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

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DARK GREEN COLOR INDEX AS A METHOD OF REAL-TIME IN-SEASON CORN NITROGEN MEASUREMENT AND FERTILIZATION

DARK GREEN COLOR INDEX AS A METHOD OF REAL-TIME IN-SEASON CORN 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 Science

By

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Hendrix College
Bachelor of Arts in Environmental Studies, 2007

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ABSTRACT

Corn (*Zea mays* L.) requires higher rates of nitrogen fertilizer than any other major U.S. crop. Soil and applied N are subject to loss through various mechanisms. A timely, accurate, and precise method for measuring in-season corn N status is needed to ensure high yield and to allow producers to increase nitrogen use efficiency.

Using appropriate software, images from a digital camera can be used to determine the greenness, or dark green color index (DGCI) of corn leaves, which is closely associated with leaf N concentration. Our objectives were: (1) to develop quantitative relationships among yield, corn leaf N concentration and DGCI measurements taken in the mid-vegetative stages of corn growth; (2) to determine the amount of N to apply to recover yield based upon DGCI measurements on 6-to-10-leaf corn (V6-V10); and (3) explore the efficacy of the DGCI method in other, non-leguminous crops.

Several regionally-adapted corn hybrids were planted for 2 years in Arkansas over a range of N treatments. Leaf chlorophyll (SPAD), DGCI, and leaf N measurements were taken prior to midseason N application. There was a significant relationship ($p \leq 0.05$) between DGCI and SPAD ($r^2=0.48$ to 0.87), DGCI and leaf N concentration ($r^2=0.56$ to 0.70), and SPAD and leaf N concentration ($r^2=0.43$ to 0.80). Combining the responses of yield to midseason N application amounts with concurrent mid-season DGCI, SPAD, or leaf N measurements allowed for the development of equations (r^2 from 0.57 to 0.83) that predicted the amount of N required to attain 90 or 95% of the yield potential.

Significant relationships between DGCI and leaf N concentrations were also found in other crops tested under varying N treatments. Winter wheat ($r^2=0.79$), bermudagrass ($r^2=0.77$), creeping bentgrass ($r^2=0.49$), and tall fescue ($r^2=0.53$) demonstrated DGCI-leaf N concentration

relationships. Flooded rice was sampled but no significant relationship was found between DGCI and leaf N concentration in those crops.

This thesis is approved for recommendation
to the Graduate Council.

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ACKNOWLEDGMENTS

"I love to study the many things that grow below the corn stalks and bring them back to the studio to study the color. If one could only catch that true color of nature - the very thought of it drives me mad."

-*Andrew Wyeth*

Without over three years of support, guidance, and patience in the extreme from Dr. Larry Purcell, this thesis could never have happened. I owe him more than I feel I can repay. Larry, you are an inspiration, professionally and personally.

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Robbie, Adriano, Ruben, Dirk, Luke, Ale, Sadal, Montse, and David: who could ask for anything more? What a team we made.

This thesis is dedicated to my father.

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Chapter 1

Introduction

Nitrogen is one of the most important nutrients for plant life and is required for many fundamental functions of plant metabolism and development such as amino acid synthesis and as a major component of chlorophyll (Fisher, 2000). Although abundant in the earth's atmosphere, the form of nitrogen most commonly found in nature, dinitrogen gas (N_2), is not available as a nutrient to plants.

To be acquired from the soil by non-legume plants, nitrogen must be in the forms of ammonium (NH_4^+) or nitrate (NO_3^-). These accessible forms can be derived from atmospheric N_2 in several ways. Biological nitrogen fixation is the process wherein atmospheric dinitrogen is converted to ammonia by the nitrogenase enzyme present in many different bacteria, most notably, *Rhizobia*. Rhizobia form a symbiotic relationship with several agriculturally important members of the family *Fabaceae* (Boonkerd et al., 1978). The Haber-Bosch process produces ammonia through the reaction of N_2 and methane with an iron oxide catalyst and is the main source of nitrogen fertilizer for non-leguminous plants. This process is now used to produce over 136,000,000 metric tons of synthetic ammonia worldwide annually, 89% of which is used for agricultural fertilizer, at an eventual market price of US$783 per metric ton (USDA, 2012).

Nitrogen is the most abundant element in plants after carbon, hydrogen, and oxygen. Nitrogen is an important building block in protein, and it is removed in large quantities in grain from the field during harvest, leaving little residual soil nitrogen for the next year's planting (Iowa State, 2007). Additionally, nitrogen is lost from soils by leaching and denitrification. As a result, nitrogen is the factor most commonly limiting crop production. In production agriculture systems, nitrogen is provided to the plant in the form of chemical fertilizers such as anhydrous ammonia and urea in order to overcome this production barrier. The addition of the fertilizer provides the nutrients needed for elevated grain yield and quality (Miao et al., 2007) and is a key component of modern agriculture.

Corn (*Zea mays* L.) is no exception in its requirement for large amounts of nitrogen fertilizer, in fact requiring and receiving nitrogen fertilizer at higher rates than any other major U.S. crop (USGS, 1999). The nitrogen demands of corn vary throughout its development, increasing greatly through late vegetative and into the reproductive stages (NDSU, 1999; Scharf et al., 2002). Binder et al. (2000) suggested that early and severe nitrogen deficiencies in corn can greatly reduce yield if undiagnosed and have a reduced chance of recovery depending on the lag between deficiency, diagnosis, and application.

In addition to the nutrient requirements of corn, there are other factors to be considered in nitrogen fertilizer application. Nitrogen fertilizer is subject to loss through volatilization, leaching beyond the rhizosphere, and denitrification (PennState, 2009). Proper timing of nitrogen fertilizer applications is a complex and important undertaking for farmers (Scharf, 2001). Farmers will often apply more than the estimated crop nitrogen needs in an attempt to reduce it as a limiting factor (Torbert, 2001), but this tactic is wasteful. Under aerobic conditions ammonium undergoes nitrification to form nitrate (Espinoza, 2009), which is highly mobile in the soil and can leach into groundwater or be lost as surface runoff (Schlesinger, 2009). Nitrate is subject to denitrification and the resultant loss as N_2 gas when soils become anaerobic after heavy rains. Urea, a common form of nitrogen fertilizer, will undergo hydrolysis after application and convert into NH_3, which is in turn lost to the atmosphere. The loss of nitrogen fertilizer from agricultural systems contributes to the problem of eutrophication in aquatic environments (Hong, 2007; Gehl, 2006; Pierzynski, 2005).

To obtain an estimate of appropriate levels of nitrogen fertilizers to be applied to their fields at a given time, farmers will often rely on systems such as the Economically Optimum

Nitrogen Rate (EONR) or a particular nitrogen application algorithm (PennState, 1999). These calculations rely on such factors as previous yield data for a particular field and highly-fertilized crop strips for calibration purposes. Some of the more advanced algorithms will even take into account soil N concentration, climate, water, and management practices (Setiyono, 2011). The actual application rate is commonly higher than calculated rates, an attempt by farmers to capitalize on the chance for a bumper crop from a particular growing year (Hong, 2007). However, practices like this lead to the pollution previously described, increased nitrogen costs, and an estimated nitrogen use efficiency rate of only 33% (Raun, 2002). Furthermore, these estimates are often tailored to very specific regional climates and seasonal production patterns, resulting in an error of up to 61 kg ha^{-1} N when attempting to estimate EONR for any given year (Setiyono, 2011).

For improved nitrogen efficiency and crop nutrient management, it is important that farmers have tools for accurately measuring the amount of nitrogen needed at any particular time by their crops (Scharf, 2001). In Arkansas, about 1 to 1½ lbs (0.45 to 0.68 kg) of N are applied to the soil for each bushel (25.4 kg) of expected yield; however, this may vary depending on the type of soil (Espinoza, 2009). For soils with greater clay contents, increased amounts of nitrogen fertilizer are recommended to overcome the nitrogen sequestration tendency of those soils compared with silt-loam soils (Espinoza, 2009). In soils with high amounts of clay, ammonium may be fixed into an unavailable form. Of the total recommended nitrogen, half is applied before planting to avoid potential ammonium salt damage to the root structure and the rest is typically sidedressed before the V8 stage.

A real-time knowledge of the amount of nitrogen needed by a particular field is not feasible, and as such farmers must rely on measurements indicative of crop nitrogen concentration and fertilize accordingly. Application recommendations may change during the season if nitrogen is lost through leaching and denitrification from excessive rainfall. Though sufficient nutrient availability is important throughout the development of the plant, the nitrogen requirements of the crop increase dramatically beginning with the V6 development stage (Iowa State, 2007). The V8 developmental stage, occurring a week or so after the V6 stage, typically coincides with a plant height that dictates the final chance for effective application of non-foliar nitrogen fertilization (NDSU, 1999). At this time, N may be applied with ground equipment, which makes this the cheaper and more effective method for farmers (Ling, 2002). The V6 development stage is also crucial in that attempts to correct extant nitrogen deficiencies via fertilizer applications beyond that point are too late to completely restore yield potential (Raun, 2005). From V6 through the tasseling phase constitute the period in development most vulnerable to nutrient deficiencies; therefore, the V6 stage is most crucial for identifying nitrogen fertilizer needs (Binder, 2000).

Furthermore, spatial variability exists within any field with concern to the amount of nitrogen required by a particular crop (Raun and Johnson, 1999). Any attempt to determine an EONR must take into account this variation. Indeed, understanding and appreciation of this variation is crucial to maintaining an EONR throughout a particular crop year. The levels of precision and resolution that are achieved in determining nitrogen requirements will dictate the fineness of the variable-rates of N to be applied. This precision and resolution should be considered in the field element size, defined by Solie et al. (1996) as “that area or resolution which provides the most precise measure of the available nutrient where the level of that nutrient changes with distance”. Variation in crop response to N applications can be detected within $1m^2$ (Solie et al., 1996).

Typical corn production methods in North and South America utilizing similar hybrids have been shown to give rise to average *plant to plant* yield differences of 4200 kg ha^{-1} (Martin, 2005). This suggests the need to make multiple measurements within a field element size of at most $1m^2$ to attain the proper resolution for greater nitrogen use efficiency and an EONR.

There are several methods for estimating crop nitrogen status. Elemental nitrogen analysis is the most precise way of measuring tissue nitrogen, but the analysis processes involved in the Kjeldahl-Rittenberg procedure (Barrie, 1995) or Micro-Dumas Combustion method (University of Georgia, 1997) are sophisticated beyond the point of practicality for the production farmer. This process can be time-consuming, especially when a testing service is not nearby, and thus the test results may not be current to the point of usefulness when they are finally received. Additionally, the cost of the test combined with the number of samples that a typical producer would need to develop a useful map of field nitrogen requirements make this method practically prohibitive.

Another option is to rely solely on the soil nitrogen concentration measurements; however, there is evidence to suggest that this approach alone is a very poor method of determining the EONR for corn production (Scharf, 2006). At present, there is no soil test for nitrogen that is used for corn in Arkansas. However, promising research has developed a soil test for nitrogen in rice production (Roberts, 2010).

Yin (2011) suggested a novel method of estimating corn yield by measuring plant height from the V6 to V12 growth stages. Significant correlations were found between corn plant height at various late vegetative stages and subsequent yield. The study further proposed that this methodology could similarly be used to assess the spatial variability of crop response to nitrogen within a field. This assessment could be used to develop high-resolution treatment maps for subsequent variable-rate N applications for the purpose of maximizing yield (Yin, 2011). This attempt to overcome spatial variability of crop response to N within a field represents a key component of practical, in-season crop N assessments; namely, larger, higher-resolution treatment “maps” will be inherently more useful to a producer wishing to attain an EONR. However, the ultrasound-distancing technology necessary to make this type of measurement practical on a field-wide basis is preliminary, and compiling a sufficient sample of individual plant height measurements would be laborious and time-consuming.

An increasingly common, practical approach is to estimate leaf nitrogen concentration via chlorophyll concentration. Because leaf nitrogen is closely correlated with leaf chlorophyll concentration, this can be an effective, non-destructive way of obtaining a nitrogen estimate for a particular corn leaf (Costa, 2001). This can be achieved in several ways. The chlorophyll meter is a handheld device that estimates leaf chlorophyll concentration based on the absorbance measurements in two wavelengths (Konica Minolta, 2009).

There are drawbacks to the chlorophyll meter as well, however. It is expensive, with a basic model costing upwards of US$2,000. It has a small sampling area, 2x3mm, which can lead to sampler bias and greatly increase the number of individual measurements required to adequately assess all the necessary field elements in a typical corn field. Additionally, studies have shown that chlorophyll meters are ineffective tools for calculating nitrogen needs during the mid-to-late season (Bullock 1998; Zhang 2008; Zhang 2009).

An alternative to the chlorophyll meter is the spectral radiometer. This piece of equipment employs the principle of reflectance measurement. Because nitrogen-stressed corn will have lower chlorophyll concentration, it will reflect more of the visible spectrum than a well-fertilized corn crop. Thus, a relative level of nitrogen-deficiency can be measured at different points over an entire field (Scharf and Lory, 2009). Reflectance-measurement equipment usually comes in the form of a Normalized Difference Vegetation Index (NDVI) meter. NDVI measures light at two wavelengths, one absorbed by chlorophyll and the other unabsorbed. By comparing the difference between the two measurements, a relative NDVI number can be generated. This method, too, is limited by its lack of precision and of algorithms necessary for nitrogen calculations among various environmental conditions (Samborksi, 2009). As with the chlorophyll meter, reflectance measurements are also ineffectual for mid-to-late season nitrogen calculations. Spectral radiometers have a cost starting around US$4,000.

A method that is both low-cost and provides real-time nitrogen diagnostics is digital-image analysis. Digital imaging has been successfully employed in the past for the purposes of plant diagnostics (Waksman, 1997), and specifically for the estimation of plant nitrogen nutritional status. Pagola (2009) used this method to develop estimates of nitrogen nutrition in barley and found that the digital image measurements were on par with, and at times more accurate than, SPAD chlorophyll meters as predictors of total yield and nitrogen deficiencies. Today's typical digital camera produces images on the order of several million pixels, providing the potential for evaluating many thousands of field elements at a sufficient resolution given adequate distance.

For this procedure, a common, low-cost digital camera with a charge-coupled device (CCD) array is used to take photographs of a leaf sample. The resulting digital image can then be used to assess crop nitrogen status. As mentioned above, leaf nitrogen concentration is closely correlated with leaf chlorophyll concentration, which in turn determines the relative greenness of a leaf. Color images are composed of three values: red, green, and blue (RGB). The RGB color scale is simple to interpret, however it is not appropriate as a means to directly measure the leaf greenness. RGB does not accurately quantify shifts in such values as hue, saturation and brightness between different color samples. For this reason, RGB values are converted into a different scale measuring hue, saturation and brightness (HSB) using a method described by Karcher and Richardson (2003). This HSB value is in turn converted into a dark green color index (DGCI) value specifically for the purposes of nitrogen concentration analysis as in the following equation:

$$DGCI = \left[\left(\frac{HUE - 60}{60} \right) + (1 - SATURATION) + (1 - BRIGHTNESS) \right] / 3$$

The resulting DGCI value is on a scale from 0 (very yellow) to 1 (dark green). Therefore, digital images of a crop can be used to produce an index for the purposes of quickly and simply estimating leaf nitrogen concentration. Rorie et al. (2011) demonstrated the relative abilities of both the chlorophyll (SPAD) meter and the digital imaging (DGCI) methods of measuring total leaf nitrogen concentration. In that particular study, the DGCI method had a correlation comparable to the SPAD meter when it came to determining the total leaf nitrogen concentration, thus demonstrating the value of the method as a means of crop nitrogen estimation.

To develop an accurate and useful dark green color index (DGCI) for evaluating plant leaf nitrogen, it is important to keep the individual images as standardized as possible. Images have been taken indoors to minimize lighting vacillations. Pagola (2009) used a flat black board with a 1cm^2 hole cut out from the center and then placed over the leaf to be measured. This allowed for a common reference point for each photograph.

Rorie et al. (2011) improved upon the digital imaging method described above. First, measurement data were collected at five different fields within the state of Arkansas. Each field was of a different soil type. A range of corn hybrids was planted at each field and then subjected to a wide range of nitrogen fertilizer treatments. At silking, photographs were taken of the entire leaf on a bright pink, felt cloth to provide greater contrast between leaves and background for ensuing image analysis. Additionally, standardized color discs of yellow and green were included in each photograph to serve as standards and to account for subtle lighting changes over the course of the process and for differences among cameras. SigmaScan Pro 5 (SyStat Software Inc., San Jose, CA) software was used to quantify greenness and determine DGCI (Karcher and Richardson, 2003). In Rorie's work, the yield and DGCI values were expressed as a fraction of the treatment receiving the highest amount of nitrogen fertilizer for each field. The resultant relative yield and relative DGCI could then be compared to each other with a single function. To further advance standardization of DGCI, it is important that a field measured by this method have a small area that is highly fertilized to establish a benchmark for relative measurements and account for environmental factors. Similar to the reflectance technique, digital color analysis will require the development of basic algorithms and standard curves to cover various environmental conditions.

An understanding of the relationship between nitrogen deficiencies and corrective measures is important for farmers and researchers. This is especially true for the late vegetative and reproductive stages of corn development, when nitrogen nutrition is crucial to eventual yields. Accomplishing this understanding in the context of digital image analysis will provide an important, effective, and inexpensive tool for farmers worldwide. An important next step is to develop appropriate technologies that will employ DGCI as a means of correcting nitrogen deficiencies in corn at specific development stages. Improved nitrogen use efficiency will benefit crop producers and consumers with lower costs of production, less environmental pollutants, and greater energy efficiency.

In an effort to develop a greater understanding of the relationships between DGCI measurement methodology, nitrogen fertilization, and crop yields, several studies were conducted in Arkansas. The objectives were: (1) to develop quantitative relationships among yield, corn leaf N concentration and DGCI measurements taken in the mid-vegetative stages of growth development; (2) to determine the amount of N to apply to recover yield based upon DGCI measurements on 6-to-10-leaf corn (V6-V10); and (3) explore the efficacy of the DGCI method in other, non-leguminous crops. Chapter 2 of this study outlines the conduct and findings of this study over a two year period. Chapter 3 examines applicability of the methods used in corn across several other agronomically important crops including wheat, rice, and turf grass species.

Chapter 2

Quantitative Relationships among Yield, Leaf Nitrogen Concentration, SPAD, and DGCI in Corn

ABSTRACT

Corn (*Zea mays* L.) is an important agronomic crop in the United States. Corn production requires high levels of nitrogen fertilization to achieve profitable yields. Increasing N costs have led to demand for greater N fertilizer use efficiency, requiring an accurate measure of current crop N status. Our aim was to quantify relationships among yield, leaf N concentration, SPAD, and the Dark Green Color Index (DGCI) method to better tailor N fertilization to crop N needs. These measurements made it possible to construct calibration curves relating observations such as DGCI or SPAD to subsequent N applications for achieving target yields. Corn was planted at five locations over 2 years in Arkansas and then subjected to varied early season and midseason N applications. The relative N sufficiencies or deficiencies were estimated using DGCI, SPAD, and leaf N. Data over both years revealed a significant relationship ($p \leq 0.05$) at midseason (V6 to V10) between DGCI and SPAD (r^2=0.48 to 0.87), DGCI and leaf N concentration (r^2=0.56 to 0.70), and SPAD and leaf N concentration (r^2=0.43 to 0.80) in corn. Crops with varying early-season N deficiencies demonstrated a non-linear, quadratic response to midseason N applications. Combining the responses of yield to midseason N application rates with concurrent mid-season DGCI measurements allowed for the development of calibration equations. These data were used to develop calibration curves for DGCI taken indoors (r^2=0.65), DGCI taken outdoors (r^2=0.83), SPAD (r^2=0.57), and leaf N concentration (r^2=0.64). These calibrations equations provide prediction tools to allow corrective, mid-season N applications to be made based on an observed value, which allows for the recovery of 90 or 95% of the crop's yield potential.

INTRODUCTION

Nitrogen is fundamental to terrestrial life. Accordingly, it is part of the complex nutrient economy that dictates the behavior of that life from the basic level of amino acid synthesis. In plants, nitrogen is a crucial component of chlorophyll (Buchanan, 2007). The prevalence of N in the cells of agronomic crops means that harvest removes large quantities of N from a field, and, in doing so, creates of a paucity of the nutrient residual in the soil for future production (Iowa State, 2007). This fact, coupled with N loss from soils by leaching, volatilization and denitrification, establishes a situation in which N is the nutritional factor most commonly limiting crop yield potential. To remedy this, plant-available N forms are provided to the crop in the form of chemical fertilizers such as anhydrous ammonia and urea. Nitrogen fertilization is a cornerstone of modern agriculture because it provides the nutrients needed for elevated grain yield and quality (Miao, 2007).

Corn (*Zea mays* L.) is a prime example of the necessity for N fertilization. Yields expected in modern production schemes require N fertilizer at higher rates than any other major U.S. crop (USGS, 1999). At the same time that N fertilizers are subject to loss into the environment, a corn crop requires widely varied amounts of N throughout development, culminating in the late vegetative through early reproductive stages (NDSU, 1999; Scharf and Lory, 2002). As a result, early and severe N deficiencies in corn will reduce yield if left uncorrected and have a reduced chance of recovery depending on the lag between the advent of deficiency and diagnosis (Binder, 2000).

The diagnosis of in-season N deficiencies must be followed by corrective N applications to recover potential yield. Vetsch and Randall (2004) suggest that at the V10 growth stage, a period about five weeks after emergence that is crucial to ear growth and development, N deficiencies as diagnosed with a chlorophyll meter can be corrected to recover some yield. Chlorophyll meter readings have also been strongly correlated to yield in corn plots having received a first N application mid-season (Scharf et al., 2006). When corn crops experience a significant lack of N in the early part of the growing season, timely corrective applications can improve yield potential to a point near that of a control (Ruiz Diaz et al., 2008).

A key to corrective N fertilization action is up-to-date knowledge of advent and degree of N deficiency. Immediate knowledge of plant N concentration is often not obtainable due to a lag time for processing. This lag time can negatively affect the value of the derived information due to the short window during which N demand is increasing and deficiencies can be most effectively corrected. Nitrogen requirements of the crop increase dramatically beginning with the V6 development stage (Iowa State, 2007), and the final chance for practical application of non-foliar N fertilization (due to increasing plant height) occurs about a week later at the V8 stage (NDSU, 1999). The V8 development stage is also the final point at which corrective applications can re-establish near complete yield potential (Raun, 2005).

In addition to timing, spatial variability exists within any field relative to the amount of N required by a particular crop (Raun and Johnson, 1999). An attempt to reduce N fertilizer losses must take this into account. As discussed later, developing a field-wide map for variable-rate N application can be achieved several ways, but must have as high a level of accuracy and resolution as possible to maximize fertilizer efficiency. This precision and resolution should consider the field element size, defined by Solie et al. (1996) as “that area or resolution which provides the most precise measure of the available nutrient where the level of that nutrient changes with distance.” Variation in crop response to N applications can be detected within $1m^2$ (Solie et al., 1996).

Crop N status can be determined by several extant methods. Total N analysis is the most accurate way of measuring leaf or soil N, but analyses such as the Kjeldahl-Rittenberg procedure (Barrie, 1995) or Micro-Dumas Combustion method (University of Georgia, 1997) require equipment and technique beyond the point of everyday production practicality. Shipping the measurements off to a qualified testing service can compromise the brief window in which the information might be of value.

Leaf N concentration is closely correlated to chlorophyll concentration. Exploiting this relationship opens the way for an increasingly common, practical, non-destructive approach to estimate leaf N concentration (Costa, 2001). The chlorophyll meter is a handheld device that estimates leaf chlorophyll concentration based on the absorbance measurements in two wavelengths (Konica Minolta, 2009) and has been shown to carry a significant correlation between chlorophyll-meter measurements and leaf N concentration in corn (Bullock and Anderson, 1998). Additionally, relative leaf chlorophyll has been found to have a strong correlation to relative grain yield in corn (Rorie et al., 2011; Vetsch, 2004).

Though information about corn N status can be attained very quickly with the chlorophyll meter, other aspects can prove burdensome. It is expensive, with a basic model costing upwards of US $2,000. It has a small sampling area, 2x3 mm, which can lead to sampler bias and greatly increase the number of individual measurements required to adequately assess all the field elements in a typical corn field. Some studies have shown that chlorophyll meters are ineffective tools for calculating N needs during the mid-to-late season (Bullock, 1998; Zhang, 2008; Zhang, 2009).

Digital image analysis is an emerging method of N status diagnosis that addresses the needs of time, cost, and data resolution. Pagola (2009) made estimates of N nutrition in barley using digital image analysis and found them at least on par with chlorophyll meter values as indicators of N deficiencies and predictors of total yield.

For this procedure, a common, low-cost digital camera is used to make color images of corn leaves. The color in images is composed of three values: red, green, and blue (RGB). The RGB color scale is easily converted into hue, saturation and brightness (HSB) using a method described by Karcher and Richardson (2003) for turfgrass analysis. This is because the relative greenness of a leaf will be used to make statements about the amount of chlorophyll it contains, which in turn is related to N concentration. RGB does not accurately quantify shifts in such values as hue, saturation and brightness among different color samples. HSB data are converted into dark green color index (DGCI) values specifically for the purposes of N concentration analysis as in the following equation:

$$DGCI = \left[\left(\frac{HUE - 60}{60}\right) + \left(1 - SATURATION\right) + \left(1 - BRIGHTNESS\right)\right] / 3$$

DGCI value is on a scale from 0 (very yellow) to 1 (dark green). Multiple digital images of a crop can quickly be developed into an index of crop leaf N concentration.

PREVIOUS RESEARCH AT THE UNIVERSITY OF ARKANSAS

Rorie et al. (2011) modified the digital imaging method of Karcher and Richardson (2003) for use in corn. Five corn hybrids were planted in fields of differing soil types in Arkansas and subjected to differing N fertilization treatments. At silking, photographs were taken of the entire leaf on a bright pink, felt cloth to provide greater contrast between leaves and background for ensuing image analysis. Image analysis software (SigmaScan Pro 5, SPSS, 1998, San Jose, CA) was used to quantify greenness and subjected to an algorithm to determine DGCI values (Karcher and Richardson, 2003). Observed relative yield and relative DGCI values were compared. It was possible to eliminate much inter-field error and create a standard index by including a small area that was fertilized at a very high rate at each location. This established a benchmark for relative measurements and accounted for environmental factors.

Corn N status is constantly in flux throughout the growing season. Early, precise, and accurate measurements of current N status are crucial for maintaining high N use efficiencies in the crop and also for creating favorable nutrient conditions for maximizing potential yield. The DGCI method has the potential to be an important tool for achieving these goals. It is important to understand the relationship between SPAD measurements, DGCI measurements, and actual corn leaf N concentrations throughout the growing season, both to validate the emerging technology and to integrate into an existing array of tools. The objectives of this research were to (1) quantify the relationship among DGCI, leaf N concentration, SPAD and yield for corn at midseason, and (2) develop a calibration curve prescribing mid-season N applications for determining yield based on DGCI, leaf N concentration, and SPAD measurements also made at midseason.

MATERIALS AND METHODS

CROP MANAGEMENT

Commercial corn hybrids (treated with Cruiser Extreme® 250 fungicide and insecticide, genetically modified to express the *cry*1F gene, and resistant to glyphosate, see Table 2.1 for hybrid information) were planted at eight fields in Arkansas over the course of 2 years. Corn was planted at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR 29 April 2010. Seed was sown at a rate of 74,000 kernels ha^{-1} in a Captina silty loam (fine-silty, siliceous, active, Mesic Typic Fragiudults). Plots consisted of four rows, 101.6 cm apart and 7.6 m in length. Prior to planting, plots received nutrient amendments to meet soil-test recommendations for all nutrients except for N. Soil N had been minimized by planting, mowing, and removing at heading a cover crop of rye (*Secale cereale* L.). Numerous soil core samples had been taken throughout the field at depths of 0-15 cm and 15-30 cm, dried, and analyzed using the Mehlich-3 procedure to establish baseline field soil nitrogen levels (Table 2.1); levels were similar across locations. Irrigation was applied using sprinklers when the soil-moisture deficit reached a deficit maximum of 32 mm as determined by an irrigation scheduling program devised by Purcell et al. (2007). The experimental design was a randomized complete block design with four replications.

Field management was similar at other locations and other years (Table 2.1). At locations other than Fayetteville, K, zinc, and sulfur (as potassium sulfate and zinc sulfate) amendments were applied concurrent with the emergence N application as determined necessary by soil test recommendations.

At maturity, the inner 5 m of the middle two rows were harvested by a plot combine. Grain was weighed and moisture content determined, and yield was expressed at a moisture content of 15.5g $100g^{-1}$.

Table 2.1 Production and management information for corn N-response experiments in 2010 and 2011 at four locations in Arkansas.

2010				
Location	**Fayetteville**	**Stuttgart**	**Marianna**	**Keiser**
lat, long	36.09, -94.17	34.46, -91.41	34.73, -90.76	35.67, -90.08
Hybrid	Pioneer 33D49	Pioneer 33D49	Pioneer 31D59	Pioneer 33D49
Planting Date	29 April	22 April	27 April	7 May
Irrigation Method	sprinkler	furrow	furrow	furrow
Row Spacing, cm	101.6	76.2	96.5	96.5
Plot Length, m	6.1	6.1	6.1	6.1
Total soil N, mg kg^{-1}	714±65	744±102	688±55	759±69
n, plots	72	72	72	84

2011				
Location	**Fayetteville**	**Rohwer**	**Marianna**	**Keiser**
lat, long	36.09, -94.17	33.81, -90.76	34.73, -90.76	35.67, -90.08
Hybrid	Pioneer 1184HR	Pioneer2023HR	Pioneer 31P42	Pioneer1615HR
Planting Date	6 May	14 April	12 May	12 May
Irrigation Method	sprinkler	furrow	furrow	furrow
Row Spacing, cm	91.4	96.5	96.5	96.5
Plot Length, m	6.1	5.8	6.1	6.1
Total soil N, mg kg^{-1}	800±19	733±146	NA‡	771±34
n, plots	84	84	84	84

‡Pre-plant soil N samples were not taken in Marianna in 2011.

N TREATMENTS

Nitrogen treatments were applied in two splits at all locations (Table 2.2.). Nitrogen was broadcast to each row by hand. Urea fertilizer (46-0-0) was used for all applications, and in every location, except Fayetteville in 2010, the urea prills were treated with dicyandiamide and N-(n-butyl) thiophosphotictriamide (Agrotain®, AGROTAIN International, St. Louis, MO). Agrotain® was applied to urea prills the subsequent year in Fayetteville due to revised experimental protocol.

At or near emergence for each location, plots received three N fertilizer treatments. In the study year 2010, the Stuttgart location was treated at approximately V5 while the Keiser location received treatment at V3. In 2011, Marianna received a similarly late initial treatment at V4. These belated treatments were due to travel delays or field conditions preventing earlier application. One third of the plots received 84 kg ha^{-1} of urea broadcast by hand. One third of the plots received 168 kg ha^{-1} of urea broadcast by hand. The final third of plots received no N fertilizer. In 2010, when the plots receiving the highest N rate reached the V6-V10 growth stage, plots from each emergence N treatment received urea applications of 0, 28, 56, 84, 112 or 168 kg ha^{-1}. At Keiser, an additional treatment rate of 224 kg ha^{-1} (2010 and 2011) was applied to ensure that sufficient N was available to maximize yield and not be immobilized by the clay soil. In 2011, the V6-V10 applications were 0, 14, 18, 70, 112, 168, and 224 kg ha^{-1}(Table 2.2). The experimental design was a randomized complete block with four replications of treatment combinations.

In 2010, urea application in Fayetteville was immediately followed by irrigation. As the other locations received urea plus Agrotain (urease inhibitor), these locations were not irrigated until after a rainfall event had occurred in order to prevent N movement via furrow flooding; if a rainfall event did not occur within several days of application, irrigation was applied to ensure nutrient availability coincident with target growth stage.

SAMPLING METHODS

Plots were sampled twice during the growing season and once again at harvest. The initial sampling was made when plants in the highest N-rate plots were rated at the V6-V10 stage. At this point, a corn plant from the center of each of the middle two rows of the plot was selected for sampling and was assumed to be representative of the plot overall. In 2011, following establishment of correct sampling protocols, an additional digital image was taken *in situ* of the upper portion of plants selected for analysis. These images were later used to make comparisons with images of the same plants taken under controlled lighting conditions. Both excised leaves and plants in the field were photographed against a plywood board that was painted pink (to provide contrast in subsequent image processing) and two internal color standards that were included in each image (Rorie et al., 2011). These internal color standards were disks colored with a paint of a known DGCI value (0.5722 for green and 0.0733 for yellow). The inclusion of the color standards in each image allowed for corrections of minor vacillations in light and shadow that may occur (Rorie et al., 2011).

Images were taken at a resolution of 320 x 240 with a Canon Powershot S5IS Digital Camera (Canon USA, Inc., Lake Success, NY). After images were taken, the uppermost collared leaf of each plant was removed. These leaves were immediately placed in a sealed plastic bag and put on ice. After the entire field had been sampled, an image was made of the selected leaves indoors under fluorescent lighting conditions. Four chlorophyll meter (SPAD-502Plus Chlorophyll Meter, Konica-Minolta Inc., Tokyo.) readings were taken from each leaf sampled and then averaged. The leaves were subsequently dried and analyzed for total N concentration via LECO FP428 N Analyzer (LECO Corporation, St. Joseph, MI) by the Soil Test and Plant Analysis Lab (University of Arkansas, Fayetteville). All sampling was conducted prior to a concurrently scheduled N fertilizer application.

Table 2.2 Nitrogen treatment information for corn N-response experiments in 2010 and 2011 at four locations in Arkansas.

2010				
Location	**Fayetteville**	**Stuttgart**	**Marianna**	**Keiser**
Emergence Treatment				
Rates, kg ha^{-1}	0,84,168	0,84,168	0,84,168	0,84,168
Date	9 May	26 May	28 May	25 May
Stage	V1	V5	V1	V3
Mid-season Treatment				
Rates, kg ha^{-1}	0, 28, 56, 84, 112, 168	0, 28, 56, 84, 112, 168	0, 28, 56, 84, 112, 168	0, 28, 56, 84, 112, 168, 224
Date	17 June	18 June	19 June	21 June
Stage	V7	V10	V10	V7

2011				
Location	**Fayetteville**	**Rohwer**	**Marianna**	**Keiser**
Emergence Treatment				
Rates, kg ha^{-1}	0,84,168	0,84,168	0,84,168	0,84,168
Date	17 May	7 May	27 May	27 May
Stage	V1	V2	V4	V1
Mid-season Treatment				
Rates, kg ha^{-1}	0, 14, 28, 70, 112, 168, 224	0, 14, 28, 70, 112, 168, 224	0, 14, 28, 70, 112, 168, 224	0, 14, 28, 70, 112, 168, 224
Date	20 June	7 June	27 June	21 June
Stage	V6	V9	V10	V6

Grain yield was determined by first removing 1m from each end of the rows within a plot to account for edge effects. The remainder of the interior two rows was then harvested. Moisture was noted and then each sample weight was adjusted for a standard 15.5% moisture content. The result was then multiplied by the two-row harvest area to attain a kg ha^{-1} value for grain yield.

A representative sample of the grain from each plot was ground and analyzed for N concentration using a LECO FP428 N Analyzer. The total N content of the grain was calculated as:

N content of grain = Yield × N concentration of grain

Grain N recovery was determined as:

Grain N recovery = [(N content of grain – N content of grain receiving no N) / N applied] × 100

Post-sampling analysis was conducted on each digital image using SigmaScan Pro 5 (SPSS, 1998, San Jose, CA) and a macro described by Karcher and Richardson (2005). Image color thresholds were set at ranges of 30 to 130 for hue and 0 to 100 for saturation. Leaf DGCI values were corrected with internal color standard values to yield a corrected DGCI value (Rorie et al., 2011).

Leaf N concentration, DGCI, and yield values showed no significant differences among different soil types and hybrids. However, relative values were used for pertinent analyses to more fully account for some of the variation that occurred among fields. The relative value for a measurement was obtained by finding the highest value of a particular measurement for a certain location and then dividing all other measurements of a similar type at that location by that value.

CALIBRATION OF N RESPONSE

Regression analysis was used to relate grain yield response to N applied at V6-V10 for each emergence N rate and at each location and year. Linear and quadratic models were both examined; in almost every instance, the quadratic model was significant and thus they were used for further calculations (Table 2.3). This was not the case for the 168 kg N ha^{-1} treatment in Stuttgart in 2010 and Keiser in 2011, which showed no significance fit to either linear or quadratic models. The quadratic model for each location was described by an equation in the form $f(x) = ax^2 + bx + c$. By setting the first derivative to zero and solving for x, the amount of N that need be applied to achieve theoretical maximum yield can be determined for that emergence N rate, location, and year (Black, 1993). In several instances, the calculated amount of N needed to achieve theoretical maximum yield was greater than that applied in the course of the experiment; in such cases, the maximum amount actually applied was used instead for further calculations. From there, the amount of N needed to attain 90% and 95% of the theoretical maximum yield was calculated by finding the respective percentages of the determined theoretical maximum yield and solving for x *(cf* Fig. 2.1).

Furthermore, because each plot receiving a mid-season N application was also sampled for DGCI, SPAD, and leaf N immediately prior to fertilizing, they represent the current N status of the corn that gave the particular yield response to the N application. It can thus be said that applying N fertilizer in amounts of x (or $0.95x$ or $0.9x$) mid-season to a corn plant having the observed DGCI, SPAD, or leaf N value would yield the theoretical maximum potential yield (or 95% or 90% of it, respectively). These latter values are of interest, because a relatively small decrease in yield of 5 to 10% requires substantially less N than that required for maximum yield.

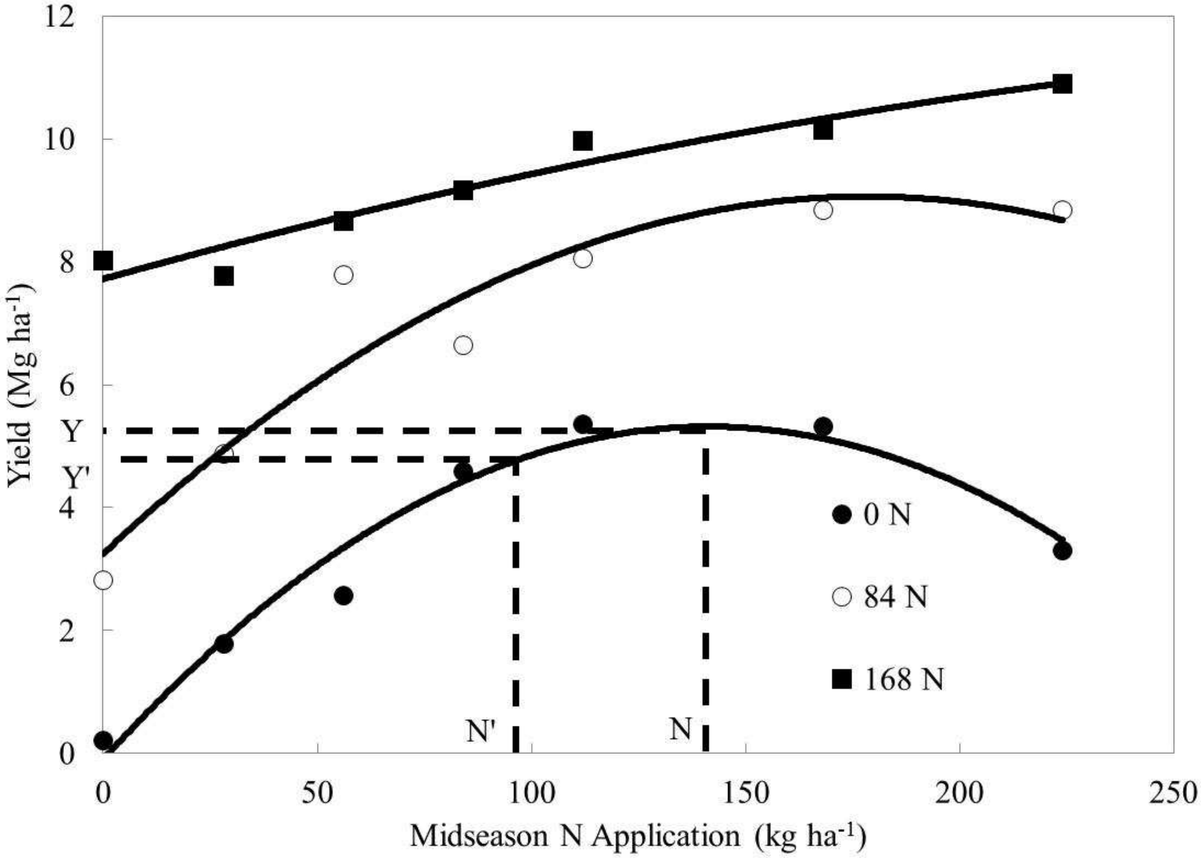
The derived data discussed in the previous paragraph was used to develop a calibration curve according to a procedure described by Black (1993). Each location and year had three different N rates applied near emergence. Combining these gives a broad range of mid-season N conditions, the corresponding DGCI measurements, and the N needed to attain a given percentage of the theoretical maximum yield. The DGCI measurements and N needed can be plotted to attain a calibration curve (*cf*Fig. 2.2). Similar calculations were made for creating calibration curves based on SPAD and leaf N concentration measurements.

Table 2.3 Quadratic equations describing yield response to midseason N application for all emergence N rates, locations, and years. The rightmost two columns describe values for each equation maxima.

Year	**Location**	**Emg N**	$y=ax^2+bx+c$				**Yield at max**	**N rate at max**
		kg ha^{-1}	***a***	***b***	***c***	**r^2**	**kg ha^{-1}**	**kg ha^{-1}**
2010	Fayetteville	0	-0.22	68.21	2,992	0.90	8,279	155
		84	-0.10	37.47	6,047	0.66	10,075	168‡
		168	-0.11	33.64	7,714	0.45	10,309	154
	Keiser	0	-0.29	76.19	-86	0.74	5,332	142
		84	-0.18	65.22	3,251	0.81	9,076	179
		168	-0.02	19.38	7,721	0.37	10,941	224‡
	Marianna	0	-0.12	55.92	61	0.52	5,726	168‡
		84	-0.14	61.47	3,554	0.66	9,714	168‡
		168	-0.08	40.35	5,539	0.54	9,767	168‡
	Stuttgart	0	-0.02	19.88	24	0.74	2,936	168‡
		84	-0.15	37.49	2,367	0.45	4,476	129
		168†	-	-	-	-	-	-
2011	Fayetteville	0	-0.17	72.70	426	0.91	8,386	219
		84	-0.15	53.60	5,840	0.69	10,693	181
		168	-0.15	47.67	6,309	0.54	10,147	161
	Keiser	0	-0.18	85.16	6,585	0.82	16,770	224‡
		84	-0.24	69.16	10,231	0.60	15,213	144
		168†	-	-	-	-	-	-
	Rohwer	0	-0.08	45.00	1,307	0.80	7,436	224‡
		84	-0.07	40.41	2,803	0.81	8,629	224‡
		168	-0.05	27.66	4,761	0.81	8,821	216

† Quadratic responses were significant, except for the 168 kg ha^{-1} emergence rates for Stuttgart in 2010 and Keiser in 2011($p<0.01$). These two locations showed no linear response as well.
‡ If calculated N rate at maximum exceeded the maximum amount of N applied at midseason (168 kg ha^{-1}), the maximum amount applied at midseason was used.

Figure 2.1 Yield response to mid-season (V6-V10) N applications for the three emergence N application rates during the study year 2010 at the Keiser, AR experimental location. Data points represent average values for all replications. The quadratic response for the 0 N emergence treatment follows the equation $y = -0.27x^2 + 76.49x - 90.86$. Adjusting yield goals from 100% of theoretical maximum yield to 90% of maximum yield would result in a yield loss of 0.53 Mg ha^{-1} (Y-Y') and a reduction in N rate of 44.5 kg ha^{-1} (N-N').



RESULTS

2010

The yield data for the 2010 season largely resembled expectations based on the extant literature reviewed by Binder (2000). Across all locations, yields were generally highest for corn fertilized with the highest N rates at emergence and at the subsequent, mid-season date (Table 2.4). The ANOVA tables for yield response to N by location are presented in the appendix (App. Table 1). As each emergence N rate was intended to simulate a relative N sufficiency or deficiency by the time the crop reached the crucial mid-season stage of N uptake, varying responses should be expected for each of the three N levels, as was illustrated by Fig. 2.1. For almost all of the locations and rates, a quadratic regression response was significant ($p \leq 0.05$), and was therefore more appropriate than a linear model for these data (Table 2.3) based on p values. This was not the case for the 168 kg ha^{-1} N rate applied near emergence at Stuttgart, which failed to show a significant linear or quadratic response, and was subsequently disregarded in further calculations. The observed quadratic responses were more pronounced within individual locations for the year (*cf* Fig. 2.1).

The 2010 results led to two suppositions, both supported within the literature (Binder, 2000). Firstly, that perhaps the emergence N rates that demonstrated the flattest response to mid-season applications may not have received a high enough mid-season rate to produce maximum yield. Secondly, that early season N availability and uptake

Figure 2.2. The calibration curve for the amount of N to be applied at midseason (V6-V10) to recover 90 or 95% of maximum yield versus dark green color index (DGCI) values measured at V6-V10. The DGCI values were made on the topmost collared leaf and photographed indoors. Data are included from both 2010 and 2011.

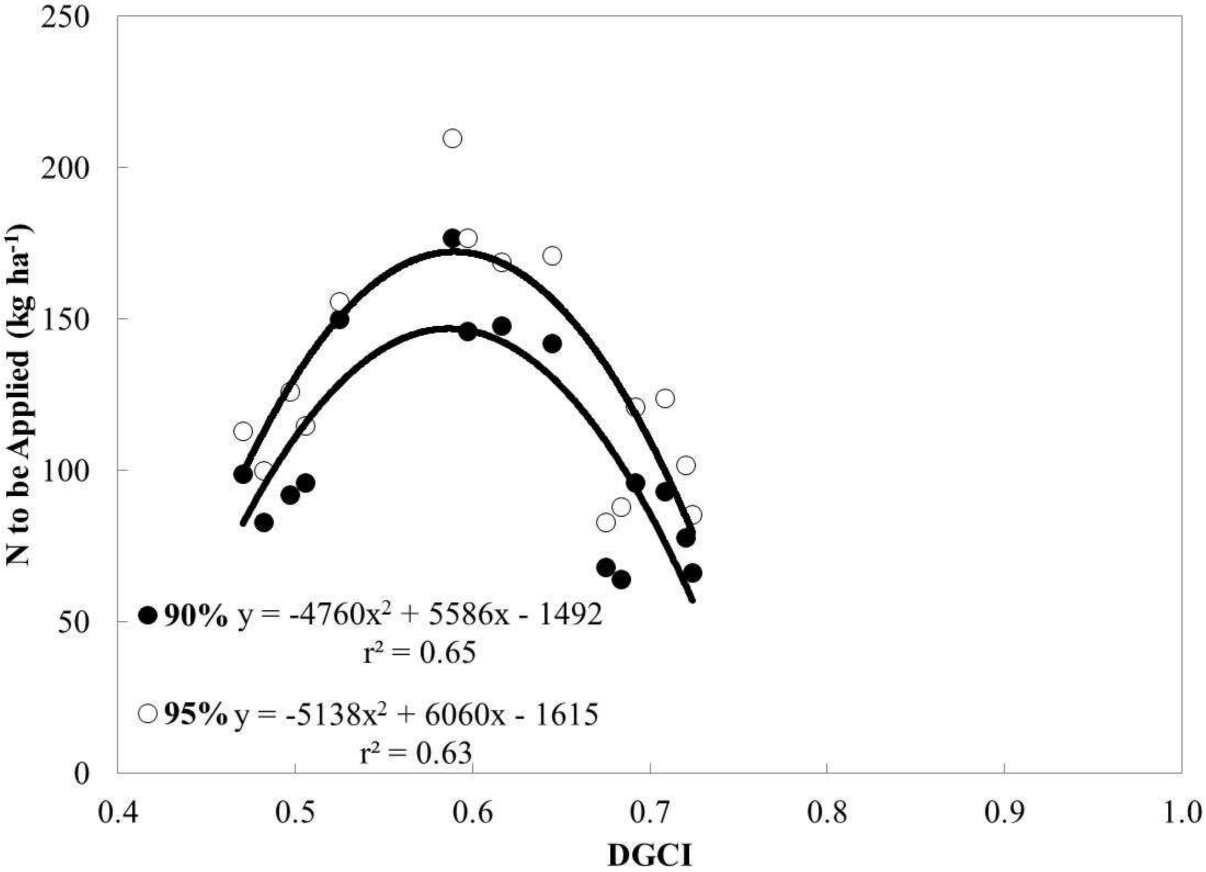


Table 2.4. Summary of grain yield and nitrogen recovery for all locations in 2010.

		Grain Yield			**Grain N Recovery**		
		Emergence N Application, kg ha^{-1}					
Location	V6-V10 N App., kg ha^{-1}	0	84	168	0	84	168
			kg ha^{-1}			%	
Fayetteville	0	2925 g†	5653 efd	7949 bc	-	34 efd	32 efd
	28	4840 f	7439 cd	8248 bc	78 a	46 becd	32 ef
	56	6145 efd	8127 bc	7456 cd	62 ba	45 ecd	25 f
	84	7099 ecd	9566 ba	9530 ba	62 ba	49 bcd	37 efd
	112	7847 bcd	8232 bc	10690 a	58 bc	35 efd	35 efd
	168	8242 bc	11048 a	10103 a	45ecd	45 ecd	30 ef
Marianna	0	3762 fde	4471 fdec	4085fdec	-	13 dc	4 dc
	28	5809 bdec	3483 fe	3755fde	116 a	-3 dc	2 dc
	56	6965 bdac	3926 fdec	5797bdec	93 ba	9 dc	17 dc
	84	5937 bdec	1312 f	4206fdec	44 bc	-19 d	6 dc
	112	4981 bdec	5333 bdec	7146 bac	23 dc	16 dc	21 dc
	168	8089 ba	9712 a	5620bdec	49 bc	45 bc	13 dc
Stuttgart	0	114 e	2567 dc	4785‡	-	27 bac	35‡
	28	776 e	5049 ba	3890‡	21 bc	44 a	27‡
	56	1163 de	5240 ba	5278‡	18 bc	35 ba	33‡
	84	1147 de	5649 a	4870‡	12 c	34 ba	28‡
	112	3130 c	5731 a	3464‡	29bac	31 ba	18‡
	168	3654 bc	6052 a	5110‡	22 bc	27 bac	22‡
Keiser	0	223 h	2837 g	8029 dc	-	39 fe	49 fed
	28	1792 hg	4878 fe	7783 dc	75 a	52 fedc	48 fed
	56	2588 g	7797 dc	8680 bc	57bdc	71 bac	42 fed
	84	4595 fe	6658 de	9172 bc	71 ba	48 fed	46 fed
	112	5367 fe	8065 dc	9983 ba	55bec	50 fed	41 fed
	168	5327 fe	8851 bc	10164 ba	42 fed	47 fed	41 fed
	224	2658 g	8857 bc	10915 a	16 g	40 fed	37 f

† Means with the same letter within a location are not significantly different as determined by an LSD ($p \leq 0.05$).

‡ Apparent sampling errors for the 168 kg ha^{-1} rate at Stuttgart led to that data being excluded from analysis of variance. Data are included here for completeness.

will affect potential yield despite mid-season-ameliorating-N applications, thus resulting in a point at which the application of additional N units would exhibit a diminishing marginal effect on grain yield.

These ideas gave rise to several minor changes in the experimental design for the second year of the study, as discussed in the materials and methods section of this chapter. The purpose of the higher addition of N fertilizer at V6-V10 was to reach the maxima of the response curve.

Grain N recovery for each location is also shown in Table 2.4. Grain N recovery was largely by corn receiving the lowest amounts of N at emergence and at V6-V10. The grain N concentration values used to determine grain N recovery as described in the previous section are shown in Appendix Table 2. The ANOVA tables for corn N recovery by location are presented in the appendix (App. Table 3)

In 2010, DGCI, SPAD and leaf N concentrations at the V6-V10 stage had a close relationship within all individual locations (r^2 values ranging from 0.74 to 0.91) except Keiser (Table 2.5). Though a significant relationship ($r^2 = 0.87$) existed between DGCI and SPAD at Keiser, neither measurement bore any strong relationship to the N concentration in the leaves sampled on the same date. This seemed unusual, because all other locations over both years demonstrated a relationship of some kind among DGCI, SPAD, and leaf N concentration (Fig. 2.3). There were several points between initial sampling and final analysis during which mislabeling of the samples could have occurred, and this seemed the most likely explanation. As a result, data relating to leaf N concentration at Keiser for the first sampling date were removed from further analysis.

Table 2.5. Linear regression data and sample size (n) for the relative dark green color index (DGCI) versus SPAD, DGCI versus leaf N concentration, SPAD versus leaf N concentration, and individual locations and combined for the V6-V10 stage for 2010.

Location		**DGCI vs SPAD**	**DGCI vs Leaf N Concentration**	**SPAD vs Leaf N Concentration**
Fayetteville				
	Slope	0.0078	0.14	16.77
	Intercept	0.21	0.23	5.34
	r^2	0.86**	0.74**	0.74**
	n	71	72	71
Marianna				
	Slope	0.0072	0.10	13.92
	Intercept	0.25	0.32	10.90
	r^2	0.91**	0.81**	0.82**
	n	71	62†	62†
Keiser				
	Slope	0.0082	0.045	5.74
	Intercept	0.19	0.405	25.39
	r^2	0.87**	0.07‡	0.08‡
	n	84	84	84
Stuttgart				
	Slope	0.0071	0.11	13.66
	Intercept	0.31	0.41	15.35
	r^2	0.91**	0.78**	0.84**
	n	60	60	52
All				
	Slope	0.66§	0.103	14.42
	Intercept	0.36 §	0.35	11.43
	r^2	0.87** §	0.68*	0.80**
	n	286 §	194	185

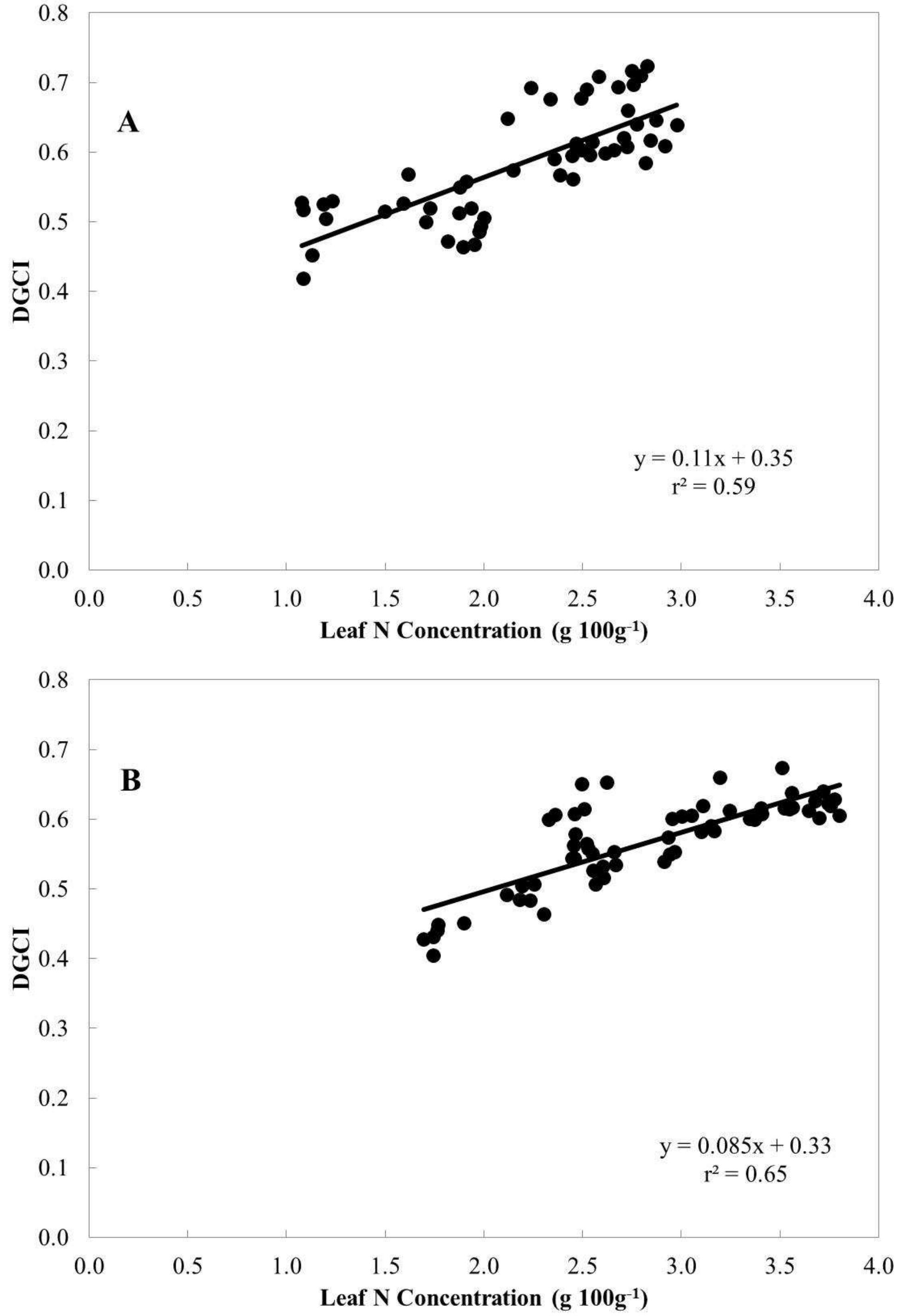
*,**, significant at $p = 0.05$ and 0.01 levels, respectively.

† Partial post-sampling loss of data resulted in an incomplete set of leaf N concentration data for Marianna.

‡ Sample mis-labeling led to problems with Keiser leaf N concentration data; Keiser leaf N concentration values not included in ‘All’ values.

§ Relative values were used to analyze DGCI vs SPAD for ‘All’.

Figure 2.3. Relationship of DGCI versus leaf N concentration over the study years 2010 (Fayetteville, Keiser, Marianna) at V6-V10 (A) and 2011(Fayetteville, Keiser, Rohwer) at V6-V10 (4 rep avgs) (B).



Data for the remaining three locations were combined, resulting in generalized observations about the measurements made within the first study year. The relationships among SPAD, DGCI, and leaf N concentrations, though significant in regression ($p \leq 0.01$), resulted in low r^2 values, especially with comparisons made concerning the leaf N concentrations (Table 2.5).

2011

A confluence of factors including timing, weather, and weed encroachment resulted in conditions at the Marianna location in 2011 that left the data gathered there unsuitable for further analysis. Spring of 2011 saw massive flooding along the Mississippi river, including the Arkansas Delta. This created a shortage of manpower and access to fields, resulting in an emergence N application at Marianna occurring at V4, closer to what should have been the second application stage than the first. Subsequent lack of rain or irrigation to incorporate the urea prills may have exacerbated this problem. Additionally, the northern half of the field was heavily infested with pigweed (*Amaranthus palmeri*) which required several applications of herbicide to effectively treat. By the time the field was approached for a mid-season N application, it was apparent that not only had the initial N application failed to create any discernible color or height variation among plots, but that it was well past the target V6 application stage. Although the application was made, the field was monitored throughout the rest of the season and determined to be unsuitable for experimental inclusion. As a result, that location was not considered the second year.

Overall, and also within individual locations, the yield responses to mid-season N applications was slightly different than those of 2010 (Table 2.6). The ANOVA tables for yield response to N for each location are presented in the appendix (App. Table 4). Those plots that did not receive any N at emergence showed less curvature in the

Table 2.6. Summary of grain yield and nitrogen recovery for Fayetteville, Rohwer, and Keiser in 2011.

		Grain Yield			Grain N Recovery		
		Emergence N Application, kg ha^{-1}					
Location	V6-V10 N App., kg ha^{-1}	0	84	168	0	84	168
			kg ha^{-1}			%	
Fayetteville	0	1363 d†	7162 bac	6289 bc	.	88 a	43 bc
	14	1958 d	7011 bac	6745 bac	44 bc	72 ba	40 bc
	28	2375 d	6767 bac	7662 bac	40 bc	54 bac	44 bc
	70	4998 dc	8058 ba	7941 ba	64 bac	58 bac	38 bc
	112	6640 bac	7295 bac	9343 a	59 bac	44 bc	41 bc
	168	7591 bac	8610 ba	8878 ba	52 bac	42 bc	36 bc
	224	7611 bac	9276 a	8220 ba	41 bc	35 bc	26 c
Rohwer	0	369 i	2156 figh	3134 feg	-	29 ba	21 ba
	14	850 igh	2011 igh	3620 fedc	21 ba	9 b	27 ba
	28	451 ih	2539 figh	4727 fedc	12 ba	23 ba	22 ba
	70	3054 fegh	3839 fed	6232 bac	43 a	25 ba	33 ba
	112	4761 fedc	5766 bdc	4779 bedc	44 a	29 ba	25 ba
	168	4295 fedc	7216 ba	7528 a	36 a	39 a	33 ba
	224	7362 bac	4824 bdec	6229 ba	32 ba	25 ba	33 ba
Keiser	0	-§	4668 c	85‡	-	14 c	14‡
	14	4672 c	7996 bac	15349‡	81 a	56 bac	72‡
	28	5299 bc	7928 bac	16941‡	70 ba	50 bac	75‡
	70	6447 bac	9072 ba	15008‡	61bac	48 bac	66‡
	112	6429 bac	8697 bac	13533‡	45 bac	40 bac	49‡
	168	8899 bac	9866 a	10830‡	50 bac	40 bc	55‡
	224	9972 a	6788 bac	6781‡	45 bac	19 c	24‡

† Means with the same letter within a location are not significantly different as determined by an LSD ($p \leq 0.05$).

‡ Apparent sampling errors for the 168 kg ha^{-1} rate at Keiser led to that data being excluded from analysis of variance. Data are included here for completeness.

§Inconclusive sampling of plots from the lowest 0 kg ha^{-1} rate at Keiser rendered the data insufficient for analysis.

quadratic response to mid-season applications. This suggests that midseason N applications to corn with severe early N deficiencies can have a dramatic effect on yield, and, when compared to the other emergence N rates for the year, illustrates the effect of emergence N availability has on midseason N application response and setting yield potential. The higher two emergence applications demonstrated decidedly more curvature in the quadratic response. The lone exception to this was the plots in Keiser receiving the highest emergence N rate, which showed neither a linear nor a quadratic response. Moreover, mean comparisons showed a greater range of yields than had been seen in the previous year (Table 2.6), indicating that the application of additional lower and higher mid-season rates to the experimental design may have had the desired outcome. By revealing the maxima of the yield response curves, more confident statements could be made regarding the calibration curves that would ultimately be developed (see next section). As in 2010, soil type and corn hybrid seemed to have no significant effect on observations made at the V6-V10 growth stage.

Grain N recovery for each location is shown in Table 2.6. Grain N recovery among experimental plots showed less variation compared to the previous year. In 2011, the most efficient plots for both Fayetteville and Rohwer were those that received no nitrogen at emergence and then a mid-level amount at the V6-V10 application. The grain N concentration values used to determine grain N recovery as described in the previous section are shown in Appendix Table 5, ANOVA of grain N recovery is shown in Appendix Table 6.

The study year 2011 was marked by relationships observed between relative DGCI, DGCI, relative SPAD, SPAD, and leaf N concentration that were somewhat weaker than those in 2010 (Table 2.7). The relationships were still found to be significant, however. As discussed

Table 2.7. Linear regression data and sample size (n) for the relative dark green color index (DGCI) versus SPAD, DGCI versus leaf N concentration, SPAD versus leaf N concentration, and individual locations and combined for the V6-V10 stage for 2011.

Location		**DGCI vs SPAD**	**DGCI vs Leaf N Concentration**	**SPAD vs Leaf N Concentration**
Fayetteville				
	Slope	0.0079	0.10	10.84
	Intercept	0.20	0.24	12.21
	r^2	0.69**	0.79**	0.77**
	n	83	82	82
Keiser				
	Slope	0.0037	0.06	11.96
	Intercept	0.40	0.40	14.9
	r^2	ns	0.47**	0.55**
	n	72	72	72
Rohwer				
	Slope	0.011	0.13	12.44
	Intercept	0.13	0.30	12.845
	r^2	0.67**	0.80**	0.63**
	n	62	61	61
All				
	Slope	0.59 †	0.10	9.91
	Intercept	0.38 †	0.35	18.24
	r^2	0.48** †	0.70**	0.62**
	n	217 †	215	215

*,**, significant at p = 0.05 and 0.01 levels, respectively.
† Relative values were used to analyze DGCI vs SPAD for ‘All’.

earlier, the field in Marianna received the first and second N application much later than would be suitable for the study and was subsequently eliminated from further analysis. The trend of weaker relationships held true when all remaining locations were combined, except for the relationships between DGCI and leaf N concentration (r^2=0.70), which had a greater fit than that of the previous year.

CALIBRATION OF YIELD DATA

For each year, location, and emergence N rate, the quadratic response of yield to N applied at V6-V10 is described in Table 2.3. In all but two instances, the quadratic response was significant at $p<0.001$. At Stuttgart in 2010 and Keiser in 2011, the corn receiving 168 kg N ha^{-1} at emergence were deemed non-significant for both quadratic and linear regression models.

The calculated values of the amount of N to attain a theoretical maximum are presented in the rightmost columns of Table 2.3. Once maximum theoretical yield has been established, percentages of that figure can be calculated to tailor to an individual yield goal; in the case of this experiment, 90% and 95% of theoretical maximum yield was calculated as an example. These adjusted theoretical yields can then be applied to the quadratic equation for the location rate to determine the amount of N to be applied to achieve those yields. These calculated yields were then paired with observed DGCI values and used to develop the calibration curve seen in Fig. 2.2.

Fig. 2.2 is comprised of data taken from photographs made indoors, under controlled lighting conditions, of corn leaf samples taken from the field just prior to the mid-season N application. Ultimately, the yield data showed strong relationships in the form of the developed calibration curve (r^2 = 0.63 and 0.65 for the 95% and 90% curves, respectively).

Sampling by means of taking photographs of the corn in the field required the development of a technique that was not perfected until the second year of the study. DGCI data derived from outdoor photographs in 2011 were used to develop a calibration curve (Fig. 2.4). Though the range of DGCI values observed in outdoor photographs (0.51-0.72) was slightly narrower than those indoors (0.49-0.73), the range of recommended N to be applied was generally similar. The outdoor calibration curve showed a closer fit (r^2=0.83 and 0.79 for achieving the 95% and 90% yield potential), it resulted in higher recommendations on the lower end of the DGCI range as compared to the indoor photographs.

SPAD (Fig. 2.5) and leaf N concentration (Fig. 2.6) calibration curves are presented for comparison. The SPAD calibration curve had the least goodness of fit compared to leaf N concentration and DGCI, though it ultimately recommended similar N rate applications (75 to 160 kg ha^{-1}) to achieve target yields across a range of mid-season N crop statuses. The leaf N concentration calibration curve bore the strongest resemblance to the DGCI curve, with equivalent r^2 values for the 95% curves and a deviation of 0.09 between the r^2 values of the 90% curve. The recommended range to attain a maximum yield was similar, between 60-160 kg ha^{-1}.

Relationships between the N recommendations made by the various calibration curves showed high r^2 values among DGCI and leaf N concentration, with no significant relationship discernible between SPAD and leaf N concentration. Figure 2.7 illustrates the DGCI and leaf N concentration relationships, with an r^2 value of 0.75 and 0.88 for the measurements taken indoor and outdoor, respectively. The outdoor chart has fewer data points, as matching sets needed for comparison were not as complete as with the indoor sets. The slope of both graphs (0.98 and 1.12 for indoor and outdoor), which demonstrates the similarities between the level of recommendations for the DGCI method compared with those derived from leaf N concentration analysis.

DISCUSSIONS AND CONCLUSIONS

Overall, the relationships between the DGCI values, SPAD values, and leaf N analysis was strong at the V6-V10 stage. This agrees with previous research with corn measured at silking (Rorie et al., 2011) and turfgrass (Karcher and Richardson, 2003). The DGCI was closely associated with leaf N concentration at the V6-V10 stages in both years (r^2 of 0.45 in 2010 and 0.70 in 2011). The diagnosis and treatment of N deficient corn at an early stage can prevent yield loss by corrective N fertilization or prevent over-application of N fertilizer. The SPAD and DGCI values were closely associated with respect to each location and each year. The SPAD meters are a useful and well-accepted tool for evaluating plant N status, and this study demonstrates that the DGCI method can be used in similar situations.

There was a marked increase in yield as the levels of emergence N rate increased indicating that early season N deficits reduced corn grain yield potential for the season, despite any amount of remedial N fertilizer that was applied as a corrective measure mid-season. Binder (2000) reported a 12% decrease from maximum grain yield when a N deficiency was observed at the V6-9 stage. Our study suggested that this yield decrease may be even greater (Tables 2.4, 2.6), with

Figure 2.4. The calibration curve for the amount of N to be applied at midseason (V6-V10) to recover 90 and 95% of maximum yield versus dark green color index values measured at V6-V10. DGCI values were made on the upper portion of the plant and photographed outdoors. Data are included from 2011.

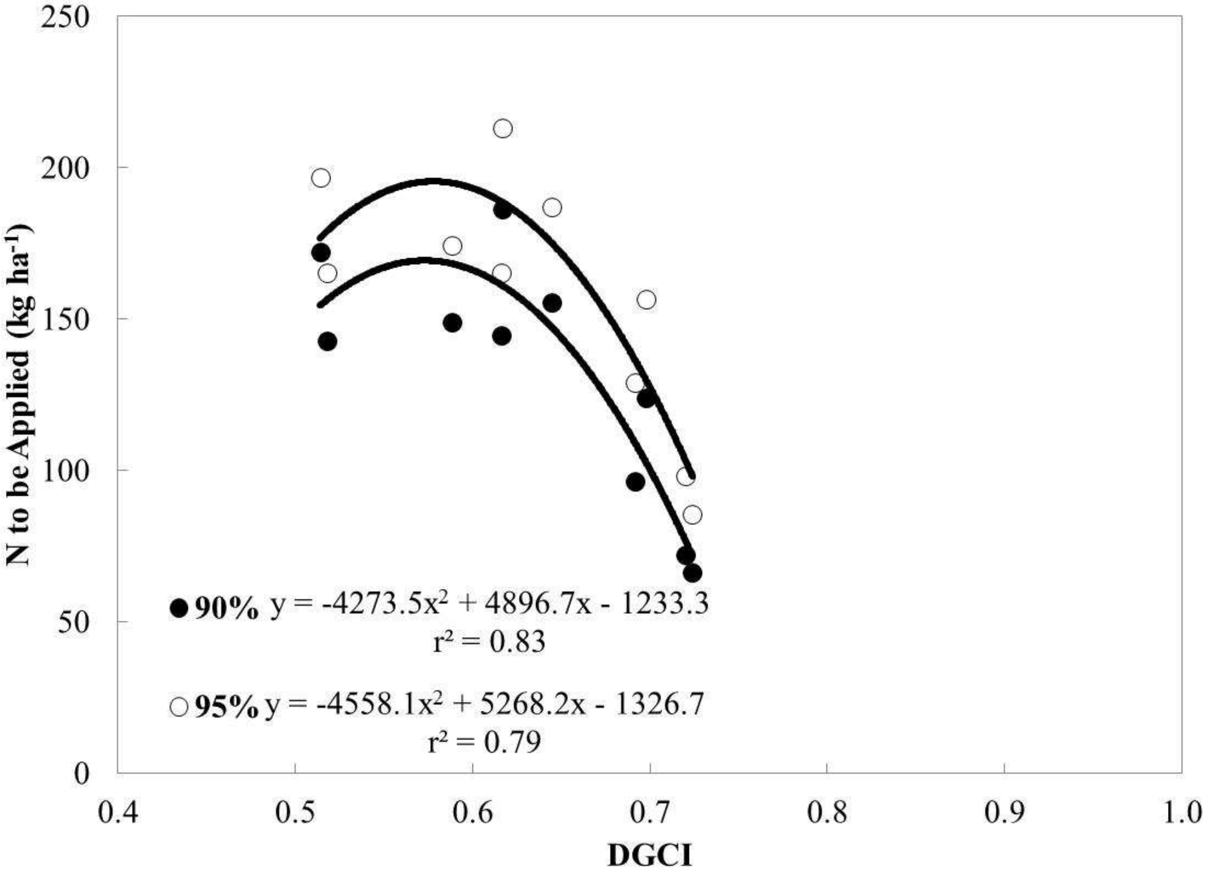


Figure 2.5. The SPAD calibration curve developed from the 2010 and 2011 data. Observed SPAD values were used to determine subsequent N mid-season applications to attain 90 and 95% of theoretical maximum potential yield. This calibration curve is based on SPAD values taken at V6-V10.

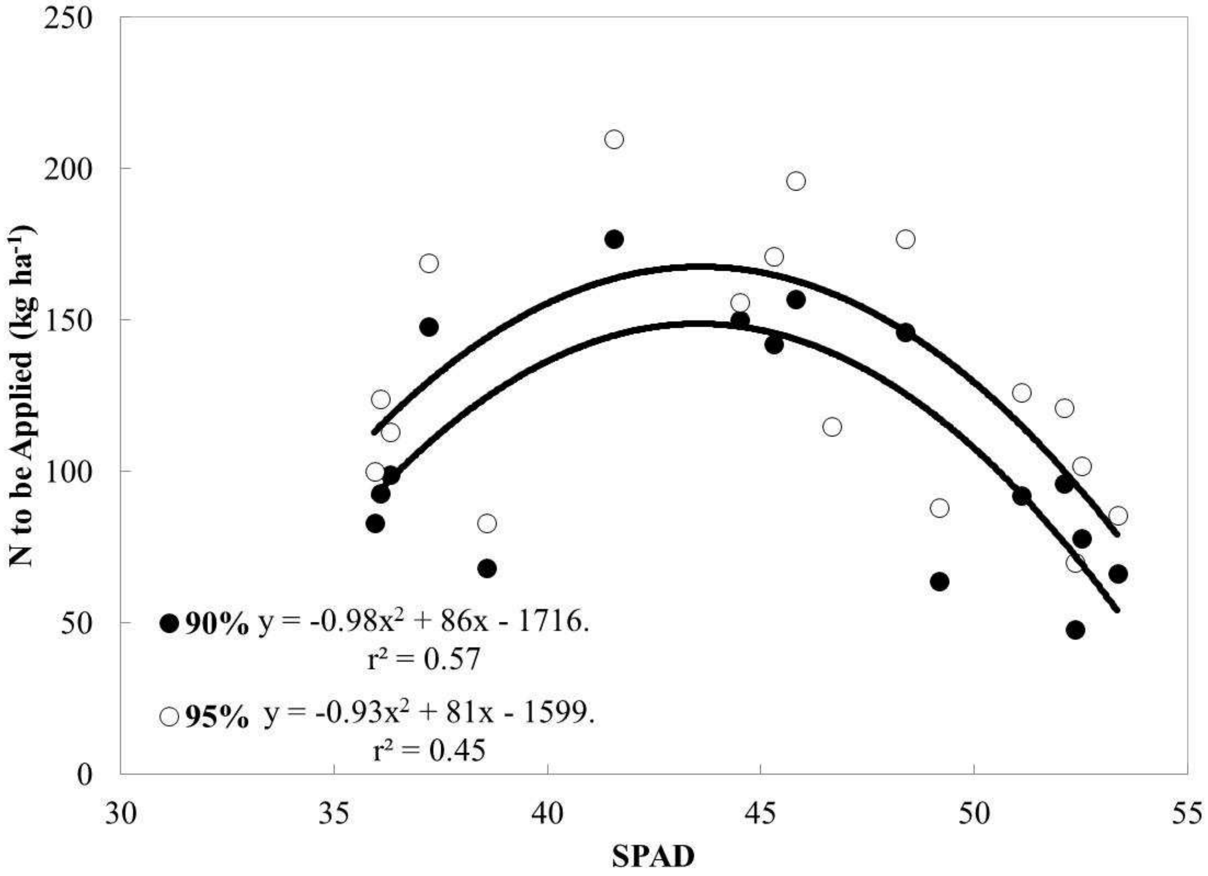


Figure 2.6. The leaf N concentration calibration curve developed from 2010 and 2011 data. Observed leaf N concentration measurements were used to determine subsequent N mid-season applications to attain 90 and 95% of theoretical maximum potential yield. This calibration curve includes data from both years of the study and is based on leaf samples taken at V6-V10. Partial data loss of leaf N samples in Marianna in 2010 account for the lower number of data points compared to the other two calibration curves.

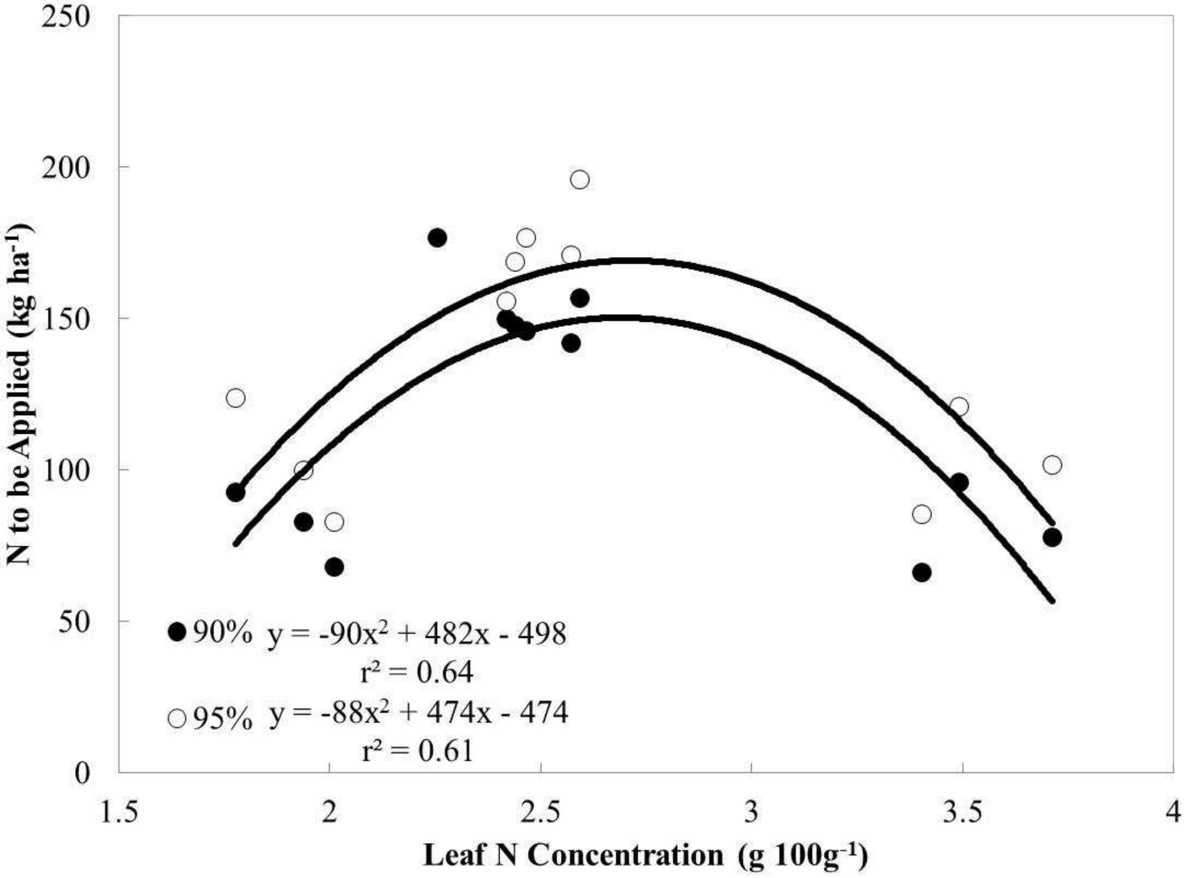
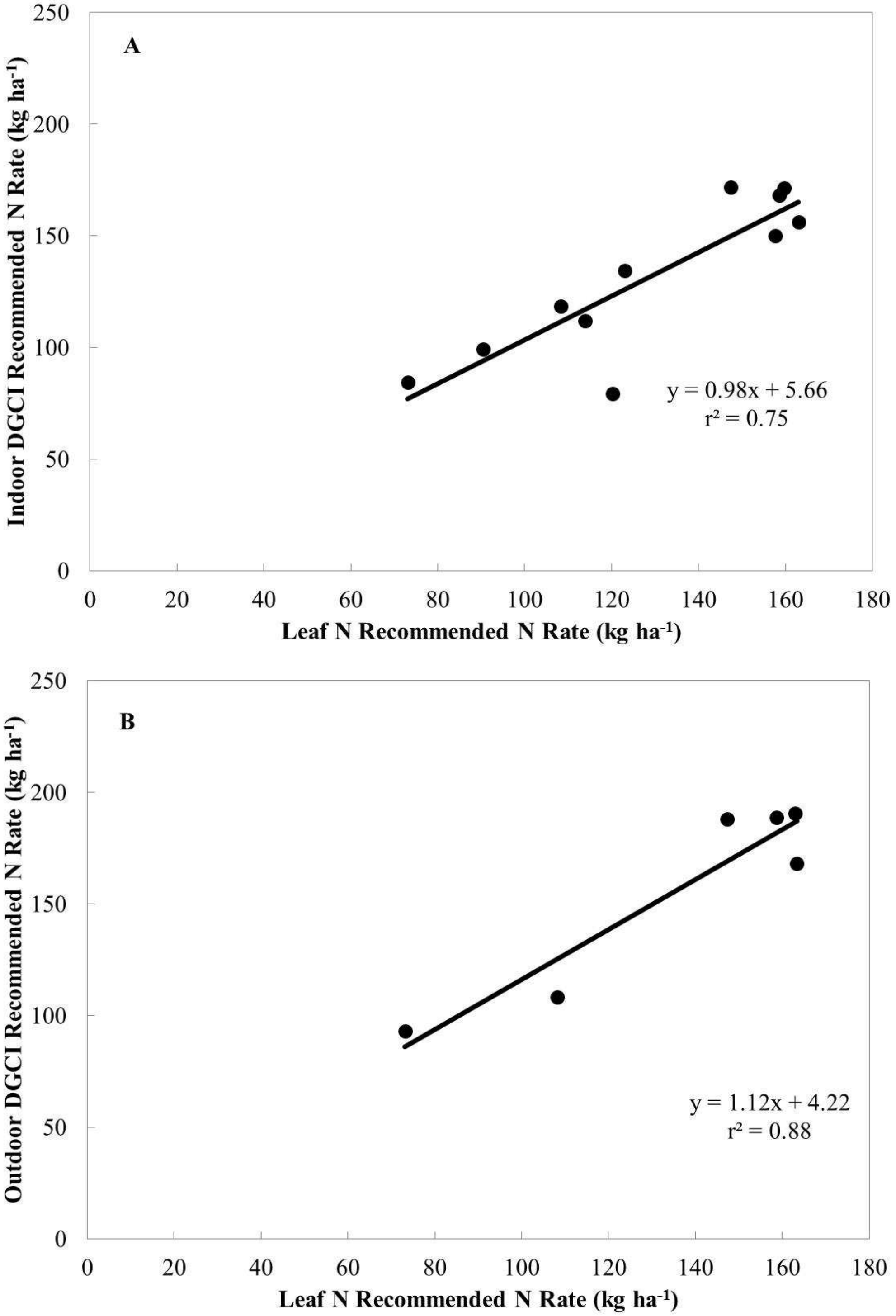


Figure 2.7. Relationships between N application recommendations made by the calibration curves (90% maximum yield) of indoor DGCI (A) and outdoor DGCI (B) with leaf N concentration.



average yield differences of 41% between the highest and lowest emergence N rates for both years.

The shape of the quadratic response and the effect that the changes in the experimental design had on it between the two study years suggest that, while yield responses to mid-season N applications may initially appear to have only slightly quadratic characteristics, this only occurs at lower rates. Sufficiently high N rates will demonstrate the overall quadratic nature of the response in corn. The quadratic model was used in this study, and indeed, increasingly higher rates would presumably eventually lead to reduced yields due to over-application (Britto and Kronzucker, 2002). From a practical standpoint, the maxima in the quadratic response curves indicate the very limit of economic return on further N applications for the producer.

Indeed, the maximum net profit will most likely occur for a producer at a point below the N rate required to attain 100% of the maximum potential yield (Black, 1993). The economics of this will of course depend on trends in N fertilizer prices, corn prices, other variables and fixed costs. The calibration curve makes these adjustments possible.

The two years of this study demonstrated strong relationships among yield, SPAD, DGCI, and leaf N concentration and thus strong potential for practical application. However, there are several reservations should be kept in mind. The study was conducted entirely within the state of Arkansas, and as such the DGCI approach was tested only under the localized climate. Although six unique soil series made up the fields used in the study, only the Fayetteville site was not located in the Mississippi and Arkansas River alluvial zones. Though not entirely lacking, none of these locations have the high levels of soil organic matter typically present in the major corn producing regions of the northern Midwest. This, coupled with the prevalence of no-till cropping systems and cooler climates in the Corn Belt, would mean a different flux of plant-available N throughout the growing season. This study was built upon corn which received two timed N applications in fields with low native N, conditions designed to highlight N deficiencies. Mineralized N from organic matter would certainly ameliorate the degree of deficiency to some extent. As discussed in the introduction, N economics are complex and vary widely across the agricultural landscape; the DGCI method appears to be an unbiased means of measuring current N content based on present, local conditions but must be tested further in that regard.

Further tests should address the DGCI method's efficacy in other locations, cropping systems, and corn hybrids. If this is indeed to become a widespread tool for evaluating corn N status, it would be important to pursue similar trials, albeit with some adjustments. For instance, the N applications in the course of this experiment were limited to two application dates and with a total of twenty-one different treatment combinations over the course of the season. This was a compromise between the time, manpower, and space available for a field experiment and the understood N metabolism in commercial corn production. As with the adjustments to experimental design undertaken between the two study years, additional evaluation of early season N rates would potentially increase the resolution of the calibration curve.

Preliminary statistical analyses for the two years of this study suggested that yield response to N is not linear. The highest yields at each location were not produced by corn receiving the highest N rates; often, mid-level applications followed by much larger ones yielded highest. This is something hinted at in the literature but can potentially be explored much more closely using the DGCI methods.

The outdoor sampling showed a great deal of promise, though three sample fields examined over one study year were not sufficient to entirely address the subject. As the outdoor sampling is a crucial axis for further, more practical applications of the DGCI method, this is an important area for refinement and confirmation.

Chapter 3

Digital Image Analysis as a Proxy for N Status in Wheat, Rice, and Turfgrass Species

ABSTRACT

Many agronomically-important crops require nitrogen fertilizer applications throughout production. Among these are rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and various turf species. Nitrogen fertilizer prices are tied to energy markets and subject to volatility; as such, timely decision-making about N applications is crucial to maintaining profitable agricultural yield. The Dark Green Color Index (DGCI) method has been used as a means of determining in-season crop N status based upon “greenness” of leaves in corn (*Zea mays* L.). Our objective in this study was to evaluate the efficacy of the DGCI method in measuring real time crop N status in several non-leguminous crops besides corn. Field experiments were conducted in 2010 and 2011 at several locations within Arkansas. Flooded rice, wheat, ‘Riviera’ Bermudagrass (*Cynodondactylon* (L.) Pers.), creeping bentgrass (*Agrostis spp.)*, and ‘Rebel Exeda’ tall fescue (*Festuca arundinacea* L.) were subjected to varying rates and timing of N fertilizer application and examined for DGCI and leaf N concentration. In wheat, measurements were also made of the normalized difference vegetation index (NDVI). NDVI (r^2 = 0.71 to 0.87) and DGCI (r^2 = 0.79) were both closely associated with leaf N concentration in winter wheat. Similar values (r^2 = 0.77) described the relationship between DGCI and leaf N concentration in bermudagrass, fescue (r^2 = 0.53), and bentgrass (r^2=0.49). No significant relationships were observed between DGCI and leaf N or NDVI and leaf N in the rice. DGCI measurements of wheat and turf may be incorporated inexpensively into a variety of production schemes to reduce time needed for crop N status diagnoses and action.

INTRODUCTION

Nitrogen plays a crucial role in metabolic processes for agronomic crops. Though the highest costs are exhibited in corn (*Zea mays* L.), N accounts for a high cost of production for all non-leguminous, agronomic crops. The costs associated with N applications must necessarily be justified by consequentially profitable yields.

Key to achieving such yields is maintaining an optimum crop N during the growing season. There is a strong correlation between biomass accumulation and critical N concentration over the growth period, which is broadly similar within major C3 and C4 cultivated species (Gastal and Lemaire, 2002). A strong relationship exists among N availability, crop growth, and yield. Crop growth and yield increase linearly with increasing N availability until an eventual plateau is reached, at which point the crop grows at the genetic potential rate (Lawlor, 2002).

Precision measurements of elemental N in leaf or soil samples are possible through the Kjeldahl-Rittenberg procedure (Barrie, 1995) or Micro-Dumas Combustion method (University of Georgia 1997). These methods are time-consuming and laborious, requiring the handling of hot acid fumes (former) or possession of expensive elemental analyzers (latter), both of which are beyond the scope of a typical agricultural operation. Even when a properly-equipped lab is nearby, there is typically a long turnaround time for sample processing, and useful data will likely arrive after a lag time unsuitable for such a time-sensitive diagnosis as crop N status. Additionally, the cost of the test combined with the number of samples that a typical producer would need to develop a useful map of field N requirements make this method expensive.

Soil N concentration measurements, whether for total soil N or soil nitrate, have been employed for a number of years to estimate crop N status. Though inexpensive, it lacks the ability to provide real time data about a given field should the need arise for an in-season evaluation. There is currently no soil test for N that is used for corn in Arkansas. However, promising research has developed a soil test for nitrogen in rice (*Oryza sativa* L.) production and may point the way towards that option in the future (Roberts, et al. 2010).

Leaf N is closely correlated with leaf chlorophyll concentration. Exploiting this relationship can provide an excellent, non-destructive approach to estimate crop N status with a short turn-around time (Costa, 2001). Typically, leaf chlorophyll concentration estimates are attained through absorbance or reflectance measurements.

Chlorophyll meters estimate leaf chlorophyll concentration based on absorbance measurements in two wavelengths (Konica Minolta, 2009). While easy to use and well-established, some studies have also shown that chlorophyll meters are ineffective tools for calculating N needs during the mid-to-late season (Bullock 1998; Zhang 2008; Zhang 2009).

Spectral radiometers measure reflectance in the visible spectrum of a crop and derive chlorophyll concentration from that. This can be especially useful for developing a N status map of a field with high data resolution (Scharf and Lory, 2009). Reflectance measurements, while useful, are somewhat hampered by a lack of current algorithms necessary for N calculations among various environmental conditions (Samborksi, 2009). Spectral radiometers have a cost starting around US$4,000.

More recently, the problems of cost, currency, and accuracy in chlorophyll concentration measurements have been addressed using digital image analysis of crops (Waksman, 1997). Pagola (2009) used this method to develop estimates of N nutrition in barley and found that the digital image measurements were on par with, and at times more accurate than, SPAD chlorophyll meters as predictors of grain yield and N deficiencies.

Digital image analysis requires a common digital camera. With it, digital images of any typical resolution can be captured. As mentioned above, leaf N concentration is closely correlated with leaf chlorophyll concentration, which in turn determines the relative greenness of a leaf. Color images are composed of three values: red, green, and blue (RGB). To account for minute variations between digital image samples, the RGB scale is converted to one measuring hue, saturation and brightness (HSB) (Karcher and Richardson, 2003). These three HSB values are then weighted and converted into a single dark green color index (DGCI) value for further analysis and indexing.

DGCI values derived from digital images of a crop can be used to produce an index for the purposes of quickly and simply estimating leaf N concentration. Rorie et al. (2011) demonstrated the relative abilities of both the chlorophyll (SPAD) meter and the digital imaging (DGCI) methods of measuring total leaf N concentration; both methods produced comparable correlations between leaf N concentration estimates and measurements determined by tissue sample analysis.

Previous research conducted at the University of Arkansas (Rorie et al., 2011.) further refined digital image analysis with regards to accurate measurements of agronomic crop leaf N concentrations. Across two years, five field locations with differing soil types were planted with commercial corn hybrids. The fields were subjected to various rates of N fertilizer application and crop N status was evaluated. At silking, photographs were taken of the entire corn leaf on a bright pink, felt cloth to provide greater contrast between leaves and background for ensuing image analysis. SigmaScan Pro 5 (SyStat Software Inc., San Jose, CA) software was used to quantify greenness and determine DGCI (Karcher and Richardson, 2003). In Rorie’s work, the yield and DGCI values were expressed as a fraction of the treatment receiving the highest amount of N fertilizer for each field. The resultant relative yield and relative DGCI could then be compared to each other with a single function. Inherent variations between sampling environments can be accounted for by including internal color standards in each digital image and sampling a small portion of the field which has been fertilized with a non-limiting rate of N to provide a benchmark.

The previous chapter of this thesis presented 2 years of additional research conducted at the University of Arkansas which used the DGCI method to both diagnose and correct N deficiencies in corn. Strong connections were shown between DGCI measurements made both indoors and outdoors at the V5-V10 stages in corn with the current leaf N concentration and subsequent yield of those crops. These observations were then used to develop calibration curves based on mid-season DGCI measurements that would allow producers to make N applications to maximize potential yield. This is an important step towards the ultimate practical application of the DGCI method for use in corn.

DGCI METHOD IN OTHER CROPS

Wheat (*Triticum aestivum* L.) is an important crop both worldwide and in the US, where some 20 million ha were under cultivation in 2009 (OSU, 2009). Sufficient N fertilization is critical to wheat production. Justes (1994) estimated that a constant critical shoot N concentration of 4.4% for the stages of development from Feekes 3 to Feekes 5 is necessary for ensuring sufficient crop biomass accumulation and yield. During active growth, a plant such as wheat needs less and less N to build each new unit of dry matter (Salette and Lemaire, 1981). However, as with any cropping situation, numerous other factors create a complex situation of N supply and demand throughout the growing season. Understanding and quantifying these changes is necessary to maintaining appropriate N levels in the wheat.

Leaf chlorophyll and N concentration in the leaf dry matter are indicators for current crop N nutrition (Hansen and Schjoerring, 2003). Furthermore, “the spatial and temporal variations in the field of these variables must be determined in order to match the crop requirements as closely as possible” (Hansen and Schjoerring, 2003). Cartelat et al. (2005) found a significant correlation (r^2=0.97) between chlorophyll concentration and N concentration in wheat.

The chlorophyll concentration of a corn leaf can be estimated spectrally by several methods. This holds equally true for wheat. Normalized difference vegetation index (NDVI) studies have shown that N fertilization of wheat altered the levels of light reflected from the plant leaf in the infrared and visible spectrum as a result of changing amounts of chlorophyll, thus allowing a method for N-stressed crops to be monitored (Hinzman et al., 1986). However, Chen et al. (2010) used a spectral indexing method for predicting plant N concentration, and found a much stronger relationship for corn (r^2= 0.72) than for wheat (r^2= 0.44). The same study also found significant correlations between SPAD and leaf N concentration for corn, wheat and the combined database (Chen et al., 2010).

It stands to reason that, given the similar connection between chlorophyll concentration and leaf N concentration in both corn and wheat, spectral assessment methods used for one could be applied to the other. This has held true for chlorophyll meters and spectral radiometers in previous studies, and could potentially be true for the DGCI method. As with corn, wheat is an important worldwide crop with relatively high N requirement; any tool to increase nutrient use efficiency is important.

Turfgrass also requires the maintenance of a critical leaf N concentration, estimated to be near 4.41% (Jarvis, 1987), similar to that of wheat in the early growth stages. Previous studies had shown that leaf color analysis using a colorimeter could be used to detect significant color differences in bentgrass (*Agrostis spp.*; Landschoot and Mancino, 2000). Seminal work in image analysis and development of the DGCI method has been done at the University of Arkansas with turfgrass (Karcher and Richardson, 2003). Since turf color is an important indicator of crop N nutrient status, digital images might be used to quantify N status. The need for developing this method is double, as visual examinations are highly subjective and exhibit relatively low correlations with N status (Karcher and Richardson, 2003). Karcher and Richardson (2003) conducted an examination of variable rate N fertility applications to zoysiagrass (*Zoysia spp.*) and bentgrass, and found that DGCI values were significantly affected by the N rate, thus they speculated that "color measurement using digital image analysis may be capable of assessing the N status of plant tissues."

Rice covers more agricultural land worldwide than corn (Oklahoma State University), and production in Arkansas accounts for nearly half of US production (Arkansas Rice Federation, 2012). Nitrogen is an important input for rice and there is a need to know the current N status and demands of the crop. Merely relying on "rule of thumb" average N applications across many locations results in over-application and a loss of economically optimum production (Watkins, 2010). Proper application amount and timing is crucial to maintain adequate N status in rice; improper application can lead to lodging, and may attract insect pests and diseases (Peng et al., 2010). Rice is a very large nutritional staple in much of the developing world (IRRI, 2012). Methods for increasing N use efficiency, especially in the developing world, must be inexpensive and widely available for farmers to adapt them (Islam, 2007).

Leaf color intensity of rice is directly related to leaf chlorophyll content and leaf N concentration (CREMNET, 1998).Therefore, various methods and tools may be utilized to estimate leaf N concentration through color analysis.

Flooding is an important aspect of rice production and requires close N management. When water is scarce, alternating wet and dry cycles may be used. For both of these methods, SPAD (r^2 = 0.61 to 0.74) and leaf color charts (r^2= 0.70) have been successfully used to estimate current leaf N concentration (Cabangon et al., 2011).

Wheat, rice, and turf are widely cultivated and receive high rates of N fertilizer. An increase in N use efficiency (NUE) would have an economically and environmentally positive effect. The means to an increase in NUE rely on an accurate, timely method for measuring current crop N status. All the better if this method is available easily and at little cost to producers. Previous research has demonstrated the feasibility of using leaf color analysis to attain measurements of leaf N concentration in all three crops. Our objectives in this study were to evaluate the ability of the DGCI method to predict leaf N status in non-leguminous crops other than corn, namely (1) wheat, (2) rice, and (3) turf.

MATERIALS AND METHODS

During 2011, the DGCI method for determining in-season N status was evaluated for efficacy in wheat, rice, and turfgrass. Methodology was similar for all crops, any differences that occurred were minor and in the realm of sampling technique, the approach to which often needed adjustment dependent on the crop in question.

Wheat

A field study was conducted to evaluate the relationship between leaf N concentration, NDVI, and DGCI values for soft red winter wheat. Delta King 9577 (Armor Seeds LLC, Jonesboro, AR) was planted 20 October 2010 at the Arkansas Agricultural Research Extension Center in Fayetteville, AR. Soil was a Captina silt loam (fine-silty, siliceous, active, Mesic Typic Fragiudults). Plots consisted of six rows, 25cm apart and 3m long.

Soil nutrient amendments were applied according to University of Arkansas Extension recommendations with the exception of N. Nitrogen treatments were applied at rates of 0, 45, 90, 135, 180, or 225 kg ha^{-1}, either as pre-emergence, post-emergence, or an even split between the two, all prior to sampling. Nitrogen was applied in the form of urea (46-0-0) prills treated with dicyandiamide and N-(n-butyl) thiophosphotictriamide (Agrotain®AGROTAIN International, St. Louis, MO). Experimental design was a randomized complete block with four replications.

Wheat was sampled twice, once at the Feekes 2-3 (Simmons et al., 1995), and again at approximately the Feekes 5 stage. The DGCI sampling was similar to the method described for corn in chapter two: during midday, digital images were taken overhead of each entire plot. Included in each image was a prepared 0.5m x1m plywood board that supplied the necessary internal color standard disks (Rorie et al., 2011). NDVI measurements were made on the same plots using a GreenSeeker® 505 Handheld Sensor (Trimble Navigation Ltd., Westminister, CO). The sensor was set to take consecutive measurements by holding the sensor 0.5m above the crop canopy in the center of the plot and walking at a steady pace for the length of the plot. Finally, ten representative upper-most leaf samples were cut from each plot, dried, and analyzed for total N concentration with a LECO FP428 Nitrogen Analyzer (LECO Corporation, St. Joseph, MI) by the Agricultural Diagnostic Laboratory (University of Arkansas, Fayetteville).

Rice

Two commercial rice varieties, Wells (University of Arkansas) and XL 723 (RiceTec Inc, Alvin, TX) were planted on 14 April 2011 at the Rohwer Research Station in Rohwer, AR. Soil was a Herbert silt loam (fine-silty, mixed, superactive, Mesic Udollicepiaqualfs). Plots were 5m in length and 2m wide with 4 rows 0.2m apart.

Soil nutrient amendments and irrigation were applied according to University of Arkansas extension recommendations with the exception of N, which was applied pre-flood at rates of 0, 50, 67, 84, 101, 118, 134, 146, 168, 174, 202, 207, or 235 kg ha^{-1}.

Samples were collected at late vegetative stages. DGCI sampling and NDVI measurements were similar to the wheat experiment described previously. Representative leaf samples were taken from each plot and analyzed for N concentration.

Turf

Commercial varieties of creeping bentgrass (variety G-2), ‘Riviera’ Bermudagrass (*Cynodon dactylon* (L.) Pers.), and ‘Rebel Exeda’ tall fescue (*Festuca arundinacea* L.) were examined to evaluate the relationship between leaf N concentration and DGCI in Fayetteville, AR. Soil was a Captina silt loam (fine-silty, siliceous, active, Mesic Typic Fragiudults).

Soil nutrient amendments and irrigation were applied according to University of Arkansas extension recommendations with the exception of N, which was applied at rates of 0, 25, 50, 75, or 100 kg ha^{-1}. The bermudagrass observed was part of a larger study that evaluated an adjuvant, NutriLife® (NLAF, Advanced Microbial Solutions, Pilot Point, TX), which was applied at three rates of 0, 4.2 (1.0x), and 6.3 (1.5x) ml kg^{-1}with the N fertilizer.

Sampling occurred 7 to 10 days after fertilizer application. DGCI data were collected by taking digital images as described previously. Leaf samples were taken concurrently and analyzed for total N concentration.

RESULTS

Wheat

Data were comparable between the two sampling dates with regards to leaf N, DGCI, and NDVI relationships. At the first sampling, Feekes 2-3 growth stage, there were good relationships between both NDVI and leaf N concentration (r^2 = 0.88, $p<0.0001$, Fig. 3.1a) and DGCI (r^2 = 0.74, $p<0.0001$, Fig. 3.2a) with leaf N concentration. For both groups of measurements taken on this first date, the data fit to a segmented linear regression with a breakpoint between 4.1 and 4.4g N 100g^{-1}. Similar observations were made on the second sampling date (Fig. 3.1b & 3.2b), though the data did not warrant a segmented regression. Though the measurements for the first sampling date showed a more narrow range of leaf N concentration, DGCI, and NDVI, the segmented regression suggests N stress differentiation may have resulted from different factors between sample dates. The DGCI and NDVI measurements were closely associated with each other, increasing in strength from the first to second sampling dates (Fig. 3.3a&b).

Turf

Measurements were conducted on a small number of samples of tall fescue (n=11) and bentgrass (n=11). Measurements of DGCI were made twice on a group of tall fescue plots, once before and once after mowing (Fig. 3.4). Leaf clippings from the mowing were immediately submitted for total N analysis. Covariate analysis revealed no significant differences in slope or intercept between the two sample groups (mowed and unmowed), which together had an r^2 of 0.53 ($p<0.0001$).

There was little difference in N concentration of bentgrass samples, with a range from 4.9 to 5.7 g $100g^{-1}$. As with tall fescue, there was a narrow range of DGCI, from 0.54 to 0.57. Nevertheless, there was a significant relationship (r^2 of 0.49, $p<0.0001$) between DGCI and leaf N concentration (Fig. 3.5).

As the bermudagrass was fertilized with an adjuvant, NLAF, the response of leaf N concentration and DGCI among the three NLAF treatments was examined using covariate analysis. No significant treatment effect was observed for NLAF application rates with regards to the leaf N-concentration response to N application rates (Appendix Table 7).Therefore, leaf N concentration response to amount of N fertilizer applied had similar intercepts and slopes regardless of the NLAF adjuvant (R^2 of 0.68, $p<0.0001$, Fig 3.6).

Covariate analysis indicated that the response of DGCI to the amount of N applied had similar slopes for the NLAF treatments but different intercepts (R^2 of 0.84, $p<0.0001$, Fig. 3.7, Appendix Table 8).The highest rate of NLAF (1.5x) resulted in higher DGCI values than the

Figure 3.1 The relationship between NDVI and leaf N concentration in winter wheat in 2011 at the Fayetteville site. Measurements are from (A) 17 Mar and (B) 6 Apr 2011.

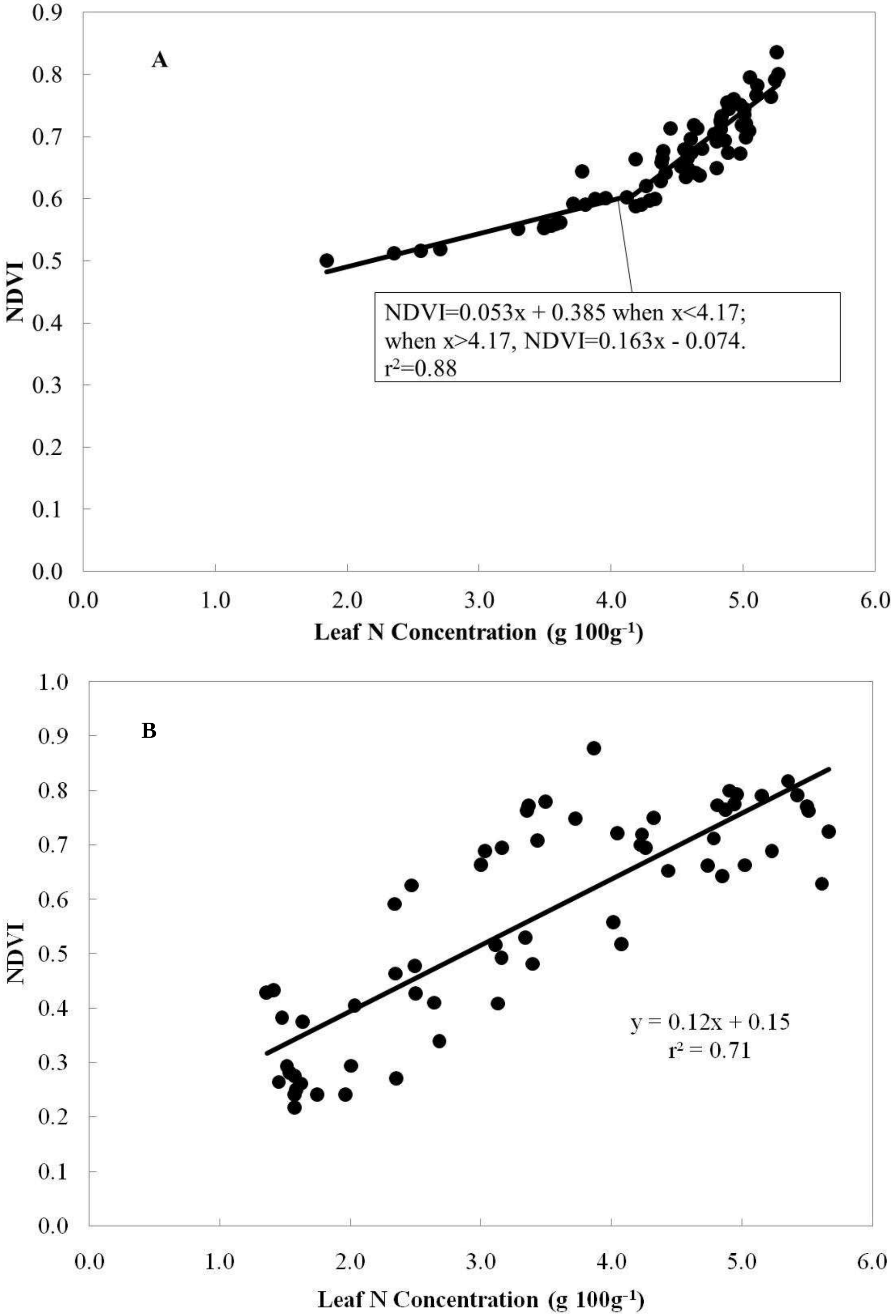


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Figure 3.2 The relationship between DGCI and leaf N concentration in winter wheat in 2011 at the Fayetteville site. Measurements are from (A) the first sampling date, 17 Mar and (B) 6 Apr 2011.

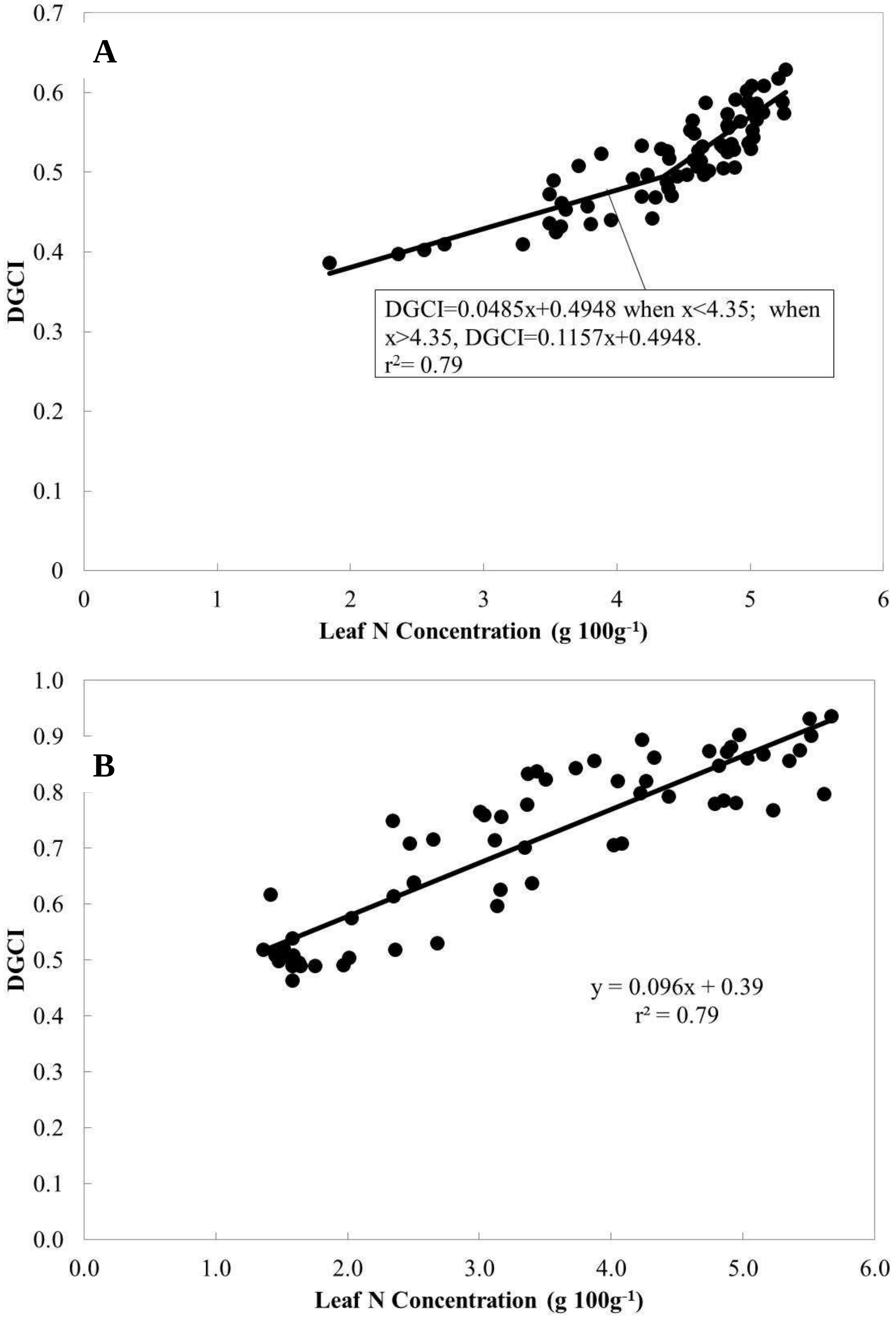


Figure 3.3 The relationship between DGCI and NDVI in winter wheat in 2011 at the Fayetteville site on 17 Mar (A) and 6 April 2011 (B).

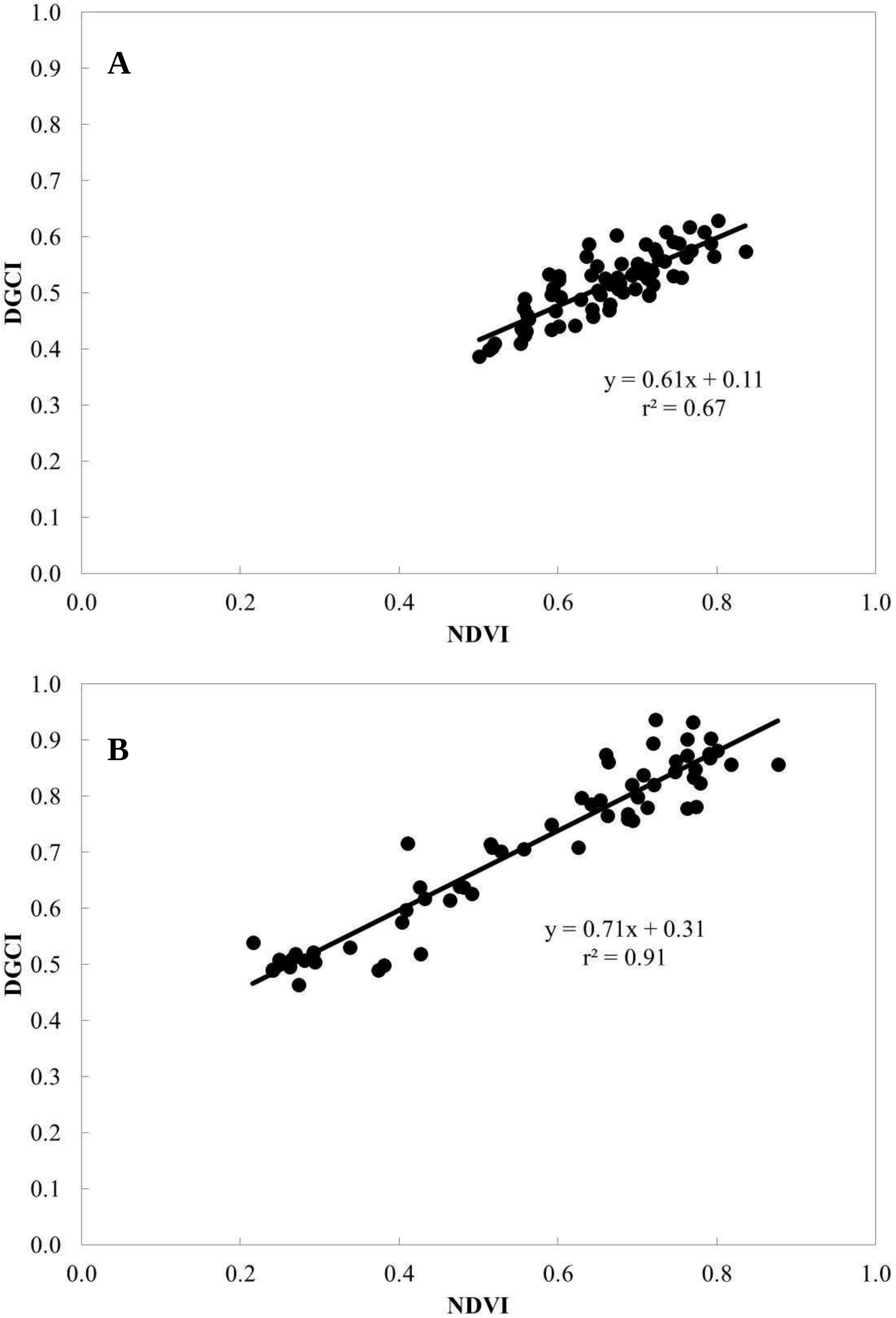
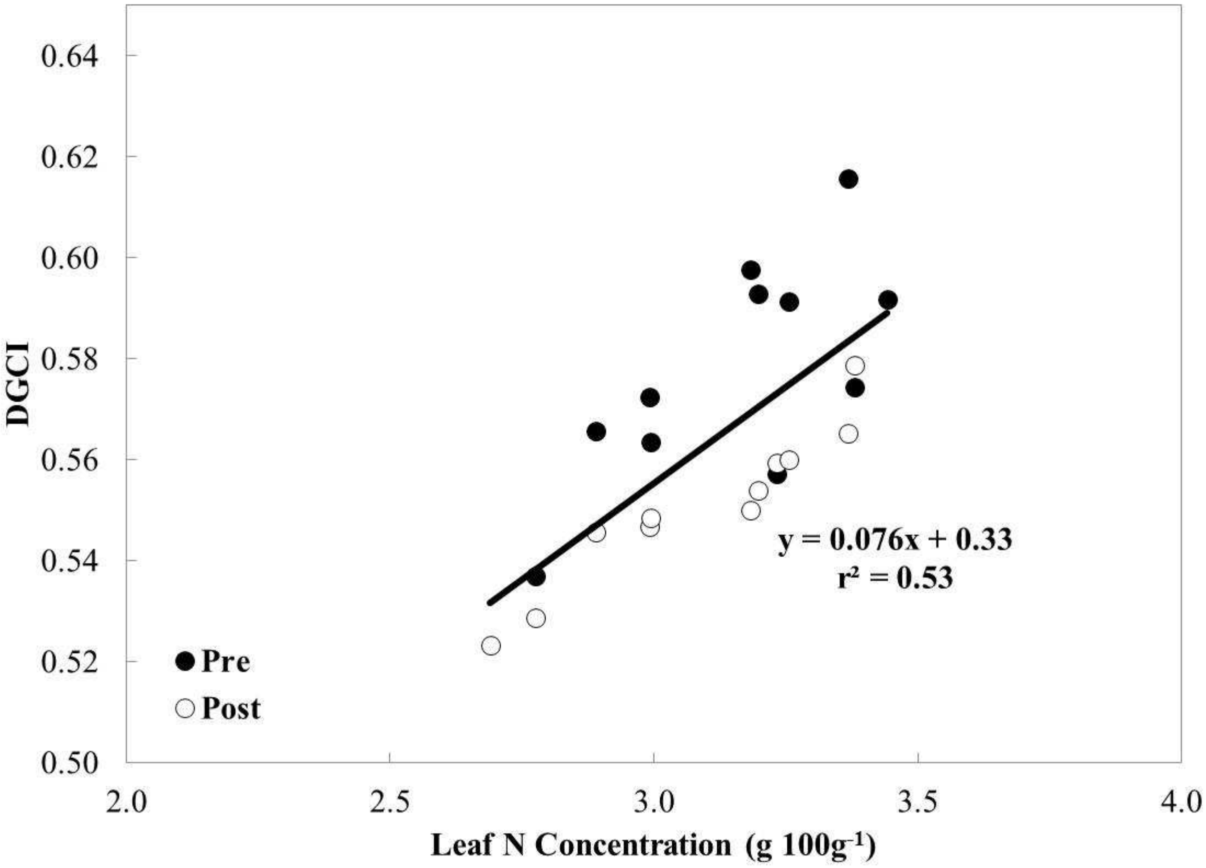


Figure 3.4 Relationship between leaf N concentration and DGCI from samples of tall fescue taken both pre- and post-mowing. Covariate analysis revealed no significant differences between the two sample groups.



control and 1.0x treatment. Covariate analysis revealed no significant effects of the three NLAF rates on the DGCI response to leaf N concentration (Fig. 3.8, Appendix Table 9). That is, there was a linear increase in DGCI as leaf N increased, regardless of NLAF treatment (R^2 of 0.77, $p<0.0001$).

Rice

The relationship between DGCI and leaf N concentration was weak for both rice varieties. Wells suggested a slight positive relationship (r^2 of 0.03, $p>0.05$, Fig. 3.9). The XL723 hybrid showed a negative relationship between leaf N concentration and DGCI values (r^2 of 0.11, $p>0.05$, Fig. 3.9). However, neither of these relationships were significant ($p\leq0.05$).

Figure 3.5 Relationship between leaf N concentration and DGCI values for bentgrass plots in 2011 at the Fayetteville site.

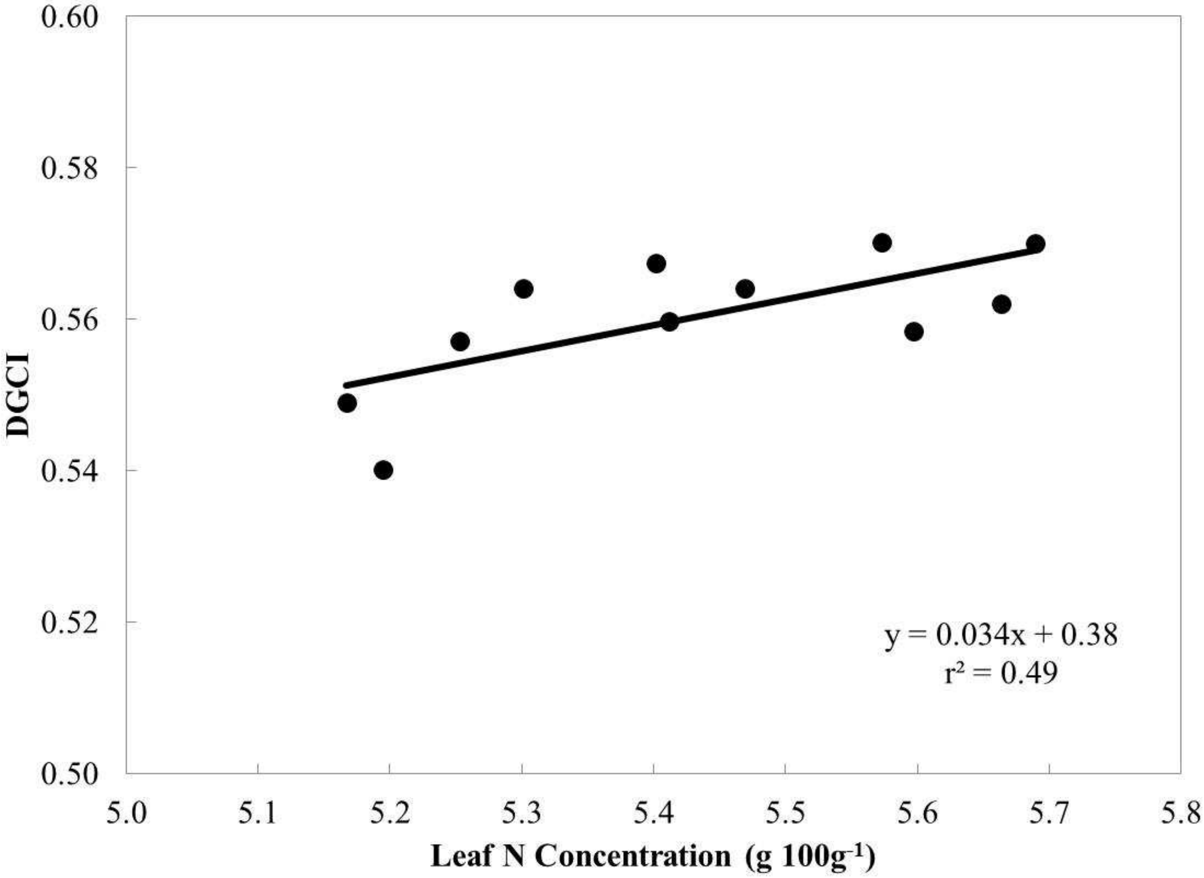


Figure 3.6 Relationship between leaf N concentration and amount of N applied to Bermudagrass in Fayetteville, 2011. Applications were made with three different levels of NutriLife (soil amendment containing 3% N and several strains of *Bacillus* bacteria intended to catalyze N uptake), though covariate analysis found no significant difference between NLAF rates. Data points are averages for all replications of each N rate.

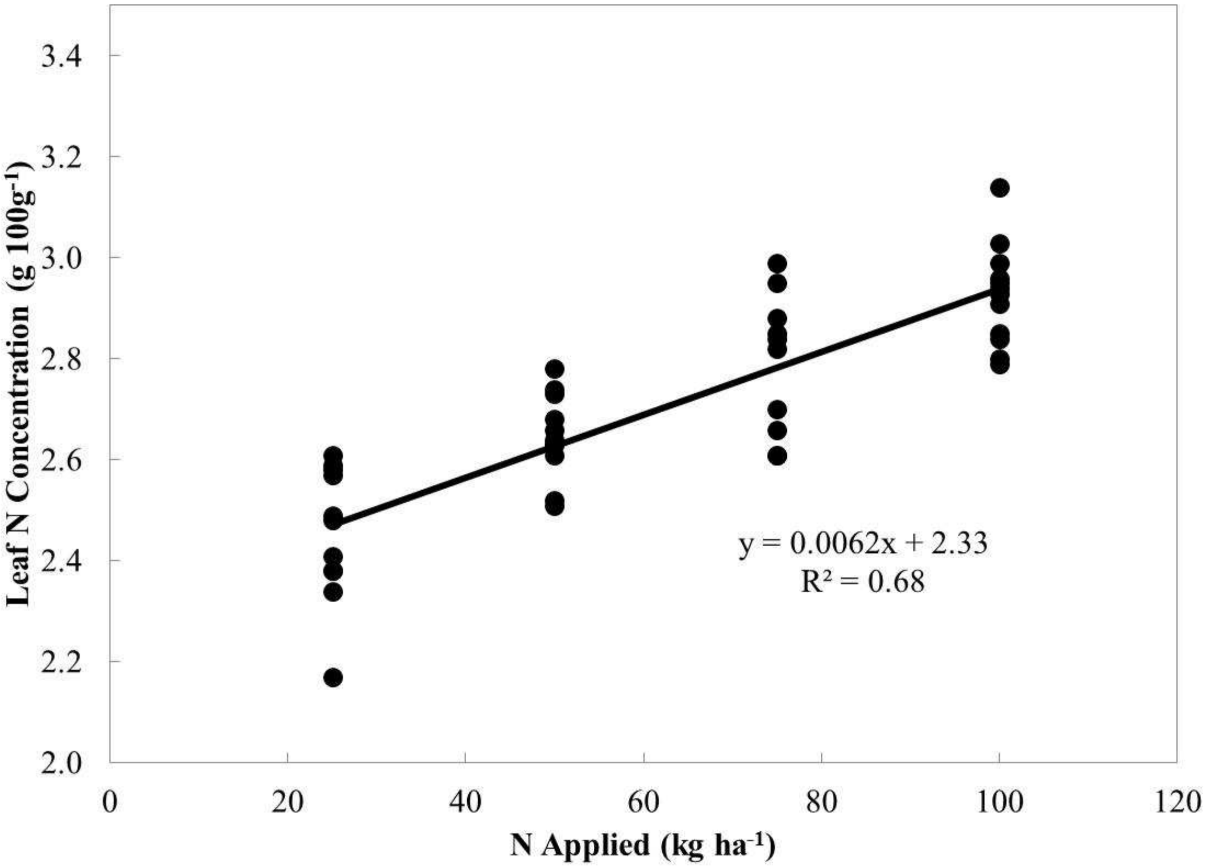


Figure 3.7 Relationship between DGCI measurements and amount of N applied to bermudagrass in Fayetteville in 2011.Treatment blocks were initially divided among those receiving various levels of NutriLife (a soil amendment containing 3% N and several strains of *Bacillus* bacteria intended to catalyze N uptake). Covariate analysis revealed responses with differing intercepts but a common slope. Data points are average values for each N rate within a treatment block.

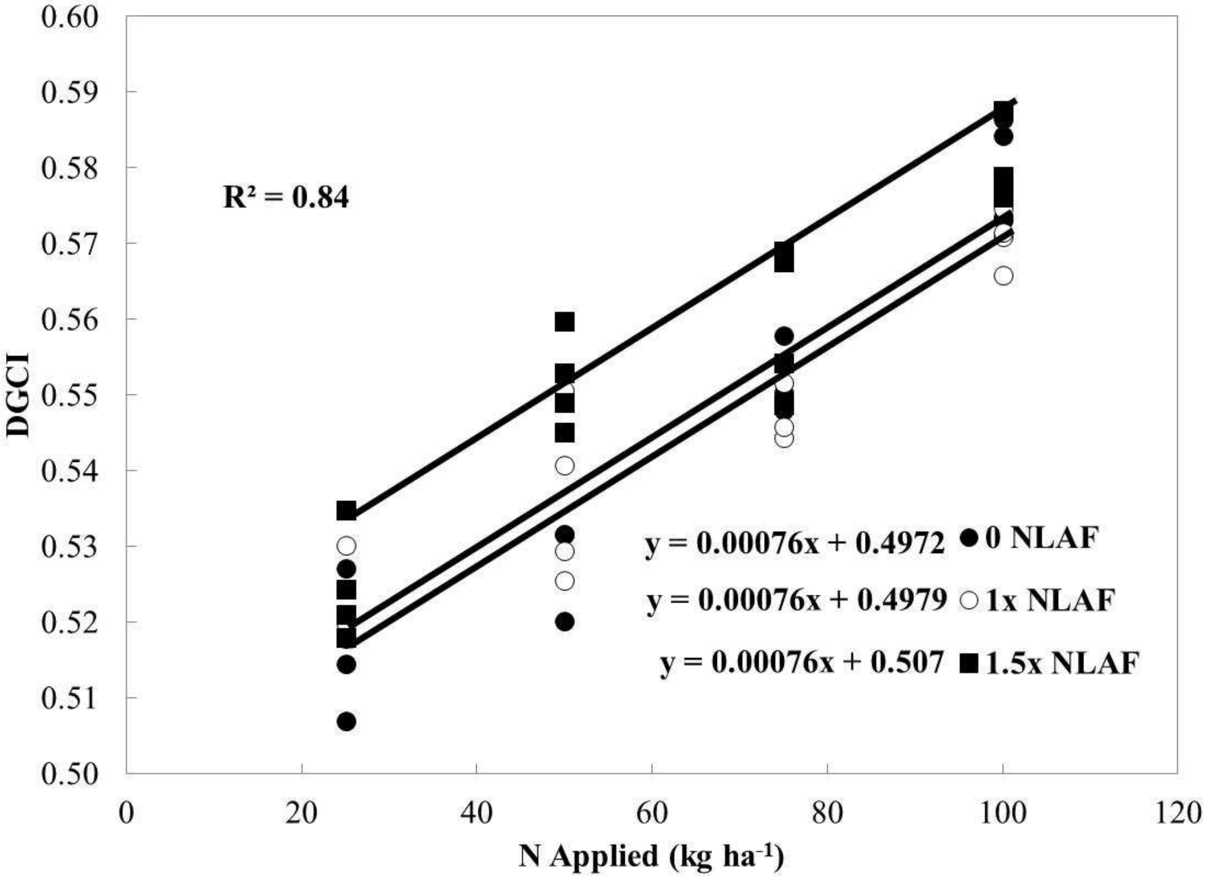


Figure 3.8 Relationship between DGCI measurements and leaf N concentration in bermudagrass from field experiments in Fayetteville in 2011. Treatment blocks were initially divided among those receiving various levels of NutriLife (a soil amendment containing 3% N and several strains of *Bacillus* bacteria intended to catalyze N uptake), though covariate analysis found no significant difference between NLAF treatment levels.

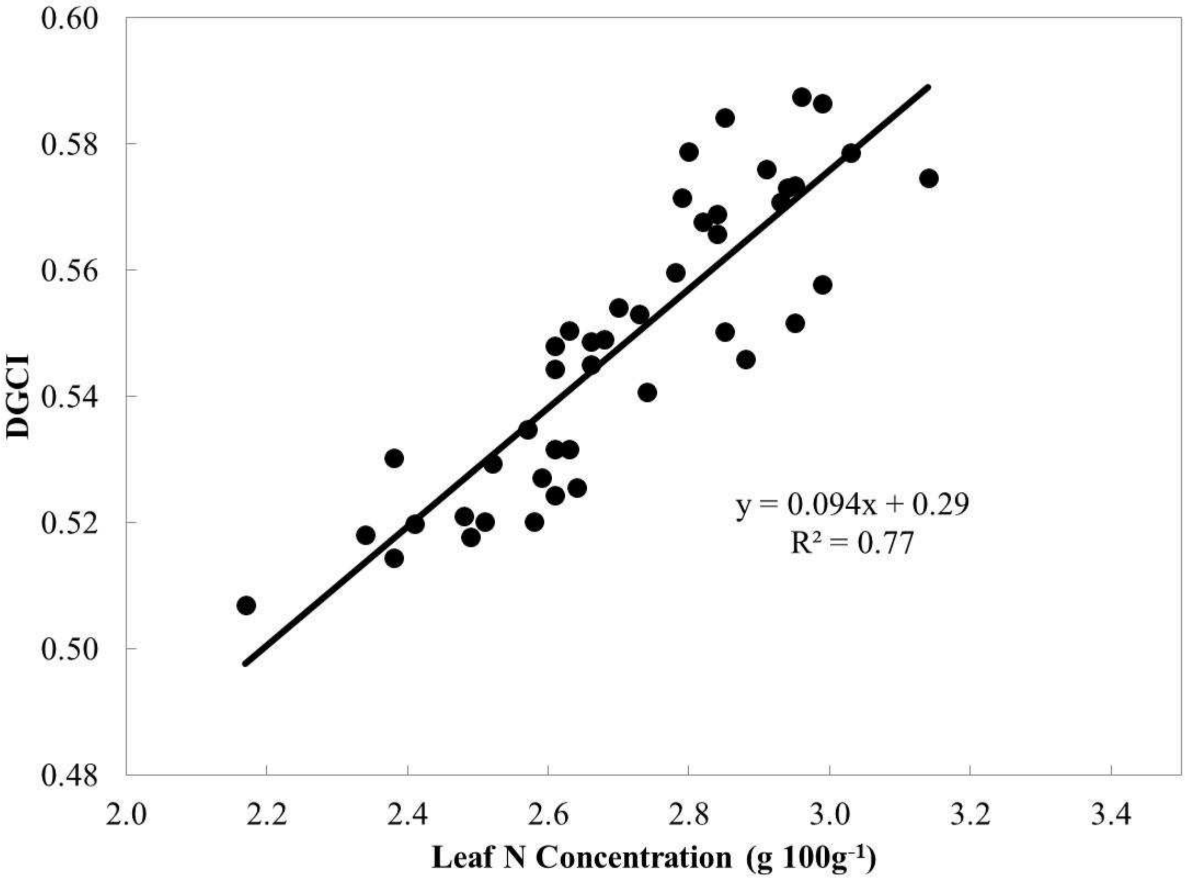
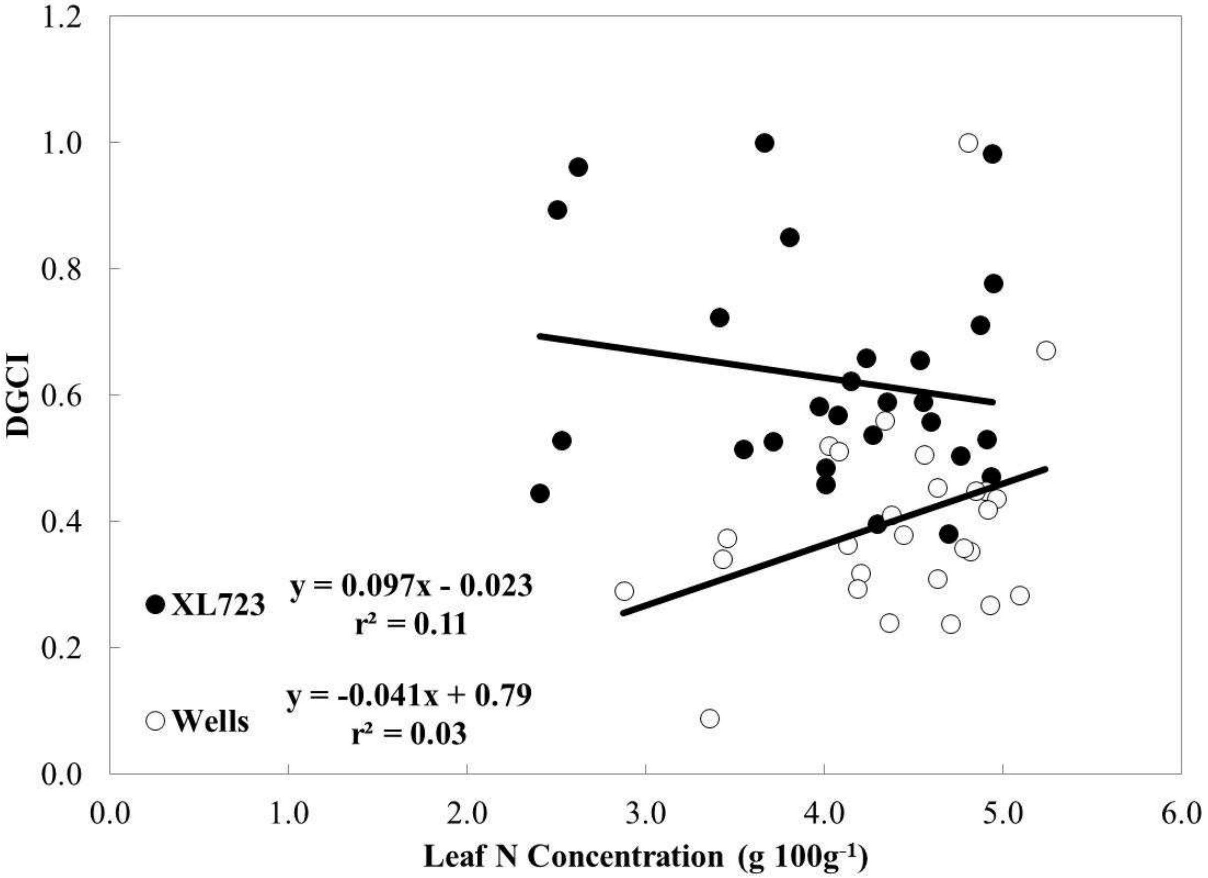


Figure 3.9 DGCI vs Leaf N concentration in rice cultivars Wells and XL723 at Rohwer in 2011. No significant relationships were observed between DGCI and leaf N concentration for either cultivar.



The wheat and turf species evaluated had strong relationships between DGCI values and leaf N concentration, similar to that observed in corn. In the case of wheat, the DGCI method compared favorably to concurrent NDVI measurements. DGCI and NDVI also had a strong relationship with each other ($r^2 = 0.91$, Fig. 3.3b) at the later sampling date, suggesting that the methods are effective in similar situations, such as when canopy coverage is greater at mid-vegetative states. Both rice cultivars examined demonstrated very poor relationships regarding the same two measurements.

These results highlight a chief limitation encountered in DGCI method during the course of this study: sampling. The digital images that capture the raw data and act as a stepping stone for further DGCI analysis are comprised of two parts. One component is pixels that are representative of the leaf tissue to be analyzed under current lighting conditions. A second component of DGCI processing is pixels that capture various sources of noise with regards to the data under analysis. This can take the form of weeds, extreme light gradients on the leaf, non-leafy plant tissue, dust on the leaf, and numerous other sources. Noise of this type will always occur in the initial sampling, and indeed is anticipated in the method. Post-sampling processing begins with image analysis that filters all but the desired yellow-to-green spectra which would encompass the internal standards and the possible ranges of the leaf tissue. This filtering greatly improves the signal-to-noise ratio of the image which will be used for further data analysis. However, some particular situations confound this filtering to different degrees, resulting in a lower final signal-to-noise ratio. This was the case with DGCI readings taken near sunrise or sunset, when incident light on the color standards was at such an angle that the recorded values were inaccurate. The lower this ratio, the less reliable the ultimate DGCI value.

Wheat and turf were especially amenable to the DGCI method and can generally be expected to have high signal-to-noise ratios. This is because they can easily be photographed from overhead and offer dense, uniform leaf canopies representative of their current nitrogen status. Even comparisons of turfgrass canopies before and after mowing showed no significant difference, thus demonstrating the robustness of measurements made at favorable perspectives.

Rice, especially when flooded, can prove more problematic. The standing water in the paddies reflects the rice leaf above it, albeit in slightly muted and distorted tones. The reflected glare off of the water also creates less uniform lighting patterns on the leaf. Finally, in this particular study, measurements were made near sunset, creating a lighting situation that gave rise to extreme gradients across paddies and individual plants. All of these issues created a sampling environment that was less than ideal for achieving a high signal-to-noise ratio in the digital image captured. However, as measurements on rice were only made outdoors under less-than-ideal lighting conditions, it cannot be stated that rice itself is unsuitable to the DGCI method, rather only that this particular approach is not appropriate.

Issues with rice sampling aside, the results of sampling wheat and turfgrass are promising with regards to the DGCI method. The strong relationships between DGCI values and current crop N status suggest possibilities for cost-effective and timely in-season measurements. Further research into this area must include study-specific fields that examine a wide variety of N treatments at various growth stages, as was conducted in the corn study discussed in the previous chapter. As mentioned, rice may be a suitable candidate for the method as well, and to discern whether or not this is the case, an experimental design geared towards testing this is needed. The core of the DGCI method—the correlation between N and greenness—suggests ease of applicability to numerous other crops and this should be examined.

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Appendix

Appendix Table 1 Analysis of variance for corn yield 2010.

Source	DF	MS	F Ratio	Prob>F	R^2
Fayetteville					
Model	20	14532264	9.69	<.0001	0.79
Error	50	1500232			
C. Total	70				
		SS			
Rep	3	4580508	1.02	0.3927	
N Emg†	2	10377278	34.5	<.0001	
N Mid	5	15115218	20.1	<.0001	
Emg*Mid	10	31139815	2.08	0.0445	
Keiser					
Model	23	33411579	22.92	<.0001	0.89
Error	60	1457825			
C. Total	83				
		SS			
Rep	3	8287903	1.9	0.1401	
N Emg	2	515269450	176.7	<.0001	
N Mid	6	192514058	22.01	<.0001	
Emg*Mid	12	52394923	3.00	0.0025	
Marianna					
Model	20	13471888	2.46	0.0050	0. 49
Error	51	5473624			
C. Total	71				
		SS			
Rep	3	14663209	0.89	0.4512	
N Emg	2	18521949	1.69	0.1943	
N Mid	5	133579945	4.88	0.0010	
Emg*Mid	10	102672666	1.88	0.0707	
Stuttgart					
Model	20	10107037	11.22	<.0001	0. 81
Error	51	900645			
C. Total	71				
		SS			
Rep	3	4570657	1.69	0.1804	
N Emg	2	144336548	80.13	<.0001	
N Mid	5	25521374	5.67	0.0003	
Emg*Mid	10	27712176	3.08	0.0039	

†N Emg and N Mid refer to the N applications at emergence and mid-season, respectively.

Appendix Table 2 Grain N concentrations for all locations 2010.

		2010		
		Emergence N Application, kg ha^{-1}		
Location	V6-V10 N App., kg ha^{-1}	0	84	168
			%	
Fayetteville	0	1.15 fed†	1.10 f	1.13 fed
	28	1.14 fed	1.14 fed	1.16 fecd
	56	1.11 fe	1.19 becd	1.20 bcd
	84	1.21 bcd	1.21 bcd	1.34 a
	112	1.26 ba	1.26 ba	1.24 bc
	168	1.33 a	1.33 a	1.33 a
Marianna	0	1.42 c	1.40 c	1.50 bc
	28	1.42 c	1.40 c	1.43 c
	56	1.49 bc	1.57 bac	1.51 bac
	84	1.46 bc	1.56 bac	1.56 bac
	112	1.54 bac	1.55 bac	1.57 bac
	168	1.66 ba	1.69 a	1.64 ba
Stuttgart	0	1.41 ba	1.21 c	1.27 bc
	28	1.19 c	1.29 bc	1.40 ba
	56	1.24 bc	1.25 bc	1.40 ba
	84	1.21 c	1.31 bac	1.47 a
	112	1.39 ba	1.39 ba	1.48 a
	168	1.32 bac	1.47 a	1.46 a
Keiser	0	1.40 ba	1.28 fged	1.21 g
	28	1.34 bedc	1.26 fge	1.21 g
	56	1.35 bdc	1.30 fgedc	1.27 fged
	84	1.31 fbedc	1.25 fg	1.30 fgedc
	112	1.32 fbedc	1.25 fg	1.27 fged
	168	1.40 ba	1.36 bdc	1.38 bc
	224	1.46 a	1.39 bac	1.35 bdc

† Means with the same letter within a location are not significantly different ($p<0.001$).

Appendix Table 3 Analysis of variance for corn nitrogen recovery 2010.

Source	DF	MS	F Ratio	Prob>F	R^2
Fayetteville					
Model	19	687.14	4.87	<.0001	0.66
Error	47	141.19			
C. Total	66				
		SS			
Rep	3	433.1	1.02	0.3912	
N Emg†	2	9322.4	33.01	<.0001	
N Mid	5	1211.4	1.72	0.1494	
Emg*Mid	9	2088.6	1.64	0.1304	
Keiser					
Model	22	601.5	4.61	<.0001	0.68
Error	47	130.5			
C. Total	69				
		SS			
Rep	3	793.9	2.03	0.1227	
N Emg	2	923.7	3.54	0.0370	
N Mid	6	6743.3	8.61	<.0001	
Emg*Mid	11	4772.2	3.33	0.0019	
Marianna					
Model	19	4191.9	2.20	0.0140	0.47
Error	48	1902.2			
C. Total	67				
		SS			
Rep	3	3716.5	0.65	0.5861	
N Emg	2	42153.9	11.08	0.0001	
N Mid	5	7987.3	0.84	0.5282	
Emg*Mid	9	25788.5	1.51	0.1729	
Stuttgart					
Model	19	227.3	1.85	0.0436	0.42
Error	48	122.8			
C. Total	67				
		SS			
Rep	3	291.1	0.79	0.5054	
N Emg	2	1789.3	7.28	0.0017	
N Mid	5	425.4	0.69	0.6315	
Emg*Mid	9	1812.7	1.64	0.1308	

†N Emg and N Mid refer to the N applications at emergence and mid-season, respectively.

Appendix Table 4 Analysis of variance for corn yield 2011.

Source	DF	MS	F Ratio	Prob>F	R^2
Fayetteville					
Model	23	16435620	4.29	<.0001	0.63
Error	57	3828796			
C. Total	80				
		SS			
Rep	3	35159154	3.06	0.0353	
N Emg†	2	143714325	18.77	<.0001	
N Mid	6	136590407	5.95	<.0001	
Emg*Mid	12	62555387	1.36	0.2115	
Keiser					
Model	23	46758195	1.71	0.0578	0.45
Error	49	27347135			
C. Total	72				
		SS			
Rep	3	64262961	0.8	0.5090	
N Emg	2	79868649	1.46	0.2421	
N Mid	6	397355505	2.42	0.0396	
Emg*Mid	12	533951365	1.63	0.1149	
Rohwer					
Model	23	15453676	9.46	0.0436	0.85
Error	39	1634248			
C. Total	62				
		SS			
Rep	3	94424214	19.26	0.5054	
N Emg	2	56605401	17.32	0.0017	
N Mid	6	180717990	18.43	0.6315	
Emg*Mid	12	23686953	1.21	0.1308	

†N Emg and N Mid refer to the N applications at emergence and mid-season, respectively.

Appendix Table 5 Grain N Concentrations for all locations 2011.

		2011		
		Emergence N Application, kg ha^{-1}		
Location	V6-V10 N App., kg ha^{-1}	0	84	168
			%	
Fayetteville	0	1.26 fgedc†	1.24 fge	1.42 bac
	14	1.20 fg	1.25 fged	1.35 bedc
	28	1.23 fge	1.16 g	1.36 bedc
	70	1.23 fge	1.33 fbedc	1.36 bedc
	112	1.26 fged	1.42 bac	1.40 bc
	168	1.38 bdc	1.43 bac	1.54 a
	224	1.44 ba	1.32 fbedc	1.46 ba
Keiser	0	1.16 d	1.21 d	1.59 a
	14	1.87 d	1.16 d	1.26 dc
	28	1.21 d	1.26 dc	1.23 d
	70	1.38 bc	1.31 dc	1.49 ba
	112	1.49 ba	1.38 bc	1.49 ba
	168	1.39 bac	1.51 ba	1.52 ba
	224	1.50 ba	1.58 a	1.57 a
Rohwer	0	1.23 e	1.25 ed	1.34 ebdc
	14	1.34 ebdc	1.37 ebdc	1.311 ebdc
	28	1.38 ebdac	1.24 e	1.31 ebdc
	70	1.29 edc	1.23 e	1.35 ebdc
	112	1.30 edc	1.29 edc	1.31 ebdc
	168	1.29 edc	1.45 ba	1.41 bdac
	224	1.44 bac	1.59 a	1.43 bdac

† Means with the same letter within a location are not significantly different.

Appendix Table 6 Analysis of variance for corn nitrogen recovery 2011.

Source	DF	MS	F Ratio	Prob>F	R^2
Fayetteville					
Model	22	977.5	1.34	0.1871	0.35
Error	56	728.6			
C. Total	78				
		SS			
Rep	3	6052	2.77	0.0500	
N Emg†	2	4943	3.39	0.0407	
N Mid	6	5437	1.24	0.2983	
Emg*Mid	11	5071	0.63	0.7931	
Keiser					
Model	22	5542	3.48	0.0002	0.65
Error	42	1590			
C. Total	64				
		SS			
Rep	3	1712	0.23	0.8785	
N Emg	2	44808	17.33	<.0001	
N Mid	6	39903	5.17	0.0005	
Emg*Mid	11	16394	0.94	0.5157	
Rohwer					
Model	22	540.81	2.74	0.0050	0.66
Error	31	197.4			
C. Total	53				
		SS			
Rep	3	8196	13.84	<.0001	
N Emg	2	728	1.84	0.1751	
N Mid	6	1591	1.34	0.2682	
Emg*Mid	11	1383	0.64	0.7835	

†N Emg and N Mid refer to the N applications at emergence and mid-season, respectively.

Appendix Table 7 Covariate analysis of the effects of NutriLife soil amendment application rate and mid-season N application rate on leaf N concentration in bermudagrass. Sample data taken 8 July 2011 in Fayetteville, AR.

	Leaf N Concentration	
NLAF Application Rate†	Intercept	Slope
0x	2.33	0.0062
1x	2.33	0.0062
1.5x	2.33	0.0062

	Source of Variation
NLAF Application Rate	NS
N Applied (kg ha^{-1})	***
NLAF x N Applied	NS
Adj. r^2	0.68

*,**,***, significant at $P< 0.05$, 0.01, and 0.001 levels, respectively

† 1x application rate: 4.2 ml kg^{-1}

Appendix Table 8 Covariate analysis of the effects of NutriLife soil amendment application rate and mid-season N application rate on DGCI in bermudagrass. Sample data taken 8 July 2011 in Fayetteville, AR.

	DGCI	
NLAF Application Rate†	Intercept	Slope
0x	0.498a	0.00076
1x	0.497a	0.00076
1.5x	0.507b	0.00076

	Source of Variation
NLAF Application Rate	**
N Applied (kg ha^{-1})	***
NLAF x N Applied	NS
Adj. r^2	0.84

*,**,***, significant at $P< 0.05$, 0.01, and 0.001 levels, respectively

† 1x application rate: 4.2 ml kg^{-1}

Appendix Table 9 Covariate analysis of the effects of NutriLife soil amendment application rate and leaf N concentration on DGCI in bermudagrass. Sample data taken 8 July, 2011 in Fayetteville, AR.

	DGCI	
NLAF Application Rate†	Intercept	Slope
0x	0.29	0.094
1x	0.29	0.094
1.5x	0.29	0.094

	Source of Variation
NLAF Application Rate	NS
Leaf N Concentration	***
NLAF x Leaf N Conc.	NS
Adj. r^2	0.77

*,**,***, significant at $P< 0.05$, 0.01, and 0.001 levels, respectively

† 1x application rate: 4.2 ml kg^{-1}