I. NITROGEN AND WATER USE EFFICIENCY AS INFLUENCED BY MAIZE HYBRID AND IRRIGATION

II. PREDICTING PRE-PLANT NITROGEN APPLICATIONS TO MAIZE USING INDICATOR CROP N-RICH REFERENCE STRIPS

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Abstract: Agriculture is an industry with continuous advancements in technology and strategies to increase production while improving efficiency. Hard red winter wheat is a staple crop for many producers in Oklahoma. However, a dramatic increase in the commodity price of maize at the beginning of 2011 resulted in a restored interest to raise maize in the less productive, semi-arid environment of Oklahoma. Provided in this dissertation are two projects that investigate the use of technology and nitrogen fertilizer management strategies to produce maize in Oklahoma. Seed companies have recently commercialized maize hybrids which are marketed as having improved drought tolerance. Both transgenic and non-transgenic approaches have been taken to exploit improved drought tolerance in maize. In chapter one, transgenic and non-transgenic maize hybrids are compared to less drought tolerant maize hybrids. Evaluation of these hybrids grown under irrigation and with different nitrogen (N) fertilizer rates on grain yield, water use efficiency (WUE), and nitrogen use efficiency were investigated. Even with the presence of irrigation and above average rainfall, drought tolerant hybrids offer improved grain yield and WUE. The transgenic drought tolerant hybrid seemed better suited for hot and dry environments. Maize producers should consider incorporating transgenic drought tolerant maize hybrid technology into water stressed farming environments. In chapter two, winter wheat and spring barley indicator crops were used to estimate the N response of the subsequent maize crop. In-season response of the indicator crops was determined (RI_{NDVI}) and provided input values to calculate N fertilizer recommendations of the subsequent maize crop using the generalized algorithm. The agronomic optimum N rate (AONR) and response of N fertilizer at harvest ($RI_{Harvest}$) were calculated from a maize N response trial. Strong correlations existed between the N fertilizer recommendation generated from the generalized algorithm and the AONR for the maize along with the RI_{NDVI} for wheat and barley and $RI_{Harvest}$ for the maize. The use of indicator crops to predict the response of a maize crop to N fertilizer is unprecedented and modifications to current maize N fertilizer recommendations could modernize N management strategies for all producers.

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CHAPTER 1

NITROGEN AND WATER USE EFFICIENCY AS INFLUENCED BY MAIZE HYBRID AND IRRIGATION

1.1 Abstract

The drought experienced in the United States in 2012 was the most severe encountered over the past 25 years and resulted in reduced maize (*Zea mays* L.) grain yields. Thus, it is important to investigate how new drought tolerant maize hybrids influence water use efficiency (WUE) in drought environments. The objective of this research was to evaluate WUE and N use efficiency (NUE) of drought tolerant and less drought tolerant maize hybrids in irrigated and dryland production systems. Beginning in 2013 and continuing through 2014, two maize hybrids designated as drought tolerant (one non-transgenic Pioneer AQUAmax hybrid and one transgenic Monsanto Droughtgard hybrid) were compared with two hybrids having less drought tolerance at two locations in north-central Oklahoma. Grain yield, WUE, and NUE were determined at harvest. Overall, irrigation had a tendency to increase grain yield, WUE, and NUE at all locations. Improved grain yield was observed with the drought tolerant Monsanto

hybrid compared to the drought tolerant Pioneer hybrid. Water use efficiency was influenced by grain yield and the interaction between WUE and NUE by hybrid proved to be weak across all eight sites. In the presence of irrigation and above average rainfall, drought tolerant hybrids increased grain yield and WUE. The transgenic drought tolerant hybrid seemed better suited for hot and dry environments. These data would suggest maize producers should consider incorporating transgenic drought tolerant maize hybrid technology into water stressed farming environments.

1.2 Introduction

The drought experienced in the United States in 2012 was the most severe encountered over the past 25 years (USDA-ERS, 2012). Areas within the Great Plains are still experiencing severe to exceptional drought (Fuchs, 2014). Despite the 2012 drought, maize (*Zea mays* L.) growers in the United States produced 274,078,246 Mg of maize grain with an estimated average grain yield of 7.74 Mg ha^{-1} (USDA-NASS, 2013). In comparison, average grain yield in 2011, without the effect of drought, was 9.23 Mg ha^{-1} and total production was 13 percent higher than in 2012 (USDA-NASS, 2013). 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 USD in crop insurance indemnity payments calculated by the Congressional Budget Office (Delisle, 2013).

Variability in precipitation is a normal part of the North American climate, but many dryland maize 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 regional 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. The authors explain that the interaction of water stress and high temperatures during pollination and grain set could be damaging to crop production and food security. As a challenge to agronomists, the authors concluded 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).

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.

Maize 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 and reduced kernel set 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 (WUE) has been a main point of emphasis for many breeding programs in the United States and abroad.

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 there is an opportunity for substantial genetic improvement.

DuPont Pioneer (DuPont Pioneer Hi-Bred Intl., Inc., Johnston, IA) is a seed company which has focused its research to develop drought tolerant maize hybrids using conventional breeding (Butzen and Schussler, 2009). Researchers from this company have developed drought tolerant maize 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 maize 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) acknowledged that the energy required to establish an improved root system may decrease above ground growth, but they were certain that extensive testing would result in higher yields in all environments. A highly efficient root system balances both shallow, immobile nutrient-mining roots and deep, watermining roots without an overly abundant root system, which would be too high of a respiratory cost for a drought stressed plant (Ho et al., 2005). Efficiencies can be achieved through root matter distribution between shallow and deep roots and also by selecting for increased parenchyma, 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 at the International Maize and Wheat Improvement Center in Mexico have worked to narrow the ASI in lowland tropical maize. Their work 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.

Scientists at the Monsanto (Monsanto Company, St. Louis, MO) and BASF (BASF Corporation, Florham Park, NJ) companies have discovered a transgene which can stabilize maize 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 maize, 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). Maize 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 maize 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 (a 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.

Water use efficiency can be defined differently depending on the objective and application. For example, plant physiologists measure WUE as the amount of photosynthesis per unit of water used in transpiration, whereas, farmers and agronomists may measure WUE as maize 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).

Many of the plant vegetative and reproductive processes are dependent upon a sufficient water supply, and so logically, adequate rainfall or irrigated production systems increase maize 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 some proteins are stored in the grain. The efficiency at which N is taken up from the soil and used to produce grain is characterized as nitrogen use efficiency (NUE). Nitrogen use efficiency is defined as the amount of maize grain produced per unit of nitrogen (N) available in the soil (Moll et al., 1982) or as the percent of N recovered in the maize 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 maize 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 maize hybrids. Research conducted in spring wheat has shown that under drought conditions, plants have the ability to improve nutrient uptake by increasing root respiration which increases nutrient solubility (Liu et al., 2004). Evaluations of maize hybrids in Indiana reported that hybrid response to increased rates of N fertilizer were similar for drought tolerant and conventional maize hybrids (Roth et al., 2013). A study conducted in Nebraska using hybrids with different NUE histories 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).

The functions of water in a maize 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 from basic research 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 maize 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 maize 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 significantly better drought tolerance for US production in the future. Thus, it is important to investigate how new drought tolerant maize hybrids influence WUE and NUE in the water limited environments of Oklahoma.

1.3 Objective

The objective of this study was to evaluate WUE and NUE of drought tolerant and less drought tolerant maize hybrids in irrigated and dryland production systems.

1.4 Materials and Methods

Field experiments were established in 2013 and 2014 at the Efaw (36.081118^{o}, -97.063270^{o}, elevation 272 m above sea level) agronomy research station near Stillwater, OK and Lake Carl Blackwell (LCB; 36.090792^{o}, -97.172486^{o}, elevation 293 m above sea level) agronomy research station west of Stillwater, OK near Lake Carl Blackwell (Table 1-1). All soil fertility parameters were managed to ensure N was the only limiting nutrient (Table 1-2). A summary of field activities for each cropping year including; soil sampling, planting, fertilization, irrigation, rainfall, and harvest are provided in Table 1-3.

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 were utilized in this study. At each location, hybrids and N rates were randomly assigned within both an irrigated and dryland production system. In 2013, two maize hybrids designated as drought tolerant (DuPont Pioneer AQUAmax brand P1498 YHR and Monsanto Dekalb Genuity DroughtGard brand DKC63-55 GENDGVT2P) were 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. In 2014, two maize hybrids designated as drought tolerant (DuPont Pioneer AQUAmax brand P1498 AM and Monsanto Dekalb Genuity DroughtGard brand DKC63-55 GENDGVT2P) were compared with two hybrids with less drought tolerance (DuPont Pioneer brand P1234 AM and Monsanto Dekalb brand DKC62-08 GENSS). Drought tolerance scores for P1498 and P1234 were 9 and predicts above average, respectively and for DKC63-55 and DKC62-08 were 1 and 3, respectively. For discussion of these hybrids, the following abbreviations will be used: P1 = P1498, P2 = P1395 or P1234, M1 = DKC63-55, and M2 = DKC62-09 or DKC62-08. Three different fertilizer N rates were used in each production system based on expected grain yield and N removal in the grain as described by Zhang and Raun, 2006. The N rates for the irrigated production system were 0 (Low), 101 (Med), and 202 (High) kg ha^{-1} and for the dryland production system were 0 (Low), 67 (Med), and 134 (High) kg ha^{-1}. Nitrogen fertilizer was applied prior to planting as broadcast and incorporated urea ammonium nitrate (UAN; 28-0-0). Planting densities for each production system were different based on best management practices. The irrigated production system was planted at 75,650 seeds ha^{-1} and the dryland production system was planted at 53,800 seeds ha^{-1}. Plots were 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 measured 3 m wide (four 0.76 m rows) by 6.1 m long.

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

Soil moisture content (SWC) of the top 1 m soil profile depth was determined prior to planting and immediately following grain harvest. Soil cores were collected using a tractor mounted Giddings hydraulic soil sampler (Giddings Machine Company, Windsor, CO) and were used to determine SWC by the direct gravimetric method (Gardner, 1986) and soil bulk density (Blake and Hartge, 1986). Volumetric soil water content ($mL\ mL^{-1}$) was calculated as the product of the gravimetric soil water content ($g\ g^{-1}$) and soil bulk density ($g\ cm^{-3}$). Prior to planting, four soil cores were collected from each production system to a depth of approximately 1 m. Soil cores were weighed directly following collection, dried in a forced air oven at 60 ^{o}C for 72 hours, and weighed for an oven-dry soil weight. A single soil core was collected from each plot following grain harvest using the same method and was used to determine the seasonal change in SWC.

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

$$ET = \pm \Delta SWC + R + I$$

where ΔSWC 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, and I is the amount of irrigation water applied (mm). Daily rainfall was recorded from an automated weather station (2 km from the field experiment) and data files were downloaded (Oklahoma Mesonet, 2014) to determine cumulative rainfall. Water use efficiency (kg ha^{-1} m^{-1}) was calculated as the ratio between grain yield (kg ha^{-1}) and evapotranspiration (m) for each plot.

A daily water balance was created for each location to interpret the influence of daily weather and irrigation on potential evapotranspiration (Fig. 1-1 and 1-2). Daily potential evapotranspiration (PET) values were determined from the American Society of Civil Engineers standardized reference evapotranspiration equation (Walter et al., 2002). Daily PET and rainfall data files were downloaded from an adjacent climate monitoring site (Oklahoma Mesonet, 2014).

At physiological maturity, mechanical grain harvest was 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 were harvested and grain yield (Mg ha^{-1}) was adjusted to 155 g kg^{-1} moisture content. A subsample of the grain harvested from each plot was collected, oven dried at 60 ^{o}C until a constant dry weight was achieved, and ground to pass through a 140 mesh screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Grain samples were 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}) was calculated as the product of grain yield (kg ha^{-1}) and grain N content. Nitrogen use efficiency was calculated using the difference method described by Varvel and Peterson (1991).

Statistical analysis was conducted by year, location, and production system for the combination of hybrid and N rate treatments. Each production system was analyzed separately due to the different management practices utilized. Analysis of variance (ANOVA) was performed using the SAS PROC GLM procedure (SAS Institute, 2011) to detect significant differences for the main and interactive treatment effects on grain yield, WUE, and NUE. Single degree-of-freedom contrasts were utilized to partition treatment means and statistical differences. A linear regression model, using the SAS PROC REG procedure (SAS Institute, 2011), was utilized to identify a relationship between WUE and NUE by hybrid for each site. The coefficient of determination (R^2) from the model was used to identify the amount of variation which could be accounted for by the NUE to predict WUE. Significant differences were declared at $\alpha = 0.05$.

1.5 Results

Efaw - Irrigated, 2013

Water Balance. Irrigation water was applied beginning at late vegetative growth (V10; Abendroth et al., 2011) and continued through pollination, ceasing at approximately maize kernel milk stage (R3). Irrigation totaled 46 mm over that four week duration and accounted for seven percent of the seasonal PET (Table 1-3).

Irrigation began at approximately the same time the water balance fell below its initial point (Fig. 1-1). Irrigation and rainfall accounted for almost 99 percent of seasonal PET.

Grain Yield. Maize grain yield values ranged from 5.4 to 9.4 Mg ha^{-1} across all treatments (Table 1-4). Effects of hybrid, N rate, and hybrid x N rate were not significant for grain yield (Table 1-4). A significant increase in grain yield (1.6 Mg ha^{-1}) was observed for the Monsanto hybrids (M1 and M2) compared to the Pioneer hybrids (P1 and P2) using single degree-of-freedom contrasts (Table 1-4).

WUE. Maize WUE values ranged from 0.96 to 1.70 kg ha^{-1} m^{-1} across all treatments (Table 1-5). Effects of hybrid, N rate, and hybrid x N rate were not significant for WUE (Table 1-5). A significant increase in WUE (0.32 kg ha^{-1} m^{-1}) was observed for the Monsanto hybrids (M1 and M2) compared to the Pioneer hybrids (P1 and P2) using single degree-of-freedom contrasts (Table 1-5).

NUE. Maize NUE values ranged from 0 to 47 percent across all treatments (Table 1-6). Effects of hybrid, N rate, and hybrid x N rate were not significant for NUE (Table 1-6). A significant increase in NUE (25%) was observed for the drought tolerant Pioneer hybrid (P1) compared to the drought tolerant Monsanto hybrid (M1) using single degree-of-freedom contrasts (Table 1-6).

WUE x NUE Interaction. The linear regression model established that NUE could not statistically predict WUE for any of the hybrids (Table 1-7). All hybrids had a positive slope, but significantly less than one (Table 1-7).

Efaw - Dryland, 2013

Grain Yield. Maize grain yield values ranged from 2.8 to 5.2 Mg ha^{-1} across all treatments (Table 1-4). Effects of hybrid, N rate, and hybrid x N rate were not significant for grain yield (Table 1-4). A significant increase in grain yield (1.2 Mg ha^{-1}) was observed for the drought tolerant Monsanto hybrid (M1) compared to the drought tolerant Pioneer hybrid (P1) using single degree-of-freedom contrasts (Table 1-4).

WUE. Maize WUE values ranged from 0.58 to 1.09 kg ha^{-1} m^{-1} across all treatments (Table 1-5). Effects of hybrid, N rate, and hybrid x N rate were not significant for WUE (Table 1-5). A significant increase in WUE (0.24 kg ha^{-1} m^{-1}) was observed for the drought tolerant Monsanto hybrid (M1) compared to the drought tolerant Pioneer hybrid (P1) using single degree-of-freedom contrasts (Table 1-5).

NUE. Maize NUE values ranged from 1.0 to 19 percent across all treatments (Table 1-6). Effects of hybrid, N rate, and hybrid x N rate were not significant for NUE, nor were the single degree-of-freedom contrast comparisons (Table 1-6). Treatment differences were difficult to discern due to high experimental error (CV, 139%).

WUE x NUE Interaction. The linear regression model established that NUE could statistically predict WUE for the less drought tolerant Monsanto hybrid (M2) and NUE accounted for 68% of the variability in WUE for that hybrid (Table 1-7). All hybrids had a positive slope, but significantly less than one (Table 1-7).

Lake Carl Blackwell - Irrigated, 2013

Water Balance. Irrigation was initiated during late vegetative growth (V12) and continued through pollination, ceasing at approximately maize kernel milk stage (R3).

Minimal irrigation water was applied (28 mm) due to significant seasonal rainfall events totaling 827 mm (Fig. 1-2). Together, irrigation and rainfall surpassed seasonal PET by 200 mm (Table 1-3). Over 250 mm of rain fell during the middle and late maize grain fill reproductive stages (R4 to R6). As a result of the unseasonable rainfall, the water balance only fell below its initial point for approximately three weeks during the entire growing season with only 2 days occurring during reproductive growth (Fig. 1-2).

Grain Yield. Maize grain yield values ranged from 5.0 to 11.2 Mg ha^{-1} across all treatments (Table 1-4). The main effect of N rate was significant for grain yield (Table 1-4). Single degree-of-freedom contrasts indicated that N rate exhibited a quadratic trend. The medium and high N rates yielded 3.0 Mg ha^{-1} more compared to the low N rate and the high N rate yielded 0.5 Mg ha^{-1} more compared to the medium N rate (Table 1-4). The main effect of hybrid and the hybrid x N rate interaction were not significant for grain yield (Table 1-4). A significant increase in grain yield (1.1 Mg ha^{-1}) was observed for the Monsanto hybrids (M1 and M2) compared to the Pioneer hybrids (P1 and P2) using single degree-of-freedom contrasts (Table 1-4).

WUE. Maize WUE values ranged from 0.64 to 1.44 kg ha^{-1} m^{-1} across all treatments (Table 1-5). The main effect of N rate was significant for WUE (Table 1-5). Single degree-of-freedom contrasts indicated that N rate exhibited a quadratic trend. The medium and high N rates increased WUE 0.40 kg ha^{-1} m^{-1} compared to the low N rate and the high N rate increased WUE 0.07 kg ha^{-1} m^{-1} compared to the medium N rate (Table 1-5). The main effect of hybrid and the hybrid x N rate interaction were not significant for WUE (Table 1-5). A significant increase in WUE (0.14 kg ha^{-1} m^{-1}) was observed for the Monsanto hybrids (M1 and M2) compared to the Pioneer hybrids (P1 and P2) using single degree-of-freedom contrasts (Table 1-5).

NUE. Maize NUE values ranged from 12 to 44 percent across all treatments (Table 1-6). Effects of hybrid, N rate, and hybrid x N rate were not significant for NUE, nor were the single degree-of-freedom contrast comparisons (Table 1-6).

WUE x NUE Interaction. The linear regression model established that NUE could not statistically predict WUE for any of the hybrids (Table 1-7). All hybrids had a positive slope, but significantly less than one (Table 1-7).

<u>Lake Carl Blackwell - Dryland, 2013</u>

Grain Yield. Maize grain yield values ranged from 0.9 to 3.3 Mg ha^{-1} across all treatments (Table 1-4). Effects of hybrid, N rate, and hybrid x N rate were not significant for grain yield, nor were the single degree-of-freedom contrast comparisons (Table 1-4). Treatment differences were difficult to discern due to high experimental error (CV, 80%).

WUE. Maize WUE values ranged from 0.12 to 0.44 kg ha^{-1} m^{-1} across all treatments (Table 1-5). Effects of hybrid, N rate, and hybrid x N rate were not significant for WUE, nor were the single degree-of-freedom contrast comparisons (Table 1-5). Treatment differences were difficult to discern due to high experimental error (CV, 80%).

NUE. Maize NUE values ranged from 1.0 to 28 percent across all treatments (Table 1-6). Effects of hybrid, N rate, and hybrid x N rate were not significant for NUE, nor were the single degree-of-freedom contrast comparisons (Table 1-6). Treatment differences were difficult to discern due to high experimental error (CV, 153%).

WUE x NUE Interaction. The linear regression model established that NUE could statistically predict WUE for all of the hybrids and NUE accounted for over 76 percent of the variability in WUE for all of the hybrids (Table 1-7). All hybrids had a positive slope, but significantly less than one (Table 1-7).

<u>Efaw - Irrigated, 2014</u>

Water Balance. Irrigation was initiated during early vegetative growth (V6) and continued through the silking growth stage (R1). Irrigation totaled 28 mm over that eight week period and accounted for four percent of the seasonal PET (Table 1-3). Irrigation began just before the water budget fell below zero (Fig. 1-1). The water budget was below zero during the reproductive growth stages, but over 200 mm of rain fell during that same timeframe resulting in a stable water balance during grain fill (Fig. 1-1).

Grain Yield. Maize grain yield values ranged from 2.1 to 9.9 Mg ha^{-1} across all treatments (Table 1-4). The main effect of N rate was significant for grain yield (Table 1-4). Single degree-of-freedom contrasts indicated that N rate exhibited a quadratic trend. The medium and high N rates yielded 3.9 Mg ha^{-1} more compared to the low N rate and the high N rate yielded 2.6 Mg ha^{-1} more compared to the medium N rate (Table 1-4). The main effect of hybrid and the hybrid x N rate interaction were not significant for grain yield, nor were the single degree-of-freedom contrast comparisons (Table 1-4).

WUE. Maize WUE values ranged from 0.56 to 2.92 kg ha^{-1} m^{-1} across all treatments Table 1-5). The main effect of N rate was significant for WUE (Table 1-5). A quadratic increase in WUE was observed for N rate. The medium and high N rates increased WUE 1.25 kg ha^{-1} m^{-1} compared to the low N rate and the high N rate increased

WUE 0.76 kg ha^{-1} m^{-1} compared to the medium N rate (Table 1-5). The main effect of hybrid and the hybrid x N rate interaction were not significant for WUE, nor were the single degree-of-freedom contrast comparisons (Table 1-5).

NUE. Maize NUE values ranged from 26 to 44 percent across all treatments (Table 1-6). Effects of hybrid, N rate, and hybrid x N rate were not significant for NUE, nor were the single degree-of-freedom contrast comparisons (Table 1-6).

WUE x NUE Interaction. The linear regression model established that NUE could statistically predict WUE for the drought tolerant and less drought tolerant Monsanto hybrids (M1 and M2) and NUE accounted for over 66 percent of the variability in WUE for those hybrids (Table 1-7). All hybrids had a positive slope, but significantly less than one (Table 1-7).

Efaw - Dryland, 2014

Grain Yield. Maize grain yield values ranged from 2.2 to 6.5 Mg ha^{-1} across all treatments (Table 1-4). The main effect of N rate was significant for grain yield (Table 1-4). Single degree-of-freedom contrasts indicated that N rate exhibited a quadratic trend. The medium and high N rates yielded 2.8 Mg ha^{-1} more compared to the low N rate and the high N rate yielded 1.4 Mg ha^{-1} more compared to the medium N rate (Table 1-4). The main effect of hybrid was significant for grain yield (Table 1-4). The less drought tolerant Monsanto hybrid (M2) yielded 1.0 Mg ha^{-1} more compared to the less drought tolerant Pioneer hybrid (P2). A significant increase in grain yield (0.8 Mg ha^{-1}) was observed for the Monsanto hybrids (M1 and M2) compared to the Pioneer hybrids (P1 and P2) using single degree-of-freedom contrasts (Table 1-4).

WUE. Maize WUE values ranged from 0.61 to 2.06 kg ha^{-1} m^{-1} across all treatments (Table 1-5). The main effect of N rate was significant for WUE (Table 1-5). Single degree-of-freedom contrasts indicated that N rate exhibited a quadratic trend. The medium and high N rates increased WUE 0.86 kg ha^{-1} m^{-1} compared to the low N rate and the high N rate increased WUE 0.42 kg ha^{-1} m^{-1} compared to the medium N rate (Table 1-5). The main effect of hybrid was significant for WUE (Table 1-5). The less drought tolerant Monsanto hybrid (M2) increased WUE 0.37 kg ha^{-1} m^{-1} compared to the less drought tolerant Pioneer hybrid (P2). A significant increase in WUE (0.26 kg ha^{-1} m^{-1}) was observed for the Monsanto hybrids (M1 and M2) compared to the Pioneer hybrids (P1 and P2) using single degree-of-freedom contrasts (Table 1-5).

NUE. Maize NUE values ranged from 26 to 39 percent across all treatments (Table 1-6). Effects of hybrid, N rate, and hybrid x N rate were not significant for NUE, nor were the single degree-of-freedom contrast comparisons (Table 1-6).

WUE x NUE Interaction. The linear regression model established that NUE could not statistically predict WUE for any of the hybrids (Table 1-7). All hybrids had a positive slope and significantly less than one with the exception of the less drought tolerant Pioneer hybrid (P2) which had a negative slope and significantly less than one (Table 1-7).

Lake Carl Blackwell - Irrigated, 2014

Water balance. Irrigation was applied only during early vegetative growth (V7 to V9) due to a significant rainfall event (105 mm) occurring directly after that point (Fig. 1-2). Irrigation only totaled 8 mm, but irrigation and rainfall accounted for almost 74 percent of seasonal PET (Table 1-3). The water balance did not fall below zero throughout the entire growing season and almost 250 mm of rain fell during the reproductive growth stages (Fig. 1-2).

Grain Yield. Maize grain yield values ranged from 3.5 to 10.7 Mg ha^{-1} across all treatments (Table 1-4). The main effect of N rate was significant for grain yield (Table 1-4). Single degree-of-freedom contrasts indicated that N rate exhibited a quadratic trend. The medium and high N rates yielded 5.2 Mg ha^{-1} more compared to the low N rate and the high N rate yielded 1.4 Mg ha^{-1} more compared to the medium N rate (Table 1-4). The main effect of hybrid and the hybrid x N rate interaction were not significant for grain yield, nor were the single degree-of-freedom contrast comparisons (Table 1-4).

WUE. Maize WUE values ranged from 0.78 to 2.39 kg ha^{-1} m^{-1} across all treatments (Table 1-5). The main effect of N rate was significant for WUE (Table 1-5). Single degree-of-freedom contrasts indicated that N rate exhibited a quadratic trend. The medium and high N rates increased WUE 1.25 kg ha^{-1} m^{-1} compared to the low N rate and the high N rate increased WUE 0.29 kg ha^{-1} m^{-1} compared to the medium N rate (Table 1-5). The main effect of hybrid and the hybrid x N rate interaction were not significant for WUE, nor were the single degree-of-freedom contrast comparisons (Table 1-5).

NUE. Maize NUE values ranged from 43 to 81 percent across all treatments (Table 1-6). Effects of hybrid, N rate, and hybrid x N rate were not significant for NUE (Table 1-6). A significant increase in NUE (22%) was observed for the medium N rate compared to the high N rate using single degree-of-freedom contrasts (Table 1-6).

WUE x NUE Interaction. The linear regression model established that NUE could not statistically predict WUE for any of the hybrids (Table 1-7). All hybrids had a positive slope and significantly less than one with the exception of the less drought tolerant Monsanto hybrid (M2) which had a negative slope and significantly less than one (Table 1-7).

<u>Lake Carl Blackwell - Dryland, 2014</u>

Grain Yield. Maize grain yield values ranged from 2.6 to 8.0 Mg ha^{-1} across all treatments (Table 1-4). The main effect of N rate was significant for grain yield (Table 1-4). Single degree-of-freedom contrasts indicated that N rate exhibited a quadratic trend. The medium and high N rates yielded 3.6 Mg ha^{-1} more compared to the low N rate and the high N rate yielded 0.1 Mg ha^{-1} more compared to the medium N rate (Table 1-4). The main effect of hybrid and the hybrid x N rate interaction were not significant for grain yield (Table 1-4). A significant increase in grain yield (0.1 Mg ha^{-1}) was observed for the drought tolerant Pioneer hybrid (P1) compared to the less drought tolerant Pioneer hybrid (P2) using single degree-of-freedom contrasts (Table 1-4).

WUE. Maize WUE values ranged from 0.56 to 1.90 kg ha^{-1} m^{-1} across all treatments (Table 1-5). The main effect of N rate was significant for WUE (Table 1-5). Single degree-of-freedom contrasts indicated that N rate exhibited a quadratic trend. The medium and high N rates increased WUE 0.87 kg ha^{-1} m^{-1} compared to the low N rate and the high N rate increased WUE 0.08 kg ha^{-1} m^{-1} compared to the medium N rate (Table 1-5). The main effect of hybrid and the hybrid x N rate interaction were not significant for WUE (Table 1-5). A significant increase in WUE (0.04 kg ha^{-1} m^{-1}) was observed for the drought tolerant Pioneer hybrid (P1) compared to the less drought tolerant Pioneer hybrid (P2) using single degree-of-freedom contrasts (Table 1-5).

NUE. Maize NUE values ranged from 37 to 82 percent across all treatments (Table 1-6). The main effect of N rate was significant for NUE (Table 1-6). A significant increase in NUE (31%) was observed for the medium N rate compared to the high N rate using single degree-of-freedom contrasts (Table 1-6). The main effect of hybrid and the hybrid x N rate interaction were not significant for NUE (Table 1-6).

WUE x NUE Interaction. The linear regression model established that NUE could statistically predict WUE for the drought tolerant Monsanto hybrid (M1) and NUE accounted for 71 percent of the variability in WUE for that hybrid (Table 1-7). Both drought tolerant hybrids (P1 and M1) had a positive slope, but significantly less than one and both less drought tolerant hybrids (P2 and M2) had a negative slope, but significantly less than one (Table 1-7).

1.6 Discussion

Cumulative rainfall throughout the 2013 growing season (first of April through the end of August) was 20 percent above the 21 year average at Efaw and 58 percent above the seven year average at LCB (Oklahoma Mesonet, 2014). However, a period of 26 days from the middle of June through the middle of July only had 8.1 mm of rainfall at Efaw and 3.0 mm at LCB. This period of dry weather coincided with maize pollination, thus having the potential to reduce grain yield (Denmead and Shaw, 1960; Grant et al., 1989). To mitigate these effects, nearly 90 percent of the irrigation water was applied during this timeframe at both irrigated production system sites. This deficit irrigation management strategy (Pandey et al., 2000) reduced maize water consumption and maintained similar grain yield levels versus well irrigated controls (Kang et al., 2000; Payero et al., 2006).

In 2014, cumulative rainfall at Efaw was 30 percent below average and nearly average at LCB (Oklahoma Mesonet, 2014). Only 20 percent of the irrigation water was applied at Efaw during the reproductive growth stages due to an 18 percent higher than average cumulative rainfall total for the months of June and July. At LCB, all of the irrigation water was applied during vegetative growth due to over 70 percent of the seasonal rain falling in the months of June and July (during pollination).

Even with above average rainfall in 2013, on average, the irrigated production system yielded nearly twice as much at Efaw and over 2.5 times as much at LCB compared to the dryland production system sites (Table 1-4). The application of irrigation water at critical growth stages has been shown to optimize grain yield (Singh and Singh, 1995). However, the above average rainfall could have masked the effects of the drought tolerant hybrids. Research and product advancement strategies employed by seed companies to improve drought tolerant hybrids are twofold. First, the drought tolerant hybrids must increase grain yield when water is limiting, but also, grain yield of drought tolerant hybrids must be competitive when growing conditions are better and water is not limiting (Castiglioni et al., 2008; Butzen and Schussler, 2009).

For the conditions in 2013 with higher than average rainfall, the drought tolerant hybrids for each company (M1 and P1) tended to yield lower (1.0 to 0.9 Mg ha^{-1}) at Efaw or have little difference (0.5 to 0.1 Mg ha^{-1}) at LCB compared to their less drought tolerant counterpart (M2 or P2) for the irrigated production systems (Table 1-4). Two other field studies exhibited similar results in similar conditions; a less drought tolerant Pioneer hybrid yielded 0.8 Mg ha^{-1} more compared to a drought tolerant Pioneer hybrid (Roth et al., 2013) and a transgenic drought tolerant hybrid yielded similar to a nontransgenic isoline (Chang et al., 2014). Drought tolerant hybrid M1 yielded 0.8 Mg ha^{-1} higher at Efaw and 0.7 Mg ha^{-1} higher at LCB compared to the less drought tolerant hybrid M2 whereas drought tolerant hybrid P1 yielded 0.8 Mg ha^{-1} less compared to the less drought tolerant hybrid P2 at Efaw in the dryland production system (Table 1-4). The Monsanto drought tolerant hybrid (M1) had a tendency to yield higher (1.6 to 0.9 Mg ha^{-1}) compared to the Pioneer drought tolerant hybrid (P1) for all sites in 2013 (Table 1-4).

In 2014, differences in grain yield between the irrigated and dryland production systems were much less pronounced with both Efaw and LCB having approximately 25 percent higher grain yields for the irrigated production system sites (Table 1-4). Variability in grain yield response to the four hybrids could be a result of the timing and duration of water stress throughout the growing season. Bruce et al. (2002) described that water stress occurring one week prior to pollination resulted in grain yield differences between drought tolerant hybrids whereas water stress three weeks prior to pollination did not result in grain yield differences. Across all sites, the drought tolerant hybrids (M1 and P1) tended to have higher yield (0.8 to 0.1 Mg ha^{-1}) compared to the less drought tolerant hybrids (M2 and P2). Although not significant, the Efaw irrigated and

LCB dryland sites, the drought tolerant Monsanto hybrid (M1) increased yield (1.6 to 1.1 Mg ha^{-1}) compared to the less drought tolerant Monsanto hybrid (M2), but the opposite occurred at the other two sites (Table 1-4). With the exception of the LCB irrigated site, the Monsanto drought tolerant hybrid (M1) had a tendency to yield higher (0.9 to 0.5 Mg ha^{-1}) compared to the Pioneer drought tolerant hybrid (P1) for three of the sites in 2014 (Table 1-4).

The application of pre-plant N fertilizer significantly increased grain yield with a quadratic trend for five of the eight sites in this experiment (Table 1-4). Similar findings from a field study in Texas found that excessive N fertilizer did not reduce grain yields with severe water stress, and that N fertilizer rates should not be decreased to reduce water stress (Eck, 1984). The remaining three sites, without a response to pre-plant N fertilizer applications, occurred in 2013. The unseasonably high rainfall totals experienced during that growing season, resulted in saturated soils, and may have promoted N fertilizer loss via denitrification and leaching. The lack of a two-way interaction suggests that all four hybrids yielded similarly across all N rates. Similar results were reported from a maize study in Indiana (having drought tolerant and less drought tolerant Pioneer hybrids) where all hybrids responded similarly to four N rates over two years (Roth et al., 2013).

Water use efficiency values obtained for all eight sites in this experiment fall within the range reported in a review of 27 global maize experiments (Zwart and Bastiaanssen, 2004). The WUE values measured in the irrigated production systems tended to be higher than the dryland production systems for any location (Table 1-5). A similar trend was described in the review by Zwart and Bastiaanssen, (2004) who explained that WUE can be maximized with fewer irrigation water applications at more precise timings, a strategy similar to deficit irrigation management. A field study in Kansas found the largest increase in WUE resulted from a single irrigation event consisting of 150 mm of water (Norwood, 2000). The 0.34 kg ha^{-1} m^{-1} increase in WUE corresponded to a 2.95 Mg ha^{-1} increase in grain yield (Norwood, 2000). Another field study in Nebraska using subsurface drip irrigation observed a strong linear relationship ($R^2 = 0.95$) between WUE and grain yield (Payero et al., 2009). The largest WUE increase obtained in this study was 0.38 kg ha^{-1} m^{-1} and corresponded to a 1.6 Mg ha^{-1} increase in grain yield between the drought tolerant Monsanto hybrid (M1) and the less drought tolerant Monsanto hybrid (M2). The application of pre-plant N fertilizer significantly increased WUE at five of the eight sites in this experiment (Table 1-4). A similar trend was observed from a field study in South Dakota that reported a 31 percent increase in WUE with the application of 112 kg N ha^{-1} (Kim et al., 2008). Water use efficiency treatment differences for hybrid, N rate, and the interaction follow the same trend as observed with grain yield (Table 1-5). The lack of treatment differences observed in ET (data not reported) resulted in differences for WUE being highly influenced by differences in grain yield.

Nitrogen use efficiency values were highly variable across all eight sites, ranging from 0 to 82 percent. The NUE values obtained in this experiment are very similar to other maize studies with pre-plant N fertilizer applications in Oklahoma (Walsh et al., 2012). As expected, irrigation had a tendency to increase NUE values compared to dryland production at all sites (Table 1-6) due to improved plant N uptake and subsequent grain yield response (Al-Kaisi and Yin, 2003). One trend observed for NUE was that the less drought tolerant hybrids (P2 and M2), when pooled together, did tend to increase NUE (4.8 to 3.4 percent) compared to the drought tolerant hybrids (P1 and M1) at all four dryland production system sites (Table 1-6). Another trend was that the less drought tolerant Monsanto hybrid (M2) increased NUE (0.5 to 3.9 percent at Efaw and 11 to 15 percent at LCB) compared to the less drought tolerant Pioneer hybrid (P2) at the irrigated production sites (Table 1-6). The largest increases in NUE occurred at the 2013 Efaw irrigated site where the drought tolerant Pioneer hybrid (P1) was 25 percent higher compared to the drought tolerant Monsanto hybrid (M1) and 22 percent higher compared to the less drought tolerant Pioneer hybrid (P2; Table 1-6). As would be expected, NUE values for the medium N rate were higher than for the high N rate at six of the eight sites (Table 1-6).

The interaction between WUE and NUE by hybrid, determined using linear regression, proved to be weak across all eight sites (Table 1-7). Only 38 percent of the regression models resulted in NUE accounting for more than 50 percent of the variability in WUE (Table 1-7). The less drought tolerant Pioneer hybrid (P2) had the best relationship, compared to the other hybrids, with a R^2 greater than 0.5 at four of the sites while the drought tolerant Pioneer hybrid (P1) had the poorest relationship with a R^2 greater than 0.5 at only one site (Table 1-7). Other work investigating the interaction between WUE and NUE in maize suggests a synergistic relationship exists between water and N (Kim et al., 2008). These authors developed a conceptual model to further explain that the addition of N fertilizer would increase WUE and the addition of water would increase NUE (Kim et al., 2008).

1.7 Conclusions

Monsanto hybrids yielded slightly better than the Pioneer hybrids, and few differences in grain yield were detected between drought tolerant and less drought tolerant hybrids at the irrigated sites. Nitrogen use efficiency was variable between all hybrids, but the less drought tolerant Monsanto hybrid was higher (up to 15 percent) compared to the less drought tolerant Pioneer hybrid. At the dryland sites, the drought tolerant Monsanto hybrid yielded better than both the less drought tolerant Monsanto hybrid and the drought tolerant Pioneer hybrid. The less drought tolerant hybrids tended to increase NUE (almost 5 percent) compared to the drought tolerant hybrids.

Overall, irrigation increased grain yield, WUE, and NUE at all four locations. Grain yield was increased with the drought tolerant Monsanto hybrid versus the drought tolerant Pioneer hybrid. Water use efficiency was highly influenced by grain yield and the interaction between WUE and NUE by hybrid proved to be rather weak. Even in the presence of irrigation and above average rainfall, drought tolerant hybrids offer improved grain yield and WUE while the transgenic drought tolerant hybrid seemed better suited for hot and dry environments. These data would suggest maize producers should consider incorporating transgenic drought tolerant maize hybrid technology into their Oklahoma farming practices.

1.8 Tables

Table 1-1 Soil map unit and taxonomic classification for each location, 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, 0-1% slope, occasionally flooded	Port: *Fine-silty, mixed, superactive, thermic Cumulic Haplustolls* Oscar: *Fine-silty, mixed, superactive, thermic Typic Natrustalfs*
2014	Efaw	Easpur loam, 0-1% slope, occasionally flooded	Easpur: *Fine-loamy, mixed, superactive, thermic, Fluventic Haplustolls*
	LCB	Pulaski fine sandy loam, 0-1% slope, occasionally flooded	Pulaski: *Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents*

† Efaw, Oklahoma Agricultural Experiment Station near Stillwater, OK;
LCB, Oklahoma Agricultural Experiment 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‡	NH_4-N§	NO_3-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	33	131	1.2	9.7
	LCB	6.1	6.2	5.3	24	139	1.1	9.5
2014	Efaw	5.4	8.9	2.6	26	126	1.1	10.4
	LCB	5.2	8.4	5.3	35	157	0.8	7.9

† Efaw, Oklahoma Agricultural Experiment Station near Stillwater, OK;
LCB, Oklahoma Agricultural Experiment 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 Field activities for each location, 2013 and 2014.

	2013		2014	
Field Activity	Efaw†	LCB	Efaw	LCB
Pre-plant soil water sampling	March 15	March 15	April 1	April 3
Pre-plant N fertilization	March 18	March 18	March 31	March 25
Planting	March 20	March 20	April 1	April 3
Start irrigation	June 13	June 14	May 19	May 20
Cease irrigation	July 9	July 9	July 9	May 22
Potential evapotranspiration (mm)	676	655	729	711
Number of irrigations	8	7	8	3
Amount of irrigation (mm)	46	28	28	8
Amount of rainfall (mm)	621	827	375	517
Harvest	September 9	September 5	September 4	August 27
Post-harvest soil water sampling	September 13	September 8	September 4	August 28

† Efaw, Oklahoma Agricultural Experiment Station near Stillwater, OK;
LCB, Oklahoma Agricultural Experiment Station west of Stillwater, OK near Lake Carl Blackwell

Table 1-4 Maize grain yield levels (Mg ha^{-1}), single degree-of-freedom contrasts, significance levels, and differences for hybrid and nitrogen fertilizer (N rate) treatments at Efaw and Lake Carl Blackwell (LCB), 2013 and 2014. Contrast comparison differences are reported in Mg ha^{-1}.

		2013†				2014			
		Efaw		LCB		Efaw		LCB	
Hybrid	N rate	Irr.	Dry.	Irr.	Dry.	Irr.	Dry.	Irr.	Dry.
		Mg ha^{-1}							
P1	Low	5.4	4.2	5.0	1.6	3.1	2.6	4.3	4.0
P2	Low	7.1	3.6	5.5	1.9	2.8	2.2	4.3	3.6
M1	Low	7.2	4.6	6.2	2.6	4.0	3.2	3.8	3.8
M2	Low	9.0	3.8	5.9	1.7	2.1	2.8	3.5	2.6
P1	Med	6.4	2.8	8.2	0.9	5.9	4.2	8.0	7.3
P2	Med	9.1	3.8	8.1	1.3	5.1	4.1	8.1	7.5
M1	Med	8.2	4.3	9.1	2.0	7.5	4.9	9.1	7.7
M2	Med	8.6	4.1	8.2	1.6	3.7	5.7	8.8	5.6
P1	High	7.6	3.1	8.0	2.5	7.2	6.2	10.7	6.8
P2	High	5.9	5.2	7.9	1.8	8.2	5.5	8.9	7.4
M1	High	8.6	4.7	8.5	3.3	7.5	6.4	9.3	8.0
M2	High	9.4	3.4	11.2	2.3	9.9	6.5	10.6	6.4
SED‡		1.9	0.8	1.2	1.3	1.8	0.5	1.7	1.2
Source of Variation§		P > F							
Hybrid		ns	@	ns	ns	ns	**	ns	ns
N rate		ns	ns	**	ns	**	**	**	**
Hybrid x N rate		ns	ns	ns	ns	ns	ns	ns	ns
CV, %¶		30	25	20	81	40	13	28	26
Contrasts		comparison differences, Mg ha^{-1}							
M1, M2 vs P1, P2		1.6*	0.4	1.1*	0.6	0.4	0.8**	0.1	-0.4
M1, P1 vs M2, P2		-1.0	-0.1	-0.3	0.4	0.6	0.1	0.2	0.8
M1 vs P1		1.6	1.2*	0.9	0.9	0.9	0.5@	-0.3	0.5
M2 vs P2		1.6	-0.4	1.3@	0.2	-0.1	1.0*	0.5	-1.3@
M1 vs M2		-1.0	0.8@	-0.5	0.7	1.1	-0.1	-0.2	1.6
P1 vs P2		-0.9	-0.8	-0.1	-0.1	0.1	0.4	0.6	-0.1*
N rate linear		ns	ns	ns	ns	**	**	ns	ns
N rate quadratic		ns	ns	**	ns	**	**	**	**
Low vs Med, High		-0.8	0.1	-3.0**	-0.1	-3.9**	-2.8**	-5.2**	-3.6**
Med vs High		0.2	-0.4	-0.5**	-1.0	-2.6*	-1.4**	-1.4**	-0.1**

† Efaw, Oklahoma Agricultural Experiment Station near Stillwater, OK;
LCB, Oklahoma Agricultural Experiment Station west of Stillwater, OK near Lake Carl Blackwell; Irr. = Irrigated production, Dry. = Dryland production
‡ SED = standard error of the difference between two equally replicated means
§ **, *, @ = significant at the 0.01, 0.05, and 0.10 probability levels, respectively,
¶ CV = coefficient of variation

Table 1-5 Maize water use efficiency (WUE) levels (kg ha^{-1} m^{-1}), single degree-of-freedom contrasts, significance levels, and differences for hybrid and nitrogen fertilizer (N rate) treatments at Efaw and Lake Carl Blackwell (LCB), 2013 and 2014. Contrast comparison differences are reported in kg ha^{-1} m^{-1}.

		2013†				2014			
		Efaw		LCB		Efaw		LCB	
Hybrid	N rate	Irr.	Dry.	Irr.	Dry.	Irr.	Dry.	Irr.	Dry.
		------------------------------ kg ha^{-1} m^{-1} ------------------------------							
P1	Low	0.96	0.84	0.64	0.21	0.87	0.81	0.96	0.94
P2	Low	1.24	0.73	0.65	0.26	0.75	0.61	0.91	0.81
M1	Low	1.32	0.91	0.76	0.35	1.11	0.94	0.85	0.99
M2	Low	1.60	0.79	0.70	0.23	0.56	0.78	0.78	0.56
P1	Med	1.12	0.58	1.04	0.12	1.88	1.22	1.84	1.70
P2	Med	1.61	0.76	1.01	0.18	1.53	1.27	1.86	1.65
M1	Med	1.50	0.89	1.14	0.27	2.17	1.38	2.17	1.90
M2	Med	1.49	0.83	1.00	0.23	1.20	1.86	2.05	1.38
P1	High	1.33	0.65	0.98	0.34	2.07	1.87	2.47	1.67
P2	High	1.01	1.09	0.98	0.25	2.46	1.58	2.04	1.72
M1	High	1.58	0.99	1.08	0.44	2.36	2.06	2.19	1.86
M2	High	1.70	0.68	1.44	0.32	2.92	1.92	2.39	1.68
SED		0.35	0.17	0.17	0.17	0.54	0.17	0.39	0.27
Source of Variation§		------------------------------ P > F ------------------------------							
Hybrid		ns	@	ns	ns	ns	**	ns	ns
N rate		ns	ns	**	ns	**	**	**	**
Hybrid x N rate		ns	ns	ns	ns	ns	@	ns	ns
CV, %¶		31	25	22	80	40	15	28	24
Contrasts		--------------- comparison differences, kg ha^{-1} m^{-1} ---------------							
M1, M2 vs P1, P2		0.32*	0.07	0.14*	0.08	0.13	0.26**	0.06	-0.02
M1, P1 vs M2, P2		-0.14	0.00	-0.02	0.05	0.17	0.04	0.07	0.21@
M1 vs P1		0.33	0.24*	0.10	0.13	0.27	0.16	-0.02	0.15
M2 vs P2		0.31	-0.09	0.17@	0.03	-0.02	0.37**	0.14	-0.19
M1 vs M2		-0.14	0.17@	-0.05	0.10	0.32	-0.06	-0.01	0.38
P1 vs P2		-0.15	-0.17@	0.01	0.00	0.03	0.15	0.16	0.04*
N rate linear		ns	ns	ns	ns	*	**	ns	ns
N rate quadratic		ns	ns	**	ns	**	**	**	**
Low vs Med, High		-0.14	0.01	-0.40**	-0.01	-1.25**	-0.86**	-1.25**	-0.87**
Med vs High		0.03	-0.09	-0.07**	-0.14	-0.76**	-0.42**	-0.29**	-0.08**

† Efaw, Oklahoma Agricultural Experiment Station near Stillwater, OK;
LCB, Oklahoma Agricultural Experiment Station west of Stillwater, OK near Lake Carl Blackwell; Irr. = Irrigated production, Dry. = Dryland production

‡ SED = standard error of the difference between two equally replicated means

§ **, *, @ = significant at the 0.01, 0.05, and 0.10 probability levels, respectively,

¶ CV = coefficient of variation

Table 1-6 Maize nitrogen use efficiency (NUE) levels (%), single degree-of-freedom contrasts, significance levels, and differences for hybrid and nitrogen fertilizer (N rate) treatments at Efaw and Lake Carl Blackwell (LCB), 2013 and 2014. Contrast comparison differences are reported in %.

		2013†				2014			
		Efaw		LCB		Efaw		LCB	
Hybrid	N rate	Irr.	Dry.	Irr.	Dry.	Irr.	Dry.	Irr.	Dry.
		%							
P1	Low	.	.	.	.	.	.	.	.
P2	Low	.	.	.	.	.	.	.	.
M1	Low	.	.	.	.	.	.	.	.
M2	Low	.	.	.	.	.	.	.	.
P1	Med	47	3.0	44	1.0	30	28	62	81
P2	Med	25	12	27	2.9	33	38	62	82
M1	Med	7.5	15	40	12	38	27	79	69
M2	Med	21	9.1	28	28	26	39	81	67
P1	High	23	1.0	19	12	29	34	54	44
P2	High	0	19	12	7.0	36	33	43	51
M1	High	11	6.8	27	12	27	28	44	37
M2	High	12	3.3	34	15	44	26	54	45
SED		16	10	16	13	12	8	14	16
Source of Variation§		P > F							
Hybrid		ns	ns	ns	ns	ns	ns	ns	ns
N rate		ns	ns	ns	ns	ns	ns	ns	**
Hybrid x N rate		ns	ns	ns	ns	ns	ns	ns	ns
CV, %¶		107	139	69	153	46	31	28	32
Contrasts		comparison differences, %							
M1, M2 vs P1, P2		-11	-0.3	6.4	11	1.9	-3.4	9.1	-10
M1, P1 vs M2, P2		7.4	-4.6	7.0	-3.9	-3.7	-4.8	-0.1	-3.4
M1 vs P1		-25*	9.0	1.7	5.8	3.4	-3.4	3.6	-9.4
M2 vs P2		3.9	-9.6	11	16	0.5	-3.4	15	-11
M1 vs M2		-7.2@	4.7@	2.3	-9.2	-2.2	-4.8	-5.7	-2.5
P1 vs P2		22	-14	12	1.4	-5.2	-4.8	5.5	-4.2
Med vs High		14	2.2	12	-0.7	-2.4	2.7	22**	31**

† Efaw, Oklahoma Agricultural Experiment Station near Stillwater, OK;
LCB, Oklahoma Agricultural Experiment Station west of Stillwater, OK near Lake Carl Blackwell; Irr. = Irrigated production, Dry. = Dryland production
‡ SED = standard error of the difference between two equally replicated means
§ **, *, @ = significant at the 0.01, 0.05, and 0.10 probability levels, respectively,
¶ CV = coefficient of variation

Table 1-7 Linear regression model parameters and analysis of variance of the model, intercept, and slope for the interaction between water use efficiency (WUE, kg ha^{-1} m^{-1}) and nitrogen use efficiency (NUE, %) by hybrid pooled across N rates (n=6) at Efaw and Lake Carl Blackwell (LCB), 2013 and 2014.

			Parameter of the model‡				Significance (Pr > F)§	
Year	Location†	Hybrid	β_0: WUE	β_1: NUE	R^2	Model	Intercept	Slope
2013	Efaw-Irrigated	P1	1.01	0.01	0.26	ns	*	*
		P2	1.00	0.02	0.64	@	**	**
		M1	1.17	0.04	0.44	ns	**	**
		M2	1.42	0.01	0.18	ns	**	**
	Efaw-Dryland	P1	0.58	0.02	0.33	ns	**	**
		P2	0.59	0.02	0.36	ns	@	**
		M1	0.83	0.01	0.44	ns	**	**
		M2	0.57	0.03	0.68	*	**	**
	LCB-Irrigated	P1	0.95	0.01	0.16	ns	**	**
		P2	0.77	0.01	0.42	ns	**	**
		M1	0.97	0.01	0.10	ns	*	**
		M2	0.79	0.01	0.54	@	*	**
	LCB-Dryland	P1	0.09	0.02	0.92	*	ns	**
		P2	0.14	0.02	0.76	*	*	**
		M1	0.17	0.02	0.87	**	*	**
		M2	0.07	0.01	0.86	*	ns	**
2014	Efaw-Irrigated	P1	1.56	0.01	0.02	ns	ns	**
		P2	0.73	0.04	0.57	@	ns	**
		M1	0.55	0.05	0.74	*	ns	**
		M2	0.39	0.05	0.66	*	ns	**
	Efaw-Dryland	P1	0.98	0.02	0.12	ns	ns	**
		P2	2.41	-0.03	0.49	ns	**	**
		M1	0.81	0.03	0.18	ns	ns	**
		M2	1.56	0.01	0.44	ns	**	**
	LCB-Irrigated	P1	1.44	0.01	0.11	ns	ns	**
		P2	1.33	0.01	0.59	@	*	**
		M1	1.08	0.02	0.48	ns	ns	**
		M2	2.39	-0.01	0.01	ns	*	**
	LCB-Dryland	P1	1.12	0.01	0.33	ns	@	**
		P2	1.83	-0.01	0.04	ns	**	**
		M1	0.92	0.02	0.71	*	@	**
		M2	1.63	-0.01	0.01	ns	@	**

† Efaw, Oklahoma Agricultural Experiment Station near Stillwater, OK; LCB, Oklahoma Agricultural Experiment Station west of Stillwater, OK near Lake Carl Blackwell

‡ β_0= intercept, β_1 = slope

§ **, *, @ = significant at the 0.01, 0.05, and 0.10 probability levels, respectively.

1.9 Figures

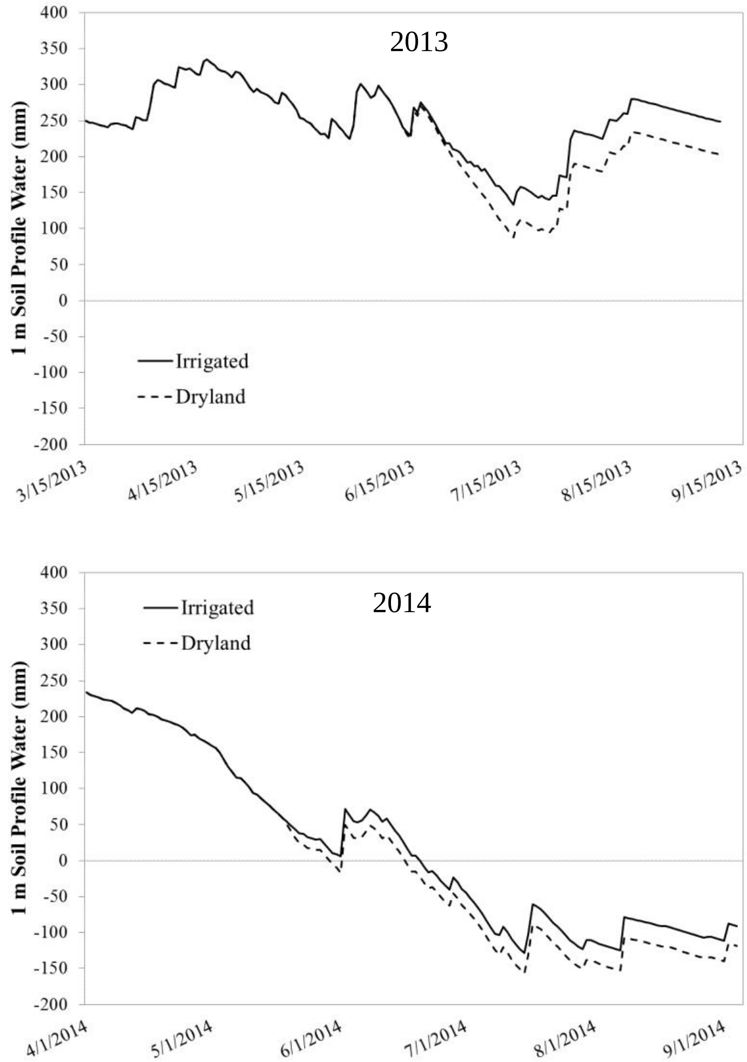

Figure 1-1. Daily water balance (rainfall plus irrigation minus potential evapotranspiration) for the 2013 (top) and 2014 (bottom) growing seasons at the Efaw experiment station near Stillwater, OK. Soil profile water begins with volumetric soil moisture content samples collected prior to planting to a depth of 1 m. Potential evapotranspiration and rainfall measured from nearby weather monitoring station.

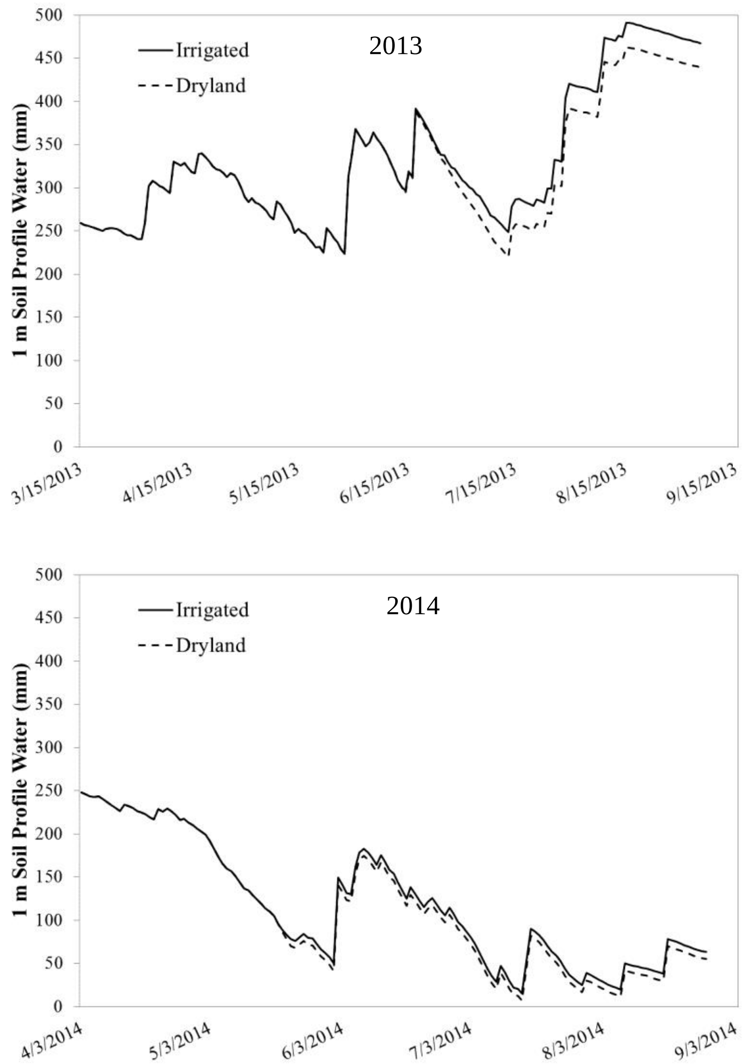

Figure 1-2. Daily water balance (rainfall plus irrigation minus potential evapotranspiration) for the 2013 (left) and 2014 (right) growing seasons at the Lake Carl Blackwell (LCB) experiment station west of Stillwater, OK. Soil profile water begins with volumetric soil moisture content samples collected prior to planting to a depth of 1 m. Potential evapotranspiration and rainfall measured from nearby weather monitoring station.

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CHAPTER 2

PREDICTING PRE-PLANT NITROGEN APPLICATIONS TO MAIZE USING INDICATOR CROP N-RICH REFERENCE STRIPS

2.1 Abstract

Use of active optical reflectance sensors has proven to accurately determine optimum nitrogen (N) fertilizer requirements and direct in-season N fertilizer applications. However, maize (*Zea mays* L.) producer adoption of this technology has been slow due to an array of agronomic, economic, and technical reasons. A study was established in 2012 at two locations in north-central Oklahoma to investigate the response of winter wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.) indicator crops to a sufficient rate (168 kg ha^{-1}) and zero rate of N fertilizer to estimate optimal early season N fertilizer application rates of the subsequent maize crop. In the spring, corn was planted between the indicator crop strips and harvested to obtain the agronomic optimum N rate (AONR) and response of N fertilizer at harvest ($RI_{Harvest}$) of six at planting N fertilizer treatments ranging from 0 to 225 kg ha^{-1} in 45 kg ha^{-1} increments. In-season response of the indicator crops was determined using the normalized difference vegetative index (RI_{NDVI}) and provided input values to calculate N fertilizer recommendations of the subsequent maize crop by using the generalized algorithm.

Strong correlations existed between the N fertilizer recommendation generated from the generalized algorithm and the AONR for the maize crop (R^2 = 0.44 and 0.80) along with the RI_{NDVI} for wheat and barley and $RI_{Harvest}$ for the maize (R^2 = 0.62 and 0.98). The use of indicator crops to predict the response of maize to N fertilizer is unprecedented and modifications to current maize N fertilizer recommendations could modernize N management strategies for all producers.

2.2 Introduction

Active optical reflectance sensors have been used to accurately determine optimum nitrogen (N) fertilizer requirements in maize (*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). The short timeframe to identify N deficiencies and apply N fertilizer mid-season using traditional sidedress application equipment is the most notable reason for maize producer's reluctance, even though substantial 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 maize with the use of active optical reflectance sensors.

Various methods have been proposed to identify optimum N fertilizer rates for maize 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:

$$RI_{NDVI} = NR_{NDVI} / FP_{NDVI}$$

where NR_{NDVI} is the NDVI collected from the N-rich strip and FP_{NDVI} 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). Identifying how the crop will respond to N fertilizer applications does not directly translate to a recommendation, but is important to determine grain yield potential (Raun et al., 2005). The relationship between in-season NDVI measurements

(RI_{NDVI}) and crop response to N fertilizer at harvest has been shown to be positively correlated (Mullen et al., 2003; Hodgen et al., 2005). Crop response to N fertilizer at harvest ($RI_{Harvest}$) can be defined by the following equation:

$$RI_{Harvest} = \text{Highest mean yield from N treatment} / \text{mean yield from check treatment}$$

In maize, early season N deficiencies are not 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 (maize 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 maize height being a limiting factor. Alternative and reliable strategies need to be investigated to identify early season N deficiencies in maize.

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 maize. This approach allows the indicator crop to demonstrate distinguishable differences in response to residual fall N and early spring N mineralization near or at the time of maize planting. These soil N pools are otherwise less recognizable during the early growth stages of maize, especially when temperatures are low and crop growth is slow.

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

2.3 Objectives

The objective of this study was to 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 maize (*Zea mays* L.) crop.

2.4 Materials and Methods

Field experiments were initiated in the fall of 2012 and continued through 2014 at the Efaw (36.081118^{o}, -97.063270^{o}, elevation 272 m above sea level) agronomy research station near Stillwater, OK and Lake Carl Blackwell (LCB; 36.090792^{o}, -97.172486^{o}, elevation 293 m above sea level) agronomy research station west of Stillwater, OK near Lake Carl Blackwell (Table 2-1). All soil fertility parameters were managed to ensure N was the only limiting nutrient (Table 2-2) based on fertilizer recommendations described by Zhang and Raun (2006). A summary of field activities for each cropping year including; N fertilizer application dates, planting dates, cultivars or hybrids, sensing dates, irrigation amounts, and harvest dates are reported in Table 2-3.

Winter wheat and spring barley were planted in late fall or early spring in strips along the outside of the eventual maize trial (Fig. 2-1) and served as the indicator crop reference strips. Each indicator crop reference strip (6 m wide by 21 m long) was split and either a sufficient rate (168 kg N ha^{-1}; N-rich reference strip) or a zero rate (farmer practice) of N fertilizer was applied prior to planting as broadcast urea ammonium nitrate (UAN; 28-0-0). Winter wheat was planted using a Kincaid model 2010 grain drill (Kincaid Equipment and Manufacturing, Haven, KS) at a seeding density of 100 kg ha^{-1} and row spacing of 18 cm (Table 2-3). Spring barley was planted using the same Kincaid model 2010 grain drill at a seeding density of 112 kg ha^{-1} (Table 2-3).

In the spring, maize was planted between the indicator crop reference strips (Fig. 2-1). A three replicate randomized complete block design was 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 was applied prior to planting as broadcast and incorporated UAN. Maize was planted at a seeding density of 65,000 kernels ha^{-1} with a 4-row John Deere 7300 Integral MaxEmerge planter (Deere & Company, Moline, IL). Individual maize plots measured 3 m wide (four 0.76 m rows) by 6.1 m long and were irrigated using a surface drip system on an as needed basis, dependent upon visual water stress symptoms. The amount of water being supplied was monitored and documented (Table 2-3). To ensure even distribution across each plot, drip tape was installed between rows 1 and 2 and between rows 3 and 4.

Crop canopy reflection measurements were collected throughout the vegetative growth stages of the indicator crops to estimate biomass accumulation and potential N deficiency. Spectral reflectance was measured from the center 1.5 m of the indicator crop reference strips using the GreenSeeker (Trimble Agriculture Division, Westminster, CO) active optical reflectance crop sensor and was 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 were collected at approximately Feekes (Large, 1954) growth stage 3, 4, 5, 7, and 10.

Grain yield was determined for all of the maize plots at physiological maturity. Mechanical grain harvest was 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 were harvested and grain yield (Mg ha^{-1}) was adjusted to 155 g kg^{-1} moisture content. Agronomic optimum N rate (AONR) was determined using the SAS PROC NLIN procedure (SAS Institute, 2011) at each location. The AONR is the N rate that produces optimum maize grain yield and was calculated using the best fit model (Cerrato and Blackmer, 1990).

In-season measurements of NDVI from the indicator crop reference strips were utilized to calculate a maize N fertilizer recommendation using the generalized algorithm (Solie et al., 2012). The most recent version of this algorithm was accessed via the online interface at the end of the 2014 growing season (Oklahoma State University, 2014). Farmer practice NDVI and N rich strip NDVI from the winter wheat and spring barley indicator crops were used as inputs, but some assumptions were required for completion of the algorithm (Oklahoma State University, 2014). 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 maize N fertilizer recommendation was calculated for each growth stage in-season measurements of NDVI were collected from the indicator crops.

2.5 Results and Discussion

Indicator Crop NDVI

Farmer practice NDVI and N rich strip NDVI values collected from the wheat indicator crop resulted in RI_{NDVI} ranging from 1.06 to 2.20 and the corresponding RI_{NDVI} from the barley indicator crop ranged from 1.02 to 1.95 for all four locations (Tables 2-4 and 2-5). Only 15% of the RI_{NDVI} values for the wheat and barley indicator crops were less than 1.1. Mullen et al. (2003) explains that if RI_{NDVI} of a winter wheat crop is less than 1.1, the probability of obtaining a response to additional N fertilizer will be low. For this trial, the large number of RI_{NDVI} values greater than 1.1 suggested that the subsequent maize crop would be responsive to additions of N fertilizer.

The maize N fertilizer recommendation from the OSU generalized algorithm ranged from 0 to 270 kg N ha^{-1} using the wheat indicator crop and 0 to 275 kg N ha^{-1} using the barley indicator crop (Tables 2-4 and 2-5). Many of the very low N fertilizer recommendations (< 10 kg N ha^{-1}) occurred during the early vegetative growth stages (Feekes 3 and 4) of the indicator crops due to a low amount of accumulated biomass, and thus, a low NDVI (<0.30) or RI_{NDVI} (<1.1). Sensor data collected at Feekes growth stages 3 and 4 of the wheat indicator crop did occur prior to corn planting while the sensor data collected for all other wheat growth stages and all of the barley growth stages occurred after corn planting, but prior to maize growth stage V6 (Abendroth et al., 2011). Nitrogen fertilizer applications made to maize at growth stage V6 or before could be accomplished using traditional sidedress equipment. The sigmoidal model utilized in the generalized algorithm predicts a low potential grain yield in response to a low NDVI of the crop due to a transitional region of NDVI between bare soil and the central region of the model (Solie et al., 2012). Therefore, it is not surprising that the N fertilizer recommendation was low when indicator crop NDVI was also low due to the limited increases in grain yield with the changes in NDVI (Solie et al., 2012). High N fertilizer recommendations (> 150 kg N ha^{-1}) occurred when RI_{NDVI} of the indicator crop was also high (>1.5) as a result of a larger denominator in grain yield potential equation (Solie at al., 2012).

Maize Grain Yield

Maize grain yields at all four sites were responsive to the addition of N fertilizer (Fig. 2-2). Maize grain yields collected in this experiment were similar to grain yields observed from other Oklahoma maize experiments using pre-plant N fertilizer (Tubaña et al., 2008; Walsh et al., 2012). A linear plateau response model was fit to the range of N rates at both locations in 2013. In 2014, the LCB location followed a quadratic trend and the Efaw location was linear (Fig. 2-2). The AONR for Efaw 2013 was 185 kg N ha^{-1} and LCB 2013 was 52 kg N ha^{-1} as a result of the linear plus plateau models. In 2014, the AONR for LCB was calculated at 95 kg N ha^{-1} and Efaw was 224 kg N ha^{-1}. The large range in AONR could be expected due to the variable environmental conditions experienced, and their impact on N demand. For example, seasonal rainfall ranged from 375 to 827 mm for the four sites (Table 2-3).

The maize crop displayed a positive response to the addition of pre-plant N fertilizer at harvest ($RI_{Harvest}$), ranging from 1.31 to 2.22 (Tables 2-6 and 2-7). All four sites had a positive $RI_{Harvest}$, indicating that increased grain yields were due to added N fertilizer rather than nonfertilizer N contributions, such as mineralization or rainfall. Nonfertilizer N contributions to the maize production system are likely reasons for low $RI_{Harvest}$ values (Mullen et al., 2003).

Agronomic Optimum N Rate vs. Generalized Algorithm

Linear regression indicated that the N fertilizer recommendations generated from the OSU generalized algorithm using both wheat and barley indicator crops could not predict the AONR for the maize crop (Fig. 2-3). However, a strong correlation was detected between the N fertilizer recommendation generated from the OSU generalized algorithm using the barley indicator crop and the AONR for the maize crop (R^2 = 0.80). Overall, the wheat indicator crop resulted in a slope closer to one and an intercept much higher than the barley indicator crop (Fig. 2-3). Even though the relationship was less using wheat (R^2 = 0.44) compared to barley (R^2 = 0.80), correlation remained high. The NDVI data collected for use in the generalized algorithm using the indicator crops occurred during the months of March, April, and May. The AONR data for the maize crop was obtained after harvest in either late August or early September. This four month span encompassed a lot of environmental variation while the N rate recommendation from the generalized algorithm using a wheat indicator crop could account for nearly 50 % of the variability in the AONR for maize.

$RI_{Harvest}$ vs. RI_{NDVI}

Linear regression established that RI_{NDVI} using the barley indicator crop could statistically predict $RI_{Harvest}$ for the maize crop and RI_{NDVI} for barley accounted for 98% of the variability in the maize $RI_{Harvest}$ (Fig. 2-4). High correlation was also found between RI_{NDVI} for wheat and maize $RI_{Harvest}$ (R^2 = 0.62). Both crops resulted in a slope greater than one, but the barley had an intercept nearly equal to zero (Fig. 2-4). The ranges in RI for both NDVI and harvest suggest that this relationship is consistent for both low and high responsive sites. Other work in Oklahoma determined that RI_{NDVI} provided good prediction for $RI_{Harvest}$ (R^2 = 0.56 to 0.75) in winter wheat (Mullen et al., 2003; Hodgen et al., 2005). Hodgen at al. (2005) also observed the slope for the relationship between RI_{NDVI} and $RI_{Harvest}$ to be greater than one due to large amounts of N taken up by the plant early in the season. The ability to predict the response of a maize crop to N fertilizer at harvest at early stages of growth using a fall or early spring planted indicator crop is unprecedented. Modifications to current maize N fertilizer recommendations as a result of this relationship would modernize N management strategies for maize producers across the United States.

2.6 Conclusions

Analysis of these four sites found that a strong correlation existed between the maize N fertilizer recommendations from the OSU generalized algorithm using wheat and barley indicator crops and the maize AONR along with the RI_{NDVI} for wheat and barley and $RI_{Harvest}$ for the maize crop. Although these relationships were a result of the best comparisons between the indicator crops and maize, the concept of using NDVI data collected from the indicator crops occurred during the months of March, April, and May to predict the response of the maize crop to N fertilizer obtained after harvest in either late August or early September is unprecedented. Producers may be tentative to use indicator crop N rich strips, but they offer an alternative approach to current maize N fertilizer recommendations which could modernize N management strategies for maize producers across the United States.

2.7 Tables

Table 2-1 Soil map unit and taxonomic classification for each location, 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	Norge loam, 3-5% slope	Norge: *Fine-silty, mixed, active, thermic Udic Paleustolls*
	LCB	Pulaski fine sandy loam, 0-1% slope, occasionally flooded	Pulaski: *Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents*

† 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‡	NH_4-N§	NO_3-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	5.1	8.7	1.7	84.1	108	0.8	7.0
	LCB	5.2	7.4	6.9	34.1	142	0.8	8.1

† 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 Field activities for each location, 2013 and 2014.

	2012/2013†		2013/2014	
Field Activity‡	Efaw	LCB	Efaw	LCB
Winter wheat				
N application	October 1	October 3	October 23	October 10
Planting	October 11	October 9	October 23	October 24
Cultivar	Doublestop	Doublestop	Iba	Doublestop
Sensing-Fk. 3	February 19	February 18	March 12	February 25
Sensing-Fk. 4	March 5	March 6	March 20	March 11
Sensing-Fk. 5/6	March 29	March 21	April 4	March 19
Sensing-Fk. 7/8	April 12	April 12	.	April 3
Sensing-Fk. 9/10	April 29	April 29	April 17	April 17
Spring barley				
N application	February 5	February 6	February 13	February 13
Planting	February 5	February 6	February 13	February 14
Cultivar	Pinnacle	Pinnacle	Pinnacle	Pinnacle
Sensing-Fk. 3	March 29	March 21	.	March 28
Sensing-Fk. 4	.	.	.	April 3
Sensing-Fk. 5	April 12	April 12	April 17	April 10
Sensing-Fk. 6/7	April 29	April 29	.	April 22
Sensing-Fk. 8/9	.	.	.	April 26
Sensing-Fk. 10	May 22	.	.	May 2
Maize				
N application	March 18	March 18	March 31	March 25
Planting	March 20	March 20	April 1	April 3
Hybrid	Pioneer P1498	Dekalb 63-55	Dekalb 63-55	Dekalb 63-55
Irrigation (mm)	50	28	0	8
Rainfall (mm)	621	827	375	517
Harvest	September 9	September 5	September 4	August 27

† Efaw, Oklahoma Agricultural Experiment Station near Stillwater, OK;
LCB, Oklahoma Agricultural Experiment Station west of Stillwater, OK near Lake Carl Blackwell

‡ Feekes (Fk.) growth stages as denoted by Large (1954)

Table 2-4 Maize 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), 2013.

Location	Crop	Growth Stage†	Farmer Practice NDVI	N Rich Strip NDVI	Response Index‡	Generalized Algorithm N recommendation§	AONR¶
Efaw	Wheat	3	0.325	0.390	1.20	9	185
		4	0.451	0.480	1.06	8	185
		5	0.649	0.774	1.19	83	185
		7	0.629	0.795	1.26	119	185
		9	0.542	0.741	1.37	150	185
	Barley	3	0.323	0.347	1.07	2	185
		5	0.612	0.662	1.08	28	185
		7	0.750	0.849	1.13	70	185
		10	0.646	0.793	1.23	102	185
LCB	Wheat	3	0.388	0.478	1.23	27	52
		4	0.410	0.631	1.54	175	52
		5	0.399	0.674	1.69	227	52
		7	0.370	0.658	1.78	239	52
		10	0.285	0.616	2.16	269	52
	Barley	3	0.219	0.264	1.20	1	52
		5	0.387	0.685	1.77	243	52
		7	0.422	0.812	1.92	275	52

† Feekes growth stages as denoted by Large (1954)

‡ Response index = N rich strip NDVI / farmer practice NDVI

§ 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 linear plus plateau model or a linear plus plateau model, kg N ha^{-1}

Table 2-5 Maize 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), 2014.

Location	Crop	Growth Stage†	Farmer Practice NDVI	N Rich Strip NDVI	Response Index‡	Generalized Algorithm N recommendation§	AONR¶
Efaw	Wheat	3	0.221	0.239	1.08	0	224
		4	0.249	0.286	1.15	1	224
		6	0.234	0.389	1.66	72	224
		9	0.255	0.397	1.56	57	224
	Barley	5	0.218	0.426	1.95	165	244
LCB	Wheat	3	0.285	0.373	1.31	14	95
		4	0.301	0.433	1.44	52	95
		5	0.288	0.504	1.75	174	95
		7	0.277	0.609	2.20	270	95
		9	0.251	0.492	1.96	210	95
	Barley	3	0.209	0.214	1.02	0	95
		4	0.201	0.224	1.11	0	95
		5	0.228	0.277	1.21	1	95
		6	0.250	0.326	1.30	7	95
		8	0.269	0.377	1.40	24	95
		10	0.274	0.442	1.61	98	95

† Feekes growth stages as denoted by Large (1954)

‡ Response index = N rich strip NDVI / farmer practice NDVI

§ 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 linear plus plateau model or a linear plus plateau model, kg N ha^{-1}

Table 2-6 Response index from the in-season sensor measurements of NDVI (RI_{NDVI}) collected from wheat and barley indicator crops along with maize grain yield response ($RI_{Harvest}$) to applied nitrogen fertilizer for Efaw and Lake Carl Blackwell (LCB), 2013.

Location	Crop	Growth Stage†	RI_{NDVI}‡	$RI_{Harvest}$§
Efaw	Wheat	3	1.20	1.31
		4	1.06	1.31
		5	1.19	1.31
		7	1.26	1.31
		9	1.37	1.31
	Barley	3	1.07	1.31
		5	1.08	1.31
		7	1.13	1.31
		10	1.23	1.31
LCB	Wheat	3	1.23	1.39
		4	1.54	1.39
		5	1.69	1.39
		7	1.78	1.39
		10	2.16	1.39
	Barley	3	1.20	1.39
		5	1.77	1.39
		7	1.92	1.39

† Feekes growth stages as denoted by Large (1954)
‡ RI_{NDVI} = N rich strip NDVI / farmer practice NDVI
§ $RI_{Harvest}$ = Highest mean yield N treatment / mean yield check treatment

Table 2-7 Response index from the in-season sensor measurements of NDVI (RI_{NDVI}) collected from wheat and barley indicator crops along with maize grain yield response ($RI_{Harvest}$) to applied nitrogen fertilizer for Efaw and Lake Carl Blackwell (LCB), 2014.

Location	Crop	Growth Stage†	RI_{NDVI}‡	$RI_{Harvest}$§
Efaw	Wheat	3	1.08	2.22
		4	1.15	2.22
		6	1.66	2.22
		9	1.56	2.22
	Barley	5	1.95	2.22
LCB	Wheat	3	1.31	1.50
		4	1.44	1.50
		5	1.75	1.50
		7	2.20	1.50
		9	1.96	1.50
	Barley	3	1.02	1.50
		4	1.11	1.50
		5	1.21	1.50
		6	1.30	1.50
		8	1.40	1.50
		10	1.61	1.50

† Feekes growth stages as denoted by Large (1954)
‡ RI_{NDVI} = N rich strip NDVI / farmer practice NDVI
§ $RI_{Harvest}$ = Highest mean yield N treatment / mean yield check treatment

2.8 Figures

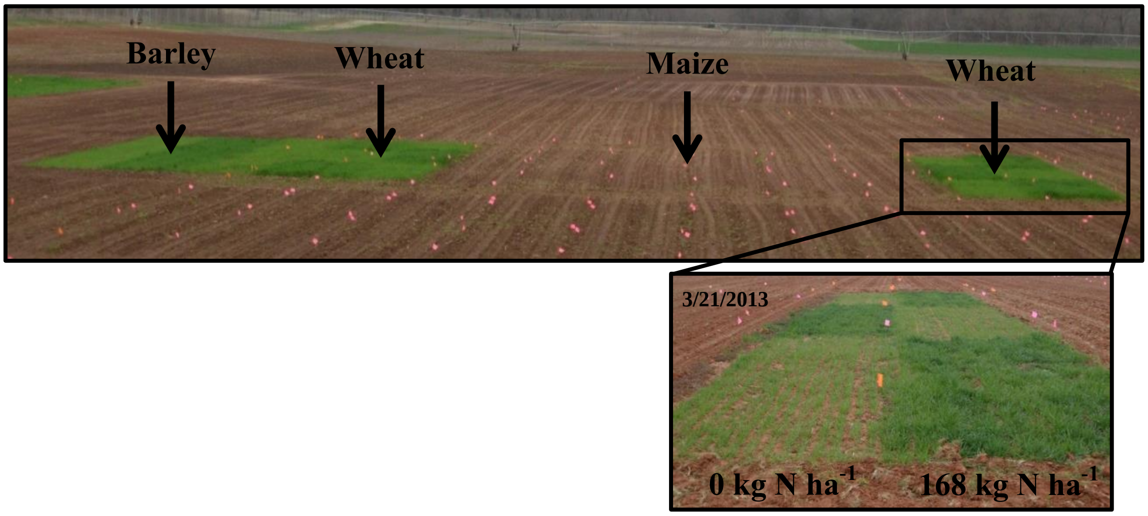

Figure 2-1. Field layout for the winter wheat and spring barley planted in late fall or early spring as strips along the outside of the eventual maize trial.

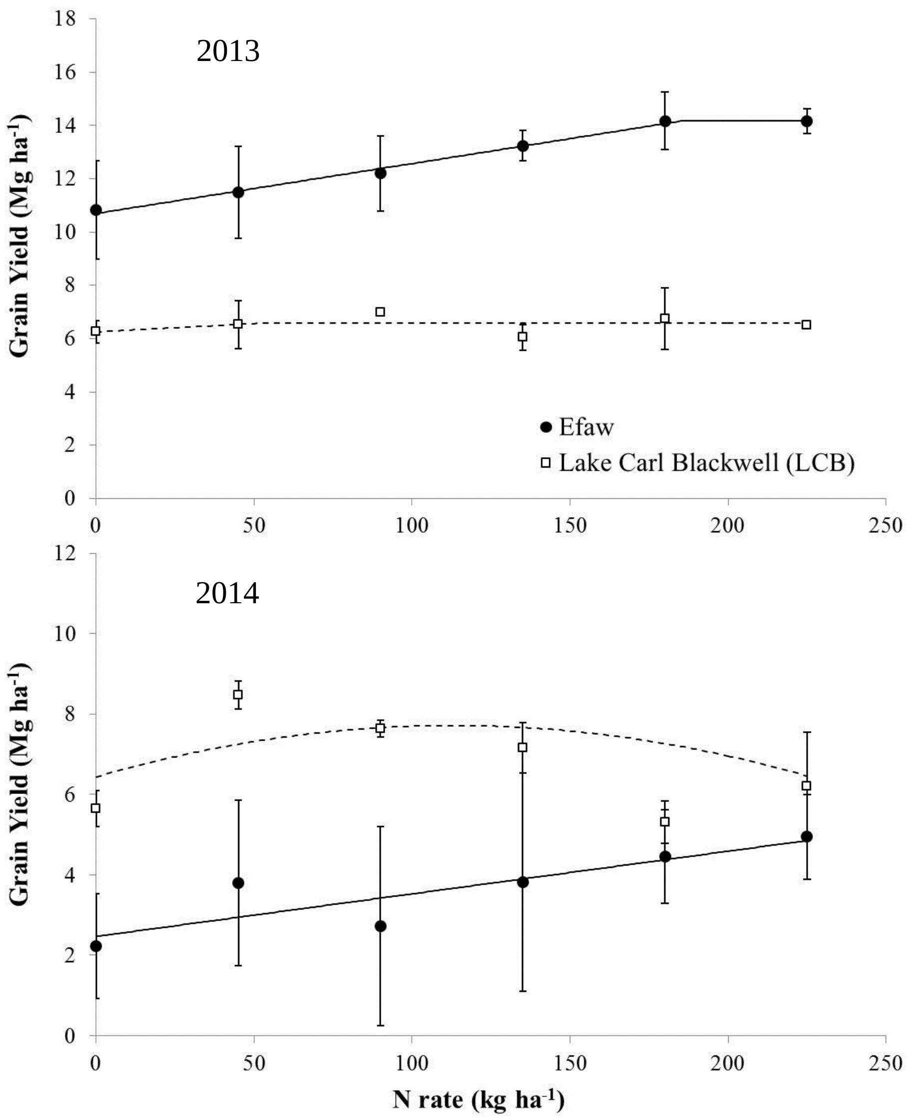

Figure 2-2. Influence of preplant N fertilizer on maize grain yield (Mg ha^{-1}) for Efaw and Lake Carl Blackwell (LCB), 2013 (top) and 2014 (bottom). Error bars represent +/- 1 standard error.

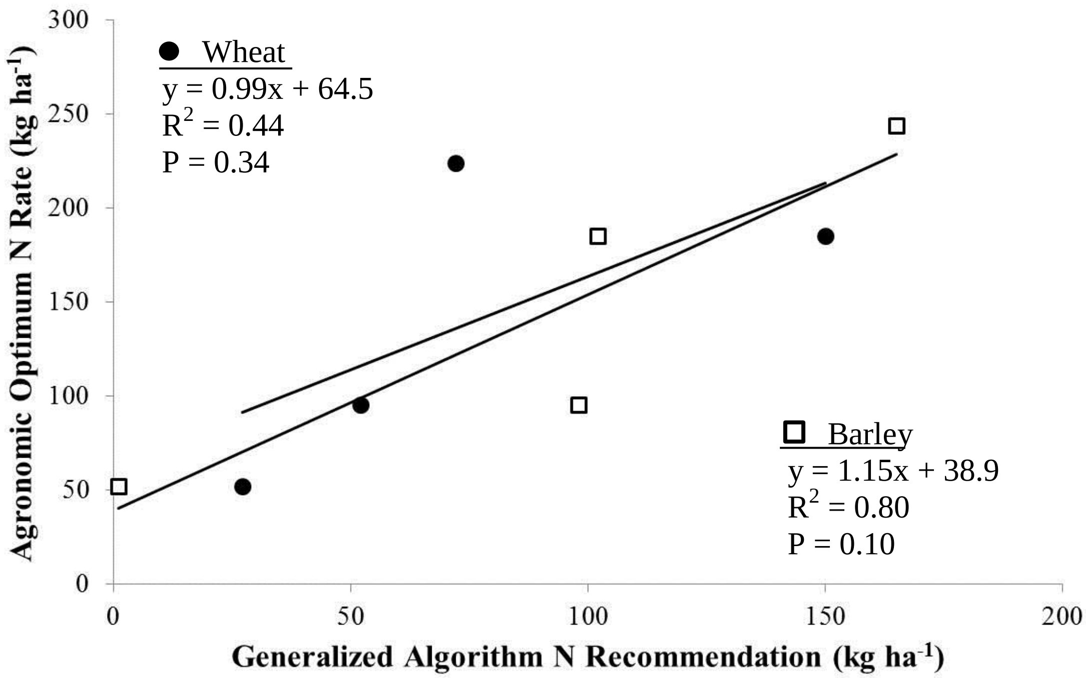

Figure 2-3. Relationship between the N fertilizer recommendation (kg ha^{-1}) generated from the OSU generalized algorithm for wheat (circles) and barley (squares) indicator crops and the maize agronomic optimum N rate (kg ha^{-1}) from four sites in 2013 and 2014.

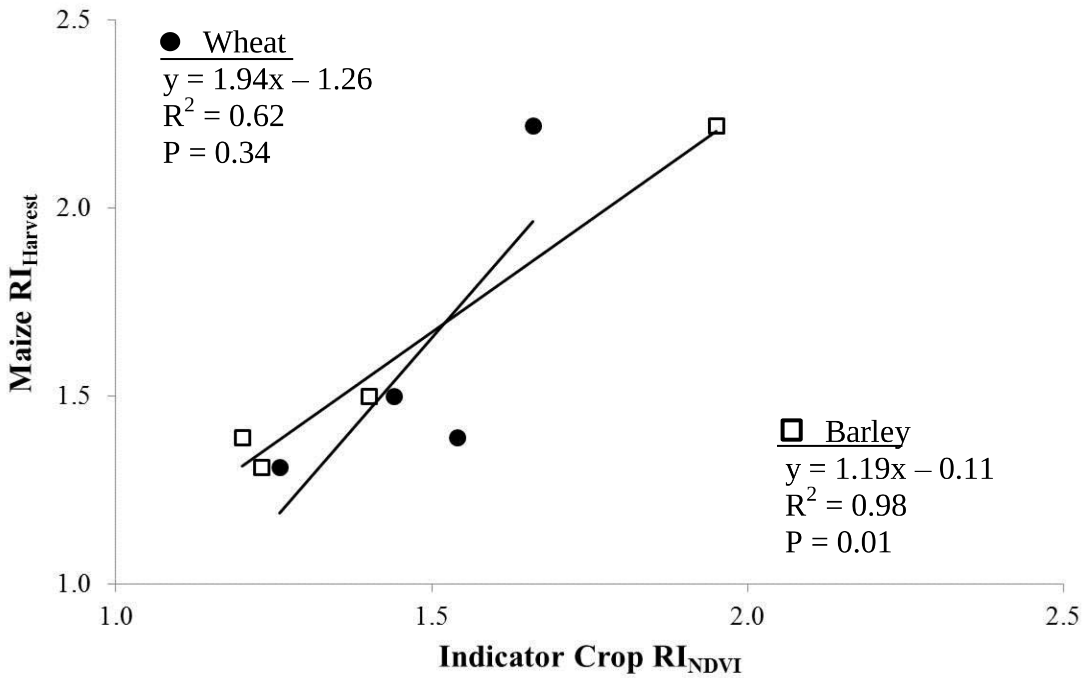

Figure 2-4. Relationship between RI_{NDVI} for wheat (circles) and barley (squares) indicator crops and the maize $RI_{Harvest}$ from four sites in 2013 and 2014.

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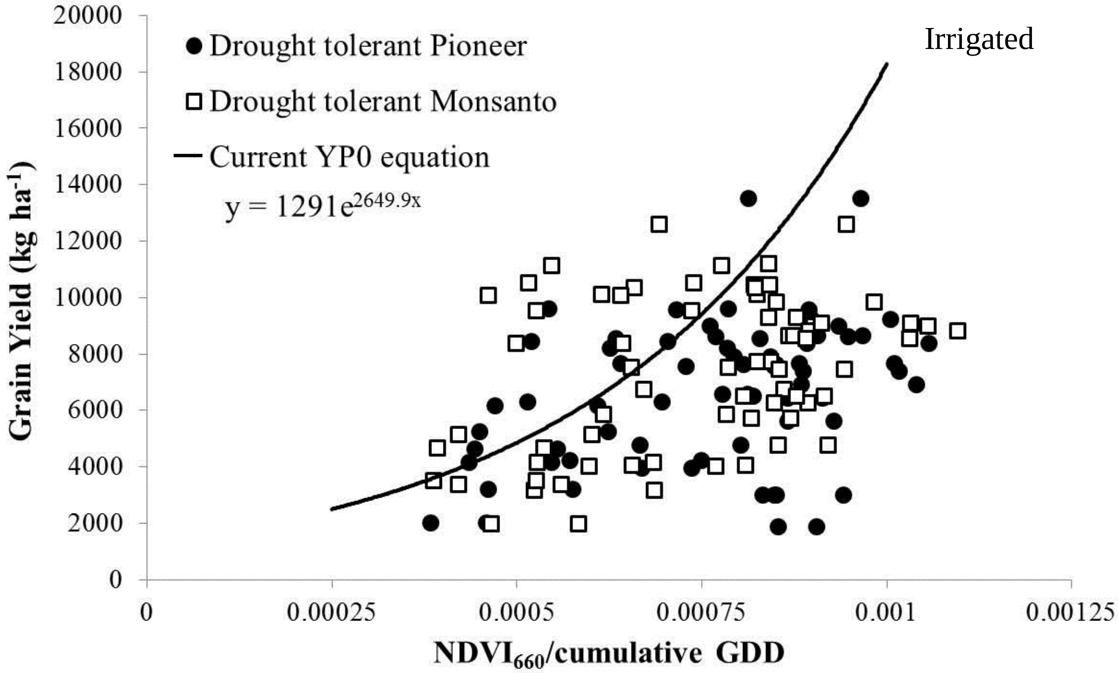
APPENDICES

In addition to the data presented in Chapter 1, plant reflectance measurements were also collected. Drought tolerant maize hybrids are a relatively new technology, and thus, no work has been done to validate current grain yield prediction approaches developed from older maize hybrids. The objective of the data presented in the appendix was to evaluate grain yield potential of drought tolerant and less drought tolerant maize hybrids in irrigated and dryland productions systems.

Crop canopy reflection measurements were collected throughout the vegetative growth stages to estimate biomass accumulation. Spectral reflectance were measured from the center two rows of each plot using the GreenSeeker (Trimble Agriculture Division, Westminster, CO) active optical reflectance crop sensor and 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 were collected at approximately growth stage V4, V6, V8, V10, and V12.



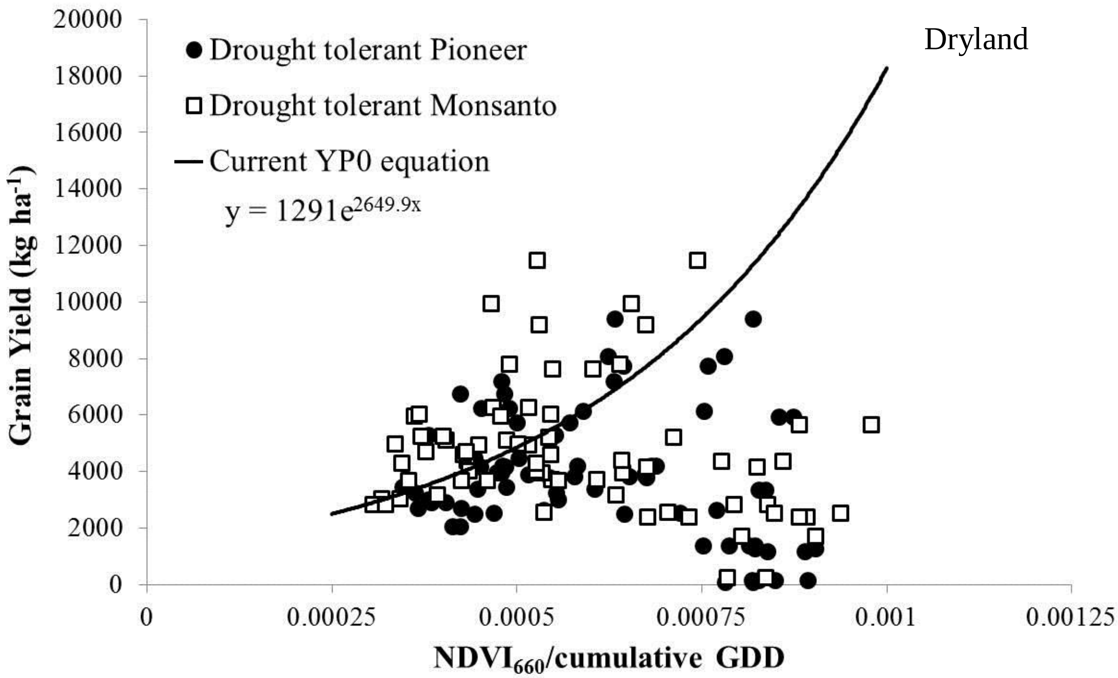


Figure A-1. Relationship between maize grain yield and NDVI divided by cumulative GDD (growing degree day) from V7 to V10 growth stage for drought tolerant Pioneer (circles) and Monsanto (squares) maize hybrids at irrigated (top) and dryland (bottom) sites over 2 years and 4 locations, 2013 and 2014.

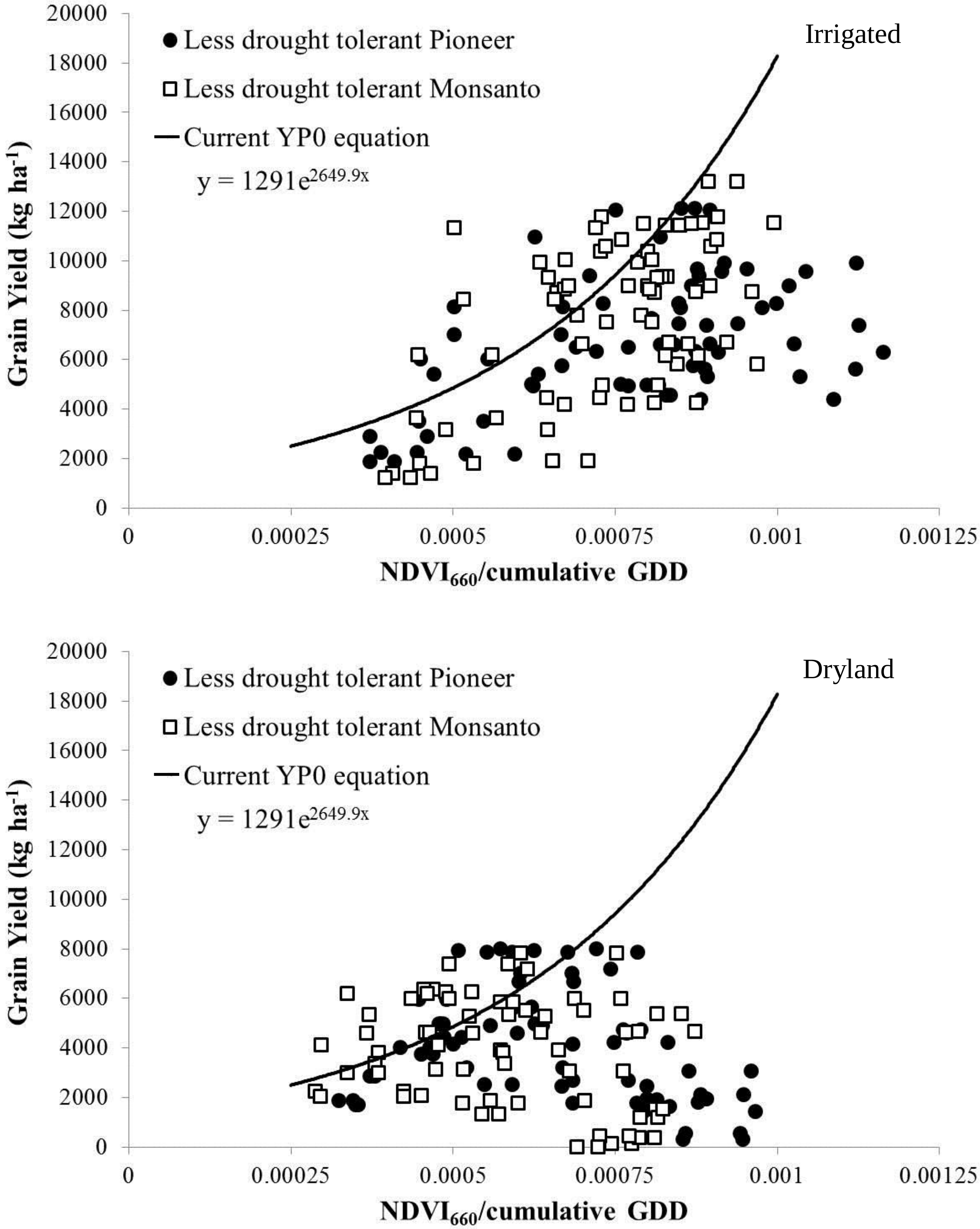


Figure A-2. Relationship between maize grain yield and NDVI divided by cumulative GDD (growing degree day) from V7 to V10 growth stage for less drought tolerant Pioneer (circles) and Monsanto (squares) maize hybrids at irrigated (top) and dryland (bottom) sites over 2 years and 4 locations, 2013 and 2014.

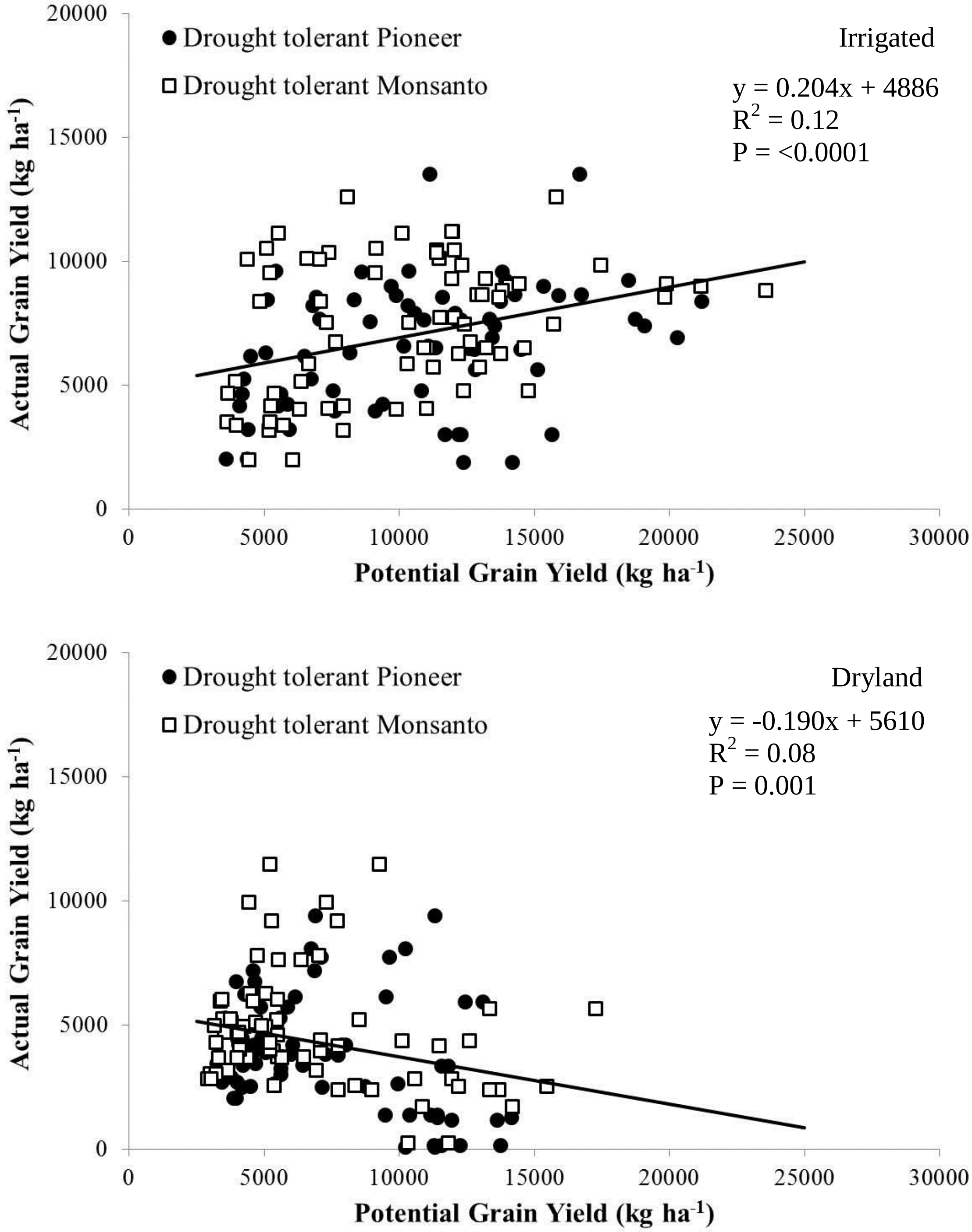
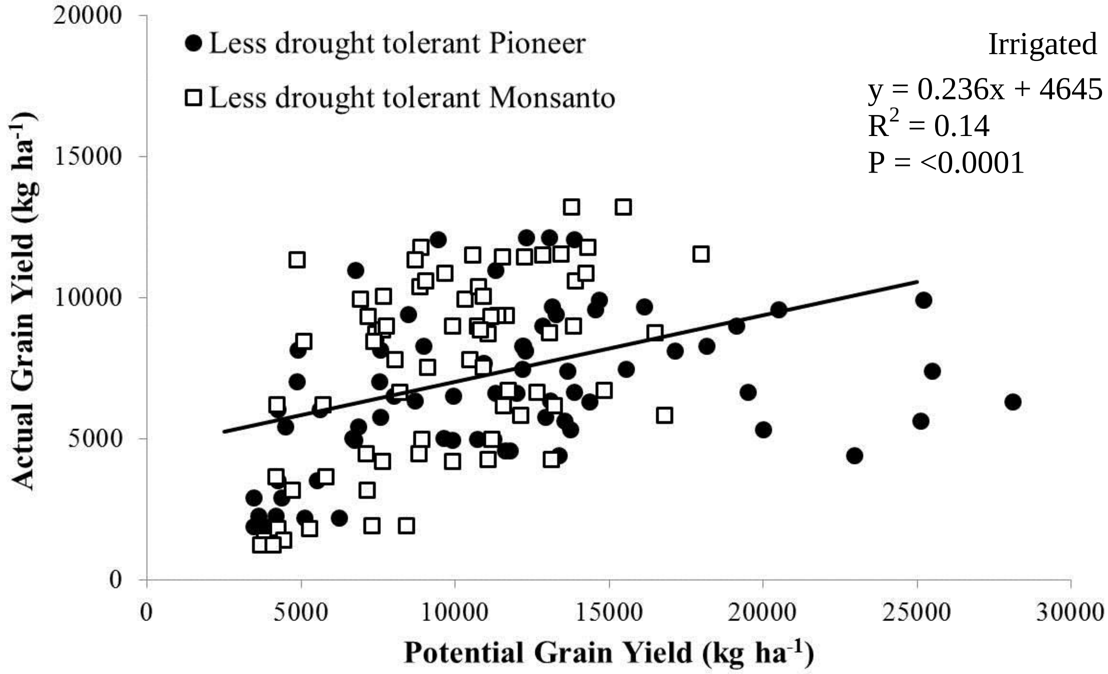


Figure A-3. Relationship between actual maize grain yield and potential maize grain yield from V7 to V10 growth stage for drought tolerant Pioneer (circles) and Monsanto (squares) maize hybrids at irrigated (top) and dryland (bottom) sites over 2 years and 4 locations, 2013 and 2014.



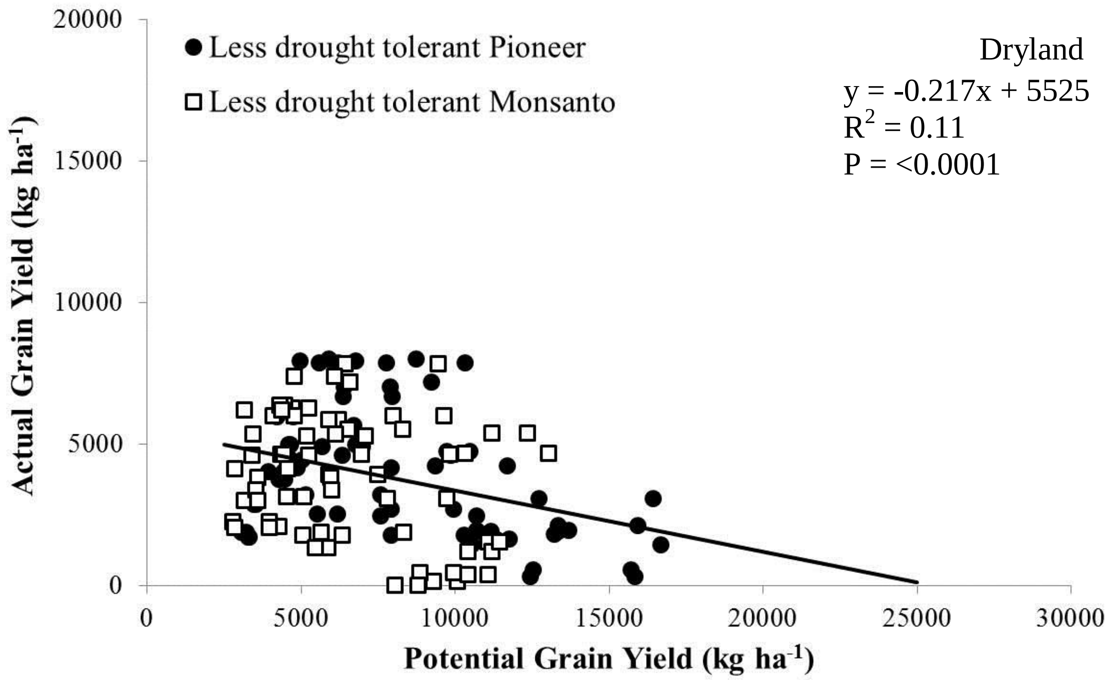


Figure A-4. Relationship between actual maize grain yield and potential maize grain yield from V7 to V10 growth stage for less drought tolerant Pioneer (circles) and Monsanto (squares) maize hybrids at irrigated (top) and dryland (bottom) sites over 2 years and 4 locations, 2013 and 2014.

VITA

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II. PREDICTING PRE-PLANT NITROGEN APPLICATIONS TO MAIZE USING INDICATOR CROP N-RICH REFERENCE STRIPS

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