**Effect of Topdress N Rates applied based on Growing Degree Days on Winter Wheat (Triticum aestivum L.) Grain Yield**

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**Abstract**

The majority of in-season nutrient management decisions for numerous crops are based on subjective morphological scales. The objective of this study was to establish whether a numerical scale based on growing degree-days (GDD> 0) utilizing weather science, could be used for nitrogen (N) management in winter wheat. An incomplete factorial within a randomized complete block design was replicated three times, over a period of three growing seasons (2017 to 2019). The locations were Efaw near to Stillwater, OK and Perkins, OK. A total of 15 treatments were included where three treatments received preplant N rates of 0, 90, and 120 kg N ha-1, and remaining treatments received topdress N of 30, 60, and 90 kg N ha-1 at 65, 80, 95, and 110 GDD’s. Data collection included grain yield, grain protein concentration, and N uptake.

 Topdress application of N at 80 to 95 GDD’s resulted in improved grain yields at three out of six site years. Grain protein concentration increased when N was applied topdress (90 kg N ha-1) at 110 GDD’s (3 out of 6 sites years). This work shows that an easier-to-use-numerical-scale based on GDD’s (80 to 115) could be utilized for efficient topdress N application in winter wheat.

**Introduction**

With the world population anticipated to be 10 billion by 2050, the demand for wheat will rise (Hitz et al., 2017). To meet this demand, researchers have estimated wheat production increase of at least 50% or more (Ray et al., 2013; Fischer et al., 2014). However, 27% of the global wheat producing area has been under yield stagnation (Grassini et al., 2013). Consistent with other wheat regions, yields have been stagnant in Oklahoma since the 1980s (Patrignani et al., 2014).

In Oklahoma, winter wheat is produced under rainfed conditions (Patrignani et al., 2014), and yields are often limited due to lack of water availability (Bushong et al., 2014). This region frequently encounters periods of extended drought, irregular rainfall, and variable temperatures (Baath et al., 2018). Hatfield et al. (2011) suggested that variation in temperature and precipitation have to be considered as a part of the production system to ensure food security.

 In cereal crop production, nitrogen (N) is considered the most limiting growth factor second to water (Sxumigalski and Van Acker, 2006). Nitrogen is essential for plant growth, production, and grain quality (Wuest and Cassman, 1992; Frink et al., 1999; Kichey et al., 2007). Additionally, Bell et al. (1995) noted 48% of wheat yield improvement is attributable to increased N application and 28% due to improved genetics. Recently, Oliveria et al. (2020) deduced that improving the plant N utilization efficiency is significant to increasing wheat grain yields. Nonetheless, the reported nitrogen use efficiency (NUE) for cereal crops that include wheat averages only 33% (Raun and Johnson, 1999), and that elucidates the need for improvement.

A major challenge for farmers is to identify ideal management practices such as the optimum fertilizer rate and application timing due to the complexity and randomness of the problem that differs yearly (Lopez-Bellido et al., 2005; Raun et al., 2019). Lopez-Bellido et al. (2005) further mentioned that NUE in winter wheat is affected by timing and splitting of N application rather than optimum N rates. Alcoz et al. (1993) noted that only 10% N is required before tillering. Strong (1995), in his review, showed low fertilizer efficiencies with fall-applied N. Besides, Sowers et al. (1994) noted increased N fertilizer recovery with spring top-dress application before stem elongation.

Irrespectively, the prediction of crop stages is essential from a management point of view, for the timing of pesticide application, harvesting (Ritchie and NeSmith, 1991) and nutrient management (Dhillon et al. 2020a). Growing Degree Day (GDD) heat units are a commonly used index to predict dates of flowering, maturity, and seasonal variation in harvest index in crops (Lu et al., 2001). Furthermore, it is used to measure the heat units in the areas of crop phenology and development (McMaster and Wilhelm, 1997). The growth of the plant depends on temperature; a specific amount of heat is required by a plant to develop through various growth stages (Miller et al., 2001; Cleland et al., 2007). Precisely, temperature affects the enzymatic activities required for plant development (Bonhomme, 2000). Various enzymes are involved in plant development, with particular temperature requirements, and as a result, we have a minimum, maximum, and optimum temperature (Bonhomme 2000). Additional uses of GDD include hybrid maturity descriptor by the seed industry (Nielson et al., 2002); quantifying crop yields as affected by planting dates (Bollero et al., 1996); Predicting N availability and losses from manure (Griffin and Honeycutt, 2000), and grain yield prediction using (Dhillon et al., 2020a; Figueredo et al 2020).

The Oklahoma Mesonet uses a cutoff method to calculate GDD values, based on the following formula:

Degree days = (Maximum Daily Air Temp + Minimum Daily Air Temp)/2 – Base Temp.

For winter wheat GDD calculation, a lower temperature threshold is 0o C; upper-temperature limit of 30oC and base temperature of 4.4o C is used. Likewise, the GDD could be used to conduct climate change research; whereby, it can be used as a climate impact index useful for management decisions (Anandhi, 2016). There is a linear relationship between the rate of plant development and GDD (Wang, 1960). Considering this direct relationship between crop development and GDD, using GDD’s for nutrient management, especially N, would be more convenient for crop nutrient management.

Recently, Figueredo et al. (2020) and Dhillon et al. (2020a) deduced that a GDD based numerical scale could be used for predicting topdress N rate in winter wheat, instead of subjective morphological scales. They further mentioned the ideal window for topdress N prediction was between 80 to 115 GDDs. The objective of this study was to identify optimum GDD’s for top-dress N rates and its subsequent effect on winter wheat grain yield, protein concentration, and N uptake. Furthermore, the idea is to adopt an easier to use numerical scale compared to the traditional morphological scale.

**Materials and Methods**

Winter wheat experiments were established in 2016-17 (2017), 2017-18 (2018), and 2018-19 (2019). These trials were located at Perkins and Efaw just north of Stillwater, Oklahoma. The soil type at Perkins is Teller sandy loam; fine-loamy, mixed, thermic Udic Agriustoll, and at Efaw is Ashport silty clay loam; fine-silty, mixed, superactive, thermic Fluventic Haplustolls.

Soil samples were taken from each site before planting. Fifteen cores per plot were taken to a depth of 15 cm. The soil samples were dried at 60oC overnight and were ground to pass a 2 mm sieve. Further, a 1:1 soil: water suspension and glass electrode was used to measure soil pH and buffer index (Sims, 1996; Sikora, 2006). A 1 M KCl solution was used for the extraction of soil NO3-N and NH4-N, which were quantified using a Flow Injection Autoanalyzer (LACHAT, 1994). Mehlich 3 solution was used to extract plant available P and K (Mehlich, 1984), where P and K were determined using a Spectro CirOs ICP spectrometer (Soltanpour et al., 1996). A detailed description of the soil analysis is reported in Table 1. To ensure N was the only limiting nutrient, both experiments were fertilized to a 100 percent level based on P and K test following regional fertilizer recommendations (Zhang and Raun 2006).

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| Table 1: Initial chemical properties of soils (0-15 cm) collected for 2017, 2018, and 2019 growing season, Efaw and Perkins, OK.  |
| Site | Year | pH | NH4-N | NO3-N | P | K |
|  | mg kg -1 |
| Efaw | 2017 | 5.77 | 9 | 12 | 17 | 109 |
|  | 2018 | 6.11 | 36 | 10 | 21 | 190 |
|  | 2019 | 5.51 | 15 | 11 | 19 | 191 |
| Perkins | 2017 | 6.96 | 14 | 1 | 13 | 132 |
|  | 2018 | 6.91 | 6 | 2 | 13 | 134 |
|  | 2019 | 6.80 | 5 | 3 | 14 | 162 |
| pH-1:1 soil:water; NH4-N and NO3-N – 2 M KCl; K and P- Mehlich III |

Herbicides and pesticides were applied as required throughout the season. A vacuum planter was used for planting. Plot dimensions were 6m long by 3m wide. A randomized complete block experimental design (RCBD) with three replications and 15 treatments was used in all trials (Table 2), except at Efaw in 2017, where treatment 3 was not included.

Three different topdress N rates were applied at four GDD dates (Table 2). Nitrogen as Urea Ammonium Nitrate (UAN) (28-0-0) (N-P-K) was used. Nitrogen was applied when 65, 80, 95, and 110 GDDs from planting had been accumulated where the GDDs were obtained from the Mesonet ([www.mesonet.org](https://www.mesonet.org/index.php/agriculture/degree_day_heat_units)), computed as follows: (Tmin+Tmax)/2-4.40C). An additional check plot was included where no N was used at any point during the growing season (Treatment 1). For treatments 2 and 3, 90 and 120 kg N ha-1 pre-plant N was applied. Treatments 4 through 15 all received midseason N at rates of 30, 60 and 90 kg N ha-1 at the 15 GDD interval, starting from 65 days to 110 days. An all-terrain vehicle (ATV) sprayer with a 3m boom using streamer nozzles was used for topdress application.

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| Table 2. Treatment structure employed to evaluate different N rates and application times of topdress fertilizer N, using the number days from planting to sensing where growth was possible, or growing degree days (GDD) that were > 0, where GDD was determined as (Tmin+Tmax)/2 – 4.4°C, Efaw, and Perkins, OK  |
| Treatment no. | The timing of fertilizer application | GDD>0 | N rate kg N ha-1 |
| 1 |  |  | 0 |
| 2 | Pre-plant |  | 90 |
| 3 | Pre-plant |  | 120 |
| 4 | Top-dress | 65 | 30 |
| 5 | Top-dress | 60 |
| 6 | Top-dress | 90 |
| 7 | Top-dress | 80 | 30 |
| 8 | Top-dress | 60 |
| 9 | Top-dress | 90 |
| 10 | Top-dress | 95 | 30 |
| 11 | Top-dress | 60 |
| 12 | Top-dress | 90 |
| 13 | Top-dress | 110 | 30 |
| 14 | Top-dress | 60 |
| 15 | Top-dress | 90 |

Grain subsamples from the harvest of each plot were collected for total N. All grain samples were ground to pass a 60-mesh screen using a Thomas micro-Wiley Laboratory Mill (Thomas Scientific, Swedesboro, New Jersey, USA). Total N analysis for grain samples was performed using LECO Truspec CN dry combustion analyzer (Leco Corp, St Joseph, Michigan, USA).

Data analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, USA), where mean separation employed the least significant difference (LSD) procedure at an alpha level of 0.05. Procedure GLIMMIX was used to explore the treatment differences, where replications were treated as a random variable. Moreover, single-degree-of-freedom contrasts were performed to evaluate specific treatment differences (Mclntosh, 2015). In addition, R statistical software was used for data visualizations.

**Results and Discussion**

The growth rates and phenological development of winter wheat are influenced by temperature and precipitation (Bauer et al., 1984). Total rainfall for all site years were highly variable, where the 2019 growing season received almost 400 mm more rain compared to the 10-year average (Table 3). Precipitation increased in May (439 and 404 mm) and June (107 and 119 mm) at both locations in 2019, in comparison to the same months in 2017 and 2018 (Table 3). An increase in precipitation during flowering and maturity (May and June) decreases the number of late growing season sunshine hours, and concurrently could reduce grain yield and grain quality (Song et al., 2019). However, high rainfall in May and June did not result in yield reduction, as mean grain yield at Efaw 2019 was higher than Efaw 2017, and Perkins 2019 yielded highest among three years at this location (Figure 1).

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| Table 3: Monthly cumulative precipitation (PT., mm), average temperature (TAVG, °C), and monthly growing degree days (GDD) for 2016-17, 2017-18, and 2018-19 growing season, Efaw and Perkins, OK. |
| Location |  | 2017 | 2018 | 2019 | 10-yr avg |
| Efaw | Planting date | Oct 14, 2016 | Oct 20, 2017 | Oct 23, 2018 |  |  |
|  | Month | PT. | TAVG | GDD | PT. | TAVG | GDD | PT. | TAVG | GDD | PT. | TAVG |
|  | Oct | 98 | 20 | 18 | 161 | 16 | 10 | 119 | 15 | 9 | 81 | 16 |
|  | Nov | 22 | 13 | 29 | 8 | 11 | 29 | 23 | 6 | 20 | 47 | 10 |
|  | Dec | 10 | 3 | 14 | 24 | 4 | 15 | 93 | 4 | 20 | 32 | 4 |
|  | Jan | 65 | 5 | 18 | 6 | 2 | 15 | 67 | 3 | 12 | 23 | 3 |
|  | Feb | 56 | 10 | 21 | 63 | 4 | 12 | 50 | 3 | 13 | 49 | 5 |
|  | Mar | 48 | 13 | 28 | 30 | 11 | 31 | 58 | 8 | 23 | 51 | 11 |
|  | Apr | 252 | 16 | 30 | 52 | 12 | 27 | 134 | 16 | 30 | 115 | 16 |
|  | May | 66 | 20 | 31 | 99 | 24 | 31 | 439 | 20 | 31 | 134 | 20 |
|  | June | 73 | 6 | 11 | 152 | 27 | 15 | 107 | 24 | 11 | 81 | 24 |
| Total | 690 | 12 | 200 | 595 | 12 | 185 | 1090 | 11 | 169 | 613 | 12 |
| Perkins  | Planting date | Oct 1, 2016 | Oct 12, 2017 | Oct 11, 2018 |  |  |
|  | Oct | 54 | 20 | 31 | 144 | 16 | 19 | 123 | 15 | 21 | 87 | 16 |
|  | Nov | 55 | 13 | 29 | 7 | 11 | 29 | 20 | 6 | 20 | 55 | 10 |
|  | Dec | 12 | 3 | 18 | 16 | 4 | 15 | 97 | 4 | 20 | 38 | 4 |
|  | Jan | 67 | 5 | 18 | 4 | 2 | 15 | 72 | 3 | 12 | 26 | 3 |
|  | Feb | 50 | 10 | 22 | 83 | 4 | 14 | 36 | 3 | 13 | 46 | 5 |
|  | Mar | 60 | - | - | 20 | 11 | 31 | 54 | 8 | 23 | 49 | 9 |
|  | Apr | 230 | - | - | 66 | 12 | 26 | 134 | 16 | 30 | 114 | 16 |
|  | May | 100 | 20 | - | 100 | 24 | 31 | 404 | 19 | 31 | 136 | 20 |
|  | June | 53 | 25 | - | 145 | 26 | 11 | 119 | 24 | 11 | 82 | 26 |
| Total | 681 | 11 | - | 596 | 12 | 191 | 1059 | 11 | 181 | 633 | 12 |

Typically, GDDs are used to quantify temperature effects and describe different biological processes (McMaster and Wilhelm, 1997; Li et al., 2012). In our study, cumulative GDD’s for the entire growing season varied, with Efaw in 2017 having a total of 200 GDDs compared to only 169 GDD’s for 2019 at the same location. Furthermore, the average monthly temperature at all site years ranged from 3 to 270C. The optimal temperature range for improved winter wheat growth is 17 to 230C with a minimum and maximum of 0 and 37°C (Porter and Gawith, 1999).

At Efaw, environmental mean grain yield ranged from 2.50 (2017) to 3.15 Mg ha-1 (2018) (Figure 1A). At Perkins mean grain yields were slightly lower and ranged from 2.07 (2017) to 3.08 Mg ha-1 (2019) (Figure 1B). All these inconsistencies across site years restricted a combined analysis of the data. Furthermore, Raun et al. (2017) recommended site years to not be combined as environmental differences that change drastically, impacts grain yields.

Figure 1: Distribution of grain yield across years with vertical dashed lines representing environmental mean for each year at Efaw (A) and Perkins (B).

Analysis of variance showed significant treatment differences in grain yield in 4 of 6 site-years (Figure 2). Growing season 2018 at both locations resulted in similar yields across treatments (Figure 2B and 2E). At Efaw 2017, grain yield ranged from 1.78 Mg ha-1 in the check plot (Treatment 1) to 3.11 Mg ha-1 with 90 kg N ha -1 preplant (Treatment 2) (Table 4). Treatment differences were not noted due to the main effects of either N rate, GDD’s or their interaction. Within individual treatment comparison, LSD at an alpha of 0.05 showed similar grain yields 3.11 Mg ha-1 (Treatment 2) and 3.00 Mg ha-1 (Treatment 12) (Table 4), with remaining treatments yielding significantly lower. During the 2018 growing season at Efaw, grain yield ranged from 2.81 Mg ha-1 in check plot (Treatment 1) to 3.49 Mg ha-1 in plots receiving 60 kg N ha-1 applied at 95 GDD’s (Treatment 11). Similar to 2017 none of the main effects had any effect on grain yield in growing season 2018. In addition, a single degree of freedom contrasts could not divulge any further information in 2017 and 2018 at the Efaw location. In 2019 at Efaw, significant treatment differences were noted due to N rate, GDDs, and interaction between GDD and N rate. The lowest yield was recorded in the check plot at 1.97 Mg ha-1 (Treatment 1), and the highest return was recorded at 3.99 Mg ha-1 when 60 kg N ha-1 was applied at 80 GDD’s (Treatment 8). Furthermore, a single degree of freedom contrasts showed that grain yields were higher with all the N rates applied at 80 GDDs compared to preplant treatments receiving 90 and 120 kg N ha-1 (Contrast 2 and 6; Table 4). Moreover, within the topdress N receiving application, 80 GDD’s yielded better in comparison to treatments at 65, 90, and 110 GDD’s as per single degree of freedom contrasts (Contrast 9, 12, and 13; Table 4).

During 2017 growing season at Perkins, check plot yield was lowest at 0.80 Mg ha-1 (Treatment 1) and 90 kg N ha-1 preplant (Treatment 2) yield was highest at 3.20 Mg ha-1. Grain yields were not different due to the main effect of N application, N rate or their interaction (Table 5). Additionally, single degree of freedom contrasts revealed that 90 kg N ha-1 preplant resulted in significantly better yield compared to other treatments (contrast 1, 2, 3, and 4; Table 5). Throughout 2018 growing season, yields ranged from 1.19 Mg ha-1 in check (Treatment 1) to 2.58 Mg ha-1 with 90 kg N ha-1 applied at 80 GDD (Treatment 8). No additional information was gathered with single degree of freedom contrasts at Perkins in 2017 and 2018. In 2019, different N rates used at four different GDD’s resulted in yield differences. The lowest yield was recorded in the check plot (Treatment 1) 2.22 Mg ha-1 and highest yield was recorded with 120 kg N ha-1 applied preplant (Treatment 3) at 4.15 Mg ha-1.

At all site years’ topdress application at 80 and 95 GDDs resulted in highest grain yield for 3 site years (Efaw 2018 and 2019, Perkins 2018). Preplant applications resulted in higher yields at 2 of the six sites (Perkins 2017 and 2019). Whereas similar yield with preplant and topdress at 95 GDD was obtained at one site (Efaw 2017). Results in our study are in agreement with many other researchers who have noted a yield increase with topdress application of N in winter wheat (Knowles et al., 1994; Mohammed et al., 2013; Liu et al., 2019; Dhillon et al., 2020b), however, all of these management decisions were based on a subjective morphological scale (Large, 1954). Dhillon et al. (2020b) noted that timing of N application has significant impact on grain yield irrespective of the method used for N application.



Figure 2: Dispersal of grain yield by treatment, color coded by N application timing for each site year with horizontal dashed line representing environmental mean grain yield for Efaw 2017 (A), Efaw 2018 (B), Efaw 2019 (C), Perkins 2017 (D), Perkins 2018 (E), and Perkins 2019 (F) growing seasons.

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| Table 4: Treatment structure, treatment means, main effect model, and single-degree-of-freedom contrasts for grain yield, protein, and N uptake for 2017, 2018, and 2019 growing season, Efaw, OK |
|  |  | 2017 | 2018 | 2019 |
| Treatments | GDD>0 | N rate kg N ha-1 | Grain yieldMg ha-1 | @Grain protein | N uptakekg ha-1 | Grain yieldMg ha-1 | Grain protein | N uptakekg ha-1 | Grain yieldMg ha-1 | Grain protein | N uptakekg ha-1 |
| 1 |  | 0 | 1.78 | 10.15 | 33 | 2.81 | 10.48 | 52 | 1.97 | 10.06 | 34 |
| 2 | Pre-plant | 90 | 3.11 | 12.99 | 71 | 3.21 | 12.77 | 72 | 2.44 | 10.35 | 44 |
| 3 |  | 120 | - | - | - | 2.95 | 12.39 | 64 | 2.88 | 10.60 | 53 |
| 4 | 65 | 30 | 2.78 | 10.57 | 52 | 3.26 | 11.61 | 66 | 1.50 | 10.67 | 28 |
| 5 | 60 | 2.63 | 11.67 | 54 | 3.24 | 12.05 | 68 | 1.91 | 11.90 | 37 |
| 6 | 90 | 2.48 | 10.95 | 48 | 3.34 | 13.18 | 77 | 3.52 | 11.18 | 69 |
| 7 | 80 | 30 | 2.37 | 11.99 | 50 | 3.32 | 10.97 | 64 | 3.90 | 10.69 | 73 |
| 8 | 60 | 2.21 | 12.53 | 48 | 3.38 | 12.48 | 74 | 3.99 | 10.93 | 77 |
| 9 | 90 | 2.63 | 11.40 | 52 | 3.12 | 14.31 | 78 | 3.07 | 10.32 | 55 |
| 10 | 95 | 30 | 1.75 | 11.18 | 34 | 2.98 | 12.67 | 67 | 1.94 | 10.48 | 36 |
| 11 | 60 | 2.85 | 12.08 | 60 | 3.49 | 12.73 | 77 | 3.31 | 11.48 | 67 |
| 12 | 90 | 3.00 | 12.29 | 65 | 3.06 | 13.58 | 73 | 3.43 | 11.16 | 67 |
| 13 | 110 | 30 | 2.21 | 11.32 | 44 | 2.85 | 13.18 | 66 | 2.35 | 10.76 | 44 |
| 14 | 60 | 2.53 | 11.08 | 49 | 2.88 | 13.04 | 66 | 2.71 | 12.09 | 57 |
| 15 | 90 | 2.54 | 12.38 | 54 | 3.36 | 13.10 | 77 | 3.20 | 12.61 | 71 |
| SED | 0.42 | 0.53 | 8.7 | 0.24 | 0.44 | 5.4 | 0.24 | 0.34 | 4.4 |
| Main Effects |
| GDD | ns | ns | ns | ns | ns | ns | \* | \* | \* |
| Nrate | ns | ns | ns | ns | \* | \* | \* | \* | \* |
| GDD\*Nrate | ns | ns | ns | ns | ns | ns | \* | ns | \* |
| Contrasts (Treatments) |  |  |  |  |  |  |  |  |  |
| 1. Pre-plant 90 (2) vs GDD-65 (4,5,6) | ns | \* | ns | ns | ns | ns | ns | ns | ns |
| 2. Pre-plant 90 (2) vs GDD-80 (7,8,9) | ns | ns | ns | ns | ns | ns | \* | ns | \* |
| 3. Pre-plant 90 (2) vs GDD-95(10,11,12) | ns | ns | ns | ns | ns | ns | ns | ns | \*\* |
| 4. Pre-plant 90 (2) vs GDD-110 (13, 14, 15) | ns | ns | \* | ns | ns | ns | ns | \* | \*\* |
| 5. Pre-plant 120 (3) vs GDD-65 (4,5,6) | - | - | - | ns | ns | ns | \*\* | ns | ns |
| 6. Pre-plant 120 (3) vs GDD-80 (7,8,9) | - | - | - | ns | ns | ns | \* | ns | \* |
| 7. Pre-plant 120 (3) vs GDD-95 (10,11,12) | - | - | - | ns | ns | ns | ns | ns | ns |
| 8. Pre-plant 120 (3) vs GDD-110 (13, 14, 15) | - | - | - | ns | ns | ns | ns | \* | ns |
| 9. GDD-65 (4,5,6) vs GDD-80 (7,8,9) | ns | \*\* | ns | ns | ns | ns | \* | ns | \* |
| 10. GDD-65 (4,5,6) vs GDD-95 (10,11,12) | ns | ns | ns | ns | ns | ns | \* | ns | \* |
| 11. GDD-65 (4,5,6) vs GDD-110 (13,14,15) | ns | ns | ns | ns | \*\* | ns | \*\* | \* | \* |
| 12. GDD-80 (7,8,9) vs GDD-95 (10,11,12) | ns | ns | ns | ns | ns | ns | \* | ns | \* |
| 13. GDD-80 (7,8,9) vs GDD-110 (13,14,15) | ns | ns | ns | ns | ns | ns | \* | \* | \* |
| 14. GDD-95 (10,11,12) vs GDD-110 (13,14,15) | ns | ns | ns | ns | ns | ns | ns | \* | ns |
| SED – standard error of the difference between two equally replicated means, Main effect excludes treatments 1, 2, and 3 where N was pre-plant applied; ns, \*, and \*\* not significant, and significant at 0.01 and 0.05 probability levels; @- Grain protein = % N in grain \*5.7  |

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| Table 5: Treatment structure, treatment means, main effect model, and single-degree-of-freedom contrasts for grain yield, protein, and N uptake for 2017, 2018, and 2019 growing season, Perkins, OK |
|  |  | 2017 | 2018 | 2019 |
| Treatments | GDD>0 | N rate kg N ha-1 | Grain yieldMg ha-1 | @Grain protein | N uptakekg ha-1 | Grain yieldMg ha-1 | Grain protein | N uptakekg ha-1 | Grain yieldMg ha-1 | Grain protein | N uptakekg ha-1 |
| 1 |  | 0 | 0.80 | 9.70 | 14 | 1.19 | 11.42 | 24 | 2.22 | 10.92 | 43 |
| 2 | Pre-plant | 90 | 3.20 | 10.62 | 59 | 2.14 | 12.61 | 47 | 3.23 | 10.03 | 57 |
| 3 |  | 120 | 1.64 | 8.69 | 25 | 2.17 | 12.87 | 49 | 4.15 | 10.87 | 79 |
| 4 | 65 | 30 | 2.29 | 9.09 | 37 | 2.00 | 11.10 | 39 | 2.78 | 10.63 | 52 |
| 5 | 60 | 2.27 | 9.02 | 36 | 2.08 | 12.68 | 46 | 3.16 | 10.58 | 59 |
| 6 | 90 | 2.12 | 9.02 | 33 | 2.02 | 13.61 | 47 | 3.37 | 10.93 | 65 |
| 7 | 80 | 30 | 2.57 | 9.37 | 43 | 1.86 | 11.70 | 38 | 2.50 | 10.32 | 45 |
| 8 | 60 | 2.38 | 9.30 | 38 | 2.33 | 12.37 | 51 | 3.03 | 10.80 | 57 |
| 9 | 90 | 1.76 | 9.00 | 28 | 2.58 | 13.59 | 61 | 3.52 | 11.05 | 68 |
| 10 | 95 | 30 | 1.79 | 9.46 | 30 | 2.08 | 11.76 | 43 | 2.76 | 10.24 | 50 |
| 11 | 60 | 2.19 | 9.63 | 37 | 2.43 | 13.07 | 56 | 3.10 | 10.49 | 57 |
| 12 | 90 | 1.78 | 9.21 | 29 | 2.57 | 12.67 | 57 | 3.32 | 10.52 | 61 |
| 13 | 110 | 30 | 1.98 | 10.05 | 35 | 2.16 | 11.61 | 44 | 2.71 | 10.44 | 49 |
| 14 | 60 | 2.17 | 10.75 | 41 | 2.23 | 12.78 | 50 | 3.44 | 11.19 | 67 |
| 15 | 90 | 2.22 | 9.22 | 36 | 2.02 | 15.08 | 53 | 2.95 | 11.64 | 60 |
| SED | 0.29 | 0.25 | 4.8 | 0.26 | 0.58 | 5.7 | 0.18 | 0.23 | 3.8 |
| Main Effects |
| GDD | ns | \* | ns | ns | ns | ns | ns | \* | ns |
| Nrate | ns | \*\* | ns | ns | \* | \* | \* | \* | ns |
| GDD\*Nrate | ns | ns | ns | ns | ns | ns | ns | ns | \* |
| Contrasts (Treatments) |  |  |  |  |  |  |  |  |  |
| 1. Pre-plant 90 (2) vs GDD-65 (4,5,6) | \* | \* | \* | ns | ns | ns | ns | \*\* | ns |
| 2. Pre-plant 90 (2) vs GDD-80 (7,8,9) | \* | \* | \* | ns | ns | ns | ns | \*\* | ns |
| 3. Pre-plant 90 (2) vs GDD-95(10,11,12) | \* | \* | \* | ns | ns | ns | ns | ns | ns |
| 4. Pre-plant 90 (2) vs GDD-110 (13, 14, 15) | \* | \*\* | \* | ns | ns | ns | ns | \* | ns |
| 5. Pre-plant 120 (3) vs GDD-65 (4,5,6) | ns | ns | ns | ns | ns | ns | \* | ns | ns |
| 6. Pre-plant 120 (3) vs GDD-80 (7,8,9) | ns | ns | ns | ns | ns | ns | \* | ns | \* |
| 7. Pre-plant 120 (3) vs GDD-95 (10,11,12) | ns | \*\* | ns | ns | ns | ns | \* | ns | \* |
| 8. Pre-plant 120 (3) vs GDD-110 (13, 14, 15) | ns | \* | \*\* | ns | ns | ns | \* | ns | \* |
| 9. GDD-65 (4,5,6) vs GDD-80 (7,8,9) | ns | ns | ns | ns | ns | ns | ns | ns | \* |
| 10. GDD-65 (4,5,6) vs GDD-95 (10,11,12) | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| 11. GDD-65 (4,5,6) vs GDD-110 (13,14,15) | ns | \* | ns | ns | ns | ns | ns | ns | ns |
| 12. GDD-80 (7,8,9) vs GDD-95 (10,11,12) | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| 13. GDD-80 (7,8,9) vs GDD-110 (13,14,15) | ns | \* | ns | ns | ns | ns | ns | ns | ns |
| 14. GDD-95 (10,11,12) vs GDD-110 (13,14,15) | ns | \*\* | ns | ns | ns | ns | ns | \* | ns |
| SED – standard error of the difference between two equally replicated means, Main effect excludes treatments 1, 2, and 3 where N was pre-plant applied; ns, \*, and \*\* not significant, and significant at 0.01 and 0.05 probability levels; @- Grain protein = % N in grain \*5.7 |



Figure 3: Dispersal of grain protein across years with vertical dashed lines representing mean protein concentration for each year at Efaw (A) and Perkins (B).

Grain protein is an essential quality for determining the market premiums and end use purposes of winter wheat. Globally a 12.5% protein content for hard red winter wheat is required; market price falls as protein content falls below this percentage (Wang et al., 2019). Mean grain protein was lower at Perkins as compared to Efaw (Figure 3). Protein levels were highly disperse in Perkins ranging from 9.48 % in 2017 to 12.59 % in 2019 (Figure 3B), whereas at Efaw the protein range was 10.96 % (2019) to 12.56 % (2018) (Figure 3A).

At all site years, grain protein was different due to treatment (Figure 4). The grain protein content increased as N rate increased for each topdress application date. This trend was most evident in Efaw 2018 (Figure 4B), Efaw 2019 (Figure 4C), and Perkins 2018 (Figure 4E). Grain protein content was not affected by the main effects of GDD, N rate and interaction between GDD and N rate during 2017 growing season at Efaw (Table 4). However, single degree of freedom contrasts indicated higher grain protein recovery when 90 kg N ha-1 was applied pre-plant compared to topdress applications at 65 GDD (Contrast 1; Table 4), whereas no such deviations were noted in comparison to other application timings. During 2018 growing season, N rates had a significant influence on protein accumulation (Table 4). Protein content increased with increment in N rate at each successive topdress N application scheduling (Table 4; Figure 4B). During 2019 growing season at Efaw, both GDD and N rate had a significant effect on grain protein concentration (Table 4; Figure 4C). Single degree of freedom contrasts revealed that N application at 110 GDDs increased protein content compared to preplant N



Figure 4: Distribution of grain protein by treatment grouped by N application timing for each site year with horizontal dashed line representing environmental mean grain protein for Efaw 2016-17 (A), Efaw 2017-18 (B), Efaw 2018-19 (C), Perkins 2016-17 (D), Perkins 2017-18 (E), and Perkins 2018-19 (F) growing seasons.

(Contrast 4, 8; Table 4), and other topdress applications (Contrast 11, 13, and 14, Table 4). At Perkins in 2017, both timing of N application (GDD) and N rate had a notable impact on grain protein concentration. According to single degree of freedom contrasts, the protein accumulation in grain improved with N application at 110 GDD’s compared to preplant applications of 90 kg N ha-1 (Contrast 4; Table 5) and 120 kg N ha-1 (Contrast 8, Table 5). Furthermore, 110 GDD’s timing of application was better than other topdress application days, 65 GDD’s (Contrast 11, Table 5), 80 GDD’s (Contrast 13, Table 5), and 95 GDD’s (Contrast 14, Table 5). During 2018, only N rates affected protein accumulation. Protein content ranged from 11.42% in check plot (Treatment 1) to 15.08% with 90 kg N ha-1 applied as topdress at 110 GDD’s (Treatment 15) (Table 5). An increasing trend in protein content was also noted with an increase in N rate within timing of N application (Figure 5E). In 2019 season at Perkins location, protein concentration was altered by GDD’s schedule and N rate application rates (Table 5). Furthermore, application timing at 110 GDD’s were better compared to preplant application of 90 kg N ha-1 as per single degree of freedom contrasts (Contrast 4, Table 5). Overall 90 kg N ha-1 application at 110 GDDs resulted in highest protein content in 3 of 6 site years. Whereas application of 60 kg N ha-1 at 110 GDDs, and 90 kg N ha-1 at 80 GDDs were better at 1 site year each. Results in this study are in agreement with several researchers where they noted a protein content increase with topdress N application (Wuest and Cassman, 1992; Bänziger et al., 1994; Mohammed et al., 2013; Dhillon et al 2020b). Moreover, Lollato et al. (2019) in a synthesis analysis of three long-term studies noted an increase in grain protein concentration with only N application, which reduced with co application of phosphorus and/or potassium.

Nitrogen uptake was different at each site year, where mean N uptake at Efaw ranged from 51 kg N ha-1 (2017) to 69 kg N ha-1 (2018), whereas these values were lower at Perkins and extended from 34 kg N ha-1 (2017) to 58 kg N ha-1 (2019) (Figure 5). Analysis of variance revealed no treatment differences at Efaw during 2017 season (Table 4; Figure 6A). During 2018 growing season at Efaw, N rate made an impact on N uptake (Table 4). Furthermore, improvement in N uptake was noted with an increase in N rate at each specific application timing (Figure 6B). However, limited differences were present when single degree of freedom contrasts were performed. During 2019 in Efaw, the main effect of GDD, N rate, and interaction between GDD and N rate were significant (Table 4). A trend in N uptake upsurged with increase in N rate within timing of N application was noted (Figure 6C). Additionally, single degree of freedom contrasts showed that topdress application were better compared to preplant applications in terms of N uptake (Contrast 2, 3, 4, and 7; Table 4). At Perkins in 2017, preplant application of 90 kg N ha-1 resulted in highest N uptake of 59 kg N ha-1. In addition, single degree of freedom contrasts showed that preplant application of 90 kg N ha-1 was significantly better compared to other treatments (Contrast 1, 2, 3, and 4; Table 5). During 2018 at Perkins, N rate resulted in significant difference in N uptake. Nitrogen uptake increased as N rate increased at different application schedules (Figure 6E). Lollato et al. (2019) noted similar results where a linear increase in N uptake was found with an increase in N application rate. At Perkins during 2019, interaction of timing and N rates were significant for N uptake. As per single degree of freedom contrasts preplant application of 120 kg N ha-1 resulted in better N recovery compared to other treatments (Contrasts 5,6,7, and 8; Table 5). Over all site years, N uptake followed a similar trend as yield, where treatments with high yields resulted in higher N uptake.



Figure 5: Dispersal of grain N uptake across site years with vertical dashed lines representing mean N uptake for each year at Efaw (A) and Perkins (B).

In this study, we have documented that GDDs could be efficiently used for topdress application management where N applied between 80 and 95 GDDs is ideal for improved winter wheat grain yield. Furthermore, topdress application resulted in higher protein and N recovery compared to preplant application. This might be due various factors affecting treatments receiving high preplant N applications. Such as excess fall tillering and biomass production resulting in late season drought stress (van Herwaarden et al., 1998), weakening of vegetative organs (Borghi, 1999), increased lodging potential (Lollato and Edwards, 2015), parasite vulnerability (Howard et al., 1994), and late spring freeze induced stress (Dhillon et al., 2019). However, all these adverse conditions could be avoided with topdress applications, where a GDD based numerical scale could be easily used for making N management decisions.



Figure 6: Nitrogen uptake by treatment grouped by N application timing for each site year with horizontal dashed line representing mean N uptake for Efaw 2016-17 (A), Efaw 2017-18 (B), Efaw 2018-19 (C), Perkins 2016-17 (D), Perkins 2017-18 (E), and Perkins 2018-19 (F) growing seasons.

**Conclusions**

This work shows that an easier-to-use-numerical-scale based on GDD’s (80 to 115) could be effectively utilized for N management strategies in winter wheat. We deduced that topdress application of N applied between 85 and 95 GDD’s resulted in increased yields and N uptake. Furthermore, we also concluded that topdress application of 90 kg N ha-1 at 110 GDD’s was best for improving grain protein content.

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