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Evaluation of Dark Green Color Index Technology as a Method of Real-time In-season Maize Nitrogen Measurement and Fertilization

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Evaluation of Dark Green Color Index Technology as a Method of Real-time In-season Maize Nitrogen Measurement and Fertilization

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Crop, Soil, and Environmental Sciences

By

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This thesis is approved for recommendation to the graduate council.

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Abstract

Nitrogen (N) is one of the most limiting factors for maize (*Zea mays* L.) production worldwide. Over-fertilization of N may decrease yields and increase NO$_3^-$ contamination of water. The Dark Green Color Index (DGCI) is a quantitative measure of greenness that is closely related to leaf N concentration. Previous research determined that DGCI values from maize at V6 to V10 could be used to predict the amount of N fertilizer to recover 90 or 95% of the potential yield. These DGCI algorithms were used by Spectrum Technologies Company to develop a smartphone application (app-method) for predicting maize N needs. Our objectives were to (1) evaluate the N recommendations based upon DGCI values made at V6 to V10 using the app-method and the previously developed method using a digital camera (camera-method) with standard N recommendation from the University of Arkansas Cooperative Extension Service (246 kg ha$^{-1}$); (2) determine if DGCI values made by the app-method agreed with values determined by the camera-method; and (3) identify potential sources of error leading to discrepancies between the camera- and the app-methods for determining DGCI. Field and laboratory experiments were conducted to answer these objectives. When residual soil N was high and the crop was unresponsive to additional N fertilizer, the app still recommended additional N to be applied. The evaluation of the camera-method at low- and medium-residual N soils showed that DGCI predicted significantly less N than the standard recommendation without affecting yield potential. There was a relatively poor agreement between DGCI values made on the same leaves, resulting in large differences in the recommended amounts of N to apply using the camera- and the app-methods ($r^2=0.52$, 0.67). Two major sources were identified that were responsible for the discrepancies between the camera and the app-methods. First, the DGCI values of the internal standards used by the two methods were different from their reported nominal values. Second, the camera-method uses the entire leaf blade in calculating DGCI
whereas the app calculates DGCI from only a small portion of the leaf. Dissection of leaf blades indicated that N concentration increased from the basal to the apical portion of leaves and that DGCI values were different across the leaf blade. Future research should improve the relationship between the camera- and the app-methods in predicting DGCI values.
ACKNOWLEDGMENTS

I would like to thank Dr. Larry Purcell for putting his faith in me and giving me this golden opportunity to study under his supervision. Sincerely, without Dr. Purcell, I could not make this big step towards getting my master's in the U.S.

Also, I would like to express my deepest gratitude to Dr. Trenton Roberts, and for his tremendous help in this project.

Likewise, to Dr. Andy King and Ms. Marilynn Davies, you were an inspiration for me.

Hua, Chester, Avi, Marianna, Montse, and Pedro: we made a great team.

This thesis is dedicated to my Mother Fatima, my Father M'hammed and my two sisters Maryame and Nouria.
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Chapter 1

Introduction
Maize (Zea mays L.) is an important crop in the U.S. with an estimated production area of 39.4 million hectares in 2013 and an average yield of 9.34 Mg ha\(^{-1}\) during the last 10 growing seasons (USDA, 2013). To achieve high yields, fertilization is a critical factor, and nitrogen (N) is considered essential in plant mineral nutrition (Shapiro and Wortmann, 2006). Currently, more than half of the world’s population depends on food production made possible by the use of N fertilizers, especially in cereal crops (Ladha et al., 2005).

Nitrogen plays an important role in cellular metabolism. The primary functions of N are to provide amino groups in amino acids as well as to contribute in the biochemistry of many non-protein compounds such as co-enzymes, photosynthetic pigments, secondary metabolites, and polyamines (Maathuis, 2009). Studies have established a strong correlation between N content and photosynthetic activity in plant cells (Wullschleger, 1993; Maathuis, 2009).

Nitrogen fertilization is a crucial factor in improving crops yields. However, over-fertilization of N does not improve crop yield and can reduce crop profitability by wasting fertilizer and leading to pollution of ground and surface waters (Hallberg, 1989; Cox et al., 1993). Raun and Johnson (1999) and Ladha et al. (2005) reported that around 67% of N fertilizer used in cereal production worldwide was lost due to denitrification, surface runoff, volatilization, and leaching. For example, the global annual amount of nitrous oxide (NO) emissions from fertilized crops due to N denitrification and ammonia volatilization was about 3300 Mg, leading to a rise of greenhouse gases in the atmosphere (Stehfest and Bouwman, 2006; Savci, 2012).

Undoubtedly, under-estimating N fertilization needs may lead to low crop yields, and N uptake is very much affected by N fertilizer rates (Shapiro and Wortman, 2006; Halvorson et al., 2006). Nitrogen fertilization influences total N uptake as well as total crop biomass production. For example, N uptake in maize increased from approximately 40 kg N ha\(^{-1}\) to 95 kg N ha\(^{-1}\), and
grain yield in maize increased from 2.3 to 4.9 Mg ha\(^{-1}\) when 120 kg N ha\(^{-1}\) was applied compared to the no-N control (Abassi et al., 2010, 2012, 2013). Shapiro and Wortman (2006) determined that yield of irrigated maize increased with increasing N applications, until maize production plateaued, and then decreased.

The challenge today is to improve the efficiency of fertilizer use to avoid the adverse environmental impacts of N fertilizer. Several methods are used to improve N use efficiency (NUE), which is the ratio of grain yield to N supply (kg grain kg-N\(^{-1}\)) (Bellido, 2001). Some of the techniques rely on agronomic practices, such as choosing the appropriate N fertilizer source, correct timing of N application, placement of N application, and the rate of N fertilizer (Abbasi et al., 2013). Some scientists have developed different strategies to reduce NO\(_3\)\(^-\) leaching and recommended N fertilization rates based on potential maize yield, soil C and N mineralization, NUE’s and N response trials (Schepers et al., 1995; Setiyono et al., 2011).

A different approach to improve NUE is the use of remote sensing tools to assess plant N and to formulate N recommendations based on leaf chlorophyll concentration or leaf greenness. For example, Samborski et al. (2009) summarized a general review of reflectance sensors like the Chlorophyll Meter, the Green Seeker, the Crop Circle, the Fieldscan, and the Dualex, and reported the advantages and limitations of each sensor in assessing plant N status. Others like Pagola et al. (2009) and Rorie et al. (2011a) determined that color image analysis from a digital camera was an effective tool to estimate plant N status. However, the digital camera technology requires more research to establish reliable N recommendations.

Purcell et al. (2015) developed algorithms for N fertilization based on color image analysis, and this approach has been utilized in an app for iDevices called Greenindex+ that is marketed by Spectrum Technologies (http://www.specmeters.com/nutrient-
management/chlorophyll-meters/chlorophyll/greenindex/). One objective of this research was to evaluate the accuracy of the Greenindex+ app in recommending appropriate N quantities to achieve maize maximum yield. The focus of this review is to describe the maize N mineral nutrition and give an overview on sensor tools for assessing maize plant N status.

Maize Nitrogen Mineral Nutrition

Nitrogen metabolism in the plant

Nitrogen stimulates root growth, and the uptake of other nutrients (Brady, 1990; Oikeha et al., 1999; Maathuis, 2009). Generally, 85% of the total N in plants is associated with proteins (Barker and Bryson, 2007). Nitrogen is taken up by the plant as NO$_3^-$ and NH$_4^+$, which are considered mineral forms and are referred to as residual soil N. Once NO$_3^-$ is absorbed, it is either stored in vacuoles or reduced to NH$_4^+$ via the intervention of two enzymes: NO$_3^-$ reductase, which converts NO$_3^-$ to NO$_2^-$, and NO$_2^-$ reductase, which reduces NO$_2^-$ to NH$_4^+$ (Evans et al., 1953). Ammonium is assimilated through glutamine synthethase and metabolized into amino acids and amides (Baron et al., 1994; Lea and Ireland, 1999).

The concentration of N in the plant has a strong relationship with its growth, and N deficiencies may alter or inhibit plant growth. The plant is unable to achieve its maximum growth if the concentration of any nutrient in tissue is below the critical concentration. Nitrogen excess leads to a phenomenon called luxury consumption. The excess of N does not contribute to an increase of yield because it is not metabolized by the plant into functional or structural compounds. The sufficiency for N in leaves is generally between 2.5 to 3.2 g N 100 g$^{-1}$ in maize at late growth stages (Barker and Bryson, 2007).

Nitrogen deficiency decreases leaf area formation, and as a consequence reduces the amount of solar radiation intercepted (Westgate et al., 2004; Leikam et al., 2010). Nitrogen
requirements are moderate at early maize growth stages but increases greatly after the V8 stage. By VT or tasseling, maize has accumulated approximately 76 % of the N found in mature plants (Table 1-1, Leikman et al., 2010). Although young maize plants need relatively small amounts of N, deficiency at early growth stages should be avoided, since yield components in maize are set between V6 and V8 (Barker and Bryson, 2007).

**Nitrogen in soils**

The total N concentration of surface mineral soils generally ranges from 0.02 to 0.5%, which is around 3.3 Mg ha⁻¹. Most of the N, however, remains in organic forms with only around 2 to 3% of the organic-N mineralized throughout the growing season. The primary N cycle reactions or transformations identified in the soil are (Brady, 1990):

- **Biological N fixation**: is a process by which N₂ in the atmosphere is converted into NH₄⁺.
- **Mineralization**: is the conversion of organic-N to NH₄⁺.
- **Nitrification**: The process of the transformation of NH₄⁺ into NO₃⁻.
- **Immobilization**: This is the conversion of mineral N (NH₄⁺ and NO₃⁻) to organic N.
- **Volatilization**: This is the transformation of CO(NH₂)₂ to NH₃ gas.
- **Denitrification**: This is the reduction of NO₃⁻ to NO and N₂.

Mineralization and immobilization rate depends on the chemical composition of the residue, specifically the C/N ratio of the residue (Bruun et al., 2006; Clément et al., 1995). A C/N ratio greater than 20 leads to the immobilization of the residual N pool while a ratio less than 15 leads to the mineralization of the organic N pool. For example, wheat (*Triticum aestivum*) straws are slow to mineralize (C/N = 80 to 90) in comparison to green leaves of oilseed rape (*Brassica napus*) or maize (*Zea mays*) (C/N = 10 to 15) which mineralize rapidly.
Ammonia is subject to loss due to volatilization especially when urea is applied to moist soils. Also, "NO₃⁻" is susceptible to denitrification and leaching that occurs in saturated soil conditions. The choice of fertilizer, application timing, and fertilizer placement method are important considerations to minimize N losses from the soil. For example, N losses are significant when fertilizers are applied at once without any partitioning and/or directly to the soil without any incorporation (Brady, 1990).

**Nitrogen management in maize**

Nitrogen management for maize production varies across the world due to differences in soil characteristics, weather conditions, hybrid availability, and yield expectations. In less industrialized regions, farmers use farmyard manure applied at a rate of about 15 Mg ha⁻¹ or apply urea as a top dressing at an average application rate between 5 and 25 kg N ha⁻¹ (Ransom et al., 2004). Sometimes farmyard manure is substituted by compost (8 Mg ha⁻¹) where available and others broadcast urea or ammonium sulfate at around 80 to 100 kg N ha⁻¹ at the time of planting (Ransom et al., 2004). In Arkansas, Espinoza and Ross (2009) determined that in general, 1 kg N ha⁻¹ returned 50 kg grain ha⁻¹. They gave detailed requirements of N fertilizer needs based on soil texture and yield goal (Table 1-2 and Table 1-3).

Shepard et al. (2011) determined that 202 kg N ha⁻¹ was sufficient to achieve the optimal yield in silty clay loam and silt loam soils, but beyond this N rate, maize grain yield leveled off. Shapiro and Wortman (2006) found during a 2-year experiment that maize yield response to N fertilization in silty clay soils fit a quadratic model, and that the optimum N rate that gave the maximum yield was 198 kg N ha⁻¹ and 145 N kg ha⁻¹ for the first and the second years, respectively.
In situations of high residual N (between 46 and 92 kg NO$_3^-$ ha$^{-1}$ in 0-30 cm depth), maize does not respond to N fertilization (Halvorson et al., 2005). The same author identified maize as a crop which quickly reduces residual N over years. Residual N in a soil profile of 30 cm may drop around 87% after 4 years of consecutive maize.

On the other hand, N application greatly affects NUE in maize (Samborski et al., 2009). Abbasi et al. (2013) determined that providing one half of the total N at sowing and the other half at V6 decreased N losses by denitrification, immobilization and leaching. In comparison to a single pre-plant N application, N plant uptake and harvest index increased by 16 and 39%, respectively. This study confirmed research by Gehl et al. (2005) who determined that a split application (one third at planting and two thirds as sidedress) was enough to maximize yield and increase NUE. Slow or controlled-release fertilizer and placement of N more precisely in the soil can also improve NUE (Samborski et al. 2009).

Pre-sidedress nitrate testing (PSNT) is another way to predict N requirements and potentially minimize NO$_3^-$ losses. This test measures soil NO$_3^-$ levels in the top 30 cm when maize plants are 30-cm tall (Heckman et al., 1995). Pre-sidedress nitrate testing is a useful tool since it can predict in-season, sidedress N requirement early in the growing season, especially on manured soils or soils that have received large applications of fertilizer (Hartz, 2006 ; Samborski et al., 2009). Pre-sidedress nitrate testing may reduce the amount of NO$_3^-$ leached by predicting small N quantities required, hence, contributing to a reduction of ground water pollution (Guillard et al., 1999). However, PSNT has some constraints such as high labor requirements as well as short timing between soil testing and the time that fertilizer should be applied, which has slowed down the adoption of this technique by farmers (Ma et al., 2007).

**Evaluating in-season N requirements using sensors**
In recent years, several different sensors have been used to assess plant N status. The chlorophyll, or SPAD meter, estimates chlorophyll concentration in the leaf, which is correlated with leaf N concentration (Dwyer et al., 1997). The SPAD meter calculates the red light absorbed by the leaf. The more absorbed red light, the greater the chlorophyll concentration in the leaf. The relationship between the SPAD meter values and leaf chlorophyll concentration is curvilinear following either exponential or polynomial functions (Markwell et al., 1995).

Hence, the chlorophyll meter is a promising tool in leaf N assessment (Dwyer et al. 1997), and identifies N deficiencies in plants (Piekielek and Fox, 1992; Blackmer and Schepers, 1995). Varvel et al. (1997) determined that to improve chlorophyll meter efficiency in predicting sidedress N needs (based on sufficiency index), maize N deficiency must be avoided during early growth stages. The best time for SPAD measurements for maize is V6, at which time chlorophyll meter readings are well correlated with leaf N concentration (Blackmer and Schepers, 1994; Piekielek et al., 1997). Unfortunately, the chlorophyll meter presents some limitations as it is expensive ($2500), has only a small sampling area of 6 mm$^2$, and does not have the ability to detect small deficiencies (Blackmer and Schepers, 1995; Zhang et al., 2008).

The Normalized Difference Vegetation Index (NDVI) is also a measurement that has been used extensively to assess N status in maize and other crops (Raun et al., 2002; Mullen et al., 2003). For instance, Crop Circle (Holland Scientifc, Lincoln NE) has the ability to predict N rates to correct in-season deficiency in corn using NDVI (Goulas et al., 2004; Varvel et al. 2007). An NDVI measurement occurs at two wavelengths; the first wavelength is associated with chlorophyll adsorption and the second wavelength is unabsorbed. An NDVI number is the result of a comparison between the two measurements (Scharf and Lory, 2002). There is a strong correlation between NDVI values and N concentration in the canopy (Schlemmer et al., 2013). In
addition, Mullen et al. (2003) determined a high correlation between NDVI and grain yield of wheat.

Two types of NDVI instruments exist: those with passive and those with active sensors. Passive-sensor instruments rely on natural energy that is reflected or emitted from the observed scene to calculate NDVI, and external sunlight is the most common external source of radiation used by passive NDVI sensors. Passive NDVI sensors are not hyper-spectral although most hyper-spectral sensors are passive. On the other hand, NDVI instruments with active sensors are equipped with light-emitting components that provide energy to illuminate the object they observe. Active sensors can be used independently of solar radiation but can only deal with specific wavelengths according to the type of light sensors (Hatfield et al., 2008).

NDVI is well correlated with plant N uptake, but it is prone to saturation with high N concentration or with LAI exceeding 2.5 to 3 (Freeman et al., 2007). Also, canopy cover affects directly the reflectance for N concentration. NDVI assessment for maize leaf N concentration is biased when canopy cover is less than 100% (Barnes et al., 2000). A comparison between an active sensor and many passive sensors reported that the active NDVI sensor was less effective than the passive NDVI sensor in estimating green cover of wheat ($r^2=0.9$ for the passive sensors vs $r^2=0.8$ for the active sensor) (Fitzgerald, 2010). In maize, for a maximum of effectiveness of sidedress N application, measurements using an active sensor should be made from V8 to V12 (Martin et al., 2007).

The Dualex (Dynamax Inc, Houston TX) sensor is also used to assess N status in the plant by calculating the amount of polyphenolics in the leaf, which are associated with leaf N concentration. Sensors using near infrared radiance were able to predict optimum N rate for maize at tasseling but not at early growth stage in maize (Sripada et al., 2005, 2006). In contrast,
Scharf and Lory (2002) determined a strong linear relationship between economic optimum N rate for maize and near infrared radiance at V7, when soil pixels were removed from the pictures.

Another technology that may give an appropriate way to assess plant N status is the intensity of green color from digital images of leaves. The camera records the image, and special software analyzes the image by measuring the greenness of the leaf based on red, green, blue (RGB) colors of the picture. The RGB values are converted into hue, saturation and brightness values using a method described by Karcher and Richardson (2003) to calculate the Dark Green Color index (DGCI).

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

The resulting DGCI value is on a scale from 0 (very yellow) to 1 (dark green). Karcher and Richardson (2003) and Rorie et al. (2011a,b) found DGCI an accurate tool to estimate N leaf concentration in turfgrass and maize, respectively.

**Previous research performed at the University of Arkansas**

Rorie et al. (2011a) quantified maize leaf greenness, with a digital camera and image-analysis software (Sigma Scan Pro 5, SPSS, 1998, San Jose) in terms of DGCI by modifying the digital-imaging method of Karcher and Richardson (2003). Rorie et al. (2011b) photographed the ear leaf of maize at tasseling against a pink board (to provide contrast in subsequent image processing) that had two internal color standards disks with known DGCI values (0.5722 for the green disk and 0.073 for the yellow disk). Color standards were included in each image to allow corrections of variations due to different conditions of light. This analysis established close relationships between leaf DGCI, leaf N concentration, SPAD and yield.

In subsequent research, Purcell et al. (2015) developed a calibration curve for the amount of N to be applied at midseason to recover 90 or 95% of maximum yield based upon DGCI.
values made using a digital camera, measured at V6-V10 from the uppermost collared leaf. Based upon algorithms from Purcell et al. (2015), Spectrum Technology (http://www.specmeters.com/nutrient-management/chlorophyll-meters/chlorophyll/greenindex/) developed an iPhone app called Greenindex+ that assessed leaf maize DGCI and determined the recommended amount of N to achieve 90-95% of maize maximum yield. Chapter 2 evaluates the N recommendations of Purcell et al. (2015) based upon DGCI values made at V6 to V10 using a digital camera and the Greenindex+ app while Chapter 3 compares DGCI values made by the Greenindex+ app with values determined by the method of Purcell et al. (2015) using a digital camera. Further research considers possible reasons for discrepancies between the method of Purcell et al. (2015) and the Greenindex+ app.


Table 1-1. Accumulation of N in maize throughout the season (Leikam et al., 2010).

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Days after emergence</th>
<th>% of N uptake of N amount applied</th>
<th>% of final N accumulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-leaf</td>
<td>32</td>
<td>0.81</td>
<td>&lt;1</td>
</tr>
<tr>
<td>8-leaf</td>
<td>44</td>
<td>2.16</td>
<td>3</td>
</tr>
<tr>
<td>12-Leaf</td>
<td>59</td>
<td>5.6</td>
<td>9</td>
</tr>
<tr>
<td>Tassel</td>
<td>72</td>
<td>15.33</td>
<td>23.9</td>
</tr>
<tr>
<td>Silk</td>
<td>84</td>
<td>14.18</td>
<td>38.7</td>
</tr>
<tr>
<td>Blister</td>
<td>108</td>
<td>15.4</td>
<td>54.1</td>
</tr>
<tr>
<td>Early dent</td>
<td>139</td>
<td>23.17</td>
<td>77.3</td>
</tr>
<tr>
<td>Maturity</td>
<td>144</td>
<td>23.31</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1-2. Fertilizer recommendations (kg ha\(^{-1}\)) for maize grown in Sandy Loams or Silt Loams, based on yield goal (Espinoza and Ross, 2009).

<table>
<thead>
<tr>
<th>Yield Goal Kg ha(^{-1})</th>
<th>Recommended N, kg ha(^{-1})</th>
<th>kg yield kg(^{-1}) N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 7875</td>
<td>134</td>
<td>69</td>
</tr>
<tr>
<td>9450</td>
<td>168</td>
<td>56</td>
</tr>
<tr>
<td>11025</td>
<td>202</td>
<td>55</td>
</tr>
<tr>
<td>12600</td>
<td>235</td>
<td>54</td>
</tr>
<tr>
<td>14175</td>
<td>268</td>
<td>53</td>
</tr>
</tbody>
</table>
Table 1-3. Fertilizer recommendations (kg ha\(^{-1}\)) and nitrogen use efficiency (NUE) for maize grown in silty clays, silty clay loams, and clays, based on yield goal (Espinoza and Ross, 2009).

<table>
<thead>
<tr>
<th>Yield Goal kg ha(^{-1})</th>
<th>Recommended N kg ha(^{-1})</th>
<th>NUE kg kg(^{-1}) N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 6300</td>
<td>140</td>
<td>45</td>
</tr>
<tr>
<td>7560</td>
<td>196</td>
<td>39</td>
</tr>
<tr>
<td>8820</td>
<td>252</td>
<td>35</td>
</tr>
<tr>
<td>10080</td>
<td>336</td>
<td>30</td>
</tr>
</tbody>
</table>
Chapter 2

Characterization of Dark Green Color Index (DGCI) technology to predict N requirements for maize
Abstract

Nitrogen fertilizer recommendations for maize (Zea mays L.) should provide adequate rates to ensure high yields but should not recommend more than is economical and environmentally sound. In previous research, the intensity of greenness from digital images of maize leaves was closely associated with leaf N concentration. The so-called dark green color index (DGCI) was used to develop algorithms that predicted N fertilizer needs from leaves of maize at the V6 to V10 development stage using a digital camera (camera-method). These algorithms were used by Spectrum Technologies to develop a smartphone app (app-method). The objective of this study was to evaluate these algorithms, using the app-and the camera-methods with N recommendations from the University of Arkansas Cooperative Extension Service (standard recommendation). In one experiment, the app-method was compared to the standard recommendation on a silt loam soil with high residual soil-N. There were no significant differences in yield between treatments receiving 0 kg N ha\(^{-1}\) and treatments receiving the standard and the app recommendations at V6, V8, V10 and V12. Although there was no need for N, the app recommendations averaged 177 kg N ha\(^{-1}\) over the V6 to V12 growth stages versus 246 kg N ha\(^{-1}\) for the standard recommendation. Additional experiments in fields with low and medium residual-N resulted in no significant differences in yield between recommendations for N using the camera-method and the standard recommendations. The camera-method averaged about 112 and 92 kg N ha\(^{-1}\) less than the standard extension recommendations in low residual-N soils. The camera-method has shown great potential in predicting appropriate N recommendations on silt loam soils. Future research should further evaluate the camera-method recommendations under a wide range of environmental conditions.
INTRODUCTION

Nitrogen (N) is an essential element for plant mineral nutrition, implicated in many metabolic processes for plant growth and development (Barker and Bryson, 2007). Nitrogen availability is a primary factor limiting yield in maize production (Maathuis, 2009). Maize N uptake varies across sites, depending on soil texture and structure, type of genotypes adopted, and soil-N management. Setiyono et al. (2010) determined that maize needs 1 kg of N to produce 40 to 83 kg of grain, whereas, in China, Liu et al. (2010) reported the return of 39.5 kg of maize grain per 1 kg of N. Under-estimating N fertilization needs may lead to mediocre yields, but yield is not improved by over-fertilization. Currently, nitrogen use efficiency (NUE, kg grain kg⁻¹ N⁻¹) by cereal crops was estimated to be 33% worldwide (Raun and Johnson, 1999). Nitrogen losses due to over-fertilization might negatively impact the atmosphere due to ammonia volatilization and denitrification and may impact groundwater quality due to NO₃⁻ leaching. Excess N in the environment increases greenhouse gases, and can lead to greater NO₃⁻ concentration in potable water than the standards set by the United States Environmental Protection Agency (USEPA) (Mitsch et al., 2001; USEPA, 1998).

The rationale of N fertilization must be based upon optimizing and reducing the economic cost of N inputs (Tang et al., 2009). Plant tissue analysis is appropriate in estimating plant N needs throughout the growing season. However, tissue analysis, when done properly, presents the problem of being expensive and time consuming (Adeptu et al., 2000). Over the last half-century considerable time and money have been spent on studies focused on developing strategies to increase NUE. Mulvaney et al. (2001) developed a methodology to classify soils, according to amino sugar fraction in the soil profile (0-30 cm), as responsive (<200 mg kg⁻¹) or not (>250 mg kg⁻¹) to N fertilization. Although the method of Mulvaney et al. (2001) was
relevant for using N fertilizers wisely, it was criticized as being difficult to implement during a growing season.

The Corn Stalk Nitrate Test (CSNT) was developed at Iowa State University as a post-season analysis tool (Binford et al., 1990; Binford et al., 1992). This technique relies on the analysis of NO$_3^-$ concentration in the lower portion of the maize stalk at maturity that should range from 700 to 2000 mg NO$_3^-$ kg$^{-1}$ (Binford et al., 1992). The NO$_3^-$ concentration in the corn stalk is highly correlated with available-N in the soil, where excessive N in the soil will result in considerable NO$_3^-$ concentration in the maize stalk (Binford et al., 1990; Brouder et al., 2000). The CSNT is a valuable tool in helping to judge the efficacy of a N fertilization strategy adopted during the growing season and to decide about the N fertilization approach for the next growing season (Brouder et al., 2000), but it has several limitations. First, the CSNT is more sensitive to detect excessive N rate on soils with medium yield potential than on soils with high yield potential. Second, the CSNT results are biased in cases where maize is grown in rotation with a legume like alfalfa (*Medicago sativa*). Finally, the NO$_3^-$ in the stalk increases in dry years while it decreases in wet years (Laboski, 2010).

On the other hand, sensing tools such as the chlorophyll meter (Spectrum technologies, Aurora IL), or sensors that use the Normalized Difference Vegetation Index (NDVI) such as the Green Seeker (Trimble, Auburn AL), the Crop Circle (Holland Scientific, Lincoln NE), the Fieldscan (Process Sensor Corp, Milford MA) and the Dualex (Dynamax Inc, Houston TX), or color image analysis (Samborski et al., 2009) might play an important role in determining maize N requirements. The use of these tools could potentially reduce the negative impacts of excessive N on the environment (Samborski et al., 2009).
The chlorophyll, or SPAD meter, estimates chlorophyll concentration in the leaf, which is correlated with leaf N concentration (Dwyer et al., 1997). Unfortunately, the chlorophyll meter presents some limitations as it is expensive ($2500), has only a small sampling area of 6 mm², and does not have the ability to detect small deficiencies (Blackmer and Schepers, 1995; Zhang et al., 2008).

The NVDI is also a measurement that has been used extensively to assess N status in maize and other crops (Raun et al., 2002; Mullen et al., 2003). The NDVI is calculated as:

$$\text{NDVI} = \frac{(\text{NIR} - \text{VIS})}{\text{NIR} + \text{VIS}}$$  (Eqn. 1)

VIS is the spectral reflectance measurement in the visible red and NIR represents reflectance in the near-infrared regions. Mullen et al. (2003) determined the efficacy of NDVI measurements at Feekes 10.5 using a hand held sensor in estimating the percentage increase in yield obtained via N fertilization in wheat (Triticum spp.). The greatest mean recorded NDVI N treatment divided by mean NDVI of check treatments (RI_{NDVI}) was strongly correlated with the highest mean yield N treatment divided by mean yield of check treatments (RI_{Harvest}). If RI_{NDVI} was less than 1.1, N fertilization was not required. If RI_{NDVI} was greater than 1.1, N fertilization was recommended. The latter method was not tested in independent experiments. Also, NDVI sensors are expensive ($2000), which could slow their adoption by farmers.

The analysis of the greenness of digital images of a crop may also allow the assessment of leaf-N concentration. Previous research determined the effectiveness of color-image analysis of maize leaves, in assessing leaf-N concentration (Rorie et al., 2011b). Images were recorded using a digital camera and analyzed with software (Sigma Scan Pro 5, SPSS, 1998, San Jose) that measured the greenness of the leaf based on hue, saturation, and brightness (HSB) of the
picture. The HSB values were used to calculate a composite value of leaf greenness termed the Dark Green Color index (DGCI) (Karcher and Richardson, 2003).

\[
DGCI = \frac{(\text{Hue} - 60)/60 + (1 - \text{Saturation}) + (1 - \text{Brightness})}{3} \quad \text{(Eqn. 2)}
\]

The resulting DGCI value is on a scale from 0 (very yellow) to 1 (dark green). Rorie et al. (2011b) determined that maize leaf DGCI was linearly related to leaf N concentration and SPAD, demonstrating the capacity of DGCI to estimate maize plant N concentration. Based upon DGCI values, a calibration curve for the amount of N to be applied to recover 90 or 95% of maximum yield measured at V6-V10 from the uppermost collared leaf, was developed by Purcell et al. (2015). Using the algorithms of Purcell et al. (2015), Spectrum Technology (http://www.specmeters.com/nutrient-management/chlorophyll-meters/chlorophyll/greenindex/) developed a smartphone app that assesses leaf maize DGCI and the recommended amount of N to achieve 90-95% of maize maximum yield.

The DGCI technology using the app was developed to predict appropriate in-season N recommendations for maize. If functional, the app would be an effective tool for farmers to determine the amount of N needed by a maize crop. For example, the app-method might replace the N-response curves (relative yield versus N doses) which do not take into account field history, soil organic matter content, and precipitation (Abbasi et al., 2013), leading sometimes to biased N predictions for maize (Vanotti and Bundy, 1994).

The main objective of this study was to evaluate the N recommendations of Purcell et al. (2015) based upon DGCI values made at V6 to V10 using the app and using a digital camera with N recommendations (246 kg ha\(^{-1}\)) from the University of Arkansas Cooperative Extension Service (standard recommendation). In the remainder of this chapter, the method developed by
Purcell et al. (2015) will be referred to as the “camera-method” and the method developed by Spectrum Technologies for an iPhone will be referred to as the “app-method”.

MATERIALS AND METHODS

Image analysis

- Camera-method

The uppermost collared leaf from one plant per row was sampled from the middle two rows of each four-row plot at V6, V8, V10, and V12 (Ritchie et al., 1993). After, leaves were removed from the plant, each leaf was cut into pieces approximately 10-cm long, placed in plastic bags on ice, and transported to the laboratory. Leaves were placed against a pink wooden board (1m×1.5m) with two disks 11-cm in diameter. One disk was painted yellow (with a standard DGCI value of 0.0733) while the second disk was painted green (with a standard DGCI value of 0.5722). Disks played the role of internal standards and served for correcting differences in lighting conditions (Rorie et al., 2011a). It was assumed that the color of the green and the yellow disks on the pink wooden board matched exactly with the internal color standards of X-rite color paper (X-Rite Inc., Kentwood, MI) used by Purcell et al. (2015) and Rorie et al. (2011b). The colored disks served as internal standards for calibration and had known Munsell color values of 6.7 GY 4.2/4.1 for the green disk, and 5Y8/ 11.1 for the yellow disk.

Pictures of leaves were taken indoors under fluorescent lighting using a camera (Canon Power Shot S51S, Canon USA, Inc. Lake Success, NY) with an image size of 3264×2448 pixels. The camera was set to an ISO of 100, a shutter speed of 1/15s, an aperture of 2.0, exposure compensation of 0, and to fluorescent light balance with the flash turned off.

Images recorded by the camera were analyzed using a commercial software package (Sigma Scan Pro5, http://www.sigmaplot.com/products/sigmascan/sigmascan.php). Images were
saved as a joint photographic expert group (JPEG) format, and threshold ranges of hue (0-100) saturation (0-100) were set in Sigma Scan to allow for complete analysis of the entire leaf without including any of the background in the final leaf color value. Each image was processed using Sigma Scan Pro software to determine the HSB values using the algorithm described by Karcher and Richardson (2003). The HSB values of each picture were transformed to uncorrected DGCI or raw DGCI values using equation 2. The internal yellow and green color standards included in each image were used to correct raw DGCI values assuming the internal standards had DGCI values of 0.0733 (yellow disk) and 0.5722 (green disk) (Rorie et al., 2011b).

- **App-method**

  The DGCI measurements using the app-method were determined for the center portion of the uppermost collared leaf when maize was at the V6, V8, V10 or V12 growth stage. The same sampling procedure used for the camera-method was adopted for the app-method. The app Greenindex+ (Version 1, Spectrum Technologies, Inc) was installed on an iPhone 5 and used to take pictures of the sampled leaves (one leaf per plot) in field conditions under ambient lighting. The central portion of a leaf was placed against the GreenIndex+ board (30 cm × 40 cm). Included in the board were green and yellow disks, 7-cm diameter on a pink background. As described for the camera-method, disks played the role of standards to correct for variations in lighting conditions where the picture was taken, and disks were assumed to have DGCI values of 0.0733 and 0.5722 for the yellow and green disks, respectively (Purcell et al., 2015). The app screen displays the corrected leaf DGCI value, the leaf SPAD equivalent, and the suggested N fertilizer amounts required to achieve 90 or 95% of their yield potential based on methods of Purcell et al. (2015).

**App-method Evaluation Field Trial**
A field experiment was conducted at the University of Arkansas research station in Fayetteville, AR (36° 5' 54.8”N; 94° 10’ 15’’W) during 2013 on a Captina silt loam (Fine-silty, siliceous, active, mesic Typic Fragiudults). Prior to planting, soil analysis was performed to determine mg NO$_3^-$ kg$^{-1}$ soil using the KCl extraction method as described by Smith and Li (1993). Amino compounds concentration (µmol L$^{-1}$) in the soil were determined as outlined by Roberts et al. (2015, Personal communication). Soil samples were taken from 0-15 cm, 15-30 cm and 30-45 cm and the resulted mean of NO$_3^-$ and amino compounds concentration from the three depths is shown in Table 2-2. The previous crop was soybean (*Glycine max* (L.) Merr.,) and the experimental field was fertilized to meet soil test recommendations for all nutrients except N. During the previous year (2012), the field received 5 Mg ha$^{-1}$ of chicken litter before planting, providing 96 kg P ha$^{-1}$ and 144 kg K ha$^{-1}$. The field was disked followed by tillage using a field cultivator, and raised beds were formed 90-cm apart to allow furrow irrigation. Plots consisted of four rows, 91.5 cm apart and 9.15 m in length, which were seeded at a depth of 5 cm using a seeding rate of 84,030 kernels ha$^{-1}$. The maize hybrid used in this study was Mycogen 2V707. The hybrid was glyphosate [N-(phosphonomethyl) glycine] tolerant and included the *Bt* trait. The experiment was planted May 27$^{th}$ and emergence occurred within 5 days after planting. Weeds were controlled with atrazine (AAtrex; 1.8 kg ai ha$^{-1}$), S-metalochlor (Dual Magnum; 1.5 kg ai ha$^{-1}$), and glyphosate (Roundup Ultra; 1.12 kg ai ha$^{-1}$). The experiment was furrow-irrigated as needed to maintain adequate soil moisture using the hand-feel method (Klocke et al., 1998). At physiological maturity, a combine was used to harvest the middle two rows of each plot. Grain was weighed and yield was adjusted to 15.5% moisture.

The experiment was a randomized complete block design (RCBD) conducted in side-by-side fields that had either 0 or 67 kg N ha$^{-1}$ applied prior to planting and incorporated into the
soil. Treatments consisted of 10 rates and timings of season total N rates equaling: 0 kg N ha\(^{-1}\), 302 kg N ha\(^{-1}\), as well as the app-method and the standard recommendations for maize at V6, V8, V10 and V12 (Table 2-1). All treatments were applied as sidedress-N applications. The app-method predictions were based on results of DGCI at the respective growth stages (V6, V8, V10, and V12) while the standard recommendation were based upon a yield goal of 14 Mg ha\(^{-1}\) and a silt-loam soil texture using the standard recommendation as developed by the University of Arkansas extension service (246 kg N ha\(^{-1}\); Espinoza and Ross, 2011) (Table 2-1). All N treatments were hand applied as urea (460 g N kg\(^{-1}\)) coated with the urease inhibitor NBPT (N-(n-butyl) thiophosphoric triamide) and incorporated timely with irrigation or rainfall within 2 days.

The app-method recommendations were compared to the standard recommendation of 246 kg N ha\(^{-1}\) using the student one-sided t-test (De Veaux et al., 2013). The effect of growth stage and preplant N on the app-method recommendations and the effect of growth stage and treatments on yield and nitrogen use efficiency (NUE) were evaluated, using analysis of variance. NUE is defined as the quotient of grain yield and the total amount of N fertilizer applied to the crop (kg grain kg\(^{-1}\) N). Replication-preplant N were combined and classified as a random effect (i.e., error term) to reduce variability in the fixed effects, which were of primary interest. Tukeys HSD procedure was used to separate means for significant effect. Statistical analyses were performed using JMP PRO 11.0 (SAS Institute, Inc., Cary, NC).

**Camera-method Evaluation Field Trials**

Two field experiments were conducted, one at the University of Arkansas research station at Fayetteville on a Captina silt loam (Fine-silty, siliceous, active, mesic Typic Fragiudults) and one at the Pine Tree Branch Station near Colt, AR (35°3’ 30.8”N; 90°48’ 44.3”W) on a Calloway
silt loam (Fine-silty, mixed, active, thermic Aquic Fraglossudalfs) during the 2014 growing season. Soybean was the previous crop for both locations. Plots in Fayetteville were sown mid-April and consisted of four rows, 90 cm apart and 8 m in length, which were seeded at 86000 kernels ha\(^{-1}\). Agronomic practices were the same as the ones adopted in 2013. However, no chicken litter was applied to the field in the previous five years. The experiment at Pine Tree consisted of four rows, 70-cm apart and 7.6-m in length, which was also seeded at 86000 kernels ha\(^{-1}\). Similarly to the app-method evaluation, soil analysis was performed before planting using procedures discussed previously to determine mg NO\(_3\) kg\(^{-1}\) soil and amino compounds of soil samples taken from 0 to 45 cm depth in Fayetteville and Pine Tree (Table 2-2). At both locations, Mycogen 2V707 was the maize hybrid used, which is glyphosate [N-(phosphonomethyl) glycine] tolerant and includes the Bt trait. The depth of sowing was approximately 5 cm; the two experiments were planted on April 24\(^{th}\) (Fayetteville) and April 27\(^{th}\) (Pine Tree), and emergence occurred 7 days after planting. Weeds were controlled with atrazine (AAtrex; 1.8 kg ai ha\(^{-1}\)), and glyphosate (RoundUp Ultra ; 1.12 kg ai ha\(^{-1}\)). Experiments were furrow-irrigated as needed to maintain adequate soil moisture using the hand-feel method (Klocke et al., 1998).

The Fayetteville site for the camera-method evaluation was different from that of the app-method. The Fayetteville and Pine Tree experimental designs were similar to the protocol adopted in 2013 to evaluate the app-method versus standard recommendation (246 kg N ha\(^{-1}\)), based upon a yield goal of 14 Mg ha\(^{-1}\) and a silt-loam soil texture (Espinoza and Ross, 2009) (Table 2-1). The experiment was a RCBD conducted in side-by-side fields that had either 0 or 67 kg N ha\(^{-1}\) applied to the field prior to planting. Treatments consisted of 10 rates and timings of N:
0 kg N ha\(^{-1}\), 302 kg N ha\(^{-1}\), and the recommendations of the camera-method and also standard extension recommendations of 246 kg N ha\(^{-1}\) at V6, V8, V10 and V12.

The camera-method recommendations were compared to the standard recommendation (246 kg N ha\(^{-1}\)) using the student one-sided t-test (De Veaux et al., 2013). The effect of growth stage and preplant N on the camera-method recommendations and the effect of growth stage and treatments on yield and NUE were tested, using analysis of variance. Tukeys HSD was used to separate means for significant effects. Replication-preplant N were combined and classified as a random effect (i.e., error term) to reduce variability in the fixed effects, which were of primary interest (Carmer et al, 1989). Statistical analyses were performed using JMP PRO 11.0 (SAS Institute, Inc., Cary, NC).

**Evaluation of N response**

The effect of preplant N and treatments on leaf N concentration at silking (R1) was tested for the camera-method experiments conducted at Fayetteville and Pine Tree in 2014. A random ear leaf was sampled from the two middle rows of each plot. Leaves were then dried at 65°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 Diagnostic Laboratory at the University of Arkansas (Fayetteville, AR). Tukeys HSD procedure was used to separate means for significance. Replication-preplant N were combined and classified as a random effect (i.e., error term) to reduce variability in the fixed effects. Statistical analyses were performed with JMP PRO 11.0 (SAS Institute, Inc., Cary, NC).

**RESULTS AND DISCUSSION**

**App-method evaluation**
The experiment conducted at Fayetteville to evaluate the app-method during 2013 was expected to have high residual-N (Table 2-2) since it received 5 Mg ha\(^{-1}\) of poultry litter in 2012 (the previous year before planting). Values of DGCI of the camera-method (DGCI\(_{\text{cam}}\)), DGCI of the app-method in the laboratory (lab DGCI\(_{\text{app}}\)), DGCI of the app-method in the field (field DGCI\(_{\text{app}}\)), leaf N concentration, recommended N and yield as averaged across year, location, preplant N and maize growth stage are shown in Appendix Table A. The analysis of variance of DGCI values measured by the app is shown in Appendix Table B and in Appendix Figure A.

The amount of N to apply using the app-method was not affected by the interaction between growth stage and preplant N (Table 2-3) but both of these main effects were significant. The amount of N recommended by the app-method increased from V6 (156 kg N ha\(^{-1}\)) to V8 (181 kg N ha\(^{-1}\)) and remained fairly constant for both V10 (184 kg N ha\(^{-1}\)) and V12 (186 kg N ha\(^{-1}\)) (Figure 2-1). Residual-N in the soil would be expected to decline over the crop’s life cycle (Halvorson et al., 2005) leading the app-method to predict greater N to apply at V8, V10 and V12 than at V6.

Before applying app-method recommendations, little visual difference in greenness at V6 was noticed between the maize plants in the 0 kg N ha\(^{-1}\) and the maize plants in the 67 kg N ha\(^{-1}\) preplant sites. Although the naked eye could not detect the subtle difference in greenness between maize from the two preplant sites, the app recommended (Table 2-3) 10 kg N ha\(^{-1}\) less for the plots that received preplant N than the plots that did not receive any preplant N application (Figure 2-1).

Analysis of variance of yield data showed an interaction between treatments and preplant N application factors (Table 2-4). There was no significant difference in yield among treatments within both the 67 kg N ha\(^{-1}\) and the 0 kg N ha\(^{-1}\) preplant sites. On the other hand, yield for
treatments consisting of the app-method and standard extension recommendations in the 67 kg N ha\(^{-1}\) preplant site was greater than the yield of the app-method and standard extension recommendations of no N preplant site (Figure 2-2). It is important to highlight that the app-method predicted about 90, 65, 62, and 60 kg N ha\(^{-1}\) less than the standard recommendation at V6, V8, V10 and V12, respectively, without compromising yield potential. The app-method recommendations were less than the standard extension recommendation (246 kg N ha\(^{-1}\)) with a mean difference of approximately 63 and 73 kg N ha\(^{-1}\) for the 0 and 67 N preplant sites, respectively.

Analysis of variance of NUE data at Fayetteville 2013 indicated an interaction between treatments and preplant N application factors (Table 2-5). There was a significant difference in NUE among treatments within both the 67 kg N ha\(^{-1}\) and 0 kg N ha\(^{-1}\) preplant sites (Figure 2-3). For 0 preplant N, the greatest NUE was recorded for app recommendation treatment at V6. App recommendations treatments were higher than the standard recommendation at all maize growth stage. For 67 preplant N, app recommendations were greater that standard recommendation in NUE only at V6.

Yield in the absence of N fertilizer was statistically similar to the yields where N fertilizer was applied, regardless of rate and the high NUE of the app recommendations. The app still recommended that N should be applied. Although N was not needed to maximize yield at this location, the app still recommended N fertilizer, which suggests that more research is needed to refine the N rate recommendations using the app on soils that have high residual N values. Our results agree with the findings of other research where yield response to N fertilization is not likely when critical NO\(_3\)^{-} concentration in the soil ranges between 20 and 30 mg NO\(_3\)^{-} kg\(^{-1}\) soil
(Roth et al., 1992; Sims et al., 1995; Mulvaney et al., 2001), which was the case of our experimental site (Table 2-2).

The scope of our study was to evaluate the app-method. Our experiment showed the app-method was unable to properly identify the yield maximizing N rate in conditions of high residual soil-N that would not be responsive to N fertilizer. Using the method of Purcell et al. (2015), DGCI measurements at V6 indicated a small amount of N was needed to recover yield potential (Figure 2-4). The DGCI values from the method of Purcell et al. (2013) predicted less N to apply than the standard recommendations did, and less N to apply than the app recommended rates, particularly at V6. This was a justification to conduct other experiments to evaluate the original technology using the camera-method as described by Purcell et al. (2015). It was hypothesized that camera- and the app-methods N recommendations were different because there were several ways in which the camera-method differs from its app counterpart. One major difference consisted of the fact camera-method measured DGCI using the whole maize leaf while the app-method calculates only the DGCI of the center portion of a maize leaf. These differences will be discussed thoroughly in Chapter 3.

**Camera-method evaluation**

After determining that the app-method was not predicting appropriate N recommendations for maize, the camera-method (the original technology) was tested. The camera method was evaluated at experiments located at Fayetteville and Pine Tree during 2014. The analysis of variance and DGCI values determined by the camera at Fayetteville and Pine Tree are shown in Appendix Table B, Appendix Figure B and Appendix Figure C.

At the Fayetteville location, an analysis of variance of the amount of N predicted to be applied using the camera-method had a significant interaction between growth stage and preplant
N factors (Table 2-3). For the 0 kg N ha\(^{-1}\) preplant site, the amounts of N recommended by the camera-method were not different for the various growth stages (Figure 2-5). However, for the 67 kg N ha\(^{-1}\) preplant site, there was a difference among maize growth stages for the camera-method N recommendations. Nitrogen recommendations using the camera were less at V10 than at V6, V8 and V12. The fact that we made DGCI measurements using leaves from different maize plants for each growth stage might be the reason for this low N recommendation value at V10 (67 kg N ha\(^{-1}\) preplant site) compared to the other growth stages.

Analysis of variance of yield data at Fayetteville identified an interaction between treatments and preplant N sites (Table 2-4). For both preplant N rates, the yield of the control treatment (0 kg N ha\(^{-1}\)) was significantly less than yields of all other treatments receiving N after maize emergence (Figure 2-6).

For both the preplant N sites and all growth stages at Fayetteville location, the camera-method predicted lower N rates to maximize grain yield than did the standard extension recommendation (246 kg N ha\(^{-1}\)) without significantly affecting yield potential. The maximum amount of N applied in the field following the camera recommendations was at V8 with 169 kg N ha\(^{-1}\) and 171 kg N ha\(^{-1}\) in the 0 and the 67 kg N ha\(^{-1}\) preplant sites, respectively. The camera-method decreased the amount of N applied by around 93 kg N ha\(^{-1}\) at V8 compared to the standard recommendation (246 kg N ha\(^{-1}\)) for both preplant N sites. Evaluation of the NUE data at Fayetteville showed a significant interaction between preplant N and treatments (Table 2-5). At both preplant N sites (0 and 67), NUE of the camera-method was higher than the standard recommendation at all maize growth stages (Figure 2-7).

At Pine Tree location, there was not a significant interaction between growth stage and preplant N (Table 2-3), but there was a significant main effect of preplant N. The rates of N
recommended by the camera-method were not different among growth stages (Figure 2-8). Although the difference in greenness between the two preplant treatments was unnoticeable, the camera recommended less N for the plots that received preplant N (134 kg N ha\(^{-1}\)) than the plots without any preplant N (154 kg N ha\(^{-1}\)).

Analysis of variance of yield data at Pine Tree identified a significant interaction between treatments and preplant N (Table 2-4). For the preplant site receiving 0 N, the control treatment, receiving no additional N, had a yield of 3.06 Mg ha\(^{-1}\), but yields of treatments receiving N at V6, V8 and V10 were similar with yields ranging from 14.97 to 15.07 Mg ha\(^{-1}\) (Figure 2-9). Yield potential decreased when N application was delayed until V12 for both the camera-method and standard recommendations suggesting that yield was lost by this point in the season, which could not be recovered. There were no statistical differences in corn grain yield at the V6, V8 and V10 growth stages when comparing the camera-method to the standard method. However, the camera-method recommended significantly lower N rates indicating that on soils with low residual-N this method can predict yield maximizing N rates.

For the preplant site at Pine Tree receiving 67 kg N ha\(^{-1}\), yield of the control treatment (10.26 Mg ha\(^{-1}\)) was less than other treatments receiving additional N (Figure 2-9). Yields of all treatments receiving N were statistically similar with yields between 16.28 and 17.76 Mg ha\(^{-1}\). Contrary to the preplant site at Pine Tree receiving 0 kg N ha\(^{-1}\), yield potential was not lost when N fertilizer was delayed until V12 due to the addition of preplant N.

Overall, the camera-method at Pine Tree location indicated a reduced N ranging from 112 to 92 kg N ha\(^{-1}\) less than the standard recommendation (246 kg N ha\(^{-1}\)) in the 67 and 0 kg N ha\(^{-1}\) preplant sites, respectively. Similar to the Fayetteville location, the yield potential of the camera
recommendations treatments was not affected even though there was a substantial difference in N between the camera-method and the standard recommendations.

At the Pine Tree location, the analysis of NUE data showed no significant interaction between preplant N and treatments (Table 2-5). There was a significant effect of treatments on NUE, and NUE of the camera-method treatments were also higher than standard recommendation at all maize growth stages (Figure 2-10).

Assuming the price of one kg of N in the U.S. is about $1.36, the camera-method would save about $110 ha⁻¹ at Fayetteville at V6 (0 N preplant) compared to the standard recommendation. Similarly at Pine Tree location (0 N preplant), N application as predicted by the camera-method would decrease N fertilizer cost by around $105 ha⁻¹.

Evaluation of the camera-method at Fayetteville and Pine Tree in 2014 indicated the camera-method was an efficient tool for N predictions. Based on maize yield records of the control (0 kg N ha⁻¹ and 0 N preplant), sites have different yield potential because of residual-N. Without any N inputs, sites could be classified as having medium yield potential (10.2 Mg ha⁻¹) (Figure 2-6) at Fayetteville and low yield potential (3.06 Mg ha⁻¹) (Figure 2-9) at Pine Tree. Indeed, amino compounds concentration for 0-45 cm soil depth before planting was greater at Fayetteville (17.54 µmol L⁻¹) than at Pine Tree location (7.38 µmol L⁻¹) (Table 2-2) for experiments conducted in 2014. At both locations, yield data indicated the effectiveness and the high NUE of the camera-method N recommendations in low and medium-yield potential silt loam soils.

It is important to note that Fayetteville site (2014) was responsive to N fertilization even though its amino compounds concentration was not different from that of Fayetteville site (2013) (Table 2-2), which was not responsive to N fertilization. Residual NO₃⁻ (mg kg⁻¹ soil) in
Fayetteville site (2014) was low (5.61) (Khan et al., 2005) and this is the reason of the responsiveness of this site to N fertilization. It appears that concentration of both soil amino compounds and residual NO$_3^-$ are necessary to evaluate the yield potential of a given site.

**Leaf N concentration**

To further evaluate the camera-method in predicting appropriate N recommendations for maize, leaf N concentration for the ear leaf at silking (R1) was determined. Leaf N concentration and yield become greater when N doses increase (Liu and Wiatrak, 2011) and yield has been reported to be correlated to leaf N concentration (Lockman, 1969) especially at silking (Brown, 1970).

Analysis of variance and values of the uppermost leaf N concentration at V6, V8, V10 and V12 collected at Fayetteville in 2013, Fayetteville in 2014 and Pine Tree in 2014, before any N treatment, are presented in Appendix Table C and Appendix Figure D, E, and F.

At Fayetteville, there were significant main effects of preplant N and N treatments on leaf N at silking but there was no significant interaction (Table 2-6). Leaf N concentration of the control was statistically similar with the camera-method and standard extension recommendations at V12 (Figure 2-11), and was significantly lower than all treatments at the V6, V8 and V10 growth stages. The 67 kg N ha$^{-1}$ preplant treatment had higher leaf N (2.97 %) being greater than the 0 kg N ha$^{-1}$ preplant site (2.64 %).

At Pine Tree, analysis of variance of leaf N concentration showed significant main effects of preplant N and treatments but no interaction between treatments and preplant N sites (Table 2-6). Leaf N concentration of the control (1.92 %) was significantly less than the N treatments at V6, V8 and V10 (Figure 2-12), but not at V12. Leaf N concentration of the 67 kg N ha$^{-1}$ preplant site (2.79%) was significantly greater than the site receiving no preplant N (2.26%).
Scientists have documented tissue and soil analyses as an appropriate tool for applying N wisely (Adeptu et al. 2000). It was determined that N concentration of the ear leaf in maize at R1 should range between 2.76 and 3.5 % (Havlin et al., 2005). At Fayetteville, leaf N concentration values of treatments consisting of camera-method and standard recommendations fall within the sufficient range proposed by Havlin et al. (2004) supporting the maize yields that were achieved. For the Pine Tree site where N rates were applied, only leaf N concentration values of camera-method and standard recommendation treatments at V12 fell below the sufficient category, which agrees with the lower yields seen in these treatments.

According to ear leaf N concentration data at R1, camera-method recommendations were appropriate. Many researchers are criticizing routine analyses in the laboratory as being expensive and time consuming (Adeptu et al. 2000; Mulvaney et al. 2001), but based on the data presented here they can be a valuable tool in predicting whether N applications are sufficient to produce maximal grain yield at the R1 growth stage. In this context, DGCI technology using the camera-method would be a promising tool to more efficiently predict yield maximizing N rates on silt loam soils.

Maize N recommendations have also been based upon SPAD and near infrared radiance (NIR) measurements. The SPAD meter was identified as an effective tool to detect N deficiencies with the V6 growth stage as the best time to perform SPAD readings (Blackmer and Schepers, 1995). Also, Varvel at al., (2007) developed a quadratic model that predicted the amount of N to apply based on SPAD reading of a treatment divided by the maximum SPAD reading belonging to a high N treatment from the same experiment (Sufficiency index). The drawback of this method is the need of a well N fertilized area to determine the sufficiency index. The NIR sensors are a potential tool to predict maize N requirement at early (V7) and late
(VT) maize growth stages (Scharf and Lory, 2002; Sripada et al. 2005; Sripada et al. 2006). However, algorithms produced from these studies were not evaluated.

**CONCLUSIONS**

The present study was the first of its kind to evaluate the DGCI technology using the app- and the camera-methods in predicting N requirements for maize. The app-method was unable to identify soils of high yield potential that do not need N inputs. However, DGCI using the camera-method demonstrated great utility in predicting appropriate N recommendations in silt loam soils. The DGCI technology was able to predict in-season N requirements for maize that were substantially less than standard recommendation (246 kg N ha\(^{-1}\)) without affecting yield. Future research should focus on evaluating DGCI technology across a wide range of environmental conditions and further investigate the origin of differences between the camera and the app-methods.
References


Table 2-1. Specification of treatments applied to the 0 and 67 kg N ha\(^{-1}\) preplant N rates in Fayetteville during 2013 and in Fayetteville and PineTree during 2014.

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<thead>
<tr>
<th>Year</th>
<th>Treatment number</th>
<th>Treatment specification</th>
<th>Year</th>
<th>Treatment number</th>
<th>Treatment specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>1</td>
<td>0 kg N ha(^{-1})</td>
<td>2014</td>
<td>1</td>
<td>0 kg N ha(^{-1})</td>
</tr>
<tr>
<td>2013</td>
<td>2</td>
<td>302 kg N ha(^{-1})</td>
<td>2014</td>
<td>2</td>
<td>302 kg N ha(^{-1})</td>
</tr>
<tr>
<td>2013</td>
<td>3</td>
<td>V6 App Rec*</td>
<td>2014</td>
<td>3</td>
<td>V6 Cam Rec**</td>
</tr>
<tr>
<td>2013</td>
<td>4</td>
<td>V6 Std Rec***</td>
<td>2014</td>
<td>4</td>
<td>V6 Std Rec***</td>
</tr>
<tr>
<td>2013</td>
<td>5</td>
<td>V8 App Rec*</td>
<td>2014</td>
<td>5</td>
<td>V8 Cam Rec**</td>
</tr>
<tr>
<td>2013</td>
<td>6</td>
<td>V8 Std Rec***</td>
<td>2014</td>
<td>6</td>
<td>V8 Std Rec***</td>
</tr>
<tr>
<td>2013</td>
<td>7</td>
<td>V10 App Rec*</td>
<td>2014</td>
<td>7</td>
<td>V10 Cam Rec**</td>
</tr>
<tr>
<td>2013</td>
<td>8</td>
<td>V10 Std Rec***</td>
<td>2014</td>
<td>8</td>
<td>V10 Std Rec***</td>
</tr>
<tr>
<td>2013</td>
<td>9</td>
<td>V12 App Rec*</td>
<td>2014</td>
<td>9</td>
<td>V12 Cam Rec**</td>
</tr>
<tr>
<td>2013</td>
<td>10</td>
<td>V12 Std Rec***</td>
<td>2014</td>
<td>10</td>
<td>V12 Std Rec***</td>
</tr>
</tbody>
</table>

* N recommendations as dictated by the app-method  
** N recommendations as dictated by the camera-method  
*** N recommendations as dictated by the University of Arkansas Agricultural Extension Service
Table 2-2. Mean values (n=4) ± SEM of amino-compounds concentration (µmol L\(^{-1}\)), and mg NO\(_3^–\) kg\(^{-1}\) soil analyses of camera and app-method evaluation sites (0-45 cm soil depth) for experiments conducted at Fayetteville and Pine Tree during 2013 and 2014.

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>amino-compounds concentration (µmol L(^{-1}))</th>
<th>mg NO(_3^–) kg(^{-1}) soil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fayetteville 2013</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-45</td>
<td>18.11±1.52</td>
<td>20.2±0.32</td>
</tr>
<tr>
<td><strong>Fayetteville 2014</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-45</td>
<td>17.54±2.93</td>
<td>5.61±0.67</td>
</tr>
<tr>
<td><strong>Pine Tree 2014</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-45</td>
<td>7.38±0.84</td>
<td>14.09±0.38</td>
</tr>
</tbody>
</table>
Table 2-3. Analysis of variance of the recommended amounts of N to apply to recover 95% of the relative yield based upon the app-method at Fayetteville in 2013 or the camera-method at Fayetteville and Pine Tree in 2014. Maize growth stage and preplant N were considered fixed effects.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Fayetteville 2013</th>
<th>Fayetteville 2014</th>
<th>Pine Tree 2014</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preplant N</td>
<td>1</td>
<td>0.0322</td>
<td>0.0001</td>
<td>0.0046</td>
<td></td>
</tr>
<tr>
<td>Growth stage</td>
<td>3</td>
<td>&lt;0.0001</td>
<td>0.0026</td>
<td>0.7761</td>
<td></td>
</tr>
<tr>
<td>Growth stage * Preplant N</td>
<td>3</td>
<td>0.7885</td>
<td>&lt;0.0001</td>
<td>0.4544</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td></td>
<td>0.43</td>
<td>0.67</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>

Table 2-4. Analysis of variance for yield as affected by the fixed effects of treatments and preplant N. The app-method evaluation experiment was conducted at Fayetteville in 2013 while the camera-method evaluation experiments was conducted at Fayetteville and Pine Tree in 2014.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Fayetteville 2013</th>
<th>Fayetteville 2014</th>
<th>Pine Tree 2014</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preplant N</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>0.262</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>9</td>
<td>0.0075</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Treatments * Preplant N</td>
<td>9</td>
<td>0.03</td>
<td>0.0091</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td></td>
<td>0.74</td>
<td>0.67</td>
<td>0.94</td>
<td></td>
</tr>
</tbody>
</table>
Table 2-5. Analysis of variance for NUE as affected by the fixed effects of treatments and preplant N. The app-method evaluation experiment was conducted at Fayetteville in 2013 while the camera-method evaluation experiments was conducted at Fayetteville and Pine Tree in 2014.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Fayetteville 2013</th>
<th>Fayetteville 2014</th>
<th>Pine Tree 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Source</td>
<td>P-values</td>
<td></td>
</tr>
<tr>
<td>Preplant N</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>0.5845</td>
<td>0.5462</td>
</tr>
<tr>
<td>Treatments</td>
<td>8</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatments * Preplant N</td>
<td>8</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.6295</td>
</tr>
<tr>
<td>$R^2$</td>
<td></td>
<td>0.96</td>
<td>0.95</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Table 2-6. Analysis of variance for leaf N concentration for camera-method evaluation data as affected by the fixed effects of treatments and preplant N. The camera-method evaluation experiments were conducted at Fayetteville and Pine Tree in 2014.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Leaf N conc. (Fayetteville)</th>
<th>Leaf N conc. (Pine Tree)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Source</td>
<td></td>
</tr>
<tr>
<td>Preplant N</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatments</td>
<td>9</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Treatments * Preplant N</td>
<td>9</td>
<td>0.3617</td>
<td>0.0597</td>
</tr>
<tr>
<td>$R^2$</td>
<td></td>
<td>0.43</td>
<td>0.65</td>
</tr>
</tbody>
</table>
Figure 2-1. The main effect of growth stage and preplant N on N recommendations by the app-method with measurements taken at Fayetteville in 2013 at V6, V8, V10, and V12. For comparison purposes, University of Arkansas extension recommendation was 246 kg N ha$^{-1}$. The interaction between growth stage and preplant N was not significant ($P>0.05$). Different letters among growth stages and between preplant N sites indicate a significant difference as determined by a HSD and LSD, respectively. At all maize growth stages, the app N recommendations were less than standard extension recommendation (246 kg N ha$^{-1}$).
Figure 2-2. Response of maize yield in 2013 at Fayetteville to N applications based on treatments consisting of 0 kg ha\(^{-1}\), 302 kg ha\(^{-1}\), standard and app-method recommendations applied as sidedress-N at V6, V8, V10 and V12 in the 0 and 67 kg N ha\(^{-1}\) preplant sites. The interaction between preplant N and treatments was significant. Different letters within a preplant N site indicate a difference as determined by a HSD. An asterisk (*) indicates a difference (\(P \leq 0.05\)) across preplant N levels within an N treatment.
Figure 2-3. Response of nitrogen use efficiency (NUE) in 2013 at Fayetteville to N applications based on treatments consisting of 0 kg ha\(^{-1}\), 302 kg ha\(^{-1}\), standard and app-method recommendations applied as sidedress-N at V6, V8, V10 and V12 in the 0 and 67 kg N ha\(^{-1}\) preplant sites. The interaction between preplant N and treatments was significant. Different letters within a preplant N site indicate a difference as determined by a HSD. An asterisk (*) indicates a difference (P≤0.05) across preplant N levels within an N treatment.
Figure 2-4. The main effect of growth stage and preplant N on N recommendations by the camera-method with measurements taken at Fayetteville in 2013 at V6, V8, V10, and V12. For comparison purposes, University of Arkansas extension recommendation was 246 kg N ha\(^{-1}\). The interaction between growth stage and preplant N was not significant (\(P>0.05\)). Different letters among growth stages and between preplant N sites indicate a significant difference as determined by a HSD and LSD, respectively. At all maize growth stages, the app N recommendations were less than standard extension recommendation (246 kg N ha\(^{-1}\)).
Figure 2-5. The main effect of growth stage and preplant N on N recommendations by the camera-method with measurements taken at V6, V8, V10, and V12 at Fayetteville in 2014. For comparison purposes, University of Arkansas Extension recommendation was 246 kg N ha$^{-1}$. The interaction between growth stage and preplant N was significant. Different letters within a preplant N site indicate a difference as determined by a HSD. An asterisk (*) indicates a difference ($P \leq 0.05$) across preplant N levels within an N treatment. At all maize growth stages, the camera N recommendations were less than standard extension recommendation (246 kg N ha$^{-1}$).
Figure 2-6. Response of maize yield in 2014 at Fayetteville to N applications based on treatments consisting of 0 kg ha\(^{-1}\), 302 kg ha\(^{-1}\), standard and camera-method recommendations applied as sidedress-N at V6, V8, V10 and V12 in the 0 and 67 kg N ha\(^{-1}\) preplant sites. The interaction between preplant N and treatments was significant. Different letters within a preplant N site indicate a difference as determined by a HSD. An asterisk (*) indicates a difference ($P \leq 0.05$) across preplant N levels within an N treatment.
Figure 2-7. Response of NUE in 2014 at Fayetteville to N applications based on treatments consisting of 0 kg ha\(^{-1}\), 302 kg ha\(^{-1}\), standard and camera-method recommendations applied as sidedress-N at V6, V8, V10 and V12 in the 0 and 67 kg N ha\(^{-1}\) preplant sites. The interaction between preplant N and treatments was significant. Different letters within a preplant N site indicate a difference as determined by a HSD. An asterisk (*) indicates a difference ($P \leq 0.05$) across preplant N levels within an N treatment.
Figure 2-8. The main effect of growth stage and preplant N on N recommendations by the camera-method with measurements taken at V6, V8, V10, and V12 at Pine Tree in 2014. For comparison purposes, University of Arkansas extension recommendation was 246 kg N ha\(^{-1}\). The interaction between growth stage and preplant N sites was not significant (P>0.05). Different letters among growth stages and between preplant N sites indicate a difference as determined by a HSD and LSD, respectively. At all maize growth stages, the camera N recommendations were less than standard extension recommendation (246 kg N ha\(^{-1}\)).
Figure 2-9. Response of maize Yield in 2014 at Pine Tree to N applications based on treatments consisting of 0 kg ha$^{-1}$, 302 kg ha$^{-1}$, standard and camera-method recommendations applied as Sidedress-N at V6, V8, V10 and V12 in the 0 and 67 kg N ha$^{-1}$ preplant sites. The interaction between preplant N and treatments was significant. Different letters within a preplant N site indicate a significant difference as determined by a HSD. An asterisk (*) means a significant difference ($P \leq 0.05$) across preplant N levels within an N treatment.
Figure 2-10. Response of NUE in 2014 at Pine Tree to N applications based on treatments consisting of 0 kg ha\(^{-1}\), 302 kg ha\(^{-1}\), standard and camera-method recommendations applied as Sidedress-N at V6, V8, V10 and V12 in the 0 and 67 kg N ha\(^{-1}\) preplant sites. The interaction between preplant N and treatments was not significant. Different letters indicate a significant difference as determined by a HSD.
Figure 2-11. Response of the ear leaf N concentration at tasseling, to N applications at Fayetteville in 2014 based on treatments consisting of 0 kg ha$^{-1}$, 302 kg ha$^{-1}$, standard and camera-method recommendations applied as sidedress-N at V6, V8, V10 and V12 in the 0 and 67 kg N ha$^{-1}$ preplant sites. The interaction between treatments and preplant N was not significant ($P>0.05$). Different letters among treatments and between preplant N sites indicate a difference as determined by a HSD and LSD, respectively.
Figure 2-12. Response of the ear leaf N concentration at tasseling, to N applications at Pine Tree in 2014 based on treatments consisting of 0 kg ha\(^{-1}\), 302 kg ha\(^{-1}\), standard and camera recommendations applied as sidedress-N at V6, V8, V10 and V12 in the 0 and 67 kg N ha\(^{-1}\) preplant sites. The interaction between treatments and preplant N was not significant (\(P>0.05\)). Different letters among treatments and between preplant N sites indicate a difference as determined by a HSD and LSD, respectively.
Chapter 3

Characterizing leaf N with greenness measurements using digital camera images and a smartphone application
Abstract

Nitrogen (N) fertilization is crucial for maximizing maize (*Zea mays* L.) yield, but optimizing the amount of N to apply for maize must also consider potential impact on the environment from NO$_3^-$ pollution. The dark green color index (DGCI) technology determines the greenness of maize leaves from digital images to assess maize N status. Spectrum Technologies used this DGCI technology to develop a smartphone app called Greenindex+. The objective of this study was to determine if DGCI values made by the app (DGCI$_{app}$) agree with values determined by a digital camera (DGCI$_{cam}$), and to identify the sources of potential discrepancies between the camera and the app for determining DGCI. Field experiments were conducted at six sites across the state of Arkansas during 2013 and 2014 with N rates ranging from 0 to 360 kg N ha$^{-1}$. Dark green color index measurements were made at tasseling both in the field using the app under ambient lighting conditions and in the laboratory under fluorescent lighting using the camera and the app. There was a significant linear-plateau relationship ($P<0.05$, $R^2=0.60$, 0.89) between DGCI$_{cam}$ and leaf N concentration. However, the relationship between DGCI$_{app}$ and leaf N concentration was more variable ($R^2=0.33$, 0.69) than between DGCI$_{cam}$ and leaf N concentration when measured in the laboratory. Under field conditions, DGCI$_{app}$ was also more variable than DGCI$_{cam}$ measured in the laboratory. It was hypothesized that one reason of the variability between DGCI$_{cam}$ and DGCI$_{app}$ is because DGCI$_{cam}$ uses the whole leaf for color analysis while DGCI$_{app}$ uses only the center portion of the leaf for color analysis.
INTRODUCTION

Nitrogen (N) is an essential element for maize growth and development (Barker and Bryson, 2007). The plant absorbs N in the form of nitrate (NO$_3^-$) and ammonium (NH$_4^+$), and N concentration in the plant ranges between 1 and 5% depending on tissue sampled and crop growth stage. Nitrogen has many metabolic functions including being a part of chlorophyll, phospho-nucleotides, and amino acids (Havlin et al., 2005). Maize (Zea mays L.) Plants under N stress exhibit leaf yellowing or necrotic symptoms compared to plants that received adequate N additions (Havlin et al., 2005). Nitrogen deficiency delays crop development by 9 days for the vegetative stages and 5 to 11 days for silking (Girardin et al., 1987; Uhart and Andrade, 1995). Nitrogen stress causes a reduction of leaf area up to 60% (Muchow, 1988; Uhart and Andrade, 1995). Uhart and Andrade (1995) reported a sensitivity of leaf area index to N shortage at the V6-V7 growth stage, whereas Girardin et al. (1987) and Cox et al. (1993) determined that the reduction of maize leaf expansion was more pronounced for N stress at V12-V18.

Maize growth is also impacted by N availability in the soil (Muchow and Davis, 1988). Compared to maize with sufficient N, plant N deficiency decreased the interception of photosynthetically active radiation (PAR) between 9 % and 40 % and radiation use efficiency (RUE) between 7 % and 51 % (Girardin et al., 1987; Muchow and Davis, 1988; Uhart and Andrade, 1995). The decline in RUE and PAR interception, especially in early plant growth stages, negatively impacts kernel number and therefore final yield (Ruget 1989; Cirilo and Andrade, 1994). Yield and kernel number increase with an increase in leaf N concentration until a threshold is reached, above which kernel number remains constant (Tollenaar et al., 1992; Uhart and Andrade, 1995). Due to this threshold relationship, yield is not improved through over-fertilization of N. Hence, it is crucial to use adequate N to optimize crop profitability,
minimize excess applications of N fertilizers and prevent the pollution of ground and surface waters (Hallberg, 1989; Cox et al., 1993).

The judicious use of N while maintaining high yield requires new tools and techniques. The chlorophyll meter (SPAD), spectral reflectance measurements (Greenseeker or Crop circle), and maize leaf color analysis are potential technologies for estimating maize N status during the growing season. These new tools are a promising substitute for routine N analysis in the laboratory, which are time consuming and expensive. The chlorophyll meter measures the amount of red light absorbed by the leaf, and there is a strong correlation between the absorbed red light and the chlorophyll concentration (Markwell et al., 1995), which is tightly associated with leaf N concentration (Dwyer et al. 1994). The chlorophyll meter is particularly useful in identifying N deficiencies (Piekielek and Fox, 1992; Blackmer and Schepers, 1995; Dwyer et al. 1991). The strongest linear relationship between the chlorophyll meter readings and leaf N concentration was at the V6 growth stage, making it the most appropriate time for measurement (Blackmer and Schepers, 1995; Piekielek et al., 1995). However, the chlorophyll meter is expensive ($2500), and presents the limitation of not detecting leaf N concentration when it is above optimum (Blackmer and Schepers, 1995; Zhang et al., 2008).

Crop spectral reflectance using the Normalized Difference Vegetation Index (NDVI) is also used to evaluate plant N status (Raun et al., 2002; Mullen et al., 2003). The principle of NDVI measurements rely on the difference between two wavelengths. The first wavelength is combined with the chlorophyll absorption while the second one is unabsorbed (Scharf, 2009). A strong relationship exists between N concentration in the canopy, plant N uptake and NDVI values. However, NDVI is saturated when exposed to high N concentration or when LAI exceeds 2.5 to 3. Previous research has shown a strong correlation between NDVI and yield (Stone et al.,
Commercial products using NDVI technology include the Green Seeker (Trimble, Auburn AL), the Crop Circle (Holland Scientific, Lincoln NE), the Fieldscan (Process Sensor Corp, Milford MA) and the Dualex (Dynamax Inc, Houston TX) (Goulas et al., 2004; Varvel et al. 2007). Costs of the Crop circle or Dualex are about $2,000 or more, thus hampering their use by farmers.

The limitations and cost of the SPAD meter and NDVI sensors prompted researchers to develop an accessible and economical technique to assess leaf N concentration. The analysis of the greenness of a leaf using digital images offers one such tool. The Dark Green Color Index (DGCI) is calculated from the hue, saturation and brightness of a digital image using the following equation (Karcher and Richardson, 2003):

\[
\text{DGCI} = \frac{\text{Hue} - 60}{60} + (1 - \text{Saturation}) + (1 - \text{Brightness}) \times 3
\]  
(Eqn. 1)

The resulting DGCI value is a unitless parameter and ranges from 0 (very yellow) to 1 (dark green).

Previous research at the University of Arkansas quantified maize leaf greenness with a digital camera and image-analysis software (Sigma Scan Pro 5, SPSS, 1998, San Jose) (Rorie et al., 2011b) in terms of DGCI by modifying the digital-imaging method of Karcher and Richardson (2003). Rorie et al. (2011a) determined a close association between DGCI and leaf N concentration, demonstrating the ability of DGCI to assess plant N status. Based upon algorithms from Rorie et al. (2011a, 2011b), Spectrum Technology (http://www.specmeters.com/nutrient-management/chlorophyll-meters/chlorophyll/greenindex/) developed a smartphone app that could assess leaf maize DGCI. The app differs from the method of Rorie et al. (2011a, 2011b) in several ways: (1) Rorie et al. (2011a, 2011b) measured DGCI using the whole leaf while the app determines DGCI from the center portion of a leaf; (2) Rorie et al. (2011a, 2011b) used a
commercial software package (Sigma Scan Pro) to calculate DGCI values whereas the app uses its own software to calculate DGCI values; (3) Rorie et al. (2011a, 2011b) used a pink board as a backdrop with color standards that were matched with known DGCI values whereas the app uses a backdrop with standards that may differ from that of Rorie et al. (2011a, 2011b).

As was cited in Chapter 2, the method developed by Rorie et al. (2011a, 2011b) will be referred to as the “camera-method” and the method developed by Spectrum Technologies using a smartphone will be referred to as the “app-method”. Similarly, DGCI measurements made using the camera-method will be designated as DGCI\textsubscript{cam}, and DGCI measurements using the app-method will be designated as DGCI\textsubscript{app}. Differences between the method of Rorie et al. (2011a, 2011b) and the app may result in different DGCI values, and, hence, different N fertilizer recommendations. The objectives of this research were to: (1) determine if DGCI values made by the app agree with values determined by the method of Rorie et al. (2011a, 2011b); (2) identify the sources leading to possible discrepancies between the camera- and the app-methods for determining DGCI.

MATERIALS AND METHODS

This study evaluated the camera- and the app-methods for assessing maize N status. The evaluation procedure using the camera- and app-methods was similar to the method described in Chapter 2. The measurement using the camera-method was determined in the laboratory under fluorescent lighting using an entire ear leaf sampled at tasseling (R1). The same sampling procedure used for the camera-method was adopted for the app-method, for the center portion of the ear leaf. Measurements for the app-method were made both in the laboratory under fluorescent lighting, and in the field under ambient lighting.
Objective 1. Determine if DGCI values made with the camera-method agree with those made by the app-method.

Field experiments were conducted at the University of Arkansas research stations. During 2013, experiments were located at two sites in Fayetteville, AR (36° 5' 54.8" N; 94° 10' 15" W) identified as Fay 3 and Fay 4, while a third experiment was conducted at the Vegetable Research Station (VRS) near Kibler, AR (35° 25' 31" N; 94° 14’ 11" W) (sown on May 25th). During 2014, experiments were located at two sites in Fayetteville, AR identified also as Fay 3 and Fay 4 (sown on April 27th), while a third experiment was conducted at the Pine Tree research station, AR (35° 3’ 30.8” N; 90° 48’ 44.3” W) (sown on April 24th).

In 2013, the soil was a Captina silt loam (Fine-silty, siliceous, active, mesic Typic Fragiudults) at Fay 3, a Pickwick silt loam (Fine-silty, mixed, semiactive, thermic Typic Paleudults) at Fay 4, and a Roxanna sandy loam (Coarse-silty, mixed, superactive, nonacid, thermic Typic Udifluvents) at Kibler. Experiments in 2014 were grown on a Captina silt loam at Fay 3 and Fay 4, and a Calloway silt loam (Fine-silty, mixed, active, thermic Aquic Fraglossudalfs) at Pine Tree. For all experiments, the previous crop was soybean. Experimental fields were fertilized to meet soil test recommendations for all nutrients except N.

In 2013, prior to the 2012 growing season, the Fay 3 site received 12.5 t ha$^{-1}$ of chicken litter, 96 kg ha$^{-1}$ P, and 144 kg ha$^{-1}$ K. In 2014, Fay 4 and Kibler experiments received 96 kg ha$^{-1}$ of P and 144 kg ha$^{-1}$ of K. Fields at all locations were disked followed by tillage using a field cultivator before planting. Maize was seeded at a depth of 5 cm. Weeds were controlled with atrazine (AAtrex; 1.8 kg ai ha$^{-1}$), S-metalochlor (Dual Magnum; 1.5 kg ai ha$^{-1}$), and glyphosate (RoundUp Ultra; 1.12 kg ai ha$^{-1}$). The experiment was furrow-irrigated as needed to maintain adequate soil moisture using the hand-feel method (Klocke et al., 1998). In all experiments, plots
consisted of four rows, 91.5 cm apart and 9.15 m in length, which were seeded at 84,030 kernels ha$^{-1}$. In 2013, hybrids used were Mycogen 2V707 for Fay 3, and Pioneer 1690HR for Fay 4 and Kibler. In 2014, hybrids used were Mycogen 2V707 for Fay 3 and Pioneer 1690HR for Fay 4, and Pine Tree. All hybrids used were glyphosate [N-(phosphonomethyl) glycine] tolerant and had the $Bt$ trait. The experimental design for each site was a randomized complete block (RCB) with four replications and six $N$ treatments per replicate. Nitrogen fertilizer rates ranged from 0 to 360 kg N ha$^{-1}$ and were broadcast by hand as urea coated with N-(n-butyl) thiophosphotictriamide (Agrotain® AGROTAIN International, St. Louis, MO). Before planting, all plots except the no-$N$ control received 36 kg N ha$^{-1}$. Treatments consisted of providing different amounts of $N$ at V6 and at V8 as shown in Table 3-1.

**Evaluation of $N$ response**

Plant sampling and image analysis were processed as explained in the Chapter 2. For all years and treatments, linear-plateau regression analysis using PROC NLIN in SAS version11 (SAS inst., Cary, NC) software was used to evaluate the relationships between the following parameters: DGCI measurements made using camera-method or with the app-method in both the field and the laboratory (dependent variable), SPAD measurements in the laboratory and leaf $N$ concentration (independent variables). In 2014, the internal standards included in each image were re-assessed, and new standards ($S T A N D_{N e w}$) were used to determine DGCI values. The Gauss-Newton method (Bjorck, 1996) was used to test the significance of the linear-plateau model. A linear model was adopted in case of the non-significance of the linear-plateau model.

**Objective2**: Identify the sources leading to discrepancies between the camera-method and the app-method in determining DGCI.

**Sigma Scan Pro5 and the app software evaluation**
The DGCI\textsubscript{app} values were compared to the DGCI\textsubscript{cam} values for a set of 12 wooden stakes (4cm×30cm) that were painted with 12 different latex paints ranging in color from light yellow to dark green. Each stake was photographed individually three times using the app-method. The app pictures were taken in the laboratory under fluorescent lighting and in the field under ambient lighting. Afterward, the app-method images were processed to get DGCI\textsubscript{app} values and were analyzed using the Sigma Scan software to determine app Sigma Scan DGCI values designated as DGCI\textsubscript{sigma app}. The DGCI\textsubscript{app} and DGCI\textsubscript{sigma app} values made under fluorescent lighting and in the field under ambient lighting were compared to DGCI\textsubscript{cam} values made under fluorescent lighting.

**Determination of the DGCI internal color standards**

The DGCI values of the internal standards were determined on the background boards that were used by the app-method and the camera-method. The DGCI values of these internal standards were compared to that of the known standards from the X-rite paper as described by Rorie et al. (2011a, 2011b). By placing the X-rite yellow (DGCI=0.0733) and green (DGCI=0.5722) standards alongside the internal standards used with the camera-method (Rorie et al., 2011a, 2011b) or alongside the standards used with the app-method, we were able to determine the true DGCI values of these internal standards. Ten pictures were taken in the laboratory in 2013 under fluorescent lighting to determine corrected DGCI values (STAND\textsubscript{New}) of the internal standards of the camera and the app-methods. The same procedure was repeated to determine STAND\textsubscript{New} of camera and the app-methods outdoors on a cloud free day at noon.

To evaluate the STAND\textsubscript{New} in eliminating differences between DGCI\textsubscript{cam} and DGCI\textsubscript{app}, stakes pictures were processed two times by Sigma Scan, using nominal DGCI values for the color standards of 0.0733 for the yellow and 0.5722 for the green as standards, and using
STAND\textsubscript{New} values of the camera-method and of the app-method. Next, an experiment was conducted in 2014 by analyzing 24 maize leaves collected from a field at the main experiment station at Pine Tree. The leaves were chosen based upon a wide range of visual differences in their greenness. Leaves were removed from the plants, photographed using the camera and the app. Pictures were analyzed for total N, for DGCI using the nominal DGCI values for the color standards (0.0733 for the yellow and 0.5722 for the green), and for DGCI using STAND\textsubscript{New} values.

**Evaluation of maize leaf section on DGCI for the camera and the app-methods**

The camera-method measures DGCI using the whole leaf while the app-method calculates only the DGCI from the center portion of a leaf, which might create a discrepancy between the two methods. An experiment was conducted on late June 2014 consisting of 20 leaves that were collected randomly from a maize field located at the main Experimental Station at Fayetteville, AR. Each leaf was sectioned into basal, middle, and apical portions. Pictures of each section were taken in the laboratory under florescent lighting using the camera-method. Next, each section was analyzed for leaf N concentration. Analysis of variance was performed to assess the effect of section on DCGI\textsubscript{cam}. Leaf N concentration was considered as a covariate to better test the effect of leaf section on DCGI\textsubscript{cam} values. Fishers’ protected LSD was used to separate means for significant effects ($P \leq 0.05$) using JMP version 11 (JMP, Cary, NC).

**RESULTS AND DISCUSSION**

**Objective 1** - Determine if DGCI values made with the camera-method agree with those made by the app-method.

**Relationship between DGCI and leaf N concentration**
We analyzed data by year because in 2014 (the second year) STAND\textsubscript{New} were used to determine DGCI values. In 2013, there was a linear-plateau relationship ($P \leq 0.05$, $r^2 = 0.60$) (Figure 3-1-A) between DGCI\textsubscript{cam} and leaf N concentration (g 100g$^{-1}$). Between 1 and 2.64 g N 100g$^{-1}$, DGCI\textsubscript{cam} increased linearly. Above a leaf N concentration of 2.64g N 100g$^{-1}$, predicted DGCI\textsubscript{cam} values were constant and equal to 0.77. In 2014, there was also a linear-plateau relationship ($P \leq 0.05$, $r^2 = 0.91$) (Figure 3-1-B) between DGCI\textsubscript{cam} and leaf N concentration. At leaf N greater than 2.58 g N 100g$^{-1}$, DGCI\textsubscript{cam} was constant at a value of 0.78. There were no significant differences between the slopes or the intercepts for 2013 and 2014. The greater $r^2$ in 2014 could be attributed to the use of STAND\textsubscript{New} and to greater range in leaf N concentration values.

Results of this study corroborate the findings of Rorie et al. (2011b) who determined a linear plateau relationship between DGCI and leaf N concentration. However, we determined a higher leaf N concentration threshold and DGCI\textsubscript{cam} plateau than Rorie et al. (2011b). This is likely because our study consisted of field grown plants (sampled at tasseling) while experiments of Rorie et al. (2011b) were conducted in a greenhouse (sampled at V5). Therefore, the environment and the time of sampling may have affected the leaf N concentration threshold above which DGCI\textsubscript{cam} became constant. Rorie et al. (2011a) reported that in situations where residual N was limiting (based upon results of field experiments), there was a strong linear relationship between DGCI\textsubscript{cam} and leaf N concentration, which supports the results of the present study. The DGCI plateaus and becomes constant when we consider values from experiments performed at sites very rich in residual-N. DGCI technology does not measure leaf N concentration changes above a given threshold because the greenness of the leaf does not change above a specific leaf N concentration. Also, the use of different hybrids may result in different
DGCI values for the same leaf N concentration. Similar to the saturation of DGCI values at high leaf N concentration, NDVI and SPAD are also prone to saturation when exposed to high leaf N concentration (Stone et al., 1996; Freeman et al., 2007; Li et al., 2005; Schlemmer et al., 2013).

A concern with the DGCI technology is that DGCI saturates when leaf N is around 2.4 to 2.6 g N 100g⁻¹. Reported critical values for leaf N concentration at R1 range from 2.76 to 3.5% (Havlin et al., 2005). Hence, DGCI technology would not be able to diagnose N requirements above the saturation value (2.4 to 2.6 g N 100g⁻¹) and the critical leaf N concentration.

Also, there was a linear-plateau relationship ($P \leq 0.05$, $r^2 = 0.33$) between DGCI_{app} measurements made in the lab and leaf N concentration in 2013 (Figure 3-2-A). Compared to measurement of DGCI_{cam}, the DGCI_{app} measurements in the laboratory were less sensitive to leaf N variation, (i.e., slope values were significantly less for DGCI_{app} than DGCI_{cam}) and had a higher degree of sample variability. Values of DGCI_{app} were constant (0.6) at leaf N concentration values greater than 2.46 g N 100 g⁻¹. Similarly in 2014, there was a linear-plateau relationship ($P \leq 0.05$, $r^2 = 0.67$) between DGCI_{app} and leaf N concentration. Leaf N between 0.75 and 2.4 g N 100 g⁻¹ resulted in a linear increase in DGCI_{app} that plateaued at leaf N concentration greater than 2.4 g N 100 g⁻¹ (Figure 3-2-B). Measurements of DGCI_{app} in the laboratory were less responsive (slope of 0.18) to leaf N variation than DGCI_{cam} measurements (slope of 0.22). The greater $r^2$ in 2014 than 2013 may be also associated with the use of STAND\textsubscript{New} and to greater range in leaf N concentration values.

There was a significant linear relationship ($P \leq 0.05$) between DGCI_{app} measured in the field and leaf N concentration in 2013 ($r^2 = 0.37$) and 2014 ($r^2 = 0.48$) (Figure 3-3-A and 3-3-B). Compared to DGCI_{cam}, DGCI_{app} measurements in the field were considerably more variable, and the DGCI_{app} measurements did not show a tendency to plateau as leaf N concentration increased.
Relationship between DGCI and SPAD

In previous work, Rorie et al. (2011a) determined a strong relationship between SPAD values and leaf N concentration at tasseling. However, Zhang et al. (2008) reported that the chlorophyll meter is sensitive to N deficiencies only when used at early maize growth stages.

In this research, we assessed the relationship between DGCI using the camera- and the app-methods and SPAD values. In 2013, there was a linear-plateau relationship ($P \leq 0.05$, $r^2=0.71$) between DGCI\textsubscript{cam} and SPAD values (Figure 3-4-A). At SPAD values greater than 50.7, DGCI\textsubscript{cam} values were constant and equal to 0.78. A similar bilinear relationship was observed in 2014 between DGCI\textsubscript{cam} and SPAD ($r^2=0.91$), with a DGCI\textsubscript{cam} plateau at 0.77 when SPAD values were greater than 47. Similarly, Rorie et al. (2011b) determined a strong relationship between DGCI\textsubscript{cam} and SPAD values at early maize growth with an $r^2$ of 0.96. Siddons (2013) also reported a strong relationship between DGCI\textsubscript{cam} and SPAD values in maize at tasseling.

Also, there was a linear-plateau relationship ($P \leq 0.05$) between DGCI\textsubscript{app} and SPAD values in 2013, in the laboratory (Figure 3-5-A, $r^2=0.51$). DGCI\textsubscript{app} for measurements in the laboratory were constant (0.61) at SPAD values greater than 50.5. Similarly, there was a linear-plateau relationship ($P \leq 0.05$, $r^2=0.78$) between DGCI\textsubscript{app} and SPAD in 2014 (Figure 3-5-B). Above a SPAD value of 46.4, values of DGCI\textsubscript{app} in the laboratory were constant and equal to 0.64.

In the field, there was a linear relationship ($P \leq 0.05$) between DGCI\textsubscript{app} and SPAD values for 2013 ($r^2=0.40$) and 2014 ($r^2=0.35$) (Figure 3-6-A and Figure 3-6-B). Overall, there was considerably more variation in DGCI\textsubscript{app} measurements than the DGCI\textsubscript{cam} measurements, and there tended to be more variability in DGCI\textsubscript{app} measurements made in the field than those made in the laboratory. In the field, the internal standards used by the app-method might not control
variation in DGCI values due to variation in lighting conditions. This may explain the greater variability of DGCI_{app} in the field.

**Relationship between DGCI_{cam}, DGCI_{app} in the laboratory, and DGCI_{app} in the field**

We were interested in the relationship of DGCI_{cam}, DGCI_{app} in the laboratory, and DGCI_{app} in the field because of the discrepancies of these parameters in predicting leaf N concentration and SPAD values. In 2013, there was a linear relationship ($P \leq 0.05$) between DGCI_{app} and DGCI_{cam} in the laboratory ($r^2=0.52$ and slope of 0.35), DGCI_{app} and DGCI_{cam} in the field ($r^2 = 0.30$ and slope of 0.58) (Figure 3-7-A). In 2014, there was also a linear relationship ($P \leq 0.05$) (Figure 3-7-B) between DGCI_{app} in the laboratory and DGCI_{cam} ($r^2=0.67$), DGCI_{app} in the field and DGCI_{cam} ($r^2=0.42$). Generally, there was relatively poor agreement between DGCI_{cam} measurements and DGCI_{app}, regardless of whether they were made in the laboratory or the field.

This study was an extension of previous research conducted at the University of Arkansas, with an aim to develop a practical tool for assessment of maize leaf N concentration. The integration of the DGCI algorithms as developed by (Rorie et al., 2011a, 2011b) into an smartphone app (the app-method) to quantify in realtime maize leaves greenness was one aspect of the practicality of this technology. DGCI technology practicability was limited at the time discrepancies were identified between the camera and the app-method (Figure 3-7). This was the reason to identify the sources leading to discrepancies between the camera- and the app-methods in determining DGCI.

**Objective2- Identify the sources leading to discrepancies between the camera- and the app-methods in determining DGCI.**
The app-method differs from its camera counterpart in three ways: the camera-method used a pink board as a backdrop with color standards that were matched with X-rite DGCI values whereas the app-method uses a backdrop with standards that may differ from that of the camera-method; the camera-method uses Sigma Scan Pro to calculate DGCI values whereas the app uses its own software; and finally the camera-method measures DGCI using the whole leaf while the app-method calculates DGCI from the center portion of a leaf. These differences might be a potential source for disagreement between the camera- and the app-methods. Our aim was to detect the cause(s) for the discrepancies between DGCI\textsubscript{cam} and DGCI\textsubscript{app} values.

**The DGCI of internal color standards**

The standard colors used as internal standards from the background board for the camera-method and for the board from the app-method were compared indoors under fluorescent lighting with the X-rite color standards. Although the standard colors used on the boards by the camera-method and the app-method were chosen to match the X-rite standards, there were discrepancies between DGCI standards from the camera-method board and the app-method board when compared to the X-rite standards (Table 3-2 and Table 3-3). The raw DGCI values in Table 3-2 represent the values of the various green and yellow discs without any corrections. The raw DGCI value of X-rite green (0.4700) and yellow (0.1050) were significantly different from their known values (0.5722 and 0.0733, respectively). Likewise, the internal standards from the board of the camera-method and the app-method had raw DGCI values significantly different from the X-rite standard true values.

After correcting the raw values with the known values of the X-rite standards, the STAND\textsubscript{New} of the camera and the app-methods boards were significantly different from the standards of the X-rite paper (Table 3-3). The STAND\textsubscript{New} of the app-method board values were
closer to the X-rite standards for the green disc than was the camera-method green disk. However, the STAND_{New} of the camera-method board were closer to the X-rite standards for the yellow disc than was the app-method yellow disk.

The standard colors used as internal standards from the background board of the camera-method board and from the app-method board were also compared with the X-rite color standards outdoors, during a cloud free day, at noon (Table 3-2 and Table 3-3). Similar to indoors measurements, the raw and STAND_{New} values of the camera-method board (0.4760 for the green and 0.0668 for the yellow) and the app-method board (0.5427 for the green and 0.1168 for the yellow) differed significantly from each other and from the corrected X-rite DGCI values (0.5722 for the green and 0.0733 for the yellow). Except for the camera-method yellow circles, the error between both the calibration circles for the camera- and the app-methods versus the X-rite standards was greater outdoors than indoors. This difference is a potential cause of discrepancies between the camera and the app-method in determining maize leaf DGCI values and may explain the greater variation in measurements taken outdoors versus indoors.

**Sigma Scan Pro 5, the app software and STAND_{New} evaluation**

- **Stakes**

  Digital images of stakes that were painted with a wide range of colors from light yellow to dark green were processed by Sigma Scan using DGCI values for the color standards of 0.0733 for the yellow and 0.5722 for the green, and using STAND_{New} values of the camera-method and of the app-method. There was a close relationship (all with $r^2 \geq 0.99$) (Table 3-4) between (1) DGCI_{cam} and DGCI_{app}, (2) DGCI_{cam} and images taken with the app but processed with Sigma Scan (DGCI_{sigma app}), and (3) DGCI_{cam} using STAND_{New} and DGCI_{sigma app} using STAND_{New}. The slopes resulting from the relationship between DGCI_{cam} and DGCI_{sigma app} using
the DGCI values of the X-rite as standards (0.5722 for the green disc and 0.0733 for the yellow disc) were 0.84 (indoors) and 0.92 (outdoors). When the STAND\textsubscript{New} were used to calculate the relationship between DGCI\textsubscript{cam} and DGCI\textsubscript{sigma app}, the slope was 1.01 (indoors) and 0.98 (outdoors). These results justify the need of the use of the new standards to calculate DGCI\textsubscript{cam} or DGCI\textsubscript{app} (Table 3-4).

- **Maize leaves**

An experiment was conducted by analyzing 24 maize leaves collected from a field at the main experiment station at Fayetteville in 2014 using DGCI values of the X-rite standards and STAND\textsubscript{New}. There was a close relationship ($P\leq0.05$) ($r^2=0.94^{**}$) between the DGCI\textsubscript{cam} and the DGCI\textsubscript{app} with a slope of 0.85 and an intercept of 0.03 when measured in the laboratory (Table 3-5). When the new standards were used to calculate the relationship between DGCI\textsubscript{cam} and DGCI\textsubscript{app} in the laboratory, the slope was 1.25 and the intercept was 0.08. On the other hand, there was a significant relationship ($P\leq0.05$) ($r^2=0.52$) between DGCI\textsubscript{cam} and DGCI\textsubscript{app} in the field (slope of 0.61 and intercept of 0.06). The use of STAND\textsubscript{New} reduced the variability between DGCI\textsubscript{cam} and DGCI\textsubscript{app} in the field ($r^2=0.67$), modifying the slope (0.76) and the intercept (0.09) values. The STAND\textsubscript{New} did not improve the slope and the intercept between DGCI\textsubscript{app} in the field and DGCI\textsubscript{app} in the laboratory with a variability remaining about the same ($r^2= 0.75$ without STAND\textsubscript{New} and $r^2=0.57$ with STAND\textsubscript{New}).

The STAND\textsubscript{New} worked appropriately in correcting differences between DGCI\textsubscript{cam} and DGCI\textsubscript{app} for the stakes. However, STAND\textsubscript{New} were unable to correct differences between the camera and the app-methods values using leaves. Indeed, the camera-method (DGCI\textsubscript{cam}) uses the whole leaf while the app-method (DGCI\textsubscript{app}) calculates DGCI of only a portion of the leaf and it
was hypothesized that leaf N concentration is not constant over a maize leaf blade which creates discrepancies between the two methods.

Evaluation of maize leaf section effect the on the relationship between the camera and the app-methods

The distribution of N which was considered in our study as a covariate was not uniform across a maize leaf blade. Nitrogen concentration increased significantly from the basal (1.51 g N 100g⁻¹) to the apical portion of the leaf (2.17 g N 100g⁻¹). As expected, there was a significant effect of leaf section on DGCI_{cam}, but there was not an interaction (P>0.05) (Table 3-6) between the leaf N concentration (covariate) and the leaf section on DGCI_{cam}. There was only a significant effect (P≤0.05) of leaf section and the leaf N concentration. The present analysis (Table 3-6 and Table 3-7) shows that DGCI_{cam} changed over leaf section. DGCI_{cam} was greater in the basal than the apical portion of the leaf which was not expected. We expected DGCI_{cam} to be greater in the apical than the basal portion of the leaf since leaf N concentration increased from the basal to the apical portion of the leaf. The slopes that represent the relationship between DGCI and leaf N concentration were similar among maize leaf sections (basal, middle and apical). However, the intercepts among sections were significantly different (P≤0.05). These results indicate that DGCI was sensitive to leaf section, which may explain the discrepancies between the DGCI_{cam} and the DGCI_{app}.

CONCLUSIONS

The primary objective of this research was to identify if the methods of determining DGCI using a digital camera (Rorie et al., 2011a, 2011b) gave similar values to an iPhone app developed by Spectrum Technologies for DGCI determination. If there were discrepancies between the methods, a second objective was to determine potential causes for these differences.
Discrepancies were identified between DGCI$_{\text{cam}}$ and DGCI$_{\text{app}}$ values. There were two major reasons behind the difference in DGCI values between the camera- and the app-methods. First, DGCI values of the internal standards for the two methods did not match exactly with the DGCI values of the X-rite paper. Second, the app-method calculates DGCI values for only a portion of a maize leaf, and DGCI values were not constant over a maize leaf blade.

This research brings a substantial improvement to the practicality of inexpensive and readily available tools for assessment of N uptake. These results can be extended in future studies. A promising focus could be on improving DGCI technology using the iPhone. The future challenge is to explore solutions to solve the problem of the effect of leaf section in the DGCI$_{\text{app}}$ values, which is due mainly to the inconsistency of leaf N concentration across a maize leaf blade.
References


Table 3-1. Treatment layout of N application timing and rate of six experiments. Two of the experiments were conducted in Fayetteville and one experiment was in Kibler in 2013. Two of the experiments were conducted in Fayetteville and one experiment was in Pine Tree in 2014.

<table>
<thead>
<tr>
<th>Application Times</th>
<th>T_1</th>
<th>T_2</th>
<th>T_3</th>
<th>T_4</th>
<th>T_5</th>
<th>T_6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen preplant</td>
<td>0</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Nitrogen Sidedress at V6</td>
<td>0</td>
<td>36</td>
<td>54</td>
<td>90</td>
<td>126</td>
<td>162</td>
</tr>
<tr>
<td>Nitrogen Sidedress at V8</td>
<td>0</td>
<td>0</td>
<td>54</td>
<td>90</td>
<td>126</td>
<td>162</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>72</td>
<td>144</td>
<td>216</td>
<td>288</td>
<td>360</td>
</tr>
</tbody>
</table>
Table 3-2. DGCI values prior to correction using internal standards (raw DGCI values) of the camera-method standard circles, the app-method standard circles, and the X-rite standard disks of pictures taken indoors under fluorescent lighting conditions and outdoors during a cloud free day at noon in 2013. Analysis of variance was performed separately for the green and the yellow circles and discs for pictures taken indoors and outdoors. Within each row (Indoors or Outdoors), different letters indicate a significant difference ($P \leq 0.05$) for standards having similar color (Green or Yellow).

<table>
<thead>
<tr>
<th></th>
<th>Camera-method green circle DGCI</th>
<th>App-method green circle DGCI</th>
<th>X-rite standard green disc DGCI</th>
<th>Camera-method yellow circle DGCI</th>
<th>App-method yellow circle DGCI</th>
<th>X-rite standard yellow disc DGCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoors</td>
<td>0.4271c</td>
<td>0.5270a</td>
<td>0.4701b</td>
<td>0.1110b</td>
<td>0.1200a</td>
<td>0.1050c</td>
</tr>
<tr>
<td>Outdoors</td>
<td>0.4031c</td>
<td>0.4585b</td>
<td>0.4820a</td>
<td>0.0635c</td>
<td>0.1050a</td>
<td>0.0683b</td>
</tr>
</tbody>
</table>
Table 3-3. The corrected camera-method DGCI values (STAND$_{\text{New}}$) of the camera-method standard circles, the app-method standard circles, and the X-rite standard disks of pictures taken indoors under fluorescent lighting conditions and outdoors during a cloud free day at noon in 2013. Analysis of variance was performed separately for the green and the yellow circles and discs for pictures taken indoors and outdoors. Within each row (Indoors or Outdoors), different letters indicate a significant difference ($P \leq 0.05$) for standards having similar color (Green or Yellow).

<table>
<thead>
<tr>
<th></th>
<th>Camera-method green circle DGCI</th>
<th>App-method green circle DGCI</th>
<th>X-rite standard green disc DGCI</th>
<th>Camera-method yellow circle DGCI</th>
<th>App-method yellow circle DGCI</th>
<th>X-rite standard yellow disc DGCI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indoors</strong></td>
<td>0.5120c</td>
<td>0.5627b</td>
<td>0.5722a</td>
<td>0.082a</td>
<td>0.05c</td>
<td>0.0733b</td>
</tr>
<tr>
<td><strong>Outdoors</strong></td>
<td>0.4760c</td>
<td>0.54277b</td>
<td>0.5722a</td>
<td>0.0668c</td>
<td>0.1168a</td>
<td>0.0733b</td>
</tr>
</tbody>
</table>
Table 3-4. Regression data and sample size for the DGCI<sub>cam</sub> and the DGCI<sub>app</sub>, DGCI<sub>sigma app</sub>, DGCI<sub>cam (STAND<sub>New</sub>)</sub> and DGCI<sub>sigma app (STAND<sub>New</sub>)</sub> for stakes pictures, ranging from light yellow to dark green, taken indoors and outdoors at Fayetteville in 2013. All regressions were significant at a probability level of 0.05.

<table>
<thead>
<tr>
<th></th>
<th>DGCI&lt;sub&gt;app&lt;/sub&gt; vs DGCI&lt;sub&gt;cam&lt;/sub&gt;</th>
<th>DGCI&lt;sub&gt;sigma app&lt;/sub&gt; vs DGCI&lt;sub&gt;app&lt;/sub&gt;</th>
<th>DGCI&lt;sub&gt;app (STAND&lt;sub&gt;New&lt;/sub&gt;)&lt;/sub&gt; vs DGCI&lt;sub&gt;cam (STAND&lt;sub&gt;New&lt;/sub&gt;)&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoors</td>
<td>Slope</td>
<td>0.84±0.03</td>
<td>1.02±0.00</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>0.02±0.01</td>
<td>-0.01±0.00</td>
</tr>
<tr>
<td></td>
<td>r²</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Outdoors</td>
<td>Slope</td>
<td>0.92±0.04</td>
<td>1.02±0.02</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>-0.02±0.02</td>
<td>-0.00±0.00</td>
</tr>
<tr>
<td></td>
<td>r²</td>
<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 3-5. Regression evaluation of DGCI measurements made with the camera (DGCI\textsubscript{cam}) and DGCI measurements made with the app (DGCI\textsubscript{app}) in the laboratory (Lab) and the field (Field) using default values of the internal standards and the revised standards (STAND\textsubscript{New}) for maize leaves pictures, ranging from light yellow to dark green, taken randomly at Pine Tree AR, in 2014. All regressions were significant at a probability level of 0.05.

<table>
<thead>
<tr>
<th></th>
<th>DGCI\textsubscript{app} (Lab) vs DGCI\textsubscript{cam} (Lab)</th>
<th>DGCI\textsubscript{app} (Lab) vs DGCI\textsubscript{cam} (STAND\textsubscript{New}) vs DGCI\textsubscript{app} (Field) vs DGCI\textsubscript{cam} (STAND\textsubscript{New})</th>
<th>DGCI\textsubscript{app} (Field) vs DGCI\textsubscript{cam} (STAND\textsubscript{New})</th>
<th>DGCI\textsubscript{app} (Field) vs DGCI\textsubscript{app} (Lab) vs DGCI\textsubscript{app} (Lab) (STAND\textsubscript{New})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Slope</strong></td>
<td>0.85±0.04</td>
<td>1.25±0.10</td>
<td>0.61±0.12</td>
<td>0.76±0.11</td>
</tr>
<tr>
<td><strong>Intercept</strong></td>
<td>0.03±0.03</td>
<td>0.08±0.06</td>
<td>0.06±0.08</td>
<td>0.09±0.06</td>
</tr>
<tr>
<td><strong>r^2</strong></td>
<td>0.94</td>
<td>0.87</td>
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<td>0.67</td>
</tr>
<tr>
<td><strong>N</strong></td>
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<td>24</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>
Table 3-6. Analysis of covariance for camera DGCI as affected by leaf section (basal, middle and apical) and the leaf N concentration (covariate). Leaf samples were taken randomly from a maize field located in Fayetteville AR, in 2014. 

\[ P_{values} \leq 0.05 \] means a significant difference at a probability level of 0.05.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>( P_{values} )</th>
<th>Adj ( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaf section</td>
<td>2</td>
<td>0.67</td>
<td>33.29</td>
<td>&lt;.0001*</td>
<td>0.74</td>
</tr>
<tr>
<td>Leaf N</td>
<td>1</td>
<td>1.96</td>
<td>193.02</td>
<td>&lt;.0001*</td>
<td></td>
</tr>
<tr>
<td>leaf section×Leaf N</td>
<td>2</td>
<td>0.02</td>
<td>0.92</td>
<td>0.34</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-7. Results of analysis of covariance for camera DGCI as affected by leaf section (basal, middle and apical) and the leaf N concentration (covariate). Leaf samples were taken randomly from a maize field located in Fayetteville AR, in 2014. Different letters for the slope and for the intercept means a significant difference at a probability level of 0.05.

<table>
<thead>
<tr>
<th>Leaf section</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>0.13a</td>
<td>0.28a</td>
</tr>
<tr>
<td>Middle</td>
<td>0.13a</td>
<td>0.26a</td>
</tr>
<tr>
<td>Apical</td>
<td>0.14a</td>
<td>0.14b</td>
</tr>
</tbody>
</table>
Figure 3-1. Relationship between DGCI<sub>cam</sub> and leaf N concentration for experiments in 2013 (A) and for experiments in 2014 (B) for measurements at tasseling. Nonlinear regression was performed to determine the breakpoint of the linear-plateau model. Regressions were significant at $P \leq 0.05$. 

A. When $x \leq 2.64$, $DGCI = 0.18x + 0.28$; when $x > 2.64$, $DGCI = 0.77$; $r^2 = 0.91$

B. When $x \leq 2.58$, $DGCI = 0.22x + 0.19$; when $x > 2.58$, $DGCI = 0.78$; $r^2 = 0.60$
Figure 3-2. Relationship between DGCI_{app} in the laboratory and leaf N concentration for experiments in 2013 (A) and for experiments in 2014 (B) for measurements at tasseling. Nonlinear regression was performed to determine the breakpoint of the linear-plateau model. Regressions were significant at \( P \leq 0.05 \).
Figure 3-3. Relationship between DGCI\textsubscript{app} in the field and leaf N concentration for experiments in 2013 (A) and for experiments in 2014 (B) for measurements at tasseling. Linear regression was performed. Regressions were significant at $P \leq 0.05$. 

For experiments in 2013:

- A. $y = 0.12x + 0.32$
- $r^2 = 0.37$

For experiments in 2014:

- B. $y = 0.15x + 0.18$
- $r^2 = 0.47$
Figure 3-4. Relationship between DGCI<sub>cam</sub> and SPAD values for experiments in 2013 (A) and for experiments in 2014 (B) for measurements at tasseling. Nonlinear regression was performed to determine the breakpoint of the linear-plateau model. Regressions were significant at P≤0.05.

A. When x≤ 50.7, DGCI= 0.013x+0.09
   when x> 50.7, DGCI= 0.78
   r²= 0.71

B. When x≤ 50.7, DGCI= 0.013x+0.09
   when x> 50.7, DGCI= 0.78
   r²= 0.71
Figure 3-5. Relationship between DGCI_{app} in the laboratory and SPAD values for experiments in 2013 (A) and for experiments in 2014 (B) for measurements at tasseling. Nonlinear regression was performed to determine the breakpoint of the linear-plateau model. Regressions were significant at $P \leq 0.05$. 

When $x \leq 50.5$, DGCI = 0.01x + 0.27  
when $x > 50.5$, DGCI = 0.61  
$r^2 = 0.51$

When $x \leq 46.4$, DGCI = 0.012x + 0.1  
when $x > 46.4$, DGCI = 0.64  
$r^2 = 0.78$
Figure 3-6. Relationship between DGCI_{app} in the field and SPAD values for experiments in 2013 (A) and for experiments in 2014 (B) for measurements at tasseling. Linear regression was performed. Regressions were significant at $P \leq 0.05$. 

Regression for 2013:

$$y = 0.01x + 0.21$$

$R^2 = 0.4$

Regression for 2014:

$$y = 0.01x + 0.07$$

$R^2 = 0.35$
Figure 3-7. Relationship between DGCI\textsubscript{app} in the lab, DGCI\textsubscript{app} in the field and DGCI\textsubscript{cam} values for experiments in 2013 (A) and for experiments in 2014 (B) for measurements at tasseling. Linear regression was performed. Regressions were significant at $P \leq 0.05$. * The regression between DGCI\textsubscript{app} in the laboratory and DGCI\textsubscript{cam}. ** The regression between DGCI\textsubscript{app} in the field and DGCI\textsubscript{cam}. **y = 0.35x + 0.32 r^2 = 0.52

* y = 0.58x + 0.2 r^2 = 0.30

** y = 0.64x + 0.14 r^2 = 0.67

** y = 0.88x - 0.05 r^2 = 0.43
GENERAL CONCLUSIONS

The dark green color index (DGCI) technology is a tool that could potentially change how farmers determine the quantity of N fertilizer to apply to maize. Previous research determined that DGCI is closely associated with leaf N concentration and could determine the amount of N needed to achieve 95% of the maximum yield in maize. These DGCI algorithms were originally developed using a digital camera (camera-method) and were modified by Spectrum Technologies Company to develop an iPhone app called Greenindex+ (app-method). The objectives of our study were to: (1) evaluate the N recommendations based upon DGCI algorithms made at V6 to V10 using the app- and the camera-methods with standard recommendations (246 kg N ha\(^{-1}\)) from the University of Arkansas Cooperative Extension Service; (2) determine if the app-method agreed with its camera counterpart in assessing DGCI values; (3) and identify the sources leading to any potential discrepancies between the camera- and the app-methods. Results showed the app-method predicted N to apply to maize in situations where residual N was high and the crop was not responsive to N fertilization. The camera-method predicted less amounts of N than the standard recommendations in situations where residual N was medium or low, without an apparent yield reduction.

Regression analysis identified variability between the camera- and the app-methods in determining DGCI values. One of the major reasons leading to discrepancies between the camera and the app-methods is the non-uniform distribution of N over a maize leaf blade. This is important because the app-method calculates DGCI of only a portion of the maize leaf while the camera-method calculates DGCI from the entire leaf. This study indicated that more work is needed to resolve problems related to the inconsistency between the camera and the app-methods.
Appendix Table A. Values of DGCI<sub>cam</sub>, lab DGCI<sub>app</sub>, field DGCI<sub>app</sub>, leaf N concentration, recommended N and yield as averaged across year, location, preplant N and maize growth stage.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Preplant N (kg ha&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Growth stage</th>
<th>DGCI&lt;sub&gt;cam&lt;/sub&gt;</th>
<th>Lab DGCI&lt;sub&gt;app&lt;/sub&gt;</th>
<th>Field DGCI&lt;sub&gt;app&lt;/sub&gt;</th>
<th>Leaf N concentration</th>
<th>Recommended N (kg ha&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Yield (Mg ha&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>Fayetteville</td>
<td>0</td>
<td>V6</td>
<td>0.73</td>
<td>0.64</td>
<td>0.66</td>
<td>3.72</td>
<td>163</td>
<td>10.7</td>
</tr>
<tr>
<td>2013</td>
<td>Fayetteville</td>
<td>0</td>
<td>V8</td>
<td>0.56</td>
<td>0.56</td>
<td>0.61</td>
<td>3.29</td>
<td>188</td>
<td>10.2</td>
</tr>
<tr>
<td>2013</td>
<td>Fayetteville</td>
<td>0</td>
<td>V10</td>
<td>0.72</td>
<td>0.59</td>
<td>0.57</td>
<td>2.95</td>
<td>189</td>
<td>10.7</td>
</tr>
<tr>
<td>2013</td>
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<td>0</td>
<td>V12</td>
<td>0.70</td>
<td>0.57</td>
<td>0.60</td>
<td>2.63</td>
<td>188</td>
<td>10.5</td>
</tr>
<tr>
<td>2013</td>
<td>Fayetteville</td>
<td>67</td>
<td>V6</td>
<td>0.75</td>
<td>0.64</td>
<td>0.67</td>
<td>4.09</td>
<td>149</td>
<td>12.1</td>
</tr>
<tr>
<td>2013</td>
<td>Fayetteville</td>
<td>67</td>
<td>V8</td>
<td>0.56</td>
<td>0.58</td>
<td>0.60</td>
<td>3.28</td>
<td>176</td>
<td>12.4</td>
</tr>
<tr>
<td>2013</td>
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<td>67</td>
<td>V10</td>
<td>0.73</td>
<td>0.57</td>
<td>0.56</td>
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<td>181</td>
<td>12.3</td>
</tr>
<tr>
<td>2013</td>
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<td>V12</td>
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<td>0.57</td>
<td>0.61</td>
<td>3.00</td>
<td>188</td>
<td>11.8</td>
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<tr>
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<td>Fayetteville</td>
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<td>V6</td>
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<td>159</td>
<td>14.2</td>
</tr>
<tr>
<td>2014</td>
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<td>--</td>
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<td>3.23</td>
<td>169</td>
<td>14.1</td>
</tr>
<tr>
<td>2014</td>
<td>Fayetteville</td>
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<td>2.69</td>
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<td>2.56</td>
<td>166</td>
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</tr>
<tr>
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<td>V6</td>
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<td>4.17</td>
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<tr>
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<td>V8</td>
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<td>3.75</td>
<td>171</td>
<td>13.1</td>
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<tr>
<td>2014</td>
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<td>V10</td>
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<tr>
<td>2014</td>
<td>Fayetteville</td>
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<td>V12</td>
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<td>2014</td>
<td>Pine Tree</td>
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<td>--</td>
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<td>3.42</td>
<td>149</td>
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<td>2014</td>
<td>Pine Tree</td>
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<td>V8</td>
<td>0.62</td>
<td>--</td>
<td>--</td>
<td>3.42</td>
<td>151</td>
<td>15.1</td>
</tr>
<tr>
<td>2014</td>
<td>Pine Tree</td>
<td>0</td>
<td>V10</td>
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<td>--</td>
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<td>2.77</td>
<td>160</td>
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</tr>
<tr>
<td>2014</td>
<td>Pine Tree</td>
<td>0</td>
<td>V12</td>
<td>0.64</td>
<td>--</td>
<td>--</td>
<td>2.35</td>
<td>157</td>
<td>13.7</td>
</tr>
<tr>
<td>2014</td>
<td>Pine Tree</td>
<td>67</td>
<td>V6</td>
<td>0.68</td>
<td>--</td>
<td>--</td>
<td>3.75</td>
<td>133</td>
<td>16.3</td>
</tr>
<tr>
<td>2014</td>
<td>Pine Tree</td>
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<td>V8</td>
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<td>--</td>
<td>--</td>
<td>3.49</td>
<td>148</td>
<td>16.6</td>
</tr>
<tr>
<td>2014</td>
<td>Pine Tree</td>
<td>67</td>
<td>V10</td>
<td>0.68</td>
<td>--</td>
<td>--</td>
<td>3.45</td>
<td>133</td>
<td>16.0</td>
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<tr>
<td>2014</td>
<td>Pine Tree</td>
<td>67</td>
<td>V12</td>
<td>0.69</td>
<td>--</td>
<td>--</td>
<td>2.95</td>
<td>122</td>
<td>16.8</td>
</tr>
</tbody>
</table>
Appendix Table B. Analysis of variance of the DGCI values based upon the app-method at Fayetteville in 2013 or the camera-method at Fayetteville and Pine Tree in 2014 before any N applications. Maize growth stage and preplant N were considered fixed effects.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Fayetteville 2013</th>
<th>Fayetteville 2014</th>
<th>Pine Tree 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P-values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth stage</td>
<td>3</td>
<td>0.0031</td>
<td>&lt;0.0001</td>
<td>0.3967</td>
</tr>
<tr>
<td>Preplant N</td>
<td>1</td>
<td>0.9941</td>
<td>0.0005</td>
<td>0.018</td>
</tr>
<tr>
<td>Growth stage * Preplant N</td>
<td>3</td>
<td>0.9302</td>
<td>0.1552</td>
<td>0.9799</td>
</tr>
<tr>
<td>R²</td>
<td></td>
<td>0.31</td>
<td>0.55</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Appendix Table C. Analysis of variance of the leaf N concentration values based upon the app-method at Fayetteville in 2013 or the camera-method at Fayetteville and Pine Tree in 2014 before any N applications. Maize growth stage and preplant N were considered fixed effects.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Fayetteville 2013</th>
<th>Fayetteville 2014</th>
<th>Pine Tree 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P-values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth stage</td>
<td>3</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Preplant N</td>
<td>1</td>
<td>0.0274</td>
<td>0.0003</td>
<td>0.0083</td>
</tr>
<tr>
<td>Growth stage * Preplant N</td>
<td>3</td>
<td>0.1943</td>
<td>0.3950</td>
<td>0.0525</td>
</tr>
<tr>
<td>R²</td>
<td></td>
<td>0.75</td>
<td>0.87</td>
<td>0.85</td>
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</table>
Appendix Figure A. Response of DGCI as determined by the app-method in 2013 at Fayetteville to N applications based on treatments consisting growth stages at V6, V8, V10 and V12 in the 0 and 67 kg N ha$^{-1}$ preplant sites. The interaction between preplant N and growth stage was not significant ($P>0.05$). The growth stage factor in DGCI$_{app}$ was significant. Different letters within a growth stage indicate a difference as determined by a HSD.
Appendix Figure B. The main effect of growth stage and preplant N on DGCI as determined by the camera-method with measurements taken at Fayetteville in 2014 at V6, V8, V10, and V12. The interaction between growth stage and preplant N was not significant ($P>0.05$). Different letters among growth stages and between preplant N sites indicate a significant difference as determined by a HSD and LSD, respectively.
Appendix Figure C. The main effect of growth stage and preplant N on DGCI as determined by the camera-method with measurements taken at Fayetteville in 2014 at V6, V8, V10, and V12. The interaction between growth stage and preplant N was not significant ($P > 0.05$). Different letters among between preplant N sites indicate a significant difference as determined by a LSD.
Appendix Figure D. The main effect of growth stage and preplant N on leaf N concentration with measurements taken at Fayetteville in 2013 at V6, V8, V10, and V12 before any N application. The interaction between growth stage and preplant N was not significant ($P>0.05$). Different letters among growth stages and between preplant N sites indicate a significant difference as determined by a HSD and LSD, respectively.
Appendix Figure E. The main effect of growth stage and preplant N on leaf N concentration with measurements taken at Fayetteville in 2014 at V6, V8, V10, and V12 before any N application. The interaction between growth stage and preplant N was not significant ($P>0.05$). Different letters among growth stages and between preplant N sites indicate a significant difference as determined by a HSD and LSD, respectively.
Appendix Figure F. The main effect of growth stage and preplant N on leaf N concentration with measurements taken at Pine Tree in 2014 at V6, V8, V10, and V12 before any N application. The interaction between growth stage and preplant N was not significant ($P>0.05$). Different letters among growth stages and between preplant N sites indicate a significant difference as determined by a HSD and LSD, respectively.