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# Midseason Nitrogen Sufficiency Guidelines for Corn Production in Arkansas Based On Tissue Analysis and Remote Sensing

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Midseason Nitrogen Sufficiency Guidelines for Corn Production in Arkansas Based On Tissue Analysis and Remote Sensing

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# ABSTRACT

There are a lack of tools to assess midseason nitrogen (N) status in corn (*Zea mays* L.) production systems and identify the need for additional N fertilization. This study was conducted to determine the ability of leaf N concentration (LN) and the Dark Green Color Index (DGCI) at the 10$^{th}$ collared leaf stage (V10), the 12$^{th}$ collared leaf stage (V12), and tasseling (VT) to predict if midseason N is required to maximize corn grain yield. From 2017 to 2019, eight field studies with 11 N treatments ranging from 0 to 258 kg N ha$^{-1}$ were conducted on silt loam soils in Arkansas. Leaf samples and digital images were collected at V10, V12, and VT growth stages. Relative grain yield (RGY) was predicted as a function of LN between V10 and VT which was described by a linear-plateau regression ($R^2$ = 0.82, P-value < 0.0001). Model predictions indicated that RGY increased linearly up to a LN concentration of 30.4 g N kg$^{-1}$ and there was no additional increase when LN was greater than 30.4 g kg$^{-1}$. Measurements of DGCI from the high N treatment (258 kg N ha$^{-1}$) were included in the regression analysis as a reference DGCI (RDGCI) to account for differences in environmental lighting when aerial images were captured. A multiple regression equation described the relationship between LN, DGCI, and RDGCI (P-value <0.0001) with a root mean square error (RMSE) of 3.49 g N kg$^{-1}$ and a bias of -0.03 g N kg$^{-1}$. Similarly, RGY was predicted as a function of DGCI and RDGCI (P-value < 0.0001) with a RMSE o 10.3% and a bias of -0.3%. Both LN and DGCI predicted corn N status accurately, demonstrating the ability to implement these tools to improve N management in corn production systems.

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# CHAPTER ONE

## Literature Review

## INTRODUCTION

Corn (*Zea mays* L.) is one of the primary cereal crops worldwide with more than 183 million hectares grown annually (USDA-NASS, 2018). The world's average production is 5,700 kg $ha^{-1}$, resulting in approximately 1 billion metric tons of corn harvested yearly. In the United States (U.S.), corn is grown on 32 million hectares with yields averaging 10,700 kg $ha^{-1}$. The total production of corn in the U.S. is around 353 million metric tons (USDA-NASS, 2018).

## HISTORICAL AND CURRENT NITROGEN USE

Among the plant nutrients, nitrogen (N) is usually required in the highest quantities. This nutrient is a component of enzymes and storage proteins, photosynthetic pigments, secondary metabolites, and defense compounds (Maathuis, 2009).

Nitrogen management has been a major concern since the domestication of plants and animals began. Even without an understanding of the chemical properties of N, over 2500 years ago, the Roman civilization used certain agronomic practices to keep the soil fertile (Sinclair & Sinclair, 2010). Barley (*Hordeum vulgare*) and wheat (*Triticum spp.*) were grown in rotation with fallow to give the soil time to mineralize N, so it would be available for the next crop. As the population increased in the Roman Empire, this rotation stopped being a priority, giving place to continuous crop production, with no fallow period. Uninterrupted crop production mined the soil N, and the soil did not have time to mineralize N for the next crop, thus resulting in lower yields. A common practice then, was to fertilize crop fields with animal manures.

Rice (*Oryza sativa*), corn, and wheat are three of the major global cereal crops and are responsible for more than half of the world's fertilizer consumption (Ladha, Pathak, Krupnik, Six, and Kessel, 2005). In 1940, the global consumption of N fertilizer was 3 Mt; by 2017, global

N use had soared to 109 Mt (FAO, 2020), showing a substantial increase in the amount of N applied to crop fields. Approximately 45% of the global N demand is supplied with synthetic fertilizers. The remaining N is supplied by biological N fixation (BNF), crop residues, animal manure, atmospheric deposition, and irrigation water (Ladha et al., 2005). Despite the importance of N to agriculture, N is used inefficiently in cropping systems. This inefficiency is noted by low average values of N use efficiency (NUE), where NUE is the ratio between the amount of N from the fertilizer accumulated by the crop and the amount of N applied via fertilizer (Raun & Johnson, 1999).

In a given region, the ratio between the average yield achieved by farmers and the yield attainable with optimal crop management practices is defined as the yield gap (Fischer, 2015). To date, some production areas still present yield gaps associated with N deficiency (Mueller et al., 2012). For instance, Sub-Saharan Africa, Eastern Europe, East and South Asia yield gap averages 71, 64 and 57% of the attainable yield, respectively. In contrast, North America averages 85%. Solely focusing on fertilizer, the yield gap for the major cereals (corn, wheat, and rice) worldwide could be narrowed to 75% of the attainable yield in 73% of the underachieving areas by increasing the input of N, $P_2O_5$ and $K_2O$ by 18, 16, and 35% respectively. On the other hand, to narrow the yield gaps to the same level while also eliminating overuse of fertilizer, the input variation would be 9 (N), -2 ($P_2O_5$) and 34% ($K_2O$) (Lassaletta, Billen, Grizzetti, Anglade, and Garnier, 2014).

## DIFFERENCES IN NITROGEN RECOMMENDATIOS AMONG STATES IN THE U.S.

The recommendation of N for corn production varies greatly among states in the U.S. In Arkansas, for instance, the recommendation is to apply 20 to 25% of the total N before planting. The remaining 75 to 80% of the N rate should be applied as sidedress, between the V6 and V8 growth stages (Ritchie, Hanway, and Benson, 1989) or be split in 50 to 65% of the N rate in sidedress between V6 and V8 followed by the remaining 15 to 25% of the total N as a pre-tassel application, between V10 and VT (Slaton et al., 2013a; b). The amount of N to be applied is defined by soil texture and yield goal. For example, for a loamy soil, and a yield goal up to 10,900 kg $ha^{-1}$, the recommended N rate is 179 kg N $ha^{-1}$. For the same soil, but with the yield goal above 10,900 kg $ha^{-1}$, the recommendation is 246 kg N $ha^{-1}$. In contrast, for a clayey soil, with the yield goal of 10,900 kg $ha^{-1}$, the recommendation is 258 kg N $ha^{-1}$, and for the yield goal above 10,900 kg $ha^{-1}$, the recommendation is 325 kg N $ha^{-1}$ (Slaton et al., 2013a).

In Nebraska, the recommended N rates are estimated based on expected yield, residual soil nitrate ($NO_3$-N), soil organic matter (SOM), timing of application, and fertilizer and corn prices. For example, for a soil with 3% of OM, soil test of 12 mg $NO_3$-N $kg^{-1}$, and a yield goal of 13,200 kg $ha^{-1}$, the recommended N rate is 118 kg N $ha^{-1}$. Given the same circumstances, but changing the SOM to 1%, the N rate recommended is 180 kg $ha^{-1}$. The application timing and fertilizer prices are taken into account by correction factors (Shapiro, Ferguson, Hergert, Wortmann, and Walters, 2008).

In Iowa, N rates are based on the previous crop, soil texture, and the price ratio between N and corn (Sawyer & Lundvall, 2018). The price ratio ranges from 0.00089 to 0.00357 \$ $kg^{-1}$N $\$^{-1}$kg $corn^{-1}$ (0.05 to 0.2 \$$lb^{-1}$ N $\$^{-1}$ bu) and is calculated as the ratio between the cost per unit mass of N and the price of the corn commodity. Soil series is taken into account by varying the recommended N rate according to the geographical region within the state. For instance, greater N rates are recommended in the southeast region of the state when compared to the rest of Iowa. The previous crop is taken into account by decreasing the recommended N rate when corn is planted in an area where the previous crop was soybean (*Glycine max*), as opposed to a continuous corn cropping system. The decrease in the N rate ranges from 46 kg N $ha^{-1}$ to 67 kg N $ha^{-1}$ depending on price ratio and soil texture. For example, in the central part of Iowa with a price ratio of 0.00089 \$ $kg^{-1}$N \$$^{-1}$kg $corn^{-1}$ in a continuous corn system, the recommended rate is 235 kg N $ha^{-1}$. However, if the previous crop was soybean, the recommended rate decreases by 56 kg $N^{-1}$ to 178 kg $N^{-1}$. In contrast, in a scenario with either high N prices or low corn prices, and where the price ratio is 0.00357 \$ $kg^{-1}$N \$$^{-1}$kg $corn^{-1}$, N rate recommendations in the central area of the state are 172 kg $N^{-1}$ and 126 kg $N^{-1}$ for a continuous corn system and corn following soybean, respectively.

## MECHANISMS OF NITROGEN LOSS

The NUE varies greatly worldwide and is strongly influenced by management and environmental conditions. For instance, in Brazil and U.S., the NUE in corn averages 65%, while NUE in India and China averages 40% (Lassaletta et al., 2014). The remaining N is lost due to soil denitrification, surface runoff, leaching and ammonia ($NH_3$) volatilization (Ladha et al., 2005).

### DENITRIFICATION

Denitrification occurs when $NO_3^-$ is present in an anaerobic environment (Brady & Weil, 2002). In rice fields, for example, the flooding irrigation system favors the denitrification process. Once most of the $O_2$ is eliminated from the soil and the redox potential is reduced, microbes use $NO_3^-$ as a terminal electron acceptor. As a result of this process, N can be lost as $NO_2^-$, NO, $N_2O$, and $N_2$. Denitrification is usually evaluated in a soil by measuring the end-products $N_2O$ and $N_2$ (Qian et al., 1997). Rates of N losses via denitrification are variable and highly influenced by environmental conditions. According to Patrick and Wyatt (1964), under continuous anaerobic conditions, denitrification is limited to $NO_3^-$ and $NO_2^-$ that were present in the soil when the anaerobic condition was established. Since the conversion of $NH_4^+$ to $NO_3^-$ (nitrification) is an aerobic process, nitrification would not occur under anaerobic soil conditions. In addition, the authors showed that intermittent wetting and drying cycles favored denitrification by renewing the $NO_3^-$ pool after dry periods, followed then by subsequent denitrification. This study also demonstrated that increasing carbon (C) availability in soil by adding rice straw to the field favored denitrification.

## LEACHING

Leaching is the vertical movement of the water in the soil profile beyond the rooting depth. This process is mainly determined by soil, crop, and N surplus (Schröder, Assinck, Uenk, and Velthof, 2010). According to Karlen et al. (2002), the predominant form of N lost by leaching is $NO_3^-$ and it is responsible for the degradation of groundwater and, eventually, surface water, such as the Mississippi River and its tributaries. In fact, N leaching and runoff from corn and soybean fields contribute to 45.6% of $NO_3^-$ contamination in the Gulf of Mexico (Alexander et al., 2008). To decrease the contamination of surface and ground water, it is necessary to use N more efficiently, reducing pollution and increasing profitability. The use of reduced N rates and cover crops decreased N loss by 59% (Syswerda, Basso, Hamilton, Tausig, and Robertson, 2012).

## AMMONIA VOLATILIZATION

Volatilization is the main N loss mechanism when $NH_3$ based fertilizers are applied to the surface of flooded soils (Smil, 1999). This process occurs when N is lost to the atmosphere as $NH_3$, being affected by soil moisture, pH, fertilizer placement, and temperature. Ernst and

Massey (1960) showed that an increase in soil pH increases $NH_3$ volatilization. Ten days after urea application, a soil with a pH of 5 volatilized around 8% of the N applied, while a soil with a pH of 7.5 volatilized 50% of the N applied. The same study showed that fertilizer placement also affects the losses via volatilization. While urea placed on the soil surface volatilized 18% of the N applied in a 13-day period, urea placed 0.04 m below soil surface volatilized 5% of the N applied. Ernst and Massey (1960) also showed that when the temperature was 7 °C, 5% of the N applied was lost was $NH_3$, when the temperature was 32 °C, 24% of the N applied was volatilized.

Soil moisture also affects $NH_3$ volatilization (Ernst & Massey, 1960). Dry soils had little measurable $NH_3$ volatilization, while saturated soils volatilized up to 18% of the N applied. Although the losses for $NH_3$ volatilization averaged around 10 to 15%, there can be considerable increase in volatilization when the pH exceeds 7 and the temperature exceeds 16 °C. For instance, when the temperature is 32 °C and the soil pH is 6.5, the losses for volatilization are around 25%, but given the same temperature and a soil pH of 7.5, the losses increase to 45%. In contrast, when the pH is 6.5 and the temperatures are 7°C and 16°C, the losses are around 5% and 10%, respectively (Franzen, 2004).

## MANAGEMENT STRATEGIES TO IMPROVE NITROGEN USE EFFICIENCY

Management strategies such as matching crop demand and N fertilizer supply, splitting N fertilizer applications, minimizing application during the wet season, and changing fertilizer sources to match the environmental conditions can increase NUE (Mosier, Syers, and Freney, 2004). The majority of N is absorbed by corn after the V8 stage (Russelle, Hauck, and Olson, 1983), which leaves N applied early in the season exposed to loss mechanisms, lowering overall NUE. Thus, synchronizing corn N demand with fertilization through split applications reduces the possibility of losses (Magdoff, 1991), increasing NUE. When N was applied to furrow-irrigated corn in a two -way split (pre-plant and sidedress application at V6 to V8), 81 to 91% of the N fertilizer was effectively absorbed by the crop (Roberts, Slaton, Kelley, Greub, and Fulford, 2016). Furthermore, in-season N applications as late as V12 to VT have resulted in no significant yield losses (Russelle et al., 1983) indicating a wide window for N midseason fertilization.

Nitrogen fertilizer source is an important management consideration. Low fertilizer recovery is frequently observed in production systems in which urea is applied to the soil surface resulting in $NH_3$ volatilization (Keller & Mengel, 1986). Urea is highly susceptible to volatilization, as it is readily soluble in water and prone to hydrolysis, forming $NH_4^+$ (Ernst & Massey, 1960), which can rapidly convert to $NH_3$. Urea hydrolysis is mediated by the urease enzyme, which is pervasive in soils and surface residues (Brady & Weil, 2002). To reduce the loss of N, it is necessary to adjust the fertilizer source to the production system. For a surface application, prilled ammonium nitrate volatilized 3.7% of the N applied, whereas granular urea volatilized 30% of the N applied 5 days after application (Keller & Mengel, 1986).

Improved efficiency fertilizers were developed to increase the synchrony between crop demand and N availability in the soil (Abalos, Jeffery, Sanz-Cobena, Guardia, and Vallejo, 2014). These can be separated in two different groups: (1) controlled release fertilizers, which are coated with inert material and slowly release the fertilizer as the coating material degrades, and (2) stabilized fertilizers, which are coated with urease or nitrification inhibitors (Shaviv & Mikkelsen, 1993). The use of improved efficiency fertilizers alone has increased the global average yield and NUE by 7.5% and 12.9%, respectively. In fact, the use of nitrification or urease inhibitors improves NUE in systems with well-drained soils and with high N input.

Urease inhibitors are the most appropriate option for systems that favor $NH_3$ volatilization (Abalos et al., 2014).

## SOIL ANALYSIS TO DETERMINE CORN NITROGEN NEEDS

In the U.S. Northwest, the problems with over or under fertilization in silage corn production encouraged the development of the Pre-sidedress Nitrate Test (PSNT) (Magdoff, Ross, and Amadon, 1984). According to Magdoff (1991), the PSNT was successful in predicting the N fertilization needs because of the climate and the production system. The Pacific Northwest climate lessens leaching loss potential during the winter and early spring, leaving more $NO_3^-$ for corn uptake and assimilation. Simultaneously, these cropping systems experience very little input of N via manure. The PSNT consists of a soil sample from the top 0.3 m of the soil, when corn is about 0.24 to 0.30 m tall. This test's advantages have been reported to increase NUE and decrease $NO_3^-$ leaching (Jaynes et al., 2004).

Similar to the PSNT, the Pre-Plant Nitrate Test (PPNT) evaluates residual $NO_3^-$ present in the soil (Bundy & Malone, 1988). This test uses the profile of $NO_3^-$ present in the top 0.9 m of the soil to make adjustments to the N rate recommendation. The PPNT reduces the risk of $NO_3^-$ leaching by decreasing the amount of $NO_3^-$ left in the soil through the recovery of the initial $NO_3^-$ present in the soil. However, due to the potential $NO_3^-$ leaching between soil sampling and crop N uptake, the PPNT has been reported to fail in distinguishing responsive from non-responsive soils in humid environments (Spargo, Alley, Thomason, and Nagle, 2009).

The search for new in-season N status assessment tools led to the development of the Illinois Soil Nitrogen Test (ISNT) (Khan, Mulvaney, and Hoeft, 2001; Mulvaney, Khan, Hoef, and Brown, 2001). Williams et al. (2007) described that this test is based on an alkaline digestion of a soil sample, followed by colorimetric analysis of the $NH_3$-N released during the digestion. The hydrolysates are fractionated in total hydrolyzable N, hydrolyzable $NH_4$-N, amino sugar and $NH_4$–N, amino acid–N, and amino sugar-N. The test was used to classify Illinois soils as responsive to N fertilization (ISNT result < 225 mg $kg^{-1}$) or non-responsive (ISNT result > 235 mg $kg^{-1}$) (Khan et al., 2001). Calculations of the economic optimum N rate (EONR) for the average fertilizer cost and corn price ratio correlated negatively and strongly with the EONR, indicating the ability to predict corn response to N (Williams et al., 2007). In contrast, the results from 80 site-years of corn response to N trials found it incapable of distinguishing responsive from non-responsive soils in Wisconsin. This study correlated the ISNT values with SOM and suggested that the ISNT is measuring a constant fraction of the SOM rather than readily mineralizable-N (Osterhaus, Bundy, and Andraski, 2008).

The Nitrogen Soil Test for Rice (N-STaR) was developed for silt loam soils in Arkansas (Roberts, Norman, Slaton, and Wilson, 2011). The N-STaR method uses direct steam distillation (DSD) to quantify amino sugar and alkaline hydrolyzable N (AH-N), predicting potentially mineralizable soil N (amino sugars, $NH_4$, and amino acids). This method's basis is the correlation between the AH-N levels and rice response parameters (total N uptake and check plot grain yield) and the calibration of N rates needed to reach 90, 95 and 100% of relative grain yield (RGY). It has accurately predicted site-specific N rates needed to achieve 95 and 100% RGY in silt loam soils in Arkansas. (Roberts, Norman, Fulford, and Slaton, 2013). Developing the same prediction system for upland row crops, such as corn, research has met a few challenges including: the need to determine the best N application strategy to achieve the highest NUE, how to assess $NO_3$-N and to determine a proper soil sampling depth based on the effective rooting depth of the crop.

## PLANT MEASUREMENTS TO DETERMINE CORN NITROGEN NEEDS

Several sensors have been used in the field as in-season evaluation tools. Crop N concentration directly affects the chlorophyll concentration in the leaf and canopy. Chlorophyll concentration affects the balance between absorbance and reflectance of the light spectrum. The more chlorophyll that is present in the leaf, relatively more green light is reflected. However, chlorophyll meters (CM), such as the SPAD-502 meter (Konica Minolta, Tokyo, Japan), base their measurements on the amount of red light absorbed. The more chlorophyll present in the leaf, the more red light is absorbed (Samborski, Tremblay, and Fallon, 2009).

In the North Central area of the USA (Illinois, Kansas, Michigan, Minnesota, Missouri, Nebraska and Wisconsin), a cooperative study was conducted to evaluate the accuracy of CM to predict corn yield response to N and to assess corn N needs (Scharf, Brouder and Hoeft, 2006). The model estimates the EONR based on relative CM readings. High N treatments were chosen as references and their CM values were used as standards. The EONR was calculated for every location, using regression analysis and an N productivity ratio of 3.4 kg corn grain $kg^{-1}$ N fertilizer, which was representative of the time period that the study was performed. At the EONR, the yield averaged 11,000 kg $ha^{-1}$ and the average response to EONR was an increase of 3600 kg $ha^{-1}$. Overall, the study concluded that CM readings were significant ($P$-value $< 0.0001$) predictors of EONR for corn.

In a similar study in Nebraska from 1995 to 2004, CM measurements were made at three growth stages (V8, V10, and V12) (Varvel, Wilhelm, Shanahan, and Schepers, 2007), and the Sufficiency Index (SI) was calculated as the ratio between the CM reading for a given treatment or region in the field and the maximum CM reading. A quadratic regression was fit to the chlorophyll meter readings (SI) and N fertilizer rates at all growth stages. This method required an area with high N input, and once this area was established this regression could be used to estimate the N rate needed to achieve maximum yield.

The normalized difference vegetation index (NDVI) has also been used to evaluate N need in cropping systems. Crop Circle ACS-210 (Holland Scientific, Lincoln, NE) and Green Seeker (N Tech Industries, Inc., Ukiah, CA) are examples of NDVI sensors used to evaluate the crop N status. These sensors determine NDVI based on red (650 ±10 nm) and near infra-red (NIR, 750 ± 15 nm) reflectance from the crop canopy. The NDVI value is calculated according to equation (1 and varies from -1 to 1 and the higher the value, the more intense the light absorption. The NDVI measurements have been reported to strongly correlate with leaf N concentration (LN) (Schlemmer et al., 2013).

$$\text{NDVI} = \frac{NIR - red}{NIR + red} \qquad (1)$$

In Oklahoma, a Nitrogen Fertilization Optimization Algorithm (NFOA) was developed using NDVI measurements (Teal et al., 2006; Tubaña et al., 2008). The algorithm uses NDVI values collected between V7 and V9 stages to calculate crop yield potential (YP). N rate is calculated as the product of N concentration in the corn grain (0.0125 kg N $kg^{-1}$) and YP, divided by the expected NUE. Using this algorithm, field trials were performed in three locations across the state. The N rate calculated with NFOA varied from 39 to 213 kg N $ha^{-1}$ (35 to 190 lb N $A^{-1}$) and even the lowest rate did not result in significant yield loss when compared to the fixed rate of 171 kg N $ha^{-1}$ (Tubaña et al., 2008). The use of the NFOA resulted in higher NUE, 65%, while the fixed rate treatments averaged 56%. Furthermore, it also resulted in higher net return, \$561 $ha^{-1}$, in contrast to \$425 $ha^{-1}$ of the fixed rate treatment.

The Dark Green Color Index (DGCI) has also been a tool to assess corn N status. An advantage of DGCI, when compared to NDVI and CM, is that it is measured from digital images, indicating that it can be utilized in aerial platforms without requiring the purchase of additional sensors. From an aerial platform, DGCI integrates measurements over an entire experimental unit compared with CM which has a sensor that covers approximately $6mm^2$. This method requires the red, green and blue (RGB) values from digital images of the crop to be converted to hue, saturation and brightness values. The DGCI value ranges from 0 (very yellow), to 1(dark green) and it is calculated according to equation (2) (Karcher & Richardson, 2003).

$$\mathrm{DGCI} = \frac{\frac{(\mathrm{Hue} - 60)}{60} + (1 - \mathrm{Saturation}) + (1 - \mathrm{Brightness})}{3} \qquad (2)$$

This index has been used in corn with the purpose of assessing LN (Rorie, Purcell, Karcher, and King, 2011a). An ear-leaf was photographed with a digital camera against a pink board to provide contrast during the image processing phase. The pink board included two circular sections, with known DGCI values. These sections served as internal standards and were used to correct DGCI values according to different lighting conditions. Yield, LN and chlorophyll concentration have been reported to directly correlate with DGCI (Rorie et al., 2011a; Rorie et al., 2011b). Additionally, DGCI values measured between V6 and V10 on the uppermost collared leaf of corn plants were used to adjust the N recommended rate (Purcell, Siddons, Karcher, and Rorie, 2015).

Further research was developed to evaluate the accuracy of the DGCI method proposed by Purcell et al. (2015) (Rhezali, Purcell, Roberts, and Greub, 2018). The recommendations were checked against the University of Arkansas Extension N rate recommendation, 250 kg N $ha^{-1}$ (Slaton et al., 2013a). Overall, N rates recommended with the DGCI method ranged from 92 to 112 kg N $ha^{-1}$ less than the recommended Extension rate while maintaining yield in all cases. The authors concluded that the DGCI method was capable of predicting in-season N requirement for corn and that future research should focus on simplifying the method and extending it to aerial platforms so it can be used directly in the field.

Although several plant and soil based tests and indirect measurements have been developed to fine-tune N rate recommendations in corn production, there is a lack of N assessment tools for midseason N fertilizations. Tissue sampling for LN determination has been used extensively in plant nutrient monitoring; however, LN is generally determined at the R1 growth stage, which is past the growth stages for which midseason N applications are recommended. This study intended to determine the relationship between final RGY and LN determined from leaves collected between V10 and VT, and the adequacy of LN to serve as a guideline for midseason N fertilizations. In addition, DGCI shows close relationship with LN and RGY, and little is known about the ability of DGCI measured from aerial platforms to predict N fertilization needs. The second objective of this study was to determine the relationships among DGCI from an aerial platform, LN, and RGY, and to evaluate the adequacy of aerial DGCI measurements to guide in-season N fertilizations.

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# CHAPTER TWO

## Leaf Nitrogen Sufficiency Level Guidelines for Midseason Fertilization in Corn

# ABSTRACT

Corn (*Zea mays* L.) is one of the primary cereal crops worldwide with a yearly production of approximately one billion metric tons. Corn yield has increased 140% over the last four decades in part because of a 40% increase in nitrogen (N) input over the same period. However, the excessive application of N for corn production raises environmental and economic concerns, emphasizing the need for agricultural practices that lead to an efficient use of N. The objective of this study was to develop a prediction system for in-season N application using plant tissue analysis. Eight site-years were planted with the hybrid P1197YHR. The treatment structure was comprised of 11 N fertilization regimes with season total N rates ranging from 0 to 257 kg N $ha^{-1}$ and with split-applications at preplant, the $10^{th}$ collared leaf stage (V10), the $12^{th}$ collared leaf stage (V12), and tasseling (VT). The uppermost fully collared leaves were sampled at V10 and V12, and ear-leaves were sampled at VT. Relative grain yield (RGY) was positively associated with leaf N concentration (LN) at V10, V12, and VT. A single regression combining data over growth stages was developed between RGY and LN indicating that RGY increased linearly as LN increased up to 30.4g N $kg^{-1}$, between V10 and VT ($R^2 = 0.82$, P-value <0.0001). When LN was greater than 30.4 g N $kg^{-1}$, there was no further increase in RGY. This relationship can be used to predict the need of additional midseason N fertilization between the V10 to VT growth stages to maximize yield.

# INTRODUCTION

Since the Green Revolution, the amount of N fertilizer applied to agricultural soils has increased substantially. In 1940, the global consumption of N fertilizer was 3 Mt; by 2017, this number had soared to 109 Mt (FAO, 2020). Of the total N used in crops globally, approximately 45% is derived from synthetic fertilizers. The remaining N is supplied by biological N fixation, crop residues, animal manure, atmospheric deposition, and irrigation water (Ladha, Pathak, Krupnik, Six, and van Kessel, 2005).

Despite the importance of N to agriculture, this nutrient has not been used efficiently in cropping systems. Nitrogen use efficiency (NUE) is defined as the ratio between the amount of N from the fertilizer accumulated by the crop and the amount of N applied via fertilizer (Raun & Johnson, 1999). Globally, NUE varies greatly and is strongly affected by management and environmental conditions. For instance, in countries such as Brazil and the U.S., the NUE in corn is around 65%; India and China present lower NUE values, averaging around 40% (Lassaletta, Billen, Grizzetti, Anglade, and Garnier., 2014). The global NUE averages 33% indicating that the remaining N is lost due to soil denitrification, surface runoff, leaching and $NH_3$ volatilization (Ladha et al., 2005).

Strategies to improve NUE include matching crop demand and N fertilizer supply, splitting N fertilizer applications, minimizing application during the wet season, and changing fertilizer sources to match the environmental conditions (Mosier, Syers, and Freney, 2004). The majority of N is accumulated by corn after the V8 stage (Russelle, Hauck, and Olson, 1983), and N applied before V8 is exposed to potential losses, which lower the overall NUE. Synchronizing the crop’s time of greatest N requirement with N fertilization through split applications reduces the probability of losses (Magdoff, 1991). In Arkansas, for example, N fertilizer recommendations for corn are to apply 20 to 25% of the total N before planting. The remaining 75 to 80% of the N should either be applied as sidedress between the V6 and V8 stages (Ritchie, Hanway, and Benson,1989) or should be split in 50 to 65% of the N as sidedress, between V6 and V8, with the remaining 15 to 25% total N applied as a pre-tassel application (Slaton et al., 2013a, 2013b).

In-season N applications as late as V12 to VT have resulted in no significant yield losses, indicating that there is a large window of opportunity in-season to apply N to corn, especially in irrigated systems (Russelle et al., 1983). Diverging from the global average NUE of 33% (Ladha et al., 2005), furrow-irrigated corn in Arkansas has been reported to recover 81% to 91% of the N fertilizer when applied at V6 and more than 80% when fertilizer was applied in a two-way split (pre-plant and sidedress application at V6 to V8) (Roberts, Slaton, Kelley, Greub, and Fulford, 2016).

Rate and timing of N application recommendations for corn production varies greatly among states in the U.S. In Nebraska, for instance, N rate is recommended based on corn and fertilizer prices, soil organic matter (SOM), timing of application, expected yield, and residual soil nitrate (Shapiro, Ferguson, Hergert, Wortmann, and Walters, 2008). In Iowa, N rate recommendations are based on soil regions, previous crop, and a ratio between the cost of N and the price of the corn commodity (Sawyer & Lundvall, 2018). In Arkansas, N rate recommendations are based on soil texture and yield goal (Slaton et al., 2013a, 2013b).

Several tests have been developed that can be used before the growing season to assess corn N needs, including the Illinois Soil Nitrogen Test (ISNT) (Khan, Mulvaney, and Hoeft, 2001; Mulvaney, Khan, Hoeft, and Brown, 2001) and the Pre-Plant Nitrate Test (PPNT) (Bundy & Malone., 1988). The Pre-sidedress Nitrate Test (PSNT) (Magdoff, Ross, and Amadon, 1984) is designed to determine the amount of N needed for corn sidedress application. Additionally, ear-leaf N concentration at the R1 growth stage can be used to monitor crop in-season N status, adjust fertilization program, and provide information on the need of a possible late season N application, as an attempt to salvage yield (Kovács & Vyn. 2017). Yield increases have been reported from late season applications between V10 and late R1 (Binder, Sanders, and Walters, 2000; Roberts et al., 2016). However, it is important to diagnose the N need early to not lose yield potential, especially when N deficiency is severe (Binder et al., 2000).Therefore, it is critically important that N fertilizer is applied in a timely fashion so that maximal yields can still be achieved.

Identifying N deficiency earlier than at the R1growth stage would provide valuable information for corn producers to adjust N fertilization programs in-season. This information could be used to support the decision to adjust a late season N application to corn fields. The objective of this research was to determine the relationship between yield and LN and to identify the sufficiency level of LN that could serve as a guideline for midseason N fertilization.

## MATERIALS AND METHODS

Between 2017 and 2019, eight field studies were conducted at three research stations in Arkansas which were all situated on loamy textured soils (Table 2.1). Three of these fields were located at the Pine Tree Research Station (PTRS), three fields were situated at the Milo J Shult Agricultural Research and Extension Center (SAREC), and the remaining two fields were located at the Rohwer Research Station (RRS). The experimental design was a randomized complete block with four replications and 11 different treatments, which represented different timings and rates of N (Table 2.2). Plots were planted with the hybrid P1197YHR (Pioneer, Johnston, IA ) at the seeding rate of 98,000 seeds $ha^{-1}$, and plots consisted of four rows, 9 m in length and with row spacing of 0.76 (PTRS), 0.91(SAREC), and 0.96 m (RRS). Pre-plant N rates (33.6 kg N $ha^{-1}$) were applied to experimental units assigned to treatments 2 through 11 and incorporated into the soil prior to sowing. The sidedress treatments were surface applied between the stages V6 and V8. The range of sidedress N treatments were meant simulate deficient, optimal, and above optimal N nutrition of corn prior to pre-tassel N applications. The pre-tassel application was applied to various treatments at V10 or V12. All N fertilizations were made with urea (46-00-00) treated with N-(n-butyl)-thiophosphoric triamide (NBPT) (Agrotain Ultra, Koch Agronomic Services LLC., Wichita, KS) at the rate of 0.89 g NBPT $kg^{-1}$ urea. A single composite soil sample (four 3-cm diam. cores) was collected from each replication at all experimental sites to a depth of 15 cm, soil nutrient analysis was performed (Mehlich-3 extractable nutrients, Zhang, Hardy, Mylavarapu and Wang, 2014) to confirm nutrients other than N were not limiting crop growth. Irrigation and pest management of plots closely followed furrow-irrigated corn recommendations established by the University of Arkansas System Division of Agriculture Cooperative Extension Service (Espinoza and Ross, 2003). Irrigation timing was scheduled according to the Arkansas Irrigation Scheduler set at a 5-cm soil profile water deficit.

The uppermost leaf with a visible collar was collected from five plants in the two middle rows of each plot at V10 and V12, and five identifiable ear-leaves were sampled at the VT stage prior to N fertilization (Table 2.3). The leaf samples were oven-dried at 70 °C until a constant weight, ground and sieved via a 20-mesh screen, and analyzed for total N using the Campbell (1992) combustion method at the Soil Test and Plant Diagnostic Laboratory at the University of Arkansas (Fayetteville, AR).

Plots were trimmed to 6 m in length at maturity, the two center rows were harvested using a small plot combine, and yield was corrected to 15.5% moisture. Relative grain yield (RGY) was calculated as the ratio between the yield of an individual plot and the maximum yield attained within each environment.

Statistical analysis was conducted in R 3.5.2 (R Core Team, Vienna, Austria). The relationship between RGY and LN was investigated by fitting a nonlinear segmented regression (Eq.1) using the least squares estimation method where $\theta_0$ is the intercept, $\theta_1$ is the increment in RGY per one unit change in LN, and $x_1$ is the joint point of the regression:

$$RGY = \begin{cases} \theta_0 + \theta_1 \times LN, & LN < x_1 \\ \theta_0 + \theta_1 \times x_1, & LN \geq x_1 \end{cases} \tag{1}$$

An initial model was fit containing different coefficients for each growth stage; this model will be referred to as the full model for the remainder of this manuscript. Subsequently, a reduced model was fit, containing common coefficients for all three growth stages. The models were subjected to an F-test to test the hypothesis that the full model explained better the relationship between RGY and LN than the reduced model (Eq. 2) (Archontoulis & Miguez, 2014). In Equation 2, $SS_{full}$ is the sum of squares of the full model, $SS_{reduced}$ is the sum of squares of the reduced model, $df_{full}$ is the degrees of freedom of the full model, and $df_{reduced}$ is the degrees of freedom of the reduced model. A nonsignificant F test would indicated that the reduced model and the full model did not differ from each other in their descriptive performance of the relationship between RGY and LN.

$$F = \frac{(SS_{full} - SS_{reduced})}{(df_{full} - df_{reduced})} \times \frac{SS_{full}}{df_{full}} \tag{2}$$

For each growth stage, only experimental units that had received all of the N determined by the treatment structure were included in the regression process. For instance, at the growth stage V10, the observations used in the regression were limited to treatments 1, 2, 5, 8, and 11. Likewise, the regression for the V12 growth stage included only observations from treatments 1, 2, 3, 5, 6, 8, 9 and 11. Similarly, at the VT growth stage, the regression included observations from all treatments.

## RESULTS AND DISCUSSION

Across all environments, yields averaged 12,534 kg $ha^{-1}$ and ranged from 1,592 to 20,676 kg $ha^{-1}$ (Table 2.1). However, yields varied greatly among environments. The most productive environment was RRS in 2019, where yields ranged from 8,813 to 20,676 kg $ha^{-1}$ and, except for treatments 1 and 2, all treatments yielded greater than 17,148 kg $ha^{-1}$. This yield level above the maximum yield of all other environments. Conversely, the least productive environment was SAREC in 2017, where the maximum yield was 13,131 kg $ha^{-1}$ and the average yield was 10,807 $kg^{-1}$, which is roughly 2,000 $kg^{-1}$ less than the average yield of the remaining environments.

The ratio between the yields of the experimental units that received no N fertilization and those that received the highest N rate is an indicator of environmental responsiveness to N. The most responsive environments were RRS in 2018 and SAREC in 2019, where experimental units with no N fertilizer yielded 11% of the yields attained by the experimental units receiving the highest N rate (Table 2.1). Conversely, RRS in 2019 was the least responsive environment, where experimental units with no N fertilizer yielded 42% of the yields attained by experimental units receiving the highest N rate. In the remaining environments, plots receiving no N fertilizer yielded on average 28% of those receiving optimum and above optimum N fertilizer.

Measurements of LN ranged from 10.5 to 36.8 g $kg^{-1}$ and averaged 28.5 g $kg^{-1}$. The environments with the lowest LN concentrations were SAREC in 2017 and RRS in 2018, which coincided with the most responsive environments to N. In these environments, LN averaged 22.2

and 22.8 g $kg^{-1}$, respectively. In contrast, at RRS in 2019, LN concentration averaged 32.3 g $kg^{-}$1 and 75% of the experimental units had LN concentrations above 30.9 g $kg^{-1}$. This high average LN concentrations also coincided with the environment that was the least responsive to N and had the highest yields.

The relationship between RGY and LN was characterized by a linear plateau regression. Growth stage was tested as a fixed factor to determine a possible change in the optimum LN concentration among growth stages. Based on the results of the F-test (Table 2.4), including growth stage in the model did not improve statistically the model performance (P-value= 0.30). Therefore, data were combined over growth stages and the model with common coefficients for all three growth stages was chosen to represent the relationship between RGY and LN.

The relationship between RGY and LN between V10 and VT was characterized by a significant (P-value < 0.0001) (Table 2.5) nonlinear model, where RGY increased linearly between LN values of 10.0 g $kg^{-1}$ and 30.4 g $kg^{-1}$ and plateaued for LN values between 30.4 g $kg^{-1}$ and 36.8 g $kg^{-1}$ (Fig. 2.1). The model predicted RGY with a root mean square error of 8.6% and a bias <0.0001%. The joint point of the regression (30.4 ± 0.8 g $kg^{-1}$) represents the minimum adequate LN concentration between V10 and VT at which no midseason N fertilization would be required to produce maximal yields. Conversely, LN levels less than 30.4 ± 0.8 g N $kg^{-1}$ represent plants that would be expected to respond to N fertilization.

The LN sufficiency concentration of 30.4 g $kg^{-1}$ agrees well with sufficiency concentrations previously reported for the Midsouth: between 28.0 g $kg^{-1}$and 30.0 g $kg^{-1}$ (Bennett, Stanford, and Dumeni, 1953), 30 g $kg^{-1}$ (Melsted, Motto, and Peck, 1969), and 31.0 g $kg^{-1}$ (Greub, Roberts, Slaton, Kelley, and Gbur, 2018). However, these indexes were determined for N concentrations analyzed from ear-leaves, at the R1 stage. Normally, the information provided by ear-leaf sampling at the R1 stage is used to adjust N fertilization programs for future crops (Kovacs & Vyn, 2017). The present research provides a sufficiency level for earlier growth stages, between V10 and VT, widening the window for tissue sampling and nutrient monitoring. The fact that the uppermost collared leaf can be used to diagnose crop N status may help producers fine tune N fertilization, improve NUE, and avoid unnecessary N applications. While N applications between V10 and late R1 can statistically increase crop yield (Binder et al., 2000; Roberts et al., 2016), it is important to supply N earlier to maximize yields, as opposed to increasing yields, especially in cases of severe N deficiency (Binder et al., 2000). Therefore, a test at growth stages earlier than R1 provides valuable information for corn growers.

## CONCLUSIONS

Current interpretations of LN concentration for corn are restricted to early vegetative growth stages (prior to V6) and early reproductive growth stages (R1). In irrigated corn production systems where N can be applied and incorporated quickly and efficiently, midseason N diagnostic tools could provide valuable information to corn growers looking to maximize yield, by helping to identify areas were N is sufficient or lacking. Tissue analysis of the uppermost collared leaf at the V10 and the V12 growth stages and of the ear-leaf at the VT growth stage demonstrated the ability to identify optimum and suboptimum levels of LN in furrow irrigated corn in Arkansas. In our experiments the time between V10 and VT ranged from 21 to 28 days, providing a wide window for corrective action. The possibility of evaluating corn N status during vegetative growth provides an opportunity to growers to determine the potential need for a pretassel N application. By providing this information to growers, there is a possibility of salvaging yield with a pretassel N application, in cases where N sufficiency levels are below optimum ($< 30.4$ g $kg^{-1}$). Furthermore, there is also the possibility to prevent N over-fertilization, in cases where N sufficiency levels are equal or above optimum levels ($\geq$ 30.4 g $kg^{-1}$). The development of these LN sufficiency guidelines has greatly increased the interpretation window for corn produced in irrigated production systems and when combined with previous research identifies critical LN concentrations from V10 through the late R1 growth stage. These LN sufficiency guidelines provide research-based tissue N concentration levels that will allow producers to better manage N applications to corn increasing NUE and reducing potential for negative environmental impacts.

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# TABLES AND FIGURE

**Table 2.1**. Soil series, geographical coordinates, sowing date, and yield range for year and location of studies

| Location | Latitude | Longitude | Year | Soil series | Sowing date | Yield range ($kg\ ha^{-1}$) |
|---|---|---|---|---|---|---|
| SAREC[1] | 36.09 N | 94.17 W | 2017 | Captina silt loam | 5/8 | 3,936 – 13,131 |
| PTRS[2] | 35.12 N | 90.92 W | 2017 | Calloway silt loam | 4/13 | 5,780 – 17,145 |
| SAREC | 36.09 N | 94.17 W | 2018 | Captina silt loam | 4/24 | 6,094 – 16,155 |
| PTRS | 35.12 N | 90.92 W | 2018 | Calloway silt loam | 4/12 | 4,445 – 16,645 |
| RRS[3] | 33.80 N | 91.26 W | 2018 | Sharkey silt loam | 4/11 | 1,592 – 14,319 |
| SAREC | 36.09 N | 94.17 W | 2019 | Captina silt loam | 4/11 | 1,676 – 14,856 |
| PTRS | 35.12 N | 90.92 W | 2019 | Sharkey silt loam | 4/29 | 2,840 – 16,237 |
| RRS | 33.80 N | 91.26 W | 2019 | Calloway silt loam | 4/24 | 8,813 – 20,676 |

[1] Shult Agricultural Research and Extension Center
[2] Pine Tree Research Station
[3] Rohwer Research Station

**Table 2.2.** Nitrogen (N) rates and times of application for different treatments

| Treatment | Nitrogen rates (kg N $ha^{-1}$) | | | Pre-tassel application timing |
|---|---|---|---|---|
| | Pre-plant | Sidedress | Pre-Tassel | |
| 1 | 0 | 0 | 0 | - |
| 2 | 33.6 | 56.4 | 0 | - |
| 3 | 33.6 | 56.4 | 50.4 | V10 |
| 4 | 33.6 | 56.4 | 50.4 | V12 |
| 5 | 33.6 | 112.7 | 0 | - |
| 6 | 33.6 | 112.7 | 50.4 | V10 |
| 7 | 33.6 | 112.7 | 50.4 | V12 |
| 8 | 33.6 | 169.2 | 0 | - |
| 9 | 33.6 | 169.2 | 50.4 | V10 |
| 10 | 33.6 | 169.2 | 50.4 | V12 |
| 11 | 33.6 | 212.7 | 0 | - |

**Table 2.3**. Dates in which leaf nitrogen (LN) samples were collected at different locations. Pretassel applications were made at the V10 and V12 growth stages on the same day that LN samples were collected according to the treatment plan.

| Location | Year | Growth stage | | |
|---|---|---|---|---|
| | | V10 | V12 | VT |
| SAREC[1] | 2017 | 28, June | 05, July | 21, July |
| PTRS[2] | 2017 | 08, June | 15, June | 21, June |
| SAREC | 2018 | 11, June | -[ǂ] | 29, June |
| PTRS | 2018 | - | - | 20 July |
| RRS[3] | 2018 | - | 07, July | 19, July |
| SAREC | 2019 | 10, June | 20, June | 28, June |
| PTRS | 2019 | 18, June | 25, June | 03, July |
| RRS | 2019 | 12, June | 18, June | 02, July |

[ǂ]Cells containing a dash represent missed sampling times
[1]JShult Agricultural Research and Extension Center
[2] Pine Tree Research Station
[3] Rohwer Research Station

**Table 2.4.** Analysis of variance comparing full and reduced models based on extra sum of squares test.

| Model | Residual DF | Residual SS | DF | SS | F-value | P-value |
|---|---|---|---|---|---|---|
| Full | 519 | 38,566 | - | - | - | - |
| Reduced | 513 | 38,028 | 6 | 538 | 1.21 | 0.30 |

**Table 2.5.** Regression coefficients for the relationships between relative grain yield (RGY) and tissue leaf nitrogen (LN) concentrations between V10 and VT.

| Parameter | $R^2$ | N | Estimate | St. Error | t-value | P-value | Confidence interval at 95% | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| Intercept | | | -26.7 | 2.41 | 11.06 | <0.0001 | -33.4 | -20.4 |
| Slope | 0.82 | 522 | 3.8 | 0.09 | 39.9 | <0.0001 | 3.56 | 4.08 |
| Joint point | | | 30.4 | 0.24 | 125 | <0.001 | 29.6 | 31.1 |

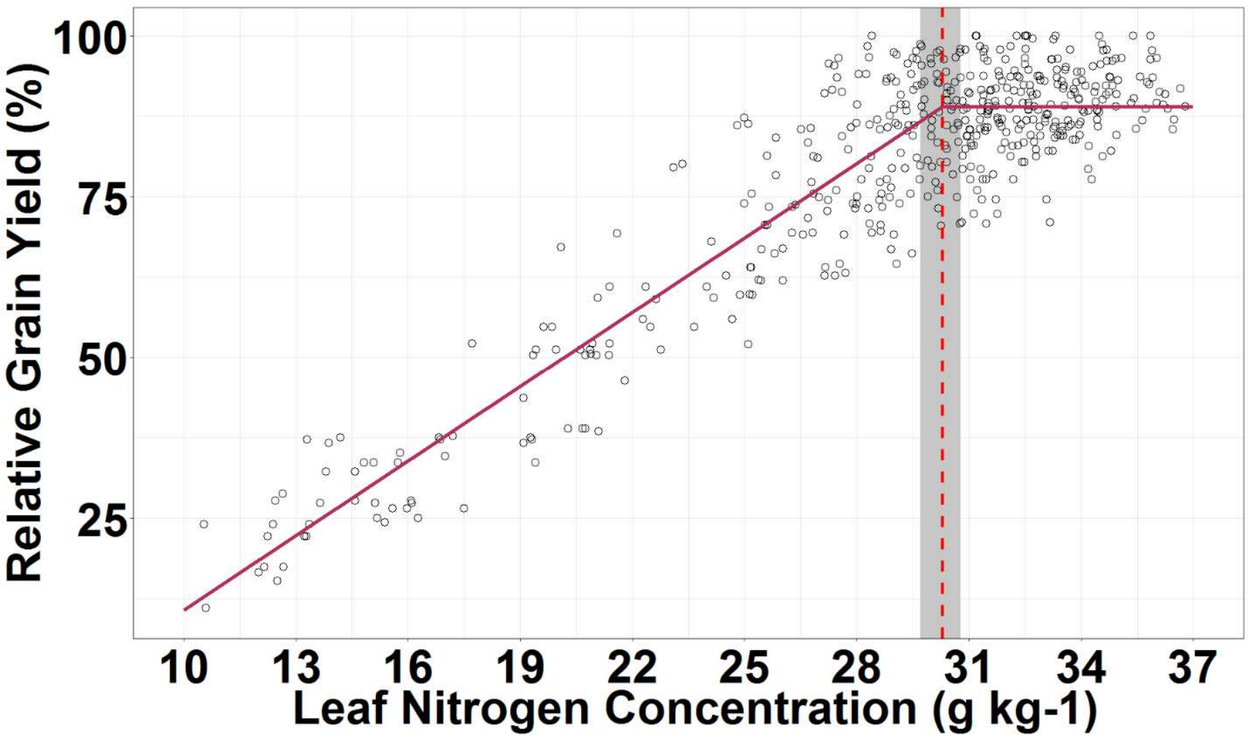


**Figure 2.1**. Relationship between relative grain yield (%) and leaf nitrogen concentration between V10 and VT (%). The red dotted line represents the joint point (30.4 g N $kg^{-1}$) and the grey shaded area represents the confidence intervals at α= 0.05.

# CHAPTER THREE

The Dark Green Color Index as a Midseason Nitrogen Management Tool in Corn Production Systems

**ABSTRACT**

The use of an in-season nitrogen (N) assessment tool, such as the Dark Green Color Index (DGCI), can improve N management. This study was conducted to determine the ability of DGCI measured from aerial digital images to provide in-season information on corn (*Zea mays* L.) N status, serving as a guideline for in-season N management. Measurements of DGCI from high N (258 kg N $ha^{-1}$) experimental units were used as a reference DGCI (RDGCI) to correct for variable lighting conditions. Ability of DGCI to assess in-season N status was investigated by predicting leaf N concentration (LN) and relative grain yield (RGY) as functions of DGCI and RDGCI. A total of eight field-studies were conducted on silt loam soils in Arkansas. Digital images and leaf samples were collected at the $10^{th}$ collared leaf stage (V10), the $12^{th}$ collared leaf stage (V12), and tasseling (VT). Across a wide range of environmental lighting conditions, LN was described by a regression (P-value < 0.0001) as a function of DGCI and RDGCI with a root mean square error of 3.49 g N $kg^{-1}$ and a bias of -0.03 g N $kg^{-1}$. Likewise, RGY was regressed against DGCI and RDGCI (P-value < 0.0001) and predicted RGY with a RMSE of 10.3% and a bias of -0.3%. Measurements of DGCI and RDGCI predicted LN and RGY accurately, demonstrating the potential to assess N status and improve N management in corn fields. These relationships provide reliable N status during late vegetative and early reproductive growth using aerial images.

# INTRODUCTION

Of the total N used in crops globally, approximately 45% is derived from synthetic fertilizers (Ladha, Pathak, Krupnik, Six, and van Kessel, 2005). Despite the massive amount of N used in agriculture every year, N fertilizer is not being used efficiently in agricultural systems. This inefficiency is noted by the low global average N use efficiency (NUE), where NUE is the ratio between the amount of N from the fertilizer accumulated by the crop and the amount of N applied via fertilizer (Raun & Johnson., 1999). The global NUE averages around 33% (Ladha et al., 2005), which indicates that approximately 67% of the N is not effectively used by the crop to which it was applied.

Several agronomic practices can minimize N losses. For example, matching crop demand with N fertilizer supply, splitting N fertilizer applications, minimizing application during the wet season, and changing fertilizer sources to match the environmental conditions (Mosier, Syers, and Freney, 2004). In a corn production system specifically, fertilizer applications near the V8 growth stage (Ritchie, Hanway, and Benson,1989) are an effective practice to decrease N losses. The majority of N uptake by corn occurs after the V8 growth stage (Russelle, Hauck, and Olson 1983), which exposes N applied before V8 to potential loss mechanisms. Thus, synchronizing the time of greatest N requirement by the corn crop with the timing of N fertilization application reduces the possibility of losses (Magdoff, 1991).

In Arkansas, recommendations are that the N rate should be split into two or three applications. In a two-way split strategy, 20 to 25% of the recommended N is applied preplant and the remaining 75 to 80% is applied as a sidedress application between V6 and V8. In the three-way split, 20 to 25% of the N recommended is applied preplant, and 50 to 65% should be applied between V6 and V8 as a sidedress application, and 15 to 25% of the N is applied between V10 and VT (Slaton et al., 2013a). Corn grown using these split-applications have achieved NUE levels of 80% (Roberts, Slaton, Kelley, Greub, and Fulford, 2016), which diverges greatly from the global average of 33%. In addition to matching corn N demand and supply, splitting the N rate allows in-season implementation of N-status assessment tools (Scharf, Wiebold, and Lory, 2002b).

Nitrogen rate recommendations for corn production systems vary greatly among states in the U.S. For instance, in Arkansas, N is recommended based on soil texture and yield goal (Slaton et al., 2013a; Slaton et al., 2013b). In Nebraska, N rates are estimated based on anticipated yield, residual soil nitrate ($NO_3$-N), soil organic matter (SOM), timing of application, as well as fertilizer and corn prices (Shapiro, Ferguson, Hergert, Wortmann, and Walters, 2008). In Iowa, N rates are based on the previous crop, soil series, and on the price ratio between N and corn (Sawyer & Lundvall, 2018).

Numerous tests based on soil and plant chemical analysis or on indirect indicators have been intended to predict optimum N rates in corn production (Miller, Bushong, Raun, Abit, and Arnall, 2017). Among the chemical tests, the Pre-sidedress Nitrate Test (PSNT) (Magdoff, Ross, and Amadon, 1984), the Pre-Plant Nitrate Test (PPNT) (Bundy & Malone., 1988), and the Illinois Soil Nitrogen Test (ISNT) (Khan, Mulvaney, and Hoeft., 2001; Mulvaney, Khan, Hoeft, and Brown, 2001) have been extensively used in corn production. An example of an indirect measurement of corn N status is the chlorophyll meter (CM), such as the SPAD-502 meter (Konica Minolta, Tokyo, Japan). The more chlorophyll that is present in the leaf, the higher the leaf-N concentration, which can be related to potential late-season N applications to corn (Samborski, Tremblay, and Fallon, 2009). Sufficiency indexes based on CM measurements have been used in several states across the U.S. to predict corn yield response to N fertilization and assess N status (Scharf & Brouder, 2006).

In addition to chlorophyll concentration, the normalized difference vegetation index (NDVI) has also been used to evaluate corn N status using sensors such as the Crop Circle ACS-210 (Holland Scientific, Lincoln, NE) and Green Seeker (N Tech Industries, Inc., Ukiah, CA). These sensors determine NDVI based on red (650 ±10 nm) and near infra-red (NIR, 750 ± 15 nm) reflectance from the crop canopy. The NDVI value ranges between -1 and 1, with higher values indicating sufficient leaf-N concentrations (LN) (Schlemmer et al., 2013). This index has been successfully used in Oklahoma to develop a Nitrogen Fertilization Optimization Algorithm for corn based on NDVI measurements made between the V7 and V9 growth stages (Teal et al., 2006; Tubaña et al., 2008).

The dark green color index (DGCI) has also been utilized to assess the plant N status. The DGCI is determined from the red, green and blue (RGB) channels of a standard digital camera, after conversion to hue, saturation and brightness values. The DGCI value ranges from 0 (yellow) to 1 (dark green) as shown in Eq. 3.1 (Karcher & Richardson, 2003).

$$\mathrm{DGCI} = \frac{\frac{(\mathrm{Hue} - 60)}{60} + (1 - \mathrm{Saturation}) + (1 - \mathrm{Brightness})}{3} \quad (3.1)$$

Rorie, Purcell, Karcher, and King (2011) found that yield, leaf-N concentration and chlorophyll concentration were directly correlated with DGCI values from the ear leaf. Purcell, Siddons, Karcher, and Rorie (2015) developed calibration curves for the amount of N to apply that would recover 90 to 95% RGY from DGCI values measured between V6 and V10 on the uppermost collared leaf. Rhezali, Purcell, Roberts, and Greub (2018) compared grain yield resulting from N application based on DGCI calibration curves from the uppermost collared leaf of plants between the V6 and V10 growth stages (Purcell et al., 2015) with the University of

Arkansas Cooperative Extension Service N rate recommendations (Slaton et al., 2013a). Overall, N rates recommended with the DGCI method ranged from 92 to 112 kg N $ha^{-1}$ less than the recommended Extension rate while maintaining yield in all cases. The authors concluded that the DGCI method was capable of predicting the in-season N requirement for corn and future research should focus on simplifying the method and extending it to aerial platforms so it can be used directly in the field. The objectives of this study were to (i) determine the relationship between LN and DGCI across a wide range of environments, (ii) determine the ability of in-season DGCI measurements to predict final RGY, (iii) determine the applicability of DGCI measurements as a midseason N management tool in corn production systems.

## MATERIALS AND METHODS

Between 2017 and 2019, eight field studies were conducted on silt-loam soils at three University of Arkansas Research Stations (Table 3.1): the Pine Tree Research Station (PTRS), Milo J. Shult Agricultural Research and Extension Center (SAREC), and the Rohwer Research Station (RRS). The PTRS and SAREC stations contained field studies in all three years, while the RRS did not have a field study in 2017. The experimental design was a randomized complete block with four replications and 11 different combinations of N rate and time of application, aiming to generate different levels of N sufficiency (Table 3.1). Experimental units were planted with the hybrid P1197YHR (Pioneer, Johnston, IA) at the seeding rate of 98,000 seeds $ha^{-1}$. Each experimental unit was 9 m long with four rows that were spaced 0.76 (PTRS), 0.91 (SAREC), and 0.96 (RRS) m apart. For treatments 2 through 11, the preplant N rate (33.6 kg N $ha^{-1}$) was applied to the field and incorporated into the soil prior to planting. Sidedress N rates were applied to experimental units when the crop was between the V6 and V8 growth stages. The different sidedress rates were meant to mimic sub-optimal, optimal, and above-optimal N rates prior to pretassel fertilization. Pretassel fertilization (51.5 kg N $ha^{-1}$) was applied to experimental units between V10 and VT according to the treatment plan (Table 3.2). All N fertilizer was applied as urea (46-00-00) treated with N-(n-butyl)-thiophosphoric triamide (NBPT) (Agrotain Ultra, Koch Agronomic Services LLC., Wichita, KS) at the rate of 0.89 g NBPT $kg^{-1}$ urea. A single composite soil sample (four 3-cm diam. cores) was collected from each replication at all experimental sites to a depth of 15 cm, soil nutrient analysis was performed (Mehlich-3 extractable nutrients, Zhang, Hardy, Mylavarapu and Wang, 2014) to confirm nutrients other than N were not limiting crop growth. Irrigation and pest management of plots closely followed furrow-irrigated corn recommendations established by the University of Arkansas System Division of Agriculture Cooperative Extension Service (Espinoza and Ross, 2003). Irrigation timing was scheduled according to the Arkansas Irrigation Scheduler set at a 5-cm soil profile water deficit.

The uppermost fully developed leaf blade from five corn plants were sampled from the two middle rows of each plot at V10, and V12, and five identifiable ear-leaves were sampled at the VT stage prior to N fertilization (Table 3). The samples were oven-dried at 70 °C until constant weight, ground and sieved via a 20-mesh screen, and analyzed for total N using combustion (Campbell, 1992) at the Fayetteville Agricultural Diagnostic Laboratory at the University of Arkansas (Fayetteville, AR).

The RGB images were collected immediately prior to or following (same day) pre-tassel fertilization (Table 3.3) at V10, V12, and VT with a Phantom 4 Pro ( DJI, Shenzen, China) using the camera that comes as standard equipment on the UAS (25.4-mm 20-megapixel CMOS sensor). Images were collected at 30.5 m above ground level and with 80% front and side overlap between the pictures. An orthomosaic of the individual images was built using

MetaShape Professional© (Agisoft LLC, St. Petesburg, Russia). The DGCI values of individual plots were determined from orthomosaic images using Field Analyzer© (http://www.turfanalyzer.com/field-analyzer) software.

Experimental units were trimmed to 6 m in length at maturity, the two center rows were harvested using a small plot combine, and yield was corrected to 15.5% moisture. Relative grain yield was calculated as the ratio between the yield of an individual plot and the maximum yield attained within each environment (Eq. 3.2).

$$\mathrm{RGY} = 100 \times \frac{\mathrm{Y_{ij}}}{\mathrm{max.\,Y_j}} \tag{3.2}$$

where $Y_{ij}$ is the yield of experimental unit "i" in environment "j" and max. $Y_j$ is the maximum yield attained within environment "j".

To account for the differences in exposure that could affect DGCI, previous research (Rorie et al., 2011a; Purcell et al., 2015; Rhezali et al., 2018) used color standards with known DGCI values in each image. In the current research, DGCI values from experimental units with high N were used as a reference or high N check. The Reference DGCI (RDGCI) value was calculated for each location and growth stage as the average DGCI for treatment 11 (33.6 kg N $ha^{-1}$ at preplant, and 212 kg N $ha^{-1}$ at sidedress) within a combination of growth stage, location, and year.

Statistical analysis was conducted in R 3.5.2. (R Core Team, Vienna, Austria) and the combination of year and location was considered as an environment. The relationship between DGCI, RDGCI, and LN was investigated by fitting a generalized linear model (GLMM), in which DGCI was the response variable, and LN and RDGCI were predictors. Since LN is a continuous non-negative response variable, a Gamma distribution was assumed for the GLMM fitting, using a log link function (Gbur et al., 2012).Observations from all treatments were included in the regression analysis. Environment and growth stage were considered as random effects. Similarly, the relationship between RGY, DGCI, and RDGCI was investigated by fitting a GLMM, in which RGY was the response variable, and DGCI and RDGCI were predictors. The GLMM was fitted assuming a beta distribution for RGY using a logit link function because RGY is a continuous proportion response variable (Gbur et al., 2012). As certain treatments received N fertilization at different growth stages, when measurements were made at V10, only observations from treatments 1, 2, 5, 8, and 11 were included in the regression analysis. For measurements made at the V12 growth stage, the regression analysis was restricted to observations from treatments 1, 2, 3, 5, 6, 8, 9 and 11. Finally, when DGCI measurements were made at VT, regression analysis included observations from all treatments.

## RESULTS AND DISCUSSION

Yields varied greatly among environments with a maximum yield of 20,676 kg $ha^{-1}$ recorded in 2019 at RRS, the minimum yield of 1,592 kg $ha^{-1}$ also occurred at RRS, but in 2018 (Table 3.1). The ratio between yields attained in experimental units assigned to treatments with the lowest N rate and the highest N rate can be used as a metric to assess how responsive to N application the corn was across varying environments. Using this ratio, RRS in 2018 and SAREC in 2019 were categorized as the most responsive environments, as experimental units with no N fertilization yielded 11% of the highest yielding experimental units in those environments. Furthermore, RRS in 2019 was the least responsive environment, in which experimental units with no N fertilization yielded 42% of the highest yielding experimental unit. Additionally, RRS in 2019 also had the highest yielding levels of all environments, with the yields ranging from 8,813 to 20,676 kg $ha^{-1}$, and a median yield of 18,654 kg $ha^{-1}$. In the remaining environments, the ratio between the yields of experimental units that received no N and the experimental units that received the highest rate of N averaged 28%.

Measurements of DGCI ranged from 0.44 to 0.83 and were highly influenced by LN concentration and ambient environmental lighting conditions at the time images were collected. Greater values of RDGCI were associated with images taken on overcast days, while lower values of RDGCI were associated with brighter pictures and days with full sunlight. Previous research reported that different lighting conditions result in variable values of DGCI (Rorie et al., 2011a). To account for the effect that environmental lighting has on DGCI measurements in the current research, RDGCI values were included as a factor in the regression analysis.

Except for RRS in 2019, LN concentrations were consistent across environments and ranged from 10.54 to 36.79 g $kg^{-1}$. At RRS in 2019 LN values ranged from 20.89 to 36.65 g N $kg^{-1}$, which was also the highest overall yielding environment across all N treatments (Table 3.1). These high LN levels indicate that there was more N available in the soil in this environment than in the remaining environments.

An initial regression predicting LN as a function of DGCI and RDGCI found that the interaction between DGCI and RDGCI was nonsignificant. Therefore, the interaction term was removed from the analysis and the model was refit. The final model was significant (F= 274, p-value < 0.0001) and described adequately LN as a function of DGCI and RDGCI (Fig. 1). Additionally, the model predicted LN concentration with a root mean square error (RMSE) of 3.49 g N $kg^{-1}$ and with a bias of -0.03 g N $kg^{-1}$. Predicted values of LN varied as a function of the combined effects of the linear coefficients. As DGCI values increased between 0.44 and 0.83, predicted values of LN increased. Conversely, as RDGCI increased, predicted values of LN decreased. The regression coefficients indicate that the relationship between LN and DGCI was maintained for RDGCI values between 0.60 and 0.79. However, the inverse relationship between LN and RDGCI indicates that the model predicts smaller values of LN as RDGCI increases. Greater values of RDGCI were associated with overcast days, and smaller values of RDGCI were associated with clear sky days. Because LN was predicted as the combined effects of DGCI and RDGCI, a given DGCI measurement can predict different LN levels, depending on RDGCI. For instance, with a DGCI of 0.65 and a RDGCI of 0.63, LN is predicted to be of 34.5 g N $kg^{-1}$. This LN would represent a plant with sufficient N to achieve optimal yield, as the sufficient LN has been reported to be between 28 and 31 g N $kg^{-1}$ (Bennett, Stanford, and Dumeni, 1953; Melsted, Motto, and Peck, 1969; Greub, Roberts, Slaton, Kelley, and Gbur, 2018). In contrast, when the DGCI value is 0.65 and RDGCI is 0.80, LN is predicted to be 13.2 g N $kg^{-1}$, representing a plant that is deficient in N. The ability of DGCI to predict LN agrees with previous research showing that DGCI was closely associated with LN and N fertilization treatments in corn (Rorie et al., 2011a) and turfgrass (Karcher & Richardson, 2003).

An initial regression was fit in which RGY was predicted as a function of DGCI and RDGCI, but this initial model found that the interaction term between DGCI and RDGCI was not significant. The model was refit without the interaction term, and the final model was significant (F-value = 399, P-value < 0.0001) with a RMSE =10.3% and bias = -0.3% (Fig. 3.2). The analysis indicated that RGY increased as DGCI values increased between 0.44 and 0.83. Likewise, the regression indicated an inverse relationship between RGY and RDGCI. For example, a DGCI value of 0.65 predicts a RGY of 25.4% when the RDGCI is 0.80, but RGY is predicted to be 95.5% with a RDGCI of 0.63.

Figure 3.3 presents the same relationship shown in Figure 3.2 as a two-dimensional graph in which the diagonal isolines represent RGY as a function of DGCI and RDGCI. Using the example discussed previously, the open circle in Figure 3.3 shows a RGY of 95.5% when DGCI is 0.65 and RDGCI is 0.63; the closed rectangle shows a RGY of 25.4% when DGCI is 0.65 and RDGCI is 0.80. The capability of DGCI to predict RGY agrees with previous research where an exponential increase in RGY was reported as a function of the increase in DGCI (Rorie et al., 2011a).

Previous research using DGCI on individual leaves to predict corn N needs and the relationship of DGCI to RGY has indicated that several factors can affect DGCI measurements; these factors include environmental lighting conditions, corn hybrid, diseases, and nutrient deficiencies besides N (Rorie et al., 2011b). To account for these cofounding factors, researchers have included internal standards in the digital images (Rorie et al., 2011a; Rhezali et al., 2018). Rorie et al. (2011b) also normalized DGCI for individual treatments relative to the highest DGCI value in a given environment; normalized DGCI values were closely associated with relative SPAD values, relative leaf N concentration, and RGY when combined over 10 different environments and with seven different hybrids. In the current research we used the DGCI measurement of a high N area in the same field to adjust for variable ambient lighting conditions and differences among corn hybrids (i.e., RDGCI). Implementing a well fertilized area as a standard or reference for improved N management can be used to adjust N rates based on hyperspectral indices, such as with NDVI (Raun et al., 2010), and has been suggested when using color bands (Scharf & Lory, 2002). This method of accounting for different light conditions facilitates the use of this tool in large scale fields, as it is important that the standards and the crop being assessed for N status are exposed to the same light conditions. One important assumption of using this method is that the high N area will be fertilized with a N rate that is not limiting for corn production (Raun et al., 2010).

## CONCLUSIONS

The present research has shown that DGCI measured from aerial digital images can be useful in determining the need for midseason N applications. The inclusion of RDGCI in the model allows for DGCI to be used across a wide range of environmental lighting conditions with out the need for color standards to be included in each image. The strong relationship between LN and DGCI shows that DGCI measurements can be used as an indirect N sufficiency assessment tool for corn. This tool has advantages over tissue sampling because it provides immediate results and allows for N monitoring across large areas, provided that there is an area within the field where N supply is nonlimiting for the crop (i.e. high N reference strip or area). Additionally, DGCI measurements taken between V10 and VT growth stages were closely associated with RGY. The assessment of N sufficiency using DGCI can help growers salvage corn yield in cases where the predicted RGY is low. Use of the DGCI to predict N sufficiency can also avoid unnecessary application of N in corn fields, ultimately reducing the risk of N movement into the landscape and decreasing overall production costs. The relationship between DGCI and RGY between V10 and VT provides a relatively quick, non-destructive, and simple tool to determine if corn RGY would respond (increase) with additional fertilizer N additions. The research presented here highlights a new approach that when coupled with a high N reference plot will allow producers to quickly and easily assess corn N status over a wide range of growth stages where corrective N applications can be made.

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## TABLES AND FIGURES

**Table 3.1.** Soil series, geographical coordinates, sowing date, and yield range for year and location of studies

| Location | Latitude | Longitude | Year | Soil series | Sowing date | Yield range (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|
| SAREC[1] | 36.09 N | 94.17 W | 2017 | Captina silt loam | 5/8 | 3,936 – 13,131 |
| PTRS[2] | 35.12 N | 90.92 W | 2017 | Calloway silt loam | 4/13 | 5,780 – 17,145 |
| SAREC | 36.09 N | 94.17 W | 2018 | Captina silt loam | 4/24 | 6,094 – 16,155 |
| PTRS | 35.12 N | 90.92 W | 2018 | Calloway silt loam | 4/12 | 4,445 – 16,645 |
| RRS[3] | 33.80 N | 91.26 W | 2018 | Sharkey silt loam | 4/11 | 1,592 – 14,319 |
| SAREC | 36.09 N | 94.17 W | 2019 | Captina silt loam | 4/11 | 1,676 – 14,856 |
| PTRS | 35.12 N | 90.92 W | 2019 | Sharkey silt loam | 4/29 | 2,840 – 16,237 |
| RRS | 33.80 N | 91.26 W | 2019 | Calloway silt loam | 4/24 | 8,813 – 20,676 |

[1] Shult Agricultural Research and Extension Center
[2] Pine Tree Research Station
[3] Rohwer Research Station

**Table 3.2.** Nitrogen (N) rates and time of application for different treatments used in eight field trials.

| Treatment | Nitrogen rates (kg N ha$^{-1}$) | | | Pre-tassel application timing |
|---|---|---|---|---|
| | Pre-plant | † Sidedress | Pre-Tassel | |
| 1 | 0 | 0 | 0 | - |
| 2 | 33.6 | 56.4 | 0 | - |
| 3 | 33.6 | 56.4 | 50.4 | V10 |
| 4 | 33.6 | 56.4 | 50.4 | V12 |
| 5 | 33.6 | 112.7 | 0 | - |
| 6 | 33.6 | 112.7 | 50.4 | V10 |
| 7 | 33.6 | 112.7 | 50.4 | V12 |
| 8 | 33.6 | 169.2 | 0 | - |
| 9 | 33.6 | 169.2 | 50.4 | V10 |
| 10 | 33.6 | 169.2 | 50.4 | V12 |
| 11 | 33.6 | 212.7 | 0 | - |

† Sidedress N applied between the V6 and V8 growth stages.

**Table 3.3.** Dates in which leaf nitrogen (LN) samples and digital images were collected at different locations. Pretassel applications were made at the V10 and V12 growth stages on the same day that LN samples were collected according to the treatment plan.

| Location | Year | Growth stage | | |
|---|---|---|---|---|
| | | V10 | V12 | VT |
| SAREC[1] | 2017 | 28, June | 05, July | 21, July |
| PTRS[2] | 2017 | 08, June | 15, June | 21, June |
| SAREC | 2018 | 11, June | -[†] | 29, June |
| PTRS | 2018 | - | - | 20 July |
| RRS[3] | 2018 | - | 07, July | 19, July |
| SAREC | 2019 | 10, June | 20, June | 28, June |
| PTRS | 2019 | 18, June | 25, June | 03, July |
| RRS | 2019 | 12, June | 18, June | 02, July |

[†]Cells containing a dash represent missed sampling times
[1]Shult Agricultural Research and Extension Center
[2] Pine Tree Research Station
[3] Rohwer Research Station

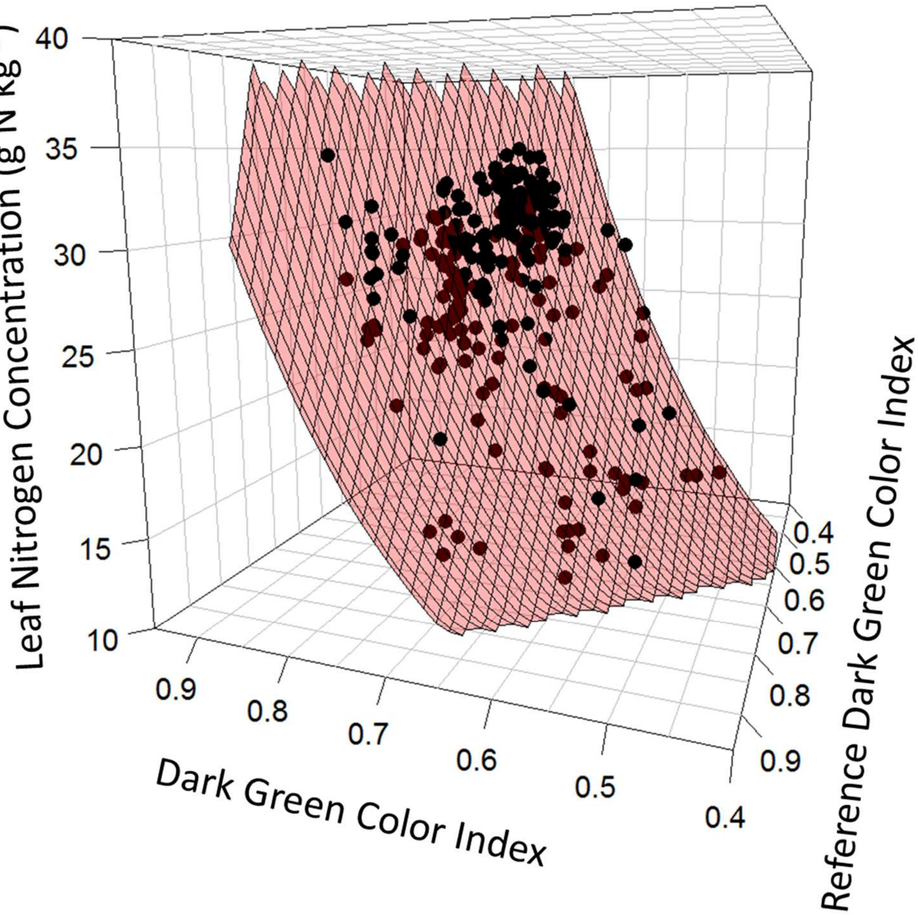


**Figure 3.1.** Relationship between Leaf Nitrogen Concentration (LN), Dark Green Color Index (DGCI), and Reference Dark Green Color Index (RDGCI)

($LN = e^{3.9+4.8xDGCI-5.6xRDGCI}$ , bias = -0.03 g N kg$^{-1}$, RMSE = 3.49 g N kg$^{-1}$, AIC= 1301).

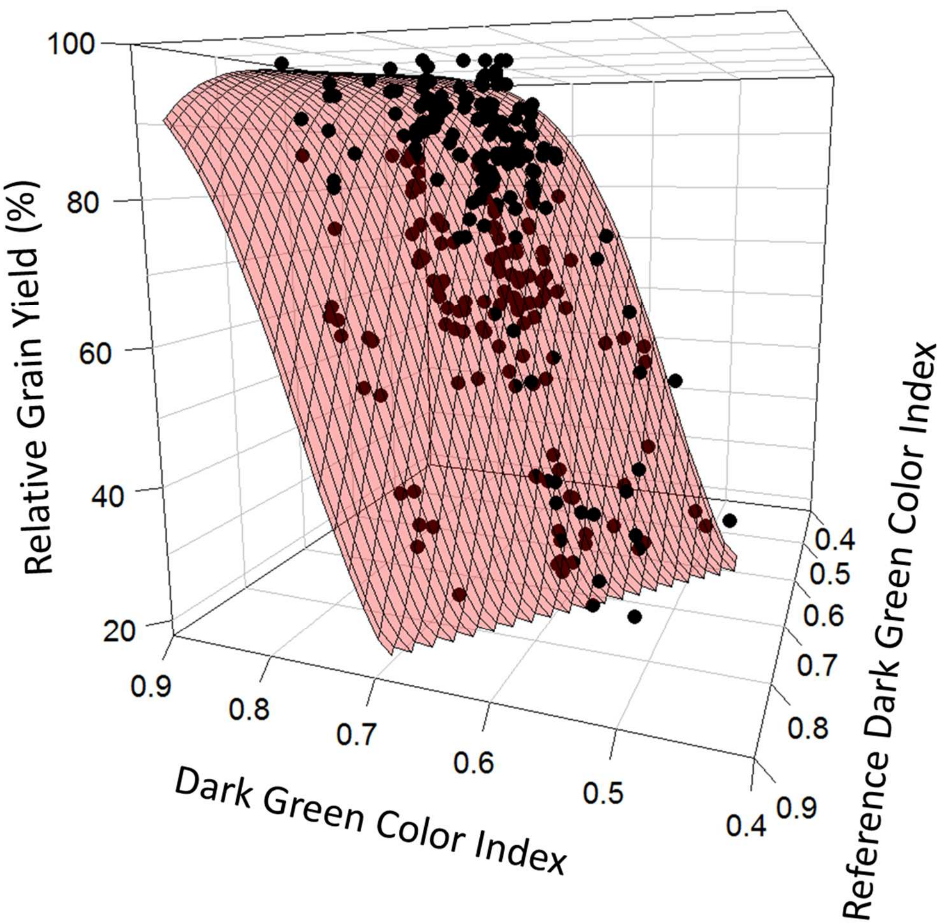


**Figure 3.2.** Relationship between Relative Grain Yield (%), Dark Green Color Index (DGCI), and Reference Dark Green Color Index (RDGCI) ($RGY = \frac{e^{0.47+19.6*DGCI-18.2*RDGCI}}{1+e^{0.47+19.6*DGCI-18.2*RDGCI}}$, bias = -0.3 %, RMSE=10.3 %, AIC=-457).

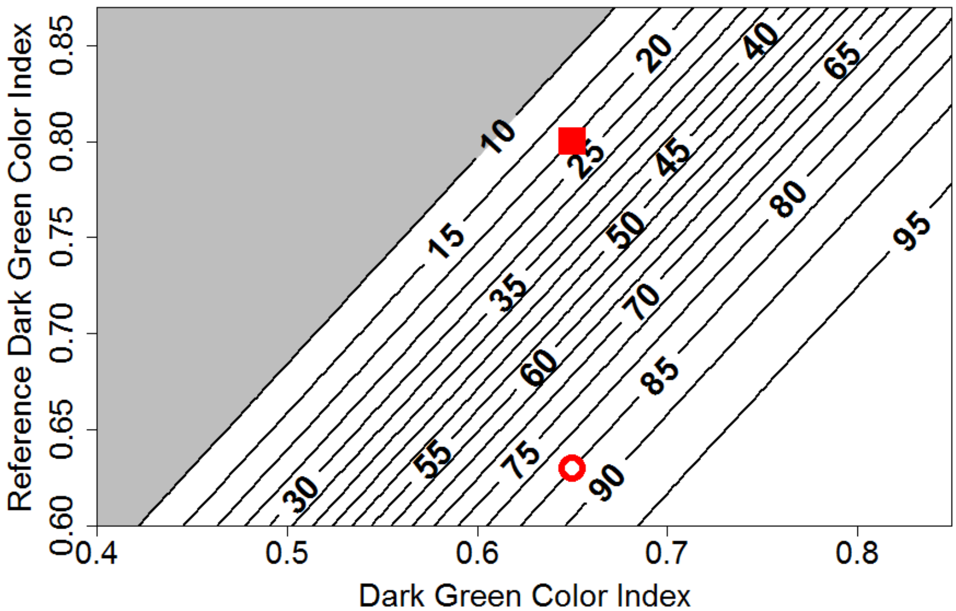


**Figure 3.3.** Relationship between Relative Grain Yield (%, RGY), Dark Green Color Index, and Reference Dark Green Color Index ($RGY = \frac{e^{0.47+19.6*DGCI\ -18.2*RDGCI}}{1+e^{0.47+19.6*DGCI\ -18.2*RDGCI}}$, bias = -0.3 %, RMSE=10.3 %, AIC= -457). The grey shaded area represents areas for which no predictions were made because it is outside of the range of the observations. The isolines represent RGY. Open circle shows a predicted RGY= 86.0% when DGCI is 0.65 and RDGCI is 0.63; the closed rectangle shows a predicted RGY= 22.3% when DGCI is 0.65 and RDGCI is 0.80.

# CHAPTER FOUR

## Conclusions

The purpose of this study was to evaluate the adequacy of leaf nitrogen (N) concentration (LN) and the Dark Green Color Index (DGCI) as in-season N status tools. The results indicate that LN from the uppermost fully collared leaf at the V10 and V12 (Ritchie, Hanway, and Benson,1989), and from the ear-leaf at VT can accurately predict final relative grain yield (RGY). This tool can assist corn producers when considering a midseason N application. Between the V10 and VT growth stages when LN is greater than 30.4 g N $kg^{-1}$ no yield increase is expected from additional fertilization. At the same time, when LN is less than 30.4 g N $kg^{-1}$ plants are deficient and an additional N fertilization could be used to maximize RGY, especially in cases of severe N deficiency.

The use of the Dark Green Color Index (DGCI) measured from digital aerial images associated with a high N area (reference strip) within the corn field accurately predicted LN and RGY. The close relationship between DGCI measurements and LN indicate that DGCI can be used as an indirect N assessment tool. Greater values of DGCI from the target area associated with smaller DGCI values from the reference area predicted greater LN and RGY values. Concurrently, when the DGCI value of the reference area was large but the DGCI of the target area was small, LN and RGY were predicted to be small, indicating N deficiency. When LN and RGY are predicted to be low, additional N fertilization can be used as a strategy to salvage yield. The advantage of assessing N status with DGCI measurements is that this tool provides immediate results and can be used across a wide range of ambient lighting conditions.

Both LN and DGCI showed potential to be used as midseason N guidelines. The main contributions of this study are: (i) to provide information on N sufficiency between the V10 and VT growth stages, allowing producers to monitor corn N status for a wide period of the growing season, (ii) to demonstrate that DGCI from aerial platforms can be used as an indirect assessment tool for midseason N status in corn production systems.

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