predicting the N status of maize to the presence of nonchlorophyll N (NO_3-N), whereas rice leaves do not contain a significant quantity of NO_3-N.

Recent research on the relationship between maize N status and chlorophyll meter readings, however, has been more favorable than results obtained by Takebe and Yoneyama. We conducted experiments to determine the feasibility of using SPAD-502 readings for evaluation of N status for maize in Alabama (Wood *et al.*, 1992a). Chlorophyll meter readings were highly correlated with tissue N concentrations at V10 and midsilk stages of

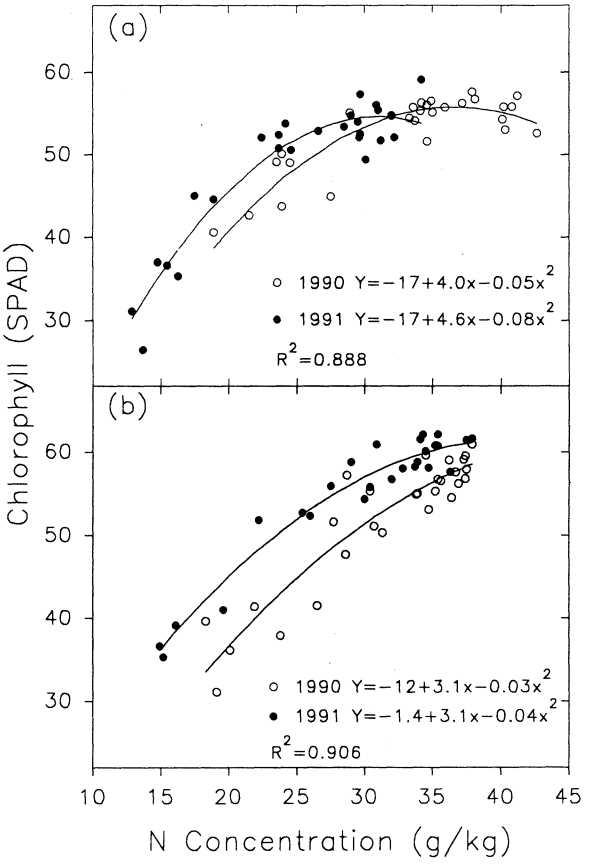

Figure 3. Relationship between maize tissue N concentrations and SPAD-502 readings in Alabama (USA) during 1990 and 1991; (a) tissue samples and SPAD-502 readings taken at V10, and (b) tissue samples and readings taken at midsilk (adapted from Wood *et al.*, 1992).

growth in field studies during 1990 and 1991 (Fig. 3). Chlorophyll meter readings had excellent grain yield prediction capabilities (Fig. 4), even at V10, which shows promise for utilization of this tool for in-season N recommendations. It should be noted that the relationship between leaf N concentration and SPAD 502 readings is curvilinear, i.e., SPAD-502 readings plateau even though leaf N concentrations continue to increase (Fig. 3). Grain yields continue to increase with SPAD-502 readings (Fig. 4), suggesting that grain yield is closely associated with chlorophyll/unit area. Moreover, it appears that grain yield is not as closely aligned with leaf N concentration at high N levels as it is with leaf chlorophyll. Curvature in the relationship between SPAD-502 readings and tissue N concentrations indicates the presence of nonchlorophyll N, and suggests that leaf NO_3-N concentrations may have to be determined, perhaps with portable NO_3-N meters, along with SPAD-502 readings to properly diagnose maize N status.

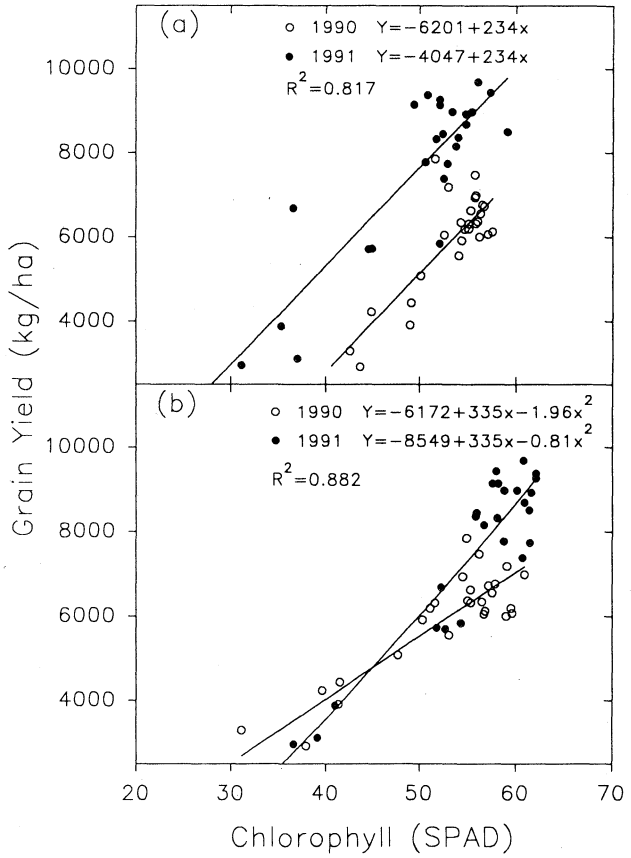

Figure 4. Relationship between SPAD-502 readings and maize grain yields in Alabama (USA) during 1990 and 1991; (a) SPAD-502 readings taken at V10, and (b) SPAD-502 readings taken at midsilk (adapted from Wood *et al.*, 1992).

Schepers *et al.* (1992) compared maize leaf disk N concentrations and SPAD-502 readings at silking from several N rate studies for a variety of hybrids at several locations in the midwestern U.S. They found that chlorophyll meter readings correlated well with leaf N concentrations for a given maize hybrid and location. They suggested that calibration of chlorophyll meters to determine crop N status may not be practical because of unique greenness of different hybrids. They further suggested, however, that normalization procedures could be used to standardize SPAD-502 readings across hybrids, locations, and growth stages: standardization can be accomplished via comparison of an adequately fertilized reference area to the field area under investigation.

Perhaps the most extensive study of chlorophyll meter use for the prediction of maize N needs was conducted by Piekielek and Fox (1992). They collected chlorophyll meter reading (at V6), soil N supplying capability (average maize N uptake in unfertilized plots minus 75% of starter N fertilizer), and grain yield data from 67 locations in Pennsylvania. Using Cate-Nelson procedures, they found that chlorophyll meter readings on the fifth leaf of maize at V6 was an accurate predictor of whether maize would respond to sidedress N fertilizer, and that chlorophyll meter readings below 43.4 indicated the need for supplemental N. They did, however, find that chlorophyll meter readings were not correlated well enough to soil N supplying capability (r = 0.59) to determine sidedress N fertilizer rates for N responsive fields.

Wheat: We also conducted studies on wheat under a range of fertilizer N rates and management practices (Reeves *et al.*, 1993). Results from these studies indicate that neither leaf N concentration, N uptake, nor SPAD-502 readings at GS3 (growth stage 3; Feekes, 1941) can predict grain yields. The GS3 appears to be too early for practical prediction of wheat grain yield response owing to N fertility under a wide array of management practices. Nitrogen uptake at GS5 was a better predictor of wheat grain yield (average r^2 = 0.85)

than SPAD-502 readings (average $r^2 = 0.50$). However, since N uptake is the product of N concentration and dry matter yield, comparison of SPAD-502 readings to N uptake is biased unless dry matter yield is used as component in a model with SPAD-502 readings. When we included dry matter in the model with SPAD-502 readings, the average R^2 was 0.81, comparing favorably to N uptake for prediction of wheat grain yield. The effectiveness of SPAD-502 readings in combination with dry matter yield at GS5 to predict wheat grain yields is encouraging, since N fertilizer applied immediately after this growth stage can meet the N requirement of wheat (Alley *et al.*, 1986). It appears that these relatively simple measurements could be combined to develop quick and reliable methods for prediction of N requirement for wheat.

Other researchers have confirmed the potential for SPAD-502 readings to determine the N status of wheat. Follett *et al.* (1992) found a positive association between chlorophyll meter readings and leaf N concentrations, soil inorganic N concentrations (the three measurements were taken at GS5) and grain yield from four N rate studies for dryland winter wheat in Colorado. However, the range of SPAD-502 readings in their study was small (< 8), which may indicate limited utility of the SPAD-502 for prediction of dryland winter wheat grown on soils of the U.S. western Great Plains, which tend to have a higher inherent N fertility status than those of humid regions (Jenny, 1980).

Cotton: In field experiments with cotton (Wood *et al.*, 1992b), we compared leaf-blade total N and petiole NO_3-N concentrations with SPAD-502 readings at the first square, first flower, and midbloom stages of growth to determine their ability to predict seed cotton yield across several N fertilizer rates. Chlorophyll meter readings were significantly correlated with leaf-blade N concentrations at all three stages of growth. However, in contrast to maize, SPAD-502 readings were not as highly correlated to seed cotton yields as leaf-blade N concentrations. Compared to petiole NO_3-N concentrations, SPAD-502 readings better predicted seed cotton yield at midbloom, but were slightly less effective in predicting yield at first square and first flower. Although the SPAD-502 appears to be a less effective predictor of cotton N status than standard tissue tests, it may offer an alternative to chemical tissue tests for monitoring cotton N status, particularly when convenience is considered.

Horticultural crops: Although much research has been done on the use of chlorophyll meters to predict amounts of extractable chlorophyll in leaves of horticultural crops (Campbell *et al.*, 1990; Fanizza *et al.*, 1991; Himelrick *et al.*, 1992; Schaper and Chacko, 1992), little research has been conducted to relate chlorophyll meter readings with the N status of such crops. We (Himelrick *et al.*, 1993) conducted a study with hydroponically grown strawberry at several N levels, and found poor correlations between SPAD-502 readings and total and NO_3-N in leaf-blades and petioles. Because of these poor relationships, the potential for SPAD-502 readings to evaluate the N status of strawberry appears questionable. Rouse (1992) reported similar results for 10 varieties of citrus grown on three different rootstocks. The reason for the decreased ability of chlorophyll meters to predict horticultural crop than agronomic crop N needs is not known. However, it is suspected that greater complexity of N source-sink relationships may diminish the ability of chlorophyll meters to determine the N status of these and other crops such as cotton.

Conclusions

It appears that hand-held chlorophyll meters offer an excellent means of replacing time consuming *in vitro* methods for estimating leaf chlorophyll concentration. Moreover, the practical application of chlorophyll meters to the determination of crop N status appears promising. However, several limitations currently exist with regard to use of chlorophyll meters for determination of plant chlorophyll concentration and crop N status. Although, chlorophyll meter readings at early growth stages appear capable of identifying N responsive sites, using them for determination of appropriate N rates at N responsive sites is more difficult. Moreover, since genetics and environmental factors influence greenness of plant leaves, standardization of chlorophyll meter readings across species, varieties, locations, and growth stages seems imperative. Future research may improve the capability of hand-held chlorophyll meters to predict plant chlorophyll concentrations, plant N status, the need for supplemental N, and appropriate N rates at N responsive sites. Chlorophyll meter technology appears to have great potential, once calibrated, for rapid on-farm determination of crop N status.

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