Wheat Grain Yield and Grain Protein Concentration Response to Nitrogen Rate During the 2018–2019 Growing Season in Kansas

Romulo P. Lollato  
*Kansas State University*, lollato@ksu.edu

Kavan E. Mark  
*Kansas State University*, kavanmark58@k-state.edu

Brent R. Jaenisch  
*Kansas State University*, bjaenisch5@ksu.edu

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Abstract
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Keywords
wheat, nitrogen rate, nitrogen concentration, flag leaf

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Wheat Grain Yield and Grain Protein Concentration Response to Nitrogen Rate During the 2018–2019 Growing Season in Kansas

R.P. Lollato, K. Mark, and B.R. Jaenisch

Summary
The objective of this project was to evaluate winter wheat grain yield and grain protein concentration responses to nitrogen (N) rate in the state of Kansas during the 2018–2019 growing season. Experiments evaluating the response of the wheat variety Zenda to four nitrogen rates (0, 50, 100, and 150 lb N/a) were established at four locations. In-season measurements included flag leaf N concentration, grain yield, yield components, and grain protein concentration. Flag leaf N concentration ranged from 2.4 to 4.1% across all environments and treatments, and increases in N rates increased flag leaf N concentration linearly. Grain yield ranged from 36.3 to 94.4 bu/a and increased with increases in N rate usually following quadratic relationships at all locations except for Belleville, where no response was observed, likely due to the high organic matter levels. Grain protein concentration ranged from 11 to 15% across all locations and treatments and increases in N rates increased grain protein concentration following a usually linear relationship; however, the quadratic yield response to N rate, coupled to the linear protein response to N rate, indicated that greater N rates might be needed to maximize protein as compared to maximizing yields. Both relative grain yield and relative grain protein concentration variables calculated relative to the maximum in each respective environment, were related to flag leaf N concentration in a linear-plateau way, suggesting that flag leaf N concentration could be used as a diagnostic tool for crop N status.

Introduction
Nitrogen is a critical component of different amino acids and proteins needed to complete a plant’s life cycle; thus, it is an essential element to crops (Taiz and Zieger, 2010). About 80% of total wheat N uptake occurs by anthesis (Waldren and Flowerday, 1979). Total N uptake at maturity depends on yield level and ranges from near zero to about 360 lb N/a (de Oliveira Silva et al., 2020a). Different aspects of N management (i.e., rate and timing) are among the leading causes behind the large yield gap in Kansas (de Oliveira Silva et al., 2020b; Lollato et al., 2019a), which is estimated at about 50% (Lollato et al., 2017). The exception to this rule is when the system is already saturated by N. In these cases, no response to N rate usually occurs and other factors, such as fungicide and seeding rate, become prevalent (Jaenisch et al., 2019).
A recent comprehensive synthesis of long-term experiments conducted in the region suggested that wheat grain yield and grain protein concentration responses to N rate depended on yield environment (Lollato et al., 2019b). In other words, while there were limited yield responses to increases in N rate at low yield environments, yield followed a quadratic response to N rate in medium, high, and very high yield environments, with an agronomic optimum N rate increasing with increases in yield environment. Higher yield environments resulted in lower protein concentrations, as expected (Lollato and Edwards, 2015), and protein concentration increased linearly with increases in N rate.

Due to the importance of N management to wheat yield and protein, the objectives of this project were to assess winter wheat grain yield, grain protein concentration, flag leaf nitrogen concentration, and yield components as affected by different nitrogen rates in the state of Kansas during the 2018–2019 growing season.

**Procedures**

Field experiments were conducted during the 2018–2019 winter wheat growing season in different locations across Kansas: Ashland Bottoms, Belleville, Great Bend, Hutchinson, and Manhattan. At all locations, plots were comprised of seven 7.5 in.-spaced rows wide and 30-ft long, for a total plot area of approximately 131 ft$^2$. A total of four treatments resulting from four N rates were evaluated in each location. The fertility treatments evaluated consisted of 0, 50, 100, and 150 lb N/acre applied as urea during the fall. Planting, harvest, and product application dates are provided in Table 1. The same wheat variety (Zenda) was evaluated at all locations. Harvest occurred using a Massey Ferguson XP8 small-plot, self-propelled combine. Plot ends were trimmed at harvest time to avoid border effect, and the portion harvested for grain was approximately 100 ft$^2$ at both locations, comprising the central portion of the plots.

**Measurements and Statistical Analyses**

A total of 15 individual soil cores (0 to 24-in. depth) were collected from each location and divided into 0–6 in. and 6–24 in. increments for initial fertility analysis. The individual cores were mixed to form one composite sample, which was later analyzed for base fertility levels (Table 2).

Measurements included flag leaf N concentration taken at heading (approximately 40 flag leaves were collected per plot); a 0.19 m$^2$ biomass sample retrieved at harvest maturity from which we measured yield components (aboveground biomass, harvest index, head number per area, kernels per head, kernels per area, and 1000-kernel weight); and grain yield, grain test weight, and grain protein concentration. Nitrogen removal in the grain was calculated using a 5.7 conversion factor from protein to nitrogen in the wheat grain, and multiplying grain N by grain yield.

Statistical analysis of the data collected in this experiment was performed using PROC GLIMMIX procedure in SAS v. 9.4 (SAS Inst. Inc., Cary, NC). Replication was treated as a random effect in the analysis for individual locations, while location and replication nested within location were random effects in the analysis across locations.
Results

Weather Conditions
The 2018–2019 winter wheat growing season in Kansas was characterized by below average temperatures and above average precipitation (Table 3). The fall had anywhere from 9.3 to 13.9 inches of precipitation in the studied locations, sometimes resulting in poor stand establishment across the state. Due to this excessive fall precipitation and its consequent waterlogging, the Great Bend location was abandoned. The studied locations received anywhere from 16.3 to 24.9 inches of precipitation during the spring (April until July) which, coupled with below average temperatures, extended the growing season and delayed harvest until early to mid-July.

Overall Treatment Significance on the Measured Variables
Table 4 shows the results from the analysis of variance for each location individually, as well as for the combined analysis across locations. At the 0.1 probability level, nitrogen rate was a significant effect for most of the measured parameters at Ashland Bottoms, followed by Manhattan, Hutchinson, and finally the least responsive location to N rate was Belleville. The combined analysis showed a significant N rate effect on all but three measured parameters (Table 4).

Grain Yield and Yield Components
Across all treatments and locations, grain yield ranged from 36.3 to 94.4 bu/a. The lowest yielding location was Ashland Bottoms (average yield: 47 bu/a) and the highest yielding location was Belleville (average yield: 88 bu/a). At all locations except for Belleville, grain yield increased with increases in N rate (Table 5), usually following quadratic relationships (increasing until about 100 lb N per acre and plateauing at greater N rates) although in some instances, the relationship was linear. The lack of a significant N rate effect at the Belleville location could result from high levels of organic matter in this location, releasing organic nitrogen during the cycle of the crop (Table 2).

The ANOVA results for the yield components are shown in Table 6. Overall, the yield components most often impacted by N rate were shoot biomass and 1000-kernel weight, although in some locations there were also significant effects on heads per area and kernels per area. Biomass ranged from 5116 to 14,262 lb/a, and usually increased with increased N rates (Table 6). Harvest index ranged from 0.39 to 0.46 and was not impacted by the treatments evaluated. Heads per square foot ranged from 44 to 83 and increased with increasing N rates in the combined analysis (although the individual site-year analysis failed to detect significant treatment effects). At a few sites, increasing N rate reduced 1000 kernel weight, which is probably explained by more kernels being produced due to more N, and thus, additional smaller/secondary kernels originated. Increases in N rate generally increased kernels per area.

Flag Leaf N Concentration, Grain Protein Concentration, and Grain Test Weight
Flag leaf N concentration ranged from 2.4 to 4.1% across all environments and treatments, and it was significantly affected by N rate at Ashland Bottoms, Belleville, Manhattan, and in the combined analysis (Table 7). Usually, increasing N rates increased flag leaf N concentration (c.a., 2.9% in the zero-N control versus 3.28% in the 150 lb
Across all sources of variation, a linear plateau model explained the relationship between relative grain yield (calculated in each location relative to the maximum yield in that respective location) and flag leaf N concentration with an overall robustness of $r^2 = 0.26$. This model suggested that relative yield increased from ~0.5 at flag leaf N of 2.4%, to 0.84 at flag leaf N of 2.97%, and plateaued afterwards for flag leaf N concentration as high as 4.1% (Figure 1).

Grain protein concentration ranged from 11 to 15% across all locations and treatments. Nitrogen rate had a significant effect on grain protein concentration at all locations, including the combined analysis (Table 7). Increasing N rates increased grain protein concentration usually in a linear way (Ashland Bottoms, Manhattan, and combined analysis), but sometimes the relationship tended to reach a plateau or quadratic relationship in which there was no increase in protein concentration beyond a given N rate (Belleville and Hutchinson). Similarly to relative grain yield, relative grain protein concentration (calculated by location relative to the maximum respective to each location) was related to flag leaf N concentration ($r^2 = 0.23$) and followed a linear-plateau shape (Figure 1). Relative grain protein concentration increased from about 0.75 at flag leaf N concentration of 2.4%, to 0.94 at flag leaf N concentration of 2.95%. Further increases in flag leaf N concentration did not increase relative grain protein content.

Grain test weight ranged from 57.3 to 64.2 pounds per bushel across all treatments and locations. There were significant N rate effects on test weight in Hutchinson and Manhattan, as well as in the combined analysis. At these locations, test weight tended to decrease with increases in N rate, likely because greater N rates originated more tillers and these secondary tillers usually are later and result in lighter kernels (although this was not measured in the current study).

**Preliminary Conclusions**

Winter wheat response to N rate is dependent on environmental conditions, including not only the weather experienced in the season (and thus the potential yield of the season), but also the amount of inorganic nitrogen made available through the soil. In this research, most of the yield response to N rate was quadratic, suggesting that the 100 lb N/a rate was sufficient to maximize yields at the yield environments here studied (though small site-to-site variations were reported). Protein tended to follow a more linear response, perhaps suggesting that more N is needed to maximize protein as compared to yield. The linear-plateau relationship developed between relative grain yield or relative grain protein as affected by flag leaf N concentration provides preliminary evidence for using flag leaf N as an in-season diagnostic tool for crop N status.

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Table 1. Dates of field activities for the nitrogen rate trials conducted in 2018–2019

| Activity            | Stage     | Ashland Bottoms | Belleville  | Great Bend  | Hutchinson | Manhattan |
|---------------------|-----------|-----------------|-------------|-------------|------------|-----------|
| Planting            | ---       | 11/1/2018       | 10/3/2018   | 10/2/2018   | 10/22/2018 | 10/23/2018|
| Nitrogen application| Feekes 1  | 1/9/2019        | 11/7/2019   | 11/20/2019  | 11/14/2019 | 12/10/2019|
| Herbicide           | Feekes 4  | 3/22/2019       | 4/2/2019    | 3/27/2019   | 3/18/2019  | 3/22/2019 |
| Flag leaf sampling  | Feekes 10 | 5/15/2019       | 5/17/2019   | ---         | 5/6/2019   | 5/15/2019 |
| Fungicide           | Feekes 10.5| 5/31/2019       | 5/16/2019   | ---         | 5/15/2019  | 5/20/2019 |
| Harvest index       | Maturity  | 7/1/2019        | 7/15/2019   | ---         | 6/26/2019  | 7/1/2019  |

The Great Bend location was abandoned due to excessive fall precipitation causing waterlogging and sub-optimal stand establishment.
| Sample Name           | Depth | Ca   | Cu   | Mg   | Mn   | Na   | OM  | P-M | meq/100 g | pH  | NO₃⁻N | NH₄⁺-N | K   | Zn   | Fe   | S   | Cl   |
|----------------------|-------|------|------|------|------|------|-----|-----|-----------|-----|--------|--------|-----|------|------|-----|------|
|                      | in.   | ppm  | ppm  | ppm  | ppm  | ppm  | ppm | ppm | meq/100 g | ppm | ppm    | ppm    | ppm | ppm  | ppm  | ppm | ppm  |
| Ashland Bottoms      | 0–6   | 3329 | 1.8  | 550  | 15.1 | 21   | 3.0 | 8.4 | 22.1      | 6.5 | 3.0    | 6.5    | 304 | 0.6  | 47.1 | 2.6 | 8.2  |
|                      | 6–24  | 3604 | 2.0  | 760  | 10.7 | 36   | 2.2 | 3.7 | 25.3      | 6.7 | 1.7    | 7.9    | 309 | 0.2  | 33.3 | 2.2 | 6.6  |
| Belleville           | 0–6   | 2056 | 2.1  | 296  | 43.1 | 17   | 3.1 | 52.4| 27.98     | 5.4 | 0.4    | 3.0    | 437 | 0.8  | 114.2| 3.4 | 7.7  |
|                      | 6–24  | 4022 | 2.2  | 555  | 15.5 | 58   | 2.4 | 7.8 | 25.96     | 6.6 | 4.0    | 5.0    | 381 | 0.3  | 52.3 | 3.0 | 8.9  |
| Hutchinson           | 0–6   | 4746 | 1.1  | 163  | 7.0  | 35   | 2.9 | 27.2| 26.05     | 8.0 | 9.7    | 3.2    | 315 | 0.3  | 19.9 | 3.3 | 8.0  |
|                      | 6–24  | 5202 | 0.8  | 162  | 4.3  | 128  | 2.2 | 4.0 | 28.41     | 8.1 | 3.2    | 4.5    | 194 | 0.1  | 14.4 | 12.5| 12.3 |
| Manhattan            | 0–6   | 2977 | 2.4  | 357  | 30.4 | 17   | 3.5 | 22.3| 26.27     | 6.2 | 3.2    | 7.3    | 162 | 0.9  | 92.1 | 2.5 | 7.5  |
|                      | 6–24  | 4477 | 2.7  | 411  | 16.3 | 26   | 2.8 | 8.9 | 26.48     | 7.0 | 2.6    | 5.9    | 217 | 0.5  | 50.9 | 3.5 | 9.1  |

Information was collected for the 0 to 6-in. depth, and 6 to 24-in. depth. Fertility level include soil pH, buffer pH, Mehlich-3 extractable phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), ammonium-(NH₄⁺-N) and nitrate-nitrogen (NO₃⁻N), chloride (Cl), sulfate-sulfur (SO₄²⁻S), organic matter (OM), and cation exchange capacity (CEC). Sampling depths were 0–6 in. and 6–24 in.
### Table 3. Average maximum (Tmax) and minimum (Tmin) temperatures, precipitation, and grass evapotranspiration (ETo) during the fall (October 1–December 31), winter (January 1–March 31), and spring (April 1–July 15)

| Location       | Season | Tmax | Tmin | Precipitation | ETo |
|----------------|--------|------|------|---------------|-----|
|                | Fall   | 52.4 | 30.5 | 9.1           | 5.1 |
|                | Winter | 41.3 | 23.2 | 5.0           | 5.2 |
|                | Spring | 77.2 | 55.0 | 22.3          | 20.4|
| Ashland Bottoms| Fall   | 49.6 | 28.4 | 8.9           | 5.3 |
|                | Winter | 37.9 | 21.3 | 2.2           | 4.6 |
|                | Spring | 75.0 | 51.7 | 17.9          | 18.8|
| Belleville     | Fall   | 44.6 | 24.6 | 3.3           | 6.1 |
|                | Winter | 78.0 | 54.4 | 19.0          | 19.5|
|                | Spring | 53.2 | 31.6 | 9.3           | 5.3 |
| Hutchinson     | Fall   | 52.3 | 30.8 | 13.9          | 6.2 |
|                | Winter | 44.6 | 24.6 | 3.3           | 6.1 |
|                | Spring | 78.0 | 54.4 | 19.0          | 19.5|
| Manhattan      | Fall   | 42.2 | 24.0 | 5.0           | 5.0 |
|                | Winter | 77.8 | 55.6 | 24.9          | 19.0|

### Table 4. Significance of nitrogen (N) rate on different measured variables at all Kansas locations where the trial was conducted, as well as the analysis combined across sites, during the 2018–2019 growing season

| Variable        | Ashland Bottoms | Belleville | Hutchinson | Manhattan | Combined |
|-----------------|-----------------|------------|------------|-----------|----------|
| Test weight     | 0.29            | 0.99       | 0.06       | 0.03      | 0.02     |
| Yield           | <0.01           | 0.32       | 0.05       | <0.01     | <0.01    |
| Protein         | <0.01           | <0.01      | <0.01      | <0.01     | <0.01    |
| N removal       | <0.01           | 0.12       | 0.01       | <0.01     | <0.01    |
| Flag leaf N     | 0.01            | <0.01      | 0.13       | <0.01     | <0.01    |

Yield components

- Biomass: 0.06, 0.97, 0.83, 0.46, 0.03
- HI: 0.41, 0.27, 0.71, 0.88, 0.36
- Heads/m²: 0.12, 0.74, 0.11, 0.07, <0.01
- 1000-KW: 0.01, 0.7, 0.49, 0.96, 0.31
- Kernels/m²: 0.04, 0.78, 0.7, 0.49, 0.01
- Kernels/head: 0.04, 0.48, 0.42, 0.06, 0.83

Bold numbers show significant effects at \( P < 0.1 \).

HI = harvest index. KW = kernel weight.
Table 5. Wheat grain yield as affected by nitrogen (N) rate at four experiments conducted in Kansas during the winter wheat season of 2018–2019

| N rate | Ashland Bottoms | Belleville | Hutchinson | Manhattan | Combined |
|--------|-----------------|------------|------------|-----------|----------|
| lb of N/a | bu/a | lb of N/a | bu/a | lb of N/a | bu/a | lb of N/a | bu/a | lb of N/a | bu/a | lb of N/a | bu/a |
| 0      | 36.3 d          | 82.5       | 69.5 bc    | 50.1 c    | 59.6 d   |
| 50     | 46.8 c          | 89.5       | 73.4 abc   | 64.6 b    | 68.6 c   |
| 100    | 52.4 a          | 85.8       | 76.1 ab    | 74.6 a    | 72.2 abc |
| 150    | 52.8 a          | 94.4       | 78.1 a     | 76.9 a    | 75.6 ab  |

Means followed by the same letter indicate no statistical difference at the 0.05 probability level.

Table 6. Wheat yield components as affected by nitrogen rate at four experiments conducted in Kansas during the winter wheat season of 2018–2019

| Location         | Nitrogen rate | Biomass lb N/a | Harvest index | Heads/ft² | 1000 KW | Kernels/f² | Kernels/head |
|------------------|---------------|----------------|---------------|-----------|---------|------------|--------------|
| Ashland Bottoms  | 0             | 5116 c         | 0.46          | 44        | 28.9 a  | 828 c      | 19.0 bc      |
|                  | 50            | 6743 bc        | 0.46          | 52        | 28.7 ab | 1122 ab    | 22.0 a       |
|                  | 100           | 7492 a         | 0.46          | 59        | 28.2 bc | 1254 a     | 20.5 ab      |
|                  | 150           | 7616 a         | 0.45          | 59        | 27.6 c  | 1300 a     | 21.5 a       |
| Belleville       | 0             | 13927 ab       | 0.45          | 75        | 30.8 a  | 2078       | 27.5         |
|                  | 50            | 14168 a        | 0.44          | 82        | 30.0 ab | 2180       | 27.0         |
|                  | 100           | 14262 a        | 0.44          | 77        | 30.2 ab | 2205       | 29.0         |
|                  | 150           | 13901 ab       | 0.46          | 79        | 29.8 b  | 2246       | 28.5         |
| Hutchinson       | 0             | 7777           | 0.39          | 53        | 32.8 b  | 972        | 18.5         |
|                  | 50            | 7924           | 0.4           | 51        | 33.0 ab | 999        | 20.0         |
|                  | 100           | 8325           | 0.39          | 55        | 33.7 a  | 1026       | 18.5         |
|                  | 150           | 8094           | 0.41          | 58        | 32.1 b  | 1061       | 18.0         |
| Manhattan        | 0             | 9159           | 0.43          | 62        | 31.6    | 1295       | 21.0         |
|                  | 50            | 9983           | 0.42          | 72        | 31.3    | 1421       | 19.5         |
|                  | 100           | 10977          | 0.42          | 80        | 31.4    | 1578       | 19.5         |
|                  | 150           | 10705          | 0.43          | 83        | 31.7    | 1512       | 18.0         |
| Combined         | 0             | 8994 d         | 0.43          | 58 c      | 31.0    | 1293 c     | 21.5         |
|                  | 50            | 9702 cd        | 0.43          | 63 bc     | 30.8    | 1430 ab    | 22.0         |
|                  | 100           | 10264 a        | 0.43          | 67 ab     | 30.9    | 1516 a     | 22.0         |
|                  | 150           | 10077 b        | 0.44          | 69 a      | 30.3    | 1529 a     | 21.5         |

KW = kernel weight (g).
Table 7. Winter wheat flag leaf nitrogen (N) concentration (%), grain protein concentration (%), and grain test weight (lb/bu) as affected by nitrogen rate at four experiments conducted in Kansas during the winter wheat season of 2018–2019

| Location         | N rate | Flag leaf N | Protein | Test weight |
|------------------|--------|-------------|---------|-------------|
|                  | lb N/a | %           | lb/bu   |             |
| Ashland Bottoms  | 0      | 2.59 b      | 12.4 c  | 64.2        |
|                  | 50     | 2.73 ab     | 12.4 cd | 63.9        |
|                  | 100    | 2.83 a      | 13.5 ab | 63.3        |
|                  | 150    | 2.85 a      | 14.3 a  | 63.0        |
| Belleville       | 0      | 3.62 c      | 14.2 c  | 58.8        |
|                  | 50     | 3.83 b      | 14.6 ab | 59.3        |
|                  | 100    | 4.06 a      | 14.6 ab | 59.0        |
|                  | 150    | 4.02 a      | 15.0 a  | 59.8        |
| Hutchinson       | 0      | 2.78        | 13.7 c  | 60.7 a      |
|                  | 50     | 2.77        | 13.8 bc | 61.1 a      |
|                  | 100    | 2.86        | 14.0 a  | 60.5 ab     |
|                  | 150    | 2.96        | 13.8 bc | 58.4 bc     |
| Manhattan        | 0      | 2.70 c      | 11.1 d  | 63.1 a      |
|                  | 50     | 3.05 b      | 11.3 dc | 62.3 abc    |
|                  | 100    | 3.30 a      | 12.0 ab | 61.9 abc    |
|                  | 150    | 3.29 a      | 12.4 a  | 61.3 c      |
| Combined         | 0      | 2.92 c      | 12.8 c  | 61.7 a      |
|                  | 50     | 3.09 b      | 13.0 c  | 61.7 a      |
|                  | 100    | 3.26 a      | 13.5 ab | 61.2 abc    |
|                  | 150    | 3.28 a      | 13.9 a  | 60.6 bc     |
Figure 1. Relative grain yield (upper panel) and relative grain protein concentration (lower panel) as affected by flag leaf nitrogen (N) concentration across all environments and treatments.