NITROGEN USE EFFICIENCY AND NITROGEN RESPONSE OF WHEAT VARIETIES COMMONLY GROWN IN THE GREAT PLAINS, USA

by

NATHANIEL D. DORSEY

B.S., Brigham Young University, 2011

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2014

Approved by:

Major Professor
Nathan O. Nelson

ABSTRACT

Increasing nitrogen use efficiency (NUE) and nitrogen response in winter wheat could help producers reduce input costs associated with nitrogen fertilizers and decrease the negative environmental impacts of N loss. The objectives of this research were to i) establish if there are genetic differences in NUE and other related parameters among wheat varieties commonly grown in the Great Plains, ii) determine if there are differences in N response among select varieties with a range of NUEs, and iii) determine if NUE influences N response. This information could be useful in future breeding efforts as researchers seek to develop more efficient varieties. This was approached by conducting two separate studies, a large NUE study with 25 winter wheat varieties, and a smaller N Rate study with 4 varieties that represented a range of NUEs based on the preliminary results of the NUE study. The NUE study was conducted over the course of several seasons and locations, with treatments of consisting of N Rate and variety. The experiment was laid out in a strip-plot design and replicated four times at each location. In the 2010-11 and 2011-12 seasons it was planted at the Kansas River Valley Experiment Field in Rossville, KS. In the 2012-13 season the experiment was planted at two locations, one at Silverlake and another at Ashland Bottoms, KS. The experiment was again planted at two locations in the fall of 2013, in Ashland Bottoms, KS, and Hutchinson, KS. The wheat varieties were grown with two N rates, 0 kg N ha^{-1} and 90 kg N ha^{-1}. Nitrogen use efficiency was calculated as the grain yield per unit of available nitrogen (sum of soil N and fertilizer N) and ranged from 22-30 kg of grain per kg of N and was strongly influenced by variety with a $p<0.001$. Several other related parameters, such as grain yield, nitrogen utilization efficiency, harvest index, and fertilizer use efficiency were also significantly affected by variety with a $p<0.05$. These data suggest there are significant genetic differences in how varieties use and transport nitrogen within their tissues to produce grain. The N Rate experiment was planted in two locations during the 2012-13 season at Silverlake and Ashland Bottoms, KS, and planted again in the fall of 2013 at Ashland Bottoms and Hutchinson, KS. This experiment was laid out in a split-plot design with four varieties and four nitrogen rates. The varieties selected for this study were Duster, Everest, Jagger, and Larned and the four N rates were 0, 33.6, 89.7, and 145.7 kg N ha^{-1}. The results showed significant

differences in yield response among the varieties at only one location, Ashland Bottoms (p=0041). Although N response at Silverlake was not significant, mean grain yields between varieties was significant ($p<0.001$). Two additional parameters, NUE and harvest index (HI), were also significantly different between varieties at Silverlake, KS with a $p<0.05$, while no additional parameters were significant at Ashland Bottoms. Those varieties that had higher response, Everest and Jagger, also tended to have higher NUEs compared to the other two varieties, Duster and Larned. However, because of the contrasting results between locations, additional research is needed to develop firm conclusions. These results provide significant evidence to support varietal differences in nitrogen use due to genetics, and provide the opportunity for breeders to begin developing varieties with higher NUE and improved N response. However, additional research will be required to determine the specific traits responsible for these varietal differences and to determine the suitability of high NUE crops for meeting the nutritional requirements of the future.

ACKNOWLEDGEMENTS

I am first and foremost grateful for my wonderful family. My wife has been a source of continual encouragement and support during my education and has provided me the drive to devote myself to this work. I am especially grateful for the joy my son brings into my life, and for his natural curiosity and creativity that have inspired me to enjoy the simple things. I am grateful for parents who have shown me what it means to be a lifelong learner and how to serve others with your talents and education. I offer sincere thanks to Dr. Nathan Nelson, who has not only been an excellent mentor, but a friend and role-model. I appreciate the time he has taken to answer my questions and the latitude he has given me to manage these projects. I offer many thanks to my other committee members, Drs. Mengel, Fritz, and Ruiz Diaz for their advice and counsel in the development and implementation of these projects. I am especially thankful for Dr. Fritz and his group for providing the wheat varieties used in these studies and for taking the time to plant the NUE study each season. I thank Drs. Murray and Assefa for their statistical expertise and the time they have taken to consult on this project with me. I am grateful for the many lab technicians, Andy, Christian, Ian, Reggeany, Tyler, and Brett who helped in this project, often in less than ideal weather, and without whom I would not have been able to finish. I am also grateful for two fellow graduate students, Ammar and James, who have always been willing to help. A special thanks to the managers of the research locations, Vernon Schaffer, Gary Cramer, and Dr. Adee for their assistance and hard work. I would also like to acknowledge the Kansas Fertilizer Research Fund for their financial support and contributions to this project. I also want to provide a sincere thanks to J. B. Pearl Sales and Services, Inc. for their support and for providing the fertilizers used in these projects. Lastly, I acknowledge my Heavenly Father for the blessings I’ve received, not only these last two years, but throughout my life.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

Chapter I

Chapter II

Chapter III

Appendix B

Appendix C

Appendix D

LIST OF FIGURES

Page

Chapter II

Chapter III

Appendix A

Chapter I

Wheat Cultivation and NUE: A Literature Review

INTRODUCTION

Nitrogen (N) is an essential nutrient for crop growth, and enormous quantities are used to supply the world's agricultural production. The current use of N in agriculture is extremely inefficient, with an average of about 30% being recovered by the crop (Cassman et al., 1998; Raun and Johnson, 1999). This equates to an average loss of around 60% of all N applied through leaching, volatilization, and runoff which represents a $15.9 billion global annual loss of nitrogen (Raun and Johnson, 1999). Improving nutrient efficiencies, especially nitrogen use efficiency (NUE), in crops such as wheat could have tremendous environmental and economic benefits.

Nourishing the world's agriculture requires substantial amounts of energy derived from fossil fuels. A majority of the nitrogen used in fertilizers results from the Haber-Bosch process, in which hydrogen atoms are combined with nitrogen in the presence of a catalyst in order to produce ammonia. This process is responsible for the consumption of 1% of the world's total energy demand (Smith, 2002). As global energy is largely derived from fossil fuels, this amounts to an incredible release of carbon dioxide. There is also substantial cost associated with transporting and applying nitrogen fertilizers. With increased NUE, the consumption of fossil fuels could be drastically reduced as less nitrogen fertilizers would be produced, transported, and consumed.

Agricultural emissions of nitrous oxide (N_2O), a greenhouse gas, account for more than 75% of annual N_2O emissions (Skiba et al., 2012). It is also the most prevalent ozone-depleting molecule in the atmosphere since the decline of CFCs (Ravishankara et al., 2009). The majority of these agricultural sources are due to fertilizer use and the production of legumes (Freney, 1997). Increasing NUE in wheat and other crops would reduce fertilizer inputs and would in turn reduce agricultural emissions of N_2O (Baggs et al., 2003; Hauck, 1984). There are many other opportunities for further research on NUE and its relationship to climate change and the ozone layer.

Utilizing high NUE crops, and thus less fertilizer, would also be a benefit to human health. There are many chemical forms of nitrogen released from fertilizers and agriculture that are detrimental to people. For example, nitrate can easily leach into groundwater or other water sources from which humans derive their drinking water (Nolan et al., 1998). As nitrogen fertilizer rates increase, the rate of nitrate leaching through the soil profile and into groundwater can also increase, demonstrating the need for controlling N inputs and improving NUE (Jian et al., 2013; XianLong et al., 2013). When consumed, nitrate adheres to the oxygen exchanging sites on red blood cells, resulting in a shortage of oxygen in the body. Serious effects to adults are rare, but infants and ruminant animals can suffer from methemoglobinemia and essentially suffocate from the lack of oxygen (Sadeq et al., 2008; Addiscott, 2007). Also, nitrogen oxide emissions from agricultural sources are a considerable cause of smog and ozone release on the earth’s surface, which have been linked to increases in asthma and even heart attacks (Addiscott, 2007; Brady and Weil, 2004; Stieb et al., 1996).

Norman Borlaug won a Nobel Peace prize for his developments in wheat production; the next development that could also have a vast effect on the worlds’ nutrition could be the production of varieties that are nitrogen use efficient. The world’s population is projected to rise 50% by the year 2050, which will more than double the demand for food (Tilman et al., 2002). This presents a problem, as nearly all arable land is already in production. Additional research and development in agriculture will be needed to produce the required amount of food to supply demand. This is compounded by the fact that as the population of the world grows, agricultural land will continue to be taken out of production to provide housing and accommodate growing cities. High NUE wheat and other crops could be the next great advancement following that of Norman Borlaug's.

The applications for identifying high NUE wheat varieties are far reaching and provide many opportunities for future research, but require an in-depth knowledge of the factors that can influence NUE. These factors are very complex as it relates to winter wheat production, and range from crop management, such as fertilizer type, rate, timing, and application methods, to advances in plant genetics and breeding. To improve NUE in wheat it is first necessary to understand the history of its cultivation and breeding and current physiology. An understanding of plant physiology, especially as it relates to N use within the tissues, will provide the best means for comprehending NUE and the methods by which it can be improved. Improvements in NUE could be akin to the advancements made during Green Revolution started by Norman Borlaug in the mid 20th century, and with the aid of modern genetics and breeding, the development of new varieties capable of using nutrients more efficiently will help to meet the global food demands of the future.

THE HISTORY OF WHEAT

For 100,000 years humans existed as nomadic hunter-gatherers subsisting on meat and to a lesser extent foraged grains and other plant products (Charmet, 2011; Cordain, 2002). Then, around 10,000 years ago, the age of agriculture began with the cultivation of wild emmer wheat in the Fertile Crescent (Araus et al., 2007; Matsuoka, 2011). With this domestication of plants came rapid population growth. Since this beginning of agriculture the world's population has risen from around 4 million to over 6 billion (Tilman, 2002). This population growth has required substantial advances in agricultural practices and plant breeding and genetics in order to meet the demand for food.

Wild emmer wheat, *Triticum dicoccoides,* is considered the ancestor of modern wheat and is still found in the Fertile Crescent today (Ozkan et al., 2010). It is an annual grass characterized by long grains, a brittle rachis, and its ability to self-pollinate (Nevo et al., 2002). It was most likely collected as an early food source by hunter-gatherers long before it became domesticated. Upon its domestication, emmer wheat began to change due to selection and then gave rise to a range of domesticated species of wheat still in existence today, most notably *Triticum aestivum* and *Triticum durum*.

One of the most important initial genetic changes to wheat was grain yield. It's hard to say whether a higher yield was purposely selected for if it resulted inadvertently, but it was certainly the result of human interaction (Evans, 1993). Larger grains provide additional food and were therefore more desirable and harvested and replanted more aggressively. The result was domesticated varieties with larger grains that had higher concentrations of starch and protein that were more suitable for producing breads (Nevo, et al., 2002).

Seed dispersal mechanisms were another key change. In mature wild wheat the spikelets, or the structure that consists of the grain and its protective glume, readily detach from the rachis, or stem, of the plant when brushed against or in the wind to effectively plant the seed (Lev-Yadun et al., 2006). This attribute poses a problem for man because by the time harvest arrives many seeds could already have been lost or would easily be lost during the harvesting process. As a result, the wheat that was harvested likely had spikelets that did not release from the rachis as easily, a portion of which was replanted and resulted in inadvertent selection and breeding. With this new characteristic one could simply pluck the entire wheat head from the stem and remove the grain later. While this trait would be unfavorable for wild-type wheat because it would not be able to propagate on its own, it has proved vital to the success of the species in a human-dominated world.

Another advance in wheat is its ability to be threshed. As mentioned before, wild wheat is encased in a glume that protects the seed. When the spikelet is dislodged it will insert the grain into the ground, planting the seed (Zohary, 1969). Separating the glume from the grain is time consuming work that requires milling and pounding. Wheat was therefore selected for a weak glume but tough rachis. The spikelet would not release from the plant before or during harvest, but during the threshing process the grains could be removed easily (Charmet, 2011; Brown et al., 2008). These developments dramatically increased the productivity of wheat and allowed it to become a crop of worldwide importance.

Brown et al. (2008) referred to the process of domestication in cereal plants as “domestication syndrome”. They divide this syndrome into several stages. One of them, and possibly the most important, is the loss of seed dispersal due to a toughened rachis that allows the grains to be harvested. Additional indicators of this syndrome include: an increase in grain size due to selection; seeds germinating upon planting instead of the crop awaiting an environmental cue; and homogenous plants that have a similar shape and size.

After these alterations, which occurred fairly early in the domestication of wheat, very little changed until the 20th century when breeding and genetics became better understood. With the aid of genetics, scientists began breeding wheat to increase disease resistance and further increase grain yields (McMahon et al., 1997). Kansas State University has a very rich history of wheat development. One of the most notable of these was the introduction of Norin 10 in 1945 by S.C. Salmon, a graduate of and former professor at K-State (Paulson, 2003). Norin 10 was a dwarf variety of wheat from Japan that was later used by Norman Borlaug in his breeding program and is the predecessor of 95% of wheat grown in Kansas (Paulson, 2003).

In the early 1960s Norman Borlaug crossed his own disease-resistant wheat varieties with Norin 10 to create several semi-dwarf disease-resistant varieties (Reitz, 1970). For his work Borlaug received the Nobel Peace Prize in 1970. His new varieties were successful for several reasons, not only for their disease resistance, but also for their shorter stature. Wheat is particularly sensitive to disease because it can affect any part of the plant including the roots, stems, leaves, and grain. Therefore, disease resistance allows varieties to avoid common infections and better create a harvestable crop (Reitz, 1970). Dwarf varieties have another key advantage: they are not as susceptible to lodging, or falling over (Smale et al., 2008). Lodging usually results in plant death or, if close to harvest, loss of the grain due to water and soil contamination. As dwarf varieties are sturdy and do not lodge as easily they can support a higher, and therefore heavier, grain yield.

Since 1917 Kansas State University has released 36 wheat varieties that were adapted specifically to the growing conditions within the state (Paulson, 2003). Wheat breeders continue to produce new varieties, selecting individuals that have disease-resistant characteristics and high yields. However, there are thousands of wheat varieties grown throughout the world. In fact, there may be upwards of 25,000 unique varieties (Nevo, et al., 2002). With growing environmental concerns and an ever-increasing global population, more breeders and researchers are beginning to turn their attention towards fertilizers and nitrogen use. Their hope is to produce varieties that use nitrogen more efficiently in order to save costs, allow those in developing countries to produce an acceptable crop with minimal fertilizer inputs, and help protect the environment from pollution.

Wheat has been a key fare since the time of its cultivation, and now accounts for more than 20% of the calories and protein consumed in the human diet (Nevo et al., 2002). To supply the current market requires 216,974,683 hectares worldwide- 34% greater that of the next largest commodity in terms of area: maize, at 161,908,449 hectares (Table 1.1, FAO, 2010). As each crop is grown and harvested, important nutrients such as nitrogen are removed from the soil. After hundreds or even thousands of years of cultivation this has caused nitrogen that naturally accumulated in the soil to become depleted. In order to meet current food demand fertilizers are required sustain soils and production.

Table 1.1. Worldwide grain production in 2010 by hectares harvested (FAO, 2010).

Commodity	Hectares
Wheat	216,974,683
Maize	161,908,449
Rice	153,652,007
Soybeans	102,386,923
Sorghum	40,508,600
Oats	9,054,956
Rye	5,327,477

Domesticated wheat has gone through many genetic changes in order to increase yield, many of which have been driven by man (Foulkes et al., 1998). However, there is a need for further genetic improvements due to the increasing human population and loss of arable land worldwide. The high yields that are expected of wheat require substantial fertilizer inputs that many throughout the world and the United States simply cannot afford. Also, high rates of fertilizer can have damaging environmental effects such as nitrogen loss through volatilization, runoff, and leaching (Anbessa et al., 2009). Therefore, there is a need to further research wheat varieties to develop ones that can use nitrogen more efficiently to make grain, thus maintaining or improving yield with less fertilizer input. This will save costs and help protect the environment from pollution.

WHEAT GROWTH STAGES

To understand how wheat uses nitrogen, it is critical to understand its growth cycle. The most commonly used scale in the US to describe the growth stages of wheat, and the one used for this study, is the Feekes scale. It classifies the development of wheat on a number system of 1 through 11, with 1 being seedling growth and 11 wheat grain maturation. The nitrogen requirements of the plant vary at different growth stages, and fertilizer applications are administered according to those needs. Herbek and Lee (2009) provide a comprehensive overview of the growth stages, from which the following is derived.

Feekes 1

Winter wheat is planted during the fall when temperatures and soil moisture best promote germination. Once the seed germinates and the first leaves become visible at the soil surface the plant is classified in the Feekes 1 stage. This usually occurs around one week after planting and is the first indication as to whether the crop will be successful. A successful crop will have rapid establishment and root growth and a uniform appearance throughout the field (Miller, 1992). During this period several leaves will emerge before entering the next growth stage.

Feekes 2

This growth stage marks the beginning of tillering. Tillers are shoots that originate from the parent root mass. Each tiller will form its own leaves and begin growing additional roots. Tillers are important as each one has the potential to produce a grain-bearing head, and with an average of three or more tillers per plant, a substantial amount of grain can be produced. Research has shown that 30-50% of grain yield comes from the main stem and 50-70% emanates from the tillers (Thiry et al., 2002). It is during the tillering stages that the first application of Nitrogen is recommended to help promote tiller growth and future grain yield (Miller, 1992).

Feekes 3

During this stage the tillers become fully developed. The colder temperatures of November and December cause the plant to go dormant and the plant usually overwinters in this stage. With the cold temperatures the tillers stay close to the ground, known as creeping. This dormant period, called vernalization, is essential for winter wheat varieties. The wheat will not enter its reproductive growth phase or produce grain without vernalizing.

Feekes 4

As warmer temperatures arrive in the late winter, the dormant period ends. The leaf sheaths at the bottom of each tiller begin to thicken, causing the tillers that grew close to the ground over the winter to grow upright (Miller, 1992).

Feekes 5

The tillers become strongly erect during this growth stage. The reproductive structure, or head, which was previously located underground to guard against the cold, now begins to grow as warmer temperatures arrive. It is recommended that the spring application of nitrogen is added during this stage. As the head begins to develop the nitrogen will improve its size and grain yield.

Feekes 6

The head begins to push above the soil surface as the stem elongates. The first node on the stem swells and becomes visible. This stage is the beginning of what is commonly called jointing.

Feekes 7

The stem continues to elongate and the second node on the stem becomes discernible. The elongation of the stem and development of nodes is known as jointing. The head continues to expand and develop throughout the jointing process.

Feekes 8

Feekes 8 is significant in that the flag leaf begins to emerge from the whorl. The flag leaf is the uppermost leaf on the stem and is critical to the production of grain. While there will be several other leaves on the stem by this point, the flag leaf produces a majority of the photosynthates responsible for grain filling. Also by this stage the third, and in some cases a fourth, and final node on the stem will become visible.

Feekes 9

The flag leaf finishes emerging during this stage. Any damage incurred from an infection on the flag leaf will drastically affect yield so it may be necessary to evaluate the wheat to see if fungicides or other pesticides are needed. Shortly after the flag leaf is fully developed, the plant enters Feekes 10, the boot stage.

Feekes 10

The developing head is located just inside the flag leaf sheath and begins to swell. As this occurs the sheath takes on a swollen appearance, known as a boot. The head begins to emerge from the sheath, and once completely uncovered begins to flower, known as anthesis. As wheat is self-pollinated, it relies on the wind to carry pollen to neighboring heads. This entire stage is relatively short in comparison to many of the others, generally occurring over the course of only a few days.

Feekes 11

By this point the plant is completely developed. All that remains is grain production. Carbohydrates from the flag leaf begin to flow into the grain, as do other products that were manufactured elsewhere and stored in the stems, leaves, and roots. This process is known as grain filling, and usually occurs over the course of a few weeks to a month. Early on, the kernels take on a milky or doughy consistency. Then as the grain ripens the moisture content decreases until the kernel becomes hard. Once it reaches a moisture content of around 15%, the grain is ready to harvest.

Understanding the growth stages is critical to any research on wheat. Seeds need to be planted at set times to establish a good crop. Nitrogen demand varies over the course of the season, and fertilizers may need to be applied accordingly. Disease may become an issue and need to be addressed at appropriate times. Finally, one must know when the crop is ready to be harvested. Understanding the growth stages allows farmers and researchers alike to create the highest quality wheat.

NITROGEN IN WHEAT

With increasing attention on the environment and a greater demand for high quality food, nitrogen and its role in wheat production is a topic of great interest in research. This research varies from nitrogen and its role in grain yield to its contributions in bread making (Nikolic et al., 2011; Gutieri et al., 2005; Guarda et al., 2004). To fully comprehend this research one must understand nitrogen, its cycle through the environment, and its importance in crop production.

Nitrogen is the most plentiful molecule in the atmosphere. As a matter of fact, 78% of the atmosphere is composed of nitrogen. The next most abundant molecule is oxygen accounting for just 21%. Nitrogen is an essential component of DNA and proteins, which are the building blocks of life. It is therefore a necessary element to sustain life on this earth.

The nitrogen cycle is the complicated process that allows this vital element to enter the biologic system. Free atmospheric nitrogen, or N_2, cannot be taken up directly by plants or animals. Therefore the important role of capturing nitrogen falls on seemingly insignificant microorganisms: bacteria (Murphy et al., 1999). The bacteria form symbiotic relationships with the roots of certain plants, often legumes. Here they form root nodules where the plant provides water and sugar to the bacteria and the bacteria in turn fixes atmospheric nitrogen into a useable form, ammonia, or NH_3 (Brady and Weil, 2004). The plant may then be consumed by other organisms to which it will pass on its nitrogen, or it will die and return the nitrogen to the soil.

Much of the nitrogen present in soil is locked in organic compounds such as proteins and humic complexes (Brady and Weill, 2002). While most plants can’t use these compounds, they prevent the nitrogen from being lost. Making these forms of nitrogen available once again falls on microbes such as bacteria and fungi, which release nitrogen in the form of ammonium ions, NH_4^+ (Murphy et al., 1999). Free ammonium in soil could potentially be lost into the atmosphere as ammonia gas, a process called volatilization. Fortunately though, ammonium has a positive charge and adheres to the negatively charged soil. There it can be taken up by plants and used again or it can be oxidized through a process called nitrification in which nitrite (NO_2^-) and subsequently nitrate (NO_3^-) are formed by soil bacteria.

Nitrate is another form of nitrogen that is useable by plants, but it is particularly prone to leaching. Both the soil and nitrate ions have a negative charge, which means they repel each other. As water moves through the soil profile, the nitrate will freely move with it, which can result in serious environmental problems. The nitrate can easily move into and contaminate groundwater and surface waters. Increased nitrates in drinking water can result in methemoglobinemia in infants and ruminant animals, a harmful condition in which nitrate replaces oxygen on red blood cells. Nitrate contamination of surface waters can result in eutrophication and harmful algae blooms. It is for these reasons, and many others, that nitrogen fertilizer needs to be applied carefully.

Denitrification is the next step of the cycle. This is when the nitrate is reduced by bacteria into gaseous forms that are released into the atmosphere hopefully as elemental nitrogen, or N_2. However, during the denitrification process other gases, such as nitric oxide (NO) and nitrous oxide (N_2O), can be released. These products are considered pollutants, as both forms can create nitric acid, which is responsible for acid rain (Brady and Weil, 2004). N_2O is particularly damaging, as it can move into the ozone layer and destroy ozone gas (O_3), which is responsible for protecting the earth from UV radiation (Brady et al., 2002). N_2O not only damages ozone, but is considered a powerful greenhouse gas linked to global climate change (Intergovernmental Panel on Climate Change, 2001). Under ideal conditions N_2 is released which can then reenter the nitrogen cycle and allow life to continue.

Nitrogen is important in crop production because it is an essential nutrient for plant growth. Wheat, like all living things, requires nitrogen and it is used at every growth stage because the plant is continually growing and producing new cells. Nitrogen is not used at a constant rate, though, as certain growth stages require more of it than others. The peak demand for nitrogen in wheat occurs just after anthesis when grain filling begins and stops around the soft dough stage (Daigger et al., 1976).

Humans have greatly influenced and changed the nitrogen cycle beginning in the 20th century by developing methods to produce commercial products that provide nitrogen to plants. This has been done primarily through the development of the Haber-Bosch process in which free atmospheric nitrogen is combined with hydrogen atoms to form ammonia (NH_3). The NH_3 can then be used to form many different fertilizer products. The Haber-Bosch process requires tremendous amounts of energy, which contribute to the rising costs of N fertilizers and the release of greenhouse gases. This has made N much more plentiful in the nitrogen cycle because of its extensive use in agricultural production. However, because of poor management practices and over application, N loss through leaching and volatilization has also greatly increased.

NITROGEN USE EFFICIENCY

The concept of nitrogen use efficiency (NUE) is relatively new. Early definitions of NUE simply stated that it was the inverse of nitrogen concentration in the plant tissue (Chapin, 1980). This was later developed into the definition most commonly used today by Moll et al. (1982) as the weight of grain yield per unit of available nitrogen in the soil (Table 1.2). However, there are several other definitions and methods used to calculate NUE (Barraclough et al., 2010).

According to the definition by Moll et al. (1982), NUE can also be considered the product of two independent calculations, nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE), and can be calculated by multiplying these two values together (Table 1.2; Foulkes et al., 2009; Barraclough et al., 2010). Other studies calculate NUE as the grain yield divided by the amount of N fertilizer applied, which in the context of this study is defined as partial factor productivity, or PFP (Cassman et al., 1996; McMahon et al., 1997; Olk et al., 1999). Another common method of calculating NUE is by estimating the difference in N uptake by a crop in fertilized and unfertilized plots, and dividing it by the fertilizer N rate (Raun and Johnson, 1999). While this was also calculated in this study, it is referred to as fertilizer use efficiency (FUE).

Table 1.2. Definitions and calculations for NUE and related parameters determined in NUE and Nrate experiments.

Term	Definition	Calculation
Nitrogen Use Efficiency (NUE)	Weight of grain per unit of available nitrogen	NUE= grain weight/ total nitrogen supply or NUpE*NUtE
Nitrogen Uptake Efficiency (NUpE)	How efficiently nitrogen is taken up by the plant from the soil	NUpE= Total N uptake/ total N supply
Nitrogen Utilization Efficiency (NUtE)	How efficiently nitrogen absorbed from the soil is used to make grain	NUtE= grain weight/ N taken up by plant or HI*BPE
Harvest Index (HI)	Weight of harvested grain as a percentage of total plant weight	HI= grain weight/ aboveground biomass
Biomass production efficiency (BPE)	Total plant weight compared to total plant N content at maturity	BPE= aboveground biomass/total N at maturity
Nitrogen harvest index (NHI)	Nitrogen content in the grain compared to total plant N content at maturity	NHI= N in grain/ total N at maturity
Nitrogen uptake after anthesis (NUpAA)	Difference in total N from anthesis to maturity	NUpAA= total N at maturity-total N at anthesis
Nitrogen remobilization efficiency (NRE)	How efficiently nitrogen at anthesis was remobilized to the grain	NRE= (N in grain-NUpAA)/total N at anthesis
Fertilizer use efficiency (FUE)	Fraction of nitrogen applied as fertilizer that was absorbed by the plant	FUE= (N uptake with fertilizer-N uptake without fertilizer)/N applied as fertilizer
Agronomic efficiency	Increase in yield per unit of applied nutrient	AgEf= (yield with fertilizer-yield without fertilizer)/ fertilizer rate
Partial factor productivity	Yield produced per unit of applied nutrient	PFP= yield/fertilizer rate
Partial nutrient balance	The fraction of nutrient that is actually used by the plant	PNB= nutrient removed/nutrient applied

One problem with calculating NUE is that it is very difficult to account for residual soil N or N that will become available over the course of the season through mineralization because there is no standardized method for its calculation (Bingham et al., 2012). Some studies approached this through soil testing for ammonium and nitrate prior to planting and ignored mineralization, assuming that it would be uniform across the field (Muurinen et al., 2006; Anbessa et al., 2009). Other studies have attempted to account for this source of N by calculating the amount of N in the biomass of control plots that were not fertilized (Paponov et al., 1996; Sylvester-Bradley and Kindred, 2009). For the purposes of this study, no attempt was made to account for mineralized N; however, future plantings will use several control plots in each block to account for mineralized N and possible spatial variability in the field.

Because of the lack of standardization in calculating NUE, it can be difficult to compare studies. The definition developed by Moll et al. (1982) is the most common, and the method used in this study. Nitrogen use efficiency was calculated as the grain yield divided by the total nitrogen supply, which included the fertilizer N and residual soil N. The two most important factors affecting NUE are NUpE and NUtE (Moll et al., 1982). Improving NUE would result in lower fertilizer expenditures and also minimize negative effects on the environment, such as leaching, eutrophication, and acid rain. There are several other parameters related to NUE, and the calculations can be found in Table 1.2.

High NUE is an indicator of a well-functioning plant-soil system as many components must work in synchronization. First, the plant must effectively remove the nitrogen from the soil through a healthy root system, also known as its nitrogen uptake efficiency (NUpE). Nitrogen uptake efficiency was calculated as total nitrogen uptake (kg ha^{-1}) per unit of nitrogen supply, or the sum of residual soil N and fertilizer N (kg ha^{-1}). It is a measure of how well the plant is able to uptake N from the soil, and is primarily a function of root traits, such as rooting depth, proliferation through the soil, and transport into plant tissues. The soil nitrogen is assumed to be fully recovered while the amount of fertilizer nitrogen used is variable (White, 2012). It is important to note that just because the plant uptakes nitrogen, it does not mean that the nitrogen remains there. A substantial amount of nitrogen can be lost from the plant during its growth. Wheat generally loses about 20% of its nitrogen (Daigger et al., 1976). The nitrogen may be lost either by leaking it into the soil from the roots or by gaseous release from the stem and leaves in the form of ammonia or nitrogen oxides. Plants that have high NUE should also be able to retain more nitrogen in the plant and subsequently use it for grain production.

From the research of Moll et al. (1982) and Lopez-Bellido (2001), it has been shown that NUpE is directly correlated to NUE. As the main purpose of any living thing is to create offspring and promote the viability of its species, devoting any nitrogen taken from the soil to its seeds is a primary objective, especially in annual crops. What limits the plant's ability the most in this regard is how effectively it can remove nitrogen from the soil matrix. A plant that is very adept at absorbing nitrogen can use it to produce a high yield and will subsequently have a high NUE.

The plant must then effectively use the nitrogen for plant growth and later reallocate some for the production of grain. The ability of the plant to use this nitrogen to make grain is known as the plants nitrogen utilization efficiency (NUtE), calculated as the grain weight over N uptake by the plant (Table 1.2). This parameter provides a value for estimating how well the plant is able to assimilate N in tissues and later translocate it to the grain to produce high yields. This parameter is a reflection of how well the biomass is able to use N to produce yield, primarily through the production of carbohydrates through photosynthesis that are later remobilized to the grain.

Biomass production efficiency was calculated as the total biomass (kg ha^{-1}) divided by the total nitrogen uptake (kg ha^{-1}), providing a measure to compare the total aboveground biomass that is produced by the wheat varieties with certain levels of nitrogen uptake. The total nitrogen uptake was determined using biomass production values from a hand-harvest and plant tissue analysis for nitrogen content.

Harvest index (HI) and nitrogen harvest index (NHI) provide a means for determining the allocation of important nutrients within the plant tissues to grain during reproductive growth. This is a common term used in agriculture and plant breeding and refers to the yield that is produced by the crop. HI was calculated by dividing grain yield (kg ha^{-1}) by the total biomass at maturity (kg ha^{-1}). It is the weight of a harvested crop expressed as a percentage of the total plant biomass. Expressing the yield in this way allows breeders and agriculturalists to compare the biological yield of a plant to its crop yield. Harvest index measurements have become a valuable tool to compare varieties within species. This provides a measure of how much dry matter, mostly in the form of carbohydrates, is allocated to the grain to produce high grain yields. NHI was calculated by using the total N in the grain (kg ha^{-1}) divided by the total N uptake (kg ha^{-1}).The nitrogen in the grain was calculated by using yield values and grain tissue analysis for N content. This value provides a measure of how much N is partitioned to the grain, and is important in understanding how the plant uses N and later allocates it to grain at the correct growth stage.

Nitrogen uptake after anthesis was calculated by subtracting the total N at anthesis (kg ha^{-1}) from the total N in the plant at maturity (kg ha^{-1}), providing a measure of the performance of the plant during the grain filling period. Nitrogen remobilization efficiency also provides an indication of plant performance during this time, and was calculate by subtraction the NUpAA value from the total N in the grain and dividing them by total N at anthesis.

Several other agronomic parameters commonly used in production agriculture were calculated, such as fertilizer use efficiency, agronomic efficiency, partial nutrient balance, and partial factor productivity. Fertilizer use efficiency was calculated by subtracting the nitrogen uptake in unfertilized plots from the nitrogen uptake from the corresponding fertilized plot, and dividing this by the total N that was applied as fertilizer. Agronomic efficiency is calculated as the difference in yield between fertilized and unfertilized plots divided by the fertilizer rate. Partial factor productivity is simply calculated by dividing the yield by the fertilizer rate that was applied. Lastly, partial nutrient balance was calculated by dividing the amount of nutrient removed by the plant by the total amount of that nutrient that was applied, which in this study was nitrogen.

FACTORS THAT AFFECT NUE

There are many factors that affect nitrogen use efficiency in plants. The most significant of these are weather, soil type, preceding crops, type of fertilizer, fertilizer timing, and different plant varieties. When you combine multiple influences they can be hard to distinguish and can cause a large variation in the NUE of the plant.

All agriculture depends on weather. Not only is it difficult to predict, but it is impossible to control and can either help or hinder crop growth and NUE. During years of drought, applying nitrogen fertilizer to wheat can actually reduce grain yield. This, coupled with fertilizer costs, can be very detrimental for a farmer (Sadras, 2002). In contrast, too much water is also problematic, as it can cause nitrogen to runoff, denitrify, or leach through the soil profile where plants cannot use it (Asseng, 2001). Favorable weather conditions will encourage mineralization, increasing the amount of soil-available nitrogen, and decrease the amount of fertilizer required to grow a satisfactory crop (Koeijer et al., 2003). The appropriate weather conditions for an area to promote high NUE are largely dependent on the soil type and management factors.

There are 12 major soil orders in the world and within each one are thousands of different types. Each of these thousands of soils differs in its physical and chemical characteristics, which can interact with management factors and NUE. A study by Villinga and Andre (1999) found only relatively small changes in NUE between different soil types. Their data also showed changes in NUE over time, and proposed they were due to the development of new wheat varieties and their adaptations to soil conditions. However, it is more widely accepted that changes in soil types, textures, and their interactions with management will influence nitrogen utilization and use efficiencies (QuanBao et al., 2007; Shu-Ping, et al., 2013; Tittonell et al., 2007).

Several studies show that NUE in wheat is also dependent upon the prior crop that was grown in the field (Rahimizadeh et al., 2010; Sahin et al., 2010). Crop rotations, especially when growing wheat, are highly recommended as they reduce the possibility of disease carryover between seasons. In a study by Rahimizadeh et al. (2010) they found evidence showing a wheat-on-wheat monoculture system had the lowest NUE compared to more diverse rotations. They grew several other crops such as corn, clover, sugarbeet, and potato. The wheat-potato rotation provided the highest NUE value, which was 24% higher than the continuous wheat system. This study is also supported by Yamoah et al. (1998) who found strong evidence that the preceding crop can significantly affect the nitrogen use efficiency of wheat.

There are several different types of fertilizer nitrogen to choose from, and each can have a different effect on NUE. Selecting the appropriate form of nitrogen largely depends on the soil type and the climate of the area. The most common fertilizers chosen for wheat will contain anhydrous ammonia, urea, and/or nitrate. Each is prone to different problems that require careful consideration. To help mitigate these problems, they each need to be applied at different times with different equipment.

Anhydrous ammonia (AA) needs to be injected into the soil because it is a gas at atmospheric pressure and temperature. The injection process requires specialized equipment that can be quite costly. Fortunately, the benefits of direct injection into the rooting zone outweigh the costs. Under the right moisture conditions, AA is quickly converted into ammonium (NH^{4+}), which adheres readily to cation exchange sites, reducing N loss (Teal et al., 2008). The moisture level needs to be within a certain range for application. In either extreme, too wet or too dry, AA is prone to volatilization (Sommer and Christensen, 1992). Anhydrous ammonia applications also have drastic effects on soil pH and AA is highly caustic, making it a safety hazard for human applicators, and also damaging to seeds and established plants.

Urea, unlike anhydrous ammonia, is solid and relatively safe to handle, making it one of the most commonly used N fertilizers in wheat. In a study by Fan et al. (2007), they showed that both conventional and controlled-release urea fertilizers increase NUE compared to a control of no nitrogen application. It is easy to apply with a broadcast surface spreader and, in optimal environmental conditions, will dissolve into the soil profile and convert into plant useable forms, namely ammonium and nitrate. However, substantial amounts of N can be lost under certain conditions.

Urea molecules cannot be used directly by plants, and to become plant available they need to be converted to ammonium through a natural process. This is primarily carried out through urea hydrolysis, which creates an intermediate form of N, ammonia. As a gas, ammonia can be lost freely to the atmosphere from the conversion of surface-applied urea, especially in high-residue environments that contain substantial amounts of the urease enzyme responsible for the hydrolysis reaction. Urea is readily soluble in water, and the best conditions for urea applications are those in which surface-applied urea can be quickly dissolved and moved into the soil profile. Then, as urea converts to ammonia, the soil will hold the gas and convert it into ammonium which is strongly held, preventing N loss. Recent research on forms of coated urea, which control the release nitrogen, have shown that grain yield and NUE can be improved with these products over normal urea fertilizers (Fan et al., 2007; Chauhan, 1999).

Another good source of plant nitrogen are fertilizers containing nitrate. Nitrate is often applied in the form of ammonium nitrate, or urea-ammonium nitrate fertilizers. Ammonium nitrate is a solid that is not at risk of volatilization, but is considered a potential security hazard because it can be used to produce powerful explosives. Urea-ammonium nitrate is a liquid fertilizer composted of a mixture of ammonium nitrate, urea, and water and cannot be used to produce explosives. Because fertilizers containing nitrates are not as prone to volatilization, they are a good option for dry areas and surface broadcast applications (Schlegel et al., 2003). They may not be the best option in wet areas with well-drained soil because nitrates are prone to leaching or being denitrified under anaerobic conditions, which would result in substantial nitrogen losses.

Properly timing the applications of these different fertilizers is also crucial to obtaining a high NUE. One strategy for improving NUE is to split-apply nitrogen by applying once early in the growing season and again during later growth stages when nitrogen is in high demand (Kharub and Chander, 2010). For wheat, studies have shown that split-applying fertilizers at the tillering stages and again during stem elongation results in higher grain yield and NUE than applying the same amount of nitrogen pre-planting (Blankenau et al., 2002; Barbieri et al., 2008). Although there is specialized machinery that can sub-surface apply anhydrous ammonia with minimal plant disturbance once the crop is established, it is an uncommon practice and therefore not commonly used for split applications. Ammonium nitrate and urea, then, are optimal fertilizers for split applications because they are solids and can easily be broadcast over a growing crop.

There is large variation in the physical characteristics of different crops and they will each have different nitrogen use efficiencies. For example, the nitrogen use efficiencies of potatoes and wheat are quite different (Zebarth et al., 2008; Raun and Johnson, 1999). Despite large differences between wheat and vegetable crops, Muurinen et al. (2006) showed that most cereal crops have very similar NUEs. The cereal crops used in their study were wheat, barley, and oats, which are similar grain-producing grasses. They found there was no statistical difference in NUE among the different species, but within the species themselves there were differences among varieties.

There are perhaps hundreds, or even thousands, of wheat varieties throughout the world. A study conducted by Mladenov et al. (2011) of 24 common wheat varieties in Serbia demonstrated the differences among varieties. The varieties were selected by their year of release, and results showed an average increase in grain yield of 41 kg per hectare per year of release, which is consistent with several other studies (Brancourt-Hulmel et al., 2003; Guarda et al., 2004). They also showed modern wheat varieties are more responsive to nitrogen inputs and have higher NUEs. Most modern wheat varieties are adapted for high nitrogen applications as breeders developed them in non-limiting conditions where resources, such as nitrogen, were supplied at high rates. Further research and breeding efforts should be aimed at increasing nitrogen use efficiency in wheat when at lower levels of fertilizer application.

Perhaps the most promising strategy to improve NUE in wheat is to select varieties that are adept at absorbing soil nitrogen and assimilating it into the grain (Koutroubas and Ntano, 2003). The only issue is very little genetic diversity among modern cultivars, despite the many unique variety names (Harlan, 1975, 1976). Wheat today is derived from purebred lines, resulting in fields that are completely homogenous. This type of situation is not desirable for plant breeding. Breeding wheat for high NUE will require substantial genetic variability that is simply not found in modern agriculture (Anbessa, et al., 2009). Past research has shown significant diversity in wild wheats, such as emmer, that have been suggested as a possible gene pool for future wheat improvement (Nevo et al., 2002; Feldman and Sears, 1981).

NITROGEN RESPONSE

Nitrogen response is measuring how plants respond to differing supplies of nitrogen with no other limiting factor. Typically, responses will be high when an addition is made to plants in low nutrient conditions, while plants that already have an adequate supply of a given nutrient, like nitrogen, will exhibit very low responses because they are already producing at or near their maximum. Much of the previous research in nitrogen response is in determining appropriate fertilizer applications to maximize economic returns (Kamprath and Watson, 1980; Reid, 2002). In these studies, the plants are treated with varying levels of nutrient supply and plant performance, such as yield, is observed at each rate to determine how the plant responds across a range of treatments. This information can then be used to make models of crop response for other producers.

There has been very little research on the differences in nitrogen response of varieties within the same species, such as wheat. This type of information could be especially useful for breeders who are seeking to develop high nutrient efficient crops that are able to achieve maximum yields with less nitrogen. This would also open the opportunity to determine whether crops with higher NUE also have higher N-response. Improvements in NUE and N-response would benefit producers who farm marginal lands, have limited access to fertilizers, or who have limited budgets for soil nutrients. This would allow producers to better meet the growing demand for food worldwide while protecting the environment.

In designing and implementing these types of experiments it is important to understand basic plant reactions to nutrient supply. One of the leading theories in this regard is Liegib’s Law of the Minimum, developed in the mid-1800s (Rubio et al. 2002). The law states that plant growth will be limited by one nutrient at a time, and always the one that is in the least supply to meet plant demand. The limiting nutrient can be anything from sunlight, water, to elements like nitrogen and phosphorus. For example, a producer could supply as much nitrogen or phosphorus to a field as desired, but without adequate supplies of water there will be no growth response from the plants until water is supplied through irrigation or a precipitation event. For studies on nitrogen response field conditions have to be such that nitrogen is the most limiting nutrient to adequately measure plant N response to differing rates.

Unfortunately, plant growth is rarely as simple as that defined in Leibig’s Law and there are economic limits where costs for fertilizer inputs will no longer provide enough return of investment. For this reason, many researchers have tried to use response curves and simple models to estimate yield for a range of input and determine an economic optimum rate. However, these models do not account well for differences between environments or years (Reid, 2002).

Creating a response curve is quite simple and requires relatively small amounts of data. They are often considered as being just as reliable as more complicated models that try to take into account nutrient flows and spatial variability as they are difficult to calibrate and validate (Reid, 2002). Response curves, in their most basic form, compare average yield to applied fertilizer (Reid, 2002; Cerrato and Blackmer, 1990; Willcutts et al., 1998). Some limitations are that they rarely account for existing soil nutrient supplies, do not account for mineralization, and only describe response to a single nutrient (Reid, 2002). However, because they require fairly small amounts of data, such as average yield values for a given rate of fertilizer, they can be easily computed in basic programs such as Excel.

RESEARCH NEEDS

There are several areas for potential research that could advance the understanding of NUE, especially as it relates to wheat. The first is to establish whether there are genetic differences in NUE and related parameters between common wheat varieties. This information would be valuable to breeders as an indication that NUE could be selected for and provide evidence for the mechanisms by which it could be improved in the future. Another area of needed research is to evaluate if NUEs in wheat have improved over time, as they have in other crops, such as corn. In order to do this, varieties that represent a range of release dates are needed. The last area in which there is little information is the effect of NUE on N-response. The most important question in this area of research is whether varieties with higher NUE also have better N-response and are able to reach their maximum yields with less N inputs. These important research needs will be addressed in the following research and will advance the understanding of NUE, allowing for potential wheat improvements in the future.

REFERENCES

Addiscott, T.M. 2007. Nitrate from agriculture: a hazard to health and the environment? CAB Rev. 2:29

Ahman, A., I. Khan, Y.P. Abrol, and M. Iqbal. 2008. Genotypic variation of nitrogen use efficiency in Indian mustard. Environmental Pollution. 154: 462-466.

Anbessa, Y., P. Juskiw, A. Good, J. Nyachiro, and J. Helm. 2009. Genetic variability in nitrogen use efficiency of spring barley. Crop Sci. 49: 1259-1269.

Araus, J.L., J.P. Ferrio, R. Buxo, and J. Voltas. 2007. The historical perspective of dryland agriculture: lessons learned from 10 000 years of wheat cultivation. J. Exp. Botany. 58: 131-145.

Asseng, S., N.C. Turner, and B.A. Keating. 2001. Analysis of water- and nitrogen-use efficiency of wheat in a Mediterranean climate. Plant and Soil. 223:127-143.

Baggs, E.M., M. Stevenson, M. Pihlatie, A. Regar, H. Cook, and G. Cadisch. Nitrous oxide emissions following application of residues and fertilizer under zero and conventional tillage. Plant and Soil. 254: 361-370.

Barbieri, P.A., H.S. Rozas, and H.E. Echeverria. 2008. Time of nitrogen affects nitrogen use efficiency of wheat in the humid pampas of Argentina. Canadian J. of Plant Sci. 88: 849-857.

Barraclough, P.B., J.R. Howarth, J. Jones, R. Lopez-Bellido, S. Parmar, C.E. Shepherd, and M. J. Hawkesford. 2010. Nitrogen efficiency of wheat: Genotypic and environmental variation and prospects for improvement. Europ. J. Agron. 33: 1-11.

Blankenau, K., H.W. Olfs, and H. Kuhlmann. 2002. Strategies to improve the use efficiency of mineral fertilizer nitrogen applied to winter wheat. J. of Agron. and Crop Sci. 188: 146-154.

Brady, N.C., and R.R. Weil. 2004. *Elements of the nature and properties of soils.* Upper Saddle River, New Jersey. Pearson Prentice Hall.

Brancourt-Hulmel, M., D. Goussinault, C. Lecomte, P. Berard, B. LeBuanec, and M. Trottet. 2003. Genetic improvement of agronomic traits of winter wheat cultivars release in France from 1946 to 1992. Crop Sci. 43: 37-45.

Brown, T.A., M. K. Jones, W. Powell, and R.G. Allaby. 2008. The complex origins of domesticated crops in the Fertile Crescent. Trend in Ecology and Evolution. 24: 103-109.

Cassman, K.G., S. Peng, D.C. Olk, J.K. Ladha, W. Reichardt, A. Dobermann, and U. Singh. 1998. Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. Field Crops Res. 56: 7-39.

Chapin, F.S., III. 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics. 11: 233-260.

Charmet, G. 2011. Wheat domestication: lessons for the future. C.R. Biologies. 334:212-220.

Chauhan, R.P.S. 1999. Efficiency of coated-urea materials in rice (*Oryza sativa*)- wheat (*Triticum aestivum*) system under partially reclaimed sodic soil. Indian J. of Agric. Sci. 69:311-316.

Cordain, L., S.B. Eaton, J.B. Miller, N. Mann, and K. Hill. 2002. The paradoxical nature of hunter gatherer diets: meat-based, yet non-atherogenic. European J. of Clinical Nutrition. 56: S42-S52.

Daigger, L.A., D.H. Sander, and G.A. Peterson. 1976. Nitrogen content of winter wheat during growth and maturation. Agron. J. 68: 815-818.

Evans, L.T. 1993. Crop evolution, adaptation and yield. Cambridge University Press, Cambridge.

Fan, X., F. Li, F. Liu, and D. Kumar. 2007. Fertilization with a new type of coated urea. Evaluation for nitrogen efficiency and yield in winter wheat. J. of Plant Nutrition. 27:853-865.

Food and Agriculture Organization, Statistics. 2010. FAOStat. http://faostat.fao.org/ (accessed May 31, 2012).

Foulkes, M.J., M.J. Hawkesford, P.B. Barraclough, M.J. Holdsworth, S. Kerr, S. Kightley, and P.R. Shewry. 2009. Identifying traits to improve the nitrogen economy of wheat: recent advances and future prospects. Field Crops Res. 114: 329-342.

Foulkes, M.J., R. Sylvester-Bradley, and R.K. Scott. 1998. Evidence for differences between winter wheat cultivars in acquisition of soil mineral nitrogen and uptake and utilization of applied fertilizer nitrogen. J. of Agric. Sci. 130:29-44.

Freney, J.R. 1997. Emission of nitrous oxide from soils used for agriculture. Nutrient Cycling in Agroecosystems. 49: 1-6.

Guarda, G., S. Padovan, and G. Delogu. 2004. Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. Europ. J. of Agron. 21: 181-192.

Guttieri, M.J., R. McLean, J.C. Stark, and E. Souza. 2005. Managing irrigation and nitrogen fertility of hard spring wheats for optimum bread and noodle quality. Crop Sci. 45: 2049-2059.

Hamid, A., and R.L. Mahler. 1994. The potential for volatilization losses of applied nitrogen fertilizers from northern Idaho soils. Communication Soil Sci. Plant Analysis. 25:361-373.

Harlan, J.R. 1975. Our vanishing genetic resources. Science. 188: 618-621.

Harlan, J.R. 1976. Genetic resources in wild relative of crops. Crop Sci. 16: 329-333.

Hauck, R. D. 1984. Technological approaches to improving the efficiency of nitrogen fertilizer use by crop plants. Hauck, R.D., editor. Nitrogen in Crop Production. ASA, Madison, WI. p. 551–560.

Herbek, J., C. Lee. 2009. ID-125: A comprehensive guide to wheat management in Kentucky. UK Ag Extension. Section 2 pages 6-12.

Intergovernmental Panel on Climate Change. 2001. *Climate change 2001: the scientific basis.* Cambridge University Press, New York.

Jian, D., W. ZhaiHui, L. Qiang, L. MengHua, and L. FuCui. 2013. Effects of nitrogen application rate on winter wheat yield and soil nitrate nitrogen during summer fallow season on dryland. Acta Pedologica Sinica. 50: 956-965.

Kamprath, E.J., Watson, M.E., 1980. Conventional soil and tissue tests for assessing the phosphorus status of soils. In: Khasawneh, F.E., Sample, E.C., Kamprath, E.J. (Eds.), The

Kharub, A.S., and S. Chander. 2010. Effect of nitrogen scheduling on wheat (*Triticum aestivum*) productivity and quality under alternate tillage practices. Indian J. of Agric. Sci. 80: 29-32.

Koeijer, T.J., A.J. de Buck, G.A.A. Wossink, J. Oenema, J.A. Renkema, and P.C. Struik. 2003. Annual variation in weather: its implications for sustainability in the case of optimizing nitrogen input in sugar beet. European J. of Agron. 19:251-264.

Lev-Yadun, S., A. Gopher, S. Abbo, A. Hartmann, M.E. Kislev, and E. Weiss. 2006. How and when was wild wheat domesticated? Science. 313: 296.

Lopez-Bellido, R.J., and L. Lopez-Bellido. 2001. Efficiency of nitrogen in wheat under Mediterranean condition: effect of tillage, crop rotation and N fertilization. Field Crop Res. 71: 31-64.

Matsuoka, Y. 2011. Evolution of polyploid *Triticum* wheats under cultivation: the role of domestication, natural hybridization and allopolyploid speciation in their diversification. Plant Cell Physiol. 52: 750-764.

McMahon, M., J.I. Ortiz-Monasterio R., S. Rajaram, and K.D. Sayre. 1997. Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Sci. 37.3: 898+

Mladenov, N., N. Hristov, A. Kondic-Spika, V. Djuric, R. Jevtiv, and V. Mladenov. 2011. Breeding progress in grain yield of winter wheat cultivars grown at different nitrogen levels in semiarid conditions. Breeding Sci. 61: 260-268.

Moll, R.H., E.J. Kamprath, and W.A. Jackson. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agron. J. 74: 562-564.

Murphy, D.V., A.J. Macdonald, E.A. Stockdale, K.W.T. Goulding, S. Fortune, J.L. Gaunt, P.R. Poulton, J.A. Wakefield, C.P Webster, and W.S. Wilmer. 1999. Soluble organic nitrogen in agricultural soils. Biology and Fertility of Soils. 30: 374-387.

Muurinen, S., G.A. Slafer, and P. Peltonen-Sainio. 2006. Breeding effects on nitrogen use efficiency of spring cereals under northern conditions. Crop Sci. 46:561-568.

Nevo, E., A.B. Korol, A. Beiles, and T. Fahima. 2002. *Evolution of wild emmer and wheat improvement.* Springer-Verlag, Germany.

Nikolic, O., T. Zivanovic, M. Kraljevic-Balalic, and M. Milanovic. 2011. Interrelationship between grain yield and physiological parameters of winter wheat nitrogen nutrition efficiency. Genetika-Belgrade. 43: 91-100.

Nolan, B.T., B.C. Ruddy, K.J. Hitt, and D.R. Helsel. 1998. A National Look at Nitrate Contamination of Ground Water. Water Conditioning and Purification. 39: 76-79.

Ozkan, H., G. Willcox, A. Graner, F. Salamini, and B. Kilian. 2010. Geographic distribution and domestication of wild emmer wheat (*Triticum dicoccoides).* Genetic Resources and Crop Evolution. 58: 11-53.

Paponov, I., W. Aufhammer, H.P. Kaul, and F.P. Ehmele. 1996. Nitrogen efficiency components of winter cereals. Eur. J. Agron. 5: 115-124.

Paulson, G.M. 2003. A history of wheat improvement at Kansas State University. Keeping up with Research. 136: 1-6.

QuanBao, Y., Z. HongCheng, W. HaiYan, Z. Yinh, W. BenFu, X. Ke, H. ZhongYang, D. QiGen, and X. Ke. 2007. Effects of nitrogen fertilizer on nitrogen use efficiency and yield of rice under different soil conditions. Front. Ag. China. 1: 30-36.

Rahimizadeh, M., A. Kashani, A. Zare-Feizabadi, A.R. Koocheki, and M. Nassiri-Mahallati. 2010. Nitrogen use efficiency of wheat as affected by preceding crop, application rate of nitrogen and crop residues. Australian J. of Crop Sci. 4:363-368.

Rahman, M.M., T. Amano, and T. Shiraiwa. 2009. Nitrogen use efficiency and recovery from N fertilizer under rice-based cropping systems. Australian J. of Crop Sci. 3: 336-351.

Raun, W.R., and G.V. Johnson. 1999. Improving nitrogen use efficiency for cereal production. Agron. J. 91:357-363.

Ravishankara, A., J.S. Danial, and R.W. Portman. 2009. Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science. 326:123-125.

Reid, J.B. 2002. Yield response to nutrient supply across a wide range of conditions 1. Model derivation. Field Crops Res. 77:161-171.

Reitz, L.P. 1970. New wheats and social progress. Science. 169: 952-955. Role of Phosphorus in Agriculture. American Society of Agronomy, Madison, WI, pp. 433–469.

Rubio, G., J. Zhu, and J. P. Lynch. 2002. A critical test of the two prevailing theories of plant response to nutrient availability. Am. J. of Botany. 90: 143-152.

Sadeq, M., C.L. Moe, B. Attarassi, I. Cherkaoui, R. ElAouad, and L. Idrissi. 2008. Drinking water nitrate and prevalence of methemoglobinemia among infants and children aged 1-7 years in Moroccan areas. Int. J. of Hygiene and Env. Health. 211: 546-554.

Sadras, V. 2002. Interaction between rainfall and nitrogen fertilization of wheat in environments prone to terminal drought: economic and environmental analysis. Field Crops Res. 77:201-215.

Sahin, S., M.R. Karaman, and O. Duzdemir. 2010. Residual effect of bacterium (*Rhizobium* spp.) inoculated chickpea (*Cicer arietinum* L.) genotypes on nitrogen (N) use efficiency of some wheat plants. African J. of Agric. Res. 5: 2215-2221

Schlegel, A.J., K.C. Dhuyvetter, and J.L. Havlin. 2003. Placement of UAN for dryland winter wheat in the central high plains. Agron. J. 95: 1532-1541.

Shu-Ping, X., Z. Juan-Juan, Y. Yang, L. Juan, W. Xiao-Hang, W. Yan-Peng, and M. Xin-Ming. Research on nitrogen metabolism characteristics and use efficiency in different winter wheat cultivars grown on three soil textures. Chinese J. Plant Ecol. 37: 601-610.

Skiba, U., S.K. Jones, U. Dragosits, J. Dewer, D. Fowler, R.M. Rees, V.A. Pappa, L. Cardenas, D. Chadwick, S. Yamulki, and A.J. Manning. 2012. UK emissions of the greenshouse gas nitrous oxide. The Royal Society. 367: 1175-1185.

Smale, M., J. Singh, S. Di Falco, and P. Zambrano. 2008. Wheat breeding, productivity and slow variety change evidence from the Punjab of India after the Green Revolution. Australian J. of Agric. and Resource Economics. 52: 419-432.

Smith, B.E. 2002. Nitrogenase reveals its inner secrets. Science. 297: 1654-1655.

Sommer, S.G., and B.T. Christensen. 1992. Ammonia volatilization after injection of anhydrous ammonia into arable soils of different moisture levels. Plant and Soil. 142:143-146.

Stieb, D.M., R.T. Burnett, R.C. Beveridge, and J.R. Brook. 1996. Association between ozone and asthma emergency department visits in Saint John, New Brunswick, Canada. Env. Health Perspectives. 104: 1354-1360.

Sylvester-Bradley, R., and D.R. Kindred. 2009. Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. J. Exp. Bot. 60: 1939-1951.

Teal, R.K., K.W. Freeman, K. Girma, D.B. Arnall, J.W. Lawles, K.L. Martin, R.W. Mullen, and W.R. Raun. 2008. Effect of tillage and anhydrous ammonia application on nitrogen use efficiency of hard red winter wheat. J. of Sustainable Ag. 30:51-67.

Tilman, D., K.G. Cassman, P.A. Matson, R. Naylor, and S. Polasky. 2002. Agricultural sustainability and intensive production practices. Nature. 418: 671-677.

Tittonell, P., S. Zingore, M.T. van Wijk, M. Corbeels, and K.E. Giller. 2007. Nutrient use efficiencies and crop responses to N,P and manure applications in Zimbabwean soils: Exploring management strategies across soil fertility gradients.

Vellinga, T.V., and G. Andre. 1999. Sixty years of Dutch nitrogen fertilizer experiments, an overview of the effects of soil type fertilizer input, management and of developments in time. Netherlands J. of Agric. Sci. 47: 215-241.

White, E. 2012. The effects of cultivar, season and site on variation in grain N concentration and N use efficiency of winter wheat in Northern Ireland. J. of Agric. Sci. 150: 460-472.

XianLong, Y., L. YongLi, T. Yan'an, L. Wen, and L. Ting. 2013. Effects of application of nitrogen fertilizer and incorporation of straw on nitrate leaching in farmland under wheat-maize rotation system. Acta Pedologica Sinica. 50:564-573.

Yamoah, C.F., G.E. Varvel, W.J. Waltman, and C.A. Francis. 1998. Long-term nitrogen use and nitrogen removal index in continuous crops and rotations. Field Crop Res. 57:15-27.

Zebarth, B.J., T.R. Tarn, H. de Jong, and A. Murphy. 2008. Nitrogen use efficiency characteristics of Andigena and diploid potato selections. Am. J. of Potato Res. 85: 210-218.

Zohary, D. 1969. The progenitors of wheat and barley in relation to domestication and agricultural dispersal in the Old World. *The domestication and exploitation of plants and animals*. Duckworth, London, pp 47-66.

Chapter II
Differences in Nitrogen Use Efficiency and Related Parameters in 25 Winter Wheat Varieties

ABSTRACT

Two increasingly important issues in modern-day agriculture are the high costs of nitrogen fertilizers and environmental concerns surrounding their application. A promising method to alleviate these problems is by developing more efficient crops. The objective of this study was to determine if there are genetic differences in nitrogen use efficiency (NUE) and related parameters in 25 wheat varieties commonly grown in the Great Plains. The experiment was a field study conducted during the 2010-2014 seasons at five locations in Kansas. The factorial experiment was a strip plot design with treatments consisting of N rate and variety. Two N rates were split-applied at each location, 0 kg N ha^{-1} and 90 kg N ha^{-1}. Nitrogen use efficiency was calculated as the grain yield per unit of available N (sum of soil and fertilizer N). There were no significant differences in the included varieties for any parameter measured at anthesis. Significant differences between the varieties developed at maturity for grain yield, biomass production, nitrogen use efficiency, nitrogen utilization efficiency, and harvest index with a $p<0.05$. Mean NUE values ranged from 22 to 30 kg grain per kg of N. Several other agronomic parameters, fertilizer use efficiency, partial factor productivity, and partial nutrient balance, were also significantly affected by variety with a $p<0.05$. However, nitrogen uptake efficiency and nitrogen remobilization efficiency were not significantly different among varieties. These results suggest that there are strong varietal differences in NUE that are not due to differences in uptake or remobilization, but instead are dependent on the utilization of N within the plant to produce higher yields. Differences in NUE were not correlated with date of variety release, suggesting that continuation of current breeding practices will not result in increased NUE of future

varieties. For progress to be made, programs will need to directly select for high NUE, and these data on varietal differences are a good place to begin identifying traits and possible parent-lines for future breeding programs.

INTRODUCTION

Nitrogen is an essential nutrient for all biological life, including agricultural crops. In many agricultural systems N is the primary nutrient that affects plant productivity. This is especially true in developing countries where many producers can't afford, or have limited access to, fertilizers. In favorable environmental conditions in terms of moisture, light, and temperature, the yields of crops closely follow N availability (Barraclough et al., 2010). While crop yields often increase at higher N rates, there can also be negative environmental consequences associated with high N inputs to agriculture, such as nitrate leaching and the release of greenhouse gases like nitrous oxide (N_2O).

Nitrogen fertilizers accounted for 59% of total nutrient consumption in the United States in 2011(FAOSTAT, 2011). Furthermore, N fertilizer prices have risen due to higher production and transportation costs (USDA ERS, 2013). Higher prices, increasing demand for food, and environmental concerns provide motivation to control the amount of N used in agricultural production.

The global population is expected to rise by 50% through the year 2050 (Rothstein, 2007). Although the predicted population increase is 50%, the increase in food demand will likely be close to 100% because of the growing middle class in developing countries, such as in China and India. This increase in demand is due to the popularity of animal products that accompany a higher standard of living, which require an enormous amount of resources to produce (Rothstein, 2007; Tilman et al., 2002). It is expected that if yields rise to meet burgeoning populations, the consumption of nitrogen will increase 2.7-3.9 times that of current rates (Tilman et al., 2001).

A promising method to minimize N applications is by increasing the nitrogen use efficiency of crops. Nitrogen use efficiency is grain weight divided by total nitrogen supply (Moll et al., 1982). It follows, then, that there are three ways to improve NUE. One is to maintain yields while decreasing the N supply, which would at least maintain food production, but would not meet the needs of a growing population. Another is to increase yield with a constant supply of N, which would better meet global food demand, but would still require substantial amounts of fertilizer. The last, and most ideal, method would be to increase yield and decrease N supply (Cormier et al., 2013). The final method would require improvements in both N management and N uptake and utilization by crops. In order to develop higher NUE crops, breeding programs will need to make breeding more efficient crops a top priority along with disease resistance, grain yield, and grain quality.

The largest advancements in wheat breeding came during the Green Revolution when dwarfing genes were bred into wheat by Norman Borlaug. The dwarfing genes allowed more resources to be devoted to grain instead of producing biomass and also decreased the chances of lodging, allowing the plant to support higher yields (Barraclough et al., 2010; Gooding et al., 2012). Today, most breeding programs conduct their activities at optimum N rates, and while there is evidence that NUE and plant performance at low N levels have also increased over time, these are the result of indirect selection (Brancourt-Hulmel et al., 2005; Cormier et al., 2013). If the same attention can be provided to NUE as has been applied to other traits, such as disease resistance and grain yield, significant advancements could be made.

For any breeding program, differences between varieties and desired traits first need to be identified. To breed for NUE, differences between varieties and the effects of nitrogen rate on NUE need to be established. Interestingly, heritabilities for traits in most crops are generally lower under limiting conditions, which could be a challenge in the development of higher NUE crops designed for low nutrient or stressful conditions (Bertin and Gallais, 2000; Sinebo et al., 2002). Unfortunately, there is no one gene that is responsible for NUE, and there is very little information on the differences in NUE among wheat varieties.

The objectives of this study are to determine if there are genetic differences in NUE and related parameters in 25 winter wheat varieties commonly grown in the Great Plains. These varieties represent a range of release dates and pedigrees, and the effect of breeding on NUE over time will also be evaluated. This information would be valuable to wheat breeders as programs begin to focus on increasing nutrient efficiencies in new varieties.

MATERIALS AND METHODS

Research was conducted from 2010-2014 at 6 site-years Kansas (Table 2.1). Soil samples were taken pre-planting at most locations; however, some were sampled after planting due to scheduling conflicts. In these situations, soil samples were taken in between planting rows to avoid the P fertilizer that was applied with the seed. Samples were taken at depths of 0-15 cm and 15-61 cm for complete profile nitrate analysis. Three composite samples were taken from each experimental block, with 10 cores per composite taken in the fall of 2010, 10 cores per composite in 2011, and 15 cores per composite in the fall of 2012 and 2013.

Table 2.1. Locations, soils, and cultural practices used for wheat production in six environments in Kansas.

Growing Season	Location	Coordinates	Soil map unit	Cultural practices
2010-2011	Rossville Exp. Field	39° 07' 07” N, 95° 55' 29” W	Eudora silt loam (coarse-silty, mixed, superactive, mesic Fluventic Hapludoll)	No-till after soybean. No irrigation.
2011-2012	Rossville Exp. Field	39° 07' 07” N, 95° 55' 29” W	Eudora silt loam (coarse-silty, mixed, superactive, mesic Fluventic Hapludoll)	No-till after soybean. 19.8 mm irrigation.
2012-2013	Silverlake Exp. Field	39° 04’ 34” N, 95° 46' 12” W	Eudora-Bismarck Grove silt loam (coarse-silty, mixed, superactive, mesic Fluventic Hapludoll)	No-till after soybean. 42.2 mm irrigation.
2012-2013	Ashland Bottoms Research Farm	39° 7' 35” N, 96° 38' 11” W	Wymore silty clay loam (Fine, smectitic, mesic Aquertic Argiudoll)	No-till after soybean. No irrigation.
2013-2014†	Hutchinson Exp. Field	37° 55' 51” N, 98° 01' 41” W	Ost loam (Fine-loamy, mixed, superactive, mesic Udic Argiustoll)	No-till after soybean. No irrigation.
2013-2014†	Ashland Bottoms Research Farm	39° 7' 23” N, 96° 38' 17” W	Wymore silty clay loam (Fine, smectitic, mesic Aquertic Argiudoll)	No-till after soybean. No irrigation.

† not included in data analysis because yield data was not available at the time of thesis defense.

Soil samples were dried, ground, and sent to the K-State Soil testing lab for analysis. The samples were then analyzed for pH, total carbon and nitrogen percent’s, P, K, and N [ammonium (NH_4^+) and nitrate (NO_3^-)]. Samples collected prior to the 2010-11 season were analyzed for extractable nitrogen in house, using KCl extraction and extracts were analyzed using a Lachat in the Soil Fertility Lab. All other soil analysis were conducted by the K-State Soil Testing Laboratory. Soil pH was determined with a 1:1 soil and water mixture by using a standard pH meter. Total carbon and nitrogen were determined by dry combustion. Extractable P was determined using the Mehlich-3 extraction method (Mehlich, 1984). Potassium was determined using an ammonium acetate (NH_4OAc) extraction (Hanlon and Johnson, 1984). Lastly, NH_4-N and NO_3^--N were determined using a potassium chloride (KCl) extraction (Keeney and Nelson, 1982). The results for these analyses at all locations are located in Table 2.2.

Table 2.2. Initial soil test results for pH, total C, total N, Mehlich III P, extractable K, and extractable inorganic N by environment.

Location	Depth	pH	Total C	Total N	P	K	NH4	NO3	Inorganic Profile N
	cm		— g kg^{-1} —		———	mg kg^{-1}		———	kg ha^{-1}
Rossville 2010-11	0-15	6.0	7.2	0.7	27.7	107.4	1.7	0.9	**20.4**
	15-61	-	-	-	-	-	1.9	0.6	-
Rossville 2011-12	0-15	7.0	8.7	0.9	26.9	172	5.1	6.6	**57.5**
	15-61	-	-	-	-	-	3.5	2.1	-
Silverlake 2012-13	0-15	7.4	7.0	0.7	14.8	115	2.3	4.5	**43.0**
	15-61	-	-	-	-	-	1.9	2.9	-
Ashland Bottoms 2012-13	0-15	6.4	13.4	1.4	5.2	300	4.0	2.2	**41.9**
	15-61	-	-	-	-	-	3.1	1.7	-
Ashland Bottoms 2013-14	0-15	6.5	14.0	1.2	20.2	401.4	3.8	6.5	**66.8**
	15-61	-	-	-	-	-	4.1	3.5	-
Hutchinson 2013-14	0-15	5.4	12.5	1.1	52.6	245.7	6.3	10.8	**131.4**
	15-61	-	-	-	-	-	4.8	11.2	-

The study was conducted in a randomized strip-plot design replicated four times at each location (Appendix A.1-A.4). Wheat varieties were planted in plots 18.3 m long and 1.7 m wide. The two nitrogen treatments were stripped across wheat varieties, creating subplots that were 9.1 m long and 1.7 m wide.

There were 25 wheat varieties included in the NUE study (Table 2.3). These varieties represent a range of release dates, pedigrees, and sources. All of the varieties were semi-dwarf hard red winter wheat. These varieties were selected based off of preliminary research by Battenfield et al. (2013) in a project that sought to demonstrate breeding gains in varieties released over the course of several decades. The varieties were no-till planted at a rate of 78.3 kg ha^{-1} into soybean residue using a 6-row cone planter at each location. Phosphorus was applied infurrow at planting using triple super phosphate (0-45-0) as starter at a rate of 87 kg product ha^{-1}, or 17 kg P ha^{-1}, in the 2010-11 and 2011-12 seasons and 50.4 kg product ha^{-1} or 9.9 kg P ha^{-1}. The difference in rates were due to differences in planter setting, but were intended to be non-limiting at all locations, even with variability in soil test levels. This would ensure the growth of wheat in this study would not be limited by P availability, and would instead be governed by nitrogen. A detailed table of important dates in these studies, such as planting, fertilizer applications, and pesticide applications can be found in Table 2.4.

Table 2.3. Wheat varieties included in the NUE study with corresponding year of release and source.

Variety	Year of Release	Developed By
2137	1995	Kansas State University
2174	1997	Oklahoma State University
2180	1988	Pioneer
Armour	2010	WestBred
Art	2007	AgriPro
Billings	2009	Oklahoma State University
Cedar	2011	WestBred
Custer	1994	Oklahoma State University
Duster	2006	Oklahoma State University
Endurance	2004	Oklahoma State University
Everest	2010	Kansas State University
Fannin	2003	AgriPro
Fuller	2006	Kansas State University
Jackpot	2008	AgriPro
Jagalene	2001	AgriPro
Jagger	1994	Kansas State University
KS020319-7-2	Not yet released	Kansas State University
Ogallala	1992	AgriPro
Overley	2003	Kansas State University
Postrock	2006	AgriPro
Santa Fe	2003	WestBred
TAM105	1979	Texas A&M University
TAM110	1996	Texas A&M University
TAM111	2003	Texas A&M University
TAM112	2005	Texas A&M University

Table 2.4. Table of important events and corresponding dates in the NUE studies from 2010-14 seasons.

Location	Soil Sampled	Planting	1st N appl.	2nd N appl.	Herbicide application	Fungicide application	NDVI	Anthesis Biomass samples	Mature Biomass harvest	Combine harvest
Rossville 2010/11	10/10†	10/15/10	10/10†	3/11†	2/23/11	5/3/11	4/20/11	5/10/11	6/23/11	6/11†
Rossville 2011/12	10/13/11	10/15/11	11/17/11	3/9/12	3/15/12	4/18/12	4/11/12	4/25/12	6/8/12	6/12/12
Silverlake 2012/13	11/1/12	10/12/12	11/1/12	4/1/13	4/2/13	5/7/13	3/15/13	5/21/13	7/9/13	Not harvested w/combine
Ashland Bottoms 2012/13	10/31/12	10/30/12	11/15/12	3/4/13	Not Applied	5/15/13	3/15/13	5/23/13	7/1/13	Not harvested w/combine
Ashland Bottoms 2013/14	10/22/13	11/18/13	12/6/13	3/21/14	NA	NA	NA	NA	NA	NA
Hutchinson 2013/14	11/12/13	10/23/13	11/14/13	3/25/14	NA	NA	NA	NA	NA	NA

† Exact dates unknown for these treatments or measurements

Abbreviations: NA, date not available at time of thesis defense.

The N treatments were a control (0 kg N ha^{-1}) and a high N treatment (either 90 or 111 kg N ha^{-1}). Nitrogen source was ammonium nitrate (34-0-0) and was split-applied at all locations. Ammonium nitrate was hand-applied to each sub-plot at 34 kg N h^{-1} shortly after planting at all locations except Rossville 2011-2012. At the Rossville 2011-2012 location, the first ammonium nitrate application was made with a drop spreader (Barber spreader) at a rate of 55 kg N ha^{-1}. At Feekes 4 to 5 growth stage a second N application was made by hand-applying ammonium nitrate to the high N sub-plots at 56 kg N ha^{-1} for all locations (see Table 2.4 for exact dates).

Herbicide and fungicide treatments were applied at appropriate times in the season to prevent yield losses from weed or disease pressure (Table 2.5). Herbicide was not applied at Ashland Bottoms in the 2012-13 season due to a scheduling mistake; therefore, there was a significant amount of early-season weed pressure. The most dominant weed species was identified as henbit (*Lamium amplexicaule*). Density was uniform throughout the study and could have influenced development and performance of the wheat. However, the henbit life cycle ended quite early in the season and appeared to be outcompeted by the wheat crop as it entered stem elongation phases of growth.

Table 2.5. Rates of herbicide and fungicide applications for each location of the NUE study.

Growing Season	Location	Herbicide	Fungicide
2010-2011	Rossville Exp. Field	0.019 kg Finesse ha^{-1}	0.67 kg Quilt Xcel ha^{-1} and 0.38 kg Trophy Gold ha^{-1}
2011-2012	Rossville Exp. Field	0.019 kg Finesse ha^{-1}	0.67 kg Quilt Xcel ha^{-1} and 0.38 kg Copper Oxychloride ha^{-1} ($Cu_2[OH]_2Cl$, or COC)
2012-2013	Silver Lake Exp. Field	0.019 kg Finesse ha^{-1}	0.57 kg Headline ha^{-1} and 0.38 kg COC ha^{-1}
2012-2013	Ashland Bottoms Research Farm	none	0.67 kg Headline ha^{-1} and 0.38 kg COC ha^{-1}
2013-2014†	Hutchinson Exp. Field	NA	NA
2013-2014†	Ashland Bottoms Research Farm	NA	NA

† not included as information was not available at the time of thesis defense.

Abbreviations: NA, information not available at time of thesis defense.

Normalized Difference Vegetation Index (NDVI) readings were taken at flag leaf emergence (Feekes 8) at all locations using a GreenSeeker® produced by Trimble®. These measurements provided an indication of biomass and were used in an effort to correlate varietal performance during vegetative growth to final yield and other parameters related to NUE, as supported by other research (Girma et al., 2006; Marti et al, 2007; Raun et al., 2002).

Biomass samples were taken at anthesis (Feekes 10) to measure aboveground biomass and carbon and nitrogen contents (Figure 2.1). These samples were taken using a hedge trimmer in the 2010-11 and 2011-12 seasons and handheld sickles in the 2012-2013 season by removing all of the aboveground biomass in a specified area. In the 2010-11 and 2011-12 seasons this was done by harvesting four rows for 0.61 m, for a total area of around 0.68 m^2. In the 2012-2013 season, in an effort to take a more representative sample, the two center rows of each plot were harvested for 3 m, for a total area of around 1.7 m^2. The biomass samples were weighed in the field and processed through a plant mulcher (Figure 2.1). A subsample of the plant tissue was then taken, weighed, and placed in a bag. The tissue samples were placed in a large oven at 60° C until thoroughly dried. They were then removed and weighed again, providing information on moisture content. The tissue samples were then ground using a Wiley-Mill, and a sample was submitted to the K-State Plant Analysis Lab to determine total carbon and nitrogen contents using a dry combustion method (Matejovic, 1995).

Figure 2.1. Pictures of biomass harvest at anthesis at Silverlake, KS in the spring of 2013.

The wheat was then grown to maturity and grain and biomass samples were taken once the plant reached Feekes 11 and appropriate grain moisture content of below 15%. During the 2010-11 and 2011-12 seasons the biomass samples were harvested by hand using sickles, again by harvesting four rows x 0.61 m starting where the last biomass sample was taken at anthesis. The remainder of the plots were then harvested using a plot research combine to remove the grain. The combine was equipped with a plot monitor and provided readings on test weight, yield, and grain moisture content that were used in later calculations. However, due to mechanical problems with the combine harvest in 2012, the grain from several plots was lost. Because of these past problems a combine was not used in the 2012-13 season to harvest the grain. Instead, a larger area was hand-harvested, again by harvesting the two center rows for 3 m on the opposite side of the plot from where the samples were taken earlier at anthesis. These samples were not processed in the field because biomass and grain would need to be processed and weighed separately to provide necessary information for parameter calculations.

The mature plant samples were placed in a large oven at 60° C until dry, then removed and weighed. In the 2010-11 season, a large industrial thresher was used to process the samples and separate the grain. The grain was weighed, and a sample of the straw and grain was removed. These samples were then ground and sent to the K-State Plant Analysis lab to determine carbon and nitrogen contents of these tissues. The large thresher that was used was inefficient and lost a significant amount of grain with the straw, and for this reason a smaller and more efficient thresher was used to process samples for the 2011-12 and 2012-13 studies. The grain from these studies was weighed and samples of straw and grain were ground and again submitted to the K-State Plant Analysis lab. Grain yield was corrected to 12% moisture for all studies.

Once yield, biomass, nitrogen and carbon contents of the plant tissue at anthesis and maturity, and grain nitrogen and carbon contents were determined, the parameters of interest could be calculated (Table 1.2). Total nitrogen uptake (kg ha^{-1}) by the plant was calculated at both anthesis and maturity by taking the nitrogen content (%) from plant analysis and multiplying them with measured biomass (kg ha^{-1}). These uptake values were then used to calculate several parameters, namely Nitrogen Uptake Efficiency at anthesis and maturity (NUpEanth and NUpE), Nitrogen Utilization Efficiency (NUtE), Biomass Production Efficiency at anthesis and maturity (BPEanth and BPE), Nitrogen Harvest Index (NHI), Nitrogen Uptake After Anthesis (NUpAA), Nitrogen Remobilization Efficiency (NRE), Fertilizer Use Efficiency (FUE), and Partial Nutrient Balance (PNB).

Grain protein content was determined using the percent nitrogen in the grain from tissue analysis. Percent nitrogen was multiplied by a standard, 5.83, to get protein content (FAO, 1949). As percent nitrogen was determined when the grain was dried, the values were corrected to a

12% moisture basis (12% m.b.), which is the standard for reporting grain protein contents in the global marketplace. This conversion was made using the following formula: Grain Protein * (1-0.12).

The calculated values of these parameters were then analyzed with SAS using the PROC Mixed procedure (SAS Institute, 2005; Appendix Table B.1) with Nrate and variety as fixed effects and environment as a random effect. For the purposes of this study, each year and site was considered a separate environment and all of the values were run through the program together. The output provided information on the main effect, variety, as well as the effect of Nrate and determined if there was an Nrate x variety interaction. Because environment was entered as a random effect, the output did not provide any information on the effect of the various locations and years of this study. Environment was then analyzed separately as a fixed effect for NUE, the main parameter of interest, to determine if there was any variety by environment interactions present (Appendix Table B.2).

Pairwise comparisons were made using the protected least significant difference (LSD) method. Because of missing data, LSDs varied by variety and N rate, therefore, LSD values listed in tables were conservatively selected as the largest LSD. Results of actual pairwise comparisons are denoted by letters, indicating significant differences in the results ($p<0.05$) as determined by the pdiff option in SAS Proc Mixed. Assignment of means groupings was facilitated by the PDMIX800 program (Saxton, 2000).

Simple linear regressions were also performed in SAS using the Proc Reg procedure (SAS Institute, 2005). This output provided the linear line and equation of best fit, as well as determined the R^2 value and p-value for the comparison. The Proc Reg procedure was used to determine any possible correlations between NUE and other related parameters.

RESULTS AND DISCUSSION

The soil nutrient statuses of the soils included in this study were very different between years and locations (Table 2.2). Most of the soil results, such as pH, P, and K were within agronomic production standards for the state of Kansas, however, there was considerable range in the inorganic nitrogen concentrations in these soils. There was a trend of increasing profile nitrogen over the course of this study, which may have impacted the level of response of these crops to nitrogen fertilizer additions.

In the 2010-11 season at Rossville, KS there was 20.4 kg N ha^{-1} and the following year at the same location there was 57.5 kg N ha^{-1}. Both locations in the 2012-13 season had very similar profile N contents, with 43.0 kg N ha^{-1} at Silverlake and 41.9 kg N ha^{-1} at Ashland Bottoms, although nitrate concentrations were higher at the Silverlake location. Mean profile N was very high for the two locations planted in the fall of 2013, with 66.8 kg N ha^{-1} at Ashland Bottoms and 131.4 kg N ha^{-1} at Hutchinson, KS. As these studies are ongoing, the end-of-season data for these locations will not be reported in this study, but due to the high residual N at Hutchinson it may be difficult to determine the effect of the two N rates on the parameters of interest because there may not be a difference in response.

Soil test P at most of the locations was near or above the Kansas threshold of 20 ppm, with the exception of Ashland Bottoms in 2012-2013. Because of the short window of time between soybean harvest and wheat planting and the time required to analyze samples, soil test results were not available until after planting and P fertilizer application. Because of this, P was applied at a rate appropriate for moderate soil tests expected for these locations. However, P at Ashland Bottoms in the 2012-13 season was very low at 5.2 ppm. Using the K-State sufficiency recommendation equation for wheat and a yield goal of 47.0 bu ac^{-1}, which was the highest mean yield for a variety at the highest N rate observed at this location, the fertilizer application rate should have been around 54.5 kg P_2O_5 ha^{-1} to supply plant demand; however, only 22.7 kg P_2O_5 ha^{-1} was applied with the planter. The low test phosphorus at this location could have impacted the study and performance of the varieties. However, this location was also impacted by low rainfall and low soil moisture (Table 2.6), which may have been a more limiting factor than P.

Table 2.6. Weather data for the NUE studies from the 2010-12 seasons.

	Min Air Temp	Max Air Temp	Mean Air Temp	Min Soil Temp	Max Soil Temp	Mean Soil Temp	Total Precip	Total Irrigation	Total Water
	°C						mm		
Rossville 2010-11	-21.5	36.5	8.2	-8.5	33.2	9.5	427.5	-	427.5
Rossville 2011-12	-14.4	36.3	10.2	-0.4	31.6	11.2	449.2	19.8	469.0
Ashland Bottoms 2012-13	-16.9	36.4	8.6	0.1	28.6	9.6	359.4	-	359.4
Silverlake 2012-13	-20.4	39.1	9.0	-6.2	42.9	10.6	436.6	42.2	478.8

Source: Kansas State University Mesonet (2014).

The weather conditions between the locations were highly variable. Table 2.6 shows in-season weather information for each study location. The studies in the Kansas River Valley experiment field area, Rossville and Silverlake, had higher in-season total precipitation and access to irrigation, while the Ashland Bottoms location had less precipitation and no irrigation access. The other measurements, such as air and soil temperatures, were fairly comparable between environments. As the study is ongoing, there is no end-of-season weather data available for the studies planted in the fall of 2013 at Ashland Bottoms and Hutchinson, KS. These environmental differences may be useful in determining which varieties perform the best in the range of conditions present in Kansas.

Results at Anthesis

Several plant measurements were taken at anthesis to determine if there were any early-season differences among the varieties that could explain differences at maturity. In this study, there were no significant differences observed between varieties for any of the parameters measured at anthesis. There were also no significant Nrate x variety interactions observed at anthesis, and only one parameter, total nitrogen uptake at anthesis (NUpanth), was significantly affected by nitrogen rate (Table 2.7). Mean values for these parameters and varieties can be found in the Appendix (Appendix Table C.1-C.2).

Table 2.7. Levels of significance (p-values) for parameters determined at anthesis.

	NDVI	BManth	BPEanth	NUpEanth	Nanth	Canth	NUpanth
Variety	0.364	0.241	0.212	0.352	0.093	0.991	0.056
Nrate	0.077	0.065	0.107	0.127	0.127	0.125	0.012
Nrate x Variety	0.562	0.473	0.457	0.491	0.459	0.318	0.120

Abbreviations: NDVI, normalized vegetation index; BManth, biomass at anthesis; NUpEanth, nitrogen uptake at anthesis; Nanth, nitrogen concentration at anthesis; Canth, carbon concentration at anthesis; NUpanth, total nitrogen uptake at anthesis.

The lack of significant results appears to be the product of high variability within the fields and between locations at this growth stage, which is supported by the results when environment was analyzed as a fixed effect. The results showed that the effect of the environment was significant, but that there were no significant variety by environment interactions for any parameter (Table 2.8). This indicates that while there were differences in wheat performance between locations, the varieties maintained the same relationships across environments, with no significant differences resulting from high variability.

Table 2.8. Levels of significance (p-values) with environment as a fixed-effect for parameters determined at anthesis.

	NDVI	BManth	BPEanth	NUpEanth	Nanth	Canth	NUpanth
Enviroment	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Environment x Variety	0.980	0.970	0.999	0.963	0.959	0.998	0.982

Abbreviations: NDVI, normalized vegetation index; BManth, biomass at anthesis; NUpEanth, nitrogen uptake at anthesis; Nanth, nitrogen concentration at anthesis; Canth, carbon concentration at anthesis; NUpanth, total nitrogen uptake at anthesis.

These are interesting results, and contrary to those found in other relevant studies where significant differences in early season biomass production and nitrogen uptake at or around anthesis in wheat were noted (Pang et al., 2014; Liao et al., 2004). Pang et al. (2014) found significant differences in these parameters in 24 wheat varieties at stem elongation. They also observed a strong correlation between nitrogen content and biomass production, but observed some variability. Although varieties exhibited no significant differences in biomass at anthesis or nitrogen uptake in this study, a similar correlation was observed (Figure 2.2). As a result, varieties with higher biomass production also tended to have higher total nitrogen uptake.

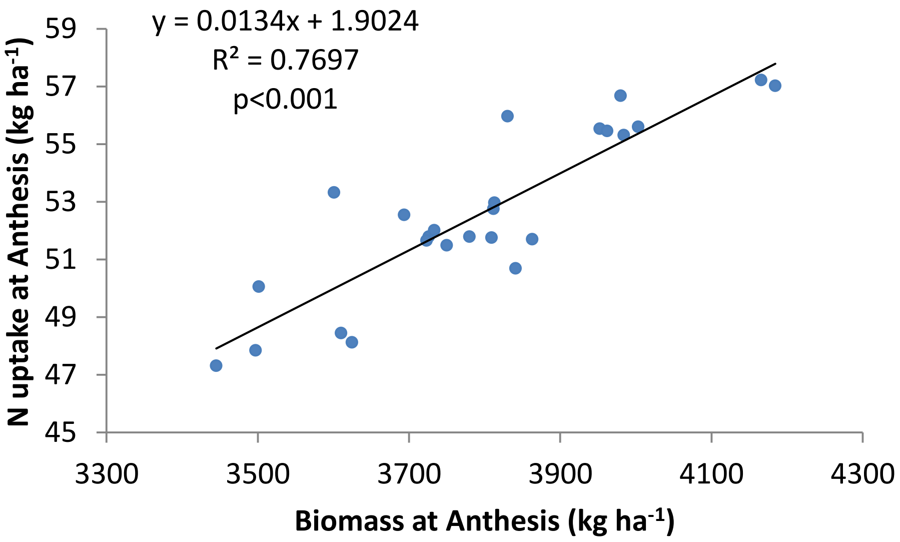


Figure 2.2. Correlation between mean biomass production and nitrogen uptake at anthesis for 25 wheat varieties (averaged across N rates).

Higher nitrogen uptake with increasing biomass is thought to be related to the increased sink in plant tissues for nitrogen, and may contribute to increased grain yields at maturity (Liao et al., 2004; Marti et al., 2007). Early season growth and nitrogen uptake are important as the amount of heads, their sizes, and subsequent grain yields are often determined during this time (Marti et al., 2007). Once grain filling has begun, the plant will devote most of its resources to grain development and the roots will start to senesce. As the roots begin to die and remobilize their resources to other plant tissues and grain, their capacity to uptake nitrogen decreases. By providing nitrogen when the plant needs it most, around tillering, yields and nitrogen use efficiency could be improved. Nitrogen use efficiency could also be improved by selecting for varieties that maintain roots longer in the season, thereby extracting more N from the soil during grain fill.

Split-applications of nitrogen, like those made in this study, have been demonstrated to improve yields and NUE (Blankenau et al., 2002; Arregui and Quemada, 2008; Lopez-Bellido et al., 2005). It is recommended that application rates in the fall be lower than those in the spring because of the risk of nitrate leaching or denitrification (Limaux et al., 1999; Lopez-Bellido et al., 2005). Although no significant differences were noted between the varieties in early-season growth, split-applications ensured that the nitrogen applied to the wheat varieties was available when needed most and also reduced the risk of N loss. It may be possible that the low amount of N supplied in the first application limited early-season growth of some varieties, concealing potential differences in growth and performance, as other research has shown significant differences among varieties when higher rates were applied early in the season (Pang et al., 2014).

Results at Maturity

Unlike at anthesis, several significant differences were observed for plant performance measurements taken at maturity. Three parameters, grain yield, biomass, and nitrogen concentration in the grain (Ngr), showed significant differences among varieties with a $p<0.05$ (Table 2.9). Two additional parameters, total nitrogen in the grain (TNgr) and total nitrogen uptake (TNup), had no significant differences in mean values between the varieties; however, there was a significant Nrate x variety interaction observed for each of these parameters, suggesting that fertilizer rate influenced total N uptake in some varieties more than others (Figure 2.3).

Table 2.9. Levels of significance (p-values) from ANOVA for plant measurements taken at maturity.

	Grain Yield	**Biomass**	**Nstov**	**Cstov**	**Ngr**	**Cgr**	**TNstov**	**TNgr**	**TNup**
Variety	**0.002**	**0.013**	0.143	0.838	**0.009**	0.910	0.090	0.323	0.177
Nrate	**0.045**	**0.030**	0.085	0.220	0.212	0.343	**0.004**	**0.021**	**0.006**
Nrate x Variety	0.294	0.225	0.661	0.311	0.467	0.568	0.150	**0.005**	**0.008**

Abbreviations: Nstov, nitrogen concentration in the stover; C stov, carbon concentration in the stover; Ngr, nitrogen concentration in the grain; Cgr, carbon concentration in the grain; TNstov, total nitrogen in the stover; TNgr, total nitrogen in the grain; TNup, total nitrogen uptake.

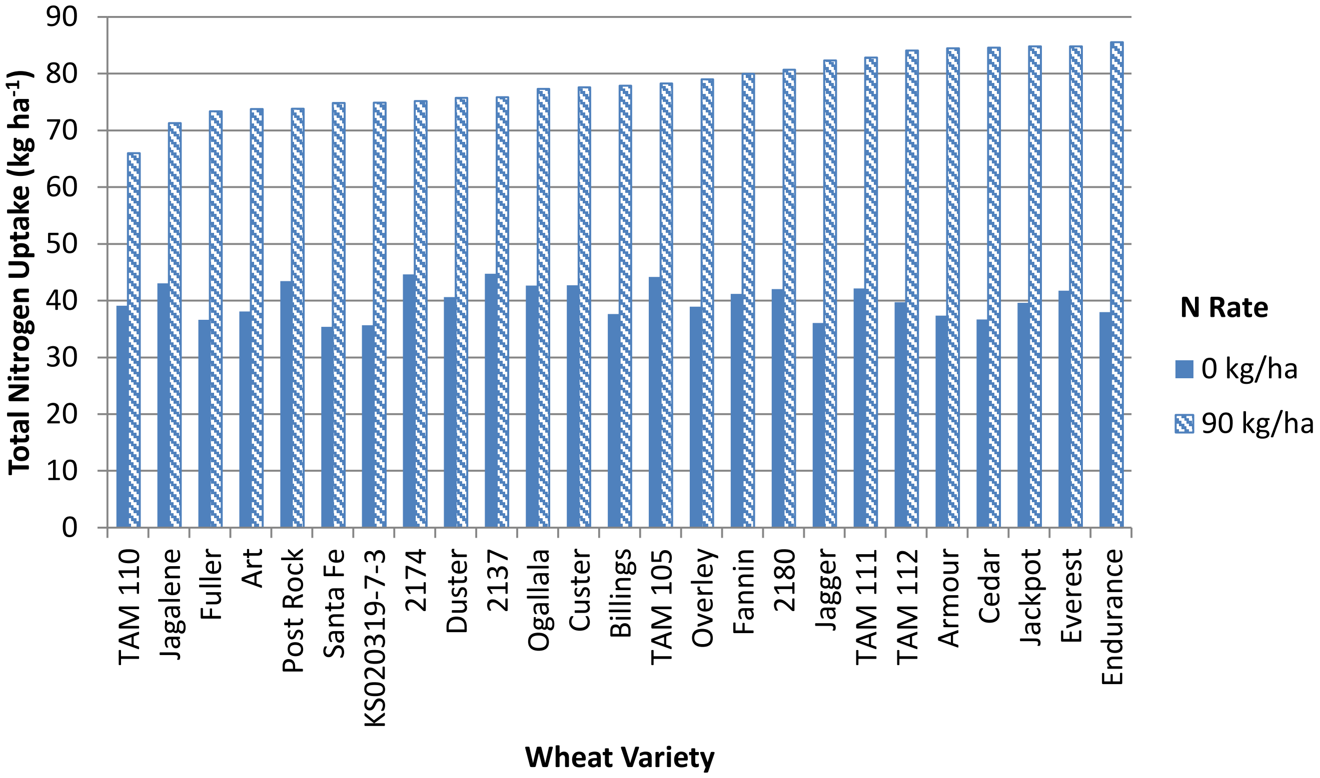


Figure 2.3. Effect of Nrate on total nitrogen uptake in 25 winter wheat varieties.

In addition, there were several parameters that were significantly affected by nitrogen rate (Table 2.9). On average the fertilizer application increased yield, biomass, total nitrogen in the stover, total nitrogen in the grain, and total nitrogen uptake by 1142, 3388, 14.2, 24.1, and 38.3 kg ha^{-1} respectively.

There was considerable range in the mean values for the parameters significantly affected by variety (Table 2.10). Mean grain yield ranged from 1989 kg ha^{-1} for TAM 110 up to 2546 kg ha^{-1} for 2137. Biomass ranged from 4896 kg ha^{-1} for Overley to 6305 kg ha^{-1} in TAM 111. Mean Ngr ranged from 19.0 g kg^{-1} for 2137 to 21.8 g kg^{-1} for 2180. Mean values for the parameters that were significantly affected by variety can be found in Table 2.10, and the performance of these varieties at each Nrate can be found in Table C.3 of the Appendix. The values for the other parameters that had no significant results can be found in the Appendix in Tables C.4-C.5.

Table 2.10. Mean grain yield, biomass, and N concentration in grain (Ngr) at maturity.

Variety	**Grain Yield**		**Biomass**		**Ngr**	
	kg ha^{-1}				**g kg^{-1}**	
2137	2546	A[†]	6242	AB	19.0	G
2174	2060	DEF	5740	ABCDE	21.1	ABCDE
2180	2066	DEF	5658	ABCDE	21.8	A
Armour	2337	ABC	5788	ABCD	20.1	DEFG
Art	2064	DEF	5376	CDEF	21.1	ABCDE
Billings	2363	ABC	5871	ABCD	19.8	FG
Cedar	2212	BCDEF	5707	ABCDE	20.3	BCDEF
Custer	2131	CDEF	5635	ABCDE	20.7	ABCDEF
Duster	2281	ABCD	5980	ABC	19.7	FG
Endurance	2261	BCDE	5963	ABC	20.4	BCDEF
Everest	2396	ABC	5608	ABCDEF	20.8	ABCDEF
Fannin	2344	ABC	5652	ABCDE	20.3	CDEF
Fuller	2061	DEF	5607	ABCDEF	20.6	BCDEF
Jackpot	2422	AB	5877	ABCD	20.3	CDEF
Jagalene	2129	CDEF	5713	ABCDE	20.1	DEFG
Jagger	2059	DEF	5066	EF	21.4	ABC
KS020319-7-3	2179	BCDEF	5205	DEF	19.9	EFG
Ogallala	2213	BCDEF	5563	BCDEF	21.0	ABCDE
Overley	2064	DEF	4896	F	21.5	AB
Post Rock	2154	BCDEF	5402	CDEF	20.8	ABCDEF
Santa Fe	1994	EF	5196	DEF	21.1	ABCD
TAM 105	2231	BCDEF	5859	ABCD	20.4	BCDEF
TAM 110	1989	F	5048	EF	20.4	BCDEF
TAM 111	2368	ABC	6305	A	20.4	BCDEF
TAM 112	2288	ABCD	5977	ABC	20.4	BCDEF
LSD[‡]	**277**	**-**	**2644**	**-**	**1.2**	**-**

†Means within a column that are followed by the same letter are not significantly different (p>0.05)
‡The least significant difference (LSD) varied by comparison due to missing data, therefore, the LSD listed is the largest LSD for any comparison. Means groupings should be used for precise means comparisons.

Several additional parameters calculated by using the plant measurement values also showed significant results (Table 2.11). Varieties showed highly significant differences for the main parameter of interest—nitrogen use efficiency (p=0.003). The mean values for NUE ranged from 22.06 kg grain kg N^{-1} for Santa Fe to 29.61 kg grain kg N^{-1} for 2137 (Table 2.13). Because there was no Nrate x variety interaction, the varieties maintained the same relationships at both Nrates, so the high and low performing varieties remained consistent. There was no significant Nrate effect because of variability, but at each location NUE values were lower for the high nitrogen treatment and higher for the low nitrogen treatment (Appendix Table C.7). When analyzed with environment as a fixed effect, only HI and NHI showed significant environment x variety interactions. The absence of an environment x variety interaction for the other parameters suggests that varieties performed the same relative to one another at all locations (Table 2.12).

Table 2.11.Levels of significance (p-values) for parameters determined at maturity.

	NUE	NUpE	NUtE	NRE	BPE	HI	NHI	NUpAA
Variety	**0.003**	0.571	**0.002**	0.344	0.898	**0.044**	0.311	0.332
Nrate	0.374	0.171	**0.041**	0.100	0.402	**0.026**	**0.024**	0.737
Nrate x Variety	0.211	0.150	0.449	**0.041**	0.600	0.780	0.422	**0.001**

Abbreviations: NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency; NRE, nitrogen remobilization efficiency; BPE, biomass production efficiency; HI, harvest index; NHI, nitrogen harvest index; NUpAA, nitrogen uptake after anthesis.

Table 2.12. The effect of environment as a fixed effect on parameters determined at maturity.

	NUE	NUpE	NUtE	NRE	BPE	HI	NHI	NUpAA
Environment	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Envrionment x Variety	0.968	1.000	0.363	0.363	1.000	0.021	**0.002**	0.974

Abbreviations: NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency; NRE, nitrogen remobilization efficiency; BPE, biomass production efficiency; HI, harvest index; NHI, nitrogen harvest index; NUpAA, nitrogen uptake after anthesis.

Table 2.13. Mean values and significant differences for parameters with significant varietal differences determined at maturity.

Variety	NUE		NUtE		HI	
	kg kg^{-1}					
2137	29.61	A[†]	42.70	A	0.38	ABC
2174	23.56	BCDEF	36.24	GH	0.35	DE
2180	23.67	BCDEF	35.52	H	0.35	E
Armour	26.02	BCD	40.63	ABCDE	0.38	ABC
Art	22.97	CDEF	38.90	BCDEFG	0.37	ABCDE
Billings	26.21	ABC	42.00	AB	0.38	ABC
Cedar	24.01	BCDEF	38.68	CDEFG	0.36	ABCDE
Custer	24.38	BCDEF	37.10	FGH	0.35	DE
Duster	24.63	BCDEF	40.55	ABCDE	0.36	ABCDE
Endurance	25.71	BCDE	39.60	ABCDEF	0.36	ABCDE
Everest	26.38	ABC	39.49	BCDEF	0.39	AB
Fannin	26.32	ABC	40.82	ABCD	0.37	ABCDE
Fuller	22.40	EF	39.45	BCDEF	0.35	DE
Jackpot	26.66	AB	39.60	BCDEF	0.38	AB
Jagalene	23.53	BCDEF	38.45	CDEFGH	0.38	ABCD
Jagger	22.35	EF	38.04	DEFGH	0.36	ABCDE
KS020319-7-3	23.66	BCDEF	41.42	ABC	0.38	ABC
Ogallala	24.37	BCDEF	37.77	EFGH	0.36	ABCDE
Overley	22.08	F	37.13	FGH	0.37	ABCDE
Post Rock	24.56	BCDEF	38.34	CDEFGH	0.36	BCDE
Santa Fe	22.06	F	38.40	CDEFGH	0.36	CDE
TAM 105	24.83	BCDEF	38.13	DEFGH	0.35	DE
TAM 110	22.64	DEF	39.27	BCDEFG	0.39	A
TAM 111	26.88	AB	38.91	CDEFG	0.36	ABCDE
TAM 112	24.56	BCDEF	38.61	CDEFG	0.38	ABCD
LSD[‡]	**3.56**	**-**	**3.15**	**-**	**0.03**	**-**

†Means within a column that are followed by the same letter are not significantly different ($p>0.05$)
‡The least significant difference (LSD) varied by comparison due to missing data, therefore, the LSD listed is the largest LSD for any comparison. Means groupings should be used for precise means comparisons.

Abbreviations: NUE, nitrogen use efficiency; NUtE, nitrogen utilization efficiency; HI, harvest index; FUE, fertilizer use efficiency; LSD, least significant difference; NS, not significant; Sig. Diff, significant differences.

The other parameters significantly affected by variety were nitrogen utilization efficiency (NUtE) and harvest index (HI) ($p<0.05$, Table 2.11). The mean values for NUtE ranged from 35.52 kg grain kg N^{-1} for 2180 to 42.7 kg grain kg N^{-1} for 2137, and HI ranged from 0.35 kg grain kg biomass^{-1} for 2174, 2180, Custer, Fuller, and TAM 105 to 0.39 kg grain kg biomass^{-1} for Everest. Two additional parameters, nitrogen remobilization efficiency (NRE) and nitrogen uptake after anthesis (NUpAA), showed no significant differences in mean varietal values, but did have significant Nrate x variety interactions with a $p<0.05$ (Table 2.11; Appendix Tables C.6-C.7). This indicates that the some varieties may uptake, lose, or remobilize more nitrogen than others depending on N availability.

Nitrogen Use Efficiency

This study provides strong evidence for genetic differences in wheat varieties for NUE. The NUE values for each variety were different at the locations because of varying environmental conditions between years and sites, but the varieties maintained the same relationships at each location as evidenced by the lack of an environment x variety interaction when environment was analyzed as a fixed effect (Table 2.12). This provides evidence that the varieties maintained the same relationships at each location regardless of environmental conditions, and the varieties 2137, TAM 111, Jackpot, and Everest had the highest consistent NUE values, while Santa Fe, Overley, Jagger, and Fuller had the lowest (Figure 2.4).

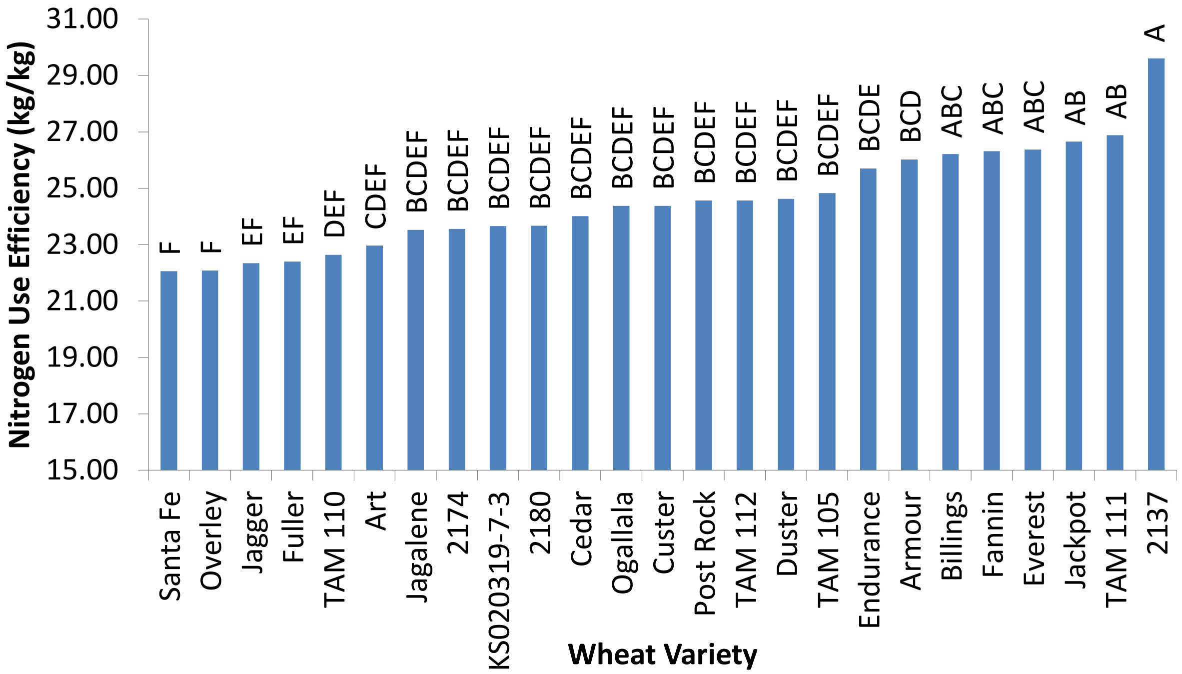


Figure 2.4. Mean nitrogen use efficiency for 25 winter wheat varieties.

Several related studies have also detected varietal differences for NUE in wheat and other related cereal crops, such as barley and rice (Anbessa et al., 2009; Cormier et al., 2013; Bingham et al., 2012; Namai et al., 2009). These studies have sought to provide evidence that future breeding efforts could produce more efficient varieties that could help conserve resources and protect the environment. In many of these studies, increased grain yield has been an accurate predictor of NUE, and breeding programs have been indirectly selecting for higher NUE as they have tried to improve grain yield (Cormier et al., 2013; Bingham et al., 2012).

In this study, there is a strong relationship between grain yield and NUE, which is not surprising given the way that NUE is calculated as grain yield divided by the total nitrogen supply (Figure 2.5). Grain yield explained 90% of the variation in nitrogen use efficiency. Because grain yield was significantly different among the varieties (Table 2.9), this resulted in significant differences in NUE as some plants produced more grain yield with the same availability of N. These differences in NUE and grain yield are likely the result of complex relationships with several parameters, namely NUtE, NUpE, HI, NUpAA, and TNup. All of these parameters, with the exception of NUpE, had significant differences for variety, or a significant Nrate by variety interaction.

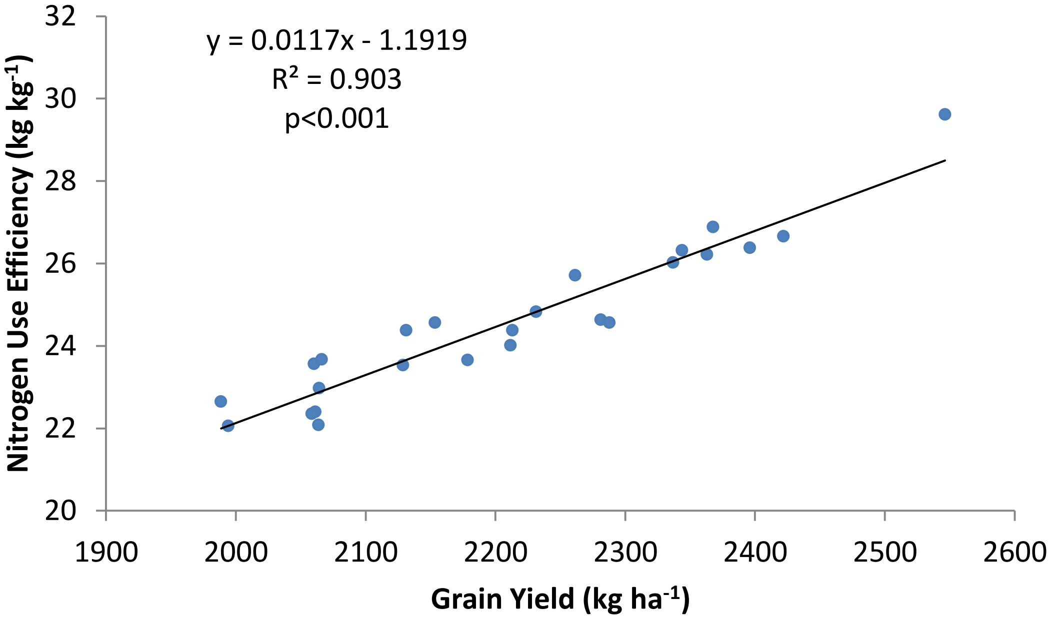


Figure 2.5. Correlation between mean grain yield and nitrogen use efficiency for 25 different wheat varieties (data averaged across two N rates and four environments).

As NUE and grain yield are intricately connected, any improvements in grain yield over time should result in increased NUEs. The varieties included in this study were selected based on their range of release dates, dating back to 1979. While other studies have shown increases in yield over time, their projects included varieties that represented a much larger range of release dates (Sadras and Lawson, 2013; Muurinen et al., 2006). As a result, their studies included both full-height and semi-dwarf varieties, and finding differences in yield and NUE among them would be expected and easily explained. This study only included semi-dwarf varieties, and interestingly there is not a significant correlation between release date and either yield or NUE (Figure 2.6).

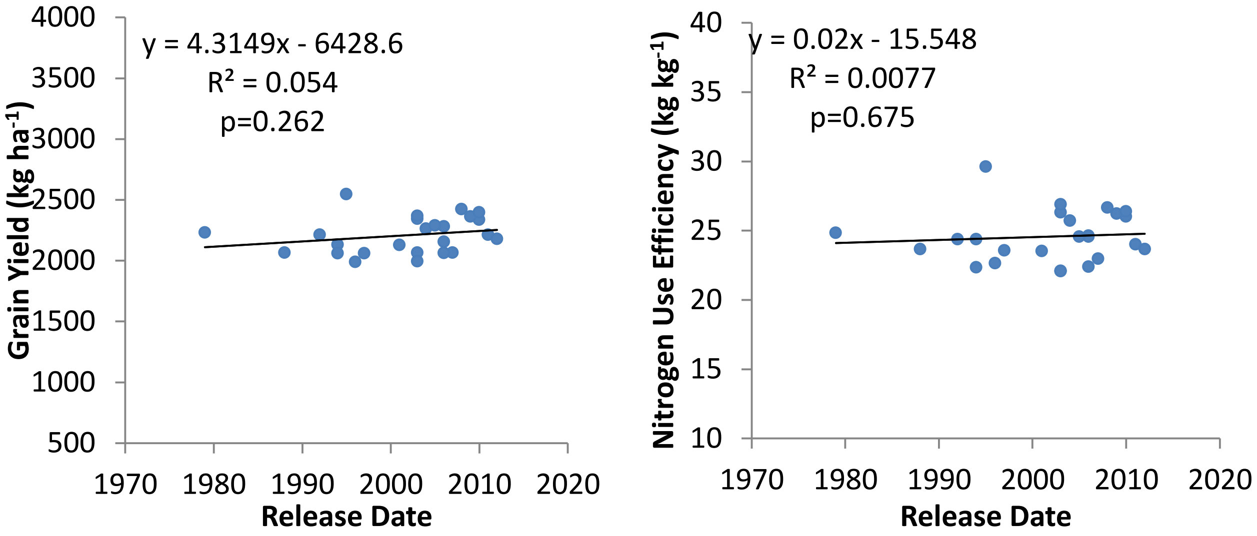


Figure 2.6. Relationship between grain yield (left) and nitrogen use efficiency (right) to wheat variety release date.

Breeding programs have spent a great deal of effort on developing other traits besides yield in these varieties, such as disease resistance, drought tolerance, and tolerance to certain soil conditions, with little direct selection for grain yield. Another reason that breeding programs concentrate more effort on these other qualities is the effect that increased yield has on grain quality. In general, increased yield is the result of more carbohydrates in the grain and results in a dilution of nitrogen. Less nitrogen concentration in the grain results in less gluten content, which is essential for bread-making. In this study, there was a strong inverse relationship observed between grain yield and grain protein content (Figure 2.7). Because of the relationship between grain yield and nitrogen use efficiency, it follows that as NUE increases, grain quality will also decrease unless nitrogen uptake and remobilization increase (Figure 2.7). In the case of this study, nitrogen uptake and remobilization efficiencies were not significantly different between varieties, indicating that increases in yield are primarily due to increased carbohydrate in the grain. In this study, those varieties with the highest grain yield, namely 2137, Armour, and Billings, also had some of the lowest nitrogen content in the grain, and hence grain protein content (Table 2.10).

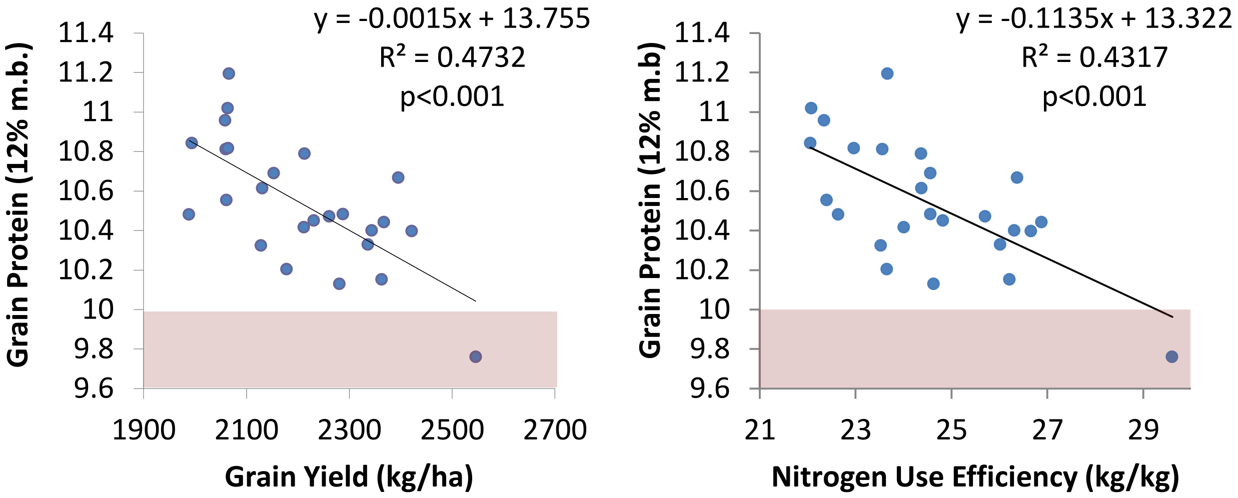


Figure 2.7. Relationship between grain yield and NUE to grain protein content in 25 winter wheat varieties.

The drought tolerance or susceptibility of these varieties was also determined and compared to the NUE values. The drought tolerance characteristics were determined based on published ratings (Watson, 2003) and personal communication with Drs. Allan Fritz and James Shroyer at Kansas State University. Drought tolerance of wheat varieties is highly subjective, but the general characteristics of these varieties are outlined in Table 2.14. According to these results, no trends observed between drought tolerance and NUE values, with both drought tolerant and susceptible varieties having high and low NUEs.

Table 2.14. Winter wheat varieties and corresponding nitrogen use efficiency values and drought tolerance characteristics.

Variety	NUE	Drought
Santa Fe	22.06	Susceptible
Overley	22.08	Average
Jagger	22.35	Tolerant
Fuller	22.4	Tolerant
TAM 110	22.64	Tolerant
Art	22.97	Susceptible
Jagalene	23.53	Tolerant
2174	23.56	Average
KS020319-7-3	23.66	Unknown
2180	23.67	Average
Cedar	24.01	Average
Ogallala	24.37	Average
Custer	24.38	Average
Post Rock	24.56	Average
TAM 112	24.56	Tolerant
Duster	24.63	Tolerant
TAM 105	24.83	Tolerant
Endurance	25.71	Tolerant
Armour	26.02	Susceptible
Billings	26.21	Susceptible
Fannin	26.32	Average
Everest	26.38	Average
Jackpot	26.66	Tolerant
TAM 111	26.88	Tolerant
2137	29.61	Average

Nitrogen Uptake and Utilization Efficiencies

Nitrogen use efficiency can also be calculated as nitrogen uptake efficiency (NUpE) x nitrogen utilization efficiency (NUtE) as defined by Moll et al. (1982) because it is the result of these two independent traits. This is supported by Figure 2.8 which shows the independence of these two parameters in this study based on the very low R^2 value (R^2=0.04) and high p-value (p=0.321). As these parameters are independent, it provides possible methods for breeding programs to improve nitrogen use efficiency. These programs can either improve NUpE, NUtE, or seek to improve both simultaneously.

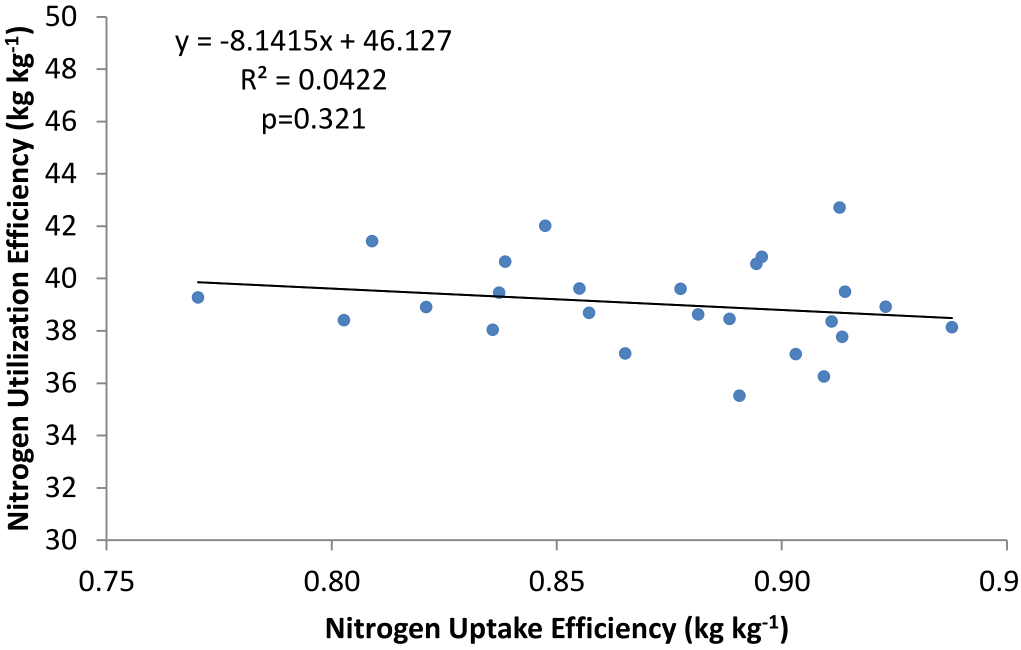


Figure 2.8. Independence of nitrogen uptake efficiency and nitrogen utilization efficiency.

In this study, only NUtE was significantly affected by variety with a $p=0.002$ and showed a considerable range of values (Table 2.11; Figure 2.9). This provides evidence that these varieties differ in how they use the same amount of nitrogen taken up from the soil to produce grain. In contrast to these results, other studies have found significant varietal differences for both NUpE and NUtE, and strong relationships in both to NUE (Hawkesford, 2012). In this study, there were no significant differences among the varieties for NUpE, indicating the varieties had roughly the same N uptake per unit of N supply. However, NUtE values were significantly affected by variety, indicating that differences in NUE were a result of how the varieties used their nitrogen to produce higher grain yields.

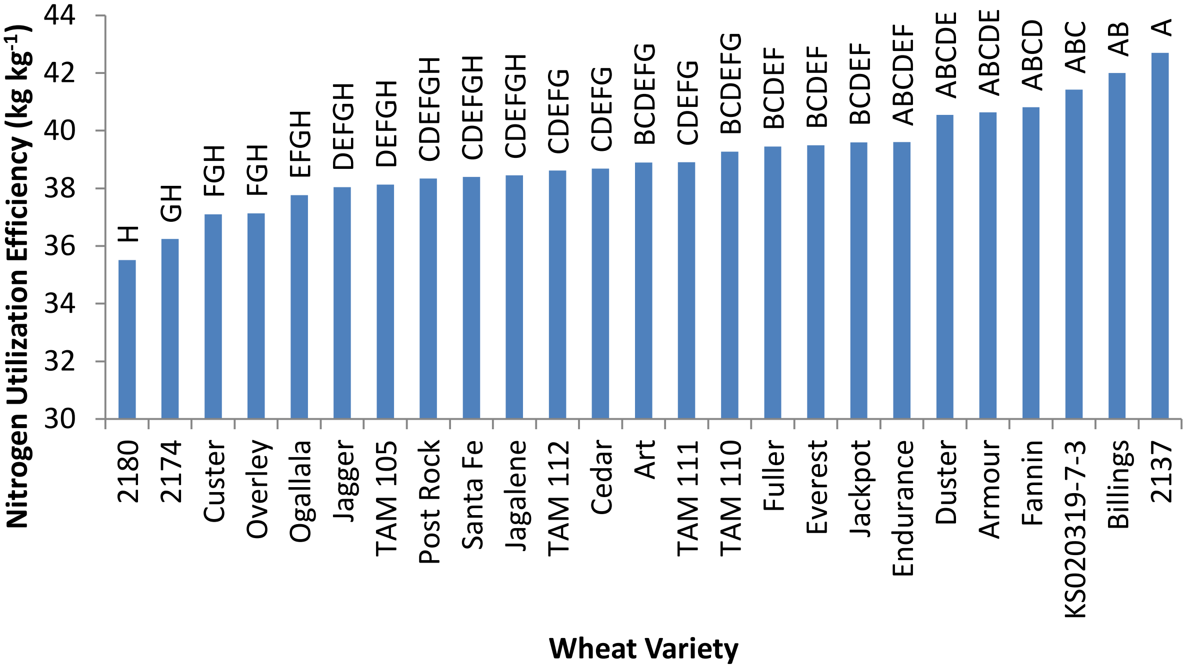


Figure 2.9. Nitrogen utilization efficiencies of 25 wheat varieties and significant differences.

Differences in N utilization could be due to several physiological factors such as differences in biomass production, photosynthesis, and remobilization of nitrogen and carbohydrates to the grain during reproductive growth. Nitrogen remobilization efficiency (NRE), nitrogen harvest index (NHI), and harvest index (HI) provide additional information on which physiological processes are responsible for improved yield, NUtE, and NUE. Harvest index was significantly affected by variety, while NRE had a significant Nrate by variety interaction (Table 2.11). As HI is most directly a measure of the amount of carbohydrates allocated to the grain from plant tissue, this indicates that improved NUtE may be the result of higher production of carbohydrates and subsequent allocation to grain. It could also be due to in part to differences in remobilization of N in some varieties depending on the rate. As nitrogen is a critical component of photosynthesis, it may contribute to improved photosynthetic ability of the plant tissue and overall plant health. In this study, NUtE explained 41% of the variability in HI (Figure 2.10).

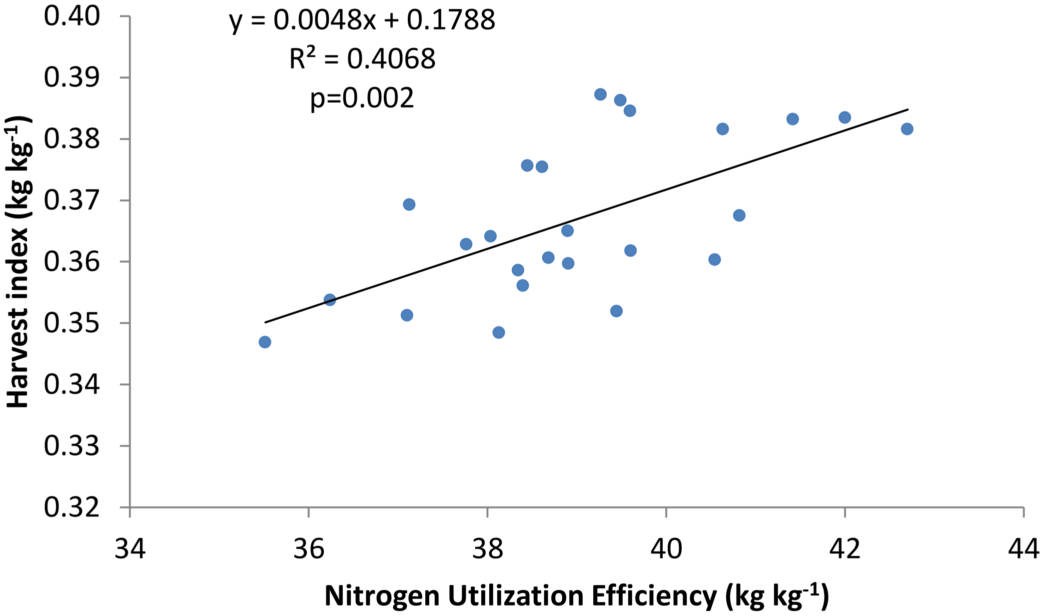


Figure 2.10. Relationship between nitrogen utilization efficiency and harvest index for 25 different wheat varieties (data averaged across two N rates and four environments).

By providing each variety with a rank for some of the important parameters related to NUE that were significantly affected by variety, specifically grain yield, NUtE, and NUE, it is possible to identify the best and poorest performing varieties. Each variety was assigned a rank from 1-25, with 25 being the highest mean value observed for each parameter. These ranks were then averaged to determine overall performance of the varieties relative to one another. The results showed that the varieties 2137, Billings, Fannin, and Jackpot were the best performing varieties, while 2174, Jagger, Overley, and Santa Fe performed the poorest (Table 2.15).

Table 2.15. Wheat varieties ranked for grain yield, nitrogen utilization efficiency, and nitrogen use efficiency, and average ranks.

Variety	Grain Yield	Rank	NUtE	Rank	NUE	Rank	Average Rank
	kg ha^{-1}		kg kg^{-1}		kg kg^{-1}		
2137	2546	25	42.70	25	29.61	25	25.0
Billings	2363	21	42.00	24	26.21	20	21.7
Jackpot	2422	24	39.60	18	26.66	23	21.7
Fannin	2344	20	40.82	22	26.32	21	21.0
Everest	2396	23	39.49	17	26.38	22	20.7
TAM 111	2368	22	38.91	14	26.88	24	20.0
Armour	2337	19	40.63	21	26.02	19	19.7
Duster	2281	17	40.55	20	24.63	16	17.7
Endurance	2261	16	39.60	19	25.71	18	17.7
KS020319-7-3	2179	12	41.42	23	23.66	9	14.7
TAM 112	2288	18	38.61	11	24.56	15	14.7
TAM 105	2231	15	38.13	7	24.83	17	13.0
Cedar	2212	13	38.68	12	24.01	11	12.0
Post Rock	2154	11	38.34	8	24.56	14	11.0
Ogallala	2213	14	37.77	5	24.37	12	10.3
Art	2064	7	38.90	13	22.97	6	8.7
Custer	2131	10	37.10	3	24.38	13	8.7
Jagalene	2129	9	38.45	10	23.53	7	8.7
Fuller	2061	5	39.45	16	22.40	4	8.3
TAM 110	1989	1	39.27	15	22.64	5	7.0
2180	2066	8	35.52	1	23.67	10	6.3
2174	2060	4	36.24	2	23.56	8	4.7
Jagger	2059	3	38.04	6	22.35	3	4.0
Overley	2064	6	37.13	4	22.08	2	4.0
Santa Fe	1994	2	38.40	9	22.06	1	4.0

†Means within a column that are followed by the same letter are not significantly different (p>0.05)

‡The least significant difference (LSD) varied by comparison due to missing data, therefore, the LSD listed is the largest LSD for any comparison. Means groupings should be used for precise means comparisons.

Abbreviations: NUtE, nitrogen utilization efficiency; NUE, nitrogen use efficiency.

Although only NUtE values were significantly affected by variety, both NUtE and NUpE were well correlated with NUE. Mean nitrogen uptake efficiency values explained about 26% of the variability in NUE with a p=0.009 (Figure 2.11). Nitrogen utilization efficiency explained slightly more of the variability observed in NUE at 34% with a p=0.002 (Figure 2.11). These results are consistent with findings by Bingham et al. (2012) in research on barley. They found that NUpE and NUtE were both significantly affected by variety, but NUtE values were more highly correlated to NUE and explained more of the variability between genotypes. However, other studies present contrasting explanations for differences in NUE. Some found that nitrogen uptake was the most significant contributor to improvements in NUE (Muurinen et al., 2006), while other research has suggested that selecting for nitrogen utilization efficiency is the most promising method for increasing NUE (Bingham et al., 2012; Cormier et al., 2013).

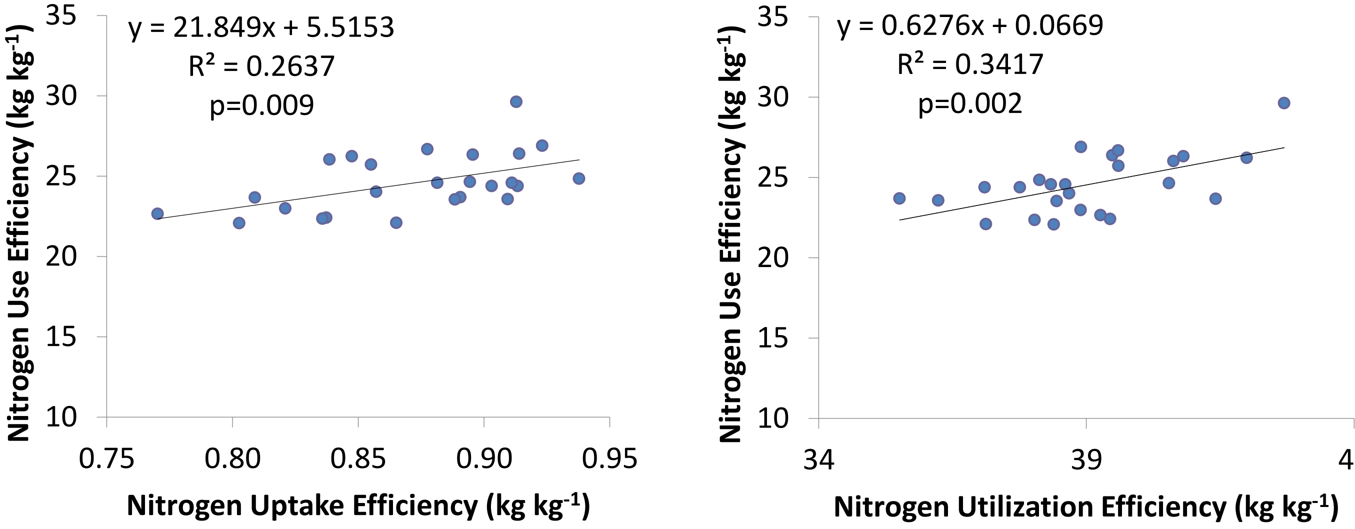


Figure 2.11. Relationship between nitrogen uptake and nitrogen utilization efficiencies and nitrogen use efficiency for 25 different wheat varieties (data averaged across two N rates and four environments).

As well as finding evidence for increasing NUE and grain yield over time, some of these studies have also found evidence for improvements in NUtE and NUpE through breeding over the course of several decades (Sadras and Lawson, 2013; Muurinen et al., 2006). In this study, no significant changes over time were observed in NUpE (p=0.072), but there was a significant improvement in NUtE over time (p=0.015) (Figure 2.12). Release date and NUtE exhibited a positive relationship, with release date explaining 23% of observed variation, indicating that NUtEs have improved in these varieties over the course of the last several decades at a rate of about 0.1 kg kg^{-1} each year (Figure 2.12). Although NUpE was not significantly affected by release date, its relationship with release date was opposite that of NUtE. Nitrogen uptake efficiency had a negative relationship with release date, providing some evidence that uptake efficiency may have decreased over the course of time in these varieties (Figure 2.12). For this reason, the absence of improvements in NUE over time may in part be due to a decreased capacity for nitrogen uptake from the soil.

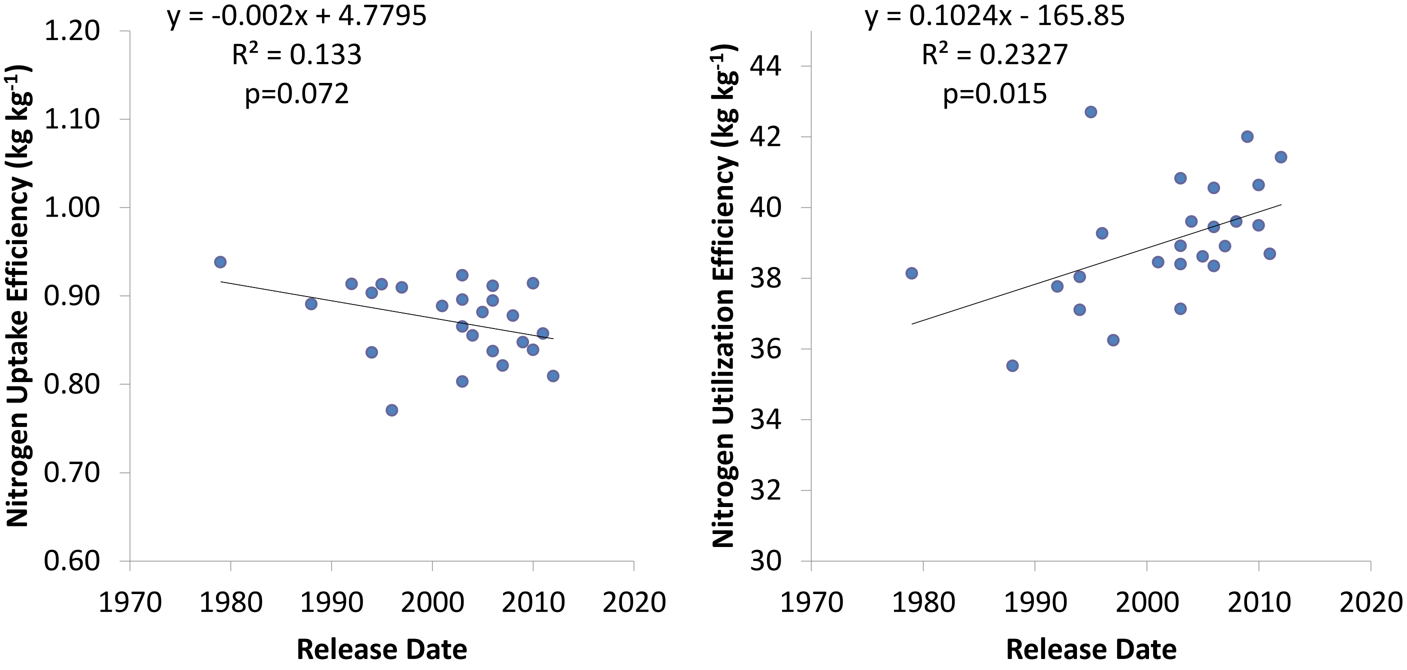


Figure 2.12. The effect of release date on nitrogen uptake and nitrogen utilization efficiency for 25 different wheat varieties (data averaged across two N rates and four environments).

Nitrogen Uptake After Anthesis

While the mean NUpAA values were not significantly affected by variety in this study (p=0.332), there was a highly significant Nrate x variety interaction (p=0.001). This interaction was due to the drastic differences in NUpAA between the two N rates included in this study. All of the varieties gained nitrogen after anthesis for the 0 N rate, but in the high N treatment several varieties lost N after anthesis while some had relatively large amounts of N uptake during the grain filling period (Figure 2.13). This difference between the two N rates resulted in no mean differences when the values were combined and analyzed in SAS.

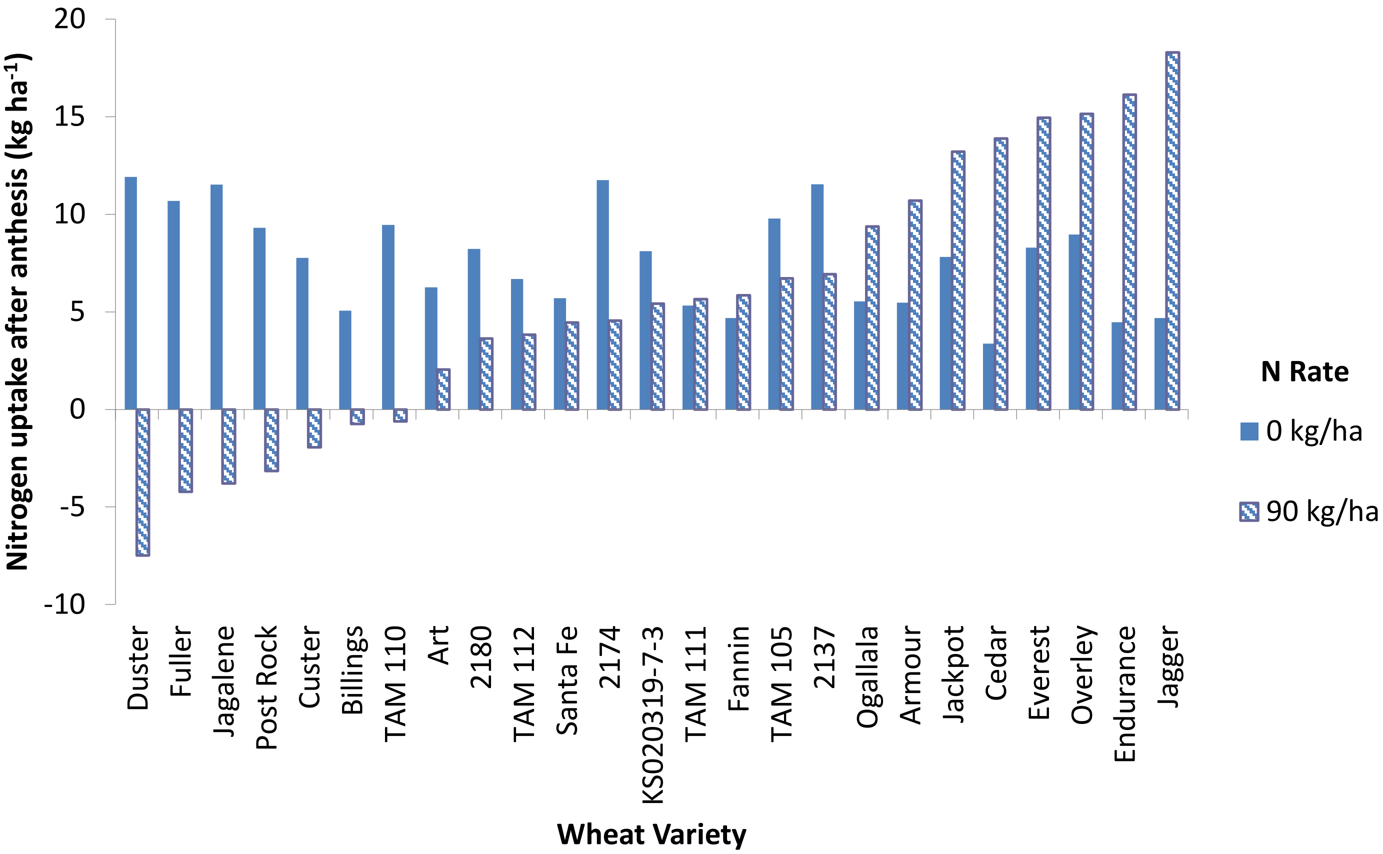


Figure 2.13. Nrate x variety interaction observed for nitrogen uptake after anthesis for 25 wheat varieties.

The varieties that lost N after anthesis at the high N rate were Duster, Fuller, Jagalene, Post Rock, Custer, Billings, and TAM 110 with losses ranging from 0.6 to 7.5 kg N ha^{-1}. Other varieties had significant N uptake after anthesis ranging from 13.9 to 18.3 kg N ha^{-1}, such as observed in Cedar, Everest, Overley, Endurance and Jagger. Interestingly, those varieties that experienced N loss at the high N rate tended to have fairly high N uptake at the low rate, and those varieties with low N uptake at the low rate tended to have greater N uptake after anthesis at the high N rate (Figure 2.14).

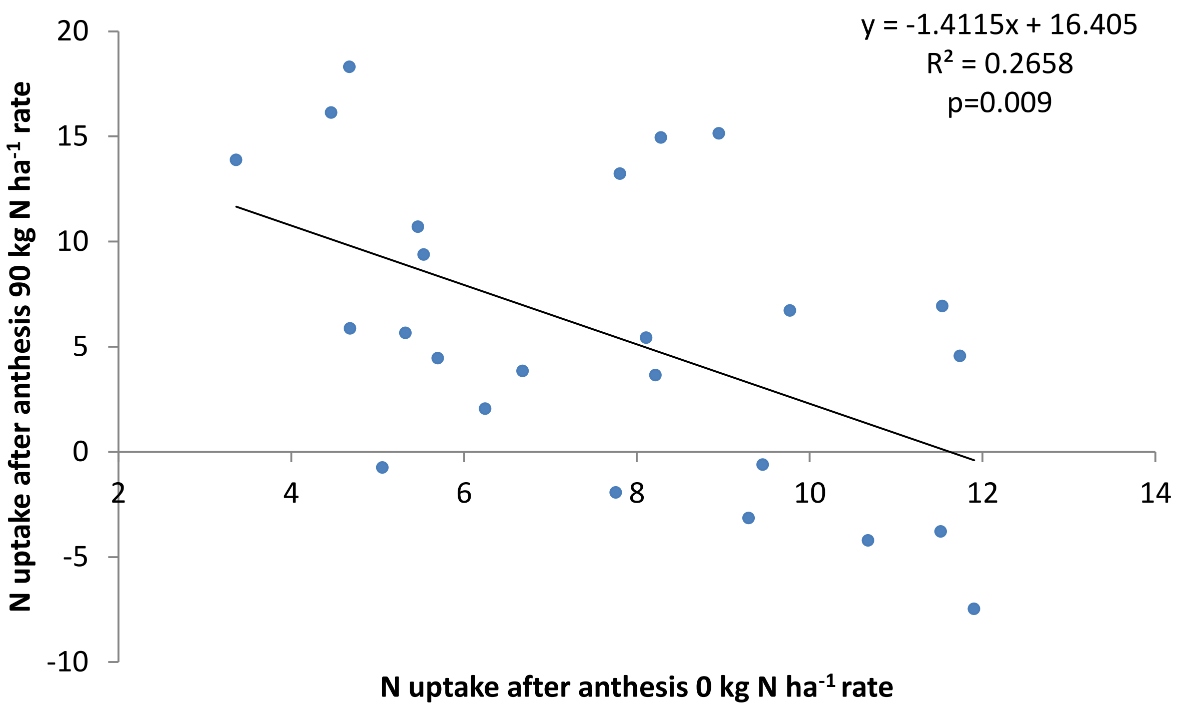


Figure 2.14. Correlation between the low and high N rates on nitrogen uptake after anthesis for 25 wheat varieties.

These results are supported by other studies in corn and wheat that have noted similar nitrogen losses after anthesis, especially in high nitrogen environments (Bahrani et al., 2011; Daigger et al., 1976; Francis et al., 1993; Harper et al., 1987; Papakosta and Gagianas, 1991). In a recent study by Bahrani et al. (2011) on wheat, significant amounts of N, up to 25%, were lost post-anthesis under non-limiting conditions with respect to nitrogen and water, while N losses were much less under water-stressed conditions with the same amount of N. These losses could be due to a luxury consumption of N early in the season and result from N losses from tissues through volatilization, as has been documented in other studies (Egle et al., 2008; Haberle et al., 2006).

There does appear to be some evidence in this study that those varieties with high N uptake early in the season at the highest N rate experienced N loss after anthesis. All of the varieties with negative NUpAA values, with the exception of TAM 110, accumulated very high amounts of total N before anthesis (NUpanth), although the uptake values were not significantly different from each other and had no Nrate x variety interaction (Table 2.8; Figure 2.15). Nitrogen uptake before anthesis contributed an average of 89% of total nitrogen uptake at maturity in this study, which is in agreement with the findings of other research, and illustrates the relative importance of pre-anthesis N uptake versus post-anthesis N uptake (Barbottin et al., 2005). While high N uptake early in the season may contribute to better plant performance through increased tillering and biomass production, the results of this study suggest varieties with very high uptake may not be as efficient at storing the nitrogen for later use in grain filling.

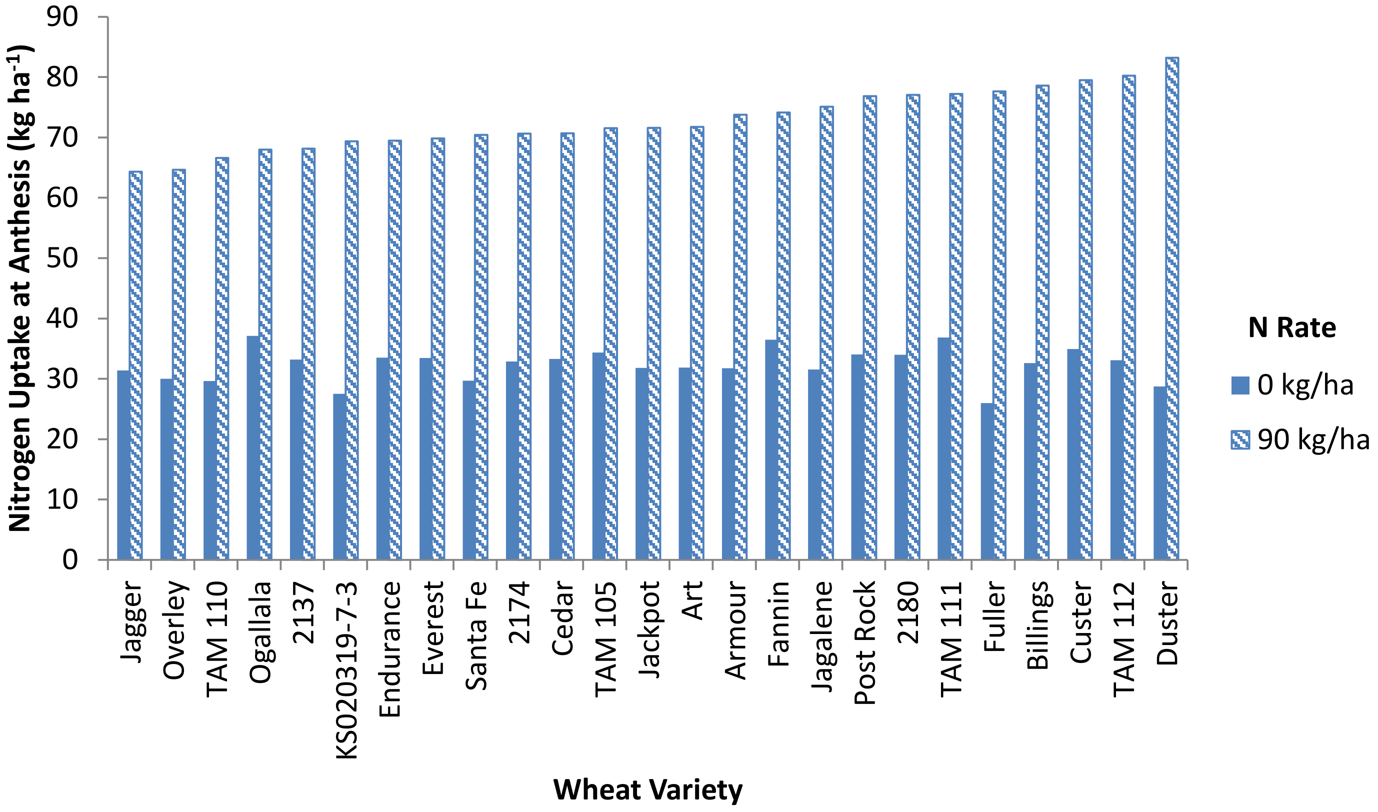


Figure 2.15. Differences in nitrogen uptake at anthesis for the 25 wheat varieties with significant Nrate effect, but no significant effect of variety or Nrate x variety interaction.

When the values at the high N rate were analyzed using a linear regression, it was found that NUpanth and NUpAA were highly correlated, with NUpanth explaining 49% of the variability in NUpAA at the high N rate (Figure 2.16). The results provide further evidence to suggest that at the high N rate, increased nitrogen uptake early in the season contributed to less N uptake later in the season, and even N loss in some varieties. In considering the overall nitrogen balance, NUpAA was not as important as nitrogen uptake before anthesis, and the amount of nitrogen lost, while significant, was only a small portion of the total nitrogen in the plants.

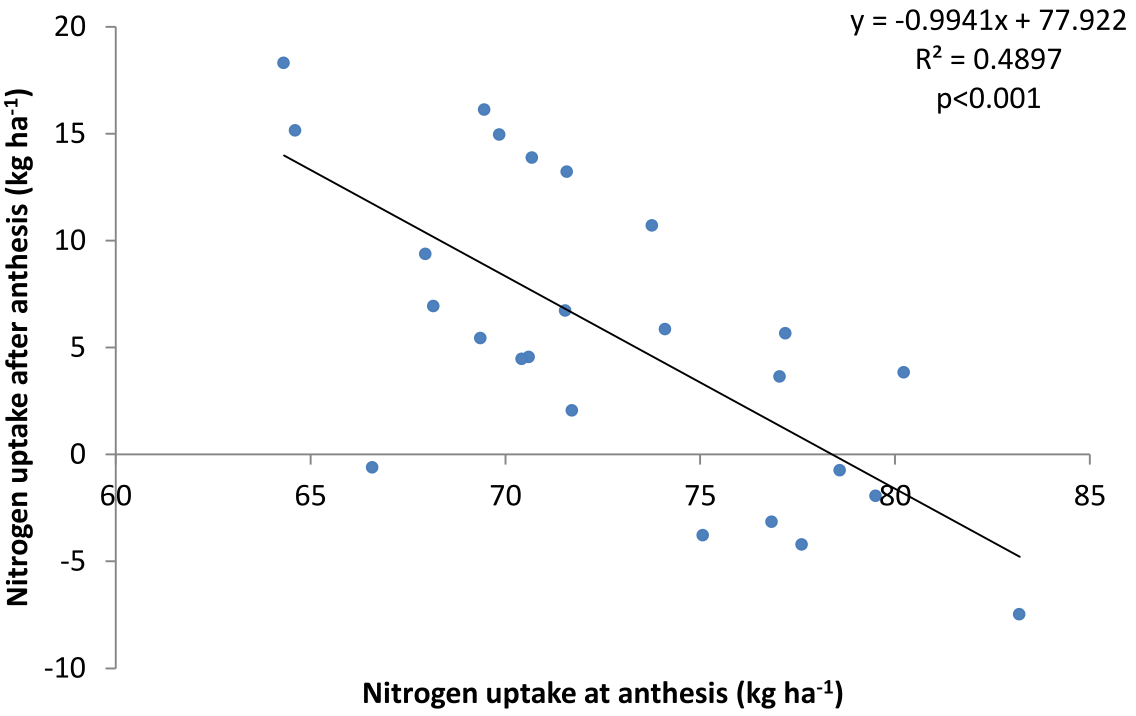


Figure 2.16. Relationship between nitrogen uptake at anthesis and nitrogen uptake after anthesis at the high N rate (90 kg N ha^{-1}) for 25 wheat varieties.

Other possible explanations for N loss could be that varieties that uptake high amounts of N early in the season also have higher biomass production and possible water use (Figure 2.2).

These varieties may use up available soil water earlier in the season, which could contribute to decreased N uptake after anthesis as temperatures warm and N loss from tissue due to earlier senescence. Another possible explanation may be that some varieties are more prone to losing grain either in the field or during combine harvest, which leaves a portion of the nitrogen unaccounted for and could result in decreased or negative uptake after anthesis values.

Although the interaction of NUpAA with Nrate and variety was a major finding in this study, there was no significant effect of NUpAA values on NUE (p=0.456) (Figure 2.17). However, improving N retention and remobilization in the varieties that were prone to losing N at the high N rates could be a potential tool for increasing NUE and improving grain quality. There appears to the sufficient genetic variation among the varieties in NUpAA that selections and improvements could be made, especially in the high N environments that these varieties are often bred in. This could result in higher NUEs in some of these varieties, conserving nitrogen and limiting N loss to the environment.

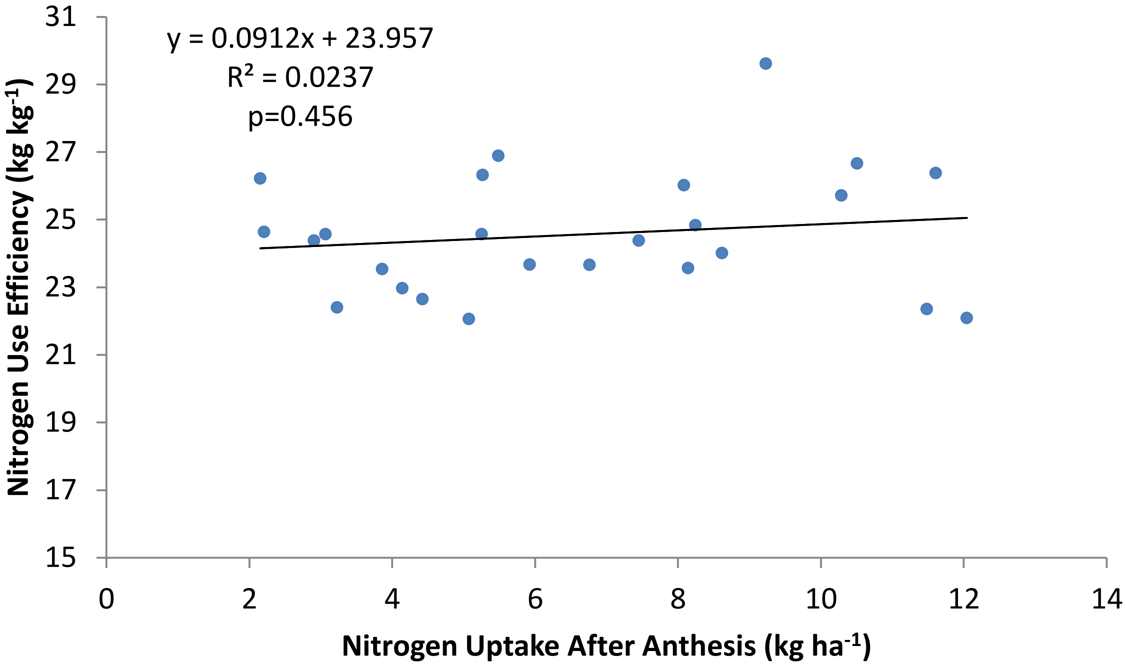


Figure 2.17. Relationship between nitrogen uptake after anthesis and nitrogen use efficiency for 25 wheat varieties (data averaged across two N rates and four environments).

Other Agronomic Parameters

Several parameters calculated in this study were included because of their economic implications for producers. These were fertilizer use efficiency (FUE), partial factor productivity (PFP), agronomic efficiency (AgEf), and partial nutrient balance (PNB). Because of the way these parameters are calculated and the fact that there were only two N rates, values could only be calculated for the high N treatment. All of these parameters had significant differences for variety with the exception of AgEf (Table 2.16).

Table 2.16. Levels of significance (p-values) for agronomic parameters determined at maturity.

	FUE	**PFP**	**AgEf**	**PNB**
Variety	**0.009**	**0.009**	0.274	**0.019**

Abbreviations: FUE, fertilizer use efficiency; PPF, partial productivity factor; AgEf, agronomic efficiency; PNB, partial nutrient balance.

Fertilizer use efficiency is often mistakenly referred to as NUE, but this study considers these two parameters separately. For the purposes of this study, FUE is a percentage of the fertilizer recovered by the plant, while NUE is a more complete assessment of available N calculated with the sum of fertilizer and residual soil N. In interpreting FUE, it's important to realize that 100% recovery is not necessarily the goal, and accounting for 100% of nitrogen would be nearly impossible (Lees et al., 2000). A portion of all fertilizers will be incorporated into residues and organic matter to replace nutrients that mineralize over the course of each season. If these nutrients were not replaced, then the organic matter of the soil would be depleted and fertilizer rate recommendations would need to be higher to supply additional resources that the soil contributed in the past.

On average, FUE values range from 30-60% for most crops and environments. When studied with ^{15}N, some research has been able to precisely measure applied fertilizers and suggests that the true range may be closer to 40-58% (Olson and Swallow, 1984; Harris et al., 1994). In this study, FUE was significantly different between the included varieties with a p=0.009 (Table 2.16). Values ranged from 0.30 kg kg^{-1} for TAM 110 up to 0.53 kg kg^{-1} for Endurance and Cedar, which is within the average ranges for a healthy system (Table 2.17). Because of the genetic variability present in these varieties, it may be possible to select for higher FUE varieties to increase fertilizer recovery and possibly NUE. However, as additional fertilizer is recovered by the plant, the possibility for N loss through plant tissues, as illustrated earlier, may increase. This would decrease NUE and contribute to N losses that the soil OM could have retained for future crops.

Table 2.17. Mean values and significant differences for agronomic parameters, FUE, PFP, AgEf, and PNB, determined at maturity.

Variety	FUE		PFP		AgEf		PNB	
			kg kg^{-1}					
2137	0.35	ABE[†]	27.75	CDE	10.21	AC	0.87	ABCD
2174	0.34	BEG	21.81	B	9.37	C	0.87	ABCD
2180	0.43	ABCDEF	23.13	AB	12.57	ABCD	0.94	ABC
Armour	0.52	DF	26.71	ACDEFG	15.75	BD	0.98	AB
Art	0.37	ABCE	22.90	BF	11.20	ABC	0.85	CDE
Billings	0.45	ABCDF	27.23	CDEH	14.36	ABCD	0.90	ABC
Cedar	0.53	F	26.81	ACDEF	15.83	BD	0.98	AB
Custer	0.39	ABCDE	23.72	ABC	11.06	ABC	0.90	ABC
Duster	0.39	ABCDEH	26.87	ACDEF	12.84	ABCD	0.88	ABCD
Endurance	0.53	FH	26.29	ACDEFG	15.14	ABD	0.99	B
Everest	0.48	ACDF	27.99	DE	14.65	ABD	0.98	AB
Fannin	0.43	ABCDEF	26.97	ACDE	13.43	ABCD	0.93	ABC
Fuller	0.41	ABCDEF	22.86	AB	11.24	ABC	0.85	CDE
Jackpot	0.50	CDF	29.30	E	16.92	D	0.98	AB
Jagalene	0.31	BE	23.76	ABC	11.03	ABC	0.83	CD
Jagger	0.51	CDF	24.44	ABCD	15.52	BD	0.96	ABE
KS020319-7-3	0.44	ABCDEF	22.66	BG	12.76	ABCD	0.86	ABCD
Ogallala	0.38	ABCDE	25.20	ABCD	12.17	ABCD	0.90	ABC
Overley	0.43	ABCDEF	23.87	ABC	13.79	ABCD	0.92	ABC
Post Rock	0.34	BEG	23.26	ABH	10.66	ABC	0.86	ACD
Santa Fe	0.44	ABCDF	23.45	ABH	13.22	ABCD	0.87	ABCD
TAM 105	0.38	ABCE	25.01	ABCD	11.68	ABC	0.91	ABC
TAM 110	0.30	E	22.06	B	11.00	ABC	0.76	D
TAM 111	0.45	ACDFG	26.26	ACDEFG	12.24	ABCD	0.96	ABE
TAM 112	0.51	CDF	25.82	ABCDE	14.58	ABCD	0.97	ABE
LSD[‡]	**0.15**	**-**	**4.18**	**-**	**N.S.**	**-**	**0.13**	**-**

†Means within a column that are followed by the same letter are not significantly different (p>0.05)

‡The least significant difference (LSD) varied by comparison due to missing data, therefore, the LSD listed is the largest LSD for any comparison. Means groupings should be used for precise means comparisons.

Abbreviations: FUE, fertilizer use efficiency; PPF, partial productivity factor; PNB, partial nutrient balance; LSD, least significant difference; Sig. Diff, significant differences.

Partial factor productivity provides a value to estimate how well a crop is producing a yield from a given amount of fertilizer. This parameter was developed by Cassman et al. (1996) as means to determine the efficiency of applied nutrients, which could include nitrogen. This parameter has significant economic implications as it is able to quantify the economic benefits of that nutrient from all sources in the cropping system, which could include fertilizer and natural soil sources (Dua et al., 2007). Partial factor productivity in this study ranged from 21.8 for 2174 to 29 kg kg^{-1} for Everest (Table 2.17). The trends observed in PFP closely followed those of NUE and indicate that increasing NUE would increase PFP and provide significant economic benefits to producers from more efficient nutrient use.

Partial nutrient balance measures the removal of a nutrient, such as nitrogen, from the total amount that was applied as fertilizer. When PNB values equal one crop removal is equal to the amount that was applied. When PNB values are less than 1, removal is less than the applied rate and a surplus of nutrient will be present in the soil. When PNB values are greater than one, crop removal exceeds the amount of applied fertilizer. Varieties were significantly different for this parameter with a p=0.019 (Table 2.16). The values ranged from 0.76 for TAM 110 up to 0.99 for Endurance (Table 2.17). These results indicate the varieties included in this study were fairly good at removing N from the soil in similar proportions to the applied fertilizer, but that there is substantial room for improvement.

FUTURE RESEARCH

To continue to improve NUE, a better understanding of the mechanisms behind the differences is necessary. This study demonstrated the importance of nitrogen utilization and uptake in determining NUE. As NUpE and NUtE are independent traits, finding methods to improve both of these would have substantial impacts on NUE. Future research should focus on finding differences in nitrogen uptake between varieties that could also be incorporated into breeding programs. By increasing nitrogen uptake and utilization efficiencies, grain yields could be improved without sacrificing grain quality and protein content. Research on NUpE should focus on differences in rooting characteristics and nitrogen uptake. Other research has shown significant differences in nitrogen uptake due to rooting characteristics (Liao et al., 2006; Bingham et al., 2012; Hawkesford, 2012). Liao et al. (2006) found significant differences in root structures of wheat varieties that contributed to differences in biomass production and nitrogen uptake. They found that varieties with higher root growth and proliferation, and not rooting depth, contributed to significantly higher N uptake and aboveground biomass. This provides a basis to study nitrogen uptake and root characteristics in more detail in these varieties in the hopes of finding significant differences that could be exploited to produce higher NUE wheat.

Future research on the pedigrees of the varieties included in this study and the specific genes responsible for controlling NUpE, NUtE, and NUE would also be very valuable to breeding programs. The varieties included in this study were bred for different characteristics, such as disease resistance, insect resistance, and bread quality, which may alter the physiology of the plants such as root depth and structure or photosynthetic abilities that may contribute to differences in NUpE or NUtE. In this study, variety 2137 was found to be superior in many of the calculated parameters. This variety has a very broad genetic base and may be one of the most unique varieties included in this study.

Future research on 2137 and what makes it superior for NUE, NUtE, and grain yield could be an appropriate starting place for a breeding program. Identifying markers and genes responsible for its high performance could result in the development of further improved varieties in the future. Crossing 2137 with varieties that performed poorly, such as Overley, Santa Fe, or Jagger, could help to identify the specific genes responsible for improved NUE, and may result in increased performance of their offspring.

CONCLUSIONS

The results provide strong evidence for genetic differences in NUE for the 25 wheat varieties included in this study. It was also shown that there have been no significant improvements in NUE over the last several decades. If producers want to continue to meet the food demands of the future, yields will need to increase. Because of the strong relationship between NUE and grain yield, improvements in one area will likely also improve the other. Concentrating on improving NUE would not only increase grain yields, but also have significant economic and environmental benefits through more efficient use of applied N fertilizers and soil N supplies.

Nitrogen use efficiency is correlated with both NUpE and NUtE, but only NUtE showed significant varietal differences in this study. Finding ways to improve nitrogen uptake and nitrogen utilization will have positive effects on NUE. Significant progress could be made if varieties with differences in NUpE could be identified. Improving NUpE would provide more nitrogen in the plant system that could help maintain or improve grain quality as yields increase. NUtE showed significant improvements over time, while NUpE had a negative relationship with release date, although it was not a significant correlation. This suggests the capacity for these wheat varieties to uptake nitrogen from the soil may have decreased over time and contributed to the lack of any improvements in NUE over the same time period.

One other factor that may contribute to low NUE in some of the varieties was the observed loss of nitrogen after anthesis at the high Nrate. The drastic differences in NUpAA, especially at the high Nrate, contributed to a significant Nrate x variety interaction in this study. Any nitrogen loss from the system contributes to a decrease in NUE, and the differences in these varieties provide evidence for genetic differences in how these varieties uptake and handle nitrogen. It was shown that those varieties that had large amount of N uptake early in the season were the most likely to lose nitrogen after anthesis. While N uptake early in the season is very important for plant development and later reproductive growth, luxury consumptions of N may lead to N loss from the plants and decrease NUE.

Improving NUE through better understandings of genetics and the physiological processes behind efficient N use will have significant benefits for producers and the rising global population. Higher NUE crops will allow producers to produce the same or higher yields with less fertilizer inputs, and open up more marginal land for production. This will also have benefits for producers in developing countries where access to fertilizers is limited and often unaffordable. The development of higher NUE crops could result in the new green revolution and allow for the support of the projected global population in the future.

REFERENCES

Anbessa, Y., P. Juskiw, A. Good, J. Nyachiro, and J. Helm. 2009. Genetic variability in nitrogen use efficiency of spring barley. Crop Sci. 49:1259-1269

Arregui, L.M. and M. Quemada. 2008. Strategies to improve nitrogen use efficiency in winter cereal crops under rainfed conditions. Agron. J. 100: 277-284.

Bahrani, A., H.H.S. Abad, and A. Aynehband. 2011. Nitrogen remobilization in wheat as influenced by nitrogen application and post anthesis water deficit during grain filling. Afr. J. of Biotech. 10:10585-10594.

Barbottin, A., C.H. Lecomte, C.H. Bouchard, and M.H. Jeuffroy. 2005. Nitrogen remobilization during grain filling in wheat. Eur. J. Agron. 25:119-128.

Barraclough, P.B., J.R. Howarth, J. Jones, R. Lopez-Bellido, S. Parmar, C.E. Shepher, and M.J. Hawkesford. 2010. Nitrogen efficiency of wheat: genotypic and environmental variation and prospects for improvement. Eur. J. Agron. 33: 1-11.

Battenfield, S.D., A.R. Klatt, and W.R. Raun. 2013. Genetic yield potential improvement of semidwarf winter wheat in the Great Plains. Crop Sci. 53: 946-955.

Bertin, P., and A. Gallais. 2000. Genetic variation for nitrogen use efficiency in a set of recombinant maize inbred lines. I. Agrophys. Results 45: 53-66.

Bingham, I.J., A.J. Karley, P.J. White, W.T.B. Thomas, and J.R. Russell. 2012. Analysis of improvements in nitrogen use efficiency associated with 75 years of spring barley breeding. Eur. J. Agron. 42: 49-58.

Blankenau, K., H.W. Olfs, and H. Kulhmann. 2002. Strategies to improve the use efficiency of mineral fertilizer nitrogen applied to winter wheat. J. Agron. Crop Sci. 188: 146-154.

Cormier, F. S. Faure, P. Dubreuil, E. Heumez, K. Beauchene, S. Lefarge, S. Praud, and J. Le Gouis. 2013. A multi-environmental study of recent breeding progess on nitrogen use efficiency in wheat (*Triticum astivum* L.). Theor. Appl. Genet. 126: 3035-3048.

Daigger, L.A., D.H. Sander, and G.A. Peterson. 1976. Nitrogen content of winter wheat during growth and maturation. Agron. J. 68:815-818.

Dua, V.K., P.M. Govindarishman, S.S. Lal, and S.M. Paul Khurana. 2007. Partial factor productivity of nitrogen in potato. Better Crops. 91:26-27.

Egle, K., H. Beschow, and W. Merbach. 2008. Assessing post-anthesis nitrogen uptake, distribution, and utilization in grain protein synthesis in barley (*Hordeum vulgare* L.) using 15N fertiliser and 15N proteinogenic and non-proteinogenic amino acids. Ann. Appl. Biol. 152: 209-221.

FAO. 1949. Food composition tables for international use. FAO Corporate Document Repository. http://www.fao.org/docrep/x5557e/x5557e04.htm#cereals (accessed 21 Mar. 2014).

FAOSTAT. 2011. Food and Agriculture Organization of the United Nations, FAOSTAT database. http://faostat3.fao.org/faostat-gateway/go/to/download/R/RF/E (accessed 27 Dec. 2013).

Francis, D.D., J. S. Schepers, and M.F. Vigil. 1993. Post-anthesis nitrogen loss from corn. Agron. J. 85:659-663.

Girma, K., K.L. Martin, R.H. Anderson, D.B. Arnall, K.D. Brixey, and M.A. Casillas. 2006. Mid-season prediction of wheat grain yield potential using plant, soil, and sensor measurements. J. of Plant Nut. 29:873-897.

Gooding, M.J., M. Addisu, R.K. Uppal, J.W. Snape, and H.E. Jones. 2012. Effect of dwarfing genes on nitrogen-use efficiency. J. of Agric. Sci. 150: 3-22.

Haberle, J., P. Svoboda, and J. Krejcova. 2006. Uptake of mineral nitrogen from subsoil by winter wheat. Plant Soil Env. 52: 377-384.

Hanlon, E.A., and G.V. Johnson. 1984. Bray/Kurtz, Mehlich-III, AB/DTPA, and ammonium acetate extractions for P, K, and Mg in four Oklahoma soils. Commun. Soil Sci. Plant Anal. 15:277-294.

Harper, L.A., R.R. Sharpe, G.W. Langdale, and J.E. Giddens. 1987. Nitrogen cycling in a wheat crop: soil, plant, and aerial nitrogen transport. Agron. J. 79:876-883.

Harris, G.H., O.B. Hesterman, E.A. Paul, S.E. Peters, and R.R. Janke. 1994. Fate of legume and fertilizer nitrogen-15 in a long-term cropping systems experiment. Agron. J. 89: 910-915.

Hawkesford, M.J. 2012. The diversity of nitrogen use efficiency for wheat varieties and the potential for crop improvement. Better Crops. 96: 10-12

Keeney, D.R. and D.W. Nelson. 1982. Nitrogen - inorganic forms. In A.L. Page (eds.) Methods of soil analysis, part 2. Agron. Monogr. 9, 2nd ed. ASA and SSSA, Madison, WI. p. 643-698.

Kansas State University Mesonet. 2014. Historical Weather. http://mesonet.k-state.edu/weather/historical/ (accessed: 15 Jan. 2014).

Lees, H.L., W.R. Raun, G.V. Johnson. 2000. Increased plant nitrogen loss with increasing nitrogen applied in winter wheat observed with ^{15}nitrogen. J. Plant Nut. 23: 219-230.

Liao, M.T., I.R.P Fillery, and J.A. Palta. 2004. Early vigorous growth is a major factor influencing nitrogen uptake in wheat. Funct. Plant Biol. 31: 121-129.

Liao, M.T., J.A. Palta, and I.R.P. Fillery. 2006. Root characteristics of vigorous wheat improve early nitrogen uptake. Aust. J. of Ag. Res. 57:1097-1107.

Limaux, F., S. Recous, J.M. Meynard, and A. Guckert. 1999. Relationship between rate of crop growth at date of fertilizer N application and fate of fertilizer N applied to winter wheat. Plant Soil. 214: 49-59.

Lopez-Bellido, L., R.L. Lopez-Bellido, and R. Redondo. 2005. Nitrogen efficiency in wheat under rainfed Mediterranean conditions as affected by split nitrogen application. Field Crops Res. 94: 86-97.

Marti, J., J. Bort, G.A. Slafer, and J.L. Araus. 2007. Can wheat yield be assessed by early measurements of Normalized Difference Vegetation Index? Ann. Appl. Biol. 150: 253-257.

Matejovic, I. 1995. Total nitrogen in plant material determined by means of dry combustion: A possible alternative to determination by Kjeldahl digestion. Commun. Soil Sci. Plant Anal. 25:943-954.

Mehlich, A. 1984. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. Commun. Soil Sci. Plant Anal. 15:1409-1416.

Moll, R.H., E.J. Kamprath, and W.A. Jackson. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agron. J. 74: 562-564.

Muurinen, S., G.A. Slafer, and P. Peltonen-Sainio. 2006. Breeding effects on nitrogen use efficiency of spring cereals under Northern Conditions. Crop Sci. 46: 561-568.

Namai, S., K. Toriyama, and Y. Fukuta. 2009. Genetic variations in dry matter production and physiological nitrogen use efficiency in rice (*Oryza sativa* L.) varieties. Breeding Sci. 59: 269-276.

Olson, R.V., and C.W. Swallow. 1984. Fate of labeled nitrogen fertilizer applied to winter wheat for five years. Soil Sci. Soc. Am. J. 48: 583-586.

Pang, J., J.A. Palta, G.J. Rebetzke, and S. P. Milroy. 2014. Wheat genotypes with high early vigour accumulate more nitrogen and have higher photosynthetic nitrogen use efficiency during early growth. Func. Plant Biol. 41: 215-222.

Papakosta, D.K., and A.A. Gagianas. 1991. Nitrogen and dry matter accumulation, remobilization, and losses for Mediterranean wheat during grain filling. Agron. J. 83:864-870.

Raun, W.R., J.B. Solie, G.V. Johnson, M.L. Stone, R.W. Mullen, K.W. Freeman, W.E. Thomason, and E.V. Lukina. 2002. Improving nitrogen use efficiency in cereal grain production with optical sensing and variable rate application. Agron. J. 94: 815-820.

Rothstein, S.J. 2007. Returning to our roots: making plant biology research relevant to future challenges in agriculture. The Plant Cell 19: 2695-2699.

Sadras, V.O., and C. Lawson. 2013. Nitrogen and water-use efficiency of Australian wheat varieties released between 1958 and 2007. Europ. J. Agron. 46: 34-41.

SAS Institute. 2005. The SAS system for Windows. Release 9.3. SAS Inst., Cary, NC.

Saxton, A. 2000. PDMIX800. http://animalscience.ag.utk.edu/facultystaff/Saxton/pdmix800.sas University of Tennesee, Knoxville, TN.

Sinebo, W., R. Gretzmacher, and A. Edelbauer. 2002. Environment of selection for grain yield in low fertilizer input in barley. Field Crops Res. 74: 151-162.

Slafer, G.A. 2003. Genetic basis of yield as viewed from a crop physiologist's perspective. Annals Appl. Biol. 142: 117-128.

The Fertilizer Institute. 2010. Fertilizer Use. http://www.tfi.org/statistics/fertilizer-use (accessed 27 Dec. 2013).

Tilman, D., J. Fargione, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, W.H. Schlesinger, D. Simberloff, and D. Swackhamer. 2001. Forecasting agriculturally driven global environmental change. Science 292: 281-284.

Tilman, D., K.G. Cassman, P.A. Matson, R. Naylor, and S. Polasky. 2002. Agricultural sustainability and intensive production practices. Nature 418: 671-677.

United States Department of Agriculture, Economic Research Service. 2013. Fertilizer Use and Price. USDA ERS. http://www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx#.UyhpZ4XB2hM (accessed 20 Mar. 2014).

Watson, S. 2003. Wheat varieties for Kansas and the Great Plains: Your best choices for 2003. Lone Tree Publishing, Topeka, KS.

Chapter 3
Nitrogen Rate Response of Wheat Varieties Representing a Range of Nitrogen Use Efficiencies

ABSTRACT

With the increasing costs of nitrogen fertilizers, producers would benefit economically from more efficient varieties with improved response to N fertilizer. Varieties with these characteristics would also help protect the environment by reducing N applications, leaching, and greenhouse gas emissions. The objectives of this study are to determine differences in nitrogen response (N response) and parameters related to nitrogen use efficiency (NUE), and evaluate the effect of NUE on N response. This Nrate experiment was a split plot design planted at two locations in the 2012-13 season at Ashland Bottoms and Silverlake, KS. The study included four varieties, Duster, Everest, Jagger, and Larned, which represented a range of NUEs based on preliminary research. The four N fertilizer rates were 0, 33.6, 89.7, and 145.7 kg N ha^{-1}. Several parameters were affected by variety or had significant Nrate x variety interactions, although the results between locations were contrasting. The only location with significant varietal differences in N response was at Ashland Bottoms ($p=0.041$). While there was no difference in N response at Silverlake, there were significant differences in mean grain yield ($p<0.001$). When NUE was evaluated, it was found that those varieties with higher NUE also tended to have improved N response. These results provide evidence that there are differences in N response between these varieties and that they are closely related to the observed nitrogen use efficiency values, however, because of the differences in results between locations, additional research will be needed to develop firm conclusions.

INTRODUCTION

From the time that wheat was first domesticated and became an agricultural staple crop, breeding for higher yield and grain quality has been a primary focus (Motzo et al., 2004). This has been performed by breeding plants that produced more and larger grain while conserving bread making characteristics. Beginning in the 20th century, yields were elevated through the use of commercial fertilizers and the release of new varieties that performed well in high-nutrient conditions. Another tactic to produce higher yields was by breeding for disease resistant traits, allowing more consistent yields and minimizing losses. With the Green Revolution beginning in the 1960s, wheat yields again made significant advances with the introduction of semi-dwarf wheat varieties capable of supporting higher grain yields by minimizing lodging losses and the introductions of additional disease resistant characteristics.

In most breeding programs, varieties are developed in non-limiting conditions to ensure maximum yield values are selected for (Brancourt-Hulmel, 2005). However, with the increasing cost of nitrogen fertilizers worldwide, there has been a great deal of concern among producers on meeting the nitrogen demands of commercial crops. Another key concern is the environmental problems that come with high rates of nitrogen fertilizer applications, such as leaching loss and greenhouse gas emissions.

One method to combat these problems is by developing varieties that have high yield responses to low nitrogen additions, which could significantly benefit farmers who have limited budgets and resources in the US and abroad. Previous research by Austin et al. (1980) and Ortiz-Monasterio et al. (1997) have found evidence that high-yielding wheat varieties performed better than traditional varieties in both high and low nutrient conditions. Their conclusions are further supported by the work of Hasegawa (2003) who found that commercial rice varieties bred in high-nutrient conditions outperformed traditional varieties at all N rates. However, the ability of these varieties to outperform traditional ones at low nutrient rates is the result of indirect, rather than direct, selection (Brancourt-Hulmel, 2005). It is likely that significant progress could be made in nitrogen response and crop performance at lower N rates if these characteristics were purposely, rather than indirectly, selected for.

Conversely, there could be some difficulty in breeding for plant response in low nutrient conditions as some research has shown that many genetic traits have poor heritability in these environments (Banziger et al, 1997; Sinebo et al., 2002). This could be a potential problem in developing varieties designed for low-nutrient or stressful conditions. These studies suggest that the low heritability of traits may be due to lower genetic variance among populations, difficulty in selecting breeding pairs for a specific trait, and high error rates. Brancourt-Hulmel et al. (2005) concluded that breeding programs interested in improving plant characteristics in stressful conditions should use these environments to maximize traits of interest.

Previous research in the NUE study indicated significant differences in NUE between varieties, but provided very little information on nitrogen response (Chapter 2). Varieties with high NUE and high yield responses to N inputs could have significant benefits for food production and the environment. These varieties would be more efficient and reach their highest yields at lower N rates, saving producers money and limiting N loss to the environment.

Relevant research on N response and the effect of NUE is lacking, especially in the area of determining genetic differences among wheat varieties and the potential for breeding crops with higher N-Response and NUE.. The objectives of this study are to determine if there are differences in nitrogen response, NUE, and other related parameters in four winter wheat varieties to multiple N rates and determine the effect of NUE on N-Response.

MATERIALS AND METHODS

This study was a field experiment planted in the 2012-13 and 2013-14 seasons, for a total of 4 environments (Table 3.1). Because the experiment planted in the fall of 2013 is ongoing, the results are not included in this thesis. The experiment included four varieties, Everest, Duster, Jagger, and Larned. These varieties were selected because of their range of NUEs, as determined from preliminary results in the NUE study (Chapter 2). Larned was not included in the NUE study because of its markedly different physiology, although it was hypothesized to have low NUE as it's a full-height variety. This variety was developed by K-State and released in 1976, and although this was after semi-dwarfing genes were gaining in popularity, they were not bred into this variety.

Table. 3.1. Locations, soils, and cultural practices used for wheat production in four environments in Kansas.

Growing Season	Location	Coordinates	Soil map unit	Cultural practices
2012-2013	Ashland Bottoms Research Farm	39° 7' 35" N, 96° 38' 11" W	Wymore silty clay loam (Fine, smectitic, mesic Aquertic Argiudoll)	No-till after soybean. No irrigation
2012-2013	Silverlake Exp. Field	39° 04' 34" N, 95° 46' 12" W	Eudora-Bismarck Grove silt loam (coarse-silty, mixed, superactive, mesic Fluventic Hapludoll)	No-till after soybean. 42.2 mm irrigation.
2013-2014	Ashland Bottoms Research Farm	39° 7' 23" N, 96° 38' 17" W	Wymore silty clay loam (Fine, smectitic, mesic Aquertic Argiudoll)	No-till after soybean. No irrigation.
2013-2014	Hutchinson Exp. Field	37° 55' 51" N, 98° 01' 41" W	Ost loam (Fine-loamy, mixed, superactive, mesic Udic Argiustoll)	No-till after soybean. No irrigation.

The experiment was a split-plot design with whole plots consisting of variety and subplots consisting of the four N treatments. Whole plots were randomized within each block and divided into four subplots and the four N treatments, 0, 33.6, 89.7, or 145.7 kg N ha^{-1}, were then randomly assigned to each. The whole plots were 6.1 m wide and 18.3 m long and subplots were 3 m wide and 9.1 m long (Appendix A.5-A.6). The experiment was replicated four times at each location.

Soil samples were taken pre-planting at most locations, however, due to scheduling and traveling conflicts some were sampled after planting. In these instances, soil samples were taken in between rows to avoid P fertilizer that was applied in-row with the planter. Three composite samples of 15 cores each were taken from each replication, or block, and averaged together to estimate soil nutrients. Sampling by block provided a means to account for soil variability within locations. Soil samples were then dried, ground, and sent to the K-State Soil Testing Lab for analysis. The Soil Testing Lab analyzed the soil samples for pH, P, K, total carbon and nitrogen, nitrate-N (NO_3^--N), and ammonium-N (NH_4^+-N). The results for these analyses are found in Table 3.2.

Table 3.2. Initial soil test results for pH, total C, total N, Mehlich III P, extractable K, and extractable inorganic N by environment.

Location	**Depth**	**pH**	**Total C**	**Total N**	**P**	**K**	**NH4**	**NO3**	**Inorganic Profile N**
	cm		— g kg^{-1} —		——— mg kg^{-1} ———				kg ha^{-1}
Ashland Bottoms 2012-13	0-15	6.6	12.9	1.4	4.8	353	4.8	2.9	**46.0**
	15-61	-	-	-	-	-	3.4	1.6	-
Silverlake 2012-13	0-15	7.2	8.9	0.9	8.0	137	3.0	6.3	**51.7**
	15-61	-	-	-	-	-	2.5	3.0	-
Ashland Bottoms 2013-14	0-15	6.0	12.3	1.5	17.1	375	3.6	6.5	**63.7**
	15-61	-	-	-	-	-	3.8	3.3	-
Hutchinson 2013-14	0-15	5.3	13.1	1.1	51.1	330	5.5	9.4	**105.3**
	15-61	-	-	-	-	-	4.0	8.2	-

The experiments were planted with a 3 m wide no-till planter. At the Ashland Bottoms location in the 2012-13 and 2013-14 seasons a John Deere no-till planter was used that allowed P fertilizer to be applied in-row with the seed. Phosphorus was applied as Triple Super Phosphate (0-46-0) at a rate of 44.8 kg material ha^{-1}, or 9.0 kg P ha^{-1} in the 2012-13 season and 59 kg material ha^{-1}, or 11.9 kg P ha^{-1} in the 2013-14 season. At Silverlake in the 2012-13 season, a Speed King CrustBuster no-till drill was used to the plant the experiment, which also allowed for the application of P fertilizer in-row at planting. Triple Super Phosphate fertilizer was applied at a rate of 31 kg material ha^{-1}, or 6.2 kg P ha^{-1}. In the 2013-14 season at Hutchinson a no-till planter was used that did not have the capability of applying P fertilizer in-row. Therefore, triple super phosphate was applied by hand with the first nitrogen application at a rate of 50 kg material ha^{-1}, or 10 kg P ha^{-1}. Notable events in this study, such as soil sampling, planting, fertilizer applications, and fungicide and herbicide treatment dates, can be found in Table 3.3.

Table 3.3. Table of important events and corresponding dates in the NRate studies from 2012-14 seasons.

Location	Soil Sampled	Planting	1st N application	2nd N application	Herbicide application	Fungicide application	NDVI readings	Anthesis Biomass samples	Mature Biomass harvest	Combine harvest
Silverlake 2012/13	11/1/12	10/18/12	11/1/12	4/1/13	4/2/13	5/7/13	3/15/13	5/21/13	7/2/13	7/8/13
Ashland Bottoms 2012/13	11/15/12	10/16/12	11/15/12	3/4/13	Not Applied	5/15/13	3/15/13	5/23/13	7/1/13	7/5/13
Ashland Bottoms 2013/14	10/9/13	10/12/13	10/17/13	3/21/14	NA	NA	NA	NA	NA	NA
Hutchinson 2013/14	11/12/13	10/24/13	11/14/13	3/25/14	NA	NA	NA	NA	NA	NA

Abbreviations: NA, date not available at time of thesis defense.

At planting the seed varieties were placed in the seed box and all plots within each block that were assigned to that variety were planted. After each was planted, the seed was then thoroughly vacuumed and removed from the seed box and the excess was returned to a storage container. The next variety was then loaded into the planter, and the process was repeated until all plots were planted with the correct varieties.

The first nitrogen applications were made shortly after planting at around seedling emergence. Equal rates were applied to the three subplots within each whole plot receiving N treatment at the first split-application. This application was applied by hand at a rate of 33.6 kg N ha^{-1}. The fertilizer used at all locations was ammonium nitrate (34-0-0).

The second application was made in the spring as temperatures warmed and the wheat resumed growth after winter vernalization. Spring fertilizer applications were only made to the 89.7 and 145.7 kg N ha^{-1} plots as the others (0 and 33.6 kg N ha^{-1}) already received their allotment in the fall. Nitrogen fertilizer was again applied as ammonium nitrate by hand to these two sub-plots.

The fields were treated with herbicide and fungicide, with the exception of Ashland Bottoms in 2012-13 (Table 3.4). At Ashland Bottoms in the 2012-13 season, herbicide was not applied due to a scheduling mistake, resulting in a large amount of early season weed growth at this location. The most dominant weed species was identified as henbit, or *Lamium amplexicaule.* Its presence may have lowered the performance of the wheat crop, but its growth cycle ended fairly early in the season and was quickly outcompeted by the wheat as temperatures warmed.

Table 3.4. Rates of herbicide and fungicide applications for each location of the Nrate study.

Growing Season	Location	Herbicide	Fungicide
2012-2013	Silver Lake Exp. Field	0.019 kg Finesse ha^{-1}	0.57 kg Headline ha^{-1} and 0.38 kg COC ha^{-1}
2012-2013	Ashland Bottoms Research Farm	none	0.67 kg Headline ha^{-1} and 0.38 kg COC ha^{-1}
2013-2014†	Hutchinson Exp. Field	NA	NA
2013-2014†	Ashland Bottoms Research Farm	NA	NA

† not included as information was not available at the time of thesis defense.

Abbreviations: NA, date not available at time of thesis defense

In the spring, NDVI readings were taken after flag leaf emergence. These readings were taken using a portable GreenSeeker® produced by Trimble®. The GreenSeeker® was mounted at the end of a pole and readings were sent to a handheld computer. The NDVI readings were taken by walking at a constant pace through the length of the center of each plot. The readings for each plot were then stored on the handheld computer using Trimble's Capture program for Windows Mobile. The program calculated average NDVI readings for each plot, which were later used in data analysis to compare readings to N-Response, NUE, and grain yield.

Biomass samples were harvested when the wheat reached anthesis (Feekes 10.5.1). This was performed by hand using small serrated sickles and harvesting 3 m of two rows on the side of each plot. The harvested rows were two rows from the edge of each the plot to remove any edge effect, and also allowed for adequate plot space to use a research combine to harvest the grain at the end of the season. The biomass samples were weighed in the field, ground through a plant mulcher, and subsamples of this tissue were then taken. These subsamples were weighed while wet, then placed in a large oven at 60° C for several days and weighed again to provide information on moisture content used to determine total dry biomass production. Samples of dried plant tissue were ground using a Wiley-Mill and submitted to the K-State Plant Analysis lab to determine N and C contents.

The plots were then allowed to grow to maturity, and once the grain had reached optimum moisture levels of below 15%, biomass samples and grain were harvested. The plots were first hand-harvested using sickles by again removing two rows of biomass for 3 meters on the same side of the plot that the samples at anthesis were taken from. These biomass samples were placed in large bags and put into an oven at 60° C for several days and then weighed. The grain was threshed from the plants using a small thresher belonging to the K-State Crop Protection and Weed Science group. The grain from each plot was then weighed, and samples of straw and grain were ground and submitted to the K-State Plant Analysis lab to determine C and N contents in the plant tissue and grain.

The remainder of the plots were harvested using a research combine. At Silverlake in the 2012-13 season a research combine fitted with a plot yield monitor was used to harvest which provided data on total grain weight, moisture, and test weight for each plot. At Ashland Bottoms in the 2012-13 season, a Hege research combine was used to harvest a 1.5-m strip down the center of each plot. All grain harvested from the plots was placed in a large sack and taken back to the lab to determine yield and other parameters of interest. The grain from both locations was analyzed using a Dickey-John to determine moisture content and test weight. Grain yield for both locations was corrected to 12% moisture, and yield values obtained from the combines were used to calculate nitrogen response and the other parameters of interest.

Although grain yields were computed both from the hand-harvest and combine harvest, only the values from the combine harvest were used to calculate the parameters of interest. This was because of the larger area the combine was able to harvest, thereby creating a more representative sample of the plot. Biomass production was determined through the hand-harvest method, and as the area harvested was consistent for each sample, the weights could be computed on a kg ha^{-1} basis. Grain yield and biomass values were then used to calculate harvest index (HI), or grain weight divided by aboveground biomass, which was then used to compute total nitrogen uptake in the straw and grain. Total N in the grain was calculated as (Biomass * HI)*(N% grain/100). Total N in the straw was calculated as Biomass(1-HI)*(N%straw/100). These values were then used to calculate the remaining parameters of interest (Table 1.2).

Of particular importance in this study was the yield response of each variety to nitrogen inputs. Treatment effects on response variables were determined by location using SAS Proc Mixed (SAS Institute, 2005; Appendix Table B.4). Contrasts were also constructed to test the nitrogen response curves to see if linear or linear plateau models best fit the data, and the results were analyzed again with locations combined and set as a fixed effect to determine any possible location interactions (Appendix Table B.3). Assigning letters according to significant differences ($p<0.05$) was facilitated by the PDMIX800 program (Saxton, 2000).

RESULTS AND DISCUSSION

Soil types, and subsequently nutrient levels, were different between locations and years (Table 3.2). In the 2012-13 season the mean profile nitrogen content was 51.7 kg ha^{-1} at Silverlake, KS and 46.0 kg ha^{-1} at Ashland Bottoms. In the 2013-14 season, the nitrogen content at Ashland Bottoms was 63.7 kg ha^{-1}, and 105.3 kg ha^{-1} at Hutchinson, KS. These values are considerably higher, and likely due to the differences in soybean performance over the summer. In the 2012 season for soybean there was a severe drought which limited crop performance and likely nitrogen fixation, whereas the 2013 season was a better year for soybean and likely contributed to increased nitrogen in the profile.

Phosphorus test values were below the Kansas soil test threshold of 20 ppm at both locations in the 2012-13 season. The challenge of soil testing and making P recommendations for these locations is due to the limited window of time between soybean harvest and wheat planting, so accurate recommendations could not be made ahead of time. As such, P was not applied in sufficient quantities at either of the locations, and may have limited wheat growth and performance. Using the K-State sufficiency recommendation equation for wheat and a yield goal of 55.7 bu ac^{-1} at Ashland Bottoms, which was the highest mean yield observed at this location, 59.1 kg P_2O_5 ha^{-1} would have been required, however only 20.2 kg P_2O_5 ha^{-1} was applied. The P concentration at Silverlake, KS was initially 8.0 ppm, and by using the same equation with a yield goal of 70.8 bu ac^{-1}, the required amount of P was 50.9 kg P_2O_5 ha^{-1}, and only 14.0 kg P_2O_5 ha^{-1} was applied at planting.

Soil test phosphorus was considerably higher the next season at both locations, and although the P was slightly lower at Ashland Bottoms, this should not have been a problem for wheat growth as sufficient amounts of P fertilizer were applied at planting. Other soil test values, such as pH and potassium, were within agronomic standards for wheat production for all locations.

Weather data are presented in Table 3.5 for the two locations in the 2012-13 season. The data show some minor weather differences between the two locations in temperature and precipitation. The fall and winter after planting were unusually dry as the region was still under drought conditions, and a majority of the precipitation occurred in the spring. As the Silverlake location had irrigation available, the fields were irrigated twice in the fall to encourage emergence and seedling establishment. This early season irrigation and slightly higher precipitation was likely responsible for better crop performance at this location compared to the dryland conditions of the Ashland Bottoms location. Environmental differences were especially notable early in the season, and emergence and establishment at Ashland Bottoms in the 2012-13 season was delayed due to very dry conditions. As the study is currently ongoing, no end of season data is available for the 2013-14 season at Ashland Bottoms and Hutchinson, KS.

Table 3.5. In-season weather and irrigation data for Nrate studies.

	Min Air Temp	Max Air Temp	Mean Air Temp	Min Soil Temp	Max Soil Temp	Mean Soil Temp	Total Precip	Total Irrigation	Total Water
	°C						mm		
Ashland Bottoms 2012-13	-16.9	36.4	9.0	0.1	28.6	10.1	361.4	0	361.4
Silverlake 2012-13	-20.4	36.7	8.8	-6.2	42.9	10.4	419.8	42.2	462

Source: Kansas State University Mesonet (2014).

Parameters at Anthesis

At anthesis, only one measured parameter was significantly affected by variety, percent carbon at anthesis (pCanth), with a p=0.001 (Table 3.6). However, the means were extremely close together, and although they were significantly different, they have little bearing on nitrogen use and response in this study. There was a significant Nrate x variety interaction for NDVI at both locations with a $p<0.05$ (Table 3.6). There was considerable range in the NDVI values, with the lowest and highest mean recorded NDVIs at Ashland Bottoms both found in Duster. These ranged from 0.41 at the low Nrate up to 0.83 at the high Nrate (Figure 3.1; Appendix D.2). At Silverlake the lowest NDVI value was 0.48 for Everest and Larned and ranged up to 0.86 for Duster (Figure 3.1; Appendix D.2). These data suggest that there are differences among varieties in the influence of N rate on early-season crop performance. However, total biomass at anthesis in kg ha^{-1} (BManth) determined through a hand-harvest method was not significantly different between varieties and had no Nrate x variety interaction, suggesting that there were no variety effects on early season biomass production response to N rates. Because there were no significant Nrate x variety interactions at either location for the other parameters, the varieties maintained similar responses at all Nrates. The mean values of these parameters can be found in Appendix D.1.

Table 3.6. Nrate experiment levels of significance (p-values) for interactions of nitrogen rate and wheat variety near anthesis.

	NDVI	BManth	BPEanth	NUpEanth	pNanth	pCanth	NUpanth
Ashland Bottoms 2012-13							
Variety	0.512	0.603	0.547	0.400	0.260	**0.001**	0.434
Nrate	**<0.001**	**<0.001**	**<0.001**	0.876	**<0.001**	**<0.001**	**<0.001**
Nrate x Variety	**0.004**	0.733	0.738	0.745	0.919	0.657	0.678
Silverlake 2012-13							
Variety	0.052	0.068	0.061	0.349	0.774	0.075	0.215
Nrate	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Nrate x Variety	**0.039**	0.491	0.525	0.490	0.202	0.788	0.227

Abbreviations: NDVI, normalized vegetation index; BManth, biomass at anthesis; NUpEanth, nitrogen uptake at anthesis; pNanth, percent nitrogen at anthesis; pCanth, percent carbon at anthesis; NUpanth, total nitrogen uptake at anthesis.

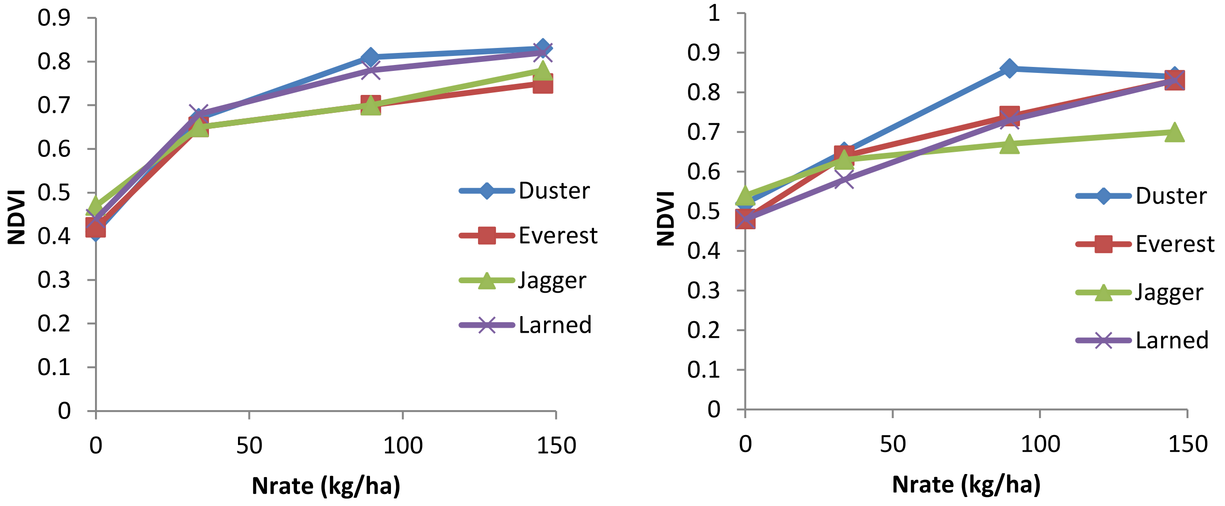


Figure 3.1. The effect of Nrate on NDVI values for 4 wheat varieties at two locations, Ashland Bottoms, KS (left) and Silverlake, KS (right).

Measured Parameters at Maturity

Several productivity measurements were taken at maturity to calculate other parameters of interest in this study and many showed significant differences for variety. However, only one measurement, mean grain yield, was significantly affected by variety at both locations with a p<0.05 (Table 3.7). The remaining parameters displayed contrasting results between locations, likely due to environmental differences. For example, there were significant differences in biomass between varieties at Ashland Bottoms but not at Silverlake, and conversely, there were significant differences between varieties in total nitrogen in the stover (TNstov) at Ashland Bottoms that were not observed at Silverlake (Table 3.7; Appendix Table D.3-D.4).

Table 3.7. Levels of significance (p-values) for variety and N rate (Nrate) treatment impacts on plant measurements taken at maturity for four winter wheat varieties at two locations.

	Grain Yield	**Biomass**	**Nstov**	**Ngr**	**TNstov**	**TNgr**	**TNup**
Ashland Bottoms 2012-13							
Variety	0.036	0.294	0.097	0.472	0.005	0.925	0.921
Nrate	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Nrate x Variety	**0.041**	0.846	0.347	0.762	0.034	0.883	0.943
Silverlake 2012-13							
Variety	**<0.001**	**<0.001**	0.831	0.789	0.094	0.488	0.186
Nrate	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Nrate x Variety	0.433	0.616	0.956	0.212	0.220	0.964	0.686

Abbreviations: Nstov, nitrogen concentration in the stover; Ngr, nitrogen concentration in the grain; TNstov, total nitrogen in the stover; TNgr, total nitrogen in the grain; TNup, total nitrogen uptake.

All of the parameters were significantly affected by nitrogen rate (p<0.05) at both locations. There were no significant Nrate x variety interactions at Silverlake, KS, suggesting that the varieties had similar N response at this location. At Ashland Bottoms, there were several parameters that had a significant Nrate x variety interactions (p<0.05). These were grain yield and TNstov, suggesting that the varieties performed differently at the various N rates applied at this location (Table 3.7).

Statistical analysis showed very few significant differences for the other parameters of interest in this study calculated at maturity for variety and Nrate x variety interactions at either location (Table 3.8). At Ashland Bottoms only one parameter, nitrogen harvest index (NHI), showed significant differences between varieties (p=0.041) with no Nrate x variety interactions for any parameter (Table 3.8-3.9). At Silverlake, NUE and partial factor productivity (PFP) showed significant differences between mean varietal values (p<0.05), but only PFP showed significant Nrate x variety interaction (p=0.015), indicating that some varieties were better at using applied nitrogen fertilizers at the different rates to produce grain (Table 3.8; Appendix Table D.7). Most of the parameters were significantly affected by N rate, but surprisingly several were not. At Ashland Bottoms the parameters not significantly affected by the multiple N rates were nitrogen utilization efficiency (NUtE), nitrogen remobilization efficiency (NRE), harvest index (HI), nitrogen harvest index (NHI), nitrogen uptake after anthesis (NUpAA), and fertilizer use efficiency (FUE) (Table 3.8; Appendix Table D.5-D.6). At Silverlake the parameters not affected by Nrate were NRE, HI, NHI, NUpAA, and FUE (Table 3.8). The mean values for these parameters with no significant effects of variety can be found in Tables D.5-D.6 of the Appendix.

Table 3.8. Levels of significance (p-values) for parameters determined at maturity in four wheat varieties at two locations in Kansas.

	NUE	NUpE	NUtE	NRE	BPE	HI	NHI	NUpAA	FUE	PFP	AgEf	PNB
Ashland Bottoms 2012-13												
Variety	0.320	0.696	0.155	0.597	0.306	0.473	**0.041**	0.390	0.729	0.216	0.338	0.728
Nrate	**<0.001**	0.074	0.102	0.955	**<0.001**	0.419	0.207	0.192	0.775	**<0.001**	**<0.001**	**<0.001**
Nrate x Variety	0.633	0.854	0.970	0.631	0.998	0.619	0.303	0.993	0.816	0.596	0.820	0.783
Silverlake 2012-13												
Variety	**0.001**	0.354	0.675	0.407	0.182	**0.039**	0.086	0.604	0.489	**<0.001**	0.688	0.833
Nrate	**<0.001**	**<0.001**	**<0.001**	0.242	**<0.001**	0.651	0.772	0.729	0.400	**<0.001**	**0.017**	**<0.001**
Nrate x Variety	0.915	0.954	0.726	0.472	0.419	0.489	0.931	0.264	0.797	**0.015**	0.473	0.969

Abbreviations: NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency; NRE, nitrogen remobilization efficiency; BPE, biomass production efficiency; HI, harvest index; NHI, nitrogen harvest index; NUpAA, nitrogen uptake after anthesis; FUE, fertilizer use efficiency; PPF, partial productivity factor; AgEf, agronomic efficiency; PNB, partial nutrient balance.

Table 3.9. Mean values for four winter wheat varieties at two locations in Kansas and differences for parameters with significant effects of variety determined at maturity.

Location	Variety	NUE		HI		NHI		PFP	
					kg kg^{-1}				
Ashland Bottoms 2012-13	Duster	23.72	A[†]	0.38	A	0.72	AB	38.95	A
	Everest	25.71	A	0.36	A	0.73	AB	43.73	A
	Jagger	25.69	A	0.36	A	0.77	A	42.97	A
	Larned	24.00	A	0.32	A	0.69	B	39.06	A
Silverlake 2012-13	Duster	40.09	A	0.30	AB	0.66	AB	66.23	A
	Everest	37.66	A	0.33	A	0.69	A	60.07	B
	Jagger	31.80	B	0.33	A	0.69	A	50.73	C
	Larned	33.16	B	0.26	B	0.62	B	53.67	C

[†] Means within a column that are followed by the same letter are not significantly different (p>0.05)

Abbreviations: NUE, nitrogen use efficiency; HI, harvest index; NHI, nitrogen harvest index; PFP, partial factor productivity.

Nitrogen Response

For the purposes of this nitrogen response study, the Nrate x variety interaction of grain yield at Ashland Bottoms was of particular interest. While Silverlake showed significant differences in mean grain yield between varieties, this does not provide useful information on differences in response, as differences in yield between varieties is widely accepted. The significant interaction at Ashland Bottoms provides evidence that some varieties may respond differently to various nitrogen rates.

At Ashland Bottoms, the varieties had very similar yields at the 0 and 33.6 kg N ha^{-1} rates with no significant differences between varieties, but developed significant differences at the higher rates (Table 3.10). Duster, Everest, and Jagger had similar responses to nitrogen rate, while Larned appeared markedly different. The Nrate x variety interaction at Ashland Bottoms is displayed in Figure 3.2. The significant Nrate x variety interaction is likely due in part to the seemingly linear increase of Larned compared to the linear plateau lines of the other varieties and the large differences at the 89.7 kg N ha^{-1} rate. At this rate, Larned was significantly different from all other varieties and Everest and Duster were also significantly different from each other (Table 3.10).

Table 3.10 Mean values and significant differences by wheat variety and Nrate for grain yield at two locations in the Nrate study.

Location	**Nrate**	**Variety**							
		Duster		Everest		Jagger		Larned	
		kg ha^{-1}							
Ashland Bottoms 2012-13	0	1107	G[†]	1086	G	1169	G	1179	G
	33.6	1918	F	2137	F	2200	F	2050	F
	89.7	3218	D	3691	ABC	3387	CD	2872	E
	145.7	3483	BCD	3854	A	3744	AB	3524	ABCD
	Mean	**2432**	**-**	**2692**	**-**	**2625**	**-**	**2406**	**-**
Silverlake 2012-13	0	2949	DEFG	2902	DEFG	2418	G	2638	FG
	33.6	3783	BC	3427	CDE	2792	EFG	3266	CDEF
	89.7	4797	A	4231	AB	3863	BC	3482	CD
	145.7	4760	A	4531	A	3801	BC	3649	BC
	Mean	**4072**	**-**	**3773**	**-**	**3219**	**-**	**3259**	**-**

[†] Means within a column that are followed by the same letter are not significantly different ($p>0.05$)

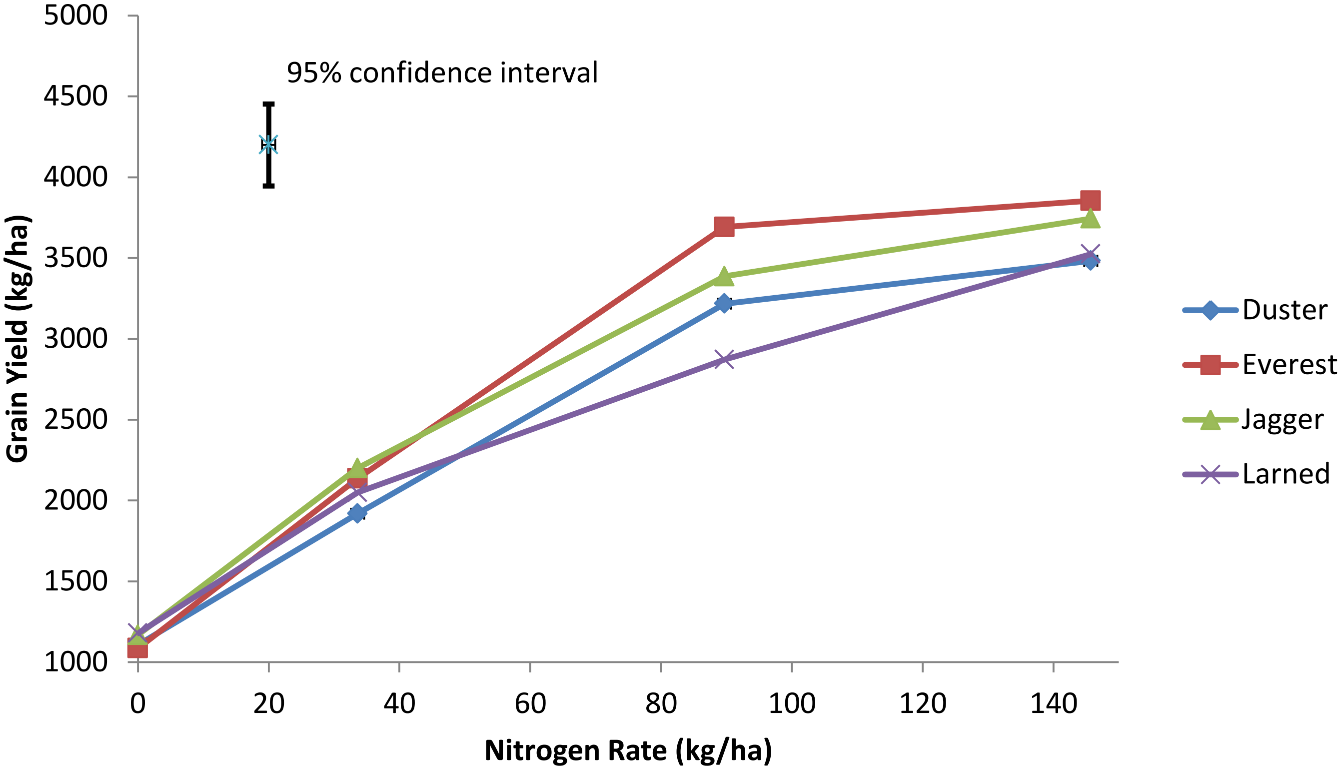


Figure 3.2. Nitrogen response of four winter wheat varieties at Ashland Bottoms, KS in the 2012-13 season.

There were no significant Nrate x variety interactions at Silverlake, KS, although mean grain yield was significantly affected by variety. At this location, Duster and Everest were the highest yielding while Jagger and Larned were the lowest (Table 3.10). The Nrate x variety interaction was not significant at this location likely because Everest, Duster, and Jagger had similar responses with very few significant differences in yield observed at each rate and between rates (Figure 3.3; Table 3.10).

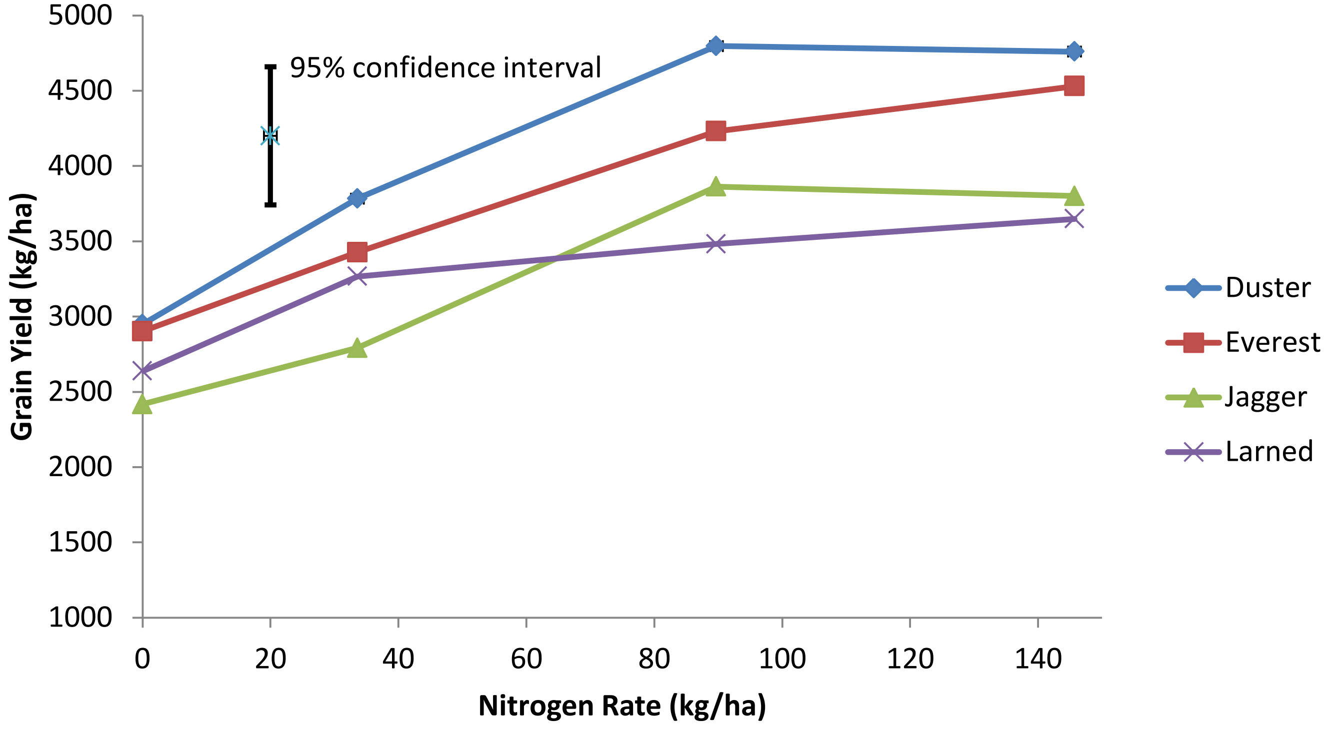


Figure 3.3. Nitrogen response of four winter wheat varieties at Silverlake, KS in the 2012-13 season.

There appears to be a linear plateau response for each variety at both locations, although it looks much less pronounced for Larned (Figure 3.2; Figure 3.3). When analyzed using contrasts in SAS it was found that both linear and linear plateau models can fit each line with a $p<0.05$. Because they do follow a linear plateau model, change points and the slope of the lines before these points could be calculated to predict economic optimum N rates for each variety and serve as a means for estimating response.

At Ashland Bottoms the change points ranged from 84.5-108.7 kg N ha^{-1} with overlapping confidence intervals, indicating that there were no significant differences in optimum Nrates (Table 3.11). However, there were some significant differences in the slopes of the lines before these points among Duster, Everest, and Larned, with Everest having the highest slope and the lowest change point, indicating that it had the highest response. Larned's high change point provides evidence that it was a large contributor to the Nrate x variety interaction observed at this location. As change points were lower than the maximum Nrate used in this study, it also indicates that the fertilizer rates were sufficiently high to maximize yield.

Table 3.11. N-response slope before change point, change points, and 95% confidence intervals for four wheat varieties at Ashland Bottoms and Silverlake, KS.

Location	Variety	Slope Before Change Point	Slope Lower 95% Confidence	Slope Upper 95% Confidence	Change Point kg N ha^{-1}	Change Point Lower 95% Confidence	Change Point Upper 95% Confidence
Ashland Bottoms 2012-13	Duster	26.3	25.9	26.8	89.9	81.6	98.1
	Everest	32.4	31.0	33.9	84.5	77.9	91.0
	Jagger	27.3	23.4	31.2	91.3	72.6	109.9
	Larned	20.7	16.1	25.3	108.7	86.8	130.7
Silverlake 2012-13	Duster	27.8	26.1	29.5	65.8	26.1	105.5
	Everest	16.6	16.0	17.1	97.7	65.9	129.5
	Jagger	18.3	14.9	21.6	80.0	58.3	101.7
	Larned	20.9	13.3	28.6	44.3	31.0	57.6

At Silverlake, the change points ranged from 44.3-97.7 kg N ha^{-1}. Only one variety at Silverlake, Larned, had a change point whose value was significantly lower than the others (Table 3.11). While Larned reached its economic optimum at a much lower rate than the others, it does not necessarily indicate that it had the highest response as it also had among the lowest yields and a fairly low slope (Table 3.11). To provide a measure of response, the values were ranked, with larger ranks indicating higher response. Nitrogen use efficiency was also ranked for each variety, with higher NUE values receiving larger ranks. These were then averaged for each variety, and provide a means for estimating response and identifying trends between response and NUE. When the varieties were ranked, it showed that at Ashland Bottoms, Everest had the highest response while Larned had the lowest (Table 3.12). The relationships were different at Sivlerlake, and here Duster had the highest response while Jagger had the lowest. Surprisingly, Larned performed quite well at this location based on its slope and change point (Table 3.12). However, the low change point value could be due to the larger amount of profile nitrate available in the soil at this location and more favorable environmental conditions, resulting in decreased response to fertilizer treatments.

Table 3.12. Nitrogen response of wheat varieties at two locations as a factor of slope before the changepoint, changepoint, and grain yield ranked according to values, with more ideal values having higher rank, with corresponding nitrogen use efficiency values.

Location	Variety	Slope Before Change Point	Rank	Change Point kg N ha^{-1}	Rank	Grain Yield	Rank	Mean Rank	NUE	Rank
Ashland Bottoms 2012-13	Duster	26.3	2	89.9	3	2432	2	2.33	23.7	1
	Everest	32.4	4	84.5	4	2692	4	4.00	25.7	4
	Jagger	27.3	3	91.3	2	2625	3	2.67	25.7	3
	Larned	20.7	1	108.7	1	2406	1	1.00	24.0	2
Silverlake 2012-13	Duster	27.8	4	65.8	3	4072	4	3.67	40.1	4
	Everest	16.6	1	97.7	1	3773	3	1.67	37.7	3
	Jagger	18.3	2	80	2	3218	1	1.67	31.8	1
	Larned	20.9	3	44.3	4	3259	2	3.00	33.2	2

The NUE values observed at the two locations were very different, with much higher values observed at Silverlake than Ashland Bottoms (Table 3.9). These differences may result from differences in management between the two locations. The key difference, and the one most likely to have effected NUE, was the difference in moisture due to irrigation. Silverlake had access to irrigation, which was especially valuable early in the season and allowed for faster germination and establishment and resulted in higher yields and NUE at this location. Another factor that may have resulted in the observed differences at these locations was that Ashland Bottoms was not treated with herbicide in the 2012-13 season. There was a high amount of weed growth that could have competed with the wheat for water, light, and other resources, again limiting grain yields and NUE values.

Because the varietal performance in yield and NUE between the two locations was contrasting, these parameters were analyzed again in SAS with environment as a fixed effect. Nitrogen use efficiency was one of the few parameters of interest significantly affected by variety and was only statistically significant at Silverlake, KS with a p=0.001 (Table 3.8). Surprisingly, NUE had no variety effect or Nrate x variety interaction at Ashland Bottoms. This is surprising because NUE is a function of grain yield, which did have a significant variety effect and Nrate x variety interaction at this location. When environment was run as a fixed effect, showed significant three-way interactions between variety, Nrate, and environment for both grain yield and NUE, suggesting that the response of NUE and yield in the varieties to the multiple fertilizer rates was different depending on the environment (Table 3.13).

Table 3.13. P-values for wheat grain yield and NUE with environment as a fixed effect.

Effect	Grain Yield	NUE
Nrate	**<0.001**	**<0.001**
Variety	**<0.001**	**0.005**
Environment	**<0.001**	**<0.001**
Variety x Environment	**<0.001**	**<0.001**
Variety x Nrate	0.110	0.916
Nrate x Environment	**<0.001**	**<0.001**
Variety x Nrate x Environment	**<0.001**	**<0.001**

The differences in change points and response of yield at the two locations indicate that optimum Nrates will be different for these varieties depending on environmental conditions. This suggests that the varietal differences in nitrogen response at the two locations have very complicated interactions, and are not as simple as differences in nitrogen rate applications. Optimum Nrates and yield response will be a function of the variability in rainfall within and between locations, soil types and water holding capacity, and the amounts, forms, and spatial variability of nitrogen in the soil. These findings are in agreement with various other studies on yield response to nitrogen and other variables (Basso et al., 2013; Nielsen et al., 2002; Sadras et al., 2012).

Also related to N response is the relationship between fertilizer rate, nitrogen uptake, and grain yield, with higher nitrogen rates generally resulting in higher grain yield and nitrogen uptake. In this study, nitrogen uptake was not significantly different between varieties at either location, however Figure 3.4 shows a significant relationship between N uptake, N rate, and subsequent yields at both locations. These data provide evidence that increasing the nitrogen rate will increase yield and N uptake. As the grain yield plateaus at the high N rate, some varieties continue to uptake nitrogen, thereby recovering more N and which could improve grain quality and NUE in some of these varieties. These findings are further supported by Barraclough et al. (2010) who found very similar results from the effect of different fertilizer rates on yield and N uptake in wheat varieties.

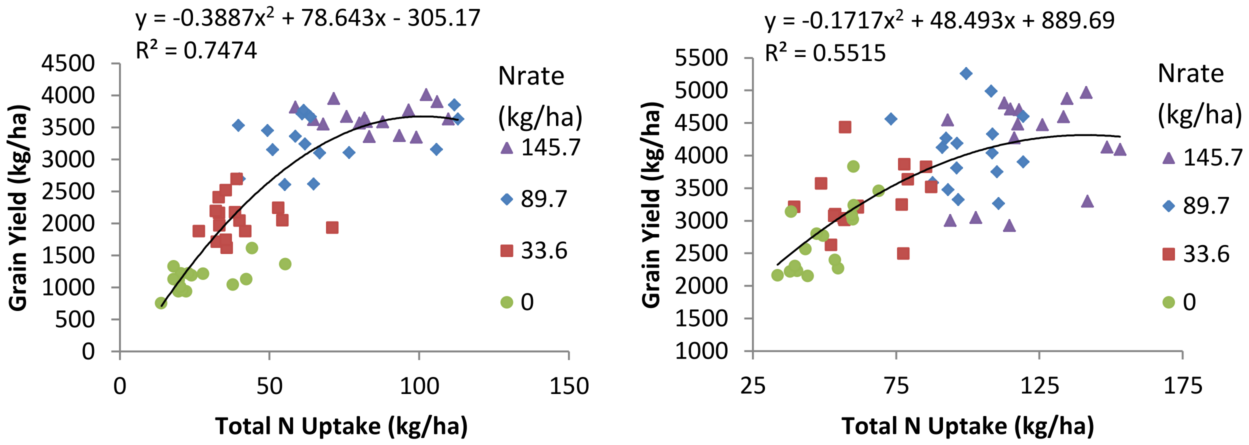


Figure 3.4. Effect of N fertilizer on N uptake and grain yield at Ashland Bottoms (left) and Silverlake, KS (right) for four winter wheat varieties.

The higher NUE values at Silverlake are likely the result of more favorable environmental conditions that led to increased grain yield. Silverlake was irrigated early in the season and also had higher in-season precipitation than Ashland Bottoms, providing more suitable conditions for crop growth (Table 3.3). While the total N in the profile was very similar at the two locations, Silverlake had a higher percentage of N as nitrate as opposed to ammonium, which could have contributed to improved crop performance, higher yields, and higher NUE (Table 3.2).

As these varieties were selected based on their range of NUEs determined from preliminary results of the NUE study (Chapter 2), evaluating the effect of NUE on N response was a topic of interest. The results showed that those varieties with higher NUE also tended to have higher N response, based on their yield response slopes, change points, and mean grain yields (Table 3.12). These are promising results and indicate that breeding efforts geared towards improving NUE could also advance N response in new varieties, allowing maximum yields to be reached with less N applications and allowing for more efficient use of applied N fertilizers. This would save producers money and result in less N loss to the environment.

Nitrogen Uptake and Utilization Efficiencies

Nitrogen use efficiency is directly related to nitrogen utilization and uptake efficiencies (Moll et al., 1982; Foulkes et al., 2009). However, despite NUE having significant varietal differences at Silverlake, KS neither NUtE nor NUpE had significant differences. Poor nitrogen use efficiency in most crops, including wheat, has been attributed to low nitrogen uptake by plants (Fillery and McInnes, 1992). The lack of significant differences in NUE at Ashland Bottoms, KS may be due to the environmental conditions that prevented efficient uptake and availability of nitrogen from the soil. Some research suggests that NUE in crops may be determined very early in the season by differences in growth and nitrogen uptake, and improving plant performance at these early growth stages could be a potential route to increasing NUE (Pang et al., 2014).

As the wheat at Ashland Bottoms, KS had poor performance early in the season because of late germination and establishment, yield values and NUE were considerably lower than at Silverlake and no significant varietal differences in NUE were noted. At Silverlake NUE did have significant differences, and although there were no significant differences in NUpE and NUtE at this location, they were both positively correlated to NUE values, with differences in NUpE contributing to 55% of the variation in NUE and differences in NUtE contributing to 39% (Figure 3.5). These findings are in accordance to recent research on NUE and barley varieties by Bingham et al. (2012), who not only found significant differences in NUpE between varieties, but also strong evidence that differences in uptake were the primary controllers of NUE. This suggests that differences in N uptake from the soil nitrogen pool at the multiple rates are contributing to varietal differences in NUE at this location. This is in contrast to the previous NUE study in which differences in NUtE were the primary contributors for differences in NUE (Chapter 2). It may be that differences in NUpE between the varieties became more apparent due to the larger range of available N supplied.

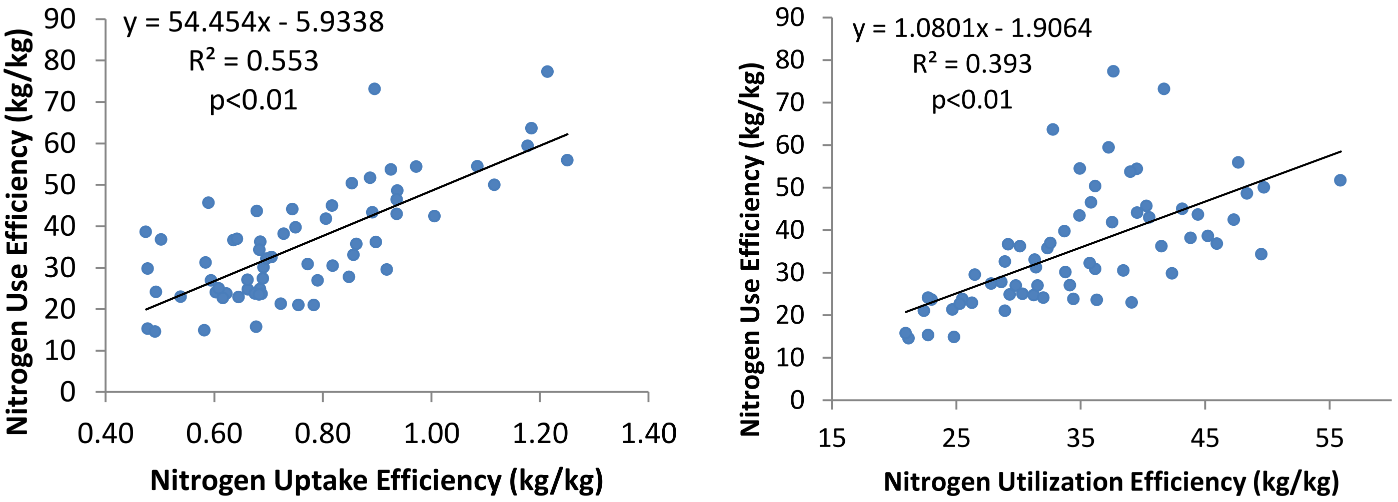


Figure 3.5. Nitrogen use efficiency linear regressions between nitrogen utilization efficiency and nitrogen uptake efficiency for four wheat varieties and four nitrogen rates at Silverlake, KS.

Varietal Differences

While the significant three-way interaction of environment, variety, and Nrate show that the N response of these varieties differed between locations, it is still evident that there were significant differences between varieties in response to N fertilizer applications. In terms of yield response, Everest was consistently the best performing variety, while Larned was the poorest. This is not surprising, as Larned is an older full-height variety while the others contain semi-dwarf genes. Because of this, Larned had very high total biomass production, nearly matching that of Duster at Silverlake, KS. It also had the highest mean biomass production at Ashland Bottoms, although it was not significantly different from the other varieties (Appendix Table D.3; Figure 3.6). The key difference at Silverlake was that a lower percentage of Larned's biomass, 32% versus Duster's 40%, was in grain. This supports previous research and the work done by many breeders throughout the Green Revolution to improve wheat varieties (Motzo et al., 2004). The semi-dwarf varieties included in this study had less biomass production, higher nitrogen harvest indices, and allocated more of their resources to grain.

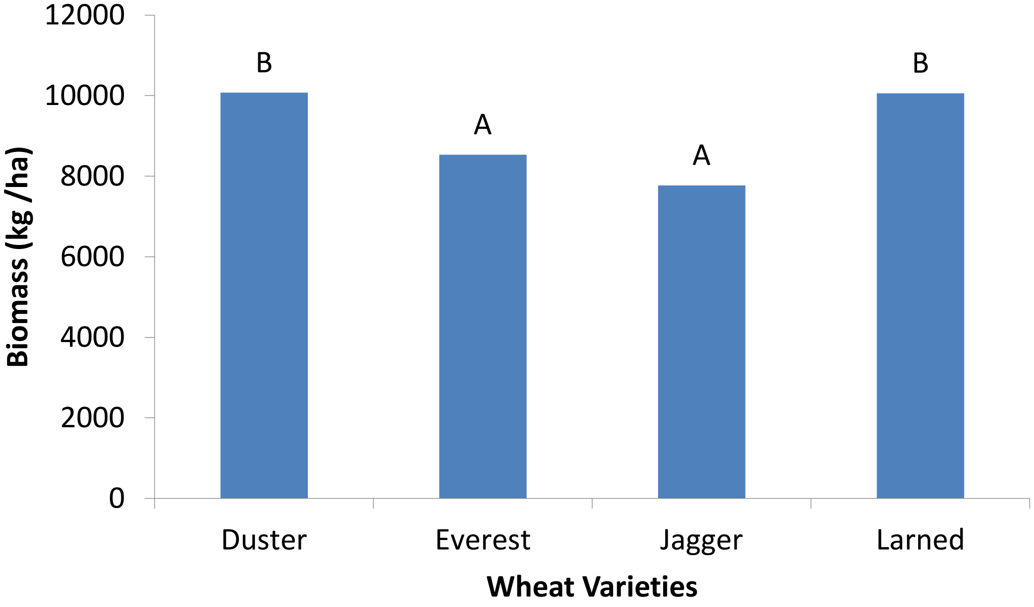


Figure 3.6. Mean biomass production and significant differences ($p<0.05$) for four winter wheat varieties at maturity at Silverlake, KS.

Larned also had the highest amount of nitrogen in the stover (kg ha^{-1}) at both locations, but was only significant at Ashland Bottoms, although total nitrogen uptake between varieties was not significantly different (Table 3.7). This is supported by the research of Motzo et al. (2004) who compared wheat varieties of several different eras, which included full-height and semi-dwarf varieties. They found that nitrogen uptake was not significantly different between these markedly different types of wheat, and that differences in yield were a result of nitrogen and biomass partitioning. As Larned is a traditional-height variety, it accumulates a much higher amount of biomass, and while its percent nitrogen concentration was not significantly different from the other varieties, it still resulted in a higher total amount of N (kg ha^{-1}) in the plants.

However, proportionately less of this nitrogen was remobilized to grain at the end of the season, resulting in decreased yields and yield response.

Larned's differences in nitrogen in the stover were especially pronounced at the highest nitrogen rate, which could be evidence of a luxury consumption that was poorly remobilized to the grain (Figure 3.7). If Larned was able to remobilize this N, its grain yields may have been substantially higher and perhaps matched those of the other varieties, but the structural integrity of the straw and its ability to support the higher yields would likely have been compromised and lodging would have been observed. Because of its poor grain yield, Larned had a very low HI, NUE, and NHI relative to the other varieties (Table 3.10). These differences arose in a short time between anthesis and maturity, and suggest that semi-dwarf varieties allocate more of their resources to grain production at the onset of reproductive growth, whereas traditional-height varieties continue invest a higher proportion of their resources to produce biomass instead of grain.

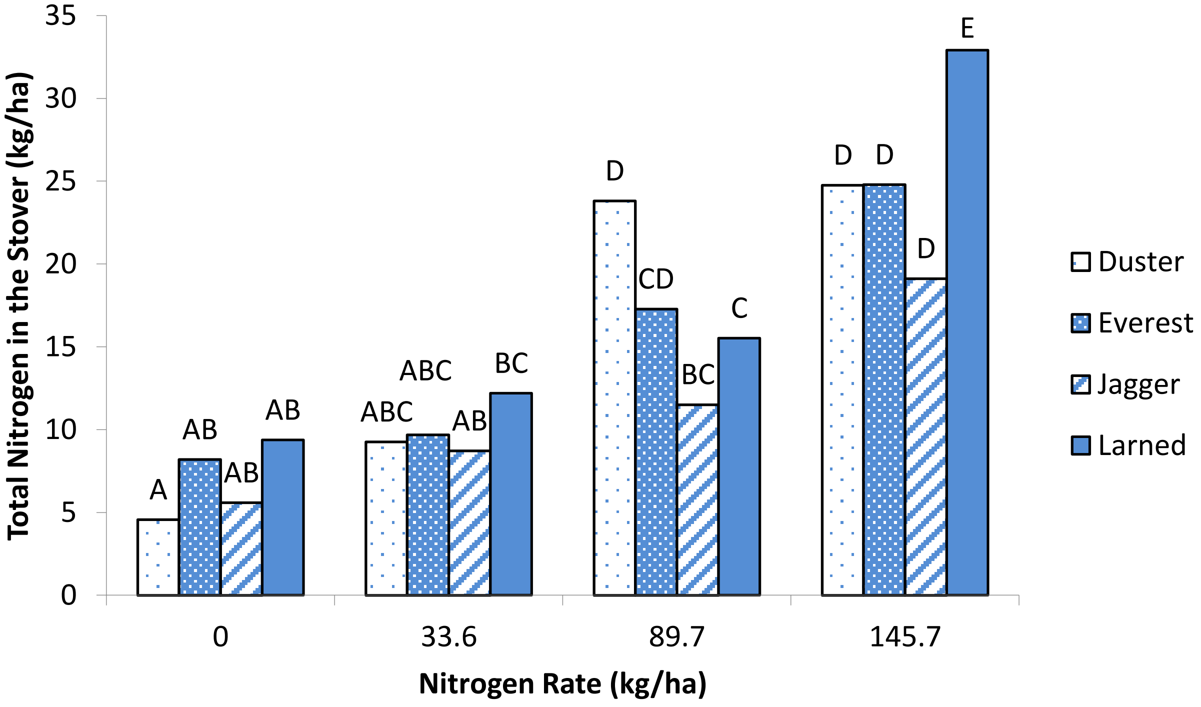


Figure 3.7. Interaction of Nrate and variety for total nitrogen in the stover in four wheat varieties with four nitrogen rates at Ashland Bottoms, KS.

Because total nitrogen uptake and nitrogen uptake efficiency were not significantly different among the varieties, it seems that nitrogen uptake has not been improved with the introduction of semi-dwarf wheat. Perhaps one of the most promising methods to increase nitrogen response, and many of the other parameters of interest such as NUE, would be by increasing nitrogen uptake. This would be particularly beneficial for semi-dwarf varieties that have been bred to allocate more resources to grain. The ability to scavenge for more nitrogen, especially at lower N rates, could significantly improve nitrogen response in these varieties, producing acceptable yields with less input.

CONCLUSIONS

The results of this study showed significant differences in winter wheat yield response to nitrogen at one location, Ashland Bottoms, in the 2012-13 season. Both locations showed significant differences in mean yield between varieties, which may have contributed to significant differences in NUE at Silverlake, KS, although no significant differences in NUE were noted at Ashland Bottoms, KS. The presence of a significant variety x Nrate x environment interaction also indicates that the varieties performed differently at the multiple N rates depending on environment and suggest the need for further research and locations to definitively determine the N response of these varieties.

Of particular interest in this study was to determine if there were differences in varietal response at lower nitrogen rates. However, the varieties included in this study showed no significant differences for yield at the two lowest rates at both locations (0 and 33.6 kg N ha^{-1}). Some significant differences developed at the higher rates, providing support for differences in nitrogen response between varieties. While the varieties performed somewhat differently between environments, Everest and Jagger tended to have the highest yields, NUE, and N response while Duster and Larned tended to have the lowest values.

The results from this study also show that varieties with higher NUE values tended to have higher nitrogen response, and that breeding for NUE could be a potential method to improve yields and response to nitrogen fertilizers. Improving N response and NUE would have many benefits. The most important would be the economic gains for producers by requiring less nitrogen fertilizers to reach maximum yields and more efficient use of applied N. The environment would also benefit from less applied N and more efficient use of fertilizers by reducing the risk of N loss to the environment through leaching or volatilization.

REFERENCES

Austin, R.B., J. Bingham, R.D. Blackwell, L.T. Evans, M.A. Ford, C.L. Morgan, and M. Taylor. 1980. Genetic improvements in winter wheat yields since 1980 and associated physiological changes. J. Agric. Sci. 94: 675-689.

Banziger. M, F.J. Betran, H.R. Lafitte.1997. Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments. Crop Sci. 37: 1103-1109.

Basso, B., D. Cammarano, C. Fiorentino, and J. T. Ritchie. 2013. Wheat yield response to spatially variable nitrogen fertilizer in Mediterranean environment. Eur. J. of Agron. 51: 65-70.

Bingham, I.J., A.J. Karley, P.J. White, W.T.B. Thomas, and J.R. Russell. 2012. Analysis of improvements in nitrogen use efficiency associated with 75 years of spring barley breeding. Eur. J. of Agron. 42: 49-58.

Brancourt-Hulmel, M., D. Goussinault, C. Lecomte, P. Berard, B. LeBuanec, and M. Trottet. 2003. Genetic improvement of agronomic traits of winter wheat cultivars release in France from 1946 to 1992. Crop Science. 43: 37-45.

Ceccarelli, S., S. Grando, and J. Hamblin. 1992. Relationship between barley grain yield measured in low- and high-yielding environments. Euphytica. 64: 49-58.

Fillery, I.R., K. J. McInnes.1992. Components of the fertiliser nitrogen balance for wheat production on duplex soils. Australian J. of Exp. Ag. 32: 887–899.

Foulkes, M.J., M.J. Hawkesford, P.B. Barraclough, M.J. Holdsworth, S. Kerr, S. Kightley, and P.R. Shewry. 2009. Identifying traits to improve the nitrogen economy of wheat: recent advances and future prospects. Field Crop. Res. 114: 329-342.

Hasegawa, H. 2003. High-yielding rice cultivars perform best even at reduced nitrogen fertilizer rate. Crop Sci. 43:921-926.

Kansas State University Mesonet. 2014. Historical Weather. http://mesonet.k state.edu/weather/historical/ (accessed: 15 Jan. 2014).

Nielsen, D.C., M.F. Virgil, R.L. Anderson, R.A. Bowman, J.G. Benjamin, and A.D. Halvorsen. 2002. Cropping systems influence on planting water content and yield of winter wheat. Agron. J. 94: 962-967.

Moll, R.H., E.J. Kamprath, and W.A. Jackson. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agron. J. 74: 562-564.

Motzo, R., S. Fois, and F. Giunta. Relationship between grain yield and quality of durum wheats from different eras of breeding. Euphytica. 140: 147-154.

Ortiz-Monasterio, R.J.I., K.D. Sayre, S. Rajaram, and M. McMahon. 1997. Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Sci. 37:898-904.

Pang, J., J.A. Palta, G.J. Rebetzke, and S.P. Milroy. 2014. Wheat genotypes with high early vigour accumulate more nitrogen and have higher photosynthetic nitrogen use efficiency during early growth. Func. Plant Biol. 41: 215-222.

Sadras, V.O., C. Lawson, P. Hooper, and G. McDonald. 2012. Contribution of summer rainfall and nitrogen to the yield and water use efficiency of wheat in Mediterranean-type environments of South Australia. Eur. J. of Agron. 36:41-54.

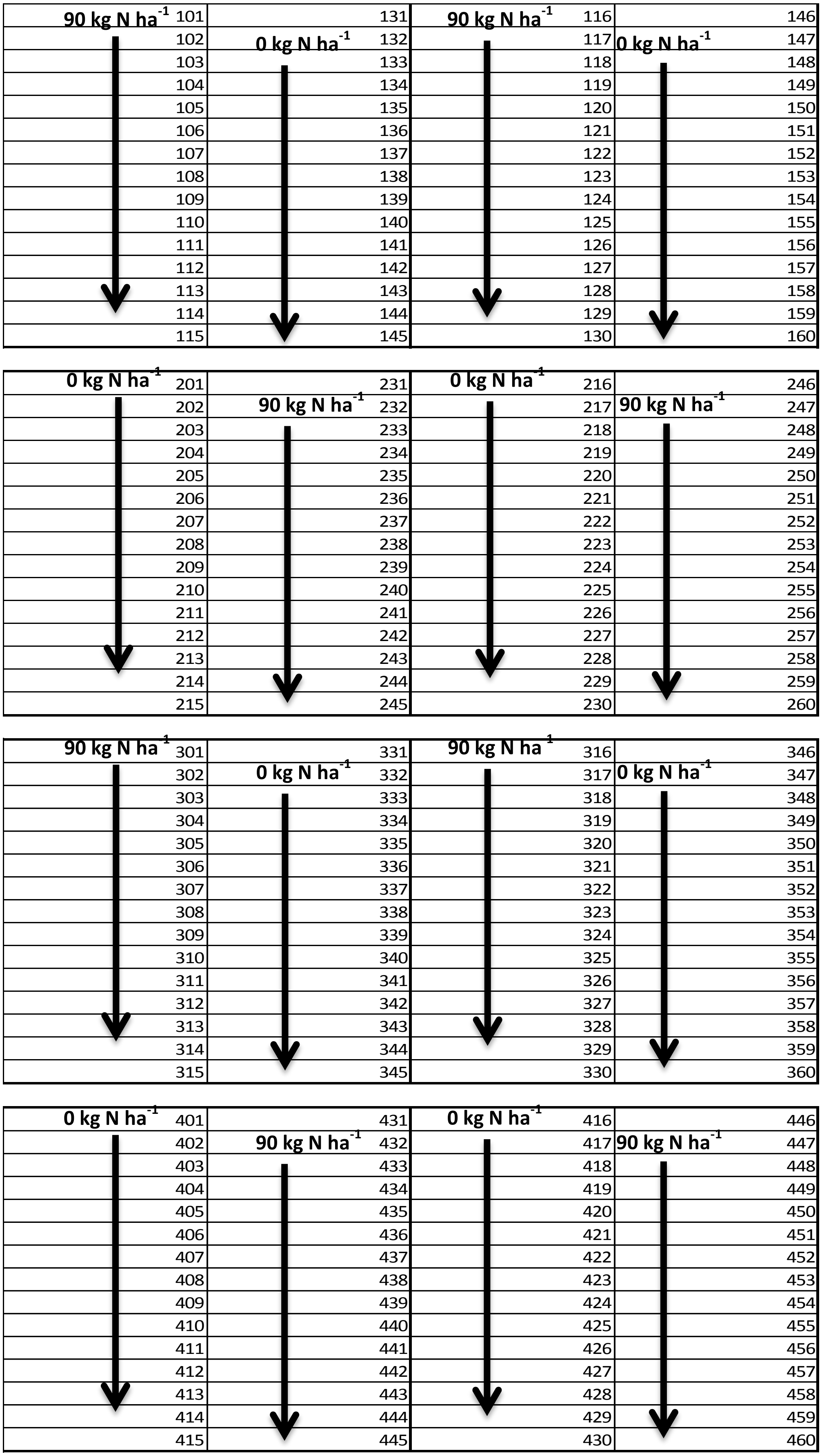
SAS Institute. 2005. The SAS system for Windows. Release 9.3. SAS Inst., Cary, NC.

Saxton, A. 2000. PDMIX800. http://animalscience.ag.utk.edu/facultystaff/Saxton/pdmix800.sas University of Tennesee, Knoxville, TN.

Sinebo, W., R. Gretzmacher, and A. Edelbauer. 2002. Environment of selection for grain yield in low fertilizer input in barley. Field Crops Res. 74: 151-162.

Appendix A

Plot Layouts for NUE and NRate Studies



90 kg N ha^{-1} 101	131	90 kg N ha^{-1} 116	146
102	0 kg N ha^{-1} 132	117	0 kg N ha^{-1} 147
103	133	118	148
104	134	119	149
105	135	120	150
106	136	121	151
107	137	122	152
108	138	123	153
109	139	124	154
110	140	125	155
111	141	126	156
112	142	127	157
113	143	128	158
114	144	129	159
115	145	130	160

0 kg N ha^{-1} 201	231	0 kg N ha^{-1} 216	246
202	90 kg N ha^{-1} 232	217	90 kg N ha^{-1} 247
203	233	218	248
204	234	219	249
205	235	220	250
206	236	221	251
207	237	222	252
208	238	223	253
209	239	224	254
210	240	225	255
211	241	226	256
212	242	227	257
213	243	228	258
214	244	229	259
215	245	230	260

90 kg N ha^{-1} 301	331	90 kg N ha^{-1} 316	346
302	0 kg N ha^{-1} 332	317	0 kg N ha^{-1} 347
303	333	318	348
304	334	319	349
305	335	320	350
306	336	321	351
307	337	322	352
308	338	323	353
309	339	324	354
310	340	325	355
311	341	326	356
312	342	327	357
313	343	328	358
314	344	329	359
315	345	330	360

0 kg N ha^{-1} 401	431	0 kg N ha^{-1} 416	446
402	90 kg N ha^{-1} 432	417	90 kg N ha^{-1} 447
403	433	418	448
404	434	419	449
405	435	420	450
406	436	421	451
407	437	422	452
408	438	423	453
409	439	424	454
410	440	425	455
411	441	426	456
412	442	427	457
413	443	428	458
414	444	429	459
415	445	430	460

Figure A.1. Plot layout for NUE study in 2010-11 season at Rossville, KS .

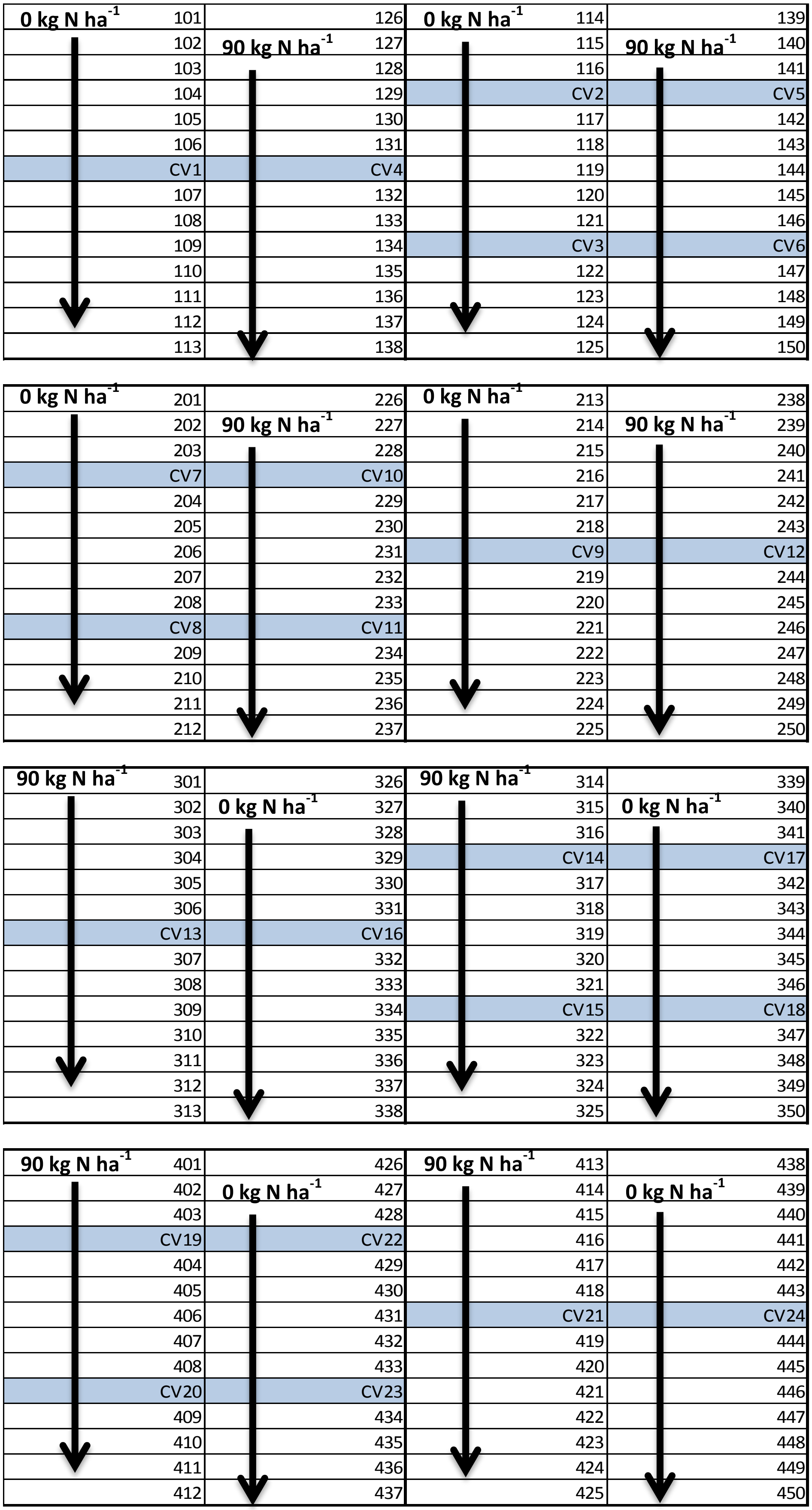
0 kg N ha^{-1} 101	131	0 kg N ha^{-1} 116	146
102	90 kg N ha^{-1} 132	117	90 kg N ha^{-1} 147
103	133	118	148
104	134	119	149
105	135	120	150
106	136	121	151
107	137	122	152
108	138	123	153
109	139	124	154
110	140	125	155
111	141	126	156
112	142	127	157
113	143	128	158
114	144	129	159
115	145	130	160

90 kg N ha^{-1} 201	231	90 kg N ha^{-1} 216	246
202	0 kg N ha^{-1} 232	217	0 kg N ha^{-1} 247
203	233	218	248
204	234	219	249
205	235	220	250
206	236	221	251
207	237	222	252
208	238	223	253
209	239	224	254
210	240	225	255
211	241	226	256
212	242	227	257
213	243	228	258
214	244	229	259
215	245	230	260

90 kg N ha^{-1} 301	331	90 kg N ha^{-1} 316	346
302	0 kg N ha^{-1} 332	317	0 kg N ha^{-1} 347
303	333	318	348
304	334	319	349
305	335	320	350
306	336	321	351
307	337	322	352
308	338	323	353
309	339	324	354
310	340	325	355
311	341	326	356
312	342	327	357
313	343	328	358
314	344	329	359
315	345	330	360

0 kg N ha^{-1} 401	431	0 kg N ha^{-1} 416	446
402	90 kg N ha^{-1} 432	417	90 kg N ha^{-1} 447
403	433	418	448
404	434	419	449
405	435	420	450
406	436	421	451
407	437	422	452
408	438	423	453
409	439	424	454
410	440	425	455
411	441	426	456
412	442	427	457
413	443	428	458
414	444	429	459
415	445	430	460

Figure A.2. Plot layout for NUE study in 2011-12 season at Rossville, KS.



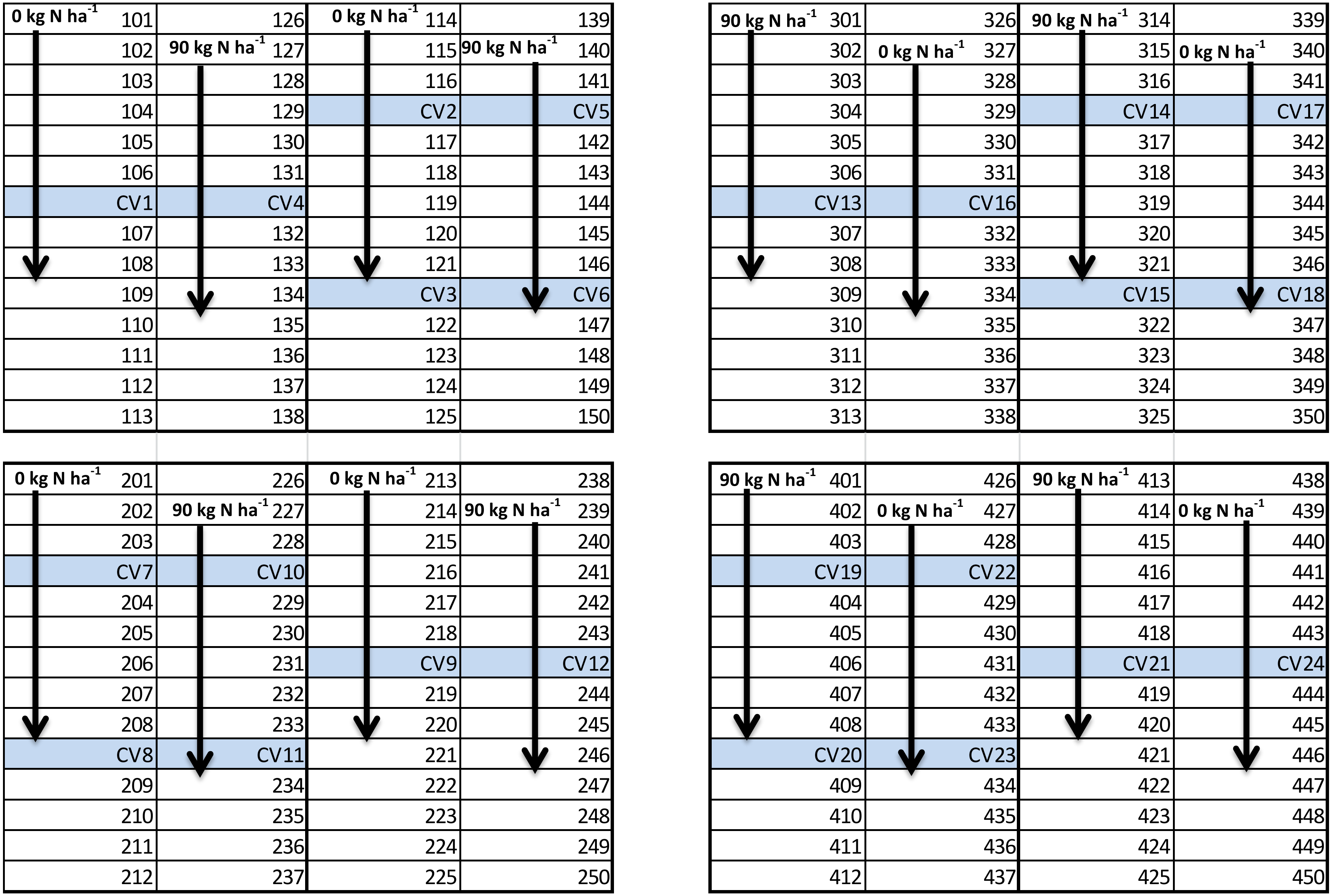
0 kg N ha^{-1} 101	126	0 kg N ha^{-1} 114	139
102	90 kg N ha^{-1} 127	115	90 kg N ha^{-1} 140
103	128	116	141
104	129	CV2	CV5
105	130	117	142
106	131	118	143
CV1	CV4	119	144
107	132	120	145
108	133	121	146
109	134	CV3	CV6
110	135	122	147
111	136	123	148
112	137	124	149
113	138	125	150

0 kg N ha^{-1} 201	226	0 kg N ha^{-1} 213	238
202	90 kg N ha^{-1} 227	214	90 kg N ha^{-1} 239
203	228	215	240
CV7	CV10	216	241
204	229	217	242
205	230	218	243
206	231	CV9	CV12
207	232	219	244
208	233	220	245
CV8	CV11	221	246
209	234	222	247
210	235	223	248
211	236	224	249
212	237	225	250

90 kg N ha^{-1} 301	326	90 kg N ha^{-1} 314	339
302	0 kg N ha^{-1} 327	315	0 kg N ha^{-1} 340
303	328	316	341
304	329	CV14	CV17
305	330	317	342
306	331	318	343
CV13	CV16	319	344
307	332	320	345
308	333	321	346
309	334	CV15	CV18
310	335	322	347
311	336	323	348
312	337	324	349
313	338	325	350

90 kg N ha^{-1} 401	426	90 kg N ha^{-1} 413	438
402	0 kg N ha^{-1} 427	414	0 kg N ha^{-1} 439
403	428	415	440
CV19	CV22	416	441
404	429	417	442
405	430	418	443
406	431	CV21	CV24
407	432	419	444
408	433	420	445
CV20	CV23	421	446
409	434	422	447
410	435	423	448
411	436	424	449
412	437	425	450

Figure A.3. Plot layout for NUE study during 2012-13 season at Silverlake, KS.



0 kg N ha^{-1} 101	126	**0 kg N ha^{-1}** 114	139
102	**90 kg N ha^{-1}** 127	115	**90 kg N ha^{-1}** 140
103	128	116	141
104	129	CV2	CV5
105	130	117	142
106	131	118	143
CV1	CV4	119	144
107	132	120	145
108	133	121	146
109	134	CV3	CV6
110	135	122	147
111	136	123	148
112	137	124	149
113	138	125	150

90 kg N ha^{-1} 301	326	**90 kg N ha^{-1}** 314	339
302	**0 kg N ha^{-1}** 327	315	**0 kg N ha^{-1}** 340
303	328	316	341
304	329	CV14	CV17
305	330	317	342
306	331	318	343
CV13	CV16	319	344
307	332	320	345
308	333	321	346
309	334	CV15	CV18
310	335	322	347
311	336	323	348
312	337	324	349
313	338	325	350

0 kg N ha^{-1} 201	226	**0 kg N ha^{-1}** 213	238
202	**90 kg N ha^{-1}** 227	214	**90 kg N ha^{-1}** 239
203	228	215	240
CV7	CV10	216	241
204	229	217	242
205	230	218	243
206	231	CV9	CV12
207	232	219	244
208	233	220	245
CV8	CV11	221	246
209	234	222	247
210	235	223	248
211	236	224	249
212	237	225	250

90 kg N ha^{-1} 401	426	**90 kg N ha^{-1}** 413	438
402	**0 kg N ha^{-1}** 427	414	**0 kg N ha^{-1}** 439
403	428	415	440
CV19	CV22	416	441
404	429	417	442
405	430	418	443
406	431	CV21	CV24
407	432	419	444
408	433	420	445
CV20	CV23	421	446
409	434	422	447
410	435	423	448
411	436	424	449
412	437	425	450

Figure A.4. Plot layout for NUE study during 2012-13 season at Ashland Bottoms, KS.

1-A 0 Duster	1-C H Duster		3-A H Jagger	3-C L Jagger
1-B L Duster	1-D M Duster		3-B 0 Jagger	3-D M Jagger
2-A M Everest	2-C 0 Everest		4-A 0 Larned	4-C H Larned
2-B L Everest	2-D H Everest		4-B L Larned	4-D M Larned

5-A 0 Jagger	5-C H Jagger		7-A L Larned	7-C H Larned
5-B L Jagger	5-D M Jagger		7-B 0 Larned	7-D M Larned
6-A 0 Duster	6-C H Duster		8-A L Everest	8-C M Everest
6-B M Duster	6-D L Duster		8-B H Everest	8-D 0 Everest

9-A M Jagger	9-C H Jagger		11-A 0lbs Duster	11-C H Duster
9-B L Jagger	9-D 0 Jagger		11-B L Duster	11-D M Duster
10-A 0 Everest	10-C L Everest		12-A M Larned	12-C 0 Larned
10-B M Everest	10-D H Everest		12-B H Larned	12-D L Larned

13-A H Everest	13-C M Everest		15-A 0 Jagger	15-C L Jagger
13-B 0 Everest	13-D L Everest		15-B H Jagger	15-D M Jagger
14-A M Larned	14-C H Larned		16-A 0 Duster	16-C H Duster
14-B L Larned	14-D 0 Larned		16-B L Duster	16-D M Duster

N Rates

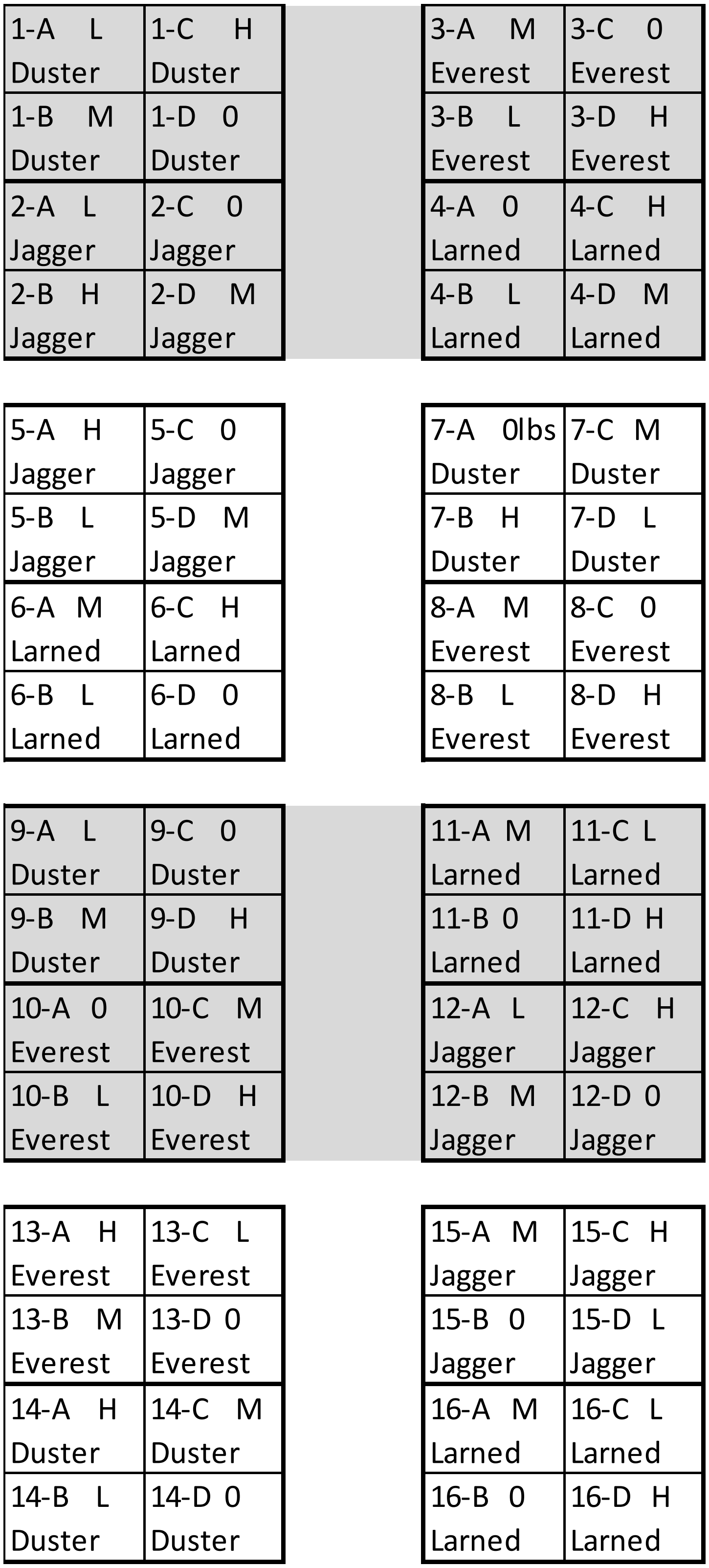
0- 0 kg N ha^{-1}

L- 33.6 kg N ha^{-1}

M- 89.7 kg N ha^{-1}

H- 145.7 kg N ha^{-1}

Figure A.5. Plot layout for Nrate study in 2012-13 season at Ashland Bottoms, KS.



1-A L Duster	1-C H Duster	3-A M Everest	3-C 0 Everest
1-B M Duster	1-D 0 Duster	3-B L Everest	3-D H Everest
2-A L Jagger	2-C 0 Jagger	4-A 0 Larned	4-C H Larned
2-B H Jagger	2-D M Jagger	4-B L Larned	4-D M Larned
5-A H Jagger	5-C 0 Jagger	7-A 0lbs Duster	7-C M Duster
5-B L Jagger	5-D M Jagger	7-B H Duster	7-D L Duster
6-A M Larned	6-C H Larned	8-A M Everest	8-C 0 Everest
6-B L Larned	6-D 0 Larned	8-B L Everest	8-D H Everest
9-A L Duster	9-C 0 Duster	11-A M Larned	11-C L Larned
9-B M Duster	9-D H Duster	11-B 0 Larned	11-D H Larned
10-A 0 Everest	10-C M Everest	12-A L Jagger	12-C H Jagger
10-B L Everest	10-D H Everest	12-B M Jagger	12-D 0 Jagger
13-A H Everest	13-C L Everest	15-A M Jagger	15-C H Jagger
13-B M Everest	13-D 0 Everest	15-B 0 Jagger	15-D L Jagger
14-A H Duster	14-C M Duster	16-A M Larned	16-C L Larned
14-B L Duster	14-D 0 Duster	16-B 0 Larned	16-D H Larned

N Rates

0- 0 kg N ha^{-1}

L- 33.6 kg N ha^{-1}

M- 89.7 kg N ha^{-1}

H- 145.7 kg N ha^{-1}

Figure A.6. Plot layout for Nrate study in 2012-13 season at Silverlake, KS.

Appendix B

Example SAS Code for NUE and NRate Studies

Table B.1. Sample SAS code for NUE study.

```
proc mixed data=aaa;
    class env rep var Nrate;
    model NUE = Nrate var Nrate*var /ddfm=satterth;
    random env rep(env) Nrate*env Nrate*rep(env) var*env var*rep(env) Nrate*var*env;
    title2 "Mixed Anova";
    lsmeans nrate var nrate*var/pdiff;
    ods output tests3=NUEtst lsmeans=NUEmeans diffs=NUEmcp;
    lsmeans var /adjust=tukey pdiff;
    ods output diffs=Yieldppp lsmeans=Yieldmmm;
    ods listing exclude diffs lsmeans;
run;
%include 'C:\Program Files\SASHome\pdmix800.sas';
%pdmix800(Yieldppp,Yieldmmm,alpha=0.05,sort=yes,mixfmt=no);
run;
    data NUEtst; set NUEtst; resp="NUE";
    data NUEmeans; set NUEmeans; resp="NUE";
    data NUEmcp; set NUEmcp; resp="NUE";
    data NUElabels; set msgrpzz; where (bygroup=2); resp="NUE";
run;
```

Table B.2. Sample SAS code for environment as a fixed effect in NUE study.

```
proc mixed data=aaa;
    class env rep var Nrate;
    model NUE = rep var env Nrate var*Nrate var*env/ddfm=satterth;
    random rep*var rep*Nrate;
run;
```

Table B.3. Sample SAS code for Nrate study for yield response analysis with contrasts.

```
proc mixed data = aaa noitprint; by env;
     class rep var nrate;
     model gYldC = var|nrate/ddfm = satterth;
     random rep rep*var;
     title2 "Mixed Anova";
     lsmeans var|nrate /pdiff;
     ods output LSmeans=gYldCmeans;
     ods output Tests3=gYldCtst;
     ods output diffs=mcp;
*/*CONTRASTS - N-Rate;
    estimate "linear   " nrate -6 -3  2  7;
    estimate "lin-plat " nrate -19 -7  13  13;
*/*CONTRASTS - N-Rate by variety;
    estimate "linear   - v1"     nrate -6 -3  2  7
                         nrate*var -6 -3  2  7  0  0  0  0  0  0  0  0  0  0  0  0;
    estimate "lin-plat - v1"     nrate -19 -7  13  13
                     nrate*var -19 -7  13  13  0  0  0  0  0  0  0  0  0  0  0  0;
    estimate "linear   - v2"     nrate -6 -3  2  7
                         nrate*var  0  0  0  0 -6 -3  2  7  0  0  0  0  0  0  0  0;
    estimate "lin-plat - v2"     nrate -19 -7  13  13
                     nrate*var  0  0  0  0 -19 -7  13  13  0  0  0  0  0  0  0  0;
    estimate "linear   - v3"     nrate -6 -3  2  7
                         nrate*var  0  0  0  0  0  0  0  0 -6 -3  2  7  0  0  0  0;
    estimate "lin-plat - v3"     nrate -19 -7  13  13
                     nrate*var  0  0  0  0  0  0  0  0 -19 -7  13  13  0  0  0  0;
    estimate "linear   - v4"     nrate -6 -3  2  7
                         nrate*var  0  0  0  0  0  0  0  0  0  0  0  0 -6 -3  2  7;
    estimate "lin-plat - v4"     nrate -19 -7  13  13
                     nrate*var  0  0  0  0  0  0  0  0  0  0  0  0 -19 -7  13  13;
ods output estimates=est;
data gYldCtst; set gYldCtst; resp="gYldC";
data gYldCmeans; set gYldCmeans; resp="gYldC";
*/*;
run;
```

Table B.4. Sample SAS code for Nrate study for parameters not analyzed with contrasts.

```
proc mixed data = aaa noitprint; by env;
     class rep var nrate;
     model NUE = var|nrate/ddfm = satterth;
     random rep rep*var;
     title2 "Mixed Anova";
     lsmeans var|nrate /pdiff;
     ods output LSmeans=NUEmeans;
     ods output Tests3=NUEtst;
     ods output diffs=mcp;
     data NUEtst; set NUEtst; resp="NUE";
     data NUEmeans; set NUEmeans; resp="NUE";
run;
```

Appendix C

Additional Tables of Results for NUE Study

Table C.1. Mean values and significant differences (p<0.05) for 25 wheat varieties at anthesis.

Variety	NDVI		BManth		BPEanth		NUpEanth	
			kg ha^{-1}		kg kg^{-1}			
2137	0.48	ABCDE[†]	3841	ABCDEF	76.2	AB	0.72	ABCDE
2174	0.49	ABCDE	3809	ABCDEF	75.1	ABC	0.70	ABCDE
2180	0.51	ABC	3953	ABCDE	73.0	ABCD	0.74	ABCDE
Armour	0.49	ABCDE	3812	ABCDEF	74.9	ABC	0.69	ABCDE
Art	0.49	ABCDE	3727	ABCDEF	73.9	ABCD	0.68	ABCDE
Billings	0.50	ABCDE	4003	ABC	74.7	ABC	0.74	ABCDE
Cedar	0.51	ABCD	3734	ABCDEF	72.6	BCD	0.73	ABCDE
Custer	0.49	ABCDE	4166	AB	75.3	ABC	0.76	AB
Duster	0.50	ABCDE	3831	ABCDEF	72.9	ABCD	0.71	ABCDE
Endurance	0.53	A	3750	ABCDEF	74.5	ABCD	0.70	ABCDE
Everest	0.48	BCDE	3724	ABCDEF	72.4	BCD	0.70	ABCDE
Fannin	0.50	ABCDE	3984	ABCD	73.9	ABCD	0.75	ABC
Fuller	0.49	ABCDE	3780	ABCDEF	77.5	A	0.65	BCDE
Jackpot	0.50	ABCDE	3863	ABCDEF	76.0	AB	0.67	ABCDE
Jagalene	0.49	ABCDE	3601	CDEF	69.8	D	0.71	ABCDE
Jagger	0.46	DE	3497	EF	76.6	AB	0.66	BCDE
KS020319-7-3	0.48	ABCDE	3610	CDEF	76.9	AB	0.63	E
Ogallala	0.49	ABCDE	3694	BCDEF	70.5	CD	0.75	ABC
Overley	0.46	E	3445	F	75.0	ABC	0.63	DE
Post Rock	0.50	ABCDE	3962	ABCDE	74.0	ABCD	0.75	ABCD
Santa Fe	0.50	ABCDE	3502	DEF	72.2	BCD	0.65	BCDE
TAM 105	0.52	AB	3813	ABCDEF	73.2	ABCD	0.72	ABCDE
TAM 110	0.47	CDE	3625	CDEF	75.6	AB	0.64	CDE
TAM 111	0.53	A	4184	A	75.1	ABC	0.79	A
TAM 112	0.51	ABC	3980	ABCDE	72.1	BCD	0.74	ABCDE

[†] Means within a column that are followed by the same letter are not significantly different (p>0.05)

Abbreviations: NDVI, normalized vegetation index; BManth, biomass at anthesis; NUpEanth, nitrogen uptake at anthesis; pNanth, percent nitrogen at anthesis; pCanth, percent carbon at anthesis; NUpanth, total nitrogen uptake at anthesis.

Table C.2. Additional mean values and significant differences ($p<0.05$) for parameters at anthesis in 25 wheat varieties.

Variety	Nanth		Canth		NUpanth	
	——— g kg^{-1}		———		kg ha^{-1}	
2137	13.7	C†	405.5	A	50.7	ABC
2174	13.9	BC	406.8	A	51.8	ABC
2180	14.4	ABC	405.9	A	55.5	AB
Armour	14.0	BC	407.5	A	52.8	ABC
Art	14.2	BC	406.7	A	51.8	ABC
Billings	14.0	BC	406.2	A	55.6	AB
Cedar	14.4	ABC	406.3	A	52.0	ABC
Custer	14.0	BC	406.7	A	57.2	A
Duster	14.4	ABC	405.6	A	56.0	AB
Endurance	14.1	BC	406.6	A	51.5	ABC
Everest	14.3	ABC	405.1	A	51.7	ABC
Fannin	14.2	BC	405.9	A	55.3	AB
Fuller	13.7	C	407.8	A	51.8	ABC
Jackpot	13.7	BC	407.9	A	51.7	ABC
Jagalene	15.2	A	405.7	A	53.3	ABC
Jagger	13.8	BC	407.8	A	47.8	C
KS020319-7-3	13.9	BC	405.0	A	48.4	C
Ogallala	15.2	A	405.7	A	52.5	ABC
Overley	14.1	BC	407.7	A	47.3	C
Post Rock	14.4	ABC	406.6	A	55.4	AB
Santa Fe	14.5	ABC	406.4	A	50.1	BC
TAM 105	14.6	AB	406.3	A	53.0	ABC
TAM 110	14.0	BC	406.7	A	48.1	C
TAM 111	14.0	BC	406.7	A	57.0	A
TAM 112	14.6	ABC	405.8	A	56.7	AB

†Means within a column that are followed by the same letter are not significantly different ($p>0.05$)

Abbreviations: Nanth, nitrogen concentration at anthesis; Canth, carbon concentration at anthesis; NUpanth, nitrogen uptake at anthesis.

Table C.3. Mean values by Nrate for the parameters measured at anthesis for 25 wheat varieties.

Variety	NDVI		BManth		BPEanth		NUpEanth		Nanth		Canth		NUpanth	
			– kg ha^{-1} –		——— kg kg^{-1} ———				——— g kg^{-1}———				– kg ha^{-1} –	
Nrate	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}
2137	0.37	0.59	2675	5008	80.1	72.3	0.93	0.51	12.7	14.6	404.2	406.7	33.2	68.2
2174	0.35	0.62	2588	5031	80.6	69.5	0.87	0.53	12.8	15.1	407.3	406.4	32.9	70.6
2180	0.39	0.64	2654	5251	79.3	66.8	0.91	0.57	13.1	15.8	404.9	406.9	34.0	77.0
Armour	0.35	0.62	2543	5080	83.5	66.3	0.84	0.55	12.5	15.6	403.6	411.4	31.7	73.8
Art	0.36	0.61	2468	4985	79.8	68.1	0.84	0.52	12.9	15.5	405.2	408.3	31.9	71.7
Billings	0.37	0.63	2658	5348	81.9	67.5	0.90	0.59	12.5	15.5	404.1	408.2	32.6	78.6
Cedar	0.41	0.61	2598	4870	78.5	66.8	0.93	0.52	13.2	15.6	403.1	409.4	33.3	70.7
Custer	0.35	0.63	2809	5523	81.6	69.0	0.92	0.60	12.9	15.2	405.1	408.3	34.9	79.5
Duster	0.38	0.61	2302	5359	82.7	63.2	0.78	0.63	12.5	16.3	402.5	408.7	28.7	83.2
Endurance	0.39	0.66	2681	4819	81.2	67.8	0.88	0.52	12.8	15.3	403.3	409.8	33.5	69.5
Everest	0.34	0.62	2455	4992	74.4	70.4	0.88	0.52	13.8	14.8	404.0	406.2	33.5	69.9
Fannin	0.37	0.62	2775	5194	77.4	70.5	0.95	0.55	13.2	15.2	404.0	407.7	36.5	74.1
Fuller	0.35	0.63	2245	5316	87.4	67.6	0.71	0.58	12.0	15.3	403.9	411.6	26.0	77.6
Jackpot	0.37	0.62	2568	5158	81.8	70.3	0.82	0.53	12.8	14.7	405.6	410.3	31.8	71.6
Jagalene	0.38	0.60	2358	4844	75.8	63.8	0.85	0.57	13.8	16.6	404.0	407.5	31.5	75.1
Jagger	0.35	0.58	2532	4463	83.7	69.5	0.85	0.47	12.4	15.2	406.2	409.4	31.4	64.3
KS020319-7-3	0.34	0.61	2323	4898	84.7	69.1	0.72	0.53	12.3	15.5	401.0	409.1	27.5	69.4
Ogallala	0.37	0.60	2803	4584	75.6	65.5	1.00	0.50	13.8	16.5	404.8	406.6	37.1	68.0
Overley	0.36	0.56	2352	4538	81.2	68.8	0.79	0.47	13.1	15.2	407.6	407.9	30.0	64.6
Post Rock	0.36	0.63	2734	5191	80.4	67.7	0.92	0.58	13.2	15.6	403.9	409.4	34.1	76.8
Santa Fe	0.38	0.62	2333	4671	79.0	65.5	0.77	0.54	13.1	15.9	403.3	409.4	29.7	70.4
TAM 105	0.41	0.63	2713	4913	79.7	66.8	0.92	0.53	13.1	16.1	404.2	408.4	34.4	71.5
TAM 110	0.35	0.59	2434	4816	80.3	70.9	0.78	0.49	13.1	14.8	406.1	407.4	29.6	66.6
TAM 111	0.42	0.64	3016	5353	80.7	69.4	0.99	0.58	12.9	15.0	404.7	408.8	36.8	77.2
TAM 112	0.37	0.65	2617	5343	78.0	66.1	0.89	0.60	13.2	15.9	404.0	407.7	33.1	80.2

Abbreviations: NDVI, normalized vegetation index; BManth, biomass at anthesis; NUpEanth, nitrogen uptake at anthesis; pNanth, percent nitrogen at anthesis; pCanth, percent carbon at anthesis; NUpanth, total nitrogen uptake at anthesis.

Table C.4. Mean values and significant differences ($p<0.05$) for measurements taken at maturity in 25 wheat varieties.

Variety	Nstov		Cstov		Cgr		TNstov		TNgr		TNup	
	g kg^{-1}						kg ha^{-1}					
2137	4.2	E†	423.8	ABC	405.5	AB	15.7	DEF	44.2	ABC	60.3	ABCD
2174	5.0	ABCD	426.5	ABC	405.8	AB	18.7	ABCD	41.2	ABCDE	59.9	ABCD
2180	5.3	A	425.3	ABC	407.7	A	19.6	AB	41.7	ABCDE	61.4	ABCD
Armour	5.0	ABCD	426.1	ABC	404.8	B	18.0	ABCDEF	43.0	ABCDE	60.9	ABCD
Art	4.7	ABCDE	426.1	ABC	406.4	AB	16.0	CDEF	39.7	BCDE	55.9	BCDE
Billings	4.4	DE	424.6	ABC	406.4	AB	15.4	EF	42.4	ABCDE	57.8	ABCDE
Cedar	4.9	ABCDE	422.1	C	405.8	AB	19.1	ABC	41.6	ABCDE	60.6	ABCD
Custer	5.1	ABC	425.8	ABC	406.8	AB	19.5	AB	40.6	ABCDE	60.1	ABCD
Duster	4.7	ABCDE	423.0	BC	405.8	AB	17.3	ABCDEF	40.9	ABCDE	58.2	ABCDE
Endurance	4.6	BCDE	423.7	ABC	405.6	AB	18.4	ABCDE	43.3	ABCD	61.8	ABCD
Everest	5.0	ABCD	424.8	ABC	405.6	AB	17.5	ABCDEF	45.8	A	63.3	A
Fannin	4.5	CDE	424.2	ABC	405.5	AB	17.0	ABCDEF	43.6	ABCD	60.6	ABCD
Fuller	4.5	CDE	425.6	ABC	406.6	AB	16.1	CDEF	38.8	CDE	55.0	DE
Jackpot	4.6	BCDE	426.1	ABC	406.3	AB	17.4	ABCDEF	44.8	AB	62.2	AB
Jagalene	5.1	ABCD	422.4	C	405.9	AB	16.8	BCDEF	40.4	BCDE	57.2	ABCDE
Jagger	5.1	ABCD	425.4	ABC	404.9	B	17.7	ABCDEF	41.4	ABCDE	59.2	ABCDE
KS020319-7-3	4.8	ABCDE	424.7	ABC	406.5	AB	15.2	EF	40.1	BCDE	55.3	CDE
Ogallala	5.1	ABCD	426.3	ABC	407.4	AB	17.7	ABCDEF	42.3	ABCDE	60.0	ABCD
Overley	5.2	AB	425.7	ABC	406.6	AB	17.3	ABCDEF	41.6	ABCDE	59.0	ABCDE
Post Rock	5.2	ABC	424.2	ABC	406.1	AB	18.2	ABCDE	40.6	ABCDE	58.6	ABCDE
Santa Fe	4.9	ABCDE	424.7	ABC	406.8	AB	16.5	BCDEF	38.6	DE	55.1	DE
TAM 105	5.1	ABC	428.0	A	406.0	AB	20.2	A	41.0	ABCDE	61.2	ABCD
TAM 110	4.9	ABCD	425.2	ABC	406.2	AB	14.7	F	37.8	E	52.5	E
TAM 111	4.6	BCDE	427.3	AB	404.8	B	18.2	ABCDE	44.3	AB	62.5	AB
TAM 112	4.7	ABCDE	426.4	ABC	407.0	AB	18.2	ABCDE	43.7	ABCD	61.9	ABC

†Means within a column that are followed by the same letter are not significantly different ($p>0.05$)

Abbreviations: Nstov, nitrogen concentration in the stover; Cstov, carbon concentration in the stover; Cgr, carbon concentration in the grain; TNstov, total nitrogen in the stover; TNgr, total nitrogen in the grain; TNup, total nitrogen uptake.

Table C.5. Mean values by Nrate for measurements taken at maturity in 25 wheat varieties.

Variety	Nstov		Cstov		Cgr		TNstov		TNgr		TNup	
	g kg^{-1}						kg ha^{-1}					
Nrate	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}
2137	3.64	4.84	424.9	422.6	405.3	405.7	9.9	21.5	34.8	53.5	44.7	75.8
2174	4.39	5.63	425.1	427.8	406.3	405.4	11.8	25.6	32.8	49.5	44.6	75.2
2180	4.85	5.82	424.6	426.0	407.0	408.5	12.5	26.8	29.5	53.9	42.0	80.7
Armour	4.37	5.59	421.9	430.4	402.9	406.7	10.5	25.4	26.9	59.0	37.4	84.5
Art	4.07	5.39	421.4	430.8	406.5	406.2	9.7	22.4	28.1	51.3	38.1	73.7
Billings	4.17	4.65	422.6	426.6	406.8	406.0	9.2	21.5	28.4	56.3	37.7	77.8
Cedar	3.89	5.86	420.0	424.2	405.2	406.4	8.9	29.2	27.7	55.4	36.7	84.6
Custer	4.40	5.87	423.1	428.5	407.9	405.6	12.2	26.9	30.4	50.7	42.7	77.6
Duster	3.89	5.52	421.4	424.5	404.5	407.1	10.1	24.4	30.5	51.3	40.6	75.7
Endurance	3.86	5.30	422.8	424.6	405.0	406.3	9.7	27.2	28.3	58.4	38.0	85.6
Everest	4.53	5.44	425.7	423.9	405.3	406.0	10.8	24.1	30.9	60.7	41.7	84.8
Fannin	3.63	5.35	424.8	423.5	405.0	406.0	9.8	24.2	31.4	55.8	41.2	80.0
Fuller	3.82	5.22	419.9	431.3	405.8	407.3	8.4	23.8	28.2	49.5	36.6	73.3
Jackpot	4.08	5.13	424.2	427.9	405.9	406.7	9.8	24.9	29.8	59.8	39.6	84.8
Jagalene	4.65	5.51	420.4	424.4	406.0	405.9	12.0	21.6	31.1	49.6	43.1	71.3
Jagger	4.49	5.63	421.9	429.0	403.5	406.2	10.2	25.3	25.9	57.0	36.0	82.3
KS020319-7-3	3.87	5.77	421.4	428.0	405.5	407.4	8.4	21.9	27.2	52.9	35.7	74.9
Ogallala	4.37	5.80	425.2	427.5	408.0	406.9	10.8	24.5	31.8	52.8	42.7	77.3
Overley	4.64	5.78	424.8	426.7	405.6	407.5	10.5	24.1	28.4	54.8	38.9	79.0
Post Rock	4.35	6.00	421.5	427.0	404.9	407.3	11.6	24.7	32.0	49.2	43.4	73.8
Santa Fe	4.37	5.42	418.8	430.5	405.3	408.2	8.8	24.2	26.6	50.6	35.4	74.8
TAM 105	4.55	5.75	425.5	430.6	405.7	406.2	13.3	27.2	30.8	51.1	44.2	78.3
TAM 110	4.37	5.48	423.7	426.8	406.4	405.9	10.0	19.4	29.1	46.6	39.1	66.0
TAM 111	4.02	5.14	425.5	429.1	403.2	406.4	10.7	25.6	31.4	57.2	42.2	82.8
TAM 112	4.08	5.32	426.5	426.2	406.3	407.8	9.4	27.0	30.3	57.1	39.7	84.1

Abbreviations: Nstov, nitrogen concentration in the stover; Cstov, carbon concentration in the stover; Cgr, carbon concentration in the grain; TNstov, total nitrogen in the stover; TNgr, total nitrogen in the grain; TNup, total nitrogen uptake.

Table C.6.Mean values and significant differences(p<0.05) for parameters calculated at maturity in 25 wheat varieties.

Variety	NUpE		NRE		BPE		NHI		NUpAA	
	kg kg^{-1}								kg ha^{-1}	
2137	0.91	AB[†]	0.66	ABCD	96.65	A	0.74	A	9.2	ABCD
2174	0.91	AB	0.59	E	94.01	A	0.71	ABC	8.1	ABCD
2180	0.89	ABC	0.60	CDE	93.29	A	0.70	BC	5.9	ABCD
Armour	0.84	ABC	0.64	ABCDE	92.52	A	0.72	ABC	8.1	ABCD
Art	0.82	ABC	0.67	ABC	93.96	A	0.73	ABC	4.1	ABCD
Billings	0.85	ABC	0.69	A	94.72	A	0.75	A	2.2	D
Cedar	0.86	ABC	0.63	ABCDE	96.31	A	0.71	ABC	8.6	ABCD
Custer	0.90	AB	0.64	ABCDE	94.91	A	0.70	BC	2.9	CD
Duster	0.89	ABC	0.66	ABCDE	98.08	A	0.72	ABC	2.2	D
Endurance	0.86	ABC	0.63	ABCDE	97.68	A	0.73	ABC	10.3	ABCD
Everest	0.91	AB	0.63	ABCDE	91.45	A	0.73	AB	11.6	AB
Fannin	0.90	AB	0.68	AB	95.87	A	0.74	A	5.3	ABCD
Fuller	0.84	ABC	0.67	ABC	98.20	A	0.72	ABC	3.2	CD
Jackpot	0.88	ABC	0.65	ABCDE	94.08	A	0.74	A	10.5	ABC
Jagalene	0.89	ABC	0.65	ABCDE	92.44	A	0.72	ABC	3.9	BCD
Jagger	0.84	ABC	0.62	BCDE	92.95	A	0.71	ABC	11.5	AB
KS020319-7-3	0.81	BC	0.64	ABCDE	96.03	A	0.73	AB	6.8	ABCD
Ogallala	0.91	AB	0.63	ABCDE	92.91	A	0.71	ABC	7.5	ABCD
Overley	0.87	ABC	0.61	CDE	91.81	A	0.72	ABC	12.0	A
Post Rock	0.91	AB	0.63	ABCDE	92.91	A	0.71	ABC	3.1	CD
Santa Fe	0.80	BC	0.64	ABCDE	94.34	A	0.72	ABC	5.1	ABCD
TAM 105	0.94	A	0.60	DE	94.61	A	0.69	C	8.2	ABCD
TAM 110	0.77	C	0.65	ABCDE	91.67	A	0.74	A	4.4	ABCD
TAM 111	0.92	AB	0.63	ABCDE	94.95	A	0.73	ABC	5.5	ABCD
TAM 112	0.88	ABC	0.67	ABC	93.26	A	0.73	ABC	5.3	ABCD

[†] Means within a column that are followed by the same letter are not significantly different ($p>0.05$)

Abbreviations: NUpE, nitrogen uptake efficiency; NRE, nitrogen remobilization efficiency; BPE, biomass production efficiency; NHI, nitrogen harvest index; NUpAA, nitrogen uptake after anthesis.

Table C.7. Mean values for 25 wheat varieties by Nrate for parameters determined at maturity.

Variety	NUE		NUpE		NUtE		NRE		BPE		HI		NHI		NUpAA	
			– kg ha^{-1} –		kg kg^{-1}				g kg^{-1}				– kg ha^{-1} –			
Nrate	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}	0 kg N ha^{-1}	90 kg N ha^{-1}
2137	35.99	23.23	1.22	0.60	45.62	39.78	0.67	0.65	99.73	93.56	0.40	0.36	0.77	0.71	11.5	6.9
2174	27.82	19.30	1.21	0.61	38.71	33.77	0.61	0.58	94.49	93.53	0.38	0.33	0.75	0.67	11.7	4.5
2180	26.56	20.78	1.12	0.66	37.87	33.16	0.61	0.60	93.62	92.96	0.37	0.33	0.73	0.67	8.2	3.6
Armour	28.37	23.66	1.00	0.68	45.91	35.36	0.68	0.61	99.44	85.60	0.40	0.36	0.75	0.70	5.5	10.7
Art	25.67	20.27	1.05	0.59	41.74	36.06	0.67	0.66	98.03	89.89	0.38	0.35	0.75	0.70	6.2	2.0
Billings	28.99	23.44	1.07	0.63	45.39	38.61	0.69	0.69	97.25	92.18	0.40	0.36	0.76	0.73	5.1	-0.7
Cedar	25.22	22.79	1.03	0.68	42.57	34.79	0.71	0.55	102.10	90.51	0.38	0.34	0.76	0.66	3.4	13.9
Custer	28.40	20.35	1.18	0.62	40.05	34.16	0.65	0.63	101.26	88.57	0.37	0.34	0.73	0.66	7.8	-1.9
Duster	26.90	22.35	1.18	0.61	43.35	37.75	0.65	0.66	103.40	92.76	0.37	0.35	0.76	0.68	11.9	-7.5
Endurance	28.54	22.87	1.03	0.68	43.86	35.35	0.69	0.56	104.59	90.76	0.38	0.34	0.76	0.69	4.5	16.1
Everest	29.14	23.62	1.15	0.68	42.79	36.18	0.66	0.59	95.54	87.35	0.40	0.37	0.75	0.72	8.3	14.9
Fannin	29.45	23.18	1.14	0.65	43.93	37.70	0.72	0.63	98.90	92.84	0.38	0.35	0.77	0.70	4.7	5.9
Fuller	24.71	20.09	1.09	0.59	44.09	34.80	0.67	0.66	102.80	93.59	0.38	0.32	0.77	0.67	10.7	-4.2
Jackpot	28.53	24.79	1.07	0.68	43.27	35.92	0.69	0.61	98.15	90.01	0.41	0.36	0.78	0.71	7.8	13.2
Jagalene	26.46	20.59	1.20	0.58	39.88	37.01	0.61	0.69	92.92	91.96	0.39	0.36	0.74	0.70	11.5	-3.8
Jagger	23.00	21.69	1.00	0.68	40.58	35.49	0.67	0.56	96.55	89.35	0.38	0.35	0.73	0.70	4.7	18.3
KS020319-7-3	26.01	21.30	1.02	0.60	45.76	37.08	0.67	0.60	104.18	87.88	0.39	0.38	0.77	0.70	8.1	5.4
Ogallala	27.21	21.54	1.20	0.63	39.71	35.82	0.67	0.58	94.47	91.35	0.38	0.34	0.75	0.68	5.5	9.4
Overley	22.98	21.18	1.09	0.64	39.77	34.49	0.64	0.57	97.93	85.68	0.39	0.35	0.74	0.69	9.0	15.1
Post Rock	28.53	20.59	1.22	0.60	40.32	36.36	0.64	0.61	94.75	91.06	0.38	0.33	0.75	0.67	9.3	-3.2
Santa Fe	23.82	20.30	0.99	0.61	41.64	35.16	0.68	0.61	95.48	93.20	0.39	0.32	0.76	0.68	5.7	4.5
TAM 105	27.98	21.68	1.24	0.63	40.10	36.16	0.61	0.58	94.60	94.62	0.37	0.33	0.73	0.65	9.8	6.7
TAM 110	25.93	19.35	1.01	0.53	41.38	37.15	0.64	0.67	93.47	89.87	0.41	0.36	0.77	0.71	9.5	-0.6
TAM 111	31.13	22.64	1.18	0.66	42.82	34.99	0.65	0.62	98.11	91.79	0.38	0.33	0.76	0.69	5.3	5.7
TAM 112	26.26	22.87	1.09	0.67	42.05	35.18	0.69	0.65	97.06	89.46	0.41	0.34	0.77	0.68	6.7	3.8

Abbreviations: NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency; NRE, nitrogen remobilization efficiency; BPE, biomass production efficiency; HI, harvest index; NHI, nitrogen harvest index; NUpAA, nitrogen uptake after anthesis.

Appendix D

Additional Tables of Results for NRate Study

Table D.1. Mean values and significant differences ($p<0.05$) for parameters measured at anthesis in four wheat varieties at two locations in Kansas.

Location	Variety	NDVI		BM anth		BPE anth		NUpE anth		N anth		C anth		NUp anth	
				kg ha^{-1}		kg kg^{-1}				g kg^{-1}				kg ha^{-1}	
Ashland Bottoms 2012-13	Duster	0.68	A†	4785.3	A	84.1	A	0.56	A	12.2	A	369.9	B	61.6	A
	Everest	0.63	A	4612.9	A	89.1	A	0.52	A	11.5	A	395.3	B	54.9	A
	Jagger	0.64	A	5290.2	A	86.2	A	0.61	A	12.0	A	400.0	A	67.2	A
	Larned	0.68	A	5016.9	A	84.8	A	0.61	A	12.2	A	399.1	A	63.7	A
Silverlake 2012-13	Duster	0.72	A	7488.2	AB	85.5	A	0.85	A	12.1	A	397.6	AB	93.1	A
	Everest	0.67	AB	6943.1	AB	85.7	A	0.80	A	12.0	A	395.5	B	85.2	A
	Jagger	0.64	B	6622.2	B	83.8	A	0.77	A	12.7	A	401.3	A	86.9	A
	Larned	0.65	B	7828.1	A	84.1	A	0.89	A	12.3	A	402.0	A	98.6	A

†Means within a column that are followed by the same letter are not significantly different ($p>0.05$)

Abbreviations: NDVI, normalized vegetation index; BManth, biomass at anthesis; NUpEanth, nitrogen uptake at anthesis; Nanth, nitrogen concentration at anthesis; Canth, carbon concentration at anthesis; NUpanth, total nitrogen uptake at anthesis.

D.2. The effect of Nrate and wheat variety on NDVI values at two locations in Kansas.

Location	Nrate	NDVI by Variety							
		Duster		Everest		Jagger		Larned	
	kg ha^{-1}								
Ashland Bottoms 2012-13	0	0.41	F†	0.42	F	0.47	F	0.44	F
	33.6	0.67	CD	0.65	DE	0.65	DE	0.68	CD
	89.7	0.81	A	0.70	BCD	0.70	BCD	0.78	AB
	145.7	0.83	A	0.75	ABC	0.78	AB	0.82	A
	Mean	**0.68**	**-**	**0.63**	**-**	**0.64**	**-**	**0.68**	**-**
Silverlake 2012-13	0	0.52	HI	0.48	I	0.54	GHI	0.48	I
	33.6	0.65	DEF	0.64	DEF	0.63	EFG	0.58	FGH
	89.7	0.86	A	0.74	BC	0.67	CDE	0.73	CD
	145.7	0.84	A	0.83	AB	0.70	CDE	0.83	AB
	Mean	**0.72**	**-**	**0.67**	**-**	**0.64**	**-**	**0.65**	**-**

†Means within a column that are followed by the same letter are not significantly different ($p>0.05$)

Table D.3. Mean values and significant differences ($p<0.05$) for parameters measured at maturity in four wheat varieties at two locations in Kansas.

Location	Variety	BM		N stov		Ngr		TNgr		TNup	
		kg ha^{-1}		g kg^{-1}				kg ha^{-1}			
Ashland	Duster	6516	A[†]	3.6	A	16.5	A	39.8	A	55.4	A
Bottoms	Everest	6646	A	3.4	A	16.5	A	41.0	A	56.0	A
2012-13	Jagger	6410	A	2.8	B	16.7	A	41.0	A	52.2	A
	Larned	7455	A	3.3	AB	16.0	A	38.0	A	55.5	A
Silverlake	Duster	10073	A	4.2	A	19.4	A	59.3	A	89.9	A
2012-13	Everest	8536	B	4.2	A	19.6	A	56.7	A	81.7	A
	Jagger	7775	B	4.5	A	20.7	A	53.6	A	77.6	A
	Larned	10062	A	4.4	A	19.9	A	54.1	A	87.7	A

†Means within a column that are followed by the same letter are not significantly different ($p>0.05$)

Abbreviations: BM, biomass; Nstov, nitrogen concentration in the stover; Ngr, nitrogen concentration in the grain; TNgr, total nitrogen in the grain; TNup, total nitrogen uptake.

Table D.4. The effect of Nrate and variety on total nitrogen in the stover (TNstov) values at two locations in Kansas.

Location	Nrate	TNstov by Variety							
		Duster		Everest		Jagger		Larned	
		kg ha^{-1}							
Ashland	0	4.6	H[†]	8.2	GH	5.6	GH	9.4	FGH
Bottoms	33.6	9.3	FGH	9.7	FGH	8.7	GH	12.2	EFG
2012-13	89.7	23.8	BC	17.3	CDE	11.5	EFG	15.5	DEF
	145.7	24.8	B	24.8	B	19.1	BCD	32.9	A
	Mean	**15.6**	**-**	**15.0**	**-**	**11.2**	**-**	**17.5**	**-**
Silverlake	0	16.5	G	15.9	G	13.2	G	18.8	G
2012-13	33.6	23.4	EFG	15.6	G	20.2	FG	23.5	EFG
	89.7	36.5	CD	29.7	DEF	30.7	CDE	38.8	BCD
	145.7	46.1	AB	39.0	BC	32.0	CDE	53.1	A
	Mean	**30.6**	**-**	**25.1**	**-**	**24.0**	**-**	**33.6**	**-**

†Means within a column that are followed by the same letter are not significantly different ($p>0.05$)

Table D.5. Mean values and significant differences ($p<0.05$) for parameters determined at maturity in four wheat varieties at two locations in Kansas.

Location	Variety	NUpE		NUtE		NRE		BPE	
		kg kg^{-1}							
Ashland	Duster	0.54	A[†]	47.09	AB	0.73	A	125.13	A
Bottoms	Everest	0.54	A	52.46	AB	0.70	A	125.27	A
2012-13	Jagger	0.48	A	55.02	A	0.78	A	131.28	A
	Larned	0.56	A	45.06	B	0.72	A	140.48	A
Silverlake	Duster	0.82	A	35.02	A	0.67	A	119.27	A
2012-13	Everest	0.74	A	36.70	A	0.70	A	111.55	A
	Jagger	0.71	A	35.02	A	0.71	A	105.42	A
	Larned	0.78	A	32.44	A	0.66	A	122.78	A

[†] Means within a column that are followed by the same letter are not significantly different ($p>0.05$)

Abbreviations: NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency; NRE, nitrogen remobilization efficiency; BPE, biomass production efficiency.

Table D.6. Mean values and significant differences ($p<0.05$) for additional parameters determined at maturity in four wheat varieties at two locations in Kansas.

Location	Variety	NUpAA		FUE		AgEf		PNB	
		kg ha^{-1}		kg kg^{-1}					
Ashland	Duster	-6.2	A[†]	36.35	A	21.33	A	0.59	A
Bottoms	Everest	1.1	A	49.46	A	26.44	A	0.67	A
2012-13	Jagger	-15.0	A	46.01	A	24.36	A	0.63	A
	Larned	-8.2	A	33.66	A	20.29	A	0.59	A
Silverlake	Duster	-3.2	A	54.65	A	19.28	A	0.91	A
2012-13	Everest	-3.4	A	39.64	A	13.88	A	0.85	A
	Jagger	-9.3	A	46.55	A	12.25	A	0.82	A
	Larned	-10.9	A	60.29	A	11.68	A	0.87	A

[†] Means within a column that are followed by the same letter are not significantly different ($p>0.05$)

Abbreviations: NUpAA, nitrogen uptake after anthesis; FUE, fertilizer use efficiency; AgEf, agronomic efficiency; PNB, partial nutrient balance.

D.7. The effect of Nrate and variety on partial factor productivity (PFP) values at two locations in Kansas.

Location	**Nrate**	**PFP by Variety**							
		Duster		Everest		Jagger		Larned	
		kg ha^{-1}							
Ashland	0	-	-	-	-	-	-	-	-
Bottoms	33.6	57.1	B[†]	63.6	AB	65.4	A	61.0	AB
2012-13	89.7	35.9	CD	41.2	C	37.8	CD	32.0	DE
	145.7	23.9	F	26.5	EF	25.7	EF	24.2	F
	Mean	**39.0**	**-**	**43.8**	**-**	**43.0**	**-**	**39.1**	**-**
Silverlake	0	-	-	-	-	-	-	-	-
2012-13	33.6	112.5	A	101.9	B	83.0	C	97.1	B
	89.7	53.5	D	47.2	DE	43.1	E	38.8	EF
	145.7	32.7	FG	31.1	FG	26.1	G	25.0	G
	Mean	**66.2**	**-**	**60.1**	**-**	**50.7**	**-**	**53.6**	**-**

[†] Means within a column that are followed by the same letter are not significantly different ($p>0.05$)