ENHANCHING PROTEIN CONCENTRATION IN HARD RED SPRING WHEAT WITH NITROGEN MANAGEMENT BASED ON PLANT PREDICTORS

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Sciences

by
Amanda Sue Schoch

In Partial Fulfillment
for the Degree of
MASTERS OF SCIENCE

Major Department:
Plant Sciences

April 2013

Fargo, North Dakota

North Dakota State University
Graduate School

Title

ENHANCHING PROTEIN CONCENTRATION IN HARD RED SPRING WHEAT WITH NITROGEN MANAGEMENT BASED ON PLANT PREDICTORS

By

Amanda Sue Schoch

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Joel Ransom
Chair

Dr. Jochum Wiersma

Dr. Hans Kandel

Dr. David Frazen

Approved:

April 19, 2013
Date

Dr. Richard Horsley
Department Chair

ABSTRACT

In recent years (2009-2011) many producers have had issues meeting the market requirements of 140 g kg^{-1} of protein concentration in hard red spring wheat (HRSW) (*Triticum aestivum* L. emend. Thell) especially with newer cultivars that are genetically prone to producing lower grain protein concentration. To address this issue, part of this study was to determine whether if plant based predictors could be used to predict grain protein content prior to anthesis. Experiments were conducted in 2011-2012 at Crookston, Minnesota (MN) and Prosper, North Dakota (ND). Another part of this study was to determine if protein concentration in HRSW can be enhanced with different sources and rates of N, while maintaining high yields and maximizing net returns. Experiments were conducted across three different locations in MN and ND in 2011-2012. Fertilizer treatments consisted of 3 sources of N, 4 growth stages, and 2 rates of N compared across three cultivars.

ACKNOWLEDGEMENTS

I am much indebted and thankful to my advisors, Dr. Joel Ransom and Dr. Jochum Wiersma, whose dedication, encouragement, guidance and support enabled me to reach my personal and professional goals. I also want to thank them for their time and patience with me.

I gratefully acknowledge Dr. Hans Kandel, Dr. Dave Franzen, Dr. Frayne Olson, for their valuable help in conducting the trials, their advice when it came to various pieces of instrumentation and their time reading this thesis and giving their critical comments about it. I want to thank Dr. James Hammond for his assistance with the statistics; especially programming and interpreting SAS output.

I especially want to thank everyone that helped in maintaining my plots and recording data: Chad Deplazes, Grant Mehring, Garrett Lungren, Courage Mudzongo, Eric Eriksmoen and Rich Olson. Also, thanks to my fellow graduate students who helped immensely in completing tasks and making graduate school an enjoyable and memorable experience: Jameson Hall, Aaron Hoppe, and Matthew Chaput.

Furthermore, I would like to thank my parents, Melvin and Silvia, for all their love, support, encouragement, and guidance. I would not be the person I am today without them. Thanks also to my family and friends who have made my life fulfilling, and for encouraging me every step along the way. I would also like to thank the Lord for giving me the strength and faith to meet any challenges that I am faced with.

I want to extend a special thank you to the Minnesota Wheat and Promotion Council, North Dakota Wheat Commission, the NDSU Experiment Station, and SBARE-Wheat Committee for funding and supporting this research.

PREFACE

This thesis was written as a series of two manuscripts that will be submitted for publication in appropriate scientific journals. The 'Introduction' provides a general review of the importance of this study and how both chapters are related to the main issue: how proper management of nitrogen, effective timing of applications and accurate predictions for the need of additional nitrogen during the growing season can assist producers in meeting production goals and maximizing profit. Following the Introduction, the thesis will be divided into two manuscripts which will contain Introduction, Material and Methods, Results, Discussion, and References Cited sections that are specific to the chapter. The references for the 'General Introduction' can be found in the 'General References Cited' section. The abbreviations for hard red spring wheat (HRSW), nitrogen (N), and Zadok's growth stage (ZGS) will be used in both articles.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

INTRODUCTION

Profitability of HRSW is based on two major components: grain quality (grain protein concentration) and grain yield. Achieving both high grain yield and high grain protein concentration is challenging due a negative relationship between protein concentration and grain yield in HRSW cultivars (Brown et al., 2005); seasonal yield differences due to weather conditions (Terman, 1979); and the availability and utilization of N.

Plant growth and metabolism is driven by the photosynthetic process, which produces assimilates, including N compounds and carbohydrates (Lawlor et al., 1989). The photosynthetic rate per unit of leaf depends on the development and maintenance of a number of components including: energy-transducing components (thylakoid membranes), enzymes of the photosynthetic carbon reduction cycle (ribulose-bisphosphate carboxylase oxygenase or rubisco), and enzymes for nitrogen assimilation (Farquhar et al., 1980). In order to achieve a high rate of photosynthesis, adequate amounts of photosynthetic components must be produced (Lawlor et al., 1989). Nitrogen promotes the formation of large, healthy leaves that have high amounts of chlorophyll and rubisco.

Nitrogen is taken up by the plant roots in the form of NO_3- and NH_4^+. Both forms are readily absorbed by the plant, but the amount depends on their quantity in the soil. Uptake of N is also dependent on the stage of growth and the growth rate of the plant (Brown et al., 2005). Once N is inside the plant it is translocated to the leaves and reduced to glutamate in the chloroplast. During grain filling this N is relocated and deposited in the inflorescences (Fageria et al., 2006). This process is known as the source-to-sink cycle, meaning that vegetative tissue that once was a depository for excess N becomes a source of N for other parts of the plant that have a greater demand for it. As the plant matures, larger amounts of photosynthates are required

to fulfill grain development needs. Thus maintenance of active photosynthesis by leaves, especially the flag leaf, throughout the grain filling period is a major requirement for optimal production of carbohydrates and amino acids that contribute to high yields and high protein concentration (Simpson et al., 1983).

The rate and duration of starch and protein deposition in the endosperm of wheat grain kernels are independent events controlled by separate mechanisms (Jenner et al., 1991). The production of grain yield and grain protein concentration occurs simultaneously; however, grain yield potential will reach it maximum prior to maximum protein concentration potential (Goos, et al., 1982). The inverse relationship between grain yield and protein concentration can be explained by differences in N uptake and the plants ability to utilize energy and nutrient reserves from the vegetative stage in kernel development (Brown et al., 2005). Most N that is taken up during early stages of crop development is used in tiller formation, leaf and spike growth which all impact grain yield potential (Jenner et al., 1991). Nitrogen that is taken up around heading will usually positively influence protein concentration of the kernel but can only marginally influence yield because the number and size of kernels are largely fixed at this time (Brown et al., 2005).

Differences in the plant's total protein concentration during vegetative stages can be minimal between high and low protein wheat cultivars (Seth et al., 1960). However, during heading (ZGS 59) (Zadoks et al., 1974), protein concentration increased more rapidly in the spikes of high protein cultivars than those of low protein cultivars. Seth et al., (1960) observed that the average mean of protein was 50 g kg^{-1} in the roots of high protein cultivars was significantly lower than that of lower protein cultivars which had an average mean of 58 g kg^{-1}. The protein concentration differences between cultivars can be explained by uptake, utilization,

and redistribution of N by the plant. Redistribution of N accounts for 80% of the N found in grain and only 20% is directly absorbed from the soil during grain filling (Dalling et al., 1976).

When moisture concentration is low throughout the growing season and excess N is available to the wheat due to reduced growth, grain yields may be low and protein concentration could be high (Neidig and Snyder, 1924). The higher protein concentration with drought conditions may be due to shriveled wheat kernels (less starch to protein ratio). When weather conditions are favorable for plant growth and yield, little plant available N is left in the soil profile during the grain filling and ripening of the grain. Plant carbohydrate production is enhanced, while total N uptake may be limited by supply, resulting in higher yields with lower protein concentration. When a plant is drought stressed or when temperatures are extremely high, the time between anthesis and harvest can be reduced by as much as seven days (Altenbach et al., 2003). The reduction in the length of grain filling can negatively impact grain yield due to inhibition of enzymes involved in the starch biosynthesis, thus reducing starch deposition. Therefore, the best way to determine genetic potential for yield and protein among cultivars is to compare them when grown under optimum conditions where differences are not related to deficiencies (Terman, 1979).

References

Altenbach, S.B., F.M. DuPont, K.M. Kothari, R. Chan, E.L. Johnson, and D. Lieu. 2003. Temperature, water and fertilizer influence the timing of key events during grain development in a US spring wheat. J. Cereal Sci. 37:9-20.

Brown, B., M. Westcott, N. Christensen, B. Pan, and J. Stark. 2005. Nitrogen Management for hard wheat protein enchancement. Ext. Publ. PNW 578. Pacific Northwest Ext. Serv., Moscow, ID.

Dalling, M.J., G. Boland, J.H. Wilson. 1976. Relation between acid proteinase activity and redistribution of nitrogen during grain development. Aust. J. Plant Physiol. 3:721-730.

Fageria, N.K., V.C. Baligar, and R.B. Clark. 2006. Physiology of crop production. Haworth Press, Binghamton, NY.

Farquhar, G.D., S. von Caemmerer, and J.A. Berry. 1980. A biochemical model of photosynthetic $C0_2$ assimilation in leaves of C_3 species. Planta. 149:78-90.

Goos, R.J., D.G. Westfall, A.E. Ludwick, and J.E. Goris. 1982. Grain protein concentration as an indicator of nitrogen sufficiency for winter wheat. Agron. J. 74:130-133.

Jenner, C.F., T.D. Ugalde, and D. Aspinall. 1991. The physiology of starch and protein deposition in the endosperm of wheat. Aust. J. Plant Physiol. 18:221-226.

Lawlor, D.W., M.Kontturi and A.T.Young. 1989. Photosynthesis by flag leaves of wheat in relation to protein, Ribulose Bisphosphate Carboxylase activity and nitrogen supply. J.

Neidig, R.E., and R.S. Snyder. 1924. The relation of moisture and available nitrogen to the yield and protein concentration of wheat. Idaho Agr. Exp. Sta. Res. Bul. 3: 173-179.

Seth, J., T.T. Hebert, and G.K. Middleton. 1960. Nitrogen utilization in high and low protein wheat varieties. Agron. J. 52:207-209.

Simpson, R.J., H. Lambers, and M.J. Dalling. 1983. Nitrogen redisturbution during grain growth in wheat (*Triticum aestivum* L.). Plant Physiol. 71:7-14.

Terman, G.L. 1979. Yields and protein concentration of wheat grain as affected by cultivar, N, and environmental growth factors. Agron. J. 71:437-440.

Wuest, S.B. and K.G. Cassman. 1992. Fertilizer-nitrogen use efficiency of irrigated wheat: I. Uptake efficiency of preplant versus late-season application. Agron. J. 84:682-688.

Zadoks, J.C., T.T. Chang, and C.F.Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res.14:415-421.

ARTICLE 1: PREDICTING IN-SEASON NITROGEN NEEDS FOR ENHANCING PROTEIN CONCENTRATION OF HARD RED SPRING WHEAT

Abstract

The standard market grain protein concentration for HRSW of 140 g kg^{-1} is not always achieved. This study was conducted to determine if final grain protein concentration could be determined prior to anthesis using a variety of measurements (normalized difference vegetative index values, chlorophyll concentration, leaf color values, and N concentration of leaf and stem samples). Field experiments were conducted at Crookston, MN, and Prosper, ND in 2011-2012, consisting of a factorial combination of N rates and cultivars. N concentration of leaf and stem samples, and chlorophyll concentration collected at Zadoks growth stage (ZGS) 37 increased linearly as the amount of N fertilizer applied increased. The plant-based predictors could be used to predict final grain protein concentration. These linear relationships between plant based predictors and final grain protein may be utilized to determine supplement N needs at the beginning of grain fill to improve final grain protein concentration.

Introduction

Nitrogen is an essential nutrient for plants; however, plant N uptake depends on several factors including its availability in the soil (Fageria et al., 2006), growth stage, and the growth rate of the plant (Brown et al., 2005). Nitrogen is a major component of the plant photosynthetic apparatus (Evans, 1983), which produces the compounds that are required for plant growth and development using light energy (Lawlor et al., 1989). Most N that is taken up during early stages of crop development helps promote increased vegetative biomass which contributes to potential yield; while N taken up after anthesis is essentially used to product grain protein concentration (Jenner et al., 1991). Differences among genotypes, variability in seasonal weather conditions, and the availability and utilization of N will all impact plant performance (Terman, 1979).

Current recommendations for determining the N fertilizer needs in many crops, including HRSW, are based on location, historical productivity, previous crop credit, and the results of soil nitrate-N test to a depth of 60 cm (Franzen, 2010). One of the weak points of applying all N prior to seeding is the variation in soil availability and loss that is possible during the first 30-60 days of each growing season when supplemental N application might still have practical benefit (Franzen, 2010).

Plants that are healthy and productive tend to have higher chlorophyll concentration than those that are N stressed. However, when vital nutrients such as N, Fe, S, and Mg are limiting, yellowing, or chlorosis can occur (Wright et al., 2002). Chlorosis can also be caused by diseases and other crop stresses. Inadequate levels of N also can reduce small grain tillering, induce stunting, cause poor kernel fill, and result in low grain protein (Cavanaugh et al., 2009). Currently, nitrogen use efficiency (NUE) of cereal crops worldwide is only 33% (Raun and Johnson, 1999). The amount of N that is not utilized in a particular year by the plant might be lost due to leaching, erosion, denitrification or volatilization, immobilized by microbes in residue breakdown intermediates, transformed into semi-permanent organic matter or even fixed in clay lattices and thus, the practice of using soil tests for making fertilizer recommendations prior to seeding is not always capable of meeting in-season N needs (Woolfolk et al., 2002). To improve NUE, current practices such as adjusting application rates based on more precise estimates of crop needs, use of products preventing N loss, applying N at the time of greatest uptake, incorporating N into the soil, and proper placement during planting are being promoted (Robertson, 2004).

Tissue N Concentration Samples

Extensive studies have been conducted to understand the relationship between N in plant tissues at various growth stages and plant response to supplemental N. Plant tissue sample collection and analysis has been considered a relatively reliable indicator for determining the nutrient status of a plant, but only when samples are properly selected, collected, handled, prepared, and shipped (Thom et al., 2000). Tissue samples can detect unseen deficiencies and confirm visual deficiency symptoms (Flynn et al., 1999). Tissue samples collected from young plants may allow producers to make corrective fertilizer applications during the growing season. Papastylianou et al. (1984) found a correlation between nitrate concentration in wheat stems at tillering and grain yield. Total N in the flag leaf about heading/flowering, was closely related to grain protein at harvest compared to tissue samples collected earlier in the growing season (Brown et al., 2005). Even though tissue sampling and analysis can be reliable predictors for both grain yield and protein concentration if taken properly, they are site-specific, expensive, labor intensive, and destructive to the plant (Wright et al., 2001). More expeditious, cheaper and more practical methods of predicting in-season N requirement would be beneficial.

Leaf Color Chart

Several instruments are currently available to assist producers in determining N status in a plant. A simple, quick, and non-destructive method for estimating N status of a plant is the leaf color chart (LCC) (International Rice Research Institute, Metro Manila, Philippines). The LCC gives a rapid evaluation of leaf N status by visually estimating the color composition of the leaf by comparing it to different intensities of green. To determine an appropriate N rate, 10-20 random LCC readings are needed from a sample area to provide an average. The LCC can be used on any cereal crop.

In wheat, Maiti and Das (2006) found a linear response between LCC values and increasing N rates. There was also a significant and positive correlation (r=0.56 to 0.59) between grain yield and LCC values across all growth stages. They concluded that LCC values can be used in indicating the need for top-dressing N and help increase NUE. A positive relationship using the LCC has also been observed in rice (*Oryza sativa*) (Yang et al., 2003).

Chlorophyll Meter

Leaf color charts are popular because they are a quick, non-destructive and an inexpensive alternative to tissue sampling (Maiti and Das, 2006; Yang et al., 2003). Another option available is the use of a chlorophyll meter that gives an indirect assessment of leaf N status by measuring the chlorophyll concentration of a leaf (Blackmer and Schepers, 1995). Comparisons of the two strategies have shown that LCC and chlorophyll meter readings have a close relationship across multiple growth stages in both wheat (Maiti and Das, 2006) and rice (Yang et al., 2003). These studies suggest that either diagnostic tool can be used in predicting N needs depending on the availability of the instrument and the preference of the user.

The chlorophyll meter uses a calibrated light-emitting diode to measure transmission of red light to infrared light through the leaf (Francis and Piekielek, 1999). The ratio between these two wavelengths is used to calibrate the chlorophyll concentration index or the actual chlorophyll concentration of the leaf. Thus plants that have a high chlorophyll concentration reading will absorb more red light and are typically greener (healthier). To determine the appropriate N rate a minimum of 30 random chlorophyll readings should be collected and averaged. The chlorophyll meter is useful because it has the ability to help detect N stress before it is visible to the human eye (Schepers et al., 2006) and is considered more reliable than visual assessment when using a LCC (Debaeke et al., 2006).

Several reports have found a strong correlation between leaf N and chlorophyll concentration (Vos and Bom, 1993; Blackmer and Schepers, 1995; Olfs et al., 2005). Research has also found a positive correlation between chlorophyll meter readings taken from wheat at the ZGS 30 and grain yield (Follett et al., 1992). Fox et al. (1994) found that SPAD-502 (Soil and Plant Analysis Development) chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan) readings taken on wheat leaves more accurately predicted wheat responses to N fertilizers than total N concentrations of plant tissue. Spaner et al., (2005) found a positive correlation between SPAD measurements at ZGS 75-79 and grain yield in wheat ($r^2 \geq 0.65$). A high correlation between SPAD readings at ZGS 71 and grain protein concentration has also been reported (Bail et al., 2005; Spaner et al., 2005).

Remote Sensors

Chlorophyll meters are relatively easy and fast to use in small fields for detecting the N status of a plant; but they are somewhat limited in practical use because they can only measure a small area of the crop canopy within each field and do not detect the variability within a field unless many readings are used (Ma et al., 1996). This issue has led to the development of canopy reflectance measurements and remote sensing techniques that theoretically can provide rapid estimates of crop N status with high spatial resolution by surveying a larger area depending on the sensor's field of view (Ma et al., 1996).

Recent advancements in precision agriculture technology have led to the development of ground-based active remote sensors (Dr. Jim Schepers, USDA-ARS, Lincoln, NE; Dr. Bill Raun, Oklahoma State University, Stillwater, OK). Active remote sensors have their own light source, which allows them to determine the normalized difference vegetative index (NDVI) throughout the growing season. These sensors work by directing visible light (VIS) (400-700 nm) as well as

near in-frared (NIR) (700-1300 nm) light at the plant canopy and then measure the amount of VIS and NIR light that is reflected back to the sensor. NDVI is calculated using the following equation: NDVI = (NIR – VIS) / (NIR + VIS)

The NDVI is related to leaf area index (LAI) and green biomass, meaning that it is highly dependent on photosynthesis efficiency (Penuelas et al., 1994). Plants that are healthier or have a larger LAI will absorb more VIS, (which is required for producing chlorophyll) and in return will reflect more NIR light than plants that are stressed by N deficiency (Shaver et al., 2011). Accordingly, there is a direct relationship between N concentration and high NDVI values. Research has found NDVI values taken at certain times to be highly correlated with grain yield of winter wheat (Raun et al., 2002), HRSW (Osborne, 2007), and corn (*Zea mays* L.) (Chang et al., 2003). These sensors can be integrated with a global positioning system (GPS) that reads the crop canopy reflectance, calculates N fertilizer rates based on reflectance, and variably applies N where needed (Shaver et al., 2010). Currently there are only two sensors on the market to assist producers in determining the N status of their crop in real-time: the GreenSeeker (GreenSeeker, NTech Inductries, Inc., Ukiah, CA) and the Crop Circle (Holland Scientific, Lincoln, NE, USA).

The GreenSeeker calculates a RED/NIR ratio by using VIS red light (660 nm) and NIR (770 nm) light at the plant canopy. Raun et al. (2002) found that the GreenSeeker can help improve NEU of winter wheat by more than 15% when a top-dressing of N fertilizer was applied based on sensor readings collected in-season. Freeman et al. (2003) found that NDVI values tended to predict grain yield, but found no correlation with total grain N. He stated that their NDVI values were not reliable metrics for predicting the protein concentration in wheat because NDVI is not capable of determining how efficient the plant is at translocating N to the kernel or how much N is lost through various pathways. Recent field studies conducted in South Dakota

have shown the GreenSeeker to be promising in predicting grain protein concentration in HRSW (Qualm et al., 2010). However, under extreme environmental stress (drought), this technology was not reliable due to stunted growth and resulting low yields.

The Crop Circle sensor uses the same principles as that of the GreenSeeker sensor. However, each sensor uses different wavelengths within the VIS (590 nm) and NIR (880 nm) spectrum (Shaver et al., 2010). The Crop Circle is also capable of emitting and detecting four different bands of light (blue, green, red, and NIR) depending on which filter is in the unit at the time. Given these differences between sensors, the Crop Circle is capable of calculating the Green Normalized Difference Vegetative Index (GNDVI) which is calculated by replacing the reflectance of red VIS light with green VIS instead (Shanahan et al., 2001). Using GNDVI to predict grain yield is more reliable then NDVI because it is sensitive to changes in chlorophyll concentration (which is absorbed by green VIS) and not by increases of vegetative biomass. When comparing NDVI and GNDVI values, Dellinger et al. (2008) found that GNDVI was a better indicator for estimating in-season N requirements in corn.

While numerous studies have been conducted to determine the efficiency of each individual diagnostic tool, only recently were they compared to each other for determining yield. Shaver et al. (2011) looked at the performance of two active sensors (GreenSeeker and Crop Circle) for determining the N status and grain yield in corn. This study also looked at how soil NO_3^- concentration, leaf N concentration, chlorophyll readings, and plant height affect these relationships. Their results found that NDVI readings from both sensors had high correlations between applied N rate and grain yield at the V12 and V14 growth stage. Results also indicated that there was no significant improvement of the r values using single or multiple regression with soil and plant variables when compared to NDVI alone. The authors concluded that both the

GreenSeeker and the Crop Circle performed well in determining N variability in corn, but at later growth stages (greater than V14) the potential of the VIS sensor was limited.

Procedures to Normalize Variation

Even though most of the diagnostic tools previously discussed are capable of predicting the N status of a plant, transferring this information into useful N fertilizer recommendations can be difficult for a number of reasons including the N-supplying ability of the soil, water supply, growth stage, sampling procedures (Olfs et al., 2005) cultivar (Debaeke et al., 2006) and seasonal effects (Hussain et al., 2000). Frost damage, herbicide injury, and deficiencies in nutrients other than N can, for example, negatively impact leaf chlorophyll concentration (Blackmer and Schepers, 1995). To standardize differences between sites, cultivars, and growth stages, Johnson and Raun (2003) suggested that measurements be normalized. To normalize data, a well fertilized reference area (N-rich strip), which covers as many soil types and growing conditions as possible within a field is compared to areas where N may be deficient in the same field. This estimates the potential crop's response to additional N.

By normalizing the data, response models can often be developed and applied across multiple fields and cultivars (Holland and Schepers, 2010), although Solari et al. (2010) warned that more generalized prediction models need to be validated locally. Furthermore in order to be considered economical to a producer, the technology needs to be based upon grain protein premiums and discounts, and the cost of N fertilizer at the time of application (Qualm et al., 2010).

In recent years (2009-2011) discounts up to \$51.40 Mg^{-1} per percent of grain protein below 140 g kg^{-1} has led to interest in and additional research on late season N applications in order to increase protein concentration. This situation has also led to the questions if accurate

predictions for the need of supplemental N prior to anthesis for enhancing protein concentration can be made using a variety of measurements. If final grain protein concentrations can be accurately predicted prior to anthesis, then producers have a tool to determine whether a late season application of N would be justifiable.

Objective

The first objective of this research was to determine if plant based measurements can reliably predict grain protein at harvest, thereby providing insight into whether additional N fertilization for improving grain protein is needed or feasible. The second objective was to determine if predictive relationships would be consistent across cultivars with different protein characteristics.

Material and Methods

General Information

Field experiments were conducted at the University of Minnesota Northwest Research and Outreach Center, Crookston, MN, (Latitude = 47.48^{o} N, Longitude = -96.36^{o} W); and the NDSU research fields near Prosper, ND, (Latitude = 47.00^{o} N, Longitude = -97.11^{o} W) in 2011 and 2012. Table 1.1 lists the soil series, soil taxonomy and slope at each location. Soil samples were collected in the spring to determine the levels of N, phosphorus (P), potassium (K), pH and organic matter at each location (Table 1.2). Five random core samples from the trial were collected and combined prior to analysis.

The experimental design was a randomized complete block (RCBD) with a split-plot restriction and four replicates. Main plot treatments were rates of N at 0, 67, 134, and 202 kg N ha^{-1}. Nitrogen treatments of dry urea were broadcasted by hand and incorporated with a field cultivator prior to planting. Sub-plot treatments were four HRSW cultivars.

Table 1.1. Soil series, taxonomy and slope at Crookston, MN and Prosper, ND in 2011-2012.

Location	Soil Series†	Soil Taxonomy‡	Slope
			%
Crookston	Wheatville–Gunclub	Coarse-silty, mixed, smectitic, superactive frigid Aeric Calciaquolls Fine-silty, mixed, superactive, frigid Aeric Calciaquolls	0-2
Prosper	Kindred–Bearden	Fine-silty, mixed, superactive, frigid Typic Endoaquolls Fine-silty, mixed, superactive, frigid Aeric Calciaquolls	0-2

† Soil data obtained from (USDA-NRCS, 2011).
‡ Soil taxonomy listed on individual lines based on hyphenated soil series name.

Table 1.2. Previous crop, available N, P, K, pH and organic matter levels by sampling depth at Crookston and Prosper, 2011-2012.

Location	PC†	Depth	N	P	K	pH	OM†
		cm	kg ha^{-1}	mg kg^{-1}	mg kg^{-1}		%
			2011				
Crookston	soybean‡	0-61	28.0	3	145	8.1	NA
Prosper	Soybean	0-15	7.8	55	250	7.6	3.9
		15-61	16.8	9	170	8.0	2.8
			2012				
Crookston	Wheat	0-61	34.0	4	137	8.3	NA
Prosper	Soybean	0-15	112.0	41	315	7.8	3.3
		15-61	238.6	21	230	7.8	2.5

†PC = Previous crop; OM = Organic matter.
‡ soybean = *Glycine max*.

The four cultivars of HRSW used in the experiment were 'Faller,' 'Glenn,' 'Samson' and 'Vantage' (Table 1.3). Faller and Samson were chosen because they are known to be high yielding with lower grain protein concentration (Ransom et al., 2012). Glenn and Vantage have opposite characteristics of Faller and Sa mson.

Table 1.3. Characteristics of four HRSW cultivars used in predicting N needs experiment.

					Average Yield Within Region†			
Cultivar	Origin‡	Year Released	Height	Days to Head	Eastern ND	Western ND	Protein§	Volumetric Weight
			cm		-----Mg ha^{-1} -----		g kg^{-1}	kg $^{-L}$
Faller	ND	2007	89	65	5.5	3.7	138	72.3
Glenn	ND	2005	94	61	4.8	3.4	149	76.4
Samson	WestBred	2007	79	63	5.1	3.8	138	73.5
Vantage	WestBred	2007	81	67	4.9	3.6	158	75.3

† Based on a three year average (2010-2012).
‡ Refers to agent or developer: ND = North Dakota State University.
§ Protein at 12% moisture.
Source: Ransom et al., 2012.

Planting and Plot Maintenance

Trials at Crookston in 2011 and 2012 were sown at a rate of 3 million viable seeds ha^{-1} using a ten row Almaco (Almaco, Nevada, IA) double disk drill with 15 cm row spacing. Before trials were planted, a uniform seed bed was prepared with a field cultivator. Due to an extremely wet spring, trials in 2011 were seeded on May 17. With a mild winter in 2012, seeding was completed on April 11.

Trials at Prosper in both 2011 and 2012 were sown using a seven row Great Plains 3P605NT drill (Great Plains Mfg Inc., Salina, KS) with 18 cm row spacing. Before trials were planted, a uniform seedbed was prepared using a field cultivator. Due to an extremely wet spring, trials in 2011 were planted on May 19. With a mild winter and favorable spring in 2012, planting was completed on April 19. Trials were sown at a rate of 4 million viable seeds ha^{-1}. Prior to planting seeds were treated with tebuconazole at the rate of 3 ml kg^{-1} seed. The seed was treated using a batch seed treatment machine (Hege II,Wintersteiger AG, Ried, Austria).

Experimental units in Crookston consisted of two adjacent solid seeded plots that measured 1.5 m wide and 10.6 m long. Experimental units and plots were spaced about 0.5 m apart while the alley between blocks was 3m. Prosper plots were 1.5 m wide and 5.2 m long. Borders between plots were 0.3 m and alleys between reps were cut mid-season at a width of 0.8 m. The harvested area was 1.5 m by 3.7 m. During the growing season, plots were trimmed from each end using a rotovator (Crookston and Prosper, 2011) or mowed (Prosper, 2012) to the lengths previously defined to create an alley. Border plots in 2011 and 2012 were planted with winter wheat at Crookston and HRSW at Prosper on the two outermost columns of plots to ensure similar competition as interior plots.

A stand count was taken in 2012 at the ZGS 12 at Crookston and Prosper. Measurement were obtained by counting the number of plants in a randomly selected 30-cm length of rows two and six at Prosper and rows two and nine at Crookston, then the number the number of plants from both rows were averaged, and adjusted to represent plants 100 cm^{-2}. Prior to harvest, lodging notes and plant heights were taken. Lodging was based on a scale of 0-10 for the entire plot length with zero having no signs of lodging to ten being completely flat. Plant heights were collected by taking the average height of the erect plants from the soil surface to the top of the awns.

Predictive Measurements

All plots were measured for plant greenness at all locations in 2011 and 2012 using an infra-red chlorophyll sensor (GreenSeeker) which records the ratio of red to infrared light emitted by the instrument and then reflected back to the sensor instrument. This ratio is the difference of NDVI values, which generally is a proxy for measuring plant health. Readings were taken twice (ZGS 16 and 37) during the growing season. Readings were obtained by walking through the plots at a consistent speed (approximately 1.34 m sec^{-1}) holding the sensor approximately 46 cm above the canopy.

The CCM-200 chlorophyll meter (Opti-Sciences, Inc., Hudson, NH) is an instrument that measures how much light is absorbed by chlorophyll molecules. Measurements were taken on twenty-five random leaves that were fully expanded and free of any disease or damage and selected from the inner rows of each plot and averaged to provide an average for the whole plot. Chlorophyll readings were obtained by placing the instrument sensor halfway between the leaf tip and collar and halfway from the leaf margin to the mid-rib. Measurements were collected at the ZGS 16 in 2012 and 37 in both 2011 and 2012.

The LCC was used to provide a rapid evaluation of leaf N status by visually estimating the color of the leaf and by comparing it to four color schemes of green. Readings were taken at the same time of every day (8-10 a.m.), because the color chart's comparative readings can be influenced by ambient light intensity (International Rice Research Institute, 2011). Readings were taken at the ZGS 16 and 37 in 2012. Ten disease free leaves were randomly selected from the inner rows of each plot. The middle part of the leaf was then placed on the chart and compared with the color panels on the LCC. These values were then averaged and a single value was used for the plot analysis.

The N concentration of leaf tissue was also used to estimate the N status of the crop. Twenty-five random leaf samples from the inner rows of each plot were gathered. Leaf samples were fully expanded and free of disease or damage. Samples were collected at the ZGS 16 and 37. Leaves were dried at 57^{o} C for several days, ground, and stored in plastic vials. Samples were ground using a Cyclone Sample mill (UDY Corporation, Fort Collins, CO) with 0.5 mm screen and a Wiley Laboratory mill model 2 (Arthur H. Thomas Co., Philadelphia, PA) with 0.8 mm screen. Nitrogen concentration was determined using the Dumas combustion method (Buckee, 1994).

The N concentration of the base of the main wheat stem was also used to estimate the N status of the crop. To accomplish this, 25 random wheat stem samples from the inner rows of each plot were gathered. Stem samples were prepared by cutting tissue just about the crown and the collar of the first living leaf (5.1cm in length). All excess biomass that was attached to the stem was removed. Samples were collected at the ZGS 37. The wheat stem tissue test was processed using the same method as the leaf tissue analysis. Due to logistical and meteorological

complications, NDVI values, chlorophyll concentration, and N concentration of leaf samples were not collected at Prosper in 2011 at the ZGS 16.

Weed Control

Broadleaf and grassy weeds at Crookston were controlled with a tank mix application of bromoxynil octanoate, and bromoxynil heptanoate and MCPA at 350 g, 350 g, and 350 g ai ha^{-1}, respectively, and pinoxaden at 39 g ai ha^{-1} applied at ZGS 14. In Prosper, weeds were controlled with an application of fenoxaprop, pyrasulfotole, and bromoxynil, at 89 g, 40 g, and 198 g ai ha^{-1}, respectively, applied at the ZGS 12. A fungicide application of propiconazole and trifloxystrobin at 55 g, 55 g ai ha^{-1}, respectively, was also applied at ZGS 12.

Harvest Methods

Plots in Prosper were harvested in both 2011 and 2012 with a Wintersteiger Classic plot combine (Wintersteiger Ag, Ried, Austria). All rows of each plot were harvested and plot lengths were recorded. Following harvest, grain was dried (if necessary) and cleaned using a Clipper Office Tester and Cleaner (Seedburo Equipment Co., Chicago, IL). After cleaning, grain yield and protein concentration were determined. Moisture was measured using a GAC 2100 moisture tester (DICKEY-John Corp., Minneapolis, MN). Yield was calculated by weighing the plot sample with a scientific scale (RS-232, Scientech Inc., Gaitherseberg, MD), and adjusting to a moisture concentration of 13.5%. Grain protein was measured using a 0.5 kg sub-sample of seed from each plot on a Diode Array 7200 NIR Analyzer (Perten Instruments, Springfield, IL).

At Crookston (2011-2012) a 1 x 1 m area from the center of one plot per experimental unit was harvested by hand. The mature crop was cut off just above the ground and bundles where threshed with a stationary laboratory thresher (Wintersteiger Inc., Salt Lake City, UT). After harvest, grain yield and grain protein were determined. Moisture and grain protein were

measured by NIT using the Tecator Infratec 1229 Grain Analyzer (Foss North American Inc., Eden Prairie, MN) following AACC method 39-10. Yield was calculated by adjusting the plot weight to a moisture concentration of 13.5% and converting the weight to kg ha^{-1}.

Statistical Analysis

Data were analyzed using the PROC Mixed procedure of SAS 9.2 (SAS Institute, Cary, NC). Location, year, and replicates were considered random effects while N rates and cultivars were considered fixed. Any fixed effect by random effect interaction was also considered random. Main effects and interactions were tested using the appropriate error terms. Means were separated using a Fisher's protected least significant differences test at the 5% level of significance.

Plant predictors were normalized (indexed) prior to statistical analysis. To normalize the data, each individual cultivar was first separated by replication at each location. Once each individual cultivar was separated it was then divided by the highest recorded value of N rate within that rep (202 kg ha^{-1} N). Orthogonal contrast and linear regression were used to determine the relationship between fertilizer rates, plant based predictors, and the grain protein concentration of the harvested grain. A multiple regression equation was also fit using all plant predictors as independent variables and protein dependent using stepwise regression. Correlation coefficients between grain protein and observed variables were calculated using the PROC Corr in SAS.

Results and Discussion

Weather Information

The 2011 and 2012 growing seasons were substantially different from each other. In 2011, much of North Dakota and western Minnesota was extremely wet (NDAWN, 2013) and

thus a large area could not be planted. Due to cold and wet conditions in April when HRSW is typically planted in this region, our trials were planted in May. At Crookston the mean air temperature for the growing season (17 May – 15 August 2011) was 18 ^{o}C, and at Prosper the mean air temperature was 19 ^{o}C for the growing season (19 May – 19 August 2011) (Table 1.4). Monthly mean air temperatures at both locations were below normal in May, but above normal for July. Rainfall during the 2011 growing season was 252 mm in Crookston and 451 mm in Prosper (Table 1.5). Excess amount of rainfall at Prosper caused waterlogged conditions multiple times throughout the season (Table 1.5).

During the 2012 growing season, much of the area was drier than normal (NDAWN, 2013) thus planting occurred in mid-April. The mean air temperature for the growing season at Crookston (11 April – 27 July) was 16 ^{o}C and the total rainfall was 226 mm (Table 1.4, 1.5). At Prosper (19 April – 31 July) the mean air temperature was 16 ^{o}C and total rainfall was 291 mm. The monthly mean air temperatures at both locations were above normal for all months during the growing season. The monthly average precipitation was normal each month during the growing season at both Crookston and Prosper.

Table 1.4. Average mean air temperature for the months of planting to harvest in Crookston and Prosper, in 2011 and 2012, along with normal (1981-2010) †.

	Crookston‡			Prosper		
	2011	2012	Normal	2011	2012	Normal
Month	----------	----------	^{o}C	----------	----------	----------
April	5	8	6	5	8	6
May	12	14	13	12	15	13
June	18	19	18	19	20	19
July	22	23	21	23	24	21
August	20	19	20	21	20	20

† Information collected from NDAWN, 2013.
‡Weather information collected from the Eldred, MN weather station which is 21 km away.

Table 1.5. Average mean rainfall for the months of planting to harvest in Crookston and Prosper, in 2011 and 2012, along with normal (1981-2010) †.

	Crookston‡			Prosper		
	2011	2012	Normal	2011	2012	Normal
Month	----------mm----------					
April	64	27	31	45	30	37
May	66	31	74	80	46	78
June	68	74	97	132	67	100
July	79	94	76	150	16	88
August	41	47	84	89	23	67
Total	319	273	361	496	183	369

† Information collected from NDAWN, 2013.
‡Weather information collected from the Eldred, MN weather station which is 21 km away.

Combine Statistical Analysis

Barlett's test for homogeneity error of variance was not significant, thus allowing for the combining of environments in the ANOVA. Nevertheless, data from the trial in Prosper 2011 were not included in the analysis across environments because excessive rainfall caused the trials to be flooded on three separate occasions resulting in low grain yield and high grain protein concentrations due to poor grain fill.

Cultivar

There were significant differences between cultivars for N concentration of leaf samples collected at ZGS 37. In the combined analysis, Faller followed by Vantage had significantly lower N concentration in leaf samples then either Samson or Glenn (Table 1.6). Differences in total N concentration levels among cultivars can be explained by how fast uptake, utilization, and redistribution of N in the plant can occur within a cultivar (Seth et al., 1960).

Cultivar x Environment

Significant environment x cultivar (E x C) interactions for grain protein, yield, and NDVI (GreenSeeker) measurements were found. These interactions resulted from differences in magnitude (rankings of cultivars did not change) across all environments except for NDVI

values. Therefore, cultivar main effects will be discussed for all agronomic traits except for the NDVI values, where the interaction will be discussed.

Vantage had significantly higher grain protein concentration than all other cultivars in all environments. Across environments the average grain protein was 154, 149, 141, and 139 g kg^{-1} for highest to lowest: Vantage, Glenn, Samson, and Faller, respectively (Table 1.6). These results in relative rank are in line with other published data (Ransom et al., 2012). Faller and Samson yielded more than Glenn and Vantage in individual environments (data not shown) and across environments.

Data indicated that the ranking of cultivars were not consistent across different environments for NDVI values; therefore no trends could be easily observed. For example at Prosper in 2012 Faller had the lowest NVDI value, but at Crookston in 2012 Faller had the highest NDVI value. Similar observations were seen with Vantage having a high NDVI value at Prosper (2012), but had the lowest NDVI value at Crookston both years. Inconsistent interactions maybe explained by differences in the rate of canopy closure across different cultivars and the same cultivar at different environments, which were visually observed.

Table 1.6. Means for normalized NDVI, normalized leaf N concentration, normalized chlorophyll concentration, normalized stem N concentration, grain protein concentration, grain yield, and total protein harvested, for four cultivars of HRSW across three environments.

	Zadoks Growth Stage 37						
Cultivar	NDVI†	N Concentration of Leaf†	Chlorophyll Concentration†	N Concentration of Stem†	Grain Protein	Yield	Total Protein Harvested
					g kg^{-1}	----------kg ha^{-1}-------	
Faller	0.97	0.96	0.77	0.96	138.9	4335	609
Glenn	0.98	0.98	0.78	0.94	149.1	3920	589
Samson	0.96	0.98	0.79	0.97	140.5	4303	610
Vantage	0.92	0.94	0.77	0.95	154.1	3792	586
Means	0.96	0.96	0.78	0.96	145.6	4087	598
CV%	10.05	4.05	10.33	11.20	4.4	15	15
LSD (0.05)	NS‡	0.01	NS	NS	8.3	347	NS

† Normalized Values.
‡ Not Significant.

Nitrogen Treatment

The ANOVA indicated that N rate by environment interactions for several variables were significant. These interactions were due to differences in magnitude for NDVI values, N concentration in the flag leaf and stalk samples, chlorophyll concentration, grain protein, grain yield, and total protein harvested. Therefore, N rates main effects will be discussed for all measurements since the interaction was due to differences in magnitude. There was a significant linear response to N rates for all variables measured based on orthogonal contrasts. All cultivars responded similarly to N treatments. It was then determined that it was appropriate to normalize (index) the data. Regression equations were then developed for each plant predictor after normalizing with the N rich treatment as previously described (Figure 1.1, 1.2, 1.3, 1.4, and 1.5). The measured values of N concentration, chlorophyll concentration, and NDVI values can be entered into the regression equations (in place of x) to determine at what value the grain protein level would be below or above the market requirements of 140 g kg^{-1}.

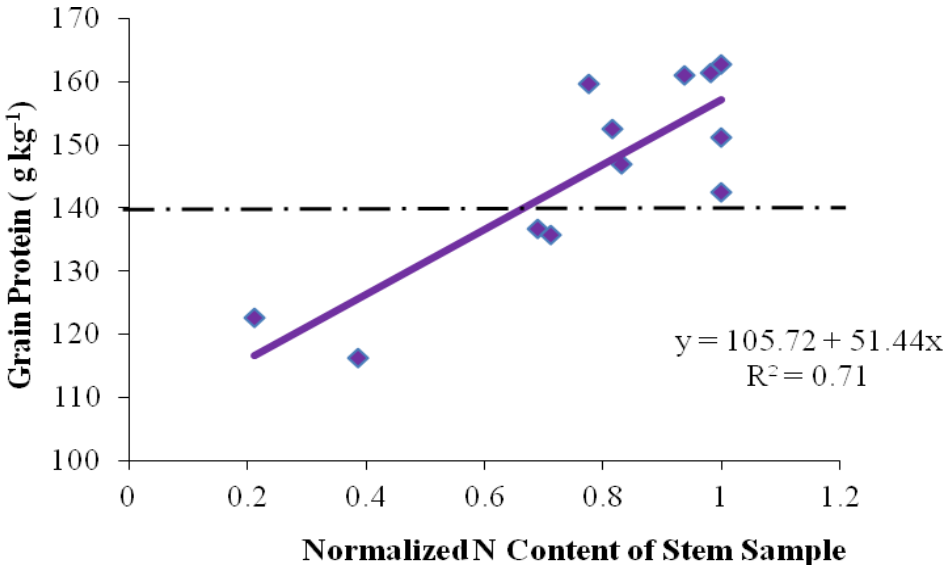

Figure 1.1. Linear regression of grain protein concentration (response variable) on normalized N concentration of stem sample (predictor) collected at ZGS 37 for all cultivars across three environments.

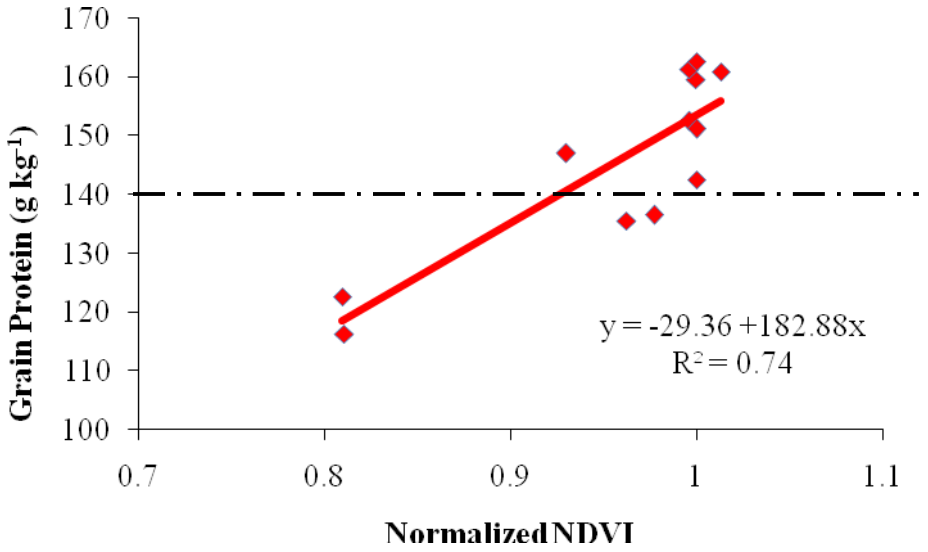

Figure 1.2. Linear regression of grain protein concentration (response variable) on normalized NDVI values (predictor) collected at ZGS 37 for all cultivars across three environments.

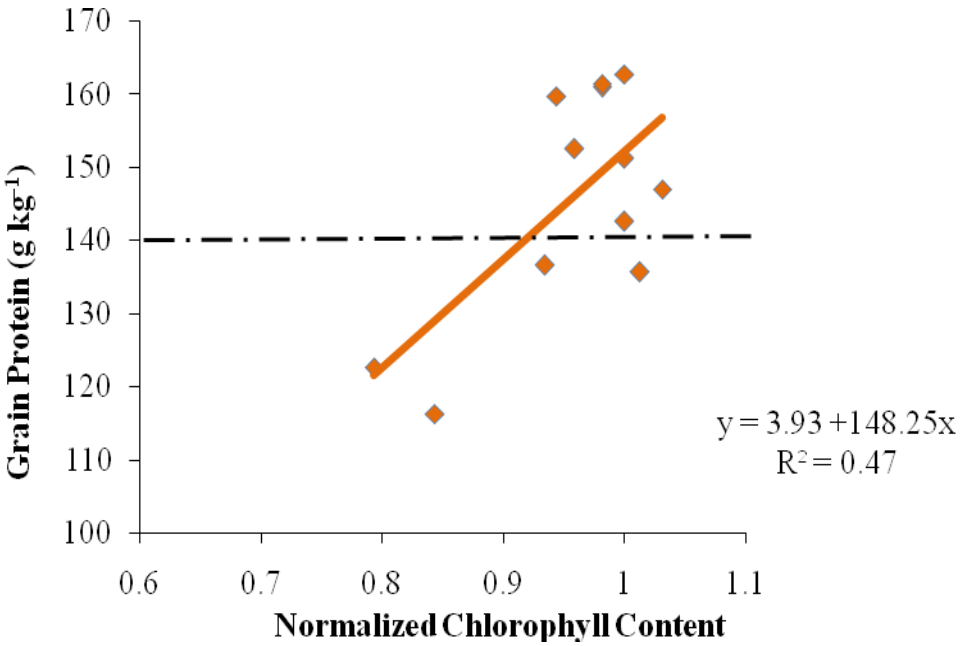

Figure 1.3. Linear regression of grain protein concentration (response variable) on normalized chlorophyll concentration (predictor) collected at ZGS 37 for all cultivars across three environments.

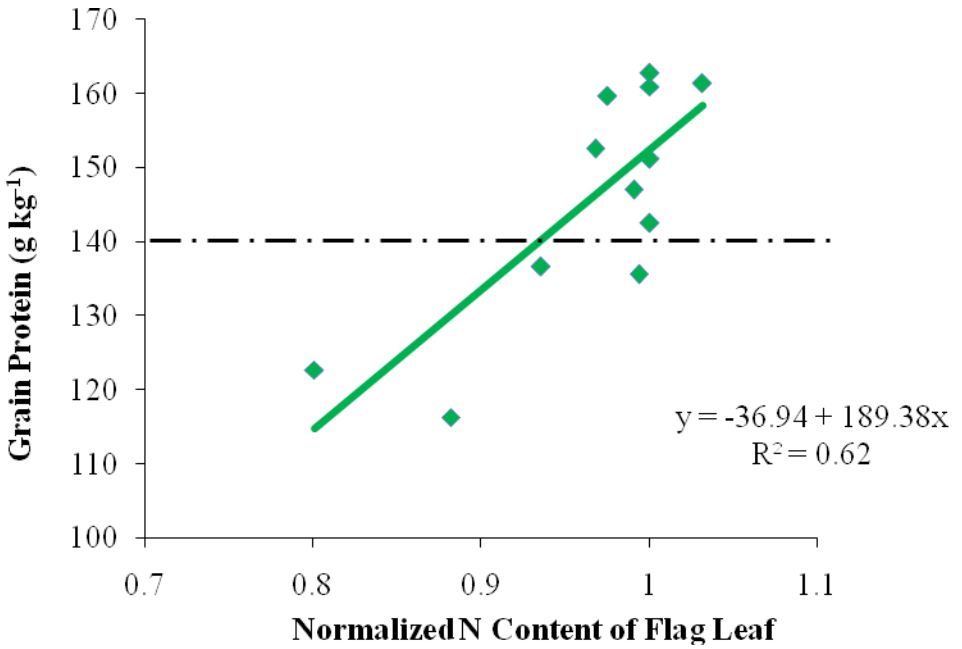

Figure 1.4. Linear regression of grain protein concentration (response variable) on normalized N concentration of leaf sample (predictor) collected at ZGS 37 for all cultivars across three environments.

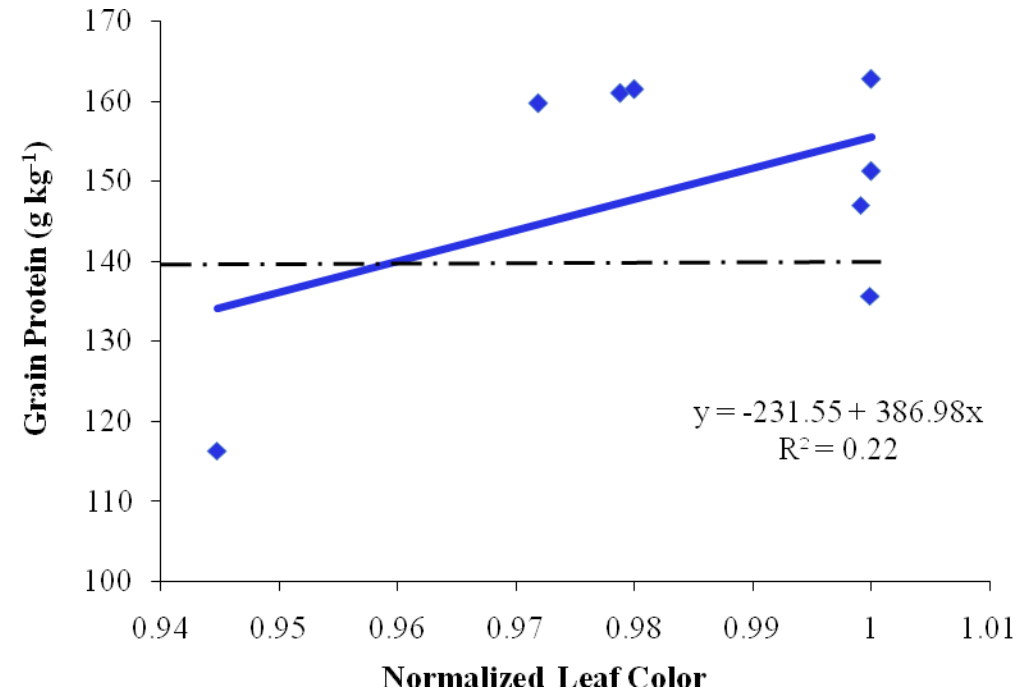

Figure 1.5. Linear regression of grain protein concentration (response variable) on normalized leaf color measurements (predictor) collected at ZGS 37 for all cultivars across three environments.

Peak stem N concentration, chlorophyll concentration, and flag leaf N concentration values measured at the ZGS 37 were found at 67 kg ha^{-1} of N with all cultivars (Table 1.7). A positive relationship between these plant predictors and grain yield and protein concentration were found at $p \leq 0.01$ (Table 1.11). Papastylianou et al., (1984), also found a significant relation between nitrate concentration in wheat stems at tillering and grain yield. Positive correlations between chlorophyll meter measurements and grain yield in winter wheat have also been observed at both the ZGS 30 (Follett et al., 1992) and ZGS 75 (Spaner et al., 2005). Brown et al. (2005) found that total N in the flag leaf around heading/flowering time, to be more closely associated with grain protein at harvest than similar measurements made earlier in the growing season. Strong correlations between chlorophyll concentration and grain protein concentration at the ZGS 71 have also been reported in wheat (Bail et al., 2005; Spaner et al., 2005).

Table 1.7. Means for normalized NDVI, normalized N concentration of leaf, normalized chlorophyll concentration, normalized N concentration of stem, grain protein concentration, grain yield, and total protein harvested, for four N rates across three environments.

	Zadoks Growth Stage 37						
N treatment (kg ha^{-1})	NDVI†	N Concentration of Leaf†	Chlorophyll Concentration†	N Concentration of Stem†	Grain Protein	Yield	Total Protein Harvested
					g kg^{-1}	--------kg ha^{-1}------	
0	0.87	0.89	0.86	0.46	132.4	3425	462
67	0.98	0.98	0.98	0.78	144.4	4117	592
134	0.97	1.00	0.99	0.88	153.6	4196	644
202	1.00	1.00	1.00	1.00	152.2	4610	695
Means	0.96	0.96	0.96	0.78	145.6	4087	598
CV%	10.05	4.05	8.39	14.15	4.4	15	9
LSD (0.05)	NS‡	0.07	0.08	0.24	NS	NS	NS

†Normalized Values.
‡ Not Significant.

Nitrogen rates also significantly influenced NDVI vales at the $p<0.10$. The correlation between NDVI values collected at the ZGS 37 and grain protein was $r = 0.48$ ($p<0.10$) (Table 1.11). Qualm et al. (2010) stated that NDVI showed promise in predicting grain protein of

HRSW under normal growing conditions with correlation coefficients between NDVI and grain protein between 0.54-0.68. These higher correlation coefficients may have been a result of the fact that NDVI measurements were made just prior to anthesis (ZGS 50-60) in that study. Others have reported poor correlations between NDVI and grain protein (Freeman et al., 2003; Wright et al., 2002). Freeman et al (2003) stated that the reason NVDI values are poor indicators of grain protein concentration are because NDVI values are not capable of determining how efficient the plant is at translocating N to the kernel.

In our study, the correlation between NDVI at ZGS 37 and grain yield was quite high ($r = 0.80$, ($p < 0.01$)) (Table 1.11). Good correlations between NDVI and grain yield have been documented by others in winter wheat (Raun et al., 2002) spring wheat (Osborne, 2007) and corn (Chang et al., 2003). NDVI values are better at predicting yield response than protein concentration because NVDI values indirectly measure vegetative biomass, which often relates to yield potential.

Nitrogen rate significantly influenced grain protein at the $p \leq 0.10$. Averaged over cultivars, protein concentration was 132, 144, 154, and 152 g kg^{-1} for 0, 67, 134, and 202 kg ha^{-1} of N rates, respectively (Table 1.7). The average protein for individual environments was 161, 139, and 138 g kg^{-1} for Prosper 2012, Crookston 2011, and Crookston 2012, respectively (data not shown). The large difference in protein concentrations between Prosper and Crookston may be related to soil N concentration differences prior to planting. At Prosper, the residual N was nearly 200 kg ha^{-1} higher than at Crookston in both years (Table 1.2). Total protein harvested had a similar trend to grain protein concentration, since this value is derived partially from grain protein concentration.

The average grain yield was not significant at Prosper in 2012 but yield was significant at Crookston both years (Table 1.8). Average grain yield was similar across all three environments. Reasons for significant responses to N rates were detected at Crookston both years maybe due to the level of residual N in the soil prior to planting. Residual soil nitrate levels at Prosper in 2012 were nearly 200 kg ha^{-1} higher than at Crookston both years. This may explain why no significant differences in yield response to N rate were detected at Prosper in 2012.

Table 1.8. Means for grain yield for four N rates collected at ZGS 37 for Prosper 2012, Crookston 2011, Crookston 2012, and Combined environments.

N treatment (kg ha^{-1})	Prosper 2012	Crookston 2011	Crookston 2012	Combined
	----------kg ha^{-1}----------			
0	4024	3062	3207	3425
67	4082	4028	4242	4117
134	4088	4673	3827	4196
202	4057	5105	4669	4610
Means	4062	4217	3986	4087
CV%	8	15	16	15
LSD (0.05)	NS	439	940	NS

Correlation between the different plant predictors were significant $p \leq 0.01$ (Table 1.9). Combining multiple plant measurements to one regression equation improved predicting protein concentration (Figure 1.6). Stepwise regression between normalized N concentration of leaf samples and chlorophyll concentration collected at the ZGS 37 provided the best prediction for grain protein ($r = 0.99$, ($p \leq 0.50$)). The multiple regression equation is: protein concentration (g kg-1) = [-143 + (N concentration of leaf sample X 857) – (chlorophyll concentration X 563)]. Others have also found a strong correlation between leaf N and chlorophyll concentration (Vos and Bom, 1993; Blackmer and Schepers, 1995; Olfs et al., 2005).

Table 1.9. Correlation between diagnostic measurements collected at the ZGS 37 for all cultivars across environments.

	N Concentration of Leaf	N Concentration of Stem	Chlorophyll Concentration	NDVI
	----------r value----------			
N Concentration of Leaf		0.92***	0.92***	0.91***
N Concentration of Stem	0.92***		0.93***	0.84***
Chlorophyll Concentration	0.92***	0.93***		0.84***
NDVI	0.91***	0.84***	0.84***	

*** Significant at (P≤0.01).

2012 Combined

In 2012, NDVI values, N concentration of leaf samples, chlorophyll concentration, NDVI values, and LCC values at ZGS 16 were collected. Leaf color chart values were also collected at ZGS 37. The combined data indicated that N rates and cultivar selection had no significant effect on these plant measurements. However, there were significant interactions between these factors and the environment.

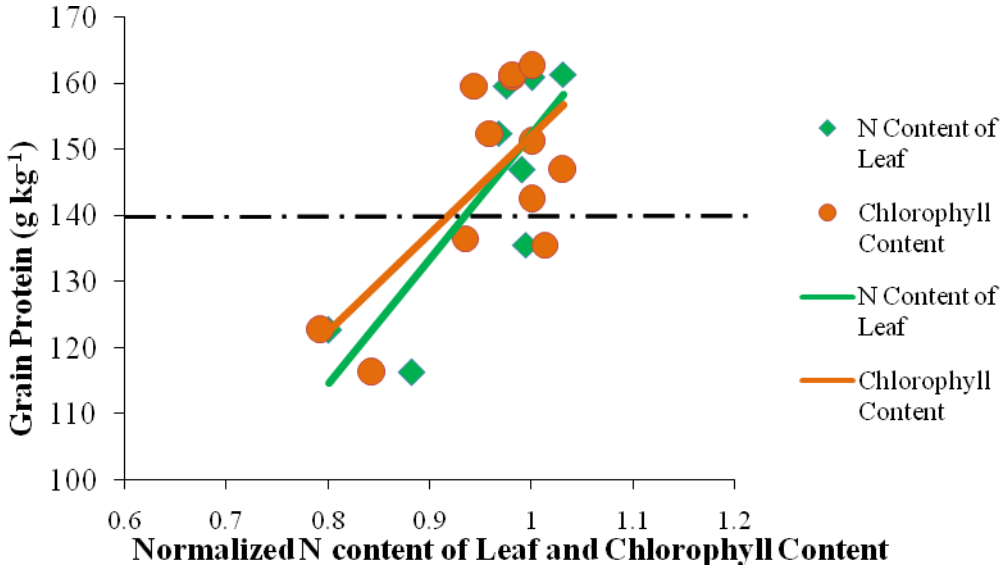

Figure 1.6. Linear regression of grain protein concentration (response variable) on normalized N concentration of leaf samples and chlorophyll concentration measurements (predictors) collected at ZGS 37 for all cultivars across three environments.

The interaction between N rate and environment can be explained by differences in magnitude for leaf N concentration, NDVI values, and chlorophyll concentration collected at

ZGS 16 (Table 1.12). Although data indicate that no significant differences at $p<0.05$ were detected across environments, the different values in N rate means for each measurement can be explained by response to individual environments (data not shown). Values at Prosper were higher than values at Crookston. This may be explained by soil N concentration prior to planting. At Prosper, the residue N was nearly 200 kg ha^{-1} higher than at Crookston both years (Table 1.2). Future experiments are needed to determine if measurements collected at the ZGS 16 can detect significant differences in final grain protein concentration.

The environment by cultivar interaction for LCC measurements collected at ZGS 37 indicated that the ranking of cultivars were not consistent across different environments; therefore no trends could be observed (data not shown). For example at Prosper, Vantage had the highest LCC value while at Crookston it had one of the lowest LCC readings. The interactions may be a consequence of the LCC lacking enough shades to detect subtle differences. Furthermore, additional replications maybe helpful in detecting significant differences.

Measurements taken at the ZGS 16 did not have as strong a correlation with grain yield and protein concentration as plant measurements collected at ZGS 37. Others found similar results for NDVI values (Raun et al., 2002; Osborne, 2007; Chang et al., 2003); N concentration of tissue samples (Papastylianou et al., 1984; Brown et al., 2005); and chlorophyll concentration (Follett et al., 1992; Fox et al., 1994; Spaner et al., 2005; Bail et al., 2005) measurements collected later in the growing season (after ZGS 30). The chlorophyll concentration and N concentration of leaf samples were the only plant predictors to have a positive relationship with protein concentration, while N concentration of leaf samples was the only plant measurement correlated to grain yield (Table 1.10). A linear response between LCC values (ZSG 16 and 37) and N rates was detected. This finding has been documented in wheat before (Maiti and Das,

2006). A correlation between LCC values collected at the ZGS 37 and protein concentration was found at $p \leq 0.01$.

Table 1.10. Correlation between measurements, grain yield and grain protein concentrations of all cultivars across single locations (Crookston and Prosper) and Combined in 2012.

	Yield			Protein		
	Prosper	Crookston	Combined	Prosper	Crookston	Combined
Variable	----------r value----------					
NDVI†	-0.218	0.354	0.302	-0.018	0.028	-0.197
Chlorophyll Concentration†	0.041	-0.053	0.566**	-0.062	0.543**	0.460*
N concentration of leaf†	0.145	0.679***	-0.212	0.040	0.602**	0.498**
Leaf color chart†	-0.044	-0.132	-0.219	0.143	0.227	0.410
Leaf color chart‡	0.049	0.537**	0.135	0.397	0.483*	0.691***

† measurements collected at the Zadoks GS 16.
‡ measurements collected at the Zadoks GS 37.
*,** , *** Significant at ($P \leq 0.10$) , ($P \leq 0.05$), and ($P \leq 0.01$) respectively.

Table 1.11. Correlation coefficients (r) plant measurements, grain yield and grain protein concentration of all cultivars across single locations (Crookston 2011, 2012 and Prosper, 2012) and Combined.

	Yield				Protein			
	Prosper 2012	Crookston 2011	Crookston 2012	Combined	Prosper 2012	Crookston 2011	Crookston 2012	Combined
Variable	r value							
NDVI	-0.56**	0.85***	0.81***	0.80***	0.51**	0.73***	NS ‡	0.48*
Chlorophyll Concentration	NS	0.90***	0.62**	0.85***	NS	0.60**	0.71***	0.68***
N Concentration of Leaf	NS	0.91***	0.81***	0.82***	NS	0.76***	0.69***	0.65***
N Concentration of Stem	NS	0.94***	0.76***	0.86***	NS	0.76***	0.87***	0.75***

*,** , *** Significant at (P≤0.10) , (P≤0.05), and (P≤0.01) respectively.
‡ Not Significant.

Table 1.12. Means for NDVI, N concentration of leaf, chlorophyll concentration for four N rates collected at ZGS 16 for Prosper 2012, Crookston 2012, and Combined environments in 2012.

	Prosper†			Crookston†			Combined†		
N treatment (kg ha^{-1})	NDVI	N Concentration of Leaf	Chlorophyll Concentration	NDVI	N Concentration of Leaf	Chlorophyll Concentration	NDVI	N Concentration of Leaf	Chlorophyll Concentration
0	1.03	0.99	1.03	0.98	0.93	0.91	1.00	0.96	0.97
67	1.00	1.00	1.06	1.10	0.95	0.93	1.06	0.98	0.99
134	1.01	1.01	0.98	1.08	0.96	1.10	1.05	0.98	1.04
202	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Means	1.01	1.00	1.01	1.04	0.96	0.99	1.02	0.98	1.00
CV%	3.04	2.30	13.30	12.70	6.40	15.30	9.70	5.00	13.60
LSD (0.05)	NS‡	NS	NS	NS	NS	NS	NS	NS	NS

†Normalized Values.
‡ Not Significant.

Conclusion

All plant predictors evaluated at the ZGS 37 were able to can predict final protein concentration, which is similar to previously published studies. The best predictors for final grain protein concentration were chlorophyll concentration, leaf N concentration, and stem N concentration. Normalized NDVI measurements lower than 0.9 suggest supplement N is required to reach sufficient protein levels.

Although chlorophyll concentration and N tissue concentration are better indicators of predicting grain protein concentration then NDVI because these measurements are direct measurements, they are somewhat limited in practical use because they can only measure a small area of the crop canopy within each field and do not detect the variability within a field like noncontact sensors, which can survey a large geographic area depending on the sensor's field of view.

The best combination of plant measurements for predicting grain protein concentration was leaf N concentration combined with chlorophyll concentration collected at the ZGS 37.

The same linear response to N fertilizer was detected among individual cultivars with different protein characteristics. This allowed for the same predictive equation for determining grain protein and grain yield to be used across multiple cultivars thereby greatly increasing their applicability.

Measurements that were collected at the ZGS 16 are inadequate at predicting supplement N needs for reaching desired protein concentration and grain yield compared to measurements collected at the ZGS 37. This observation is similar to previous studies. Measurements collected at the ZGS 37 still give producers time to apply a late-season application of N if measured values indicate protein concentration will be low. Plant based predictors hold promise to determine the

need for supplement N to improve grain protein concentration, although validation of the regression models is still needed.

References

Bail, M.L., M.H. Jeuffroy, C. Bouchard, and A. Barbottin. 2005. Is it possible to forecast the grain quality and yield of different varieties of winter wheat from Minolta SPAD meter measurements. Europ. J. Agron. 23:379-391.

Blackmer, T.M., and J.S. Schepers. 1995. Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. J. Prod. Agric. 8:56-60.

Brown, B., M. Westcott, N. Christensen, B. Pan, and J. Stark. 2005. Nitrogen management for hard wheat protein enhancement. Ext. Publ. PNW 578. Pacific Northwest Ext. Serv., Moscow, ID.

Buckee, G.K. 1994. Determination of total nitrogen in barley, malt and beer by Kjeldahl procedure and the Dumas combustion method. J. Inst. Brew. 100:57-64.

Cavanaugh, K.J., B.R. Durgan, R.K. Zollinger, and W.A. Selberg. 2009. Herbicide and nonherbicide injury symptoms on spring wheat and barley. Ext. Publ. WW-06967. Univ. of Minnesota Ext. Serv., St. Paul.

Chang, J., D.E. Clay, K. Dalsted, S. Clay, and M. O'Neill. 2003. Corn (*Zea mays* L.) yield prediction using multispectral and multidate reflectance. Agron. J. 95:1447-1453.

Debaeke, P., P. Rouet, and E. Justes. 2006. Relationship between the normalized SPAD index and the nitrogen nutrition index: Application to durum wheat. J. Plant Nutr. 29:75-92.

Dellinger, A.E., J.P. Schmidt, and D.B. Beegle. 2008. Developing nitrogen fertilizer recommendations for corn using an active sensor. Agron. J. 100:1546-1552.

Evans, J.R. 1983. Nitrogen and photosynthesis in the flag leaf of wheat. Plant Physiol. 72:297-302.

Fageria, N.K., V.C. Baligar, and R.B. Clark. 2006. Physiology of crop production. Haworth Press, Binghamton, NY.

Flynn, R., S.T. Ball, and R.D. Baker. 1999. Sampling for plant tissue analysis. Ext. Publ. Guide A- 123. Available at http://aces.nmsu.edu/pubs/_a/a-123.html. [verified 26 Nov. 2012]. New Mexico State Univ. Ext. Serv., Las Cruces, NM.

Follet, R.H., R.K. Follet, and A.D. Halvorson. 1992. Use of a chlorophyll meter to evaluate the nitrogen status of dryland winter wheat. Commun. Soil Sci. Plant Anal. 23:687-697.

Fox, R.H., W.P. Piekielek, and K.M. Macneal. 1994. Using a chlorophyll meter to predict nitrogen fertilizer needs of winter wheat. Commun. Soil Sci. Plant Anal. 25:171-181.

Francis, D.D., and W.P. Piekielek. 1999. Assessing crop nitrogen needs with chlorophyll meters. Site-specific management guide SSMG-12:1-4. Potash and Phosphate Inst., Norcross, GA.

Franzen, D.W. 2010. North Dakota fertilizer recommendation tables and equations. Ext. Publ. SF-882:1-16.North Dakota State Univ. Ext. Serv., Fargo.

Freeman, K.W., W.R. Raun, G.V. Johnson, R.W. Mullen, M.L. Stone, and J.B. Solie. 2003. Late-season prediction of wheat grain yield and grain protein. Commun. Soil Sci. Plant Anal. 34:1837-1852.

Holland, K.H., and J.S. Schepers. 2010. Derivation of a variable rate nitrogen application model for in-season fertilization of corn. Agron. J. 102:1415-1424.

Holland Scientific. 2008. Crop circle mapping/VRT system. Available at http://www.hollandscientific.com/CC_VRA-mapping.html. [verified 26 Nov. 2012]. Holland Scientific, Lincoln, NE.

Hussain, F., K.F. Bronson, S. Yadvinder, S. Bijay, and S. Peng. 2000. Use of chlorophyll meter sufficiency indices for nitrogen management of irrigated rice in Asia. Agron. J. 92:875-879.

International Rice Research Institute (IRRI). 2011. Site specific nutrient management. Available at http://irri.org/index.php?option=com=k2&view=item&id=10980+Itemid =100331&lang=en. [verified 6 Mar. 2012]. International Rice Research Institue, Metro Manila, Philippines.

Jenner, C.F., T.D. Ugalde, and D. Aspinall. 1991. The physiology of starch and protein deposition in the endosperm of wheat. Aust. J. Plant Physiol. 18:221-226.

Johnson, G.V., and W.R. Raun. 2003. Nitrogen response index as a guide to fertilizer management. J. Plant Nutr. 26:249-263.

Lawlor, D.W., M. Kontturi, and A.T.Young. 1989. Photosynthesis by flag leaves of wheat in relation to protein, Ribulose Bisphosphate Carboxylase activity and nitrogen supply. J. Exp. Bot. 40:43-52.

Ma, B.L., M.J. Morrison, and L.M. Dwyer. 1996. Canopy light reflectance and field greenness to assess nitrogen fertilization and yield of maize. Agron. J. 88:915-920.

Maiti, D., and D.K. Das. 2006. Management of nitrogen through the use of leaf colour chart (LCC) and soil plant analysis development (SPAD) in wheat under irrigated ecosystem. Archives Agron. Soil Sci. 52:105-112.

North Dakota Agricultural Weather Network. 2013. Monthly data table form. Available at http://ndawn.ndsu.nodak.edu/monthly-table-form.html [verified 8 Feb 2013]. Fargo, ND.

NTech Industries. 2009. NTech Industries: GreenSeeker frequently asked questions, chlorophyll sensors perform variable rate fertilizing of wheat and corn crops. NTech Industries, Ukiah, CA.

Olfs, H.W., K. Blankenau, F. Brentrup, J. Jasper, A. Link, and J. Lammel. 2005. Soil and plant-based nitrogen fertilizer recommendations in arable forming. J. Plant Nutr. Soil Sci. 168:414-431.

Osborne, S.L. 2007. Utilization of existing technology to evaluate spring wheat growth and nitrogen nutrition in South Dakota. Commun. Soil Sci. Plant Anal. 38:949-958.

Papastylianou, I., R.D. Graham, and D.W. Puckridge. 1984. Diagnosis of nitrogen statues of wheat at tillering and prognosis for maximal grain yield. Commun. Soil Sci. Plant Anal. 15:1423-1436.

Penuelas, J., J.A. Gamon, A.L. Fredenn, J. Merino, and C.B. Field. 1994. Reflectance indices associated with physiological changes in nitrogen and water-limited sunflower leaves. Remote Sensing Envir. 48:135-146.

Qualm, A.M., S.L. Osborne, and R. Gelderman. 2010. Utilizing existing sensor technology to predict spring wheat grain nitrogen concentration. Commun. Soil Sci. Plant Anal. 41:2086-2099.

Ransom, J.K., M. Mergoum, S. Simsek, M. Aceveda, T. Friesen, M. McMullen, S. Zhong, R. Olson, E. Eriksmoen, B. Hanson, G. Martin, G. Bradbury, and B. Schatz. 2012. North Dakota hard red spring wheat variety trail results for 2012 and selection guide. Ext. Publ. A-574-12:1-8. North Dakota State Univ. Ext. Serv., Fargo.

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

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 applications. Agron. J. 94:815-820.

Robertson, G.P. 2004. Abatement of nitrous oxide, methane and other non-CO_2 greenhouse gases: The need for a systems approach. p. 493-506. *In* C.B. Field and M.R. Raupach (ed.) The global carbon cycle. Integrating humans, climate, and the natural world. SCOPE 62, Island Press, Washington, DC.

Schepers, J.S., D. Francis, and G. Varvel. 2006. Chlorophyll meters. USDA-ARS, Gov. Print. Office, Washington, DC.

Seth, J., T.T. Hebert, and G.K. Middleton. 1960. Nitrogen utilization in high and low protein wheat varieties. Agron. J. 52:207-209.

Shanahan, J.F., J.S. Schepers, D.D. Francis, G.E. Varvel, W.W. Wilhelm, J.S. Tringe, M.R. Schlemmer, and D.J. Major. 2001. Use of remote sensing imagery to estimate corn grain yields. Agron. J. 93:583-589.

Shaver T.M., R. Ferguson, and J. Shanahan. 2010. Crop canopy sensors for in-season nitrogen management. Crop Production Clinics Proceedings. Univ. of Nebraska-Lincoln Ext. Serv., Lincoln.

Shaver, T.M., R. Khosla, and D.G. Westfall. 2011. Evaluation of two crop canopy sensors for nitrogen variability determination in irrigated maize. Precision Agric. 12:892-904.

Solari, F., J.F. Shanahan, R.B. Ferguson, and V.I. Adamchuk. 2010. An active sensor algorithm for corn nitrogen recommendations based on a chlorophyll meter algorithm. Agron. J. 102:1090-1098.

Spaner, D., A.G. Todd, A. Navabi, D.B. McKenzie, and L.A. Goonewardene. 2005. Can leaf chlorophyll measures at differing growth stages be used as an indicator of winter wheat and spring barley nitrogen requirements in eastern Canada? J. Agron. Crop Sci. 191:393-399.

Terman, G.L. 1979. Yields and protein concentration of wheat grain as affected by cultivar, N, and environmental growth factors. Agron. J. 71:437-440.

Thom, W.O., J.R. Brown, and C.O. Plank. 2000. Sampling for corn plant tissue analysis. Ext. Publ. NCH-15:1-2. Iowa State Univ. Ext. Serv., Ames, IA.

USDA-Natural Resources Conservation Service. 2012. Web Soil Survey. Available at http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm (verified 7 Dec. 2012). USDA_NRCE Washington, DC.

Vos, J., and M. Bom. 1993. Hand-held chlorophyll meter: A promising tool to assess the nitrogen status of potato foliage. Potato Res. 36:301-308.

Woolfolk, C.W., W.R. Raun, G.V. Johnson, W.E. Thomason, R.W. Mullen, K.J. Wynn, and K.W. Freeman. 2002. Influence of late-season foliar nitrogen applications on yield and grain nitrogen in winter wheat. Agron. J. 94:429-434.

Wright. D.L., R.D. Ramsey, V.P. Rasmussen Jr., and J.W. Ellsworth 2002. Midseason nitrogen application using remote sensing. *In*: Proceedings of the Sixth International Conference on Precision Agriculture. ASA-CSSA-SSA, Madison, WI.

Wright, D.L., V.P. Rasmussen, C.M.U. Neale, K.Harman, G. Searle, D. Grant, and C. Holle. 2001. A comparison of nitrogen stress detection methods in spring wheat. ERIM 3rd Int. Conf. Geospatial Inf. Agric. For. 5-7 Nov. 2001. Denver, CO.

Yang, W.H., S. Peng, J. Huang, A.L. Sanico, R.J. Buresh, and C. Witt. 2003. Using leaf color charts to estimate leaf nitrogen status of rice. Agron. J. 95: 212-217.

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res.14:415-421.

ARTICLE 2: NITROGEN SOURCE AND APPLICATION TIMING AFFECTS PROTEIN CONCENTRATION IN HARD RED SPRING WHEAT

Abstract

Standard market grain protein concentration for HRSW is 140 g kg^{-1}. This study was conducted to determine the best method for increasing grain protein with an in-season application of nitrogen (N), while maintaining yield and maximizing net return. Field experiments were conducted at Crookston, MN, Hettinger, ND, in 2011- 2012, and Prosper, ND in 2012 and consisted of a factorial combination of cultivars receiving N fertilizer treatments. Supplemental N caused leaf burn and increased grain protein concentration. The best management practice remains to apply all recommend N fertilizer prior to seeding. Spring wheat growers are advised to only consider additional N at early grain fills to increase grain protein concentration when grain yield is likely to exceeded yield considerations when preplant N recommendations were determined.

Introduction

Grain protein concentration is often an important factor when marketing HRSW. High protein concentration is associated with increased gluten strength and loaf volumes, which are both important factors in characterizing the milling and baking quality of wheat (Woolfolk et al., 2002). In the Upper Midwest of the USA, the standard market grain protein concentration for hard red winter wheat (HRWW) and HRSW is 120 g kg^{-1} and 140 g kg^{-1}, respectively (Bly and Woodard, 2003). When producers do not meet these market standards they may receive discounted prices, while a premium might be paid for grain protein concentration above 140 g kg^{-1}. The extent of these discounts and premiums vary greatly from year to year and within the year (Frayne Olson, personal communication). For example, in the fall of 2009, discounts ranged

from \$7.30 Mg^{-1} at the beginning of September to a \$51.40 Mg^{-1} discount by late September (Olson, 2009). Although these deductions can vary year-to-year, timing at greatest price fluctuation usually occurs during spring wheat harvest.

Currently the nitrogen use efficiency (NUE) of cereal crops worldwide is 33%, as a majority of the available N is lost due to leaching, erosion, denitrification or volatilization (Raun and Johnson, 1999). Maximizing NUE has become increasingly important in crop management systems for both economic and environmental reasons (Mahler et al., 1994). A split-application of N between planting and flowering can be one way to increase NUE (Strong, 1982). Split applications may allow for higher yield and grain protein responses compared to a single application in certain situations.

Applying all of the N fertilizers preplant or at seeding has been a common practice for wheat producers in North Dakota and Minnesota (Rehm and Franzen, 2005). A fall application of anhydrous ammonia (82-0-0) or dry urea (46-0-0) can also be effective; however, applications should not begin before October 1 or until soil temperatures measured at the 10.2 cm depth fall below 10 ^{o}C between 8 and 10 a.m. in North Dakota (Franzen, 2010). This is due to rapid conversion of urea and ammonia to nitrate by soil microbes at warmer temperatures. When applying urea (fall or spring) or urea ammonia nitrate (UAN) (spring) the rate of ammonia volatilization is less when it is banded rather than broadcasted on the surface. The risk of ammonia volatilization greatly increases if no measureable amount of precipitate occurs within several days after a surface application particularly if the pH of the soil is greater than seven.

Dry fertilizers can be applied as a top-dress (broadcasted) or a side-dress. Liquid N fertilizers can be applied to the foliage or side-dressed with streamer bars. It should be noted that liquid forms of fertilizer N can be phytotoxic when they contact leaf tissue because of the high

salt concentration (Garcia and Hanway, 1976; Rader et al., 1943). Studies have shown that a urea solution causes less tissue burn than UAN; however, a urea solution is more susceptible to volatilization (Gooding and Davis, 1992; Bremner, 1995). To help reduce loss through volatilization, others have looked at adding urease inhibitors such as dihyric phenols and quinones to the urea solutions (Bremner and Douglas, 1971, 1973). Krogmeier et al. (1989) found that by adding a urease inhibitor to urea fertilizer when applied to soybeans (*Glycine max*) actually increased foliar burn due to increased accumulations of toxic urea instead of NH_3. The degree of leaf burn depends on the timing of application and the cultivar (Gooding 1988). There are several tactics to help reduce the risk of tissue burn. One option is to use streamer bars (Anon, 1987). This reduces the contact between the solution and the foliage. However, a late-season application with streamer bars may be less effective than a foliar application because uptake by the roots has diminished and a rain event is needed to move the N to the roots (Seth and Mosluh, 1981). Another recommendation is to apply the fertilizer in the cool of the day and when the humidity is high (Garcia and Hanway, 1976).

Yield responses to in-season applications of N depend on timing (Gooding and Davies, 1992). A foliar application of urea at anthesis (ZGS 60) can significantly increase yield (Gholami et al., 2011; Ottman et al., 2000; Varga and Svecnjak, 2006). However, other studies have shown that yield responses start to decline when a foliar application of N is applied after the flag leaf has emerged (ZGS 37) (Finley et al., 1957; Strong, 1982). Inconsistent yields responses were recorded in Oklahoma over years and locations after late-season foliar N applications (Woolfolk et al., 2002).

When comparing grain yield responses to a late season foliar N applications, a positive grain protein concentration response is more consistently and frequently reported (Gooding and

Davis, 1992). Foliar N applied immediately following anthesis (ZGS 69) gave the best response in increasing protein concentration (Finney et al., 1957; Bly and Woodard, 2003). Strong (1982) also found that liquid N resulted in substantially higher grain protein concentration compared with dry granular N when applied at late growth stages. Endres and Schatz (1993) at Carrington, ND, found that using a foliar application of liquid N solution of UAN (28-0-0) at a rate of 34 kg N ha^{-1} resulted in the highest concentration of grain protein when applied immediately after anthesis (ZGS 69). This application resulted in a 0.5-1.0% increase in protein concentration. Although cultivar selection, planting dates, soil type, and growing conditions can affect protein concentration (Brown, 2000), a late-season N application at anthesis (ZGS 69) can increase protein concentration (Rawluk et al., 2000). Overall, the best management practice to achieve the desired grain yield and protein concentration levels is to target and manage N early in the season. A late season N application might be considered to optimize grain protein concentration, if anticipated grain yields are likely to exceed yield expectations when preplant N recommendations were determined (Wuest and Cassman, 1992).

Large price discounts have been received by producers when they have not met market standards in recent years (2009-2011). Wheat producers are seeking methods to improve protein concentration while maintaining high grain yields. This study considered different N rates, different forms of N, and different N application timings to determine which treatments provides the best response in increasing protein concentration. An economic evaluation of treatments was performed to determine the most economic N fertilization methods.

Objective

The objective of this research was to determine the best method to increase grain protein concentration with an in-season application of N, while maintaining grain yield and maximizing net return.

Materials and Methods

General Information

Field experiments were conducted at the University of Minnesota Northwest Research and Outreach Center, Crookston, MN, (Latitude = 47. 48° N, Longitude = -96.36° W); the NDSU research fields near Prosper, ND, (Latitude = 47.00° N , Longitude = -97.11° W) and the Hettinger Research Extension Center in Hettinger, ND, (Latitude = 46. 03° N, Longitude = -102.38° W) in 2011 and 2012. Table 2.1 lists the soil series, soil taxonomy and slope at each location.

Table 2.1. Soil series, taxonomy and slope at Crookston, MN, Prosper and Hettinger, ND in 2011 and 2012.

Location	Soil Series†	Soil Taxonomy‡	Slope
			%
Crookston	Wheatville –Gunclub	Coarse-silty, mixed, smectitic, superactive frigid Aeric Calciaquolls Fine-silty, mixed, superactive, frigid Aeric Calciaquolls	0-2
Prosper	Kindred–Bearden	Fine-silty, mixed, superactive, frigid Typic Endoaquolls Fine-silty, mixed, superactive, frigid Aeric Calciaquolls	0-2
Hettinger	Belfield–Savage–Daglum	Fine, smectitic, frigid Glossic Natrustolls Fine, smectitic, frigid Vertic Argiustolls Fine, smectitic, frigid Vertic Natrustolls	0-2

† Soil data obtained from (USDA-NRCS, 2012).
‡ Soil taxonomy listed on individual lines based on hyphenated soil series name.

Soil samples were collected in the spring to determine the levels of N, phosphorus (P), potassium (K), pH and organic matter at each location (Table 2.2). Five random core samples from the trial were collected and combined prior to analysis.

Table 2.2. Previous crop, available N, P, K, pH and organic matter levels by sampling depth at Crookston, Prosper, and Hettinger in 2011 and 2012.

Location	PC†	Depth	N	P	K	pH	OM†
		Cm	kg ha^{-1}	mg kg^{-1}			%
			2011				
Crookston	soybean	0-61	28.0	3	145	8.1	NA
Prosper	soybean	0-15	7.8	55	250	7.6	3.9
		15-61	16.8	9	170	8.0	2.8
Hettinger	field pea‡	0-15	63.8	32	650	6.4	3.6
		15-61	57.1	4	305	8.0	2.1
			2012				
Crookston	soybean	0-61	34.0	4	137	8.3	NA
Prosper	soybean	0-15	112.0	41	315	7.8	3.3
		15-61	238.6	21	230	7.8	2.5
Hettinger	wheat	0-15	49.3	26	375	6.1	2.8
		15-61	33.6	3	100	7.9	1.9

†PC = Previous crop; OM = Organic matter
‡ Field pea = *Pisum sativum*

Treatments consisted of a factorial combination of cultivars and N fertilizer treatments arranged in a randomized complete-block design with four replicates. The treatment structure employed at all sites is reported in Table 2.3. The recommended rate of N was based on a historical yield expectation of 4035 kg ha^{-1}. The urea solution was prepared by mixing dry urea with lukewarm water to provide a solution that was 50% urea by weight, resulting in a solution with a composition of 23-0-0. For the treatment requiring a urease inhibitor, (N-(n-butyl) thiophosporic triamide) (NBPT) (Agrotain, Koch Agronomic Services, LCC, Wichita, KS) was added to the urea solution at the label recommended rate of 4.2 ml kg^{-1}.

In treatments receiving foliar fertilizer, solutions were applied at a rate of 187 l ha^{-1} using XR TeeJet 8002 VS nozzles (TeeJet Technologies, Wheaton, IL). Applications were applied using a hand-held backpack sprayer with a pressure of 207 kPa and a constant speed of 4.8 km h^{-1}. The boom was kept 46 cm above the crop canopy. Average temperature, relative humidity, bare soil temperatures, average wind speed, wind direction, and weather conditions at the time of application were recorded for each location (Table A.4, A.5, and A.6). One week

after the treatments were applied, the percent of injury (leaf burn) to the plant foliage was estimated visually. This observation was based on symptoms across the entire plot using a scale of 0 % (no injury) to 100 % (complete crop destruction).

Table 2.3. Treatment structure employed that included N source, N rate, and time of application, Crookston, MN, Prosper and Hettinger, ND, (2011-2012).

Nitrogen Treatment (kg ha^{-1})
1) 123 urea applied pre-plant
2) Base rate (90 kg ha^{-1} urea applied pre-plant) †
3) Base rate + 34 UAN‡ applied ZGS 69 (post anthesis)
4) Base rate + 34 urea-water solution applied ZGS 69
5) Base rate + 34 UAN applied ZGS 45 (boot stage)
6) Base rate + 34 urea applied ZGS 45
7) Base rate + 34 UAN applied ZGS 92 (physiological maturity)
8) Base rate + 34 urea-water solution+ NBPT§ applied ZGS 69

† 90 kg ha^{-1} urea PP
‡ UAN = urea ammonium nitrate (28-0-0)
§NBPT = N-n-butyl thiophosphoric triamide urease inhibitor

Three HRSW cultivars were planted at an optimal planting date based on recommendations from the NDSU Extension and the UMN Extension Services. The three selected cultivars were 'Faller,' 'Glenn,' and 'RB07.' Faller was chosen because it is known to be high yielding with a lower protein concentration. Glenn has generally a higher protein concentration compared with Faller while RB07 has intermediate protein concentration and medium yield (Ransom et al., 2012). The agronomic characteristics of these cultivars are described in Table 2.4.

Table 2.4. Characteristics of three HRSW cultivars chosen for late season N foliar applications.

					Average Yield Within Region†			
Cultivar	Origin‡	Year Released	Height	Days to Head	Eastern ND	Western ND	Protein§	Volumetric Weight
			cm		-----Mg ha^{-1} -----		g kg^{-1}	kg $^{-L}$
Faller	ND	2007	89	65	5.5	3.7	138	72.3
Glenn	ND	2005	94	61	4.8	3.4	149	76.4
RB07	MN	2007	81	62	5.0	3.8	142	74.1

† Based on a three year average (2010-2012).
‡ Refers to agent or developer: ND = North Dakota State University, MN = University of Minnesota.
§ Protein at 12% moisture.
Source: Ransom et al., 2012.

Planting and Plot Maintenance

Trials at Crookston in 2011 and 2012 were sown at a rate of 3 million viable seeds ha^{-1} using an Almaco double disk plot drill with 15-cm row spacing and ten rows. Before trials were planted, a uniform seedbed was prepared with a field cultivator. Due to an extremely wet spring, seeding was delayed in 2011until May 17. In 2012 with a mild winter and favorable spring, planting was completed on April 11.

Trials at Prosper in both 2011 and 2012 were sown using a seven row Great Plains 3P605NT drill (Great Plains Mfg Inc., Salina, KS) with 18 cm row spacing. Before trials were planted, a uniform seedbed was prepared using a field cultivator. Due to an extremely wet spring, seeding was delayed until May 19. With a mild winter and a favorable spring, trials were planted on April 19, 2012. Trials were sown at a rate of 4 million viable seeds ha^{-1}. Prior to planting, seeds were treated with tebuconazole at the rate of 3 ml kg^{-1} seed, using a batch seed treatment machine Hege II (Wintersteiger AG, Ried, Austria).

Trials at Hettinger in both 2011 and 2012 were planted using a custom-made seven row no-till double-disc opener seeder (Fabro Enterprises Ltd, Swift Current, Sask. Canada) and a seven row custom made self-propelled cone seeder (Fabro Enterprises Ltd, Swift Current, Sask. Canada), with 18 cm row spacing. In 2011 trials were planted on May 9. In the fall of 2011, 112 kg ha^{-1} of 46-0-0 was broadcasted and incorporated into the soil where plots for 2012 were planted. Fifty-six kg ha^{-1} of 11-52-0 fertilizer was placed with the seed during planting both years. Trials for 2012 were planted on April 4th. Seed treatment and sowing rate were the same as trials in Prosper.

Experimental plots at Crookston were 2 m wide and 7 m long. Borders between plots were 0.5 m and alleys between reps were cut mid-season at a width of 2.4 m. Harvest area was

1.5 m wide by 4.6 m long. Prosper plots were 1.5 m wide and 5.2 m long. Gaps between plots were 0.3 m and alleys between replications were cut mid-season at a width of 1.5 m. The harvested area was 1.5 m by 3.7 m. Hettinger plots were 1.5 m wide by 8.5 m long. Borders between plots were 0.4 m and alleys between reps were cut mid-season at a width of 2.4 m. The harvest area was 1.5 m wide by 5.2 m long. Plots were trimmed from each end using a rotovator (Crookston and Prosper (2011)) or mowed (Hettinger and Prosper (2012)) to the lengths previously mentioned to produce an alley. Border plots of wheat were planted around the outside to ensure similar competition as interior plots.

Stand counts were taken in 2012 at the ZGS 12 at Crookston and Prosper by counting the number of plants in a randomly selected 30-cm area of rows two and six at Prosper and rows two and nine at Crookston, then the number the number of plants from both rows were averaged, and converted to plants m^{-2}. Prior to harvest, lodging notes and plant heights were taken. Lodging was based on a scale of 0-10 for the entire plot length with zero having no signs of lodging to ten being completely flat. Plant heights were collected by taking the average height of the erect plants from the soil surface to the top of the awns.

Weed Control

Broadleaf and grassy weeds at Crookston were controlled with a tank mix application at the ZGS 14 of bromoxynil and MCPA at 350 g, and 700 g ai ha^{-1}, respectively, and pinoxaden at 39 g ai ha^{-1}. In Prosper weeds were controlled with an application of fenoxaprop, pyrasulfotole, bromoxynil at 89 g, 40 g, and 198 g ai ha^{-1}, respectively, at the ZGS 12. A fungicide application of propiconazole and trifloxystrobin at 55g and 55g ai ha^{-1}, respectively, was also applied at the ZGS12. In Hettinger weeds were controlled with an application of fenoxaprop , pyrasulfotole,

bromoxynil at 89 g, 40 g, and 198 g ai ha^{-1} , respectively, at the ZGS12. Clopyralid, and fluroxypyur, at 138 g and 138 g ai ha^{-1}, respectively, were applied at the ZGS 15.

Harvest Methods

At Prosper trials were harvested using a Wintersteiger Classic plot combine (Wintersteiger Ag, Ried, Austria). Following harvest, seed was dried (if necessary) and cleaned using a Clipper Office Tester and Cleaner (Seedburo Equipment Co., Chicago, IL). Data collected included: yield, test weight, protein and 1000 kernel weight. Moisture and test weight were recorded using a GAC 2100 moisture tester (DICKEY-John Corp., Minneapolis, MN). Yield was calculated by weighing the plot sample with a scientific scale (RS-232, Scientech Inc., Gaitherseberg, MD), adjusting to a moisture concentration of 13.5% and adjusting for the individual length of each plot. Grain protein concentration was measured using a 0.5 kg sub-sample of seed from each plot on a Diode Array 7200 NIR Analyzer (Perten Instruments, Springfield, IL). Total protein was also calculated by multiplying the grain yield by the protein concentration of the plot and dividing by hundred. Thousand-kernel weight of the samples was determined by counting five hundred seeds with a with a seed counter (Model 850-3, International Marketing and Design Corp., San Antonio, TX), weighing them with the RS-232 Scientech Scale, adjusting them to a moisture concentration of 13.5%, and multiplying by two.

In 2011 and 2012, HRSW at Crookston was harvested using a Zurn 150 (Zurn Harvesting GmbH & Co., Schontal-Westernhausen, Germany) plot combine. After harvest, grain yield, test weight (volumetric weight), and grain protein were determined. Test weight was measured using the official GIPSA method with the Seedburo filling hopper and pint cup (Seedburo Equipment Co., Des Plaines, IL). Moisture and grain protein concentration were measured by NIT using the Tecator Infratec 1229 Grain Analyzer (Foss North American INC., Eden Prairie, MN) following

AACC method 39-10 (USDA-ARS, 2010). Grain yield was calculated by adjusting the plot weight to a moisture concentration of 13.5% and converting the weight to kg ha^{-1}. Procedures for determining thousand kernels were similar to the method used for trials at Prosper.

At Hettinger, HRSW was harvested using a Kincaid 8XP plot combine (Kincaid Equipment Manufacturing, Haven, KS). Moisture, test weight, and grain yield were determined with a Grain Gauge weighing system (Wintersteiger Ag, Ried, Austria) installed on the plot combine. Grain yield samples were adjusting to a moisture concentration of 13.5% and individual length of each plot. Grain protein concentration was measured using a 0.5 kg sub-sample of seed from each plot on a Diode Array 7200 NIR Analyzer (Perten Instruments, Springfield, IL). Procedures for determining thousand kernels were similar to the method used for trials at Prosper.

Statistical Analysis

Data were analyzed using the PROC Mixed procedure of SAS 9.2 (SAS Institute, Cary, NC). Year, location, and replicates were considered random effects while N treatment and cultivars were considered fixed. Any fixed effects by random effects were also considered random effects. Main effects and interactions were tested using the appropriate error terms. A square root transformation was applied to percent leaf burn data prior to analysis to obtain a normal distribution of the data. Means were separated using Fisher's protected least significant differences at the 5% level of significance.

Results and Discussion

Weather Information

The 2011 and 2012 growing seasons were substantially different from each other. In 2011, much of North Dakota and western Minnesota was extremely wet (NDAWN, 2013) and

thus a large area could not be planted. Due to cold and wet conditions in April when HRSW is typically planted in this geographical location, planting of the trials was delayed until May. At Crookston the mean air temperature for the growing season (17 May – 15 August 2011) was 18 ^{o}C, at Hettinger (9 May – 11 August 2011) it was 17 ^{o}C and at Prosper it was 19 ^{o}C (19 May – 19 August 2011) (Table 2.5). Monthly mean air temperatures were below normal averages in May for all locations, but above normal for July at all three locations (Table 2.6). Rainfall during the 2011 growing season was 252 mm, 290 mm, and 451 mm for Crookston, Hettinger, and Prosper, respectively.

During the 2012 growing season, much of the area was drier than normal (NDAWN, 2013). The mean air temperature for the growing season at Crookston (11 April – 27 July) was 16 ^{o}C and the total rainfall was 226 mm (Table 2.5, 2.6). At Hettinger (4 April – 27 July) the mean air temperature was 16 ^{o}C and total rainfall was 291 mm, and at Prosper (April 19 – 31 July) 17 ^{o}C and total rainfall was 160 mm. The monthly mean air temperature at all three locations was above normal for all months during the growing season. The monthly average precipitation was below normal every single month during the growing season at both Crookston and Prosper. At Hettinger, the months of April and July were well above the normal amount of precipitation.

Combined Analysis

Barlett's test for homogeneity error of variance was not significant, thus allowing for the combining of environments in the ANOVA. Results from the trials in Prosper 2011 were not included in the analysis across environments because excess rainfall caused the trials to be flooded on three separate occasions resulting in uncharacteristic grain yield and grain protein concentrations.

Table 2.5. Average mean air temperature for the months of planting to harvest in Crookston, Hettinger, and Prosper in 2011-2012 along with normal (1981-2010) †.

	Crookston‡			Hettinger			Prosper		
	2011	2012	Normal	2011	2012	Normal	2011	2012	Normal
Month	----------°C----------								
April	5	8	6	4	8	6	5	8	6
May	12	14	13	10	12	12	12	15	13
June	18	19	18	16	19	17	19	20	19
July	22	23	21	22	24	21	23	24	21
August	20	19	20	21	20	21	21	20	20

† Source: NDAWN, 2013.
‡Weather information collected from the Eldred, MN weather station which is 21 kilometers away.

Table 2.6. Average mean rainfall for the months of planting to harvest in in Crookston, Hettinger, and Prosper in 2011-2012 along with normal (1981-2010) †.

	Crookston‡			Hettinger			Prosper		
	2011	2012	Normal	2011	2012	Normal	2011	2012	Normal
Month	----------mm----------								
April	64.0	27.4	30.5	58.2	74.9	38.1	45.0	30.0	36.8
May	66.3	31.2	73.9	112.5	55.9	62.5	80.0	46.2	77.5
June	68.1	73.9	96.5	81.5	59.6	81.3	131.6	67.3	100.3
July	79.0	93.7	76.2	42.8	100.1	58.2	150.1	16.3	87.9
August	41.4	47.0	83.6	53.4	56.6	19.3	88.9	22.9	66.5
Total	318.8	273.2	360.7	348.4	347.1	259.4	495.6	182.7	369.0

† Source: NDAWN, 2013.
‡Weather information collected from the Eldred, MN weather station which is 21 kilometers away.

Cultivars

Across all environments, cultivars were significantly different for test weight, 1000 kernel weight, grain protein, and height. There were significant environment x cultivar (E x C) interactions for all agronomic traits measured except heading date. Except for yield and total protein harvested the E x C interactions were due to differences in magnitude.

Glenn had significantly higher test weight than Faller and RB07. Glenn is known for having exceptionally high test weight, while RB07 and Faller typically have average test weight (Ransom et al., 2012). The combined average test weight for all three cultivars was 759 kg m^{-3} (Table 2.7). In 2011, the average test weight for Glenn, Faller, and RB07 was 730, 690, and 680 kg m^{-3}, respectively, at Hettinger, and at Crookston the cultivars were 820, 780, and 770 kg m^{-3}

(data not shown). The 90 kg m^{-3} difference in average test weight between the same cultivars grown at Hettinger and Crookston can be explained by the differences in precipitation received during the grain filling process thus substantially impacting grain quality characteristics. Moisture stress during grain filling can shorten the duration of starch accumulation, thus resulting in lower test weight (Altenbach et al., 2003).

Across environments, Glenn had the highest protein concentration followed by RB07 and then Faller. The grain protein concentration was 164, 158, and 155 g kg^{-1} for Glenn, RB07, and Faller, respectively (Table 2.7). These results are as expected with higher protein concentration cultivars having higher protein concentration than lower protein concentration cultivars (Ransom et al., 2012). Weather conditions for 2011 caused major differences in grain protein concentration between cultivars at Crookston where the average values were 158, 150, and 147 g kg^{-1} for Glenn, RB07, and Faller, respectively; while in Hettinger values were 173, 172, and 167 g kg^{-1}(data not shown). Grain protein concentration averages were above 140 g kg^{-1} at Crookston and Hettinger because the average temperature for July (grain filling period) was above the normal (Table 2.5). Lower precipitation received at Hettinger during the grain filling period may have caused higher protein concentration levels there compared to Crookston because of alternations to the biosynthesis reactions of both starch and protein and the shortened accumulation of starch during kernel development (Altenbach et al., 2003).

Data for 1000 kernel weight showed that trends between the combined and individual environments were similar (data not shown). The general trend from lightest to heaviest kernel weight was Faller, Glenn, and RB07, respectively. Again, weather conditions in 2011 caused dramatic differences in 1000 kernel weight between Hettinger and Crookston where the average 1000 kernel weight across cultivars was 26 and 59 g, respectively (data not shown). Differences

between these two environments can be explained by the environmental conditions that altered the biosynthesis reactions of both starch and protein accumulations, thus impacting grain quality characteristics (Altenbach et al., 2003). In Crookston, cultivars had a longer grain filling period than Hettinger, thus resulting in heavier kernel weight.

Faller yielded the highest within and across environments except for Hettinger in 2012 (data not shown). In 2012 at Hettinger, RB07 was significantly higher yielding then Glenn and Faller. Faller is later maturing than RB07, and does not usually perform as well as some of the earlier maturing cultivars in western North Dakota. This has been observed in several cultivar trails across North Dakota (Ransom et al., 2012) and is possibly due to the impact that heat and drought effects can have on grain yield later in the growing season, particularly on later maturing cultivars. This is especially important because temperatures in July were high, which could have had an impact on the grain filling process. When a plant is stressed due to lack of water or N, or when temperatures are extremely high, the time between anthesis and harvest can be reduced by as much as seven days (Altenbach et al., 2003). The reduction in the length of grain filling can negatively impact yield due to inhibition of enzymes involved in the starch biosynthesis, thus reducing starch deposition. Later maturing cultivars can be at an even greater disadvantage than earlier-heading cultivars when these unfavorable conditions occur during grain filling (Tewolde et al., 2006).

Total protein is derived from grain yield and grain protein. The dominance of grain yield in this research resulted in ranking of total protein that was similar to the ranking of grain yield. The differences in agronomic traits for 1000 kernel weight, test weight, grain yield, total protein, and percent protein were consistent with the results from current and previous cultivar trials in North Dakota (Ransom et al., 2012).

Table 2.7. Means for test weight, heading date, 1000 kernel weight, grain yield, total protein harvested, height, and grain protein concentration for three cultivars of HRSW across all five locations.

Cultivar	Test Weight	Heading Date (Julian Days)†	1000 Kernel Weight	Yield	Total Protein Harvest	Height	Grain Protein
	$kg\ m^{-3}$		g	--------$kg\ ha^{-1}$--------		cm	$g\ kg^{-1}$
Faller	748.0	187	31.5	3629	594.3	74.4	155.3
Glenn	780.8	175	29.6	3281	569.9	80.6	164.3
RB07	749.5	175	28.3	3481	577.3	73.8	158.1
Mean	759.4	179	29.8	3464	580.5	76.3	145.9
CV %	3.7	38	3.8	9	8.4	4.3	2.4
LSD (0.05)	14.3	NS	1.2	NS	NS	5.7	2.3

†A heading date of Julian Day 179 equates to June 27 in a leap year and June 28 in a non-leap year.

Grain Protein Concentration

Significant differences between N treatments and grain protein concentration were found. The four N treatments that had significantly higher protein concentration than all other treatments were a split N treatments of 90 kg ha^{-1} of dry urea applied prior to planting + 34 kg ha^{-1} of a urea aq-solution applied at ZGS 45, 90 kg ha^{-1} of dry urea applied prior to planting + 34 kg ha^{-1} of a UAN applied at ZGS 45, 90 kg ha^{-1} of dry urea applied prior to planting + 34 kg ha^{-1} urea aq-solution + NBPT applied at ZGS 69, and 90 kg ha^{-1} of dry urea applied prior to planting + 34 kg ha^{-1} UAN applied at ZGS 69 (Table 2.8). The average protein concentration for these treatments was 160.2, 160.5, 159.8, and 162.1g kg^{-1}, respectively. Even though these treatments were similar, the additional application of 34 kg ha^{-1} UAN applied at ZGS 69 had in the highest grain protein concentration of all treatments. Similar observation has been documented before (Finney et al., 1957; Endres and Schatz, 1993; Bly and Woodard, 2003). This N treatment had a 0.5% increase in protein concentration over the 90 kg ha^{-1} of dry urea applied prior to planting alone, which also had the lowest protein concentration of all treatments. To satisfy the N requirements of a wheat crop, Strong (1982) suggested that N be split into separate applications,

one at planting and another around flowering, stating this strategy allows for higher yield and grain protein responses compared to a single application.

The environment x N treatment interaction for grain protein concentration was significant. The average protein concentration for all eight treatments across all environments was 159.2 g kg^{-1} (Table 2.8). Data indicated that UAN applied at ZGS 69 had the highest protein concentration in three of the five environments. This treatment did not have the highest protein concentration at Crookston and Hettinger in 2012. Nevertheless, it was still one of the best for having high protein concentration.

The average protein concentration in 2011 was 150.4 g kg^{-1} at Crookston, and 170.6 g kg^{-1} at Hettinger (Table 2.8). The major differences in protein concentration across locations in 2011 can be explained by environmental conditions that impacted grain quality. The late-season application of UAN applied post anthesis had the highest protein concentration across both locations; however the large span in protein concentration between locations maybe explained by high temperatures and rainfall events that occurred throughout the growing season, especially during grain filling, that might impact N uptake from the soil and redistribution of N within the plant (Altenbach et al., 2003; Jenner et al., 1991).

The average protein concentration for 2012 was 152.4, 164.8, and 158.8 g kg^{-1} at Crookston, Hettinger, and Prosper, respectively. Data indicated that different sources and timings of application of N resulted in different protein concentration across all three locations. In Crookston a split N treatment of 90 kg ha^{-1} of dry urea applied prior to planting + 34kg ha^{-1} of UAN applied at ZGS 45 had the highest grain protein concentration, while at Hettinger 90 kg ha^{-1} of dry urea applied prior to planting + 34 kg ha^{-1} of a dry urea applied at ZGS 45 was the best treatment. And at Prosper, a split application of 90 kg ha^{-1} of dry urea applied prior to planting +

34 kg ha^{-1} UAN applied at ZGS 69 resulted in the highest grain protein concentration. Findings to this study are contrasting to previous research that has indicated that N applied foliar at ZGS 60 gave the best response in increasing protein concentration (Finney et al., 1957; Bly and Woodard, 2003; Endres and Schatz, 1993). Research has also found that liquid N resulted in substantially higher grain protein concentration compared with dry granular N when applied at late growth stages (Strong, 1982). Different responses in protein concentration to N treatments across environments suggests that additional research needs to be conducted to help determine which N treatment will increase protein concentration the most across multiple environments. Overall, combined data confirmed that a late-season foliar application of N (regardless of source) either at the ZGS 45 or ZGS 69 can reliably raise protein concentration (Table 2.8).

Table 2.8. Grain protein concentration influenced by N treatment across individual and combined environments.

	Crookston		Hettinger		Prosper	Combined
	2011	2012	2011	2012	2012	2011-2012
Nitrogen Treatment (kg ha^{-1})	----------g kg^{-1}----------					
1) 123 urea PP†	149.9	153.4	167.4	164.4	156.0	158.2
2) Base rate‡	148.0	151.6	169.1	159.9	156.4	157.0
3) Base rate + 34 UAN§ ZGS 69	153.7	153.4	175.5	167.0	161.1	162.1
4) Base rate + 34 urea-aq¶ ZGS 69	151.8	151.5	171.8	165.4	160.6	160.2
5) Base rate + 33.6 UAN ZGS 45	149.1	155.0	170.1	165.5	155.8	159.1
6) Base rate + 34 urea ZGS 45	151.0	154.2	170.3	170.6	156.6	160.5
7) Base rate + 34 UAN ZGS 92	150.1	148.3	168.8	161.0	155.8	156.8
8) Base rate + 34 urea-aq+ NBPT# ZGS 69	150.8	152.0	171.5	164.9	160.0	159.8
Mean	150.4	152.4	170.6	164.8	158.8	159.2
CV %	1.3	3.3	1.9	2.3	1.7	2.4
LSD (0.05)	1.6	4.1	2.7	3.1	2.2	2.3

† PP = pre-plant
‡ 89.6 kg ha^{-1} urea PP
§ UAN = urea ammonium nitrate (28-0-0)
¶ aq = water solution
# NBPT = N-n-butyl thiophosphoric triamide, a urease inhibitor

Leaf Burn

There was significant environment x N treatment x cultivar interaction for percent leaf burn. The application UAN applied at ZGS 69 resulted in the highest percent of leaf burn across

all three cultivars (Table 2.9). The percent leaf burn was 35.8 %, 25.7%, and 23.2% for Faller, Glenn, and RB07, respectively. Faller also had the highest percent leaf burn with N treatments of UAN applied at the ZGS 45 and urea aq-solution + NBPT applied at ZGS 69. It was noted that Glenn had the highest percent leaf burn with UAN applied at the ZGS 45, and RB07 had a highest percent leaf burn with the urea aq-solution + NBPT solution applied at ZGS 69.

Burning of the leaf tips caused by foliar N applications has been mentioned often in wheat, rice, and corn research (Gooding, 1988; Thom et al., 1981; Chesnin and Shafer, 1953). However, in some cases no foliar discoloration has been seen (Hanley et al., 1966; Sylvester-Bradley et al., 1990). The difference in damage is likely due to the form of N in the liquid fertilizer (Garcia and Hanway, 1976; Rader et al., 1943). Some forms of N fertilizer, such as urea has a lower salt concentration compared to UAN. Thus, desiccation of leaf cells with urea through osmosis is reduced (Gary, 1977). Studies have also shown that adding a urease inhibitor can actually increase leaf burn due to increase toxic urea levels instead of NH_3 (Powlson et al., 1989). The severity of burning among cultivars may also depend on the timing of application and cultivar interaction (Gooding 1988). One recommendation to help reduce the severity of burning is to apply the solution in the cool of the day and when the humidity is high (Garcia and Hanway, 1976). However, due to distances between locations and time management it was difficult to apply some treatments at the ideal timing, especially when some cultivars, such as Faller, mature later then Glenn and RB07. Also, weather conditions during application timing may have had an impact on the severity of leaf burn. These circumstances explain why the degree of burning to Faller, applied with UAN at post anthesis, was significantly higher than all other treatments and cultivar interactions.

Table 2.9. Effects of cultivar and N treatment on leaf burn in HRSW cultivars across five environments.

Nitrogen Treatment (kg ha^{-1})	Faller	Glenn	RB07
	----------% leaf burn----------		
1) 123 urea PP†	0.0 (0.7)‡	0.0 (0.7)	0.0 (0.7)
2) Base rate§	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)
3) Base rate + 34 UAN¶ ZGS 69	35.8 (6.0)	25.7 (5.1)	23.2 (4.9)
4) Base rate + 34 urea-aq# ZGS 69	8.7 (3.0)	4.8 (2.3)	6.4 (2.6)
5) Base rate + 34 UAN ZGS 45	10.9 (3.4)	10.8 (3.4)	6.4 (2.6)
6) Base rate + 34 urea ZGS 45	0.0 (0.7)	0.0 (0.7)	0.0(0.7)
7) Base rate + 34 UAN ZGS 92	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)
8) Base rate + 34 urea-aq + NBPT†† ZGS 69	12.9 (3.7)	5.7 (2.5)	11.1 (3.4)
LSD (0.05) = 1.8 to compare different N treatments within the same cultivar			
LSD (0.05) = 0.5 to compare different cultivars within the same N treatment			
LSD (0.05) = 1.9 to compare different cultivars among different N treatments			

† PP = pre-plant.
‡ numbers in ( ) are the transformed value of the % leaf burn which are used to calculate the LSD values.
§ 90 kg ha^{-1} urea PP.
¶ UAN = urea ammonium nitrate (28-0-0).
# aq = water solution.
†† NBPT = N-n-butyl thiophosphoric triamide, a urease inhibitor.

Economic Return on Nitrogen Treatment

An economic analysis was conducted to determine which N treatments would provide the best economic return for producers. This comparison was based on local current prices: \$1.30 kg^{-1} of N as urea, \$1.61 kg^{-1} of N as UAN, \$0.14 kg^{-1} of NBPT inhibitor added to every kg of N, and application cost of \$14.20 ha^{-1} (Frayne Olson, personal communications). All other expenses were assumed equal. The average yield and protein concentration of the eight N treatments were used for determining return over the total cost of N ha^{-1}. Three different comparisons were made. One based on the historical market (2007-2011) of \$0.25 kg^{-1} for grain, another based on the 2009-2010 average market prices of \$0.18 kg^{-1} for grain and a premium of \$0.042 kg^{-1} for protein over 140 g kg^{-1}, and one based on 2012-2013 average market prices of \$0.31 kg^{-1} for grain and a premium of \$0.003 kg^{-1} for protein over 140 g kg^{-1} (Haugen et al., 2013). Values from 2009-2010 were chosen as an example of more extreme prices and discounts. Thus many producers received a large discount if they did not meet market requirements for protein and

those that did exceed the benchmark received a large premium. A comparison with minimal differences in premium and discount prices was also included (2012-2013 values).

Data showed that the economic return for the five year average ranged from \$670-\$745 ha^{-1} with an average return across treatments of \$694 ha^{-1} (table 2.10). In 2009-2010 the economic return was ranged from \$640-\$720 ha^{-1} and had an average return of \$693 ha^{-1} across treatments. In 2012-2013 the economic return across treatments ranged from \$880-\$950 ha^{-1} with an average return of \$900 ha^{-1}. Comparisons found that 90 kg ha^{-1} urea applied prior to planting provided the best economic return after subtracting N expenses for both the five-year average and 2012-2013 market average. However, 2009-2010 market averages indicated that 90 kg ha^{-1} of UAN applied at ZGS 92 had sustainably lower economic return compared to the other seven treatments.

When comparing these N treatments across different market prices, the data suggests that applying lower rates of N prior to planting provided the best economic return (Table 2.10). This maybe occurred because protein levels were well above 140 g kg^{-1} for each treatment, thus applying an additional application of N (regardless of form and timing) was not necessary. Therefore, one application of N applied at planting was sufficient in meeting grain yield and protein concentration needs.

Of course, before making a final decision on how much nitrogen to apply there are several factors to consider. First knowing how much N is in the soil profile prior to planting is beneficial in determining how much N to apply at planting (Franzen, 2010). Also assessing late-season N needs for reaching maximum grain yield and protein concentration can be achieved by taking in-season measurements that can predict the need for supplement N. Environmental stress throughout the growing season can also impact grain yield and protein concentration responses

(Altenbach et al., 2003). In order for a supplement N treatment for protein concentration to be economical depends greatly on the year due to discount and premium variability within a year and between years (Frayne Olson, personal communications).

Conclusion

Nitrogen had a significant effect on grain protein. Across environments a split application of 90 kg ha^{-1} of dry urea applied prior to planting + 34 kg ha^{-1} UAN applied at ZGS 69 resulted in the highest grain protein concentration. Applying an additional 34 kg ha^{-1} of UAN at ZGS 69 to the original 90 kg ha^{-1} of dry urea applied prior to planting resulted in a 0.5% increase in protein concentration.

Similar agronomic responses to N treatments were detected among cultivars expect for leaf burn. Faller was the most susceptible cultivar to leaf burn. Overall the data indicated that a foliar application of UAN at ZGS 69 had the highest percent of leaf burn across all cultivars. Thus, the timing of the application and the crop growth stage are critical in preventing severe leaf burn.

When comparing these N treatments across different market prices, the data suggests that applying all N at planting seems to have the highest economic return when grain protein concentration is above 140 g kg^{-1}. Of course there are several factors to consider when determining fertility management throughout the season in order to reach maximum grain yield and protein concentration levels. The best management practice to achieve desired grain yield and protein concentration levels is to target and manage N early in the season to optimize economic yield return. A late season N application might be considered to optimize grain protein concentration, if anticipated grain yields are likely to exceed yield considered when preplant N recommendations were determined (Wuest and Cassman, 1992).

Table 2.10. Economic return per ha for HRSW based on prices from the five year average (2007-2011), 2009-2010 average, and 2012-2013 average across all environments.

			Return over total N cost ha^{-1}†			
Nitrogen Treatment (kg ha^{-1})	Yield	Protein	Nitrogen cost ha^{-1}	Five year average (2007-2011)	2009-2010 average	2012-2013 average
	kg ha^{-1}	g kg^{-1}	----------$----------			
1) 123 urea PP‡	3411	158.2	160.30	690.92	681.49	892.76
2) Basal rate§	3454	157.0	116.58	745.41	713.74	948.39
3) Basal rate + 34 UAN¶ ZGS 69	3440	162.1	170.83	673.42	709.46	879.77
4) Basal rate + 34 urea-aq# ZGS 69	3495	160.2	160.30	697.85	711.97	906.10
5) Basal rate + 34 UAN ZGS 45	3464	159.1	170.83	679.40	679.83	884.93
6) Basal rate + 34 urea ZGS 45	3497	160.5	160.30	698.39	716.66	907.02
7) Basal rate + 34 UAN ZGS 92	3468	156.8	170.83	680.34	641.24	883.65
8) Basal rate + 34 urea-aq+NBPT†† ZGS 69	3482	159.8	165.12	689.60	690.79	896.58
LSD (0.05) = 35.75 for five year average (2007-2011)						
LSD (0.05) = 36.84 for 2009-2010 average						
LSD (0.05) = 42.86 for 2012-2013 average						

†Market prices: five year average = \$0.25 kg^{-1} for yield; 2009-2010 average = \$0.18 kg^{-1} for yield and premium of \$0.042 kg^{-1} for protein; 2012-2013 average = \$0.31 kg^{-1} for yield and premium of \$0.003 kg^{-1} for protein.

‡ PP = pre-plant.

§90 kg ha^{-1} urea PP.

¶ UAN = urea ammonium nitrate (28-0-0).

aq = water solution.

†† NBPT = N-n-butyl thiophosphoric triamide, a urease inhibitor.

References

Anon. 1987. Nitrogen fertilizers. Alnwick: Ministry of Agri., Fisheries and Foods.

Altenbach, S.B., F.M. DuPont, K.M. Kothari, R. Chan, E.L. Johnson, and D. Lieu. 2003. Temperature, water and fertilizer influence the timing of key events during grain development in a US spring wheat. J. Cereal Sci. 37:9-20.

Bly, A.G., and H.K. Woodard. 2003. Nitrogen management: Foliar nitrogen application timing influence on grain yield and protein concentration of hard red winter and spring wheat. Agron. J. 95:335-338.

Bremner, J.M. 1995. Recent research on problems in the use of urea as a nitrogen fertilizer. Fert. Res. 42:321-329.

Bremner, J.M., and L.A. Douglas. 1973. Effects of some urease inhibitors on urea hydrolysis in soils. Proc. Soil Sci. Soc. Am. 37:225-226.

Bremner, J.M., and L.A. Douglas. 1971. Inhibition of urease activity in soils. Soil Biol. Biochem. 3:297-301.

Brown, B. 2000. Increasing wheat protein: Nitrogen management for hard wheat protein enhancement. Misc. Publ:1-6. Univ. of Idaho., Moscow, ID.

Chesnin, L. and N. Shafer. 1953. Foliage application of urea solutions to grain and forage crops. Agron. J. 45:576.

Endres, G., and B. Schatz. 1993. Foliar N applied post-anthesis to enhance wheat grain protein. Misc. Publ. North Dakota State Univ., Carrington Res. Ext. Center., Carrington.

Finney, K.F., J.W. Meyer, F.W. Smith, and H.C. Fryer. 1957. Effect of foliar spraying on Pawnee wheat with urea solutions on yield, protein concentration, and protein quality. Agron J. 49:347-347.

Franzen, D.W. 2010. North Dakota fertilizer recommendation tables and equations. Ext. Publ. SF-882:1-16. North Dakota State Univ. Ext. Serv., Fargo.

Garcia, L.R. and J.J. Hanway. 1976. Foliar fertilization of soybeans during the seed-filling period. Agron. J. 68:653-657.

Gary, R.C. 1977. Foliar fertilization with primary nutrients during the reproductive stage of plant growth. Proc. Fert. Soc. London no. 164.

Gholami, A., S. Akhlaghi, S. Shahsavani, and N. Farrokhi. 2011. Effects of urea foliar application on grain yield and quality of winter wheat. Commun. Soil Sci. Plant Anal. 42:719-727.

Gooding, M.J. 1988. Interactions between late-season foliar applications of urea and fungicide on foliar disease, yield and breadmaking quality of winter wheat. PhD thesis. Harper Adams Agric Coll., Salop, UK.

Goodling, M.J., and W.P. Davies. 1992. Foliar fertilization of cereals: a review. Fert. Res. 32:209-222.

Hanley, F., W.J. Ridgman, and J.L. Beveridge. 1966. A comparison of the effects of liquid and solid nitrogenous top-dressed for winter wheat. Expl. Husbandry 13:79-84.

Haugen, R., T. Petry, F. Olson, and D. Aakre. 2013. Plotting a course 2013.Ext. Publ.EC-1090:1-4. North Dakota State Univ. Ext. Serv., Fargo.

Jenner, C.F., T.D. Ugalde, and D. Aspinall. 1991. The physiology of starch and protein deposition in the endosperm of wheat. Aust. J. Plant Physiol. 18:221-226.

Krogmeier, M.J., G.W. McCarty, and J.M. Bremner. 1989. Phytototoxicity of foliar-applied urea. Proc. Natl. Acad. Sci. USA. 86:8189-8191.

Mahler, R.L., F.E. Koehler, and L.K. Lutcher. 1994. Nitrogen source, timing of application, and placement: effects on winter wheat production. Agron. J. 86:637-642.

NDSU Foundation Seed. 2005. NDSU Agriculture Glenn Hard Red Spring Wheat. Available at http://www.ag.ndsu.nodak.edu/aginfo/seedstock/Brochures/Glenn%20HRSW.html. (Access: 3 March, 2013).

North Dakota Agricultural Weather Network. 2013. Monthly data table form. Available at http://ndawn.ndsu.nodak.edu/monthly-table-form.html [verified 8 Feb 2013]. Fargo, ND.

Olson, F.2009. Market Advisor: when will spring wheat protein discounts soften? [online]. Available at https://www.ext.nodak.edu:8089/news/columns/cropsma/market-advisor-when-will-spring-wheat-protein-discounts-soften. (Accessed: 14 August, 2012).

Ottman, M.J., T.A. Doerge, and E. Martin. 2000. Durum grain quality as affected by nitrogen fertilization near anthesis and irrigation during grain fill. Agron. J. 92: 1035-1041.

Powlson, D.S., P.R. Poulton, N.E. Moller, M.V. Hewitt, A. Penny, and D.S. Jenkinson. 1989. Uptake of foliar applied urea by winter wheat (*Triticum aestivum*): the influence of application time and use of a new N technique. J. Sci. Food Agri. 48:429-440.

Rader, L.F., L.H. White, and C.W. Whittaker. 1943. The salt index : a measure of the effect of fertilizers on the concentration of the soil solution. Soil Sci. 55:201-218.

Ransom, J.K., M. Mergoum, S. Simsek, M.Aceveda, T. Friesen, M. McMullen, S. Zhong, R. Olson, E. Eriksmoen, B. Hanson, G.Martin, G. Bradbury, and B.Schatz. 2012. North Dakota hard red spring wheat variety trail results for 2012 and selection guide. Ext. Publ. A-574-12. North Dakota State Univ. Ext. Serv., Fargo.1-8.

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

Rawluk, C.D.L., G.J. Racz, C.A. Grant. 2000. Uptake of foliar or soil application of N-labeled urea solution at anthesis and its effect on wheat grain yield and protein. Can. J. Plant Sci. 80:331-334.

Rehm, G., and D. Franzen. 2005. Fertility management of wheat. P. 60-64. In J.J. Wiersman and J.K. Ransom (ed.) The small grains field guide. North Dakota State University and the University of Minnesota.

Seth, J., and K.I. Mosluh. 1981. The effects of urea spray on wheat in Iraq. Expt. Agric. 17:333-336.

Strong, W.M. 1982. Effect of late application of nitrogen on the yield and protein concentration of wheat. Aust. J. Exp. Areic. Anim. Husb. 22:54-61.

Sylvester-Bradley R., A.D. Rochford, and J.S. Rule. 1990. Effects of canopy disturbance while spraying urea on grain yield and nitrogen uptake by winter wheat. In: G.F.J. Milford, P.S. Kettlewell, J.H. Orson, W.T.B. Thomas, P.E. Pritchard, and C. Myra meds. Aspects of applied biology 25. Cereal Quality. pp.91-91. Warwick: Association of Applied Biologists.

Tewolde, H., C.J. Femandez, and C.A. Erickson. 2006. Wheat cultivars adapted to post-heading high temperature stress. J. Agron. Crop Sci. 192:111-120.

Thom. W.O., T.C. Miller, and D.H. Bowman. 1981. Foliar fertilization of rice after midseason. Agron. J. 73:411-414.

USDA-ARS. 2010. Western Wheat Quality Laboratory. Available at http://public.wsu.edu/~wwql/php/wheat-was-showmeth.php?m_id=14 (verified 2 Jun. 2013). USDA_ARS Washington, DC.

USDA-Natural Resources Conservation Service. 2012. Web Soil Survey. Available at http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm (verified 7 Dec. 2012). USDA_NRCE Washington, DC.

Varga, B., and Z. Svecnjak. 2006. The effects of late-season urea spraying on grain yield and quality of winter wheat cultivars under low and high basal nitrogen fertilization. Field Crop Res. 96:125-132.

Woolfolk, C.W., W.R. Raun, G.V. Johnson, W.E. Thomason, R.W. Mullen, K.J. Wynn, and K.W. Freeman. 2002. Influence of late-season foliar nitrogen applications on yield and grain nitrogen in winter wheat. Agron. J. 94:429-434.

Wuest, S.B. and K.G. Cassman. 1992. Fertilizer-nitrogen use efficiency of irrigated wheat: I. Uptake efficiency of preplant versus late-season application. Agron. J. 84:682-688.

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res.14:415-421.

CONCLUSION

Plant measurements collected at the ZGS 37 provide the best time in predicting grain yield and protein concentration. The best measured values for predicting protein concentration are chlorophyll concentration, N concentration of leaf tissue, and N concentration of stem samples. By combining multiple measurements, the strength for predicting N needs in HRSW in order to reach maximum protein concentration levels is greatly increased. The data-set from this experiment is small and additional verification is needed, but plant measurements seem to be promising in determining supplement N needs for reaching desired protein concentration and grain yield. Therefore, incorporating plant measurements throughout the growing season to help manage N needs of the plant would be would be beneficial to producers to know if applying a late season application of N would be justifiable.

Across environments a split application of 90 kg ha^{-1} of dry urea applied prior to planting + 34 kg ha^{-1} UAN applied at ZGS 69 resulted in the highest grain protein concentration. Applying an additional 34 kg ha^{-1} of UAN at ZGS 69 to the original 90 kg ha^{-1} of dry urea applied prior to planting resulted in a 0.5% increase in protein concentration. This treatment also caused the greatest leaf burn across all cultivars. The severity to leaf burn caused by foliar applications depends on the individual cultivar, timing of application, and the crop growth stage. Thus proper management when applying foliar applications is critical in preventing severe leaf burn. When comparing these N treatments across different market prices, the data suggests that applying all N at planting seems to have the highest economic return when grain protein concentration is above 140 g kg^{-1}. Overall, the best management practice to achieve desired grain yield and protein concentration levels is to target and manage N early in the season to optimize economic return. A late season N application might be considered to optimize grain protein

concentration, if anticipated grain yields are likely to exceed yield considered when preplant N recommendations were determined (Wuest and Cassman, 1992).

APPENDIX

Table A.1. Mean squares for the analysis of variance for agronomical traits evaluated across three environments (Crookston, MN, (2011-2012)) and (Prosper, ND (2012)). All plant measurements were normalized.

Source of variation	df	Mean Squares						
		GS†	TS†	CM†	SS†	GP†	Y†	TPH†
		Flag Leaf						
Environment (E)	2	0.101	0.092	0.060	1.052	11327.00	887810	156222
Rep [E]	9	0.094	0.003	0.018	0.056	133.44	597661	15826
Treatment (A)	3	0.158*	0.138**	0.203**	2.576***	4271.17*	11373604	449968*
Linear	1	0.71***	0.028***	0.100***	0.332***	432.77**	3379860**	96531***
Quadratic	1	0.018	0.008*	0.030*	0.022	83.66	99183	5377
Cubic	1	0.013	0.000	0.005	0.008	6.03	228884	1024
A x E	6	0.042***	0.022***	0.007	0.236***	1024.79***	3548356***	116427***
Cultivar (B)	3	0.033	0.013***	0.007	0.007	2449.87**	3550532**	8202
B x E	6	0.019***	0.000	0.007	0.014	268.17***	477995**	3890
A x B	9	0.005	0.002	0.002	0.011	25.20	305574	8044
A x B x E	18	0.005	0.002	0.006	0.014	46.49	168555	4549
Error (b)	132	0.009	0.002	0.006	0.011	40.22	381474	8535

† GS = greenseeker; TS = tissue sample; CM = chlorophyll meter; SS = stalk sample; GP = grain protein; Y = yield; TPH = total protein harvested.
*,** , *** Significant at (P≤0.10) ,(P≤0.05), and (P≤0.01), respectively.

Table A.2. Correlation between NDVI values collected at ZGS 37, grain yield and grain protein concentration in individual cultivars.

Cultivars	Yield				Protein			
	Prosper 2012	Crookston 2011	Crookston 2012	Combined	Prosper 2012	Crookston 2011	Crookston 2012	Combined
	R Value							
Faller	0.50	0.85	0.64	0.72	0.60	0.82	0.56	0.66
Glenn	-0.93*	0.84	0.92*	0.90*	0.30	0.88	0.90*	0.94*
Samson	0.45	0.93*	0.99***	0.99***	0.91*	0.85	0.72	0.84
Vantage	0.31	0.98**	0.98**	0.99***	-0.40	0.97**	0.86	0.95**

*,** , *** Significant at (P≤0.10) , (P≤0.05), and (P≤0.01) respectively.

Table A.3. Mean squares for the analysis of variance for agronomical traits evaluated across two environments (Crookston, MN, and Prosper, ND) in 2012. All plant measurements were normalized.

Source of variation	Df	Zadoks GS 16				Zadoks GS 37
		Greenseeker	Tissue Sample	Chlorophyll Meter	Leaf Color Chart	Leaf Color Chart
Environment (E)	1	0.0268	0.0490	0.0282	0.0020	0.0003
Rep [E]	6	0.0316	0.0096	0.0379	0.0159	0.0082
Treatment (A)	3	0.0258	0.0090	0.0238	0.0026	0.0104
Linear	1	0.0000	0.0112	0.0044	0.0326*	0.0138*
Quadratic	1	0.0206	0.0000	0.0046	0.0000	0.0018
Cubic	1	0.0000	0.0004	0.0028	0.0216	0.0015
A x E	3	0.0316***	0.0056***	0.1115**	0.0008	0.0040
Cultivar (B)	3	0.0163	0.0002	0.0149	0.0119	0.0095
B x E	3	0.0120	0.0015	0.0255	0.0165	0.0160**
A x B	9	0.0087	0.0005	0.0270	0.0063	0.0018
A x B x E	9	0.0046	0.0006	0.0221	0.0117**	0.0038*
Error (b)	90	0.0099	0.0024	0.0185	0.0053	0.0022

*,** , *** Significant at (P≤0.10) ,(P≤0.05), and (P≤0.01), respectively.

Table A.4. Growth stage, date, time, average temperature, relative humidity, bare soil temperature, average wind speed, direction of wind, and weather conditions at the time of application of N treatments at Crookston, MN in 2011 and 2012†.

Stage‡	Date	Time	Avg Air Temp.	Avg Relative Humidity	Avg Bare Soil Temp.	Avg Wind Speed	Avg Wind Direction	Weather Conditions
			C°	%	C°	m/s		
				2011				
ZGS 45	June 30	0830	26	89	20	7.5	southeast	sunny
ZGS 69	July 9	0900	23	90	21	3.8	southwest	sunny
ZGS 92	August 3	1400	26	58	25	2.2	southwest	partly cloudy
				2012				
ZGS 45	June 6	1750	30	35	26	5.2	south	partly cloudy
ZGS 69	June 12	1830	21	46	20	5.7	south	clear
ZGS 92	July 18	1430	30	41	25	3.7	southeast	clear

† Data collected form NDAWN.

‡ Stage = ZGS 45 is Boot stage; ZGS 69 is Post Anthesis; ZGS 92 is Physiological Maturity.

Table A.5. Growth stage, date, time, average temperature, relative humidity, bare soil temperature, average wind speed, direction of wind, and weather conditions at the time of application of N treatments at Prosper, ND in 2011 and 2012†.

Stage‡	Date	Time	Avg Air Temp.	Avg Relative Humidity	Avg Bare Soil Temp	Avg Wind Speed	Avg Wind Direction	Weather Conditions
			C°	%	C°	m/s		
				2011				
ZGS 45	July 5	0900	23	70	22	5.1	northwest	clear
ZGS 69	July 12	1500	23	48	28	4.7	north	partly cloudy
ZGS 69 (Faller)	July 13	1500	24	53	28	5.7	southeast	Clear
ZGS 92	August 5	0900	24	82	22	2.7	southeast	clear
				2012				
ZGS 45	June 15	2000	25	47	28	1.6	southeast	clear
ZGS 69	June 25	2100	22	52	28	2.8	southeast	clear
ZGS 69 (Faller)	June 26	2100	24	58	28	3.8	southeast	partly cloudy
ZGS 92	July 23	0900	23	79	25	3.8	north	clear

† Data collected form NDAWN, 2011.
‡ Stage = ZGS 45 is Boot stage; ZGS 69 is Post Anthesis; ZGS 92 is Physiological Maturity.

Table A.6. Growth stage, date, time, average temperature, relative humidity, bare soil temperature, average wind speed, direction of wind, and weather conditions at the time of application of N treatments at Hettinger, ND in 2011 and 2012†.

Stage‡	Date	Time	Avg Air Temp.	Avg Relative Humidity	Avg Bare Soil Temp.	Avg Wind Speed	Avg Wind Direction	Weather Conditions
			C°	%	C°	m/s		
				2011				
ZGS 45	July 1	1000	20	82	21	2.8	northwest	partly cloudy
ZGS 69	July 12	1600	23	62	30	5.0	north	cloudy
ZGS 92	August 5	1030	25	64	24	1.9	southeast	clear
				2012				
ZGS 45	June 11	2000	15	43	23	4.6	northwest	clear
ZGS 69	June 18	1800	25	33	30	2.7	north	partly cloudy
ZGS 69 (Faller)	June 21	2000	22	46	27	2.3	north	clear
ZGS 92	July 20	1000	28	56	26	4.0	northeast	Partly cloudy

† Data collected form NDAWN, 2011.
‡ Stage = ZGS 45 is Boot stage; ZGS 69 is Post Anthesis; ZGS 92 is Physiological Maturity.

Table A.7. Mean squares for the analysis of variance for agronomical traits evaluated across five environments ((Crookston, MN (2011, 2012) Hettinger, ND (2011, 2012) and Prosper, ND (2012)).

Source of variance	df	Mean squares†							
		LF	HT	GP	HD	TW	1000 KWT	Y	TPH
Environment (E)	4	65.53	8037.8	6804.8	10544	125181	699.0	79184497	2272598
Rep [E]	15	0.72	93.1	70.3	4842	1225	5.2	880874	17555
Treatment (A)	7	174.12***	15.4	198.5***	4657	498	2.4	49544	4234
E x A	28	24.05***	12.4	39.3***	4708	744	2.0	141891*	2972*
Cultivar (B)	2	5.74	2232.6**	3378.3***	7446	54829***	429.9***	4875045*	24939
E x B	8	4.23***	493.2***	83.4***	4676	3196***	23.2***	1170760***	27959***
A x B	14	2.27**	8.7	22.2*	4708	773	1.6	169245*	2968
E x A x B	56	1.05***	12.8	13.3	4699	829	1.5	93478	1844
Error	345	0.47	10.6	12.5	4681	774	1.3	105185	2403

† LF=leaf burn; HT=height; GP=grain protein; KWT=kernel weight; Y=yield; TPH=total protein harvested.
*,** , *** Significant at (P≤0.10) , (P≤0.05), and (P≤0.01) respectively.

Table A.8. Means for test weight, heading, 1000 kernel weight, grain yield, and total protein harvested, for nitrogen treatments across all five locations.

N Treatment (kg ha^{-1})	Test Weight	Julian Day of Heading†	1000 Kernel Weight	Yield	Total Protein Harvest	Height
	kg m^{-3}		g	----------kg ha^{-1}----------		cm
1) 123 urea PP‡	760.6	176.6	29.9	3411	570.5	76.3
2) Base rate§	761.2	176.0	29.9	3454	571.3	76.4
3) Base rate + 34 UAN¶ ZGS 69	756.8	176.0	29.7	3440	585.9	75.9
4) Base rate + 34 urea-aq# ZGS 69	760.2	176.0	29.9	3495	588.9	76.1
5) Base rate + 34 UAN ZGS 45	753.5	201.0	29.5	3464	579.1	75.5
6) Base rate + 34 urea ZGS 45	760.4	175.9	30.1	3497	591.6	77.0
7) Base rate + 34 UAN ZGS 92	762.3	176.1	29.8	3468	572.2	76.9
8) Base rate + 34 urea-aq+ NBPT†† ZGS 69	760.5	176.0	29.5	3481	584.6	76.1
Mean	759.4	157.2	29.8	3464	580.5	76.3
CV %	3.7	38	3.8	9	8.4	5.7
LSD (0.05)	NS	NS	NS	NS	NS	NS

†A heading date of Julian Day 179 equates to June 27 in a leap year and June 28 in a non-leap year.
‡PP = pre-plant.
§90 kg ha^{-1} urea PP.
¶ UAN = urea ammonium nitrate (28-0-0).
# aq = water solution.
†† NBPT inhibitor = N-n-butyl thiophosphoric triamide urease inhibitor (Agrotain).