Association of "Greenness" in Corn with Yield and Leaf Nitrogen Concentration

Robert L. Rorie, Larry C. Purcell,* Morteza Mozaffari, Douglas E. Karcher, C. Andy King, Matthew C. Marsh, and David E. Longer

ABSTRACT

Efficient use of N fertilizer has become crucial due to fertilizer costs and the impact of excessive N on the environment. Diagnostic tools for estimating plant N status have an important role in reducing N inputs while maintaining yield. The objective of our study was to quantify corn (*Zea mays* L.) leaf greenness with a digital camera and image-analysis software and establish the relationship with yield, leaf N concentration, and chlorophyll meter (or SPAD, soil plant analysis development) values. In 2008 and 2009, field experiments were conducted at five sites with N treatments ranging from 0 to 336 kg N ha^{-1}. At tasseling, the ear leaf was sampled for color analysis and SPAD measurements, and then analyzed for total N. Hue, saturation, and brightness (HSB) values from digital images were processed into a dark green color index (DGCI), which combines HSB values into one composite number. Including calibration disks in images and changing the background color in photographs to pink greatly improved DGCI precision in 2009 over 2008. There was a close relationship (typically $r^2 \geq 0.70$) of SPAD and DGCI with leaf N concentration. Within a location, yield increased linearly in most cases with both SPAD (average $r^2 = 0.79$) and DGCI (average $r^2 = 0.78$). Digital-image analysis was a simple method of determining corn N status that has potential as a diagnostic tool for determining crop N needs.

Nitrogen is an important and costly input for nonleguminous grain crops, and producers are applying N fertilizer in large amounts to ensure high yields over a range of environmental conditions (Kyveryga et al., 2007). However, excessive N fertilization may lead to runoff, leaching, and nitrate pollution. A delayed N application and the use of remote sensing tools might allow a producer to apply a more economically beneficial N rate to their fields. Scharf and Lory (2002) gave several reasons to delay N applications, including avoiding extra work during the busy planting season and lowering the in-season N loss during wet years. They also suggested that diagnostic tools for plant N might increase fertilizer use efficiency, and these tools include the SPAD meter, reflectance measurements, and color analysis.

The SPAD meter is used to make an optimum fertilizer N-rate decision by measuring N stress relative to an optimum N-rate strip within a field (Hawkins et al., 2007). The SPAD meter is well documented as an accurate measure of the N status of corn at different developmental stages (Piekielek and Fox, 1992; Blackmer et al., 1994; Schepers, 1994). Piekielek et al. (1995) showed that SPAD values expressed relative to SPAD values from a high-N strip (relative or normalized SPAD) could be compared over a wide range of sampling times when using a common critical value. Normalized SPAD values lessen the effect of differences in hybrid, soil type, growth stage, or environmental conditions (Piekielek et al., 1995). Scharf et al. (2006) found that the relationship between SPAD values and economically optimum N rate was much stronger when using normalized values as opposed to absolute values. The SPAD meter is a useful tool, but it has some potential limitations. The SPAD meter costs about $1500 USD, has a small sampling area (6 mm^2), is subject to operator bias, and Zhang et al. (2008) showed that SPAD meters have difficulty in estimating chlorophyll levels when they are near or above optimum. Their observations indicate that increases in chlorophyll are not necessarily associated with increases in yield.

Spectral reflectance of crop leaves can be a valuable tool to estimate plant N status (Li et al., 2005). Spectral reflectance is the reflectance of certain plant components that are controlled by their visual properties and radiant energy exchange in a canopy (Huete, 1988). The reflectance of certain wavelengths is related to different amounts of chlorophyll a and b, which can be used to estimate the N status of certain crops (Huete, 1988). This method shows great potential because it offers a method to deliver variable-rate N applications from a vehicle-mounted sensor (Kitchen et al., 2010). Tools for measuring reflectance, however, can be expensive (>$4,000 USD), which is likely to slow the adoption by producers.

Leaf color has been recognized as one of the most sensitive indicators of nutrient deficiencies (Blinn et al., 1988). Nitrogen is directly related to leaf color because it is a key component of the chlorophyll molecule (Tracy et al., 1992). Most research that uses digital images to estimate N status of crops has used red, green, and blue (RGB) color components (Kawashima and Nakatani, 1998; Jia et al., 2007; Pagola et al., 2009); however, Karcher and Richardson (2003) found that amounts of red and blue may alter

R.L. Rorie, L.C. Purcell, C.A. King, M.C. Marsh, and D.E. Longer, Dep. of Crop, Soil, and Environmental Sciences, Univ. of Arkansas, 1366 W. Altheimer Dr., Fayetteville, AR 72704; M. Mozaffari, Dep. of Crop, Soil, and Environmental Sciences, Univ. of Arkansas Soil Testing and Research Lab at Marianna, P.O. Drawer 767, Marianna, AR 72360; D.E. Karcher, Dep. of Horticulture, Plant Science Bldg., Univ. of Arkansas, Fayetteville, AR 72701. Received 2 July 2010. *Corresponding author (lpurcell@uark.edu).

Published in Agron. J. 103:529–535 (2011)
Published online 7 Feb 2011
doi:10.2134/agronj2010.0296

Abbreviations: DGCI, dark green color index; HSB, hue, saturation, and brightness; RGB, red, green, and blue; SPAD, soil plant analysis development.

Table 1. Corn production information for experiments conducted at two sites in Fayetteville (Fay 1 and Fay 2), Keiser, Marianna, and Rohwer, AR in 2008 and 2009.

Year	Location	Hybrid	Soil series	Planting date	Replications	N rates
						kg N ha^{-1}
2008	Fay 1	Pioneer 31P42	Captina silt loam†	21 Apr. 2008	5	0, 28, 56, 84, 112, 140, 196, 280
2008	Fay 2	Pioneer 31G96	Captina silt loam†	25 May 2008	3	0, 56, 112, 168, 224, 280
2008	Keiser	Pioneer 32B29	Sharky-Steel complex‡	1 May 2008	4	0, 60, 134, 201, 269, 336
2008	Marianna	Pioneer 32B29	Calloway silt loam§	22 Apr. 2008	4	0, 60, 134, 201, 269, 336
2008	Rohwer	Pioneer 32B29	Herbert silt loam¶	4 Apr. 2008	4	0, 60, 134, 201, 269, 336
2009	Fay 1	Pioneer 31P42	Captina silt loam†	21 Apr. 2008	5	0, 28, 56, 84, 112, 140, 196, 280
2009	Fay 2	Pioneer 33M57	Captina silt loam†	21 May 2009	3	0, 56, 112, 168, 224, 280
2009	Keiser	DKC67–23	Sharky-Steel complex§	19 May 2009	4	0, 67, 134, 201, 269, 336
2009	Marianna	10289464, CBX	Loring silt loam#	25 Apr. 2009	2	0, 67, 134, 202, 269, 336
2009	Rohwer	Stine 9806	Herbert silt loam¶	4 Apr. 2009	4	0, 67, 134, 202, 269, 336

† Captina silt loam: fine-silty, siliceous, active, mesic Typic Fragiudults.
‡ Sharkey-Steel complex: Sharkey very-fine, smectitic, thermic Chromic Epiaquerts.
§ Calloway silt loam: fine-silty, mixed, active, thermic Aquic Fraglossudalfs.
¶ Herbert silt loam: fine-silty, mixed, superactive, Mesic Udollicepiaqualfs.
# Loring silt loam: Loring fine-silty, mixed, active, thermic Oxyaquic Fragiudalfs.

how green an image appears. They suggested using a dark green color index (DGCI), which is derived from values of hue, saturation, and brightness (HSB). They found significant DGCI differences due to N treatments and that DGCI was a more consistent measure of dark green color than were individual HSB values.

Digital-image analysis may offer advantages over SPAD measurements and spectral reflectance. Digital cameras are relatively inexpensive, require little technical expertise, and the images could allow sampling a greater leaf area than that used for SPAD measurements. The objectives of the following research were to determine if a digital camera and image-analysis software could accurately quantify the color of corn leaves that had received various N treatments, to serve as an indicator of N deficiency, and to establish possible relationships of DGCI with grain yield. Digital images were taken of leaves to determine the relationships among DGCI, chlorophyll concentration (SPAD), leaf N concentration, and yield.

MATERIALS AND METHODS

Crop Management

Nitrogen rate experiments were established at two sites in Fayetteville (Fay 1 and Fay 2, 36°4' N, 94°9' W), Marianna (34°46' N, 90°45' W), Keiser (35°40' N, 90°6' W), and Rohwer (33°45' N, 91°16' W), Arkansas with N fertilizer treatments ranging from 0 to 336 kg ha^{-1} (Table 1). The maximum N rate at each of the locations represents an excess of 25 kg N ha^{-1} that was expected to give a yield response based on previous experience at these locations and on these soils. In 2008 and 2009 the experimental design for all locations was a randomized complete block with two to five replications. Fields at all locations were disked followed by light tillage with a field cultivator before planting. Plots consisted of four rows, 101 cm apart and 9.3 m in length, which were planted at a seeding rate of about 86,500 kernels ha^{-1}. All hybrids used in these experiments ranged in maturity from 115 to 120 d, and all hybrids were transgenic with genes for glyphosate [*N*-(phosphonomethyl)glycine] tolerance and for a gene from *Bacillus thuringiensis* that decreases larval feeding by *Lepidoptera* spp. Urea was hand applied to 3-leaf corn (Ritchie et al., 1993) at all locations, and plots were irrigated when the soil moisture reached a maximum deficit of 32 mm as determined by an irrigation scheduling program (Purcell et al., 2007). Weeds were controlled by preemergence herbicides followed by postemergence applications of glyphosate and hand-weeding. Plots were fertilized to meet soil-test recommendations for all nutrients except N. Soil type, hybrid, and other relevant field information for all locations are recorded in Table 1.

Plant Sampling

The ear leaf from one plant per row was sampled at tasseling (VT, Ritchie et al., 1993) from the middle two rows of each four-row plot. Leaves were removed from the plants, cut in half with scissors, and placed in plastic bags on ice to facilitate ease of transport to the laboratory. Three SPAD measurements were taken per leaf on either side of the midvein, approximately at the midpoint of the leaf length, and SPAD values were averaged. After SPAD measurements, digital images were made of the same leaves, and leaves were then dried at 80°C until weight was constant, ground through a 20-mesh screen, and analyzed for total N via Dumas combustion using a LECO FP-428 Determinator (LECO Corporation, St. Joseph, MO) at the Soil Test and Plant Analysis Laboratory at the University of Arkansas. At physiological maturity, the middle two rows were trimmed to 6.1 m and hand harvested at the Fayetteville locations and harvested with a plot combine at other sites. Following hand harvest, grain was weighed and moisture was determined using a Harvest Hand moisture tester (Dickey-John Corporation, Auburn, IL). Yield was adjusted to 15% moisture.

Image Processing

Images were collected in JPEG (joint photographic experts group) format, renumbered using Faststone Image Viewer (v.3.6, www.fastone.org), and amounts of RGB light determined using SigmaScan Pro (v. 5.0, Chicago, IL). Threshold ranges were set from 30 to 120 for hue and from 27 to 100 for saturation. Hue, saturation, and brightness values were calculated automatically from RGB values by a macro that allowed batch processing of images and that saved data on a spreadsheet. The macro converted the average RGB values as measured by SigmaScan Pro to percentage RGB by dividing red, green, and blue values by 255. Then the percentage RGB values were converted to HSB values using the following algorithms (Adobe Systems, Inc., San Jose, CA):

<u>Hue</u>

If max(RGB) = R, 60*{(G – B)/[(max(RGB) – min(RGB)]}

If max(RGB) = G, 60*{2+{(B – R)/ [max(RGB) – min(RGB)]}}

If max(RGB) = B, 60*{4+{(R – G)/ [max(RGB) – min(RGB)]}}

<u>Saturation</u>

{[max(RGB) – min(RGB)]/max(RGB)}

<u>Brightness</u>

max(RGB)

The macro used HSB values to determine DGCI values (Karcher and Richardson, 2003; Rorie, 2010) whereby:

$$\text{DGCI} = [(\text{Hue} - 60)/60 + (1 - \text{Saturation}) + (1 - \text{Brightness})]/3 \quad [1]$$

Camera Settings and Calibration

In 2008, we used an Olympus C3030 camera with an image size of 1280 × 960 pixels (Olympus America Inc., Melville, NY), and in 2009 we used a Canon Power Shot S51S with an image size of 3264 × 2448 pixels (Canon U.S.A., Inc. Lake Success, NY). Cameras were set to an ISO of 100, a shutter speed of 1/15 s, an aperture of 2.0, an exposure compensation of 0, and to fluorescent white balance with the flash turned off. In 2008, leaves were photographed on a black felt background; however, the background caused small reflections in the photographs that were detected in the color-threshold settings of the software. A pink felt background was used in 2009 that increased contrast and eliminated reflections that were detected by the software. Photographs were taken under fluorescent lighting of the upper leaf surface from a camera height of 58 cm with (2009) and without (2008) the addition of green and yellow color disks alongside of the leaves. The disks served as internal standards and were used for correcting differences in lighting conditions (Rorie, 2010). The two leaves that were removed from each plot were photographed in one image immediately after SPAD measurements. The time between leaf removal from the plant and photographing the leaves was typically less than 1 h, during which time leaves were in plastic bags on ice.

Standards disks were 9 cm in diameter and had Munsell color values of 6.7GY 4.2/4.1 for the green disk and 5Y 8/11.1 for the yellow disk, which resulted in HSB values of 91/38/42 (green disk) and 66/88/100 (yellow disk). The macro used in the experiment was created in SigmaScan Pro (v. 5.0, Chicago, IL) and was developed to analyze each object in the image separately and give individual values on a separate line in a spreadsheet.

For each image, the calibration disks served as internal standards and were used in a two-point regression of actual DGCI values of the calibration disks versus observed values of the calibration disks. The DGCI values of the standard discs were 0.073 for the yellow disk and 0.572 for the green disk. To calibrate images, the first step was to determine differences in slope between the known DGCI values and the observed DGCI values.

$$\text{Slope} = (\text{Known Green DGCI} - \text{Observed Green DGCI}) \times (\text{Known Yellow DGCI} - \text{Observed Yellow DGCI})^{-1} \quad [2]$$

Next, the Y intercept was determined:

$$Y \text{ intercept} = \text{Known Yellow DGCI} - (\text{Slope} \times \text{Observed Yellow DGCI}) \quad [3]$$

DGCI values for leaves in the images were corrected using the linear relationship from the slope and intercept whereby:

$$\text{Corrected leaf DGCI} = (\text{Slope} \times \text{Observed Leaf DGCI}) + Y \text{ intercept} \quad [4]$$

Statistical Analysis

We initially used a general linear model for analysis of variance (SAS, v. 9.2, Cary, NC) to evaluate yield, DGCI, SPAD, and leaf N concentration responses to N application rate and block by year and location. If these response variables were not significantly affected by N treatment, they were excluded from analysis that combined data from multiple locations. Relative values of DGCI, SPAD, yield, and leaf N concentration were calculated by dividing individual values for a location by the largest value for that location and year. Relative values, therefore, have a range from 0 to 1.

For each year and location combination, we used simple linear regression to evaluate the relationships between variables. Residuals were inspected visually from the linear models for nonuniformity and bias. When data were combined over locations, simple linear regression was also used to describe relationships between variables. The one exception where a linear model did not fit data was for the relationship between relative yield and relative DGCI when combined over all locations in 2009. For this exception, an exponential model was used.

RESULTS

Relationship of DGCI and SPAD in 2008

There was a close relationship of DGCI with SPAD ($r^2 > 0.85$ for all locations) with the exception of Fay 1 and Rohwer. At Fay 1, the r^2 value for the relationship between DGCI and SPAD was 0.24 (Table 2). At the Fay 1 experiment, there was considerable residual N in the soil and there was no response of DGCI, SPAD, or yield to N treatment as indicated by analysis of variance (data not shown). At Rohwer, r^2 values were also low ($r^2 = 0.16$) because of poor lighting conditions where images were taken, and DGCI values were not significantly affected by N treatment as determined by analysis of variance (data not shown). Poor lighting caused images to be dark and added variation to final image values. Specific slopes, intercepts, r^2 values, and number of samples for each location are given in Table 2. After disregarding data from Fay 1 and Rohwer,

Table 2. Regression results and sample size for the dark green color index (DGCI), leaf chlorophyll (SPAD, soil plant analysis development) values, leaf N concentration (Nc, g N 100g^{-1}), and grain yield (kg ha^{-1}) from corn experiments by location in 2008. Leaf measurements were made on the ear leaf at tasseling.

Location	DGCI vs. SPAD	DGCI vs. leaf Nc	SPAD vs. leaf Nc	Yield vs. DGCI	Yield vs. SPAD	Yield vs. leaf Nc
Fayetteville 1						
Slope	0.001	0.02	10.2	18,276	127.8	1796
Intercept	0.342	0.377	24.31	2678	3677	5303
r^2	0.24*	0.20	0.53**	0.04	0.15	0.18
n	40	40	40	40	40	40
Fayetteville 2						
Slope	0.005	0.073	13.16	52,362	297.3	4501
Intercept	0.192	0.2	3.547	–13,713	–4373	–4748
r^2	0.90**	0.83**	0.83**	0.76**	0.78**	0.86**
n	18	18	18	18	18	18
Keiser						
Slope	0.005	0.075	12.82	na†	na†	na†
Intercept	0.191	0.236	7.34	na†	na†	na†
r^2	0.85**	0.74**	0.88**	na†	na†	na†
n	28	28	28	28	28	28
Marianna						
Slope	0.006	0.06	0 9.283	71,276	544.3	5449
Intercept	0.168	0.267	16.17	–21,931	–13,851	–6227
r^2	0.86**	0.81**	0.90**	0.70**	0.85**	0.90**
n	28	28	28	28	28	28
Rohwer						
Slope	0.0001	0.01	9.467	10,709	466.3	5318
Intercept	0.463	0.473	16.6	–44,799	–12,003	–6977
r^2	0.16	0.18	0.75**	0.21	0.69**	0.79**
n	28	28	28	28	28	28

* Indicates regression significance at $P \leq 0.05$.

** Indicates regression significance at $P \leq 0.01$.

† Yield data were not collected at Keiser due to equipment failure.

relative DGCI and SPAD values were combined across locations. Despite differences in hybrid and soil type there was a close relationship between relative DGCI and relative SPAD (Fig. 1).

Relationship of DGCI and SPAD to Leaf Nitrogen and Yield in 2008

Relative SPAD and relative DGCI were linearly associated with leaf N concentration for all locations with the exception of DGCI at Fay 1 and Rohwer for reasons mentioned previously (Fig. 2). The r^2 values for other locations ranged from 0.74 to 0.90 (Table 2). Corn grain yield was also closely related to SPAD and DGCI for most individual locations with the exception of Fay 1 ($r^2 < 0.15$) and Rohwer ($r^2 = 0.21$). Yield at Rohwer showed a close relationship to SPAD values ($r^2 = 0.69$) because these measurements were not affected by the poor lighting conditions; however, the relationship between yield and SPAD values at Fay 1 were still low due to the lack of N treatment effect. Yield data for Keiser were not collected due to a malfunction in harvesting equipment. Relative yield was linearly associated with relative DGCI ($r^2 = 0.72$) and relative SPAD ($r^2 = 0.76$) values when combined over locations (Fig. 3).

Yield Response to Leaf Nitrogen Concentration at Tasseling in 2008

Yield was linearly associated with leaf N concentration at tasseling for all locations regardless of hybrid or soil type. The range of r^2 values was from 0.79 to 0.90 with the exception of the Fay 1 location (Table 2). Relative leaf N concentration and relative yield were used to combine data over locations (Fig. 4). Relative values removed differences in hybrid response, soil conditions, and environment resulting in a highly linear relationship ($r^2 = 0.86$).

Relationship Among DGCI, SPAD, and Leaf Nitrogen Concentration in 2009

For all locations, DGCI and SPAD agreed well with r^2 values ranging from 0.72 to 0.95 (Table 3). The ability to use calibration standards greatly increased the regression fit between SPAD and DGCI from 2008 (Table 2) to 2009 (Table 3). Relative values of DGCI and SPAD were combined over multiple locations for analysis (Fig. 5). The agreement among locations indicates that relative values lessen the effect of hybrid, soil type, or environmental conditions. Regression data for individual locations are listed in Table 3.

Values of DGCI agreed well with leaf N concentration for all locations with average r^2 values of 0.80 for DGCI and 0.83 for SPAD (Table 3). Relative DGCI (Fig. 6) response to relative leaf N concentration was closely associated ($r^2 = 0.70$) when combined over five different hybrids and multiple soil types. However, when relative leaf N concentration was less than 0.5 there was a poor association with relative DGCI (Fig. 6), and relative values were generally less than the trend line. These severely N deficient plants (circled values in Fig. 6) were delayed

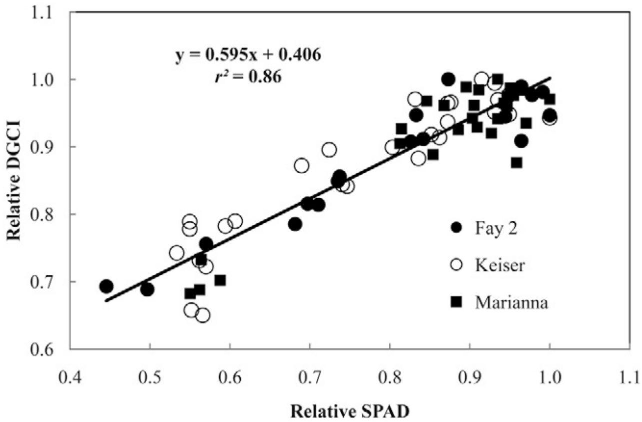

Fig. 1. Relationship in corn between relative dark green color index (DGCI) and relative chlorophyll meter (SPAD, soil plant analysis development) values for Marianna, Keiser, and Fayetteville Site 2 (Fay 2) in 2008. Relative values were calculated by dividing all values within a location by the highest value for that location. Measurements were made on the ear leaf at tasseling. Regression was significant at $P \leq 0.01$.

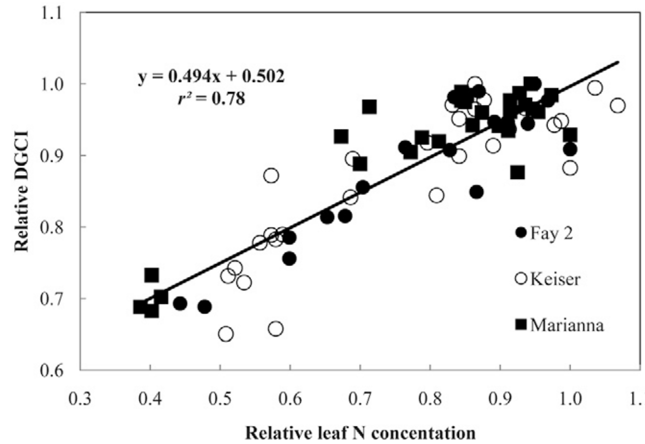

Fig. 2. The relationship in corn between relative dark green color index (DGCI) and relative leaf N concentration for Marianna, Keiser, and Fayetteville site 2 (Fay 2). Relative values were determined by dividing individual values within a location by the highest value for that location. Measurements of DGCI and leaf N concentration were made on the ear leaf at tasseling. Regression was significant at $P \leq 0.01$.

in development, and the responsiveness of DGCI to leaf N changes during ontogeny (Rorie, 2010).

Relationship of DGCI, SPAD, and Leaf Nitrogen Concentration to Yield in 2009

Measurements of DGCI and SPAD taken at tasseling were closely associated with yield with r^2 values ranging from 0.67 to 0.88 for DGCI, and from 0.56 to 0.90 for SPAD (Table 3). The two lowest r^2 values for DGCI and SPAD were from the Fay 1 location. At this location the field was sloped and plots were noticeably greener at the lower landscape position, resulting in high DGCI and SPAD values regardless of N treatment.

When yield was regressed against DGCI and SPAD, r^2 values averaged over all locations were 0.80 for both DGCI and SPAD (Table 3). Relative yield values were also regressed against relative DGCI for combined locations (Fig. 7). Although the relationship between yield and absolute values of DGCI for an individual location was linear (Table 3), relative yield values increased exponentially with increasing values of relative DGCI when data were combined over locations. Similar responses were observed for the relationship of relative yield and relative SPAD when combined across locations (data not shown).

DISCUSSION AND CONCLUSIONS

Digital images and image-analysis software were able to quantify color differences in corn leaves that had received different N treatments. This is consistent with previous work by Karcher and Richardson (2003) in which DGCI was used to accurately quantify turfgrass color that had received various N treatments. Values of DGCI were closely associated with leaf N concentration in 2009 for all locations (average r^2 = 0.80). Relationships of DGCI with leaf N, SPAD, and yield were generally stronger in 2009 than 2008 across locations because of the change in background color and the implementation of internal standards. Changing the background increased contrast and allowed standards to account for differences in lighting among locations (Rorie, 2010). The change in background color increased the r^2 values for the relationship between DGCI and leaf N concentra-

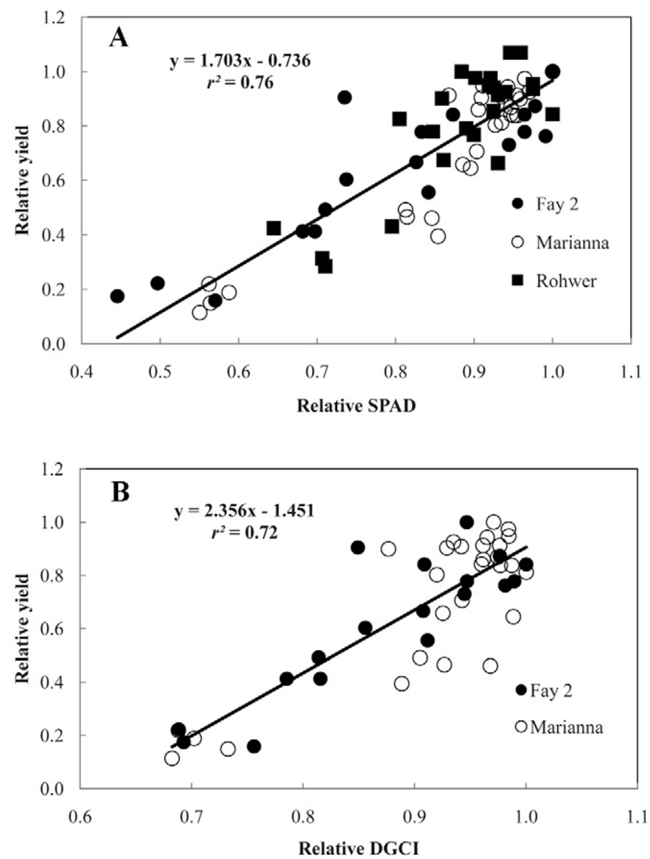

Fig. 3. The response of relative corn yield to relative dark green color index (DGCI) (A) or relative corn grain yield to relative chlorophyll meter (SPAD, soil plant analysis development) values (B) for leaves sampled at tasseling in 2008. Experiments were conducted at Fayetteville Site 2 (Fay 2), Marianna, and Rohwer. Relative values were determined by dividing individual values within a location by the highest value for that location. Measurements of DGCI and SPAD were made on the ear leaf at tasseling. Regressions were significant at $P \leq 0.01$.

Table 3. Regression results and sample size for the dark green color index (DGCI), leaf chlorophyll (SPAD, soil plant analysis development) values, leaf N concentration (Nc, g N 100g^{-1}), and grain yield (kg ha^{-1}) from corn experiments by location in 2009. All regressions were significant ($P \le 0.01$).

Location	DGCI vs. SPAD	DGCI vs. leaf Nc	SPAD vs. leaf Nc	Yield vs. DGCI	Yield vs. SPAD	Yield vs. leaf Nc
Fayetteville 1						
Slope	0.004	0.083	14.08	46,663	240.2	4795
Intercept	0.347	0.382	14.89	–19,825	–4436	–3999
r^2	0.72	0.74	0.67	0.66	0.56	0.75
n	40	40	40	40	40	40
Fayetteville 2						
Slope	0.007	0.138	21.17	27,217	206.6	4715
Intercept	0.247	0.28	1.07	–9099	–3038	–3675
r^2	0.95	0.80	0.92	0.83	0.90	0.87
n	18	18	18	18	18	18
Keiser						
Slope	0.008	0.165	18.79	28,340	244.5	5339
Intercept	0.195	0.214	2.596	–10,858	–5254	–6485
r^2	0.94	0.73	0.77	0.84	0.84	0.86
n	28	28	28	20	20	20
Marianna						
Slope	0.006	0.134	20.91	42,420	280.5	6138
Intercept	0.29	0.301	2.029	–17,127	–5207	–5221
r^2	0.93	0.86	0.92	0.88	0.86	0.87
n	12	12	12	12	12	12
Rohwer						
Slope	0.004	0.065	12.7	65,302	344.8	4871
Intercept	0.341	0.423	19.65	–29,055	–8440	–2705
r^2	0.82	0.85	0.87	0.81	0.83	0.90
n	28	28	28	28	28	28

tion at Rohwer from 0.18 in 2008 to 0.82 in 2009 for images taken in the same location under the same lighting conditions.

Chlorophyll meter values were closely associated with leaf N concentration in both 2008 (average $r^2 = 0.78$) and 2009 (average $r^2 = 0.83$) for all locations. This close relationship agrees with previous work by Rostami et al. (2008) who found an r^2 value of 0.84 for the relationship between SPAD and leaf N concentration in the ear leaf. Our SPAD measurements at tasseling agreed well with yield (average $r^2 = 0.79$) across both years. Scharf et al. (2002) found that N application to N deficient corn at tasseling positively affected grain yield; however, Binder et al. (2000) found that to avoid substantial yield losses, N must be applied before tasseling. It may be possible to use chlorophyll meters and digital color analysis to correct N deficiencies at, or before, tasseling; however, this was beyond the scope of our research.

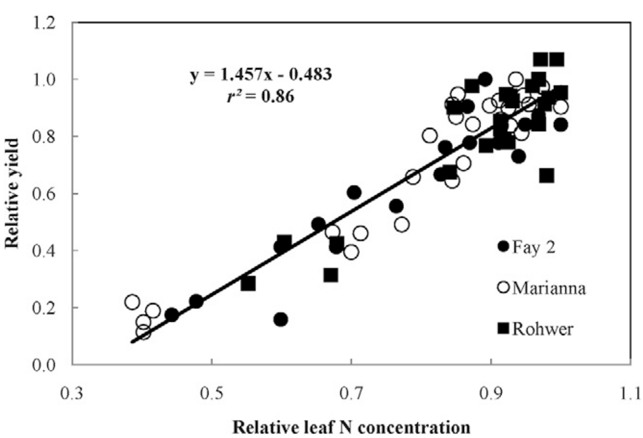

Fig. 4. Relative corn grain yield response to relative leaf N concentration for Marianna, Rohwer, and Fayetteville site 2 (Fay 2) in 2008. Relative values were determined by dividing individual values within a location by the highest value for that location. Leaf N measurements were made on the ear leaf at tasseling. Regression was significant at $P \le 0.01$.

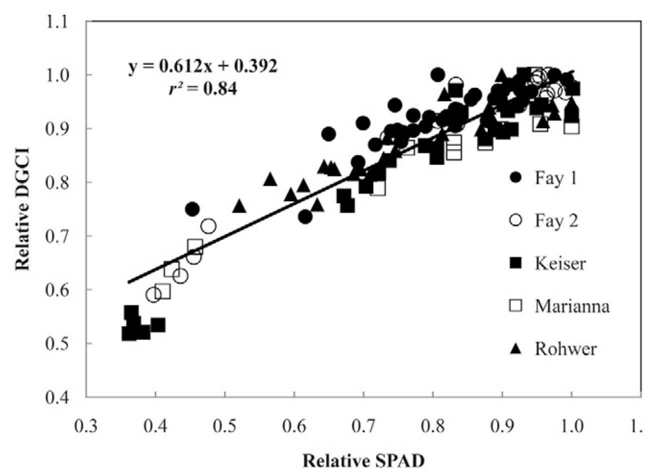

Fig. 5. Relationship in corn between relative dark green color index (DGCI) and relative chlorophyll meter (SPAD, soil plant analysis development) measurements for Fayetteville Sites 1 and 2 (Fay 1 and Fay 2), Keiser, Marianna, and Rohwer in 2009. Relative values were calculated by dividing individual values in a location by the highest value for that location. Measurements were made on the ear leaf at tasseling. Regression was significant at $P \le 0.01$.

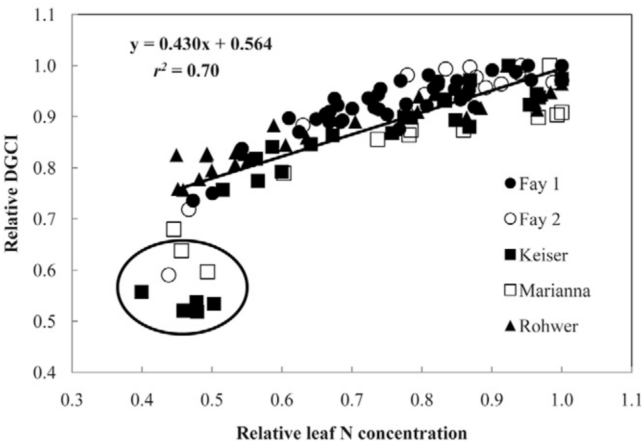

Fig. 6. Relative dark green color index (DGCI) response in corn to relative leaf N concentration. Relative values were determined by dividing individual values within a location by the highest value for that location. Experiments were conducted at Fayetteville Sites 1 and 2 (Fay 1 and Fay 2), Keiser, Marianna, and Rohwer in 2009. Circled data points in the figure were excluded from regression. Measurements were made on the ear leaf at tasseling. Regression was significant at *P* ≤ 0.01.

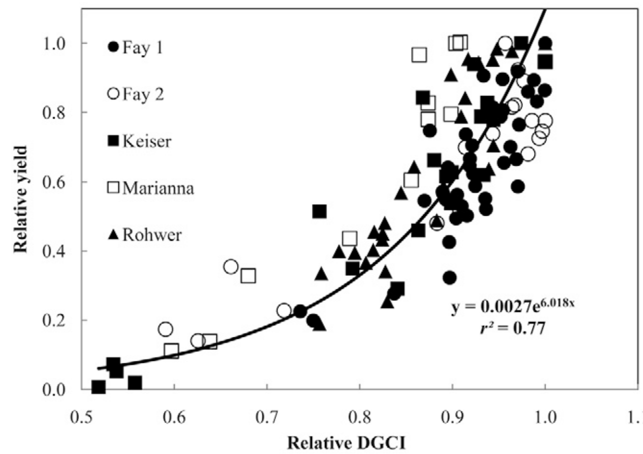

Fig. 7. Response of relative corn grain yield to relative dark green color index (DGCI) sampled for experiments conducted at Fayetteville Sites 1 and 2 (Fay 1 and Fay 2), Keiser, Marianna, and Rohwer in 2009. Relative values were determined by dividing individual values within a location by the highest value for that location. Measurements of DGCI were made on the ear leaf at tasseling. Regression was significant at *P* ≤ 0.01.

In general, DGCI values were linearly associated with SPAD (average $r^2 = 0.87$) measurements and yield (average $r^2 = 0.80$) for samples taken at tasseling. This relationship indicates that DGCI may be used similar to SPAD measurements to assess N status of corn. Zhang et al. (2008) found that SPAD had difficulty in estimating N status when chlorophyll levels were near optimum or above. Our results showed that the association of grain yield with relative DGCI (and SPAD) values became more variable as DGCI values became large (Fig. 7), indicating that factors other than leaf N concentration may have had a greater impact on grain yield. Blackmer and Schepers (1995) suggested that the plateau reached by chlorophyll meters at high N levels show that nutrients other that N are affecting chlorophyll production and the sensitivity of SPAD to detect N luxury consumption permits the producer to set a limit to the level of N required for maximum yield.

Unlike SPAD and reflectance measurements, digital-image analysis would require no specialized equipment other than a digital camera. Producers could take pictures of corn leaves alongside of calibration disks. Pictures from a high-N strip and other areas of interest could be uploaded to a server or sent to a researcher for relative DGCI determination at little cost and with a quick response.

Leaf "greenness," however, may be affected by many factors other than leaf N concentrations such as environment, disease, hybrid, or deficiencies of nutrients other than N. A preplant soil test would be needed to eliminate deficiencies of nutrients besides N, and high-N reference strips would need to be established in-field to calibrate DGCI values with yield and aid in reaching an economically optimum N fertilizer rate on a site-specific basis. Additional research is required to determine the specific growth stages at which DGCI would be most useful to determine economical optimum N rates to correct for N deficiencies and to calibrate the amount of N needed to prevent potential yield losses.

ACKNOWLEDGMENTS

The authors extend appreciation to Marilynn Davies, Mike Duren, and Scott Hayes for excellent technical assistance and to Claude Kennedy for his support.

REFERENCES

Binder, D.L., D.H. Sander, and D.T. Walters. 2000. Maize response to time of nitrogen application as affected by level of nitrogen deficiency. Agron. J. 92:1228–1236.

Blackmer, T.M., and J.S. Schepers. 1995. Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. J. Prod. Agric. 8:729–733.

Blackmer, T.M., J.S. Schepers, and G.E. Varvel. 1994. Light reflectance compared with other nitrogen stress measurements in corn leaves. Agron. J. 86:934–938.

Blinn, C.R., A. Lyons, and E.R. Buckner. 1988. Color aerial photography for assessing the need for fertilizers in loblolly pine plantations. South. J. Appl. For. 12:270–273.

Hawkins, J.A., J.E. Sawyer, D.W. Barker, and J.P. Lundvall. 2007. Using relative chlorophyll meter values to determine nitrogen application rates for corn. Agron. J. 99:1034–1040.

Huete, A.R. 1988. A soil adjusted vegetation index (SAVI). Remote Sens. Environ. 25:295–309.

Jia, L., X. Chen, F. Zhang, A. Buerkert, and V. Roemheld. 2007. Optimum nitrogen fertilization of winter wheat based on color digital camera images. Commun. Soil Sci. Plant Anal. 38:1385–1394.

Karcher, D.E., and M.D. Richardson. 2003. Quantifying turfgrass color using digital image analysis. Crop Sci. 43:943–951.

Kawashima, S., and M. Nakatani. 1998. An algorithm for estimating chlorophyll content in leaves using a video camera. Ann. Bot. (Lond.) 81:49–54.

Kitchen, N.R., K.A. Sudduth, S.T. Drummond, P.C. Scharf, H.L. Palm, D.F. Roberts, and E.D. Vories. 2010. Ground-based canopy reflectance sensing for variable-rate nitrogen corn fertilization. Agron. J. 102:71–84.

Kyveryga, P.M., A.M. Blackmer, and T.F. Morris. 2007. Alternative bench-marks for economically optimal rates of nitrogen fertilization for corn. Agron. J. 99:1057–1065.

Li, F., L. Liu, J. Wang, X. Li, C. Zhao, and W. Cao. 2005. Detection of nitrogen status in FCV tobacco leaves with the spectral reflectance. p. 25–29. *In* Geoscience and remote sensing symposium, IGARSS 2005 Proceedings. IEEE International, Seoul, Korea.

Pagola, M., R. Ortiz, I. Irigoyen, H. Bustince, E. Barrenechea, P. Aparicio-Tejo, C. Lamsfus, and B. Lasa. 2009. New method to assess barley nitrogen nutrition status based on image colour analysis comparison with SPAD-502. Comput. Electron. Agric. 65:213–218.

Piekielek, W.P., and R.H. Fox. 1992. Use of a chlorophyll meter to predict sidedress N requirements for maize. Agron. J. 84:59–65.

Piekielek, W.P., R.H. Fox, J.D. Toth, and K.E. MacNeal. 1995. Use of a chlorophyll meter at the early dent stage of corn to evaluate N sufficiency. Agron. J. 87:403–408.

Purcell, L.C., J.T. Edwards, and K.R. Brye. 2007. Soybean yield and biomass responses to cumulative transpiration: Questioning widely held beliefs. Field Crops Res. 101:10–18.

Ritchie, S.W., J.J. Hanway, and G.O. Benson. 1993. How a corn plant develops. Iowa State Univ. Coop. Ext. Serv. Spec. Rep. 48. Ames, IA.

Rorie, R.L. 2010. Characterizing leaf N with digital images in corn and the association of "greenness" with yield. M.S. thesis. Univ. of Arkansas, Fayetteville.

Rostami, M., A.R. Koocheki, M.N. Mahallati, and M. Kafi. 2008. Evaluation of chlorophyll meter (SPAD) data for prediction of nitrogen status in corn (*Zea mays* L.). Am.-Eurasian J. Agric. Environ. Sci. 3:79–85.

Scharf, P.C., S.M. Brouder, and R.G. Hoeft. 2006. Chlorophyll meter readings can predict nitrogen need and yield response of corn in the north central U.S. Agron. J. 98:655–665.

Scharf, P.C., and J.A. Lory. 2002. Calibrating corn color from aerial photographs to predict sidedress nitrogen need. Agron. J. 94:397–404.

Scharf, P.C., W.J. Wiebold, and J.A. Lory. 2002. Corn yield response to nitrogen fertilizer timing and deficiency level. Agron. J. 94:435–441.

Tracy, P.W., S.G. Hefner, C.W. Wood, and K.L. Edmisten. 1992. Theory behind the use of instantaneous leaf chlorophyll measurements for determining mid-season cotton nitrogen recommendations. p. 1099–1100. *In* D.J. Herber and D.A. Richter (ed.) Proc. Beltwide Cotton Conf., National Cotton Council of America, Memphis, TN.

Zhang, J., A.M. Blackmer, J.W. Ellsworth, and K.J. Koehler. 2008. Sensitivity of chlorophyll meters for diagnosing nitrogen deficiencies of corn in production agriculture. Agron. J. 100:543–550.