Communications in Soil Science and Plant Analysis
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcss20

In-Season Prediction of Nitrogen Use Efficiency and Grain Protein in Winter Wheat (Triticum aestivum L.)

Natasha Macnack[a], Bee Chim Khim[b], Jeremiah Mullock[a] & William Raun[a]

[a] Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, Oklahoma, USA

[b] Department of Crop and Soil Environmental Science, Virginia Tech, Blacksburg, Virgina, USA

Accepted author version posted online: 13 May 2014.Published online: 16 Sep 2014.

Communications in Soil Science and Plant Analysis, 00:1–15, 2014

ISSN: 0010-3624 print / 1532-2416 online
DOI: 10.1080/00103624.2014.904337

In-Season Prediction of Nitrogen Use Efficiency and Grain Protein in Winter Wheat (*Triticum aestivum* L.)

NATASHA MACNACK,[1] BEE CHIM KHIM,[2]
JEREMIAH MULLOCK,[1] AND WILLIAM RAUN[1]

[1]Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, Oklahoma, USA
[2]Department of Crop and Soil Environmental Science, Virginia Tech, Blacksburg, Virgina, USA

In a 3-year study, grain yield, nitrogen use efficiency (NUE), and grain protein (GP) were evaluated as a function of rate and timing of nitrogen (N) fertilizer application. Linear models that included preplant N, normalized difference vegetation index (NDVI), cumulative rainfall, and average air temperature from planting to sensing (T-avg) were evaluated to predict NUE and GP in winter wheat. GreenSeeker readings were collected at Feekes (F) 3, 4, 5, and 7 growth stages. Combined with rainfall and/or T-avg, NDVI alone was not correlated with NUE. However, NDVI and rainfall explained 45% ($r^2 = 0.45$) of the variability in GP at F7 growth stage. Preplant N, NDVI, rainfall and growing degree days (GDD) combined explained 76% ($r^2 = 0.76$) of the variability in GP at F3. Mid-season climatic data improved the prediction of GP and should therefore be considered for refining fertilizer recommendations when GP levels are expected to be low.

Keywords grain protein, nitrogen use efficiency, NDVI, winter wheat

Introduction

With the world population projected to rise to 8.9 billion by 2050 (United Nations, 2004), and a subsequent increase in food demand, producers worldwide have to find more efficient ways to utilize agricultural resources. Fertilizer is one of the most expensive inputs in crop production (Baligar et al., 2001). As nitrogen (N) fertilizer prices increase, it becomes important to monitor the efficiency with which N is used by wheat and other crops.

The algorithm for N fertilizer recommendations developed at Oklahoma State University utilizes predicted yield potential (YP0) and the response index (RI) to predict yield potential when N is applied (YPN). Yield potential is defined as the highest possible yield obtainable with ideal management for specific soil and weather conditions. The RI is calculated as the maximum grain yield divided by the grain yield of the unfertilized plot (Johnson and Raun, 2003). Fertilizer rates are calculated by dividing the difference in grain N uptake of YPN and YP0 by the estimated use efficiency (Raun et al., 2005). Fertilizer

Received 16 August 2013; accepted 13 September 2013.
Address correspondence to Natasha Macnack, Department of Plant and Soil Sciences, 052 Agricultural Hall, Oklahoma State University, Stillwater, OK 74078. E-mail: macnack@okstate.edu

N recovery in global cereal production is estimated at 33% (Raun and Johnson, 1999). When applied, fertilizer can be lost from the plant-soil system through several pathways. Raun and Johnson (1999) noted that N fertilizer losses due to gaseous plant emission, soil denitrification, surface runoff, volatilization, and leaching are the main contributors to low nitrogen use efficiency (NUE) in cereal grain production worldwide. In addition, nitrogen use efficiency (NUE) is also affected by the rate and timing of N fertilization. Several researchers reported increasing NUEs with low levels of applied N and decreasing NUEs with increasing levels of applied N (Gauer et al., 1992; Campbell et al., 1977). Campbell et al. (1993) found that NUE increased with cropping years at fertilizer rates smaller than 50 kg N ha^{-1} but would decrease when rates exceeded 50 kg N ha^{-1}. Gauer et al. (1992) noted that NUE decreased with increasing N rates; ranging from 40 to 200 kg N ha^{-1}in spring wheat. This work reported an average NUE of 32% at 40 kg N ha^{-1} and an NUE of 15% at 200 kg N ha^{-1}under moderate moisture conditions.

Grain protein (GP) is an important quality component in cereal grains and is receiving increased attention due to protein discounts at the elevator. Hard red winter wheat (HRW) has medium to high GP levels usually ranging from 10% to 13% (U.S. Wheat Associates, 2007). The Kansas City Board of Trade (KCBT) stipulated that deliverable grades of HRW should contain not less than 11% GP and that HRW with GP levels between 10.5% and 11% will be penalized $0.10 off the contract price upon delivery (KCBT, 2010). This has been reason for concern as growers need to manage N in such a way that both grain yields and GP are optimized. Nitrogen is critical in the synthesis of amino acids, which are the main components of all proteins (Brown, 2000) and as such GP is greatly affected by N fertilization. In addition, the timing of N application greatly affects GP content. According to Ellen and Spiertz (1980), N availability late in the season increases GP and yield. Wright et al. (2003) has shown that midseason N application at anthesis increased GP content by 0.3 to 0.4%.

In addition to N fertility, environmental variables such as temperature (Finney and Fryer, 1958), solar radiation (Casagrande et al., 2009), and soil moisture (Guttieri et al., 2005), also affect protein concentration in wheat.

In a study in Mexico, Fernandez and Laird (1959) found that when available moisture was 90%, the addition of 151 kg N ha^{-1} increased GP from 12.9% to 15.2%. Another study conducted by Guttieri et al. (2005) showed that reducing the supply of irrigation water increased GP. These findings are in agreement with a study by Campbell et al. (1977), which showed that under conditions of moisture stress GP levels increased.

Several researchers have investigated the effect of air temperature on GP (Finney and Fryer, 1958; Smika and Greb, 1973; Triboi et al., 2003). Work by Triboi et al. (2003) showed that when post-anthesis temperatures increased by 7 °C, GP increased from 8.9% to 13.8% and when temperatures increased by 9 °C, GP increased from 11.8% to 14.6%.

A study by Finney and Fryer (1958), assessing the effect of high temperatures on the milling and baking quality of several hard winter and spring wheat varieties, showed that maximum air temperatures above 32 °C during the last 15 days of growth caused a decline in GP.

The development of remote sensing has been essential for agriculture. Over many years of research, scientists have developed optical sensors that have enabled the analyses of different biophysical crop parameters (Jensen, 2000). Sensors and indices have been developed and are being used to determine crop parameters such as yield (Osborne et al., 2002), chlorophyll content (Wright et al., 2004; Munden et al., 1994), plant greenness (Pinter et al., 1987), N status (Wright et al., 2003), and biomass (Peñuelas et al., 1993). Freeman et al. (2003) found that the normalized difference vegetation Index (NDVI) collected at F9 and F10.5 growth stages was well correlated with grain yield, grain N uptake, straw N uptake, and total N uptake. However, the authors concluded that across years and locations NDVI did not reliably predict grain N. This was partially attributed to the inability to detect N translocation efficiency within the plant and the inability to detect how much N is lost through various pathways.

In a study evaluating the response of yield and GP to added fertilizer, Robinson et al. (1999) compiled historical climate records from 1960 to 1993. The authors noted that optimal responses to N fertilizer would most likely occur under low soil available N and abundant soil moisture levels. They further noted that low average rainfall can result in low crop response to added fertilizer. In this study we evaluated preplant N, NDVI, rainfall, and average air temperature (T-avg) as predictors of NUE and GP. In addition, the effect of N fertility on grain yield, GP, and NUE was investigated.

Materials and Methods

For this study, three winter wheat field experiments were established in 2009–10, 2010–11, and 2011–12 growing seasons. These experiments were located at Lake Carl Blackwell, Lahoma and Hennessey, Oklahoma. The experimental site at Lake Carl Blackwell is located on a Port silt loam; fine-silty, mixed, thermic Cumulic Haplustolls. The experimental site at Lahoma is located on a Grant silt loam; fine-silty, mixed, superactive, thermic Udic Argiustolls and the site at Hennessey is located on a Bethany silt loam; fine, mixed, superactive, thermic Pachic Paleustolls. The sites were planted in the fall of 2009, 2010, and 2011 using a 3 meter (m) Kincaid™ drill (Kincaid Equipment Manufacturing, Haven, Kansas, USA) with a row spacing of 15.24 cm. Plots were 6 m long and 3 m wide. Treatment structure was a randomized complete block design with 10 treatments and 4 replications. Treatment groupss 2 through 10 all received a preplant treatment with urea ammonium nitrate, (UAN; 28-0-0). Preplant N rates were 0, 28, 56, 112, and 168 kg ha^{-1}. Treatment groupss 4 through 9 received an additional topdress application at rates of 28, 56, 84, 112, and 140 kg N ha^{-1} applied at F5 growth stage. From cropping season 2010–11, 4 additional treatments were added (Table 1). Treatment groupss 11, 12, 13, and 14 received preplant N rates of 56, 84, 140, and 224 kg ha^{-1}, respectively with no additional topdress application. Urea ammonium nitrate was applied with an all-terrain vehicle (ATV) sprayer with a 3 m boom. Topdress applications were made at growth stage F5. Normalized Difference Vegetation Index measurements were collected with the GreenSeeker™ Hand Held Sensor (Trimble Navigation, Sunnyvale, CA) at growth stages F3, F4, F5, and F7 (Large 1954) . The GreenSeeker calculates NDVI as follows:

$$\frac{\rho NIR - \rho Red}{\rho NIR + \rho Red}$$

where, ρNIR and ρRed respectively are the fractions of emitted near infrared (NIR) (770 $\pm$ 15 nm) and red (650 $\pm$ 10 nm) radiation reflected back from the sensed area.

At maturity, plots were harvested using a Massey Ferguson 8XP (Massey Ferguson 8XP, Haven, KS) self-propelled combine. Grain subsamples from each plot were collected and dried at 75 °C for 2 days. Using a Thomas Wiley Laboratory mill (Thomas Scientific, Swedesboro, New Jersey, USA) the dried samples were then ground for analyses. Total N analysis was accomplished using a LECO Truspec CN dry combustion analyzer (Leco Corp., St Joseph, Michigan, USA) (Schepers et al., 1989).

Table 1
Treatment structure including N applied preplant and topdress, Lahoma, Lake Carl Blackwell, and Hennessey, OK, 2010–11, and 2011–12

Treatment[†]	N Preplant (kg ha^{-1})	N Topdress (kg ha^{-1})	N Total (kg ha^{-1})
1	0	0	0
2	168	0	168
3	28	0	28
4	28	28	56
5	28	56	84
6	28	84	112
7	28	112	140
8	28	140	168
9	56	56	112
10	112	0	112
11	56	0	56
12	84	0	84
13	140	0	140
14	224	0	224

[†]Treatments 11 to 14 were first added to the experiment in cropping season 2010–11.

Nitrogen use efficiency was calculated using the formula:

$$\left(\frac{\textit{Grain N uptake treated} - \textit{Grain N uptake check}}{\textit{N Rate applied}}\right) \times 100$$

Rainfall and T-avg data during the growing season were downloaded from the Mesonet (mesonet.org). For Hennessey data, the Lahoma Mesonet station was used, since there is no Mesonet station located in Hennessey. Statistical analysis was performed using the regression procedure in SAS (SAS, 2003). Different multiple linear models were evaluated that included all locations and years to predict NUE and final GP content. Predictor variables used included preplant N, NDVI, growing degree days (GDD) from planting to sensing, T-avg, and cumulative rainfall from planting to sensing. Treatments with additional topdress N application were not included since topdress N treatments altered growing conditions and thus final grain yields, GP, and NUE. Growing degree days are those days where growth was possible (Temperature > 4.4 °C), computed as follows:

$$\left[(Tmin + Tmax)/2 - 4.4°\mathrm{C}\right].$$

where, Tmin is the minimum daily temperature and Tmax is the maximum daily temperature.

Results and Discussion

Weather Conditions

Climatic conditions for the three cropping seasons differed greatly, which ultimately caused a difference in response to added fertilizer and crop growth. Air temperatures during cropping season 2009–10 at Lahoma ranged from 11 °C in October to 19 °C in May (Figure 1). Moisture supply from booting through grain filling is critical in wheat growth. Rainfall at Lahoma from February to May increased from 34 to 124 mm. During the same growing season Lake Carl Blackwell got as much as 108 mm of rainfall in April and 156 mm in May. Total rainfall at Lahoma in February 2011 was only 7 mm compared with 41 mm at Lake Carl Blackwell (Figure 2). Average temperatures from February 2011 to May 2011 ranged from 3 °C to 20 °C at Lahoma. Similarly, air temperatures at

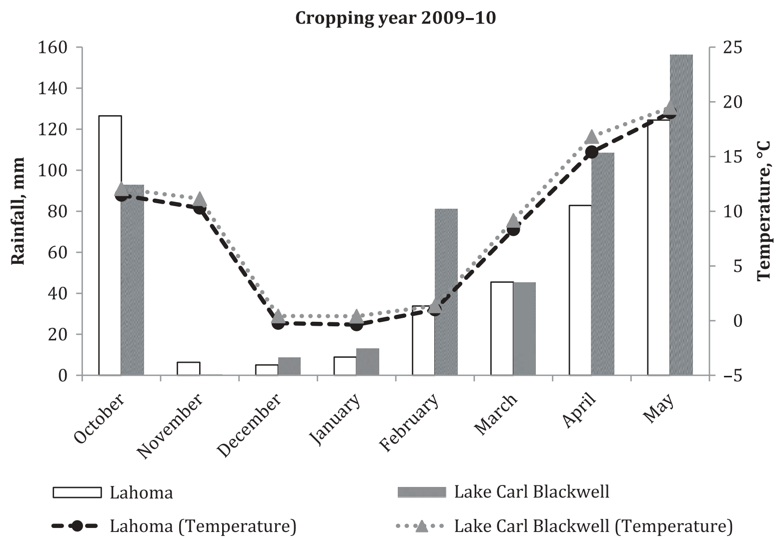

Figure 1. Monthly rainfall (mm) and average air temperature (°C) at Lahoma and Lake Carl Blackwell, OK from October 2009 to April, 2010.

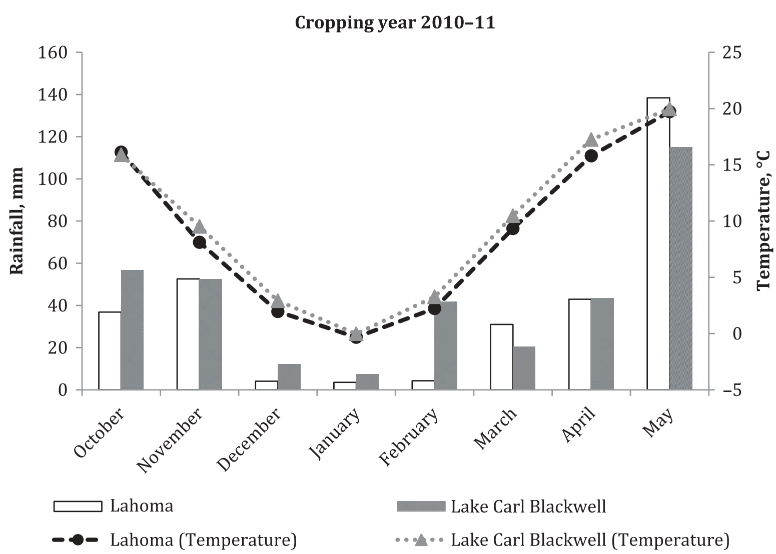

Figure 2. Monthly rainfall (mm) and average air temperature (°C) at Lahoma and Lake Carl Blackwell, OK from October 2010 to April, 2011.

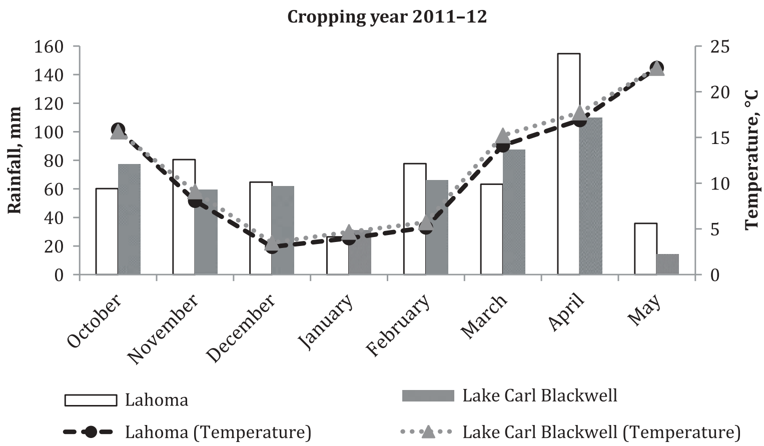

Figure 3. Monthly rainfall (mm) and average air temperature (°C) at Lahoma and Lake Carl Blackwell, OK from October 2011 to April, 2012.

Lake Carl Blackwell ranged from 2 °C to 20 °C. In April 2012, Lahoma had as much as 154 mm of rainfall and 35 mm in May, while Lake Carl Blackwell had only 14 mm of rainfall in May (Figure 3). Average air temperatures ranged from 16 °C to 23 °C from February to May at both locations.

Cropping Season 2009–10

For two out of three sites, Lake Carl Blackwell and Hennessey, the optimum N rate during the 2009–10 season was 168 kg N ha^{-1}. At Lake Carl Blackwell maximum yield was obtained with a preplant N rate of 168 kg ha^{-1}only. However, at Hennessey the highest grain yield, 4131 kg ha^{-1}, was obtained with a preplant N rate of 28 kg ha^{-1} and an additional topdress rate of 140 kg N ha^{-1}. At Lahoma the optimum N rate was 56 kg N ha^{-1} preplant in combination with a topdress application of 56 kg N ha^{-1}. Further increases in N rates had no effect on yield (Figure 4). On average the lowest yields during the 2009–10 cropping year were found at Lahoma, ranging from 1724 kg ha^{-1} (low N rate) to a maximum of 2674 kg ha^{-1} (high N rate) for a response index (RI) of 1.55. The RI is calculated as the maximum grain yield divided by the grain yield of the unfertilized plot (Johnson and Raun, 2003). At Hennessey grain yields increased from 2581 kg ha^{-1} without any fertilizer to 4131 kg ha^{-1} at 28 kg N ha^{-1} preplant with a topdress application of 140 kg N ha^{-1}, resulting in a RI of 1.60.

Low average grain yields at Lahoma resulted in high GP, ranging from 11.9 to 15.7% (Figure 4). These findings are in agreement with other research showing an inverse relationship between grain yield and GP (Terman et al., 1969; Campbell et al., 1977; Clarke et al., 1990). At Lake Carl Blackwell GP increased linearly from 9.8% (0N) to a maximum of 13.5% at a rate of 28 kg N ha^{-1} applied preplant with a topdress application of 140 kg N ha^{-1}; GP increased to levels beyond the 11% threshold only with the addition of 28 kg N ha^{-1} preplant with 56 kg N ha^{-1} topdress. At Hennessey however, 28 kg N ha^{-1} preplant with 28 kg N ha^{-1} topdress increased GP to 11.3 % but further increases in GP with increasing N rate was not consistent (Figure 4).

The lowest N rate (28 kg N ha^{-1}) yielded the highest NUE at Lake Carl Blackwell (86%) and at Hennessey (53%) (Figure 5). Nitrogen use efficiency at these sites decreased

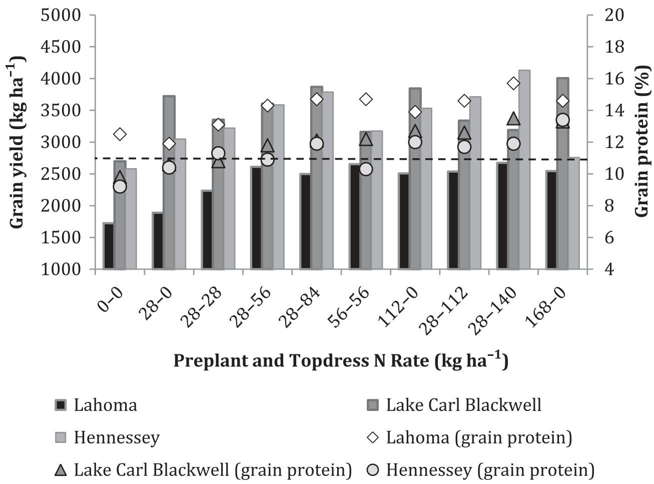

Figure 4. Grain yield and GP as affected by preplant N and additional topdress N at Lahoma, Lake Carl Blackwell, and Hennessey in cropping year 2009–10. Grain yields at Lahoma and Hennessey were significantly different between treatments at $\alpha = 0.05$ and 0.1, respectively.

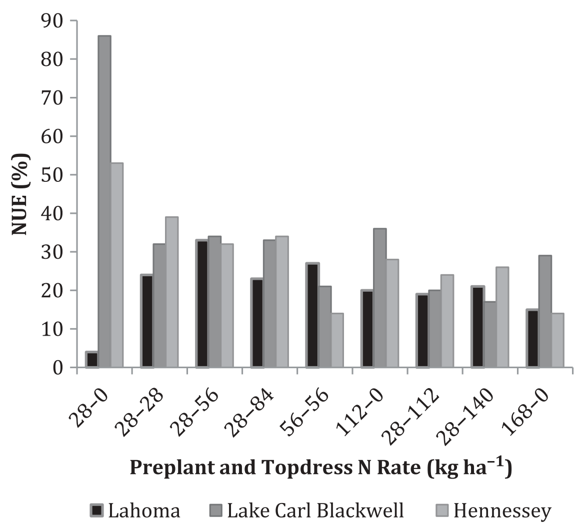

Figure 5. Mean NUE as a function of preplant and additional topdress N at Lahoma, Lake Carl Blackwell, and Hennessey, OK. in cropping year 2009–10. Nitrogen use efficiency at Lake Carl Blackwell was significantly different between treatments at $\alpha = 0.05$.

with increasing preplant N rate and no additional topdress application. At Lake Carl Blackwell NUE decreased from 86% with 28 kg N ha^{-1} preplant to 29% with 168 kg N ha^{-1} preplant (Figure 5). At Lahoma 28 kg N ha^{-1}, applied preplant, yielded the lowest NUE (4%), suggesting that most of this applied N was lost from the system. These findings indicate the importance of timing of fertilizer application. Wuest and Cassman (1992) attributed the greater fertilizer efficiency for late season versus preplant fertilizer to a better developed root system and increased photosynthetic and sink capacity.

Cropping Season 2010–11

Average grain yields during the 2010–11 cropping season were found to be lower than during the 2009–2010 and 2011–12 season at all three sites. These results were expected since rainfall numbers recorded from February to April 2011 were significantly lower and maximum temperatures significantly higher (data not shown) than during the two other seasons. Analysis of variance showed that there was no significant difference in yield between treatments at Lake Carl Blackwell. Also, Lake Carl Blackwell had an RI of 1.26, the lowest compared to the other sites (Figure 6); average grain yield at 0N was 2020 kg ha^{-1}. The highest grain yield, 2554 kg ha^{-1}, was recorded with an N rate of 168 kg ha^{-1}preplant only, as further increase in N rate caused a decrease in grain yield. High yield at 0N and low responsiveness to added N indicates that residual N was sufficient to satisfy crop needs.

Grain protein at Lake Carl Blackwell and Hennessey were consistently above 11% (Figure 6). Higher than average maximum temperatures and low rainfall during this season resulted in low average yields, and thus high GP. Analysis of variance showed that GP was significantly different between N treatments for all locations. Lowest GP at Lake Carl Blackwell was 14.3 % without any applied N and increased to as much as 16.8 % with a preplant application of 28 kg N ha^{-1} and an additional 112 kg N ha^{-1} topdress.

Response indices computed for the three sites indicate a higher response to added fertilizer at Lahoma and Hennessey than at Lake Carl Blackwell. Overall these two sites also had higher NUE's when compared with Lake Carl Blackwell (Figure 7). These findings are in agreement with Gauer et al. (1992) who stated that NUE would be greatest where yield response to added fertilizer is highest. A decreasing trend in NUE was observed at Hennessey with increasing preplant N, and no topdress N. The lowest N rate (28 kg N ha^{-1}) yielded an NUE of 37% and at 140 kg N ha^{-1} all preplant, NUE was 13% (Figure 7).

Cropping Season 2011–12

Grain yields during the 2011–12 cropping season were significantly higher than the previous season. Rainfall numbers were higher from October 2011 to April 2012 when

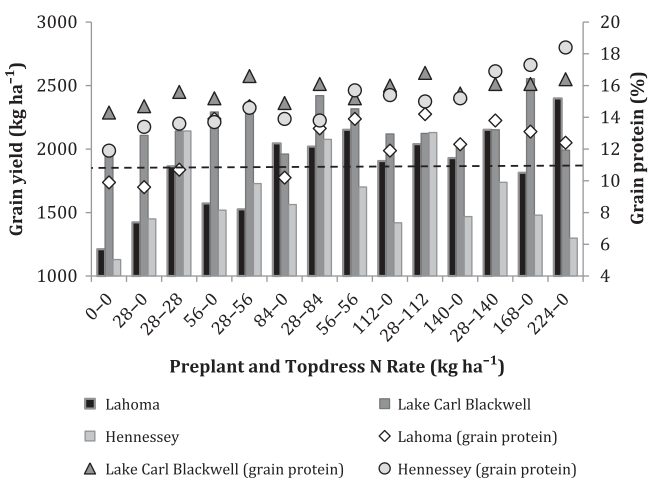

Figure 6. Grain yield and GP as affected by preplant and additional topdress N at Lahoma, Lake Carl Blackwell, and Hennessey in cropping year 2010–11. Grain yields at Lahoma and Hennessey were significantly different between treatments at $\alpha = 0.05$ and 0.1, respectively.

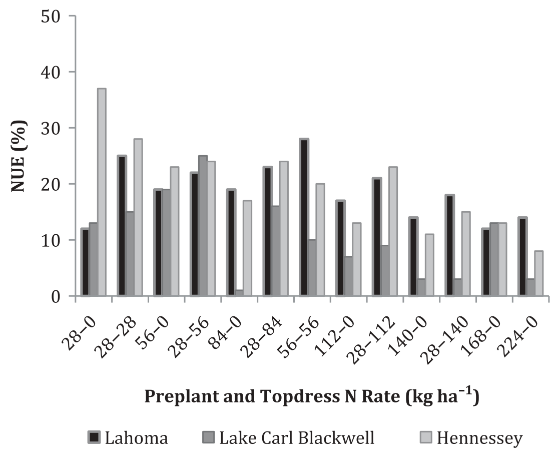

Figure 7. Mean NUE as a function of preplant and additional topdress N at Lahoma, Lake Carl Blackwell, and Hennessey, OK. in cropping year 2010–11. Nitrogen use efficiency at Lake Carl Blackwell and Hennessey was significantly different at $\alpha = 0.05$.

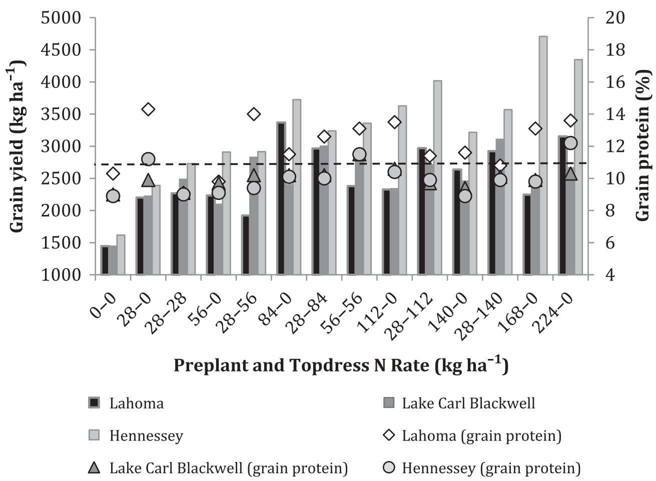

Figure 8. Grain yield and GP as affected by preplant and additional topdress N at Lahoma, Lake Carl Blackwell, and Hennessey in cropping year 2011–12. Grain yields at all locations were significantly different between treatments at $\alpha = 0.05$.

compared to the previous season at both weather stations. Average grain yields were highest at Hennessey (Figure 8); grain yields increased from 1619 kg ha^{-1} with no applied N to 4708 kg ha^{-1} with 168 kg N ha^{-1}preplant. In comparison with previous years, higher RI's were found at all three sites. Except for Lake Carl Blackwell, soil test results at the beginning of the cropping season, showed significantly lower nitrate (NO_3^-) levels for Lahoma and Hennessey (Table 2). This could explain the high response to added N during the 2011–12 cropping season. However, as in previous years, the N rate producing maximum yields differed between sites. At Lahoma the maximum yield, 3369 kg ha^{-1}, resulted from a preplant N rate of 84 kg N ha^{-1} with no additional topdress application, while at Lake Carl Blackwell a preplant application of 28 kg N ha^{-1} and an additional 140 kg N ha^{-1}

Table 2
Soil test results in the surface 0 to 15 cm at Lahoma, Lake Carl Blackwell, and Hennessey, Oklahoma for cropping years 2010–11 and 2011–12

		mg kg $^{-1}$				g kg^{-1}	
	pH[a]	NH_4-N[b]	NO_3-N[b]	P[c]	K[c]	Total N	C
Cropping year 2010–11							
Lahoma	6.3	6.3	14.3	4	201	0.70	6.90
Lake Carl Blackwell	6.7	3.6	10.7	55	101	0.50	5.30
Hennessey	5.4	4	21.9	135	558	1.00	5.40
Cropping year 2011–12							
Lahoma	5.4	7.3	4.2	11	210	0.83	7.90
Lake Carl Blackwell	4.6	13.9	16.0	29	105	0.67	5.24
Hennessey	5.2	11.9	14.3	79	396	1.09	11.60

[a]pH: 1:1 soil: Water.
[b]NH_4-N and NO_3-N: 2M KCl extraction.
[c]P and K: Mehlich III extraction.
[d]Total N and organic C: Leco Truspec CN dry combustion analyzer.

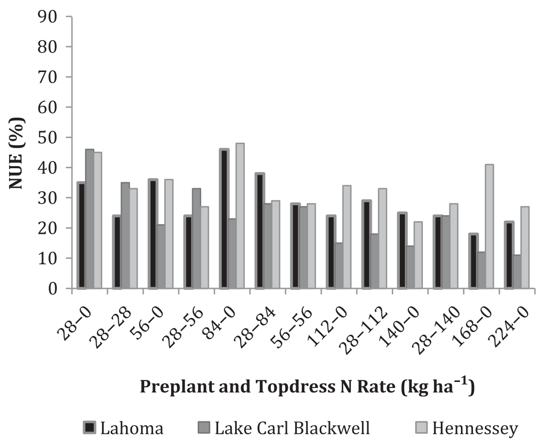

Figure 9. Mean NUE as a function of preplant and additional topdress N rates at Lahoma, Lake Carl Blackwell, and Hennessey, OK. in cropping year 2011–12. Nitrogen use efficiency.

were required to achieve maximum yield (Figure 8). At Hennessey however, maximum yield was obtained with 168 kg N ha^{-1} all preplant applied. Further increase in N rate did not increase grain yield and decreased NUE.

Grain protein levels at Lake Carl Blackwell and Hennessey were mostly under the 11% threshold (Figure 8). At Lake Carl Blackwell only a split application of 56 kg N ha^{-1} preplant and 56 kg N ha^{-1} topdress increased GP above 11% to 11.5%. At Hennessey the addition of 28 kg N ha^{-1} preplant increased GP from 9% to 11.2%, while adding 224 kg N ha^{-1} resulted in 12.2% GP.

Nitrogen use efficiency showed a decreasing trend at Lake Carl Blackwell with increasing preplant N rate (Figure 9). With an application of 28 kg N ha^{-1} only, NUE was 46%, which decreased to 11 % with an application of 224 kg N ha^{-1}.

Predicting Grain Protein and Nitrogen Use Efficiency

In addition to N fertility, climatic variables such as moisture and air temperature affect grain yield, and thus GP. Rainfall, the main source of soil moisture, and T-avg were evaluated for their use in predicting grain yield, GP, and NUE. These environmental components could then be used to refine current fertilizer recommendations. Linear models were generated for the dependent variables grain yield, GP, and NUE using the predictor variables preplant N, NDVI, GDD, cumulative rainfall, and T-avg from planting to sensing. In order to create a robust model that would work across locations and years, data from the three locations and the three cropping seasons were combined. Stepwise selection and the r^2 (coefficient of determination) selection procedure with a significance level of 0.05 were utilized to select the best fitting models. When examining r^2, NDVI at F8 growth stage was the best single predictor of grain yield ($r^2 = 0.56$) across growth stages (Table 3). It needs to be noted however, that NDVI at F8 was only collected at a single location (Lake Carl Blackwell) for 1 year. The low r^2 for the linear relationship between NDVI and yield from F3 to F7 might have been caused by combining data across years and sites and thereby ignoring the differing environments between years and locations. At early growth stages NDVI is also poorly correlated to grain yield because at these growth stages NDVI is influenced by the underlying soil. At later growth stages however, as the plant stand develops, the underlying soil becomes less of a factor. At F7 growth stage cumulative rainfall and NDVI combined in a model proofed to be the best two variable model for grain yield with an r^2 of .0.46 (Table 3). Interestingly, the 2, 3, and 4 predictor models all included rainfall as a predictor variable. These results indicate that rainfall as the main source of soil moisture has a significant effect on plant stand development and dissolving of fertilizer N (Robinson et al. 1999), and is thus critical in yield determination. Also, the best models for predicting grain yield were found at F5 growth stage, confirming findings by Lukina et al. (2001) who found NDVI at early growth stages, F4 and F5 to be an excellent predictor of grain yield. Work by Girma et al. (2005) further confirms these findings, reporting that NDVI and chlorophyll measurements at F5 and F7 were highly correlated with grain yield. Cumulative rainfall and T-avg from planting to sensing at F5 accounted for 41% ($r^2 = 0.41$) of the variability in grain yield. However, adding NDVI to the model significantly improved the predictive power of the model ($r^2 = 0.60$) (Table 3). The 4 variable predictor model that included preplant N, T-avg, cumulative rainfall, and NDVI at F5 explained 66% of the variability in grain yield (Table 3). Including GDD in the model ($r^2 = 0.66$) did not

Table 3

Predictor variables and coefficient of determination (r^2) for linear regression models for predicting grain yield

Number of Variables	Growth Stage	Predictors in Model	$r^{2\pm}$
1	F8ˆ	NDVI	0.68
2	F7	Rainfall, NDVI	0.46
3	F5	Rainfall, T-avg, NDVI	0.60
4	F5	Rainfall, T-avg, NDVI, Preplant	0.66

$^{\pm}$the proportion of variability in the dependent variable explained by the independent variables in the model.

ˆFeekes 8 NDVI collected at Lake Carl Blackwell in cropping year 2011–12.

Table 4
Predictor variables and coefficient of determination (r^2) for linear regression models for predicting grain protein

Number of Variables	Growth Stage	Predictors in Model	$r^{2\pm}$
1	F8ˆ	NDVI	0.56
2	F7	NDVI, Rainfall	0.45
2	F7	NDVI, Rainfall, GDD	0.57
4	F3	NDVI, Preplant, Rainfall, GDD	0.76

± the proportion of variability in the dependent variable explained by the independent variables in the model.
ˆFeekes 8 NDVI collected at Lake Carl Blackwell in cropping year 2011–12.

improve the prediction of grain yield. Growing degree days (GDD) was not significant as a predictor variable at a 0.05 significance level. Similarly, Lukina et al. (2001) found no improvement in grain yield prediction when GDD was added as a divisor of NDVI.

The best single predictor of GP was NDVI at F8 growth stage with an r^2 of 0.56 (Table 4). Similar to the prediction of grain yield, rainfall was significant in the prediction of GP. Best predictor models for GP were found at F3 and F7 growth stages. However, NDVI only was poorly correlated with GP at these growth stages (F3, $r^2 = 0.30$ and F7, $r^2 = 0.23$). The poor linear relationship between NDVI and GP can be attributed to poor ground coverage at earlier growth stages. Also, NDVI by itself cannot distinguish how much N is translocated to the grain (Freeman et al., 2003). A linear model including rainfall and NDVI at F7 explained 45% of the variability in GP (Table 4). Adding GDD to the model improved the relationship significantly to an r^2 of 0.57. The variables NDVI, rainfall, GDD at F7 and preplant N resulted in an r^2 of 0.63. Adding T-avg to the model did not improve the prediction of GP.

The stepwise selection method also showed that at F3, NDVI, preplant N, and rainfall combined explained 56% ($r^2 = 0.56$) of the variability in GP. Adding GDD to the model resulted in the best 4 predictor model with an r^2 of 0.76 (Table 4). The r^2 selection method revealed that a linear model that included preplant N, cumulative rainfall, and GDD at F3 explained 74% of the variability in GP. These results indicate that in addition to early season N availability, environmental conditions are essential in determining final GP. Growing conditions early in the season are critical to emergence and crop establishment (Mahdi et al., 1998). The predictor variable T-avg from planting to sensing at F3 was not significant at the 0.05 significance level.

All the models tested showed poor relationship with NUE between F3 and F7 growth stages. The three variable model preplant N, cumulative rainfall, and GDD at F3 growth stage explained 34% of the variability in GP. Preplant N and NDVI at F8 growth stage, combined in a model resulted in an r^2 of 0.45 (results not shown). However, NDVI only was poorly correlated with NUE ($r^2 = 0.09$). The poor correlation between NDVI and NUE can be attributed to the fact that NDVI measurements cannot distinguish how much of the applied N is taken up and how much is lost from the soil-plant system. The NDVI therefore does not reflect how much of the N applied (preplant) is taken up into vegetative tissue. Incorporating rainfall and T-avg into the model did not improve the prediction of NUE.

Conclusions

In this 3-year study, grain yield, NUE, and GP were evaluated as a function of rate and timing of fertilizer N application. In addition, NDVI, rainfall, and T-avg were evaluated as parameters for the prediction of GP and NUE. Results did show that N rate significantly affected grain yield, NUE, and GP. However, response to added fertilizer differed greatly between sites and years. In cropping years with suboptimal growing conditions, low yields resulted in high GP. In cropping year 2009–10, grain yields at Lahoma were consistently below 2700 kg ha^{-1} resulting in high GP levels ranging from 11.9% to 15.7%. In the following cropping year, grain yields across all sites were below 2600 kg ha^{-1}. During this cropping year, at two sites GP was consistently above 11%. At Lake Carl Blackwell GP ranged from 14.3% to 16.8% and at Hennessey GP ranged from 11.9% to 18.4%. In cropping year 2011–12 growing conditions were ideal. Grain yields at Hennessey ranged from 1619 kg ha^{-1} to 4708 kg ha^{-1} and GP ranged from 8.9% to 12.2%. Nitrogen use efficiency did not show a consistent decrease with increasing N rate. GreenSeeker NDVI readings collected between F3 and F7 growth stage were poorly correlated with grain yield and GP across sites and years. However, NDVI collected at F8 at Lake Carl Blackwell was well correlated with grain yield ($r^2 = 0.68$) and GP ($r^2 = 0.56$). Results also show that early season N fertility combined with NDVI, cumulative rainfall and GDD might be useful to predict GP. This work shows the importance of including environmental factors in N fertilizer algorithms. From this work it is elucidated that ambient temperature and rainfall in particular should be taken into consideration when predicting GP and should thus be included in current algorithms for mid-season N fertilizer application for GP adjustment.

References

Baligar, V. C., N. K. Fageria, and Z. L. He. 2001. Nutrient use efficiency in plants. *Communications in Soil Science and Plant Analysis*. 32: 921–950.

Brown, B. 2000. Nitrogen management for hard wheat protein enhancement. University of Idaho Winter Commodity school proceedings. Available at: http://www.extension.uidaho.edu/swidaho/Nutrient%20Management/increasing_wheat_protein.htm (verified 19 March 2012).

Campbell, C. A., D. R. Cameron, W. Nicholaichuk, and H. R. Davidson. 1977. Effect of fertilizer N and soil moisture on growth, N content, and moisture use by spring wheat. *Canadian Journal of Soil Science*. 57: 289–310.

Campbell, C. A., R. P. Zentner, F. Seller, B. G. McConkey, and F. B., Dijck. 1993. Nitrogen management for spring wheat grown annually on zero-tillage: yields and nitrogen use efficiency. *Agronomy Journal* 85: 107–114.

Casagrande, M., C. David, M. Valantin,-Morison, D. Makowski, and M. Jeuffroy. 2009. Factors limiting the grain protein content of organic winter wheat in south-eastern France: a mixed-model approach. *Agronomy for Sustainability Development* 29: 565–574.

Clarke, J.M., C.A. Campbell, H.W. Cutforth, R.M. De Pauw, and G.E. Win-Kleman. 1990. Nitrogen and phosphorus uptake, translocation, and utilization efficiency of wheat in relation to environment and cultivar yield and protein levels. *Canadian Journal of Plant Science* 70: 965–977.

Ellen, J., and J.H.J. Spiertz. 1980. Effects of rate and timing of nitrogen dressings on grain yield formation of winter wheat. *Fertilizer Research* 1:177–190.

Fernandez, R. G., and R. J. Laird. 1959. Yield and protein content of wheat in Central Mexico as affected by available soil moisture and nitrogen fertilization. *Agronomy Journal* 51: 33–36.

Finney, K. F., and H.C. Fryer. 1958. Effect on loaf volume of high temperatures during the fruiting period of wheat. *Agronomy Journal* 50: 28–34.

Freeman, K. W., W. R. Raun, G. V. Johnson, R. W. Mullen, M. L. Stone, and J. B. Solie. 2003. *Communications in Soil Science and Plant Analysis* 34: 1837–1852.

Gauer, L. E., C. A.Grant, D. T. Gehl, and L. D. Bailey. 1992. Effects of nitrogen fertilization on grain protein content, nitrogen uptake, and nitrogen use efficiency of six spring wheat (Triticum aestivum L.) cultivars, in relation to estimated moisture supply. *Canadian Journal of Plant Science* 72:235–241.

Girma, K., K. L. Martin, R. H. Anderson, D. B. Arnall, K. D. Brixey, M. A. Casillas, B. Chung, B. C. Dobey. S. K. Kamenidou, S. K. Kariuki, E. E. Katsalirou, J. C. Morris, J. Q. Moss, C. T. Rohla, B. J. Sudbury, B. S. Tubana, and W. R. Raun. 2005. Mid-season prediction of wheat grain yield potential using plant, soil, and sensor measurements. *Journal of Plant Nutrition* 29: 873897.

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

Jensen, J. R. 2000. Remote sensing of vegetation. In *Remote sensing of the environment: an earth perspective*. Clarke, K. C. (ed.). Prentice Hall, New Jersey. pp. 333–377.

Johnson, G. V., and W. R. Raun. 2003. Nitrogen response index as a guide to fertilizer management. *Journal of Plant Nutrition* 6: 249–262.

KCBT 2010. Kansas City Board of Trade. Contract specifications. Available at http://www.cmegroup.com/trading/agricultural/kcbt.html. (Verified 22 July 2013).

Large, E. C. 1954. Growth stages in cereals. *Plant Pathology* 3: 128–129.

Lukina, E.V., K. W. Freeman, K. J. Wynn, W. E. Thomason, R. W. Mullen, G. V. Johnson, R. L. Elliott, M. L. Stone, J. B. Solie, and W. R. Raun. 2001. Nitrogen fertilization optimization algorithm based on in-season estimates of yield and plant nitrogen uptake. *Journal of Plant Nutrition* 24: 885–898.

Mahdi L., C.J. Bell, and J. Ryan. 1998. Establishment and yield of wheat (Triticum turgidum L.) after early sowing at various depths in a semi-arid Mediterranean environment. *Field Crops Research* 58: 187–196.

Munden, R., P. J. Curran, and J. A. Catt. 1994. The relationship between red edge and chlorophyll concentration in the Broadbalk Winter Wheat Experiment at Rothamsted. *International Journal of Remote Sensing* 15:705–709.

Osborne, S.L., J. S. Schepers, D. D. Francis, and M.R. Schlemmer. 2002. Use of spectral radiance to estimate in-season biomass and grain yield in nitrogen and water-stressed corn. *Crop Science* 42: 165–171.

Peñuelas, J., J. A. Gamon, K. L. Griffin, and C. B. Field. 1993. Assesseing community type, plant biomass, pigment composition, and photosynthetic efficiency of aquatic vegetation from spectral reflectance. *Remote Sensing of the Environment* 46: 110–118.

Pinter, P. J., G. Zipoli, G. Maracchi, and R. J. Reginato. 1987. Influence of topography and sensor view angles on NIR/Red ratio and greenness vegetation indices of Wheat. *International Journal of Remote Sensing* 8:953–957.

Raun, W. R and G.V Johnson. 1999. Improving nitrogen use efficiency for cereal production. *Agronomy Journal* 91: 357–363.

Raun, W. R., J. B. Solie, M. L. Stone, K. L. Martin, K. W. Freeman, R. W. Mullen, H. Zhang, J.S. Schepers, and G.V. Johnson. 2005. Optical sensor- based algorithm for crop nitrogen fertilization. *Communications in Soil Science and Plant Analysis* 36: 2759–2781.

Robinson, J.B., D.M. Freebairn, J.P. Dimes, R.C. Dalal, G.A. Thomas, and E.J. Weston. 1999. Modelling wheat production from low rainfall farming systems in Northern Australia. *Environment International* 25: 861–870.

SAS Institute. 2003. *The SAS system for windows version 9.2*. SAS Inst., Cary, NC.

Schepers, J.S., D. D. Francis, and M. T. Thompson. 1989. Simultaneous determination of total C, total N, and ^{15}N on soil and grain material. *Communications in Soil Science and Plant Analysis* 20: 949–960.

Smika, D. E., and B. W. Greb. 1973. Protein content of winter wheat grain as related to soil and climatic factors in the semiarid Central Great Plains. *Agronomy Journal* 65: 433–436.

Terman, G.L., R. E. Ramig, A. F. Dreier, and R. A.Olson. 1969. Yield-protein relationships in wheat grain as affected by nitrogen and water. *Agronomy Journal* 61: 755–759.

Triboï, E., P. Martre, and A. Triboï- Blondel. 2003. Environmentally induced changes in protein composition in developing grain of wheat are related to changes in total protein content. *Journal of Experimental Botany* 54: 1731–1742.

United Nations 2004. World population to 2300. Department of Economic and Social affairs. Population division. Available at http://www.un.org/esa/population/publications/longrange2/WorldPop2300final (Verified 2 August 2013).

U.S. Wheat Associates. 2007. Overview of U.S. wheat inspection. Available at http://www.uswheat.org/uswPublic2009.nsf/3f1e3b91ed7dc84a852575e400579f77/9cede08317adbd7c852575e5004fc6a7/FILE/ATTX8SLN.pdf/fgis2007.pdf (Verified 8 July 2013).

Wuest, S. B., and K. G. Cassman. 1992. Fertilizer- nitrogen use efficiency of irrigated wheat: II. Partitioning efficiency of preplant versus late season application. *Agronomy Journal* 84: 689–694.

Wright, D. L., G.L. Ritchie, V.P. Rasmussen, R.D. Ramsey, and D.J. Baker. 2003.Managing grain protein in wheat using remote sensing. *Online Journal of Space Communication*: 3. (unpaginated).

Wright, D. L, V. P Rasmussen, R. D Ramsey, and D. J. Baker. 2004. Canopy reflectance estimation of wheat nitrogen content for grain protein management. *GIS and Remote Sensing* 41: 287–300.