**Dissertation Research Proposal**

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# Study 1. Evaluation of nitrogen and water use efficiencies on corn hybrids with and without drought tolerance in irrigated and dryland production systems

Nitrogen and water use efficiency as influenced by maize hybrid and irrigation

## 1.1 Introduction

The drought experienced by the United States in 2012 was the most severe and extensive drought in the past 25 years (USDA-ERS, 2012). Much of the Great Plains are still experiencing extreme to exceptional drought (Fuchs, 2013). Despite the 2012 drought, maize (*Zea mays* L.) growers in the United States produced 10,780,296,000 bushels of corn grain with an estimated average grain yield of 123.4 bushels per acre (USDA-NASS, 2013). In comparison, average grain yield in 2011, without the effect of drought, was 147.2 bushels per acre and total production was 13 percent higher than in 2012 (USDA-NASS, 2013). The devastation of the 2012 drought may have a dramatic impact on animal feed supply and retail food prices in 2013 (USDA-ERS, 2012). On January 9, 2013, the USDA declared 597 counties in 14 states primary natural disaster areas due to drought (USDA, 2013) resulting in over $14 billion in crop insurance indemnity payments calculated by the Congressional Budget Office (CBO, 2013).

Variability in precipitation is a normal part of the North American climate, but many dryland corn producers are vulnerable to catastrophic losses due to untimely drought conditions during the growing season. Irrigation offers one management tool which can minimize the impact of drought conditions. However, the largest groundwater reserve in the High Plains, the Ogallala aquifer, has been depleted over the past several decades due to over irrigation (Peterson and Ding, 2005) and has caused many producers to return to dryland production practices. If the current drought persists, this may be a growing trend across much of the Corn Belt.

In a review, Hatfield et al. (2011) explains how climate change will have an impact on agricultural systems over the next 30 years. They explain that the interaction of water stress and high temperatures during grain set and pollination could be quite damaging to crop production and food security. As a challenge to agronomists, the authors conclude a need to couple physiological responses with genetic traits to provide an opportunity for better cropping systems to manage seasonal variability in precipitation (Hatfield et al., 2011).

### 1.1.1 The Role of Water in the Plant

Water is used in many facets of a plant’s life cycle. Most notably, water is used as a reactant in photosynthesis which supports plant growth. Water is absorbed from the soil by roots and can transport dissolved nutrients throughout the plant. Water pressure, called turgor, is the structural support for plant tissues. Plants can control the opening and closing of the stomata, through which water vapor is exchanged with atmospheric carbon dioxide, in response to environmental conditions such as temperature and relative humidity. Carbon dioxide is used to produce sugars and proteins that provide protection to the plant under stress and can be important in heat stress response. The stomata also release water vapor, which increases transpiration (the movement of water through the leaves), cooling the plant through evaporation. In response to drought, plants may keep the stomata closed in an effort to conserve water, but this also results in decreasing the supply of carbon dioxide, effectively starving the plant of sugars and depriving the plant of the cooling effects of transpiration. The interaction of physiological responses to heat and drought are a subject of current research.

Corn is most susceptible to drought one week before and two weeks after flowering (Denmead and Shaw, 1960; Grant et al., 1989). Effects of drought include kernel abortion (Boyle et al., 1991) and reduced kernel set (Nielsen, 2011). Reduced kernel set is most likely to occur at the tip of an ear where the ovules are unfertilized due to reduced opportunity for fertilization during the abbreviated silking period. Kernel abortion due to drought has been documented to occur two weeks after silking (Westgate and Boyer, 1986). The yield loss from kernel abortion cannot be recovered later in the season. Drought can also increase the anthesis-silking interval (ASI) due to a delay in silk emergence (Bolaños and Edmeades, 1996). Anthesis-silking interval is the period of time between pollen shed and silk emergence and is an indicator of ear growth rate (Carena et al., 2009). Physiological stress due to drought, heat or a combination thereof can result in increased ASI and therefore reduced ear growth rate. Therefore, achieving better water use efficiency has been a main point of emphasis for many breeding programs in the United States and abroad.

### 1.1.2 Drought Tolerant Traits

Drought tolerance is a quantitative trait that has complex and polygenic inheritance mechanisms. Expression of drought tolerance is associated with epistatic effects and therefore has large genotype by environment interactions. Genotype by environment factors affecting drought tolerance include; timing and duration of water stress, soil type, temperature, and humidity. Breeding to exploit polygenic effects is desirable and is an opportunity for practically unlimited genetic improvement.

### 1.1.3 Improving Drought Tolerance with Conventional Breeding

Pioneer Hi-Bred is a seed company which has focused its research to develop drought tolerant hybrids using conventional breeding (Butzen and Schussler, 2009). Researchers from this company have developed drought tolerant corn hybrids using native drought tolerant traits through marker assisted selection. The native drought tolerant traits were identified as linked to genetic markers. The markers were then used to make advancement selections based on the known desirable genetic traits, thereby saving time and resources that would otherwise be spent on less-specific phenotypic selections. In this way, marker assisted selection can be used to quickly integrate desirable traits into market-ready hybrids. This approach has enabled breeders to stack multiple drought-related traits into successive lines, introducing more than one gene affecting drought tolerance and partially capturing the complex polygenic drought response.

Pioneer’s goal is to improve the corn plant’s ability to capture and utilize water, sunlight, and nutrients under water limited conditions (Butzen and Schussler, 2009). Specific trait goals include a more efficient root system and more aggressive silk emergence, which will theoretically result in fewer aborted kernels during drought. Butzen and Schussler (2009) acknowledge that the energy required to establish an improved root system may decrease above ground growth, but ensure extensive testing will result in higher yields in all environments. A highly efficient root system balances both shallow, immobile nutrient-mining roots and deep, water-mining roots without an overly abundant root system, which would be too high of a respiratory cost for a drought stressed plant (Lynch, 1995; Ho et al., 2005). Efficiencies can be achieved through root matter distribution between shallow and deep roots and also by selecting for increased aerenchyma, air spaces within roots which allow the plant to physically expand the root system while avoiding the respiratory cost of supporting cells within the roots (Postma and Lynch, 2011).

Breeding programs with the International Maize and Wheat Improvement Center in Mexico have worked to narrow the ASI in lowland tropical maize. Researchers there have reported increased grain yields of 30 to 50 percent under water stressed environments (Edmeades et al., 1999). Increases in grain yield were attributed to a shorter anthesis-silking interval (Chapman and Edmeades, 1999). Additionally, improvements in grain yield were also observed under unstressed environments.

### 1.1.4 Improving Drought Tolerance with Transgenic Traits

Scientists at the Monsanto and BASF companies have discovered a transgene which can stabilize corn yields during periods of inadequate water supply. Transgenes are genes which are moved from one organism to another through biotechnology instead of through traditional breeding methods. Researchers have identified a cold shock protein B gene called CspB which is originally from the *Bacillus subtilis* bacterium (Castiglioni et al., 2008). Cold shock proteins rapidly accumulate in cold shocked bacterial cells and act as RNA chaperones to facilitate the normal process of translation during protein synthesis. In corn, the CspB genes help to maintain growth and development during water stress by binding and unfolding tangled RNA molecules to promote normal function (Castiglioni et al., 2008). Corn expressing the CspB protein experiences reduced growth during times of drought stress but preserves a portion of the yield that would be lost in isogenic lines not bearing the transgene.

Field trials were conducted to evaluate corn hybrids containing CspB. When compared to nontransgenic control hybrids, the CspB transgenic hybrid demonstrated grain yield improvements of up to 15 percent under dryland growing conditions (Castiglioni et al., 2008). However, more research will need to be conducted to confirm yield stability under well watered conditions. Nonetheless, there is great potential for transgenetic advancements in drought tolerance through modifying the physiological responses to drought and heat.

Transgenic traits offer exceptional opportunities to identify and manipulate many genes and traits which affect drought tolerance. Genomic approaches will expand the possibilities to improve genetic variation in elite germplasm. Identification of specific quantitative trait loci (QTL) is the first step to identify and isolate molecular material (polymorphism) of the genetic variation at the sequence level (Tuberosa et al., 2007). However, some researchers suggest that quantitative traits are better explained by polygenes rather than QTLs (Carena and Wicks III, 2006). Still, any method that increases the frequency of favorable alleles for traits that are quantitatively inherited while maintaining genetic variability will continue to improve genetic advancement.

### 1.1.5 Water Use Efficiency, Nitrogen Use Efficiency, and the Interaction

Water use efficiency (WUE) can be defined differently depending on the objective and application. For example, plant physiologists could measure WUE as the amount of photosynthesis per unit of water used in transpiration, whereas, farmers and agronomists may perhaps measure WUE as corn grain yield per unit of water, measured as precipitation and/or irrigation (Condon et al., 2004). Commonly, evapotranspiration (ET) is the measure of water used in WUE calculations and is the summation of evaporation from soil and non-stomatal plant surfaces and transpiration from plant stomates. Variation in ET due to environmental dynamics, plant factors, and management practices can result in differences in WUE (Stone et al., 1987).

With many of the plant vegetative and reproductive process dependent upon a sufficient water supply, logically, adequate rainfall or irrigated production systems increase corn grain yield response to N fertilizer (Eck, 1984) and N uptake (Russelle et al., 1981). Once in the plant, N is assimilated into proteins and stored in the grain. The efficiency at which N is taken up from the soil to produce grain is characterized as nitrogen use efficiency (NUE). Nitrogen use efficiency is defined as the amount of corn grain produced per unit of nitrogen (N) available in the soil (Moll et al., 1982) or as the percent if N recovered in the corn grain (Varvel and Peterson, 1991). However, NUE is dependent on soil and plant interactions (Huggins and Pan, 1993).

Inorganic forms of N (nitrate and ammonium) have high mobility in the xylem of plants and long distance xylem transport of solutes, such as N, is driven by a water potential gradient generated by transpiration. Accepting this premise, if modern drought tolerant corn hybrids utilize water more efficiently will they utilize N more efficiently too? Currently, there are no known research publications addressing the WUE and NUE interaction of modern drought tolerant corn hybrids. Research conducted in spring wheat has shown that under drought conditions, plants have shown the ability to improve nutrient uptake by increasing root respiration which increases nutrient solubility (Liu et al., 2004). Evaluations of corn hybrids in Indiana reported that hybrid response to increased rates of N fertilizer were similar for drought tolerant and conventional corn hybrids (Roth et al., 2013). A study conducted using hybrids with different NUE history in Nebraska reported that different water regimes did not influence the NUE of those hybrids and suggested that hybrid selection for NUE will result in simultaneous selection for WUE (Eghball and Maranville, 1991).

### 1.1.6 Conclusion

The functions of water in a corn plant are myriad and not fully understood. This intricate relationship between water and harvestable yield speaks to the complexity of the genetic response to drought stress within the plant. Selection for drought tolerance requires accurate identification and characterization of the many underlying traits under controlled field conditions. The biggest challenge facing researchers in genomics is the translation into application. Therefore, a multi-dimensional approach using sophisticated phenotyping data, transgenic resources and conventional breeding is the best strategy to address the many facets of drought response within the plant. The genetics of individual corn hybrids affect the many various physiological processes which influence water and N use within the plant. The epigenetic relationship between the individual hybrid and its environment means that no single corn hybrid will work well across all environments. Conventional breeding along with integration of transgenic events in a comprehensive crop improvement program has potential for achieving significant drought tolerance for US production in the future. Thus, it is important to investigate how new drought tolerant corn hybrids influence WUE and NUE in the water limited environments of Oklahoma.

## 1.2 Objective

Evaluate WUE and NUE of drought tolerant and less drought tolerant corn hybrids in irrigated and dryland production systems.

## 1.3 Methods and Materials

### 1.3.1 Site Description

Field experiments will be established in 2013 and 2014 at the Efaw (36.081118o, -97.063270o, elevation 272 m above sea level) agronomy research station near Stillwater, OK and Lake Carl Blackwell (LCB; 36.090792o, -97.172486o, elevation 293 m above sea level) agronomy research station west of Stillwater, OK near Lake Carl Blackwell (Table 1-1). Total precipitation for the 2010, 2011, 2012 growing seasons, and the 18 year average were 103, 40, 67, and 86 cm, respectively, for Stillwater, OK based on the period from October of the previous year to September of the current year. All soil fertility parameters will be managed to ensure N is the only limiting nutrient (Table 1-2). A summary of field activities for each cropping year including; tillage, previous crop, and weed control will be reported.

### 1.3.2 Experimental Design

A three replicate randomized complete block design with treatments arranged as a two way factorial with 4 levels of hybrid and 3 levels of N rate will be utilized in this study. At each site, hybrids and N rates will be randomly assigned within both an irrigated and dryland production system. Two corn hybrids designated as drought tolerant (DuPont Pioneer AQUAmax brand P1498 YHR; DuPont Pioneer Hi-Bred Intl., Inc., Johnston, IA and Monsanto Dekalb Genuity DroughtGard brand DKC63-55 GENDGVT2P; Monsanto Company, St. Louis, MO) will be compared with two hybrids with less drought tolerance (DuPont Pioneer brand P1395 YHR and Monsanto Dekalb brand DKC62-09 GENVT3P). Drought tolerance scores as determined by DuPont Pioneer (1 = poor, 9 = outstanding) were 9 and 7 for P1498 and P1395, respectively and scores determined by Monsanto Dekalb (1 = excellent, 9 = poor) were 1 and 3 for DKC63-55 and DKC62-09, respectively. For discussion of these hybrids, the following abbreviations will be used: P1 = P1498, P2 = P1395, M1 = DKC63-55, and M2 = DKC62-09. Three different N rates will be used in each production system based on expected N removal in the grain. The N rates for the irrigated production system will be 0 (Low), 101 (Med), and 202 (High) kg ha-1 and for the dryland production system will be 0 (Low), 67 (Med), and 134 (High) kg ha-1. Nitrogen fertilizer will be applied prior to planting as broadcast and incorporated urea ammonium nitrate (UAN; 28-0-0). Planting density for each production system will be different based on best management practices for each environment. The irrigated production system will be planted at 75,650 seeds ha-1 and the dryland production system will be planted at 53,800 seeds ha-1. Plots will be planted with a 4-row John Deere 7300 Integral MaxEmerge planter (Deere & Company, Moline, IL) at a planting depth of approximately 5 cm. Individual plots will measure 3 m wide (four 0.76 m rows) by 6.1 m long. A summary for all treatments in each production system are provided in Table 1-3.

The irrigated production system will be irrigated using a surface drip system on an as needed basis, dependent upon visual water stress symptoms. The amount of water being supplied will be monitored and documented. To ensure even distribution across each plot, drip tape will be installed between rows 1 and 2 and between rows 3 and 4 (Fig. 1-1).

### 1.3.3 Soil Moisture Measurements

Soil moisture content (SWC) of the top 1 m soil profile depth will be determined prior to planting and directing following grain harvest. Soil cores will be collected using a tractor mounted Giddings hydraulic soil sampler (Giddings Machine Company, Windsor, CO) to determine SWC by a direct gravimetric method (Gardner, 1986) and soil bulk density (Blake and Hartge, 1986). Volumetric soil water content (Θv; mL mL-1) will be calculated as the product of the gravimetric soil water content (Θg; g g-1) and soil bulk density (BD; g cm-3). Prior to planting, four soil cores will be collected from each production system to a depth of approximately 1.2 m and exact lengths of each core will be recorded in the field for an accurate core volume. Soil cores will be weighed directly following collection, dried in a forced air oven at 60 oC for 72 hours, and weighed for an oven-dry soil weight. A single soil core will be collected from each plot post grain harvest using the same method and used to determine the seasonal change in SWC.

The amount of soil moisture within the 1 m soil profile depth will be used to obtain evapotranspiration (ET) of the production system. Evapotranspiration will be estimated using the soil water balance method proposed by Heermann (1985) and can be expressed in the following equation:

ET = +∆SWC + R + I - D

where ∆SWC is the change in volumetric soil water content (mm) of the 1 m soil profile from plant to harvest, R is the cumulative rainfall (mm) from planting to harvest, I is the amount of irrigation water applied (mm), and D is the amount of drainage (mm). It will be assumed that water loss due to drainage will be negligible. Daily rainfall will be recorded from an automated weather station (2 km from the field experiment) and data files will be downloaded (Mesonet, Norman, OK) to determine cumulative rainfall. Water use efficiency (kg m-1) will be calculated as the ratio between grain yield (kg ha-1) and evapotranspiration (m) for each plot.

### 1.3.4 Crop Measurements

Crop canopy reflection measurements will be collected throughout the vegetative growth stages to estimate biomass accumulation. Spectral reflectance will be measured from the center two rows of each plot using the GreenSeeker (Trimble Agriculture Division, Westminster, CO) active optical reflectance crop sensor and will be expressed as a plot averaged normalized difference vegetative index (NDVI). The GreenSeeker crop sensor utilizes red (660 nm) and near infrared (NIR; 780 nm) wavelengths and calculates NDVI as: NDVI = NIR(780) – red(660) / NIR(780) + red(660). Reflectance measurements will be collected at approximately growth stage V4, V6, V8, V10, and V12 (Abendroth et al., 2011).

At physiological maturity, mechanical grain harvest will be accomplished using a Massey Ferguson 8-XP self-propelled research plot combine (Kincaid Equipment and Manufacturing, Haven, KS) equipped with a HarvestMaster (Juniper Systems, Inc., Logan, UT) plot harvest data system calibrated to collect individual plot grain weight and moisture. The center two rows of each plot will be harvested and grain yield (Mg ha-1) will be adjusted to 155 g kg-1 moisture content. A subsample of the grain harvested from each plot will be collected, oven dried at 60 oC until a constant dry weight is achieved, and ground to pass through a 140 mesh screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Grain samples will be analyzed for total N content (mg g-1) using a LECO Tru-Spec CN automated dry combustion analyzer (LECO Corporation, St. Joseph, MI; Schepers et al., 1989). Total grain N uptake (kg ha-1) will be calculated as the product of grain yield (kg ha-1) and grain N content. Nitrogen use efficiency will be calculated using the difference method described by Varvel and Peterson (1991).

### 1.3.5 Statistical Analysis

Statistical analysis will be conducted by site and year for the combination of hybrid and N rate nested within the production system (Table 1-4) to determine the best management practices for irrigated and dryland corn production in Oklahoma. Analysis of variance (ANOVA) will be performed using the SAS PROC GLM procedure (SAS Institute, 2011). Differences between treatment means will be identified using a protected Fisher’s least significant difference (LSD) and contrasts with significant differences being declared at α = 0.05.

## 1.4 Tables

|  |  |  |  |
| --- | --- | --- | --- |
| Table 1-1 Soil map unit and taxonomic classification for each site, 2013 and 2014. | | | |
| Year | Location† | Soil Mapping Unit | Major Component Soil Taxonomic Classification |
| 2013 | Efaw | Norge loam,  3-5% slope | Norge: *Fine-silty, mixed, active, thermic Udic   Paleustolls* |
| LCB | Port-Oscar Complex, occasionally flooded,  0-1% slope | Port: *Fine-silty, mixed, superactive, thermic   Cumulic Haplustolls* Oscar: *Fine-silty, mixed, superactive, thermic  Typic Natrustalfs* |
| 2014 | Efaw | TBD | TBD |
| LCB | TBD | TBD |
| † Efaw, Oklahoma State University Agronomy Research Station near Stillwater, OK;   LCB, Oklahoma State University Agronomy Research Station west of Stillwater, OK near   Lake Carl Blackwell | | | |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 1-2 Pre-plant soil sample (0-15 cm) chemical properties, 2013 and 2014. | | | | | | | | |
| Year | Location† | Soil  pH‡ | NH4-N§ | NO3-N§ | P¶ | K¶ | Total N# | Organic C# |
|  |  |  | --------------- µg g-1 --------------- | | | | ----- mg g-1 ----- | |
| 2013 | Efaw |  |  |  |  |  |  |  |
| Irrigated | 5.0 | 15.0 | 17.3 | 106 | 129 | 1.3 | 11.4 |
| Dryland | 4.9 | 13.4 | 9.8 | 32.8 | 131 | 1.2 | 9.7 |
| LCB | 6.1 | 6.2 | 5.3 | 24.2 | 139 | 1.1 | 9.5 |
| 2014 | Efaw | TBD | TBD | TBD | TBD | TBD | TBD | TBD |
| LCB | TBD | TBD | TBD | TBD | TBD | TBD | TBD |
| † Efaw, Oklahoma State University Agronomy Research Station near Stillwater, OK;   LCB, Oklahoma State University Agronomy Research Station west of Stillwater,  OK near Lake Carl Blackwell  ‡ 1:1 soil water  § 2 M KCl extract (Mulvaney, 1996)  ¶ Mehlich III extract (Mehlich, 1984)  # Dry combustion (Schepers et al., 1989) | | | | | | | | |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 1-3 Treatment structure for the irrigated and dryland production systems, 2013 and 2014. | | | | | | | | |
| Treatment | Hybrid† | | Pre-plant N rate‡ | | | | Seeding rate | |
| # |  | | Irrigated | | Dryland | | Irrigated | Dryland |
|  |  | | ----- kg N ha-1 ----- | | | | ----- seeds ha-1 ----- | |
| 1 | P1498 | (P1) | 0 | (Low) | 0 | (Low) | 75,682 | 53,818 |
| 2 | P1395 | (P2) | 0 | (Low) | 0 | (Low) | 75,682 | 53,818 |
| 3 | DKC63-55 | (M1) | 0 | (Low) | 0 | (Low) | 75,682 | 53,818 |
| 4 | DKC62-09 | (M2) | 0 | (Low) | 0 | (Low) | 75,682 | 53,818 |
| 5 | P1498 | (P1) | 101 | (Med) | 67 | (Med) | 75,682 | 53,818 |
| 6 | P1395 | (P2) | 101 | (Med) | 67 | (Med) | 75,682 | 53,818 |
| 7 | DKC63-55 | (M1) | 101 | (Med) | 67 | (Med) | 75,682 | 53,818 |
| 8 | DKC62-09 | (M2) | 101 | (Med) | 67 | (Med) | 75,682 | 53,818 |
| 9 | P1498 | (P1) | 202 | (High) | 134 | (High) | 75,682 | 53,818 |
| 10 | P1395 | (P2) | 202 | (High) | 134 | (High) | 75,682 | 53,818 |
| 11 | DKC63-55 | (M1) | 202 | (High) | 134 | (High) | 75,682 | 53,818 |
| 12 | DKC62-09 | (M2) | 202 | (High) | 134 | (High) | 75,682 | 53,818 |
| † Drought tolerance scores: DuPont Pioneer (1 = poor, 9 = outstanding), P1498 = 9 and P1395 = 7; Monsanto Dekalb (1 = excellent, 9 = poor), DKC63-55 = 1 and DKC62-09 = 3  ‡ Nitrogen fertilizer will be applied prior to planting as broadcast and incorporated urea ammonium nitrate (UAN; 28-0-0) | | | | | | | | |

not sure if “low, med, high” are needed within the table. Could delineate in the table caption

|  |  |
| --- | --- |
| Table 1-4 Statistical model and degrees of freedom included in the analysis of variance; 2013 and 2014. | |
| Source | df |
| Replicate | 2 |
| Production system | 1 |
| Error A | 2 |
| Hybrid | 3 |
| Hybrid x Production System | 3 |
| Error B | 12 |
| N rate | 2 |
| N rate x Production System | 2 |
| Error C | 8 |
| Hybrid x N rate | 6 |
| Hybrid x N rate x Production System | 6 |
| Error D | 24 |
| Total | 71 |
| Each site and year will be analyzed separately. | |

## 1.5 Figures

**Photo Courtesy of Jacob Bushong**



### Figure 1-1 Surface drip irrigation system positioned between rows 1 and 2 and between 3 and 4 of each plot of the irrigated production system.

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# Study 2. Predicting Pre-plant Nitrogen Applications to Corn Using Indicator Crop N-Rich Reference Strips

## 2.1 Introduction

The use of active optical reflectance sensors has proven to accurately determine optimum nitrogen (N) fertilizer requirements and direct in-season N fertilizer applications in corn (*Zea mays* L.). However, the adoption of this N fertilizer application technology has been slow due to an array of agronomic, economic, and technical reasons (Schepers, 2013). Agronomically, the short timeframe to identify N deficiencies and apply N fertilizer mid-season using traditional sidedress application equipment is the most notable reason for corn producer’s reluctance, even though a substantial amount of work has gone into identifying the earliest growth stage at which N deficiencies can be detected (Teal et al., 2006; Martin et al., 2007). Thus, it is important to investigate alternative strategies to widen the window for applying N fertilizer to corn with the use of active optical reflectance sensors.

## 2.1.1 Nitrogen Fertilizer Rate Recommendations

Various methods have been proposed to identify optimum N fertilizer rates for corn grain production including; yield goal (Stanford, 1973), maximum return to N (Sawyer et al., 2006), soil sampling (Magdoff et al., 1984; Bundy et al., 1993, Khan et al., 2001), chlorophyll meters (Schepers, 1994), and crop reflectance (Solari et al., 2008; Tubaña et al., 2008). Of these techniques, crop reflectance measurements collected from active optical reflectance sensors offer both an on-the-go and mid-season evaluation of the plant’s nutritional status. The transformation of the crop reflectance measurements, often times expressed as the normalized difference vegetative index (NDVI), into fertilizer N recommendations has proven to improve nitrogen use efficiency of cereal grain production (Raun et al., 2002). These N fertilizer management techniques which encompass spatial soil variability and decrease uniform applications will decrease environmental degradation.

All Oklahoma-developed N fertilizer management strategies using active optical reflectance sensors require an area in each field that has a non-limiting amount of N fertilizer applied prior to planting or directly following planting (Raun et al., 2005; Solie et al., 2012). These areas are referred to as N-rich strips and are compared to an unfertilized area of the field (farmer practice area) to calculate a response index (RI); expressed in the following equation:

RINDVI = NRNDVI / FPNDVI

where NRNDVI is the NDVI collected from the N-rich strip and FPNDVI is the NDVI measured from an adjacent area with fertilizer applied at the farmer practice rate. The RI identifies N deficiencies which are then used to measure the response of the crop to additional N fertilizer along with the yield level of that field (Johnson and Raun, 2003; Mullen et al., 2003).

In corn, early season N deficiencies have shown not to be readily evident until growth stage V7 to V9 in Oklahoma (Teal et al., 2006; Martin et al., 2007) and V11 in Nebraska (Solari et al., 2008). Beyond growth stage V7 (corn height of approximately 0.5m), N fertilizer applications using traditional sidedress equipment is problematic due to clearance constraints. High clearance N applicators offer one option, but many producers do not have access to this equipment nor are these applicators compatible to apply anhydrous ammonia, which is often a more cost effective N fertilizer source. Thus, the window of opportunity to identify and correct N deficiencies must be widened for producers to adopt N management strategies using active optical reflectance sensors without corn height being a limiting factor. Alternative and reliable strategies need to be investigated to identify early season N deficiencies in corn.

## 2.1.2 Indicator Crop Reference Strips

Monitoring N availability throughout the winter and early spring using cereal grain indicator crops offers one option to widen the window to apply N fertilizer to corn. This approach allows the indicator crop to demonstrate distinguishable differences in response to residual fall N and early spring N mineralization near at the time of corn planting. These soil N pools are otherwise less recognizable during the early growth stages of corn, especially when temperatures are low and crop growth is slow.

Two indicator crop reference strips (approximately 3 m wide) will be planted during mid to late fall, after grain harvest of the preceding crop. These strips will be used as a measure of winter and early spring N carryover for the subsequent corn crop. One of the indicator crop reference strips will have N fertilizer applied at a non-limiting amount and will serve as the indicator crop N-rich reference strip. The other strip will not receive N fertilizer and will function as the indicator crop farmer practice strip. Crop reflectance measurements will be collected using active optical reflectance sensors from both indicator crop reference strips and will provide the information needed to calculate N fertilizer application rates by the use of the generalized algorithm (Solie et al., 2012). The RI of the indicator crops will mimic the RI of the corn crop that will show up later in the corn growing season. The suitability of indicator crop reference strips will be determined at the conclusion of this research.

## 2.1.3 Conclusion

The use of indicator crop reference strips will advance the detection of N deficiencies in corn. Producers could initially balk at having a secondary crop in their corn fields. However, once it is noted that the indicator crop reference strips will show N deficiency, far ahead of it being observed in corn, acceptance of this approach will soon follow. The proposed system will provide farmers with much greater flexibility to use of active optical reflectance sensors apply N fertilizer to corn.

## 2.2 Objective

Evaluate the response of winter wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.) indicator crops to applied N over winter and early spring to estimate optimal early season N fertilizer application rates of the subsequent corn (*Zea mays* L.) crop.

## 2.3 Methods and Materials

### 2.3.1 Site Description

Field experiments will be initiated in the fall of 2012 and will continue through 2014 at the Efaw (36.081118o, -97.063270o, elevation 272 m above sea level) agronomy research station near Stillwater, OK and Lake Carl Blackwell (LCB; 36.090792o, -97.172486o, elevation 293 m above sea level) agronomy research station west of Stillwater, OK near Lake Carl Blackwell (Table 2-1). All soil fertility parameters will be managed to ensure N is the only limiting nutrient (Table 2-2). A summary of field activities for each cropping year including; tillage, previous crop, and weed control will be reported.

### 2.3.2 Experimental Design

Winter wheat and spring barley will be planted in late fall or early spring in strips along the outside of the eventual corn trial (Fig. 2-1) and will serve as the indicator crop reference strips. Each indicator crop reference strip (6 m wide 21 m long) will be split and either a sufficient rate (168 kg N ha-1; N-rich reference strip) and a zero rate (farmer practice) of N fertilizer will be applied prior to planting as broadcast urea ammonium nitrate (UAN; 28-0-0). Winter wheat cultivar ‘Doublestop’ CL Plus will be planted in October of 2012 and 2013 using a Kincaid 2010 grain drill (Kincaid Equipment and Manufacturing, Haven, KS) at a seeding density of 100 kg ha-1 and row spacing of 18 cm. Spring barley cultivar ‘Pinnacle’ will be planted in February of 2013 and 2014 using the same Kincaid 2010 grain drill at a seeding density of 112 kg ha-1 and row spacing of 18 cm. Seeding depth of the winter wheat and spring barley will be approximately 1.3 cm.

In the spring of 2013 and 2014, corn will be planted between the indicator crop reference strips (Fig. 2-1). A three replicate randomized complete block design will be used to evaluate six at planting N fertilizer treatments ranging from 0 to 225 kg ha-1 in 45 kg ha-1 increments. Nitrogen fertilizer will be applied prior to planting as broadcast and incorporated UAN. Corn will be planted at a seeding density of 65,005 kernels ha-1 with a 4-row John Deere 7300 Integral MaxEmerge planter (Deere & Company, Moline, IL) approximately 5 cm deep. Individual corn plots will measure 3 m wide (four 0.76 m rows) by 6.1 m long. All corn plots will be irrigated using a surface drip system on an as needed basis, dependent upon visual water stress symptoms. The amount of water being supplied will be monitored and documented. To ensure even distribution across each plot, drip tape will be installed between rows 1 and 2 and between rows 3 and 4 (Fig. 2-2).

### 2.3.3 Crop Measurements

Crop canopy reflection measurements will be collected throughout the vegetative growth stages of both the indicator and corn crops to estimate biomass accumulation and N deficiencies. Spectral reflectance will be measured from the center 1.5 m of the indicator crop reference strips and the center two rows of each corn plot using the GreenSeeker (Trimble Agriculture Division, Westminster, CO) active optical reflectance crop sensor and will be expressed as a plot averaged normalized difference vegetative index (NDVI). The GreenSeeker crop sensor utilizes red (660 nm) and near infrared (NIR; 780 nm) wavelengths and calculates NDVI as: NDVI = NIR(780) – red(660) / NIR(780) + red(660). Reflection measurements will be collected at approximately Feekes (Large, 1954) growth stage 3, 4, 5, 7, 10, and 10.5 for the indicator crops and at approximately growth stage V4, V6, V8, V10, and V12 (Abendroth et al., 2011) for the corn plots.

Grain yield will be determined for all of the corn plots at physiological maturity. Mechanical grain harvest will be accomplished using a Massey Ferguson 8-XP self-propelled research plot combine (Kincaid Equipment and Manufacturing, Haven, KS) equipped with a HarvestMaster (Juniper Systems, Inc., Logan, UT) plot harvest data system calibrated to collect individual plot grain weight and moisture. The center two rows of each plot will be harvested and grain yield (Mg ha-1) will be adjusted to 155 g kg-1 moisture content.

### 1.3.5 Data Analysis

The corn’s agronomic optimum N rate (AONR) will be determined using the SAS PROC NLIN procedure (SAS Institute, 2011) at each location. The AONR is the N rate that produces optimum corn grain yield and will be calculated using a quadratic plus plateau model (Bullock and Bullock, 1994) or a linear plus plateau model (Cerrato and Blackmer, 1990).

In-season measurements of NDVI from the indicator crop reference strips will be utilized to calculate a corn N fertilizer recommendation using the generalized algorithm (Solie et al., 2012). Farmer practice NDVI and N rich strip NDVI from the wheat and barley indicator crops will be used as inputs on the generalized algorithm interface (Fig. 2-3). Some assumptions will need to be made for completion of the algorithm. These assumptions include; bare soil NDVI = 0.18, Max Yield for the region = 13.44 Mg ha-1, weight per bushel = 56 lb/bu, Grain N = 1.2%, and nitrogen use efficiency = 50 %. A corn N fertilizer recommendation will be calculated for each growth stage in-season measurements of NDVI were collected from the indicator crops. Farmer practice NDVI and N rich strip NDVI from the indicator crops, corn N fertilizer recommendation from the generalized algorithm, and the corn’s AONR will populate a data table similar to the one depicted in Table 2-3. Calibration and correlation regression curves will be utilized to summarize AONR and generalized algorithm N rates for each growth stage of each indicator crop at the end of this experiment to determine the suitability of indicator crop reference strips.

## 2.4 Tables

|  |  |  |  |
| --- | --- | --- | --- |
| Table 2-1 Soil map unit and taxonomic classification for each site, 2013 and 2014. | | | |
| Year | Location† | Soil Mapping Unit | Major Component Soil Taxonomic Classification |
| 2013 | Efaw | Easpur loam,  occasionally flooded,  0-1% slope | Easpur: *Fine-loamy, mixed, superactive, thermic   Fluventic Haplustolls* |
| LCB | Port-Oscar Complex, occasionally flooded,  0-1% slope | Port: *Fine-silty, mixed, superactive, thermic   Cumulic Haplustolls* Oscar: *Fine-silty, mixed, superactive, thermic   Typic Natrustalfs* |
| 2014 | Efaw | TBD | TBD |
| LCB | TBD | TBD |
| † Efaw, Oklahoma State University Agronomy Research Station near Stillwater, OK;   LCB, Oklahoma State University Agronomy Research Station west of Stillwater, OK near   Lake Carl Blackwell | | | |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 2-2 Pre-plant soil sample (0-15 cm) chemical properties, 2013 and 2014. | | | | | | | | |
| Year | Location† | Soil  pH‡ | NH4-N§ | NO3-N§ | P¶ | K¶ | Total N# | Organic C# |
|  |  |  | --------------- µg g-1 --------------- | | | | ----- mg g-1 ----- | |
| 2013 | Efaw | 6.0 | 8.4 | 1.7 | 18.4 | 106 | 1.2 | 10.2 |
|  | LCB | 6.1 | 6.2 | 5.3 | 24.2 | 139 | 1.1 | 9.5 |
| 2014 | Efaw | TBD | TBD | TBD | TBD | TBD | TBD | TBD |
| LCB | TBD | TBD | TBD | TBD | TBD | TBD | TBD |
| † Efaw, Oklahoma State University Agronomy Research Station near Stillwater, OK;   LCB, Oklahoma State University Agronomy Research Station west of Stillwater,  OK near Lake Carl Blackwell  ‡ 1:1 soil water  § 2 M KCl extract (Mulvaney, 1996)  ¶ Mehlich III extract (Mehlich, 1984)  # Dry combustion (Schepers et al., 1989) | | | | | | | | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Table 2-3 Corn N fertilizer recommendations from the OSU generalized algorithm based on farmer practice NDVI and N rich strip NDVI from wheat and barley indicator crops compared to the agronomic optimum N rate for Efaw and Lake Carl Blackwell (LCB). | | | | | | |
| Location | Crop | Growth Stage† | Farmer Practice NDVI | N Rich  Strip  NDVI | Generalized Algorithm N recommendation‡ | AONR§ |
| Efaw | Wheat | 3 | TBD | TBD | TBD | TBD |
|  |  | 4 | TBD | TBD | TBD | TBD |
|  |  | 5 | TBD | TBD | TBD | TBD |
|  |  | 7 | TBD | TBD | TBD | TBD |
|  |  | 10 | TBD | TBD | TBD | TBD |
|  |  | 10.5 | TBD | TBD | TBD | TBD |
|  | Barley | 3 | TBD | TBD | TBD | TBD |
|  |  | 4 | TBD | TBD | TBD | TBD |
|  |  | 5 | TBD | TBD | TBD | TBD |
|  |  | 7 | TBD | TBD | TBD | TBD |
|  |  | 10 | TBD | TBD | TBD | TBD |
|  |  | 10.5 | TBD | TBD | TBD | TBD |
| LCB | Wheat | 3 | TBD | TBD | TBD | TBD |
|  |  | 4 | TBD | TBD | TBD | TBD |
|  |  | 5 | TBD | TBD | TBD | TBD |
|  |  | 7 | TBD | TBD | TBD | TBD |
|  |  | 10 | TBD | TBD | TBD | TBD |
|  |  | 10.5 | TBD | TBD | TBD | TBD |
|  | Barley | 3 | TBD | TBD | TBD | TBD |
|  |  | 4 | TBD | TBD | TBD | TBD |
|  |  | 5 | TBD | TBD | TBD | TBD |
|  |  | 7 | TBD | TBD | TBD | TBD |
|  |  | 10 | TBD | TBD | TBD | TBD |
|  |  | 10.5 | TBD | TBD | TBD | TBD |
| † Feekes growth stages as denoted by Large (1954)  ‡ N recommendations, kg N ha-1; Assumptions: Bare soil NDVI = 0.18, Max Yield for the region = 13.44 Mg ha-1, weight per bushel = 56 lb/bu, Grain N = 1.2%, and NUE = 50 %  § AONR = agronomic optimum N rate, determined using either a quadratic plus plateau model or a linear plus plateau model, kg N ha-1 | | | | | | |

## 2.5 Figures



**Corn**

**Barley**

**Wheat**

**Wheat**



**Photo Courtesy of Jacob Bushong  
3/21/2013**

**0 kg N ha-1**

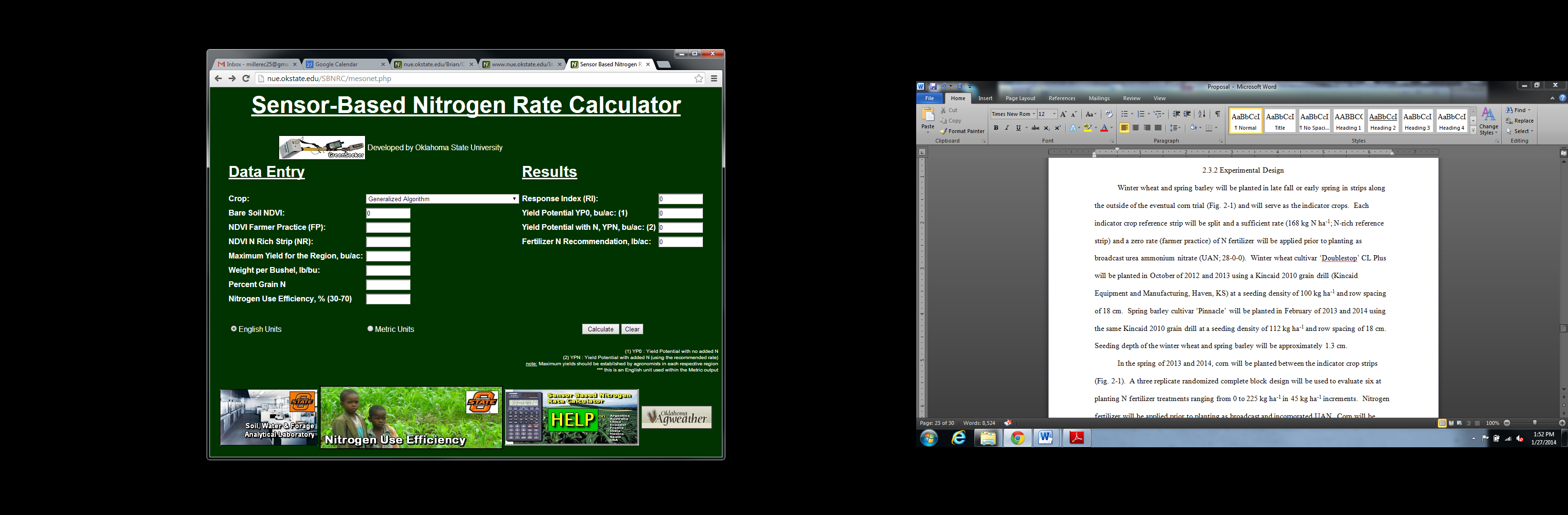
**168 kg N ha-1**

### Figure 2-1 Experimental design and field layout for the winter wheat and spring barley planted in late fall or early spring in strips along the outside of the eventual corn trial.

**Photo Courtesy of Jacob Bushong**



### Figure 2-2 Surface drip irrigation system positioned between rows 1 and 2 and between 3 and 4 of each plot of the irrigated production system.



### Figure 2-3 Generalized algorithm interface used to determine corn N rate recommendations based on farmer practice NDVI and N rich strip NDVI from wheat and barley indicator crops. (Available at <http://nue.okstate.edu/SBNRC/mesonet.php>)

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