

THESIS

VARIETIES OF WINTER WHEAT IN EASTERN COLORADO REQUIRE DIFFERENT N  
RECOMMENDATIONS FOR ACHIEVING GRAIN YIELD AND PROTEIN CONTENT

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## ABSTRACT

### VARIETIES OF WINTER WHEAT IN EASTERN COLORADO REQUIRE DIFFERENT N RECOMMENDATIONS FOR ACHIEVING GRAIN YIELD AND PROTEIN CONTENT

Nitrogen (N) and sulfur (S) fertility are important for achieving high wheat yields and desirable grain protein content, but N and S have been found to be inefficiently applied and/or utilized by wheat globally. This inefficiency may be reflected in state fertilizer recommendations. For example, Colorado State University's wheat fertilizer recommendations have not been updated in over 20 years, yet cultivars have drastically changed over this period; many wheat cultivars vary in nutrient uptake efficiency. Thus, the experimental objectives were to: 1) determine if the three dominant Colorado-grown winter wheat varieties respond differently to N and what the optimum N rate would be for each of variety; 2) determine if we could improve N fertilizer rate recommendations; 3) determine the effect of N and variety on grain yield and quality parameters such as test weight, thousand kernel weight, and protein at 18 environments in Eastern Colorado; and 4) determine how S fertilization affects winter wheat grain yield and protein content at multiple locations in Eastern Colorado.

Nitrogen application rates were 45, 90, or 135 lb N ac<sup>-1</sup>, the varieties studied were Canvas, Langin, and Snowmass 2.0, and the experiment occurred over two growing seasons. Data collected included pre-plant soil nutrient composition, NDVI, wheat growth stage dates, post-harvest residual soil nitrate-N, grain yield, protein content, and field-level climatic data during the growing season. A total of 18 environments were studied. Split plot ANOVA was conducted on the N application rates and varieties by environment, year, and both years

combined; the two years varied climatically and in response, thus they were analyzed separately. Polynomial regression of grain yield, protein, test weight, thousand kernel weight, post-harvest residual nitrate-N, and NDVI were plotted against residual nitrate-N from the fall soil test results plus additional N applications applied on the trial from treatments and by the grower-cooperators. The S treatment of 90 lbs N ac<sup>-1</sup> plus 20 lbs S ac<sup>-1</sup> was compared to 90 lbs N ac<sup>-1</sup> in two sample T-tests assuming equal variance for yield and protein by environment, variety, year, and both years combined.

The 2019 year was a relatively high-yielding year with more precipitation and cooler temperatures as compared to the 2020 year which was relatively warmer and drier. In 2019, wheat yields averaged 80.7 bu ac<sup>-1</sup> compared to 49.6 bu ac<sup>-1</sup> in 2020. The ANOVA for grain yield showed that increasing N rates affected grain yield and protein. In the drier year and two-years combined, the greatest N application rate reduced yield. Meanwhile protein increased with increasing N applications in both years and the two-years combined. Variety was significant for both grain yield and protein. Langin had the greatest yields in the high yielding year and the two-years combined. However, Canvas, the lowest yielding variety, had the greatest protein in the higher yielding year in comparison to Langin and Snowmass 2.0.

Yield regressed against applied N plus residual soil N resulted in different response curves by variety each year. In the high yielding year, grain yield had a significant, positive, curvilinear response to N. Canvas required greater N applications to reach optimum yields in both years in comparison to Langin and Snowmass 2.0. However, Canvas required less N to achieve higher grain protein levels. Canvas reached 12% grain protein in 2019 at the lowest N rate at 229 lbs N ac<sup>-1</sup>, while Snowmass 2.0 reached 12% grain protein at 250 lbs N ac<sup>-1</sup>. Langin did not achieve 12% grain protein in 2019. It is important to note that when yields are high,

protein is diluted. In 2020, grain yield had a low response to soil N plus applied N due to water-limited conditions. Protein increased with increasing N but did not reach a maximum according to regression analysis. Canvas reached 12% protein at 180 lbs N ac<sup>-1</sup>, Snowmass 2.0 at 185 lbs N ac<sup>-1</sup>, and Langin at 211 lbs N ac<sup>-1</sup>. These responses indicate that different varieties have different optimum N requirements for protein.

Test weight and thousand kernel weight were affected by N application rate, variety, and environments. The greatest N rate reduced test weight in 2019, 2020, and the 2-years combined. Test weight was also affected by variety in 2019 and 2020 but not in the two-years combined. Snowmass 2.0 was able to maintain greater test weight, grain protein, and thousand kernel weights at higher yields in response to N when compared to Langin at similar N rates and yield. Targeting these components of grain quality in future breeding efforts for higher yielding varieties will be important.

Environmental conditions within a year affected protein content. Precipitation was found to affect N utilization and evidence of this was supported by year in terms of grain yield, post-harvest residual NO<sub>3</sub>-N, and NDVI. NDVI was affected by variety and soil N plus applied N in both years. The results from the two years showed that the average NDVI was greater in the high yielding year while the NDVI index was 0.2 points lower in the dry year. The NDVI responded to increasing soil N across all environments but the actual yield response in the dry year was much lower than predicted by NDVI. It is concluded that NDVI may not be as useful in dry years for predicting yield response to N.

No significant differences were found for grain yield and protein for S in 2019, 2020, and the 2-years combined. However, future research on S rates, application timing, and the effect of

S under certain environmental conditions such as soils having high sand content and low organic matter levels for improving grain yield and protein is encouraged in Colorado.

Due to excessive N fertilizer applications, we were not able to develop new N recommendations for yield in Colorado. However, this research gave insight to the importance of environments and variety in maximizing yields using N and the opportunity to use variety response to determine optimum N rates. The results from this project show the importance of how N application rates may need to be adjusted for each variety. This research also found that understanding N utilization by variety is important to further develop varieties that are able to maintain grain quality parameters at higher yields. Future fertilizer trials could study updated N rates that better bracket N rates used by wheat producers in Colorado.

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## DEDICATION

I dedicate this thesis to my parents Dan and Jessica.

My parents are my biggest support. I am grateful for the sacrifices they have made for my sister and me. Without them, I would not be where I am at today. I am very blessed to have these wonderful people at my side.

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## CHAPTER 1

### LITERATURE REVIEW

#### **Background and Importance**

Wheat is an important global commodity; it is the major crop grown in the United States (U.S.) Great Plains, accounting for 60% of total U.S. wheat production. Eastern Colorado, part of the U.S. Great Plains, produces hard red and hard white winter wheat on approximately 2 million acres with a production value of over \$190 million dollars (USDA-NASS, 2020); winter wheat dominates Eastern Colorado's production and cropland acres (Paulsen & Shroyer, 2008).

Achieving high wheat yields and desirable grain protein content requires fertilizer inputs, especially nitrogen (N). However, N inputs have been found to be inefficiently applied and utilized by wheat globally (Raun & Johnson, 1999). Inefficient N application rates may be due to, in part, outdated N fertilizer recommendation guidelines. For example, the Colorado State University (CSU) N fertilizer recommendation fact sheets are out of date by over 20 years due to a lack of current fertility trials (Davis & Westfall, 2014; Goos et al., 2015). Kansas State University's wheat production handbook has not been updated since 1997 (Paulsen, 1997). Furthermore, new varieties may have improved nitrogen use efficiency (NUE) or unique N requirements that may require unique recommendations for each cultivar.

Studies suggest that N uptake and NUE is affected by genotype x varietal interactions (Hawkesford, 2014; Rasmussen et al., 2015). Yet, this approach was not followed during many of the studies for wheat fertilizer recommendations currently in place. For example, basing N recommendations for modern, high yielding varieties in Colorado has not been researched since

1980 (Goos, 1980). The data presented by Goos (1980) essentially provides the fertilizer guidelines for winter wheat NUE, N uptake, and grain protein content used for current recommendations (Ludwick et al., 2015). However, cultivars have been improved over the past 30 years, with some evidence suggesting that differences in N utilization efficiencies, uptake needs, and effects between cultivars of wheat may occur (Hawkesford, 2014). Therefore, it is of interest to study the winter wheat N by cultivar effect to improve winter wheat NUE, yields and protein content.

In order to improve winter wheat fertilizer recommendations, adjusting the outdated N fertilizer recommendations for the improved cultivars raised currently should provide practical fertilizer recommendations for farmers in Eastern Colorado and surrounding states. Currently used, yet outdated N fertilizer recommendations are based on attaining certain yield goals (e.g., bu ac<sup>-1</sup>). However, current evidence suggests yield-based N recommendations may not be the most accurate way to predict crop N needs (Rodriguez et al., 2019). Thus, improving winter wheat N fertilizer recommendations is desperately required. Unfortunately, crop N requirement determinations are complex and intimately coupled with other essential nutrients, such as sulfur (S).

Soil S availability is known to affect N uptake, grain protein, and wheat grain quality parameters (Wilson et al., 2020). A soil S deficiency may negatively affect wheat quality by increasing grain asparagine concentration. Asparagine negatively affects baking quality and, during baking, produces acrylamide – a potential cancer-causing compound (Herrera et al., 2012; Wilson et al., 2020). Except for inherent soil S or S fertilizer applications, atmospheric S deposition to cropland has been reduced due to the Clean Air Act of 1985 and 1990, and decreases in S impurities in pesticides (Schnug, 1998; Herrera et al., 2012; Wilson et al., 2020).

Sulfur fertilization has typically not been recommended in Eastern Colorado in the past. However, as a result of decreased atmospheric and impurity S deposition mentioned above, soil S deficiencies may be present in Eastern Colorado. Furthermore, S fertilization is being recommended to producers with seemingly little if any information to substantiate its necessity (Jim Ippolito, Associate Professor, Colorado State University, personal communication). Therefore, it is important to identify if S fertilizer applications are truly required for Eastern Colorado (and regional) winter wheat.

The objectives of this research were to determine: (1) if varieties respond differently to N and what the optimum N rate would be for each of the three studied winter wheat varieties currently raised in Colorado; (2) if we could make new and better new N fertilizer rate recommendations; (3) the effect of N and variety on grain yield, protein content, test weight, and thousand kernel weight at 18 environments in Eastern Colorado; and (4) how S fertilization affect wheat yield and grain quality in multiple locations in Eastern Colorado.

### **Wheat in Eastern Colorado**

Wheat (*Triticum aestivum* L.) is the third most produced and may arguably be the most important crop grown globally for direct human use. It is a primary source of carbohydrate energy for a significant portion of the human population, with the wheat consumption trend increasing (Shewry & Hey, 2015). The U.S. is one of the top three wheat grain exporter countries, along with wheat being an important crop for domestic consumption. The U.S. Great Plains region accounts for 60% of total U.S. wheat production (Paulsen & Shroyer, 2008). In particular, hard red winter wheat is a major wheat class grown in the US, encompassing the majority of Eastern Colorado winter wheat acreage (Paulsen & Shroyer, 2008). The wheat belt present in Colorado is primarily under dryland conditions, relying on precipitation because

irrigation is not viable. This semi-arid to arid environment, coupled with water often being a limiting crop growth factor, makes raising wheat a challenge. However, the area has benefitted from CSU's Wheat Breeding and Genetics Program that develops region-specific cultivars that yield well while being adapted to difficult climatic conditions.

CSU has improved hard winter wheat varieties specifically for Eastern Colorado's climate since 1963 ("CSU Wheat Breeding and Genetics Program", n.d.). Hard red winter wheat dominates regional acreage. Hard white winter wheat is also an important specialty winter wheat class with Colorado a leader in its production (Paulsen & Shroyer, 2008). Both hard red and hard white winter wheat have been improved for Eastern Colorado's climate by CSU's Wheat Breeding and Genetics program; varietal improvements have helped yield trends increase in the region.

Increasing yields is a frequently mentioned means for reducing world hunger in the face of an ever-increasing human population. Recent forecasts predict the growing human population will demand a 60-110% increase in food demand by the year 2050, as compared to current food productivity (Ray et al., 2013). This is significant pressure for crop breeders, agronomists, and farmers to further improve wheat yields while also maintaining nutritional value and quality for baking and processing. Global wheat yields are still increasing but the rate of increase is steadily slower each year (Slafer & Satorre, 1999; Ray et al., 2013). Among other Great Plains areas, Eastern Colorado is showing a small but significant decrease in average wheat yield over time (Ray et al., 2013). This could be attributed to various abiotic and biotic stressors, especially as most of Colorado's wheat is in dryland production and thus subjected to yearly variations in climatic conditions. Meanwhile, some wheat acres have been lost due to conversion into the

Conservation Reserve Program, which obviously influences total wheat production in the state (Parton et al., 2003).

To address these needs for increasing Colorado winter wheat yields, recent CSU Wheat Breeding and Genetics Program efforts have focused on improving hard red and hard white wheat varieties germplasm. The current CSU wheat breeder objectives are to: (1) develop improved hard red and hard white wheat varieties and germplasm adapted for the diverse production conditions in Colorado and the west central Great Plains; and (2) conduct both basic and applied research to improve understanding of genetic and environmental factors that affect wheat yield and end-use quality (“Wheat Breeding and Genetics Program”, n.d.).

Eastern Colorado growing conditions are challenging for dryland crop production due to harsh and variable growing conditions and low precipitation. This area is characterized by low relative humidity, abundant sunshine, infrequent rains and snow, moderate to high winds, and a large daily and seasonal range in temperature (Doesken et al., 2003). Specifically, precipitation patterns vary across the region, ranging between 7 to 20 inches (18 to 51 cm) annually while the estimated seasonal wheat water requirement can vary between 15.5 and 20 inches (39-51cm) annually (Schneekloth & Andales, 2003). Evapotranspiration (ET) rates also vary significantly, with ET rates increasing from northeastern to southeastern Colorado. Southeastern Colorado also receives the lowest average precipitation, and combined with the greatest ET, this often leads to a water deficit for crop productivity. It has been found that ET alone accounts for more than 50% of water loss in the Great Plains (Tracy, 1986). This fact makes southeastern Colorado an especially challenging region for dryland farming. In contrast, northeastern Colorado has relatively greater precipitation and lower ET, making dryland farming less of a challenge as compared to southeastern Colorado.

Regardless of eastern Colorado location, increasing yields in dryland winter wheat production requires an understanding of genetic and environmental factors that affect wheat yield and end use quality (“Wheat Breeding and Genetics Program”, n.d.). These factors, in combination, are known as genotype by environment interactions (GxE). GxE interactions affect wheat yields at different locations (Yan & Hunt, 2001). Some abiotic factors that are known to affect wheat yield and quality are freezing, precipitation, evapotranspiration rates, and heat stress (Telfer et al., 2008). On-site management (tillage, cropping rotation, planting date, etc) can also affect crop response to the GxE interactions (Munaro et al., 2020); biotic stressors can compromise wheat yield as well. Common, current biotic stressors for eastern Colorado wheat are wheat stem sawfly, stripe rusts, tan spot, wheat streak mosaic virus, etc. Some of these vary in impact across regions and are affected by environmental conditions and breeding efforts behind currently grown Colorado winter wheat varieties.

Several important wheat varieties have been developed for Colorado conditions. One of the more important Colorado winter wheat varieties is Langin, a high yielding hard red variety developed by CSU’s Wheat Breeding and Genetics Program. As of 2020, it was the top variety planted in Eastern Colorado at 20.1% of winter wheat planted acres (Johnson et al., 2020). This winter wheat variety is an early maturing variety with stripe rust resistance, excellent baking quality, and excellent drought tolerance (Haley et al., 2018). Langin (CO11D446) is desirable for high yields and high protein in Eastern Colorado. Canvas (CO12D1770) is another hard red variety released in 2018 by CSU. It was developed to be resistant to diseases that are economically damaging in Eastern Colorado, such as wheat streak mosaic virus, stripe rust, and stem rust. Resistance to these diseases makes Canvas a desirable variety for a farmer with intensive management; it also is drought resistant and yields well under high fertility. Snowmass

2.0 (CO13D1299) is a hard white variety released in 2018 and is part of the Colorado Wheat Research Foundation's Premium Program ("Premium Program", 2020). It is a high yielding variety that has an improved ability to earn protein premiums and has very good drought tolerance (Snowmass 2.0, 2018). Meeting protein premiums (i.e., a protein content threshold) is an aspect that can improve wheat quality for a number of factors.

Hard red and white winter wheats are specifically used for leavened breads, with this wheat class requiring protein percentages at 14% for sufficient flour quality (Freeman et al., 2003). To produce quality breads, grain quality and protein is an important determinant of baking success for food processors, with success dependent on gluten (a protein) content. Gluten allows for flour to be versatile when hydrated by creating a flexible dough. Dough flexibility aids in trapping gas bubbles created during the bread-making fermentation process, maintaining dough stretchability while retaining shape and strength. Specifically, gluten is a wheat flour protein network consisting of gliadin and glutenin proteins. Glutenin is a protein that contributes to dough strength and elasticity, while gliadin is protein that contributes to viscosity and extensibility (Barak et al., 2013, Fuertes-Mendizábal et al., 2010).

### **Nitrogen for crop production**

Nitrogen (N) is an essential element for plants yet is often restricted in agroecosystems because plant N demands can be large while the majority of soil N is organically complexed and unavailable for plant uptake. Therefore, (in)organic N fertilizer applications to crops often create a positive yield response. However, it can be challenging to predict if a N requirement exists, and if so, how much N should be applied. The challenge behind predicting crop N requirements is a function of soil N transformations and loss pathways. The N cycle complexity is important to

understand to make more informed decisions about N fertilizer applications and the effects N has on the environment and crop production. A brief description of the N cycle is outlined below.

The N cycle begins with atmospheric N<sub>2</sub> gas, comprising ~ 80% of the Earth's atmosphere (Barbarick, 2006); the remainder of N is other inert gaseous forms or in rock/mineral phases. Gaseous N<sub>2</sub> has a triple covalent bond that is not readily broken; plants cannot directly use atmospheric N and thus it needs to be transformed into plant-available forms (Brady & Weil, 2010). Plants primarily take up soil N as nitrate (NO<sub>3</sub><sup>-</sup>) or ammonium (NH<sub>4</sub><sup>+</sup>). These plant-available N forms are ultimately created via N<sub>2</sub> fixation, a process that converts atmospheric N to plant-useable forms. Nitrogen fixation occurs via three pathways: anthropogenic fixation, biological fixation, and lightning (Brady & Weil, 2010).

In the early 1900s, the Haber-Bosch process was developed. This discovery of anthropogenically fixing atmospheric N into ammonia (NH<sub>3</sub>) later became realized as the process for producing all inorganic N fertilizers currently used in commercial agriculture (Gruber & Galloway, 2008). Today, synthetic N<sub>2</sub> fixation is a process that is heavily relied upon for crop production, with N fertilizer one of the major costs for winter wheat farming in the U.S. (Vocke & Ali, 2013).

Biological N<sub>2</sub> fixation is an important natural process whereby certain bacteria (i.e., rhizobia) form symbiotic relationships with leguminous plant roots. Plants supply rhizobia with energy in the form of carbohydrates (i.e., carbon in the general formula CH<sub>2</sub>O) and water, and in return the rhizobia fix atmospheric N and supply NH<sub>4</sub><sup>+</sup> to the plant (Brady & Weil, 2010). While biological N<sub>2</sub> fixation was the major pathway for N fixation in ecosystems in natural history, since the invention of the Haber-Bosch process, the amount of biological N<sub>2</sub> fixation is less than total inorganic N fertilizer applied in the U.S. (Gruber & Galloway, 2008). The process of N<sub>2</sub>

fixation can also occur via lightning. Lightning fixes the least  $N_2$  amount compared to anthropogenic and biological fixation, yet still plays an important role in natural systems (Barbarick, 2006).

Plants that have utilized fixed N will eventually senesce, decompose, and return N to the soil. Alternatively, plants will be consumed by other organisms that will use N and release it in the form of manures, or eventually die and release N to soil via decomposition. The majority of N returned to soils remains in unusable form for plant uptake, such as humic complexes and proteins. Further N cycling must occur via the intervention of fungi and bacteria that eventually convert complex N forms to  $NH_4^+$ , a plant-available form (Brady & Weil, 2010); this process is known as mineralization.

Fungi and bacteria-released  $NH_4^+$  may be held by negatively charged clay particles. Ammonium also has potential to be lost to the atmosphere (e.g., volatilization) via the conversion to  $NH_3$  gas (Barbarick, 2006); volatilization losses are greater when  $NH_4^+$  is present near the soil surface or if soil pH is relatively high. Soil  $NH_4^+$  can be absorbed by plants or undergo nitrification, whereby it is oxidized by bacteria to form nitrite ( $NO_2^-$ ) and successively  $NO_3^-$ . The  $NO_3^-$  compound is negatively charged, repelled by negative soil particles, and thus can potentially be leached from the soil profile via free moving water (Brady & Weil, 2010).

Nitrate leaching is of concern for human health as it can contaminate drinking water and waterways, resulting in pollution and harm to humans and livestock. The concern with excess drinking water  $NO_3^-$  (typically  $> 10 \text{ mg L}^{-1}$ ) is methemoglobinemia. Methemoglobinemia, or blue baby syndrome, is especially harmful for infants and ruminants that are sensitive to the nitrate entering the blood stream where it replaces oxygen on the red blood cells; this may result in suffocation and death (Barbarick, 2006). Nitrate in waterways can also result in algal blooms and

eutrophication that constricts the ecosystem due to the reduction of resources, specifically oxygen (Brady & Weil, 2010).

Denitrification is another N cycle process whereby bacteria reduce nitrate into gaseous forms including nitric oxide (NO), N<sub>2</sub>, and nitrous oxide (N<sub>2</sub>O). This process occurs when soils lack oxygen and microorganisms obtain oxygen from nitrate, thereby converting nitrate to gases that escape the soil (Barbarick, 2006). N<sub>2</sub> is the ideal result as it is inert and can enter the N cycle again where it can be re-fixed. Regardless, none of these gases can be utilized by plants until they are again transformed (Brady & Weil, 2010).

Understanding the N cycle provides insight when making responsible N fertilization decisions, as N is often the most limiting and thus most commonly applied nutrient in the Great Plains (Schlegel & Grant, 2006). In order to make sound N fertilizer requirements, it is also suggested to obtain soil samples to a two-foot (61 cm), instead of one-foot (30.5 cm) depth increment. By obtaining a two-foot soil sample, a more robust NO<sub>3</sub><sup>-</sup> analysis and a N credit can be applied for the subsequent growing season. Credits include residual soil N at planting and N released throughout the growing season via organic matter (OM) degradation. Soil OM decomposition and thus N mineralization rates vary by soil type, and on various environmental and management factors (Brady & Weil, 2010). It is generally assumed that for every 1% of OM in the surface soil layer, about 30 lbs of N ac<sup>-1</sup> (34 kg of N ha<sup>-1</sup>) is mineralized and will be plant-available throughout the growing season (Davis & Westfall, 2014). Soils in Eastern Colorado under dryland management typically have 2% or less OM. The commonly grown crops in Eastern Colorado (wheat, sorghum, and corn) require more than 66 lbs of N (30 kg) per hectare for sufficient yields (Schlegel & Grant, 2006). Therefore, it can be assumed that N in the soil

may be insufficient for abundant wheat yield and thus N fertilizer applications may be needed based on management, climate, and cropping rotations in dryland production systems.

Dryland production systems typically experience less N losses than in irrigated agroecosystems, and this coupled with lower yields leads to lower N fertilizer needs and recommendations. The decrease of available water in dryland soils, as compared to irrigated soils, can decrease N transformation rates and losses. Soil texture also affects N applications, with sandy-textured soils potentially leaching N more quickly compared to heavier-textured soils, due to larger pores and greater downward preferential flow. Management practice may also affect fertilizer recommendations, with changes in water storage under no-till and reduced tillage systems as compared to conventional tillage systems. Nitrogen losses in no-till or reduced tillage may be realized due to alterations in the soil water profile, or if N fertilizer is surface-applied with no immediate incorporation. Surface N fertilizer applications under no-till or reduced tillage may leave N stranded where plant roots cannot access the nutrient, while surface application of ammonium-based fertilizers may lead to substantial N volatilization losses. Thus, it is important to couple understanding of the N cycle with management decisions to make sound N fertilizer recommendations.

### **Wheat response to nitrogen**

For winter wheat, N is one of the most important nutrients for production as it is a major component of proteins, enzymes, nucleic acids, and chlorophyll. As a result, having sufficient plant-available soil N is a major determinant of yield and adequate protein content in wheat grain. It is thought that sufficient N fertility increases yield due to an increase in leaf surface area, which increases photosynthesis efficiency and promotes dry matter accumulation and yield (Belete et al., 2018). A N deficiency in winter wheat typically results in chlorosis in older leaves;

when extreme, the entire plant may become chlorotic. Older leaves show N deficiencies first because N is mobile in plants, with N translocated from older to newer growth. Deficiency symptoms in most grass species manifest themselves as V-shaped chlorosis starting at the leaf tip and follow the leaf midrib back to the point of contact with the stem. This symptom may be more difficult to distinguish in small grains, but still may be present. Nitrogen deficient plants will be stunted, have limited tillering, a sparse canopy, and quicker maturation than N sufficient plants (Schlegel & Grant, 2006); these symptoms negatively affect wheat yield. In contrast, healthy wheat plants typically have dark green leaves throughout the plant (Borghi, 1999).

To reduce N deficiencies in wheat, N fertilization optimization is recommended. In order to optimize winter wheat N fertilizer applications, it is critical to consider the total N amount needed for optimal grain yield, the total N credits, and the efficiency of N applied to satisfy N needs (Borghi, 1999). The current N use efficiency (NUE) in wheat cropping systems is less than 40% (Belete et al., 2018). To optimize N fertilization and increase NUE, N application depends on the timing and type of fertilizer. The recommended approach to fertility is outlined by The Fertilizer Institute as the 4R approach (<https://nutrientstewardship.org/4rs/>). The concept outlines that the right nutrient should be applied at the right rate, right place, and right time for the “right” crop. This idea takes crop type, environment, and management practices into account to make sustainable fertilizer decisions by each farmer or agronomist.

The physiology of winter wheat results in fluxes of N needed for growth depending on the growth stage. This creates complications as to when and how much N to apply, as soil N transformations can result in losses before the plant is able to utilize it. Due to the fluctuations of wheat N needs and variable N transformations, it is recommended to split apply N for wheat. Split applying N refers to the method of applying fertilizer in increments throughout the growing

seasons at appropriate times rather than applying the total N needed for the entire season all at once. A split N application is efficient for wheat plant N utilization and for preventing losses. It also is beneficial to apply starter N at planting among other nutrients to promote tillering and robust root growth to prevent winter kill (Diaz & Weber, 2019). Nitrogen needs are minimal in the winter, with demand increasing dramatically in the spring when growth occurs. Studies have found that N applied in the spring for winter wheat results in better yield and protein composition (Boman et al., 1995; Johansson et al., 2004). The amount of N that wheat needs decreases after flowering so N applications are not typically applied from this growth stage forward. To ensure good yields and protein, it is essential to prevent deficiencies at critical wheat growth stages such as shooting, jointing, tillering, and grain fill (Borghini, 1999).

In winter wheat production, protein is important for wheat quality and elevator premiums. Protein content is typically responsive to increasing N fertilizer application rates, as N is an essential and abundant element in protein structures. Grain protein concentration often follows an inverse relationship with grain yield. Protein becomes concentrated in the grain with low yields; the opposite is typically true for high yields as protein percent becomes diluted with an increase in grain starch (Borghini, 1999). However, too much protein is found to negatively affect quality of the dough by increasing gliadin to an undesirable proportion against glutenin that weakens the dough. There is a sensitive balance for winter wheat protein needs in winter wheat grain (Johansson et al., 2004), and thus a production challenge exists between maximizing winter wheat yields and protein contents. Fortunately, it is uncommon for protein levels to be relatively too high. Fertilizing with ample N typically results in higher grain protein content. Wheat grain protein concentrations that are too low may result in lower grain elevator price, while protein concentrations that are above a determined threshold value may result in a price premium. For

example, hard white winter wheat varieties are offered a premium at Ardent Mills elevators of \$0.05 for every 0.5% increase in protein above 11.0% for those farmers in the 2020 premium program (“Premium Program”, 2020). Sufficiently applying N is an economic benefit that is often under realized in Eastern Colorado.

A common mechanism for making fertility recommendations in the Great Plains are “yield-based” algorithms. They provide a straightforward and easy-to-implement method for farmers and crop advisors to make nutrient management decisions. To make yield-based recommendations, crop yield at harvest must be estimated in order to identify the target N rate for that yield. Then, soil N credits are subtracted from the N rate for that yield estimate. Nitrogen credits include residual soil  $\text{NO}_3^-$ , potentially mineralized N, and any legume credits to calculate total N needed (Davis & Westfall, 2014).

Although yield-based fertilizer recommendations have been widely popular, they are problematic for efficiency in crop production. The limitations of yield-based fertilizer recommendations were highlighted by Rodriguez et al. (2019) who found that the original development of yield-based recommendations was based on little data, potentially faulty research, and provided only “ballpark” estimations for corn. While this approach has been relatively useful, studies investigating yield-based algorithms have had little success proving their utility. The over-confidence in yield-based algorithms may have harmed agriculture efficiency by both under and over fertilizing crops (Rodriguez et al., 2019). Another issue with yield-based fertilizer recommendations is that estimating yield at harvest can be inaccurate. Many environmental and genotypic factors can lead to variations from accurate yield predictions. The inability to accurately predict fertilizer requirements based on estimated yield and in-soil N

credits suggests that a better way to provide N fertilizer recommendations is desperately required.

In Colorado, CSU provides recommendations for wheat fertilization requirements by using the yield-based fertilizer approach (see the CSU “Fertilizing Wheat” fact sheet; Davis and Westfall, 2014). This requires farmers to assume the yield they expect for the field they are fertilizing, in bu/ac for dryland winter wheat production. Once the amount of N for the season is found, N credits are subtracted from N fertilizer needed (Davis & Westfall, 2014). However, to improve winter wheat recommendations, something other than a yield-based fertilizer method could potentially be utilized, perhaps based on grain protein content.

Concomitantly with yield, grain protein content is the other important consideration for winter wheat fertilizer recommendations. CSU has provided the fact sheet “Grain Protein Content and N Needs” (Goos et al., 2015). The fact sheet states that to increase wheat protein content, an additional 22-34 kg N ha<sup>-1</sup> is suggested to be applied for each additional protein percent desired. The fact sheet also provides a measure for farmers to estimate if they had sufficient fertility for the crop depending on grain protein concentrations. The authors suggest that when grain protein is below 11.1%, yield potential is lost due to insufficient N fertility. Protein content between 11.1-12% may need additional N but there may be a GxE interaction present. Above 12%, the authors estimate that N is sufficient, and more N would likely increase protein and not yield.

Recommendations for fertilizing winter wheat have not been tested in Colorado since the late 1970s. The dissertation of Dr. Jay Goos (1980) established these recommendations from trials conducted in Eastern Colorado on semi-dwarf and tall winter wheat varieties. It appears that, since the late 1970s, the Colorado fact sheet recommendations have been routinely

analyzed, updated, and improved based mostly on information gleaned from surrounding states (e.g., Kansas, Nebraska). However, no major N application trials on winter wheat have been conducted over the past 40 years in Eastern Colorado. This raises concern regarding the current N recommendations provided by CSU. Winter wheat varieties have increased in yields since the 1970s and further wheat improvement may have changed the physiology of CSU varieties. It has been found that modern wheat cultivars are more responsive in yield to N fertilizer (Aula et al., 2019).

Fertilizer recommendations by variety have not been common practice in wheat. However, there is interest in understanding if cultivars in Eastern Colorado have different N requirements, as wheat response to nitrogen and NUE may vary by variety (Barracough et al., 2010). The question is, are these differences between varieties significant enough to justify unique recommendations by variety? Johansson et al. (2004) found differences in wheat N utilization and protein content but yield by variety did not vary significantly. However, Dorsey (2014) found significant differences in N utilization related to genotype among 25 wheat varieties. Therefore, it is of interest for the CSU Wheat Breeding and Genetics Program to see if differences exist in N response between newly released cultivars and for updating Colorado's fertilizer recommendations.

To display the differences between winter wheat varieties, N response curves are of interest for fertilizer recommendations for winter wheat production in Eastern Colorado. Response curves are an easy-to-understand tool and require minimal data for their creation. They provide optimum fertilizer estimates for yield, and can also identify the economic optimum fertilizer rate based on return on investment. Response curves take average yields and plot them along a gradient of applied fertilizer. There is potential to use them to plot average yield against

total soil N and consider the estimated soil N mineralization throughout the season based on the mineralization rule provided by CSU (30 lbs N ac<sup>-1</sup> per 1% soil organic matter). Response curves are also practical to use as they are easily created in programs such as Excel and R.

### **Sulfur for crop production**

Sulfur is an important macronutrient for plant nutrition. Sulfur is an essential component of plant proteins, several amino acids, and plays a role in chlorophyll formation. The reduction in atmospheric S deposition in the last two decades (2000s through 2010s) may lead to S deficiency in soils (Kaiser et al., 2019). Therefore, soil S availability for crop production in Eastern Colorado may be decreasing. Decreasing S in soils may also be due to less S in non-S fertilizers, fungicides, and herbicides, and higher yielding crop varieties that remove greater soil nutrients, including S (Scherer, 2019; Jamal et al., 2010). Sulfur fertilizer applications for maximizing soil fertility in crop production should be considered as an interest in current agronomic research for Eastern Colorado.

For S fertility research, it is essential to understand the soil S cycle. Sulfur has a cycle similar to that of N, and thus S and N are often intimately linked to each other in soils (Fageria, 2001) and plants. For example, S undergoes terrestrial transformations such as mineralization, mobilization, immobilization, oxidation/reduction, and volatilization (Schnug, 1998), much like N.

The start of the S cycle begins like N, in the atmosphere. Natural sources that release S into the atmosphere include volcanoes, ocean emissions, and bacterial sources. Humans have altered atmospheric S concentrations through industrial processes that release sulfur dioxide (SO<sub>2</sub>) and hydrogen sulfide (H<sub>2</sub>S) gases. It is estimated that the majority of atmospheric S is anthropogenic (Lefohn et al., 1999). Regardless of the action by which S enters the atmosphere,

eventually S returns to the Earth. Sulfur deposition can occur via acid rain deposition in the form of sulfuric acid ( $\text{H}_2\text{SO}_4$ ), or simply by fallout whereby atmospheric S molecules or compounds settle on land or water (Schnug, 1998).

Atmospherically deposited S can be utilized by terrestrial microorganisms and plants when in the form of sulfate ( $\text{SO}_4^{2-}$ ) (Tracy, 1986). Some atmospheric S can be absorbed by plant leaves in the form of sulfur dioxide ( $\text{SO}_2$ ). Upon senescence, plants will return S to the soil during decomposition (Brady & Weil, 2010). Soil bacterial reduction of  $\text{SO}_4^{2-}$  to hydrogen sulfide ( $\text{H}_2\text{S}$ ) gas releases S back to the atmosphere. Sulfur can be removed from terrestrial ecosystems via runoff, erosion, and leaching, deposited into waterways and eventually to the ocean (Dhillon et al., 2019; Schnug, 1998).

The bulk of S in agricultural soils is unavailable for plant uptake as it is present in OM. Most soil OM-bound S is in the form of amino acids and proteins (Brady & Weil, 2010). Within the relatively small available soil S pool,  $\text{SO}_4^{2-}$  is either absorbed by plants (Schnug, 1998) or immobilized to organic forms by soil microorganisms. As with plant death, microbially-immobilized S will eventually be mineralized to the inorganic form, sulfate ( $\text{SO}_4^{2-}$ ), upon microbial tissue death and decomposition. Immobilization and mineralization rates are affected by the microbial pool and fluxes in the N:S ratio of the soil. These fluxes can be affected by temperature, plant residues, moisture, and fertilizer (Jamal et al., 2010).

Sulfur availability for plants can be affected by a variety of environmental factors, including tillage type, precipitation, soil type and fertilizer type. Tillage is known to reduce OM and thus reduce S mineralization (Tracy, 1986). Sulfur may be deficient in sandy soils with low OM, and therefore S fertilization to plants grown in sandy, irrigated soils may show growth responses (Kaiser et al., 2019). Thus, Eastern Colorado croplands with low OM and sandy

textures may respond to S. However, S is sometimes applied without soil tests that would prove whether or not the soil needs an application. Further S fertilization research is needed in Eastern Colorado for CSU to update their S fertilizer guidelines.

### **Wheat response to sulfur**

Sulfur sufficiency is important for winter wheat production. Adequate soil S fertility will help the crop achieve desirable wheat quality (Wilson et al., 2020). Sulfur is essential for wheat grain protein development, playing an integral role in grain quality parameters for bread making; S is an integral component of gluten. High protein concentrations are a necessary quality for hard red and white winter wheat production in Eastern Colorado. However, studies on wheat response to S in Eastern Colorado have not been conducted recently. The decrease in S deposition in soil signals the potential need for S fertilizer applications to produce high quality winter wheat in Eastern Colorado; lack of plant-available soil S may lead to deficiency symptoms. Sulfur deficiencies manifest as chlorosis in younger leaves; S is not mobile in plants and thus it cannot readily be translocated from older to newer growth (Lamond, 1997). The stress of the lack of S in the tissue will lower overall wheat yield and protein.

Specifically, S deficiencies negatively affect N assimilation and thus detrimentally affect the wheat plant. Therefore, adequate S is needed to maximize N fertilizer efficiency and uptake. This has led to the suggestion of using an N:S ratio for determining nutrient sufficiency (Fageria, 2001). Determining in-season plant S deficiencies commonly utilizes the wheat flag leaf to identify the within-plant N:S concentration ratio. A flag leaf N:S ratio > 17:1 is evident of S deficiency; optimum yields may be achieved below a 16:1 ratio (Kaiser et al., 2019).

Grain S deficiencies likely result in low protein and gluten content that reduces extensibility, elasticity, and loaf volume (Dhillon et al., 2019). Thus, wheat protein has often

been found to positively respond to S applications. Maximum yields need sufficient S but additional S has not been shown to increase yields (Kaiser et al., 2019). Kaiser et al. (2019) also suggest that varieties may respond differently to S applications. The authors found that varieties had different N:S threshold values in-season, yet also noted that S application amounts for optimum yields did not vary significantly.

Sulfur deficiencies can also negatively affect other wheat grain characteristics. Lack of S can lead to increased asparagine concentrations in wheat grain; it is thought that relatively high grain asparagine concentrations are indicative of plant stress and low N and S utilization efficiency. The major concern regarding asparagine formation is that asparagine contributes to acrylamide formation during the baking process. Acrylamide is considered a Group 2a carcinogen by the World Health Organization and International Agency for the Research on Cancer (Curtis & Halford, 2014). Therefore, optimizing S and N soil nutrition in Eastern Colorado (and elsewhere) is important for reducing the acrylamide consequences during the baking process. Thus, it is of interest to analyze the response of yield, protein, and quality within major eastern Colorado wheat varieties in terms of how responses to S fertilization vary by location. If wheat responds to S, the CSU recommendations may need to be updated to ensure that wheat quality and yields can reach their potential and reduce acrylamide formation in the grain for public health protection.

## **Conclusion**

Conducting N fertilizer response research on the current three major wheat varieties raised in eastern Colorado (in 2019 and 2020: Canvas, Langin, and Snowmass 2.0) will provide Colorado producers valuable information for efficient N fertilizer applications. It will also provide insight into whether each variety requires unique N fertilizer recommendations or if

varieties can simply be grouped together. This research will also guide CSU soil N fertility recommendations and determine whether current recommendations need to be updated; the research behind the current recommendations have not been updated in likely 40 years.

The current need for S fertilization in Eastern Colorado is unknown, as there is scant evidence regarding Eastern Colorado's soil S availability coupled with atmospheric S deposition decreases. Many farmers in Eastern Colorado do not test their soil for S deficiencies and thus may be applying inadequate S amounts. Therefore, understanding the wheat response to S will provide information for CSU to update their S fertilizer recommendations.

In order to address the above concerns, we are utilizing two years of data, collected from 10 locations per year, across eastern Colorado to provide valuable information for making winter wheat N and S fertilizer recommendations. Abiotic and biotic factors are known to affect wheat yield and response, thus utilizing multiple locations and monitoring yield, other responses, and within-location climatic conditions should provide a more robust dataset for enhancing state fertilizer recommendations. Based on this approach, our objectives aim to determine optimum agronomic and economic nitrogen fertilizer rates for the three main winter wheat cultivars raised in Colorado (Canvas, Langin, Snowmass 2.0). The effects of varying N fertilizer rates on winter wheat grain test weight, single kernel characterization, flour protein, and flour yield in Colorado will be determined. Weather and other climatic variables will also be analyzed to understand how they may affect grain yield and protein content across sites. Lastly, the effect of S on wheat yield and grain quality, including protein, in Eastern Colorado will be analyzed to determine if S fertilizer applications are required.

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## CHAPTER 2: MANUSCRIPT

### INTRODUCTION

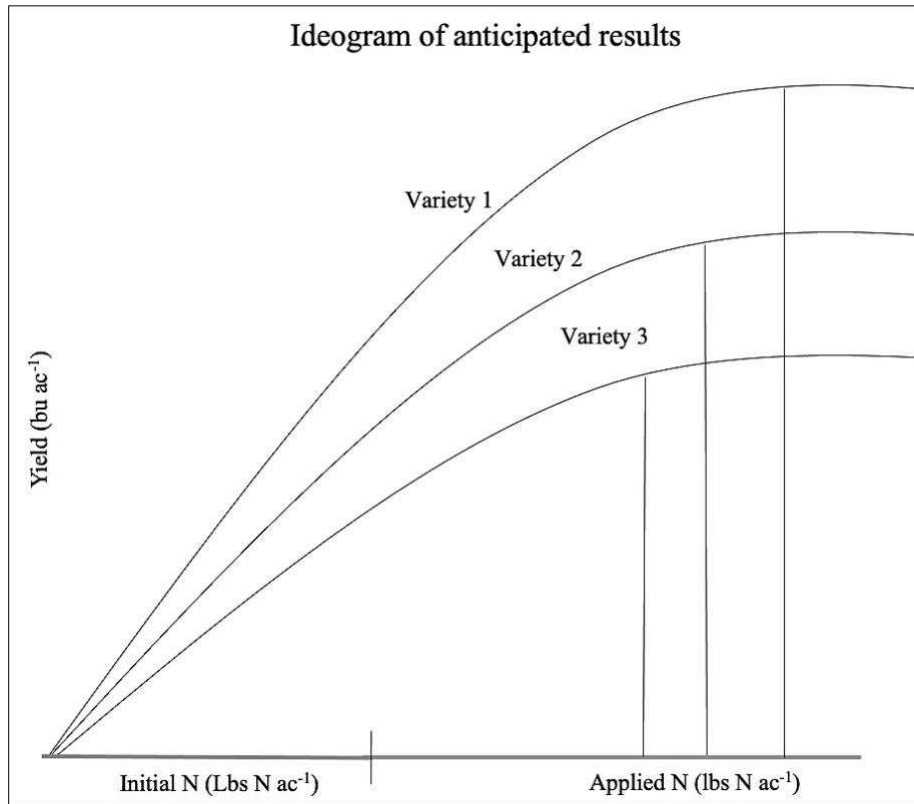
Winter wheat (*Triticum aestivium*) is an important dryland crop in eastern Colorado. In 2020, two million acres of planted winter wheat brought in over \$387 million into the state's economy. Successful winter wheat production often requires N fertilization to achieve high grain yields and protein content. Colorado State University's current winter wheat recommendations (Davis & Westfall, 2015) for applying N fertilizer are:

$$(2.6 \text{ lbs N ac}^{-1} * \text{yield goal bu ac}^{-1}) - \text{soil residual nitrate N} - \text{SOM N credits} - \text{legume N credits} \\ = \text{lbs N fertilizer recommended ac}^{-1}$$

Additionally, protein recommendations from Colorado State University from Goos et al. (2014) suggest that fields with grain protein content consistently below 11.1% likely have N deficiencies. Field history grain protein content greater than 12% probably have adequate N. Fields that have consistently produced grain protein contents between 11.1-12% may respond to N. Therefore, the recommendations suggest that applications for fields with consistently low grain protein content require an additional 20-30 lbs N ac<sup>-1</sup> to raise grain protein content by 1%.

The current recommendations outlined above for N fertilization for winter wheat in Colorado may need to be updated with current research from trials in Colorado. Data for these recommendations was established by Goos (1980) using common varieties raised in the late 1970's. Current varieties of wheat have improved ability to achieve higher grain yields and potentially are more efficient at utilizing N. Evidence of differences in N utilization and response by variety has been found in Barraclough et al. (2010), Dorsey (2014, and Johansson et al. (2004). However, yield by variety has had mixed response in the research. Our hypothesized

differences in N need by variety yield is displayed in Figure 1. Optimum N rates would be at the top of the curves.



**Figure 1.** Ideogram of anticipated yield response by variety to increasing N

Sulfur is another important nutrient for wheat growth and a component of plant proteins. It has been found by Kaiser et al (2019) that fields with low soil organic matter levels, high soil sand content, and low sulfate levels in the soil that wheat may respond to S fertilizer applications. Currently, there are no specific recommendations on rates and timing for S fertilizer applications in Colorado.

Determining if the three studied winter wheat varieties currently raised in Colorado respond differently to N and what the optimum N rate would be for each was the main objective of the study. With that information, our second objective was to determine if we could make new and better N fertilizer rate recommendations for winter wheat. The following objective was to determine the effect of N and variety on grain yield and quality parameters; test weight, thousand

kernel weight, and protein at 18 environments in Eastern Colorado. Our final objective was to determine how S fertilization affects winter wheat grain yield and protein content in multiple locations in Eastern Colorado.

## MATERIALS AND METHODS

Research was conducted from 2019-2020 in Eastern Colorado. Specifically, the experiment was conducted on 20 dryland fields (10 in 2019 and 10 in 2020) with various management strategies and environmental conditions. All trials were on grower-cooperator fields except the Akron trials that were conducted at the USDA Central Great Plains Research Station. This research was conducted during the winter wheat growing season of 2018-2019 and 2019-2020. The 2019 crop received significantly more precipitation than 2020, and thus was classified as a “wet year”. The 2020 growing season was slightly drier and warmer than average for eastern Colorado, and thus was classified as a “dry year”.

### **Study Design**

Trials at each location followed a split-plot design. The main plot consisted of four treatments of three N application rates at 45, 90, 135 lbs N ac<sup>-1</sup>, and one S treatment at 90 lbs N ac<sup>-1</sup> plus 20 lbs S ac<sup>-1</sup>. The sub-plot consisted of three semi-dwarf winter wheat varieties developed by CSU’s Winter Wheat Breeding Program, including Canvas (CO12D1770), Langin (CO11D446), and Snowmass 2.0 (CO13D1299). Each treatment was replicated three times. CSU Crops Testing Winter Wheat Variety Trials were conducted next to the fertility trials. Samples from the CSU Crops Testing’s variety trial’s varieties Langin, Canvas, and Snowmass 2.0 were

collected to use as a control in select analyses. Locations, soils, and cultural practices from each trial are shown in Table 1.

The main N treatment plots were fertilized with liquid ammonium nitrate top-dressed in two evenly split applications of the N treatments in September and October of 2018 and March of 2019 for the 2018-2019 growing season and in December 2019 and January 2020 and March 2020 for the 2019-2020 growing season (45, 90, and 135 lbs N ac<sup>-1</sup>) (Table 2). The main S treated plots were fertilized with liquid ammonium nitrate and thio-sulfate top-dressed (90 lbs N ac<sup>-1</sup> and 20 lbs S ac<sup>-1</sup>). All plots were planted with a start fertilizer of eight lbs N ac<sup>-1</sup> and 28 lbs P ac<sup>-1</sup>. The wheat varieties used for the subplot represented high yielding winter wheat varieties commonly planted in Eastern Colorado's dryland wheat cropping systems. Canvas is a hard red winter wheat variety that was released in 2018. Canvas was developed to be resistant to diseases that are economically damaging in Eastern Colorado, such as wheat streak mosaic virus, stripe rust, and stem rust; it is also rated very good for drought resistance (Canvas, 2020). Langin was released in 2016 and is rated excellent for yield and drought tolerance across the state in variety performance trials (Haley et al., 2018). Recently, it has been a top-planted variety in Eastern Colorado and in 2020 it was the most popular variety covering 20.1% of winter wheat planted acres (Johnson et al., 2020). Snowmass 2.0 is a hard white variety released in 2018 that is rated excellent in yield and drought resistance and has an improved ability to earn protein premiums (Snowmass 2.0, 2020). It is part of the Colorado Wheat Research Foundation's Premium Program (Colorado Wheat, 2020).

CSU Crops Testing Program planted and maintained the trials and applied all fertilizer to the N study. Pest control and additional fertilizer was managed by the grower-operator at each site and by CSU Crops Testing at Akron. Each trial was planted in September and October with

**Table 1.** Locations, soils, and cultural practices used for winter wheat production in 18 environments in Eastern Colorado.

Location	County	Year	Latitude	Longitude	Soil map unit	Cultural practices
Akron	Washington	2019	40.149	-103.137	Rago silt loam (Fine, smectitic, mesic Pachic Argiustolls)	No-till after proso millet and chemfallow
		2020	40.149	-103.139	Rago silt loam (Fine, smectitic, mesic Pachic Argiustolls)	No-till after proso millet and chemfallow
Arapahoe	Cheyenne	2019	39.002	-102.246	Keith-Richfield silt loam (Fine-silty, mixed, superactive, mesic Aridic Argiustolls)	Vertical tillage after corn and chemfallow
		2020	38.907	-102.314	Wiley silt loam (Fine-silty, mixed, superactive, mesic Aridic Haplustalfs)	Vertical tillage after corn and chemfallow
Burlington	Kit Carson	2019	39.285	-102.280	Kuma-Keith silt loam (Fine-silty, mixed, superactive, mesic Aridic Argiustolls)	Tilled after corn and chemfallow
		2020	39.315	-102.258	Kuma-Keith silt loam (Fine-silty, mixed, superactive, mesic Aridic Argiustolls)	Tilled after corn and chemfallow
Genoa	Lincoln	2019	39.352	-103.509	Weld silt loam (Fine, smectitic, mesic Aridic Argiustolls)	Tilled twice after chemfallow and oil sunflowers
Julesburg	Sedgewick	2020	40.825	-102.306	Keith-Kuma silt loam (Fine-silty, mixed, superactive, mesic, Aridic Argiustolls)	Tilled after corn and chemfallow
Lamar	Prowers	2019	38.003	-102.614	Briggsdale clay loam (Fine, smectitic, mesic Ustic Paleargids)	No-till after wheat and chemfallow
		2020	38.006	-102.615	Manvel silt loam (Fine-silty, mixed, superactive, mesic Ustic Haplocalcids)	No-till after wheat and chemfallow
Orchard	Morgan	2019	40.482	-104.11	Weld silt loam (Fine, smectitic, mesic Aridic Argiustolls)	No-till after proso millet and chemfallow
		2020	40.48	-104.11	Platner loam (fine, smectitic, mesic Aridic Paleustolls)	No-till after wheat and chemfallow
Roggen	Weld	2019	40.073	-104.302	Wiley silt loam (Fine-silty, mixed, superactive, mesic Aridic Haplustalfs)	No-till after corn and chemfallow
		2020	40.084	-104.301	Weld silt loam (Fine, smectitic, mesic Aridic Argiustolls)	No-till after wheat and chemfallow
Sheridan Lake	Kiowa	2019	38.535	-102.471	Wiley silt loam (Fine-silty, mixed, superactive, mesic Aridic Haplustalfs)	No-till after grain sorghum and chemfallow
		2020	38.534	-102.436	Fort Collins sandy loam (Fine-loamy, mixed, superactive, mesic Aridic Haplustalfs)	No-till after grain sorghum and chemfallow
Yuma	Yuma	2019	40.191	-102.661	Haxtun sandy loam (Fine-loamy, mixed, superactive, mesic Pachic Argiustolls)	Min-till after proso millet and fallow
		2020	40.187	-102.654	Haxtun sandy loam (Fine-loamy, mixed, superactive, mesic Pachic Argiustolls)	No-till after wheat and chemfallow

starter fertilizer put down with the seed (8-28-0 lbs NPK ac<sup>-1</sup>). The fertilizer rates for the N trial were split applications applied in the fall of 2019, January in 2020, and the end of March for both years. At each site, a weather station was installed with anemometers, air temperature sensors, soil moisture and temperature sensors at 15 cm, 30 cm, and 76 cm, air moisture sensors, and a rain gauge, with data collected throughout the season. Refer to Table 2 for information about important event dates and Table 3 for information about weather data collected in season from the weather stations and from CoAgMET.

**Table 2.** Timing of important events.

Location	Year	Planting	First appl. of treatments	Second appl. of treatment	Average heading date	Harvest
Akron	2019	10/3/18	10/3/18	3/26/19	6/4/19	7/26/19
	2020	9/19/19	12/31/19	3/25/20	5/24/20	7/2/20
Arapahoe	2019	9/13/18	9/28/18	3/27/19	5/26/19	7/16/19
	2020	9/13/19	1/3/20	3/26/20	5/19/20	7/3/20
Burlington	2019	9/13/18	9/28/18	3/27/19	5/28/19	7/17/19
	2020	9/12/19	1/3/20	3/26/20	5/18/20	7/8/20
Genoa	2019	9/18/18	9/28/18	3/27/19	6/2/19	7/18/19
Julesburg	2020	9/17/19	1/3/20	3/30/20	5/29/20	7/7/20
Lamar	2019	9/12/18	9/28/18	3/27/19	5/16/19	7/8/19
	2020	9/13/19	1/2/20	3/26/20	5/14/20	7/1/20
Orchard	2019	10/2/18	10/19/18	3/28/18	6/9/19	7/29/19
	2020	9/17/19	1/9/20	3/30/20	5/25/20	6/30/20
Roggen	2019	10/1/18	10/19/18	3/28/18	6/5/19	7/25/19
	2020	9/25/19	1/9/20	3/20/20	5/26/20	7/10/20
Sheridan Lake	2019	9/12/18	9/28/18	3/27/19	5/20/19	7/12/19
	2020	9/13/19	1/2/20	3/26/20	5/17/20	7/1/20
Yuma	2019	9/19/18	9/27/18	3/26/19	5/30/19	7/13/19
	2020	9/18/19	1/20/20	3/25/20	5/22/20	7/3/20

## Data Collection

Soil samples were obtained prior to trial planting. Samples were specifically obtained from the 0-12 and 12-24 inch depths for soil nutrient analysis. Five core samples were

composited from each location. Soil samples were sent to The American Agricultural Laboratory, Inc. to be analyzed for pH, nitrate-N ( $\text{NO}_3^-$ -N), P, K, Ca, cation exchange capacity (CEC), soil organic matter, soluble salts, soil texture, and micronutrient content at both depths. Refer to Table 4 for information about additional fertilizer application rates applied by the grower co-operator, and Table 5 for information on the fall soil test results for macronutrients.

**Table 3.** Weather information at each trial.

Location	Year	Precipitation (April 1 to June 30) (")	Days where temperature reached above 90° F (April 1 to June 30)	GDD from (Jan 1 to June 30)	Last freeze date after April 1
Akron	2019	9.25	6	1945	5/22/19
Arapahoe	2019	6.99	9	1941	n/a
Burlington	2019	7.56	7	1892	n/a
Genoa	2019	8.22	4	1726	5/22/19
Lamar	2019	5.76	10	1896	5/22/19
Orchard	2019	8.42	5	1924	5/22/19
Roggen	2019	7.40	5	2069	5/22/19
Sheridan Lake	2019	9.07	9	1880	5/22/19
Yuma	2019	5.97	5	1761	n/a
2019 Averages		7.63	7	1893	5/22/19
Akron	2020	4.10	17	1662	4/14/20
Arapahoe	2020	4.50	26	1846	4/14/20
Burlington	2020	6.40	26	1976	4/14/20
Julesburg	2020	1.60	26	1642	4/14/20
Lamar	2020	3.60	30	2046	4/14/20
Orchard	2020	2.10	26	1613	4/14/20
Roggen	2020	2.10	32	1954	4/14/20
Sheridan Lake	2020	3.20	28	1995	4/14/20
Yuma	2020	3.10	26	1693	4/14/20
2020 Averages		3.41	26	1825	4/14/20

**Table 4.** Additional application rates of nutrients applied to the trial by the grower co-operators.

<b>Location</b>	<b>Year</b>	<b>Farm N appl. rate (lbs/ac)</b>	<b>Farm P appl. rate (lbs/ac)</b>	<b>Farm S appl. rate (lbs/ac)</b>	<b>Farm Zn appl. rate (lbs/ac)</b>
Akron	2019	70	0	0	0
	2020	70	0	0	0
Arapahoe	2019	65	18	7	1
	2020	55	0	4	0
Burlington	2019	84	21	0	0
	2020	77	0	0	0
Genoa	2019	54	10	0	0
Julesburg	2020	40	0	1	0
Lamar	2019	36	8	0	0
	2020	42	0	0	0
Orchard	2019	51	9	8	0
	2020	54	0	0	0
Roggen	2019	0	0	0	0
	2020	0	0	0	0
Sheridan Lake	2019	46	0	8	0
	2020	52	0	11	0
Yuma	2019	29	0	2	0
	2020	69	0	0	0

**Table 5.** Soil test results by environments from samples obtained in the fall before planting.

Location	Year	Depth (cm)	OM %	NO <sub>3</sub> -N (kg ha <sup>-1</sup> )	Olsen P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> (mg kg <sup>-1</sup> )
Akron	2019	0-30	1.5	67	15	611	3
		30-60	1.5	40	5	647	4
	2020	0-30	1.5	171	22	702	6
		30-60	1.4	105	6	640	5
Arapahoe	2019	0-30	1.6	106	7	557	5
		30-60	1.4	62	5	542	4
	2020	0-30	1.8	121	4	511	5
		30-60	1.3	56	2	375	3
Burlington	2019	0-30	1.9	103	9	812	9
		30-60	1.9	80	10	703	10
	2020	0-30	1.7	96	18	675	4
		30-60	1.6	63	11	557	3
Genoa	2019	0-30	1.1	41	20	613	4
		30-60	1.1	74	12	737	4
Julesburg	2020	0-30	1.4	41	23	621	4
		30-60	1.3	35	8	683	3
Lamar	2019	0-30	1.1	37	5	446	5
		30-60	1.1	40	2	253	4
	2020	0-30	1.3	52	6	487	5
		30-60	1	37	2	243	5
Orchard	2019	0-30	1.2	44	5	325	2
		30-60	0.8	21	4	170	3
	2020	0-30	1.2	110	32	529	9
		30-60	1	55	9	206	6
Roggen	2019	0-30	1.4	47	10	367	4
		30-60	1	40	5	175	3
	2020	0-30	1.2	105	14	551	5
		30-60	1	72	5	203	3
Sheridan Lake	2019	0-30	1.3	47	10	504	5
		30-60	1.3	36	4	498	4
	2020	0-30	1.2	59	6	560	5
		30-60	1.2	41	3	306	6
Yuma	2019	0-30	1.4	74	18	724	4
		30-60	1.5	41	9	734	4
	2020	0-30	1.8	55	26	837	4
		30-60	1.7	55	11	557	4

The wheat canopy spectra of normalized difference vegetation index (NDVI) was measured using a handheld Greenseeker Model 505 (Trimble Navigation Limited, Sunnyvale, CA, USA). Normalized difference vegetation index readings were taken between Feekes 6

through the heading stage. All readings were taken on days without cloud cover and minimal wind. The sensor probe was passed over each plot at approximately two and a half feet above the wheat canopy. The average NDVI and NIR readings from each plot were used for subsequent analysis. Table 6 provides information for the NDVI dates and growth stage for each environment.

**Table 6.** Dates of NDVI readings and growth stage.

Location	Year	GS Reading Date	Wheat Stage (Feekes)
Akron	2019	5/26/19	8-9
Arapahoe	2019	5/29/19	10
Burlington	2019	5/29/19	10
Genoa	2019	5/21/19	10
Lamar	2019	5/29/19	Heading
Orchard	2019	5/25/19	8
Roggen	2019	5/21/19	10.4-10.5
Sheridan Lake	2019	5/25/19	9
Yuma	2019	5/26/19	Heading
Akron	2020	4/29/20	6
Arapahoe	2020	5/21/20	Heading
Burlington	2020	5/21/20	6-7
Julesburg	2020	4/20/20	6
Lamar	2020	5/14/20	10.3-10.5
Orchard	2020	4/30/20	7
Roggen	2020	5/1/20	6
Sheridan Lake	2020	5/16/20	10.4-10.5
Yuma	2020	4/30/20	7

Individual plot sizes were 1.8 x 9.14 m long, and at harvest the entire plot area was harvested with a small plot combine. The combine utilized the Harvest Master H2 GrainGauge (Juniper Systems, Logan, UT) to collect plot grain weight, grain moisture, and grain test weight. Individual plot yields, in bu ac<sup>-1</sup>, were corrected for moisture content to 12%. The N trial plots were harvested the same day as the CSU Crops Testing’s winter wheat variety trials except at Yuma where, in 2020, the N trial was harvested a day before the variety trials. Approximately, one pound of grain from each plot was collected during harvest from the combine. Some trials

had missing samples due to some plots being accidentally mixed. Missing data is noted in Table 7.

**Table 7.** Table of missing plot samples

<b>Environment</b>	<b>Plot</b>	<b>Variety</b>	<b>Treatment</b>	<b>Samples Missing</b>
Burlington 2019	230	Snowmass 2.0	0 N	SKCS
Orchard 2019	109	Langin	45 N	Protein, SKCS
Orchard 2019	209	Canvas	0 N	Protein, Yield, TW, SKCS
Orchard 2019	337	Canvas	0 N	SKCS
Akron 2020	239	Snowmass 2.0	0 N	Protein, SKCS
Arapahoe 2020	102	Snowmass 2.0	135 N	Protein
Arapahoe 2020	201	Snowmass 2.0	90 N 20 S	SKCS
Burlington 2020	301	Canvas	45 N	SKCS
Burlington 2020	305	Canvas	135 N	Protein
Julesburg 2020	111	Snowmass 2.0	90 N 20 S	Protein
Lamar 2020	101	Langin	45 N	Protein, Yield, TW, SKCS
Lamar 2020	102	Snowmass 2.0	45 N	Protein, Yield, TW, SKCS
Lamar 2020	110	Snowmass 2.0	90 N	Protein, Yield, TW, SKCS
Lamar 2020	202	Canvas	135 N	Protein
Lamar 2020	301	Canvas	90 N	Protein
Orchard 2020	310	Canvas	90 N	Protein, TW, SKCS
Roggen 2020	112	Langin	90 N 20 S	Protein, TW, SKCS

\*Abbreviations: 1,000 Kernel Weight (SKCS), Test weight (TW)

Grain samples, gathered on the combine at harvest, were cleaned to remove chaff and other debris, and then protein content was analyzed using near-infrared reflectance spectroscopy (NIRs) with a Foss NIRST<sup>TM</sup> DA1650 Feed and Forage analyzer (Foss, Hilleroed, Denmark). All protein was corrected for the standard 12% moisture basis for winter wheat. The AACC method (55-31) was used to measure single kernel weight of average gram weight of a kernel/1000 kernels.

After harvest, two composite, 2-foot depth soil samples were collected from each plot using a hydraulic soil probe. Each core was 2 inches in diameter. After collection, the soil was air-dried, ground to pass a 2-mm sieve, and then extracted with 2M KCl to determine residual nitrate-N (NO<sub>3</sub>-N) using the vanadium (III) chloride method (Doane & Horwath, 2003).

## Data Analysis

Data analysis treats all trials from different years and locations as separate environments based on the varying climatic, location, management, and agronomic factors. Two environments were removed from the analyses: Julesburg in 2019 due to heavy disease pressure, and Genoa in 2020 because of poor emergence due to drought (subsequently not harvested).

Polynomial regressions of grain yield, grain protein, test weight, thousand kernel weight, post-harvest residual nitrate-N, and NDVI were plotted on residual nitrate-N from the fall soil test results plus any additional N from the N applications and fertilizer applied by the grower-cooperators. The equation of fit,  $R^2$ , and p-value are displayed on all graphs presented in the Results and Discussion section. The responses between years showed significantly different results, thus the responses were graphed by year. Wheat varieties were also analyzed separately to observe differences in response between varieties.

Analysis of variance (ANOVA) was conducted in the SAS Version 9.4 (SAS Institute, 2020) using the GLIMMIX procedure and adjusted with Tukey-Kramer test. Split plot analysis of variance (ANOVA) was conducted on the N application rates of 45, 90, and 135 lbs N ac<sup>-1</sup> by environment, year, and both years combined. ANOVA was conducted on each environment individually treating replication as the random variable. The output by year and two years combined provided information on the main effect of the N rate, variety, and N x variety interaction. In the analysis, replication, environment, and replication x environment interaction effects were treated as random variables. The farmer-applied fertilizer rate was not used in the ANOVA as it was not randomized in with other fertilizer application rates within the study. ANOVA was conducted on grain yield, grain protein content, test weight, single kernel weight, residual nitrate-N, and NDVI readings by environment, year, and data combined over the two

years. Pairwise comparisons were made using LS MEANS in SAS. Results of the pairwise comparisons were assigned to signify differences in the results ( $p < 0.05$ ) for the means of N rates and varieties. Significance for all analyses was determined using a  $p < 0.05$ .

The control treatment of 90 lbs N  $\text{ac}^{-1}$  and S treatment of 90 lbs N  $\text{ac}^{-1}$  plus 20 lbs S  $\text{ac}^{-1}$  were tested against each other in two sample T-tests assuming equal variance for yield and protein by environment, variety, year, and years combined. Comparisons were made for yield and protein by year in groupings of soil pH, residual sulfate (from the fall soil test, in lbs S  $\text{ac}^{-1}$ ), organic matter (%), sand (%), and residual sulfate from the fall soil test plus additional sulfate rate (lbs S  $\text{ac}^{-1}$ ) applied by the grower co-operators.

## RESULTS AND DISCUSSION

### **Climate**

In the 2018-2019 growing season, April 1 to harvest precipitation averaged 7.6 inches across all environments. In the 2019-2020 growing season, April 1 to harvest precipitation across all environments averaged only 3.4 inches. Therefore, 2019 was classified as a high-yielding year due to sufficient moisture, while 2020 could be classified as a yield-limited year due to dry conditions. The last freeze date in 2019 was May 22 at six environments: Akron, Genoa, Lamar, Orchard, Roggen, and Sheridan Lake. The last freeze date in 2020 was April 14 at all environments. Between April 1<sup>st</sup> and June 30<sup>th</sup>, 2019, there was an average of 7 days when daily temperatures were above 90° F. In 2020, there was an average of 26 days between April 1<sup>st</sup> and June 30<sup>th</sup> when daily temperatures were above 90° F.

### **Effect of N application rate and variety on grain yield and protein**

Increasing N application rates did not affect grain yield in 17 of the 18 environments over two years (Table 8). Increasing N application rates did affect overall grain yield in 2020 and over the two years combined. Specifically in 2020, 135 lbs N ac<sup>-1</sup> of applied N reduced grain yield compared to the 45 lb N ac<sup>-1</sup> application rate. Over the two years combined, plots receiving 135 lbs N ac<sup>-1</sup> were lower yielding than plots receiving lower amounts of N fertilizer. Excess plant-available soil N has been shown to reduce grain yields as excess N increases vegetative growth that could deplete available soil moisture and thus negatively impact grain fill (Walsh & Walsh, 2020; Girma et al., 2006).

Grain yield was significantly affected by variety at 13 of the 18 locations. Overall variety yield differences were evident in 2019, 2020, and over the two years combined. Langin was higher yielding than Canvas and Snowmass 2.0 in 2019, while Langin and Snowmass 2.0 were higher yielding than Canvas in 2020. Over both years combined, Langin was the highest yielding variety, followed by Snowmass 2.0 and Canvas. The N application rate x variety interaction was mostly non-significant.

Langin typically outperformed Snowmass 2.0 and Canvas. This finding has also been observed in the CSU Crops Testing's 2-year and 3-year summaries of dryland winter wheat variety performances (Johnson et al., 2020). However, Langin and Snowmass 2.0 performed equally as well in 2020, a limited moisture year. Gauer et al. (1992) found greater varietal grain yield differences when relatively greater precipitation was received, with varieties significantly responding to added N. Similar results were found between the high yielding (2019) and moisture limited year (2020).

**Table 8.** Split plot analysis of variance for grain yield 2019 and 2020.

Year	Location	Average grain yield by N application rate			Average grain yield by variety			Significance		
		45 lbs N ac <sup>-1</sup>	90 lbs N ac <sup>-1</sup>	135 lbs N ac <sup>-1</sup>	Canvas	Langin	Snowmass 2.0	N application rate	Variety	N application rate x Variety
		(bu ac <sup>-1</sup> )								
2019	Akron	63.9 a	66.7 a	64.8 a	<b>57.8 c</b>	<b>72.1 a</b>	<b>65.6 b</b>	0.640	<b>&lt;0.001</b>	0.161
	Arapahoe	113.4 a	115.1 a	111.4 a	<b>104.1 c</b>	<b>123.5 a</b>	<b>112.3 b</b>	0.591	<b>&lt;0.001</b>	0.226
	Burlington	109.6 a	105.4 a	107.1 a	<b>98.1 b</b>	<b>111.4 a</b>	<b>112.6 a</b>	0.461	<b>&lt;0.001</b>	0.760
	Genoa	70.1 a	70.4 a	64.4 a	65.5 a	69.6 a	69.7 a	0.135	0.216	0.249
	Lamar	57.9 a	62.2 a	64.4 a	<b>60.6 ab</b>	<b>58.3 b</b>	<b>65.6 a</b>	0.102	<b>0.015</b>	0.994
	Orchard	54.2 a	55.5 a	49.5 a	<b>48.0 b</b>	<b>56.5 a</b>	<b>54.7 a</b>	0.116	<b>0.008</b>	0.277
	Roggen	80.9 a	84.9 a	83.8 a	<b>78.7 a</b>	<b>85.0 a</b>	<b>85.9 b</b>	0.686	<b>&lt;0.001</b>	0.280
	Sheridan Lake	<b>78.7 b</b>	<b>87.7 a</b>	<b>87.7 a</b>	84.1 a	82.8 a	87.3 a	<b>0.030</b>	0.250	0.893
	Yuma	91.8 a	90.1 a	86.3 a	89.1 a	89.9 a	89.2 a	0.333	0.970	0.560
2019 Combined ANOVA		80.1 a	82.0 a	79.9 a	<b>76.2 b</b>	<b>83.3 a</b>	<b>82.5 b</b>	0.075	<b>&lt;0.001</b>	0.850
2020	Akron	48.6 a	50.2 a	46.4 a	<b>42.8 b</b>	<b>51.7 a</b>	<b>51.3 a</b>	0.823	<b>0.003</b>	0.513
	Arapahoe	35.0 a	36.0 a	35.8 a	33.3 a	36.2 a	35.7 a	0.271	0.150	0.090
	Burlington	70.6 a	67.7 a	66.9 a	<b>65.5 b</b>	<b>67.3 ab</b>	<b>72.4 a</b>	0.305	<b>0.014</b>	0.433
	Julesburg	67.4 a	61.6 a	60.3 a	<b>59.0 b</b>	<b>63.7 a</b>	<b>66.5 a</b>	0.375	<b>&lt;0.001</b>	<b>0.030</b>
	Lamar	29.5 a	29.0 a	28.4 a	<b>26.1 b</b>	<b>29.4 ab</b>	<b>32.1 a</b>	0.745	<b>0.017</b>	0.445
	Orchard	45.6 a	42.6 a	40.6 a	<b>40.9 b</b>	<b>42.2 b</b>	<b>45.7 a</b>	0.725	<b>0.006</b>	0.147
	Roggen	64.4 a	67.3 a	64.6 a	<b>60.9 b</b>	<b>68.3 a</b>	<b>67.1 a</b>	0.260	<b>0.002</b>	0.265
	Sheridan Lake	37.6 a	40.0 a	37.0 a	<b>29.7 c</b>	<b>39.3 b</b>	<b>45.7 a</b>	0.306	<b>&lt;0.001</b>	0.418
Yuma	72.2 a	69.7 a	61.9 a	64.5 a	68.6 a	70.6 a	0.250	0.547	0.291	
2020 Combined ANOVA		<b>53.2 a</b>	<b>51.4 ab</b>	49.1 b	<b>47.0 b</b>	<b>52.1 a</b>	<b>54.7 a</b>	<b>0.024</b>	<b>&lt;0.001</b>	<b>0.029</b>
2-yr Combined ANOVA		<b>66.8 a</b>	<b>66.8 a</b>	64.5 b	<b>61.6 c</b>	<b>67.6 a</b>	<b>66.0 b</b>	<b>0.009</b>	<b>&lt;0.001</b>	0.094

\*Means in a row followed by the same letter are not significantly different (p<0.05).

**Table 9.** Grain yield ANOVA using fixed effects combined over two years.

Effect	Significance
<b>Year</b>	<b>&lt;0.001</b>
<b>Environment</b>	<b>&lt;0.001</b>
<b>Variety</b>	<b>0.001</b>
<b>Environment x Variety</b>	<b>0.040</b>
N application rate	0.230
Environment x N application rate	0.971
Variety x N application rate	0.924
Environment x Variety x N application rate	0.998

Tukey-Kramer mean separation test for differences in grain yield for the two years ( $p \leq 0.05$ ).	
Year	Estimate (bu ac <sup>-1</sup> )
<b>2019</b>	<b>80.7 a</b>
<b>2020</b>	<b>49.6 b</b>

As shown in Table 9, year, environment, variety, N application rate, and environment x variety all affected grain yield. The Tukey-Kramer mean separation test showed that in the water limited year (2020), grain yields were 31 bu ac<sup>-1</sup> lower than the high-yielding year (2019).

Increasing N application rates affected grain protein at seven of the 18 environments over two years (Table 10). Overall, increasing N application rates significantly increased protein content in the 2019, 2020, and two years combined. The lowest N rate (45 lbs N ac<sup>-1</sup>) had the least grain protein content, while the highest N rate (135 lbs N ac<sup>-1</sup>) significantly increased grain protein content despite lower grain yield. Nitrogen is known to increase grain protein (Pan et al., 2020; Smika et al., 1974; Walsh & Walsh, 2020), but substantial increases in applied N produced only small and often statistically insignificant increases in grain protein within environments. However, the results of grain protein responding to N differing by environmental conditions such as different soils, climates, and years have been observed in other studies (Gauer et al., 1992;

Smika et al., 1974). Zörb et al. (2018) found that grain protein content differed variety, and that as compared to older varieties, modern varieties were more efficient at utilizing N to assimilate proteins. The authors also found that increased N applications changed protein composition and thus grain quality for baking, in addition to observing that grain protein content varied by management systems and differing available soil N concentrations.

Grain protein content was significantly affected by variety at seven of the 18 individual trial locations (Table 10). Overall variety differences were found for grain protein in the 2019, 2020, and two years combined. Canvas had significantly greater grain protein content as compared to Langin and Snowmass 2.0 in 2019. Canvas and Snowmass 2.0 had greater grain protein content as compared to Langin in 2020. Canvas had significantly greater grain protein content as compared to Langin and Snowmass 2.0 in the two years combined. N application rate x variety interaction was mostly non-significant. As shown in Table 11, year, environment, variety, N application rate, and environment x N application rate all affected grain protein. The Tukey-Kramer mean separation test showed that in the water limited year (2020), grain protein concentrations were 1.5% greater than in the high-yielding year (2019).

Year was a main effect for the differences in grain yield and grain protein response to N application across all environments (Tables 9 and 11). This was expected as the higher yielding year (2019) had more precipitation and cooler temperatures in the growing season, and thus moisture and temperatures were less limiting to grain yield in response to N application rate. Contrastingly, the water-limited year (2020) had lower moisture and higher temperatures, both of which can limit grain yield. In fact, in 2020, grain yield response to increasing N application rates was low and grain yield decreased at the highest N rate. Thus, excessive N application rates applied under these types of climatic conditions can actually limit winter wheat yields.

**Table 10.** Split plot analysis of variance for grain protein 2019 and 2020.

Year	Location	Average grain protein by N application rate			Average grain protein by variety			Significance		
		45 lbs N ac <sup>-1</sup>	90 lbs N ac <sup>-1</sup>	135 lbs N ac <sup>-1</sup>	Canvas	Langin	Snowmass 2.0	N application rate	Variety	N application rate x Variety
		(%)								
2019	Akron	12.9 a	12.9 a	13.0 a	<b>13.3 a</b>	<b>12.5 b</b>	<b>13.0 a</b>	0.740	<b>&lt;0.001</b>	0.332
	Arapahoe	<b>9.5 b</b>	<b>10.2 b</b>	<b>11.3 a</b>	10.6 a	10.2 a	10.2 a	<b>0.004</b>	0.242	0.231
	Burlington	11.9 a	11.9 a	12.1 a	12.0 a	12.0 a	11.9 a	0.206	0.565	0.897
	Genoa	<b>12.0 b</b>	<b>12.0 b</b>	<b>14.0 a</b>	12.9 a	12.8 a	13.1 a	<b>0.003</b>	0.495	0.430
	Lamar	11.1 a	11.6 a	12.3 a	<b>11.8 ab</b>	<b>11.9 a</b>	<b>11.3 b</b>	0.070	<b>0.020</b>	0.653
	Orchard	12.9 a	13.3 a	13.3 a	13.4 a	12.9 a	13.1 a	0.116	<b>0.010</b>	0.617
	Roggen	<b>10.1 b</b>	<b>10.6 b</b>	<b>12.0 a</b>	11.1 a	10.7 a	10.9 a	<b>0.010</b>	0.508	0.467
	Sheridan Lake	<b>10.3 b</b>	<b>11.0 ab</b>	<b>11.2 a</b>	<b>11.0 a</b>	<b>10.9 ab</b>	<b>10.6 b</b>	<b>0.050</b>	<b>0.040</b>	<b>0.050</b>
Yuma	<b>11.7 b</b>	<b>11.9 b</b>	<b>12.7 a</b>	<b>12.8 a</b>	<b>12.0 b</b>	<b>12.1 b</b>	<b>0.010</b>	<b>0.040</b>	0.210	
2019 Combined ANOVA		<b>11.4 c</b>	<b>11.8 b</b>	<b>12.4 a</b>	<b>12.0 a</b>	<b>11.7 b</b>	<b>11.8 b</b>	<b>&lt;0.001</b>	<b>0.001</b>	0.810
2020	Akron	13.9 a	14.3 a	14.4 a	<b>14.3 ab</b>	<b>13.9 b</b>	<b>14.5 a</b>	0.264	0.210	0.669
	Arapahoe	13.6 a	13.9 a	14.0 a	13.9 a	13.6 a	14.0 a	0.156	<b>0.050</b>	<b>0.050</b>
	Burlington	12.6 a	12.8 a	13.3 a	12.9 a	12.7 a	13.0 a	0.060	0.200	0.653
	Julesburg	<b>9.3 c</b>	<b>10.3 b</b>	<b>11.1 a</b>	<b>10.5 a</b>	<b>9.8 b</b>	<b>10.4 a</b>	<b>&lt;0.001</b>	<b>0.002</b>	0.499
	Lamar	12.9 a	12.6 a	12.6 a	12.9 a	12.5 a	12.6 a	0.478	0.167	0.689
	Orchard	14.5 a	14.5 a	15.4 a	14.8 a	14.7 a	15.0 a	0.215	0.590	0.464
	Roggen	13.2 a	13.4 a	13.9 a	13.7 a	13.3 a	13.6 a	0.080	0.315	0.627
	Sheridan Lake	12.0 a	12.3 a	12.6 a	<b>12.8 a</b>	<b>12.0 b</b>	<b>12.1 b</b>	0.306	<b>&lt;0.001</b>	0.486
Yuma	<b>10.9 b</b>	<b>11.7 b</b>	<b>12.7 a</b>	11.8 a	11.7 a	11.8 a	<b>0.003</b>	0.763	0.602	
2020 Combined ANOVA		<b>12.5 c</b>	<b>12.9 b</b>	<b>13.3 a</b>	<b>13.0 a</b>	<b>12.7 b</b>	<b>13.0 a</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.732
2-yr Combined ANOVA		<b>11.8 c</b>	<b>12.3 b</b>	<b>12.9 a</b>	<b>12.5 a</b>	<b>12.1 b</b>	<b>12.3 b</b>	<b>&lt;0.001</b>	<b>0.002</b>	0.992

\* Means in a row followed by the same letter are not significantly different (p<0.05).

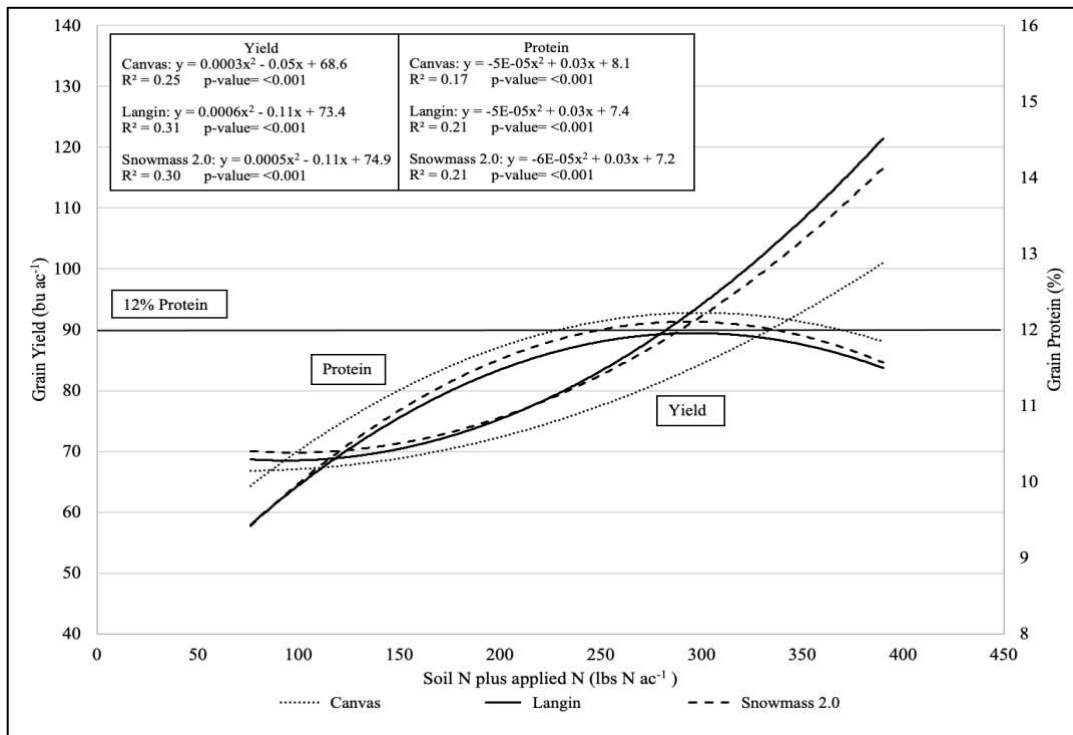
**Table 11.** Grain protein ANOVA using fixed effects combined over two years.

Effect	Significance
<b>Year</b>	<b>&lt;0.001</b>
<b>Environment</b>	<b>&lt;0.001</b>
<b>Variety</b>	<b>0.002</b>
Environment x Variety	0.893
<b>N application rate</b>	<b>&lt;0.001</b>
<b>Environment x N application rate</b>	<b>0.031</b>
Variety x N application rate	0.992
Environment x Variety x N application rate	0.999

Tukey-Kramer mean separation test for differences in grain protein for the two years ( $p \leq 0.05$ ).

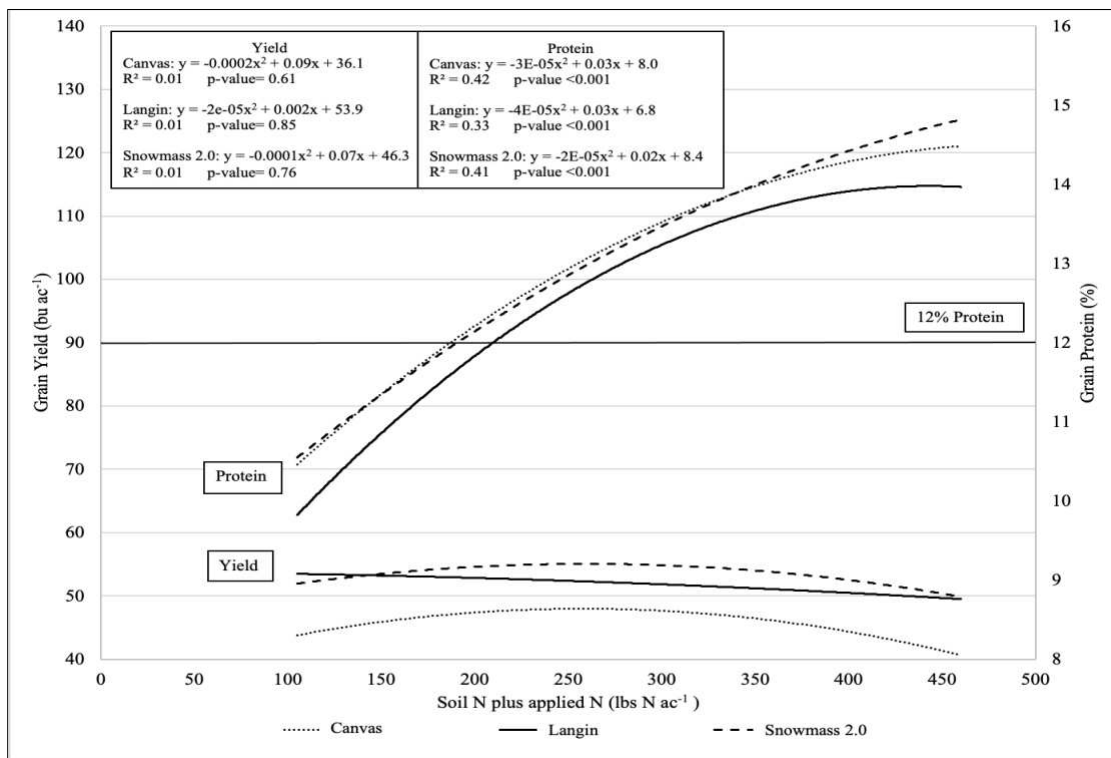
Year	Estimate (%)
<b>2019</b>	<b>11.6 b</b>
<b>2020</b>	<b>13.1 a</b>

**Grain yield and protein response curves in relation to soil N plus applied N**



**Figure 2.** 2019 grain yield and grain protein response to increasing N fertilizer application rates by year and variety across all environments.

In 2019, grain yield had a significant, positive, curvilinear response by variety across all environments to soil N plus applied N, according to regression analysis (Figure 2). The 2019 grain protein content followed a quadratic response with respect to increasing N fertilizer rates. Canvas resulted in the greatest protein content, while Langin the lowest, in response to increasing N fertilizer rates. Based on regression analysis, the maximum protein content attained was 12.2% at 298 lbs N ac<sup>-1</sup> for Canvas, 11.9% for Langin at 296 lbs N ac<sup>-1</sup>, and 12.1% at 250 lbs N ac<sup>-1</sup> for Snowmass 2.0. According to the Colorado State University fact sheet “Grain



**Figure 3.** 2020 grain yield and grain protein response to N by year and variety across all environments.

Protein Content and N Needs” for winter wheat, fields with a history of grain protein concentrations below 12% may respond to N applications (Goos et al., 2015). Canvas reached 12% grain protein in 2019 at the lowest N rate of varieties at 229 lbs N ac<sup>-1</sup>, and Snowmass 2.0 reached 12% grain protein at 250 lbs N ac<sup>-1</sup>. Langin did not achieve 12% grain protein in 2019. That year was a high yielding year due to the climatic conditions favoring high yields. When

yields are high, protein is diluted and this led to lower grain protein levels. All regressions were significant ( $p < 0.001$ ) for grain protein for each variety.

In 2020, grain yield had a low response to soil N plus applied N and the regressions were not significant (Figure 3). Conclusions cannot be drawn based on the 2020 grain yield data. The lack of grain yield response was likely due to the water-limited conditions in 2020.

In 2020, grain protein increased with increasing N but did not reach a maximum according to regression analyses (Figure 3). Canvas and Snowmass 2.0 had greater grain protein response than Langin. Based on regression analysis, Canvas reached 12% protein at 180 lbs N ac<sup>-1</sup>, Snowmass 2.0 at 185 lbs N ac<sup>-1</sup>, and Langin at 211 lbs N ac<sup>-1</sup>. This response indicates that different varieties have different optimum N requirements for protein. The regressions of protein content within each variety were significant, and quadratic curves fit the data better than linear regressions. Snowmass 2.0 maintained its protein content with high yields while a negative inverse relationship of yield to protein characterized the responses of Canvas, with low grain yields and high grain protein. Langin had the highest yields but low protein.

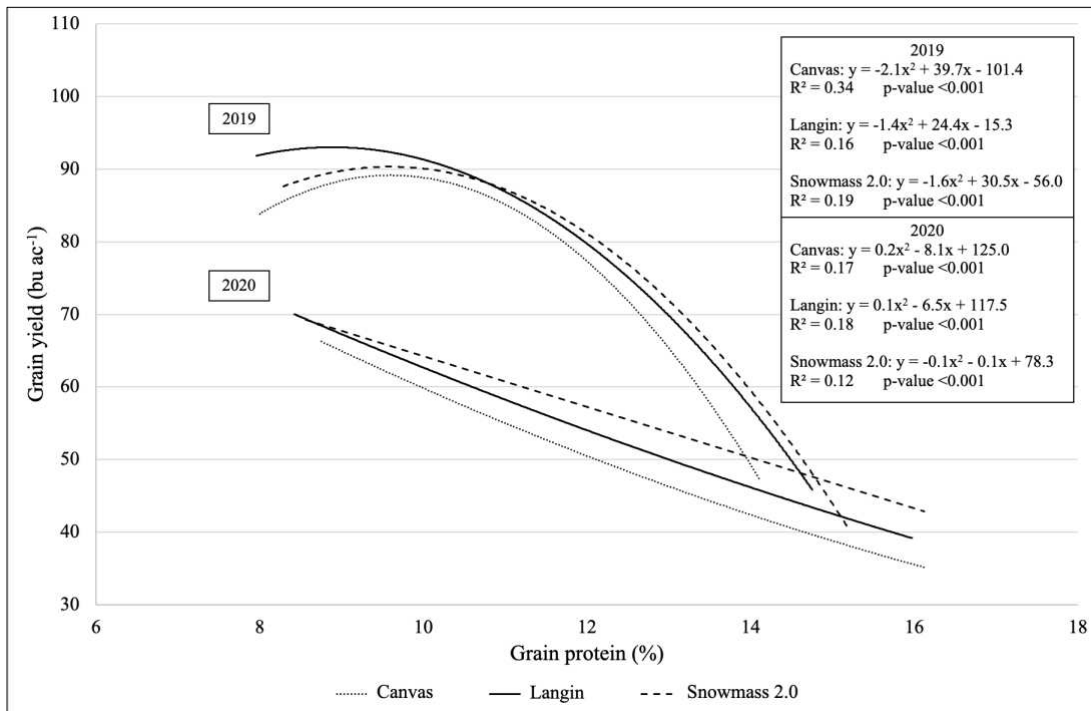
Years with relatively greater precipitation tend to negatively influence grain protein content, even given more than sufficient supplemental N. In our study, the higher yielding year (2019) had protein concentrations that were considered low as the maximum protein was around 12% for all varieties. This is compared to the water-limited year (2020) where protein was greater and increased with N up to ~ 14.5%. In the water limited year, 2020, protein responded positively to N. The positive response of protein to N in the water limited year has been shown by Walsh & Walsh (2020) and Pan et al. (2020), who both noted a protein concentrating effect where, as yields decrease, grain protein concentrates.

The relationship between grain protein and yield in both years could be supported by the findings of Justes et al. (1994), where greater yields produce more kernels per wheat head, which reduces the ratio of grain protein content to grain mass. Therefore, in years where conditions support higher yields due to above-average precipitation, it likely will be more challenging to achieve a desired grain protein content without supplemental N, this is especially true for some varieties.

The Colorado State University fact sheet on “Grain Protein Content and N Needs” for dryland winter wheat shows that if fields produce consistently lower than 12.0% grain protein, additional N may be needed (Goos et al., 2015). The fact sheet also states if grain protein contents are typically below 11.1%, additional N may be needed to not only increase grain protein content but also grain yield. However, the 2020 grain yield did not respond to increasing N application rates, likely due to lack of sufficient moisture throughout the growing season. The fact sheet should be updated to include potential climatic effects on grain yield and protein content.

### **Relationship between grain yield and protein**

The relationship between grain yield and grain protein by year and variety is shown in Figure 4. Results indicated that as yield increased, grain protein content decreased. However, the ‘by-year’ relationships have different slopes. In 2019 and between 8-10% protein content, both yield and protein content increased. When wheat yields were at or below 85 bu ac<sup>-1</sup>, the relationship between yield and protein content followed that as observed above; decreasing grain yields enhanced protein content. Similar to at or below 85 bu ac<sup>-1</sup> in 2019, decreasing grain yields resulted in increasing grain protein content in 2020. Each of the yearly set of quadratic curves were significant ( $p < 0.001$ ) with quadratic equations fitting the data better than linear equations.



**Figure 4.** Relationship between grain yield and protein by year and variety.

#### Effect of N application rate and variety on test weight

N application rate affected test weight (lbs bu<sup>-1</sup>) at five of the 18 environments over two years (Table 12). The 2019, 2020, and two-year combined ANOVA were significant ( $p \leq 0.001$ ) for N application rate. The greatest N application rate reduced test weight in both years separately as well as when data for both years were combined.

Wheat variety affected test weight at 12 of the 18 environments over two years (Table 12). In the overall 2019 and 2020 data, differences were significant ( $p \leq 0.004$ ) for variety. In the 2019 combined data, Langin had the lowest test weight as compared to Canvas and Snowmass 2.0, both of which had similar test weights. In the 2020 combined data, Canvas again had the lowest test weight, while Langin had the highest test weight; Snowmass 2.0 was not significantly different from Canvas or Langin. When the 2019 and 2020 data were combined, however, wheat variety did not affect test weight.

**Table 12.** Split plot analysis of variance for test weight 2019 and 2020.

Year	Location	Average grain test weight by N application rate			Average grain test weight by variety			Significance		
		45 lbs N ac <sup>-1</sup>	90 lbs N ac <sup>-1</sup>	135 lbs N ac <sup>-1</sup>	Canvas	Langin	Snowmass 2.0	N application rate	Variety	N application rate x Variety
		(lbs bu <sup>-1</sup> )								
2019	Akron	59.2 a	59.3 a	59.1 a	<b>58.8 b</b>	<b>59.9 a</b>	<b>58.8 b</b>	0.848	<b>&lt;0.001</b>	0.878
	Arapahoe	61.9 a	62.1 a	61.5 a	<b>62.1 a</b>	<b>61.1 b</b>	<b>62.4 a</b>	0.169	<b>&lt;0.001</b>	0.479
	Burlington	63.7 a	63.8 a	63.5 a	<b>63.9 a</b>	<b>63.1 b</b>	<b>64.0 a</b>	0.634	<b>0.014</b>	0.161
	Genoa	<b>62.0 a</b>	<b>61.3 a</b>	<b>60.4 b</b>	61.0 a	61.1 a	61.6 a	<b>0.006</b>	0.059	0.591
	Lamar	62.4 a	62.3 a	60.5 a	63.5 a	61.2 a	60.5 a	0.508	0.219	0.541
	Orchard	60.4 a	60.5 a	60.0 a	60.2 a	60.5 a	60.1 a	<b>0.076</b>	0.109	0.470
	Roggen	63.4 a	63.2 a	61.8 a	62.7 a	62.7 a	63.0 a	0.120	0.663	0.734
	Sheridan Lake	59.6 a	59.8 a	59.7 a	<b>60.6 a</b>	<b>58.2 b</b>	<b>60.3 a</b>	0.763	<b>&lt;0.001</b>	0.207
	Yuma	<b>61.1 a</b>	<b>60.8 a</b>	<b>59.8 b</b>	<b>60.8 a</b>	<b>59.7 b</b>	<b>61.1 a</b>	<b>0.017</b>	<b>&lt;0.001</b>	0.356
2019 Combined ANOVA		<b>61.5 a</b>	<b>61.4 a</b>	<b>60.7 b</b>	<b>61.5 a</b>	<b>60.8 b</b>	<b>61.3 a</b>	<b>0.001</b>	<b>0.004</b>	0.370
2020	Akron	52.8 a	51.3 a	51.9 a	<b>50.8 c</b>	<b>53.1 a</b>	<b>52.0 b</b>	0.375	<b>&lt;0.001</b>	0.128
	Arapahoe	60.7 a	60.7 a	60.5 a	<b>61.2 a</b>	<b>60.4 b</b>	<b>60.4 b</b>	0.444	<b>&lt;0.001</b>	0.288
	Burlington	<b>56.7 a</b>	<b>55.9 b</b>	<b>55.6 b</b>	<b>55.7 b</b>	<b>56.2 a</b>	<b>56.2 a</b>	<b>0.004</b>	<b>0.009</b>	0.249
	Julesburg	<b>58.3 a</b>	<b>57.3 a</b>	<b>56.1 b</b>	57.1 a	57.4 a	57.1 a	<b>0.005</b>	0.287	0.213
	Lamar	43.8 a	55.9 a	56.7 a	<b>50.5 a</b>	<b>56.7 a</b>	<b>49.3 b</b>	0.265	<b>0.001</b>	0.300
	Orchard	53.5 a	53.0 a	52.9 a	<b>52.6 b</b>	<b>53.3 a</b>	<b>53.5 a</b>	0.398	<b>0.005</b>	0.309
	Roggen	58.1 a	58.2 a	57.8 a	<b>58.4 a</b>	<b>57.4 b</b>	<b>58.3 a</b>	0.642	<b>&lt;0.001</b>	0.525
	Sheridan Lake	56.2 a	56.6 a	55.7 a	<b>55.4 b</b>	<b>56.3 a</b>	<b>56.8 a</b>	0.284	<b>0.006</b>	0.847
Yuma	<b>54.1 a</b>	<b>52.5 ab</b>	<b>50.7 b</b>	51.7 a	53.4 a	52.2 a	<b>0.036</b>	0.124	0.802	
2020 Combined ANOVA		<b>56.32 a</b>	<b>55.7 b</b>	<b>55.3 b</b>	<b>54.8 b</b>	<b>56.0 a</b>	<b>55.1 ab</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.649
2-yr Combined ANOVA		<b>58.2 a</b>	<b>58.6 a</b>	<b>58.0 b</b>	58.2 a	58.4 a	58.2 a	<b>&lt;0.001</b>	0.819	0.886

\*Means in a row followed by the same letter are not significantly different (p<0.05).

**Table 13.** Test Weight ANOVA using fixed effects combined over two years.

Effect	Significance
<b>Year</b>	<b>&lt;0.001</b>
<b>Environment</b>	<b>&lt;0.001</b>
Variety	0.819
<b>Environment x Variety</b>	<b>0.012</b>
<b>N application rate</b>	<b>&lt;0.001</b>
Environment x N application rate	0.620
Variety x N application rate	0.886
Environment x Variety x N application rate	1.000

Tukey-Kramer mean separation test for differences in test weight for the two years ( $p \leq 0.05$ ).

Year	Estimate (lbs bu <sup>-1</sup> )
<b>2019</b>	<b>61.4 a</b>
<b>2020</b>	<b>55.9 b</b>

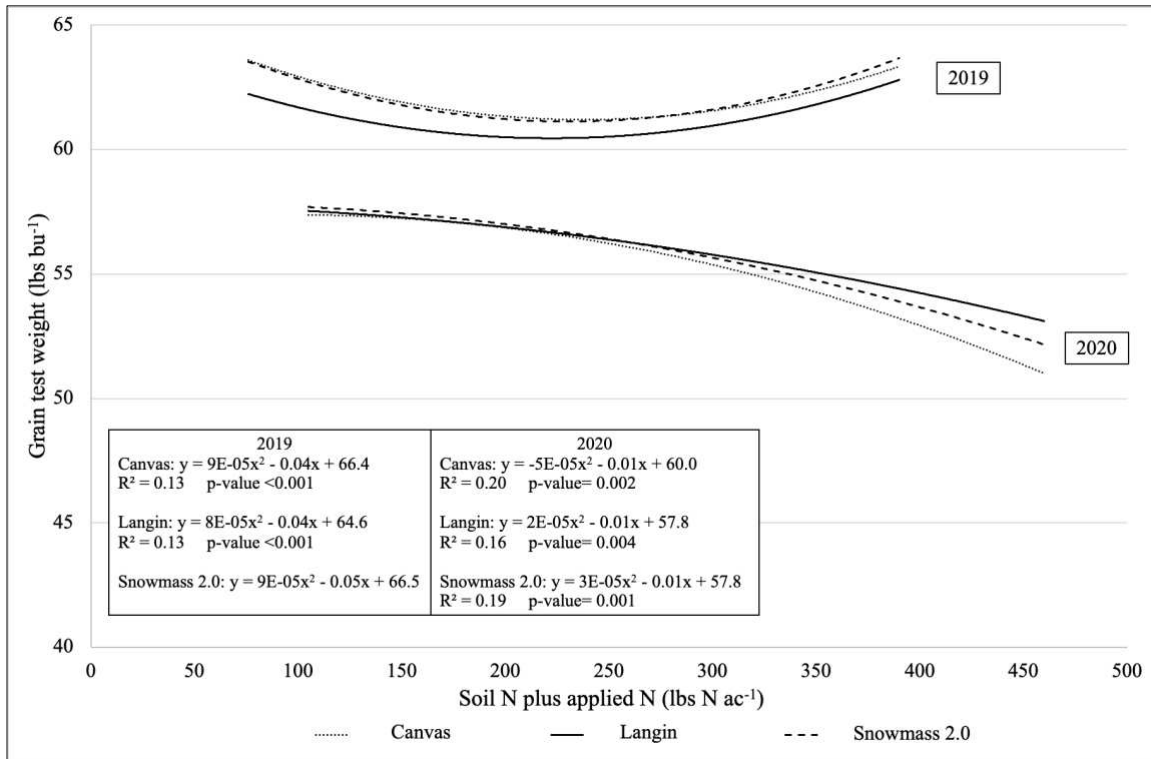
Wheat grain test weight was significantly affected by year, environment, environment x variety, and N application rate (Table 13). The Tukey-Kramer means separation test for year showed that 2019 (the higher yielding year) had a significantly greater test weight (61.4 lbs bu<sup>-1</sup>) compared to the 2020 (a water limited year; 55.8 lbs bu<sup>-1</sup>). Low test weights typically occur when the wheat plant has experienced stress during the grain filling period or if a frost occurs before physiological maturity. In 2020, the crop was under drought and heat stress, likely impacting starch accumulation and thus decreasing test weight. In 2019, the wheat crop had little environmental stress and thus the grain filled to a greater extent as compared to 2020.

#### **Test Weight Response to soil N plus applied N**

The response of test weight to increasing N application rates is shown in Figure 5. In 2019, test weights were greater at the lowest and greatest N application rates. However, test weights curvilinearly decreased in response to soil N plus applied N, and were lowest for: a)

Canvas, at 242 lbs N ac<sup>-1</sup> and a corresponding test weight of 61.1 lbs bu<sup>-1</sup>; b) Langin, at 231 lbs N ac<sup>-1</sup> and a corresponding test weight of 60.3 lbs bu<sup>-1</sup>; and c) Snowmass 2.0 at 231 lbs N ac<sup>-1</sup> and a corresponding test weight of 61.1 lbs bu<sup>-1</sup>. In 2020, increasing N decreased test weight. The response by variety was equal until after 250 lbs N ac<sup>-1</sup>. After 250 lbs N ac<sup>-1</sup>, Langin tended to have a greater test weight while Canvas has the lowest test weight. All curves were significant, and a quadratic response was the best fit.

In years with high drought and heat stress, as well as excessive N, the above pattern has been noted by others (Isleib, 2012). Although 2019 was not a high stress year, excessive soil N application rates could explain the reduction in test weight at the highest N application rate, similar to that noted by Isleib (2012). In 2020, test weight was the highest in relation to grain yield at the highest grain yields. In 2019, test weight increased with grain yield.



**Figure 5.** Wheat grain test weight response to N by year and variety across all environments.

### **Effect of N application rate and variety on thousand kernel weight**

N application rate was not significant for thousand kernel weight at 17 of the 18 environments. Yet in the 2019, 2020, and in both years combined, thousand kernel weight differences were evident with respect to increasing N fertilizer application rate (Table 14). The 2019 combined data showed that 135 lbs N ac<sup>-1</sup> reduced thousand kernel weights as compared to lesser N application rates. Similar trends were observed in the 2020 and both years combined data.

Variety differences were significant for thousand kernel weights in 17 of the 18 environments, and in the 2019, 2020, and two-year combined data the varieties followed the order of Canvas < Langin < Snowmass 2.0 (Table 14). The Tukey-Kramer mean separation test showed that the thousand kernel weight was greater in 2019 than 2020. It has been found in years with high heat stress, thousand kernel grain weight is reduced (Mohammadi, 2012; Telfer et al, 2018). Thus, the lower thousand kernel weight estimate in 2020 could be explained by the limited moisture conditions.

Thousand kernel weight was significantly affected by year, environment, variety, environment x variety, and N application rate (Table 15). Guarda et al. (2004) found that thousand kernel weight decreased with increasing N application rates, which is the same trend found in the current study. Guarda et al. (2004) also found that thousand kernel weight varied by variety, which was also reflected in the current results. Varieties that maintain higher kernel weight during drought stressed years tend to be more resilient to heat (Mohammadi, 2012).

### **Thousand kernel weight response to soil N plus applied N**

Thousand kernel weights did not significantly increase in response to increased soil N plus applied N. There were no significant differences among varieties for increasing N. Data is not shown due to lack of significance.

**Table 14.** Split plot analysis of variance for thousand kernel weight 2019 and 2020.

Year	Location	Average thousand kernel weight by N application rate			Average thousand kernel weight by variety			Significance		
		45 lbs N ac <sup>-1</sup>	90 lbs N ac <sup>-1</sup>	135 lbs N ac <sup>-1</sup>	Canvas	Langin	Snowmass 2.0	N application rate	Variety	N application rate x Variety
		(Avg. g /1000 seeds)								
2019	Akron	26.9 a	26.0 a	26.3 a	<b>24.5 c</b>	<b>26.5 b</b>	<b>28.2 a</b>	0.167	<b>&lt;0.001</b>	0.149
	Arapahoe	30.9 a	31.1 a	29.1 a	<b>28.1 c</b>	<b>30.4 b</b>	<b>32.7 a</b>	0.129	<b>&lt;0.001</b>	0.928
	Burlington	29.2 a	29.2 a	29.2 a	<b>27.4 b</b>	<b>28.6 b</b>	<b>31.5 a</b>	0.990	<b>&lt;0.001</b>	0.220
	Genoa	27.5 a	26.6 a	26.0 a	<b>24.7 c</b>	<b>26.1 b</b>	<b>29.2 a</b>	0.073	<b>&lt;0.001</b>	<b>0.010</b>
	Lamar	33.5 a	33.3 a	33.0 a	<b>30.8 b</b>	<b>34.8 a</b>	<b>34.3 a</b>	0.790	<b>&lt;0.001</b>	0.581
	Orchard	28.3 a	28.1 a	28.0 a	<b>27.0 b</b>	<b>27.8 b</b>	<b>29.5 a</b>	0.861	<b>&lt;0.001</b>	0.165
	Roggen	28.5 a	27.4 a	25.8 a	<b>25.3 b</b>	<b>27.4 a</b>	<b>29.0 a</b>	0.086	<b>&lt;0.001</b>	0.588
	Sheridan Lake	31.5 a	31.5 a	31.3 a	<b>29.5 c</b>	<b>31.1 b</b>	<b>33.8 a</b>	0.754	<b>&lt;0.001</b>	0.868
	Yuma	26.7 a	26.3 a	25.3 a	<b>24.6 b</b>	<b>24.9 b</b>	<b>28.8 a</b>	0.123	<b>&lt;0.001</b>	0.908
2019 Combined ANOVA		<b>29.2 a</b>	<b>28.8 a</b>	<b>28.2 b</b>	<b>26.9 c</b>	<b>28.6 b</b>	<b>30.8 a</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.061
2020	Akron	22.9 a	22.0 a	22.6 a	<b>21.3 b</b>	<b>22.2 b</b>	<b>23.9 a</b>	0.326	<b>&lt;0.001</b>	0.458
	Arapahoe	30.5 a	31.1 a	30.7 a	<b>29.0 b</b>	<b>30.9 a</b>	<b>32.3 a</b>	0.681	<b>&lt;0.001</b>	0.897
	Burlington	26.5 a	25.9 a	25.9 a	<b>24.0 c</b>	<b>26.1 b</b>	<b>28.1 a</b>	0.606	<b>&lt;0.001</b>	0.719
	Julesburg	<b>26.7 a</b>	<b>25.0 ab</b>	<b>24.4 b</b>	<b>24.7 b</b>	<b>25.3 ab</b>	<b>26.1 a</b>	<b>0.030</b>	<b>0.010</b>	0.948
	Lamar	25.5 a	25.7 a	24.8 a	<b>24.2 b</b>	<b>26.0 a</b>	<b>25.6 a</b>	0.438	<b>0.009</b>	0.861
	Orchard	22.7 a	22.2 a	23.0 a	<b>21.6 b</b>	<b>22.9 a</b>	<b>23.4 a</b>	0.230	<b>&lt;0.001</b>	0.080
	Roggen	27.0 a	26.8 a	26.4 a	<b>24.9 b</b>	<b>26.9 a</b>	<b>28.5 a</b>	0.673	<b>&lt;0.001</b>	0.936
	Sheridan Lake	25.7 a	26.1 a	24.8 a	<b>23.8 b</b>	<b>26.1 a</b>	<b>26.7 a</b>	0.148	<b>&lt;0.001</b>	0.224
	Yuma	23.4 a	24.6 a	23.5 a	23.4 a	23.7 a	24.4 a	0.501	0.610	0.328
2020 Combined ANOVA		<b>25.7 a</b>	<b>25.5 ab</b>	<b>25.1 b</b>	<b>24.1 c</b>	<b>25.6 b</b>	<b>26.6 a</b>	<b>0.045</b>	<b>&lt;0.001</b>	0.986
2-yr Combined ANOVA		<b>27.4 a</b>	<b>27.0 ab</b>	<b>26.5 b</b>	<b>25.4 c</b>	<b>27.0 b</b>	<b>28.6 a</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.570

\*Means in a row followed by the same letter are not significantly different (p<0.05).

**Table 15.** Thousand kernel weight ANOVA using fixed effects combined over two years.

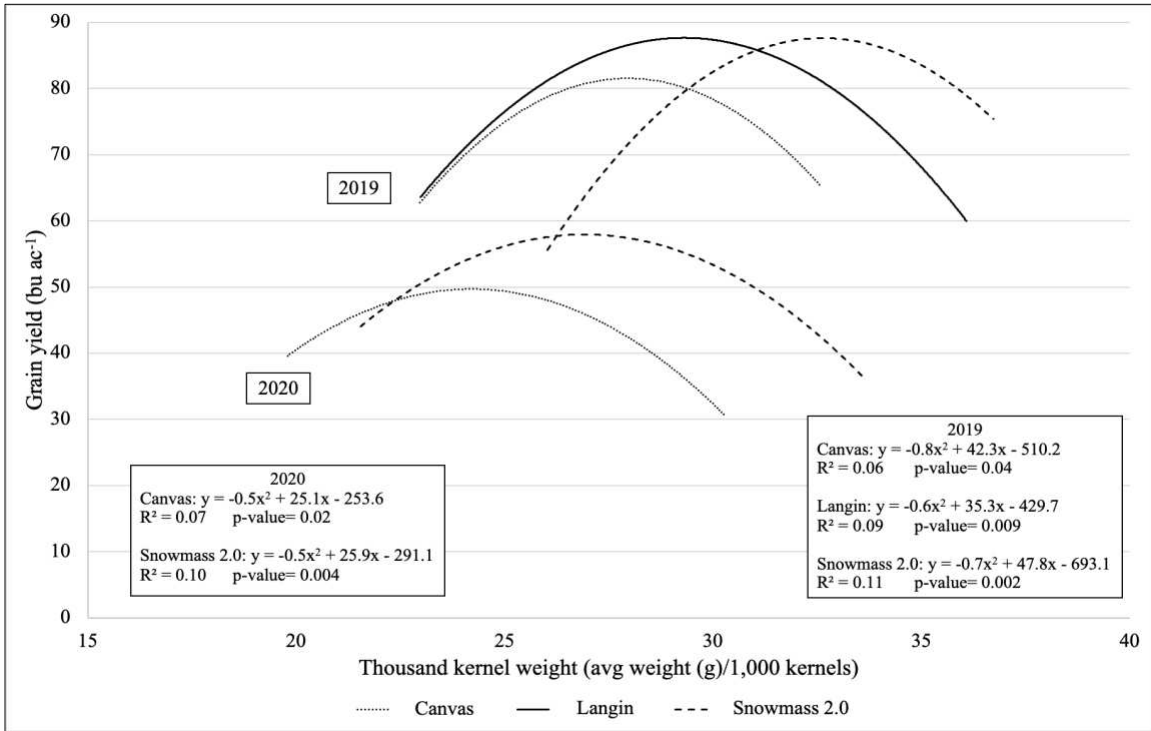
Effect	Significance
<b>Year</b>	<b>&lt;0.001</b>
<b>Environment</b>	<b>&lt;0.001</b>
<b>Variety</b>	<b>&lt;0.001</b>
<b>Environment x Variety</b>	<b>0.046</b>
<b>N application rate</b>	<b>0.001</b>
Environment x N application rate	0.622
Variety x N application rate	0.752
Environment x Variety x N application rate	1.000

Tukey-Kramer mean separation test for differences in thousand kernel weight for the two years ( $p \leq 0.05$ ).

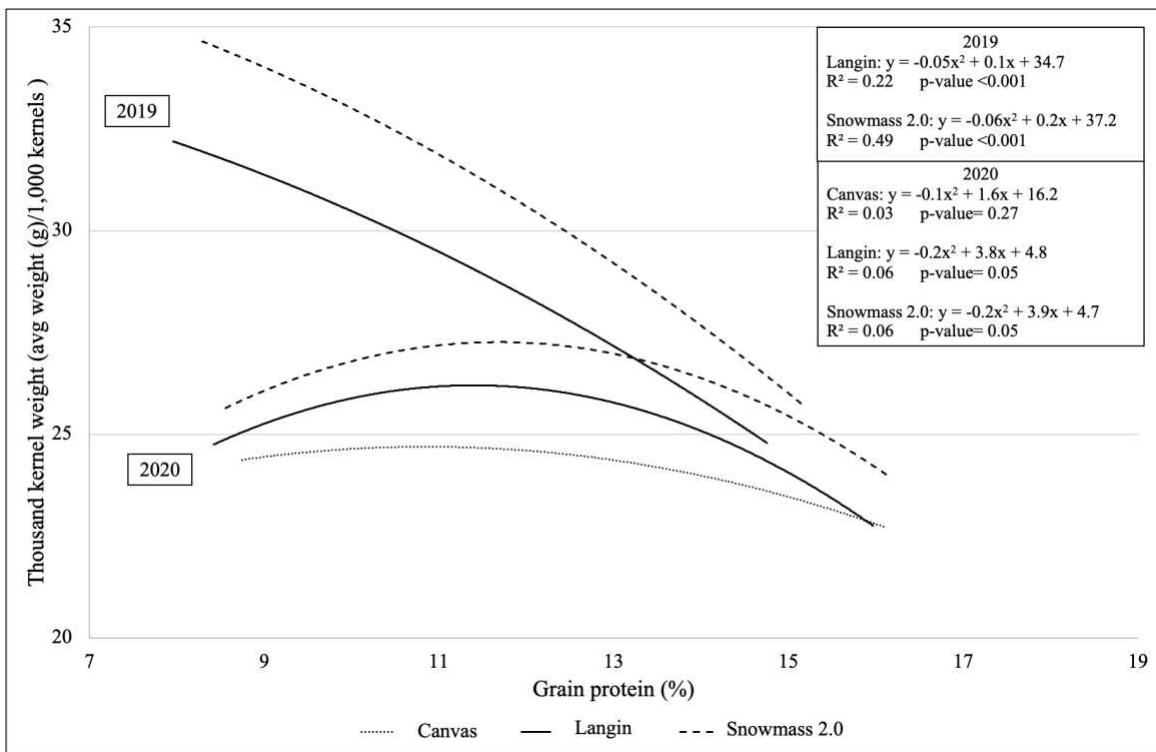
Year	Estimate (Avg. g /1000 seeds)
<b>2019</b>	<b>28.8 a</b>
<b>2020</b>	<b>25.2 b</b>

### **Relationship between thousand kernel weight and grain yield**

The relationship between thousand kernel weight and grain yield by year and variety across all locations is presented in Figure 6. It has been found by Fjell et al. (1985) that higher yields are typically correlated with higher kernel weights and that stable harvested kernel weight is an important component of yield. In 2019, yields were greater and had a higher thousand kernel weight as compared to 2020. In 2019, the maximum points for yield and thousand kernel weight were: a) Canvas, at 82 bu ac<sup>-1</sup> and 28 g thousand kernel weight; b) Langin, at 88 bu ac<sup>-1</sup> and 29 g thousand kernel weight; and c) Snowmass 2.0, at 88 bu ac<sup>-1</sup> and a 32 g thousand kernel weight. In 2020, the maximum points for yield and thousand kernel weight were: a) Canvas, at 50 bu ac<sup>-1</sup> and a 24 g thousand kernel weight; b) Langin, with non-significant results so the relationship could not be determined; and c) Snowmass, at 58 bu ac<sup>-1</sup> and a 27 g thousand kernel weight.



**Figure 6.** The relationship between thousand kernel weight and grain yield by year and variety across all environments. No significant relationship for Langin in 2020 thus it is not shown.



**Figure 7.** The relationship between thousand kernel weight and grain protein by year and variety across all environments. No significant relationship existed for Canvas in 2019, and thus it is not shown.

### **Relationship between thousand kernel weight and grain protein**

In 2019, single kernel weight decreased with increasing protein, but only Langin and Snowmass 2.0 were significantly affected (Figure 7). In 2020, the thousand kernel weight content increased with increasing protein for all curves until a maximum was achieved at 10.9% protein and 24.7 g thousand kernel weight for Canvas, 11.4% protein at 26.2 g thousand kernel weight for Langin, and 11.7% protein at 27.3 g thousand kernel weight for Snowmass 2.0.

### **Effect of N application rate and variety on NDVI**

Increasing N application rates affected the NDVI index at two of the 18 environments over two years (Table 16). The overall, combined 2019 and 2020 NDVI data both showed that  $135 \text{ lbs N ac}^{-1} \geq 90 \text{ lbs N ac}^{-1} \geq 45 \text{ lbs N ac}^{-1}$ . Variety affected the NDVI index in almost all 18 environments over the two-year study, with Langin tending to have the greatest NDVI in 2019 and 2020. Langin also had the greatest NDVI index in the overall, combined 2019 and 2020 data; a similar trend existed when all data from both years were combined. The N application rate x variety interaction NDVI term was not significant at any environment or the 2019, 2020, or two-year combined ANOVA. Other studies have shown conflicting results regarding varieties and the effect on NDVI. For example, Dorsey et al. (2014) found that NDVI by winter wheat variety to be non-significant. However, Sultana et al. (2014) found that the potential to differentiate NDVI between varieties at different growth stages was significant for wheat yield estimation.

As shown in Table 17, year, environment, and variety affected NDVI. The Tukey-Kramer mean separation test showed that in the water-limited year (2020), NDVI was 0.17 lower than under the more ideal climatic conditions of 2019. Results from this study indicate that NDVI used in-season could be a better predictor for in-season N fertilizer application needs during

**Table 16.** Split plot analysis of variance for NDVI in 2019 and 2020.

Year	Location	Average NDVI by N application rate			Average NDVI by variety			Significance		
		45 lbs N ac <sup>-1</sup>	90 lbs N ac <sup>-1</sup>	135 lbs N ac <sup>-1</sup>	Canvas	Langin	Snowmass 2.0	N application rate	Variety	N application rate x Variety
		NDVI Index (0-1)								
2019	Akron	0.71 a	0.73 a	0.74 a	<b>0.68 c</b>	<b>0.78 a</b>	<b>0.72 b</b>	0.201	<b>&lt;0.001</b>	0.556
	Arapahoe	0.85 a	0.85 a	0.97 a	0.85 a	0.97 a	0.84 a	0.397	0.333	0.463
	Burlington	0.85 a	0.86 a	0.86 a	<b>0.85 b</b>	<b>0.87 a</b>	<b>0.85 b</b>	0.444	<b>0.010</b>	0.445
	Genoa	0.76 a	0.76 a	0.76 a	<b>0.76 ab</b>	<b>0.78 a</b>	<b>0.74 b</b>	0.862	<b>0.034</b>	0.144
	Lamar	<b>0.74 b</b>	<b>0.79 a</b>	<b>0.79 a</b>	<b>0.76 b</b>	<b>0.80 a</b>	<b>0.76 b</b>	<b>0.006</b>	<b>&lt;0.001</b>	0.143
	Orchard	0.51 a	0.54 a	0.56 a	<b>0.49 b</b>	<b>0.59 a</b>	<b>0.53 ab</b>	0.373	<b>0.021</b>	0.455
	Roggen	<b>0.70 b</b>	<b>0.75 a</b>	<b>0.74 ab</b>	<b>0.73 ab</b>	<b>0.74 a</b>	<b>0.71 b</b>	<b>0.034</b>	<b>0.024</b>	0.751
	Sheridan Lake	0.81 a	0.83 a	0.81 a	0.81 a	0.82 a	0.82 a	0.249	0.304	0.169
	Yuma	0.86 a	0.86 a	0.86 a	<b>0.85 b</b>	<b>0.87 a</b>	<b>0.85 b</b>	0.766	<b>&lt;0.001</b>	0.342
2019 Combined ANOVA		0.75 b	<b>0.77 ab</b>	<b>0.79 a</b>	<b>0.75 b</b>	<b>0.80 a</b>	<b>0.76 b</b>	<b>0.013</b>	<b>&lt;0.001</b>	0.729
2020	Akron	0.61 a	0.63 a	0.64 a	<b>0.59 b</b>	<b>0.64 a</b>	<b>0.65 a</b>	0.509	<b>0.013</b>	0.638
	Arapahoe	0.40 a	0.40 a	0.37 a	<b>0.37 b</b>	<b>0.44 a</b>	<b>0.35 b</b>	0.104	<b>&lt;0.001</b>	0.760
	Burlington	0.70 a	0.72 a	0.73 a	<b>0.71 b</b>	<b>0.75 a</b>	<b>0.70 b</b>	0.101	<b>&lt;0.001</b>	0.465
	Julesburg	0.50 a	0.50 a	0.50 a	<b>0.46 b</b>	<b>0.49 b</b>	<b>0.55 a</b>	0.942	<b>0.004</b>	0.904
	Lamar	0.36 a	0.36 a	0.36 a	<b>0.34 b</b>	<b>0.40 a</b>	<b>0.34 b</b>	0.963	<b>0.001</b>	0.825
	Orchard	0.77 a	0.78 a	0.80 a	<b>0.75 b</b>	<b>0.79 a</b>	<b>0.81 a</b>	0.350	<b>&lt;0.001</b>	0.382
	Roggen	0.67 a	0.67 a	0.68 a	<b>0.65 b</b>	<b>0.66 ab</b>	<b>0.70 a</b>	0.667	<b>0.046</b>	0.917
	Sheridan Lake	0.46 a	0.47 a	0.48 a	<b>0.44 b</b>	<b>0.53 a</b>	<b>0.45 b</b>	0.680	<b>&lt;0.001</b>	0.298
	Yuma	0.75 a	0.77 a	0.78 a	0.76 a	0.77a	0.76 a	0.075	0.839	0.396
2020 Combined ANOVA		<b>0.59 b</b>	<b>0.60 ab</b>	<b>0.60 a</b>	<b>0.58 c</b>	<b>0.62 a</b>	<b>0.60 b</b>	<b>0.013</b>	<b>&lt;0.001</b>	0.729
2-yr Combined ANOVA		0.66 a	0.68 a	0.68 a	<b>0.65 b</b>	<b>0.67 ab</b>	<b>0.70 a</b>	0.378	<b>0.014</b>	0.992

\*Means in a row followed by the same letter are not significantly different (p<0.05)

**Table 17.** NDVI ANOVA using fixed effects combined over two years.

Effect	Significance
<b>Year</b>	<b>&lt;0.001</b>
<b>Environment</b>	<b>&lt;0.001</b>
<b>Variety</b>	<b>0.014</b>
Environment x Variety	0.953
N application rate	0.378
Environment x N application rate	1.000
Variety x N application rate	0.992
Environment x Variety x N application rate	0.999

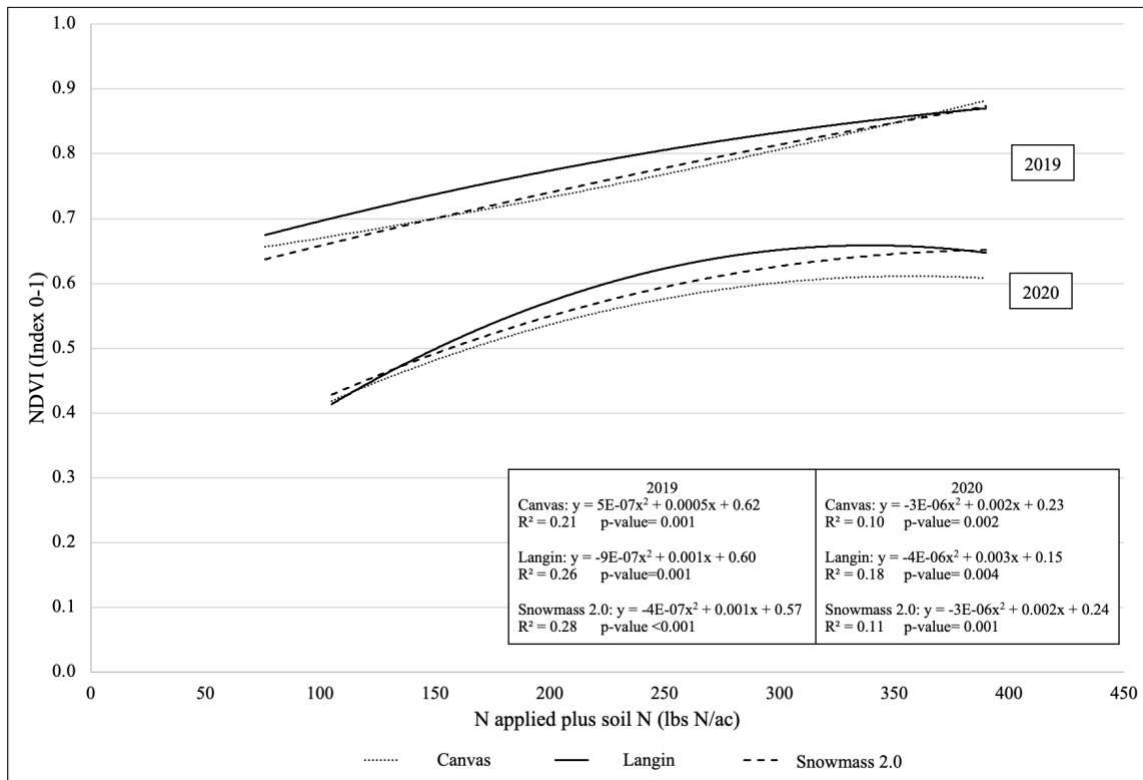
Tukey-Kramer mean separation test for differences in NDVI for the two years ( $\alpha \leq 0.05$ ).

Year	Estimate (NDVI Index 0-1)
<b>2019</b>	<b>0.76 a</b>
<b>2020</b>	<b>0.59 b</b>

normal years. However, in a water limited year, the NDVI response predicted higher yield potential than what was achieved.

#### **NDVI response to soil N plus applied N**

All regressions in 2019 and 2020 were significant for variety NDVI in response to increasing N fertilizer application rates (Figure 8), with Langin always having the greatest NDVI response. A maximum NDVI response to increasing N fertilizer rates was not attained in 2019. However, in 2020, the NDVI response to N increased until a maximum point was reached. Canvas has the lowest NDVI index and reached a maximum at 367 lbs N ac<sup>-1</sup> and a corresponding NDVI index of 0.63. Langin has the greatest NDVI index and reached a maximum at 375 lbs N ac<sup>-1</sup> and a corresponding NDVI index of 0.71. Snowmass 2.0 fell in between the other two varieties and reached a maximum at 350 lbs N ac<sup>-1</sup> and a corresponding NDVI index of 0.61.

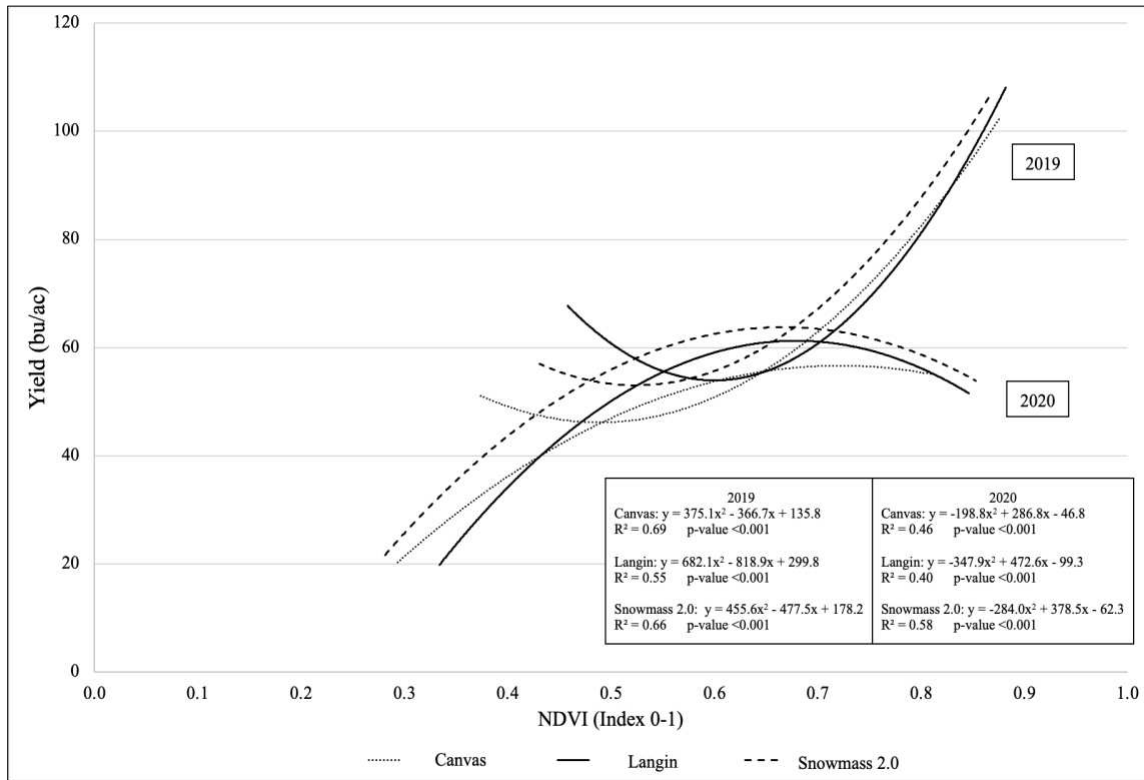


**Figure 8.** The NDVI response to N by year and variety across all environments.

### Relationship between NDVI and grain yield

Generally, increased soil N plus applied N increased NDVI in both years but the difference between years were quite different (Figure 8). NDVI and yield show distinctly different relationship in the two years (Figure 9). After the minimum NDVI point is reached in 2019, the yields increase. Snowmass 2.0 has a lower NDVI in response to yield. Langin and Canvas have a similar NDVI in relationship to grain yield. Each of the quadratic curves were significant ( $p < 0.05$ ). In 2020, there was a bell-shaped response of yield to increasing NDVI. The maximum points were: a) 0.72 NDVI at 56.7 bu ac<sup>-1</sup> grain yield for Canvas; b) 0.68 at 61.2 bu ac<sup>-1</sup> for Langin; and c) 0.67 NDVI at 63.8 bu ac<sup>-1</sup> for Snowmass 2.0. After these maximum point wer reached, the NDVI in relation to grain yield decreased. Grain yield increased with increasing NDVI, supporting the use of NDVI as a predictor of wheat yield response to N in normal to high moisture years. Other studies have confirmed this finding. Girma et al. (2006) suggests that in

season prediction of yield could be utilized using NDVI, plant height, chlorophyll content, and estimating N uptake to make better yield potential predictions for making fertilizer recommendations.



**Figure 9.** The relationship between grain yield and NDVI by year and variety across all environments.

### Effect of N application rate and variety on post-harvest residual nitrate-N

Increasing N application rates affected the post-harvest residual soil  $\text{NO}_3\text{-N}$  at only one of the 18 environments over two years (Table 18). However, the overall 2019, 2020, and the two-year combined data showed that residual soil  $\text{NO}_3\text{-N}$  was always lowest for the 45 lbs  $\text{N ac}^{-1}$  application rate as compared to greater N fertilizer application rates. It was not surprising that the lowest N application rate had the least residual soil  $\text{NO}_3\text{-N}$ , yet what was surprising was that residual soil  $\text{NO}_3\text{-N}$  was the same in both the 90 and 135 lbs  $\text{N ac}^{-1}$  application rates.

The average post-harvest residual soil  $\text{NO}_3\text{-N}$ , by variety, was significant at four of the 18 environments over two years. Variety was not found to have a meaningful effect for post-harvest

**Table 18.** Split plot analysis of variance for post-harvest residual soil NO<sub>3</sub>-N 2019 and 2020.

Year	Location	Average post-harvest residual soil NO <sub>3</sub> -N by N application rate			Average post-harvest residual soil NO <sub>3</sub> -N by variety			Significance		
		45 lbs N ac <sup>-1</sup>	90 lbs N ac <sup>-1</sup>	135 lbs N ac <sup>-1</sup>	Canvas	Langin	Snowmass 2.0	N application rate	Variety	N application rate x Variety
		lbs NO <sub>3</sub> -N ac <sup>-1</sup>								
2019	Akron	100.62 a	145.89 a	129.51 a	126.4 a	120.4 a	129.39 a	0.065	0.685	0.088
	Arapahoe	5.2 a	40.1 a	14.3 a	23.3 a	12.5 a	23.8 a	0.256	0.752	0.446
	Burlington	49.0 a	44.2 a	53.9 a	<b>68.7 a</b>	<b>40.5 b</b>	<b>37.9 b</b>	0.660	<b>0.018</b>	0.302
	Genoa	24.0 a	47.7 a	61.7 a	42.0 a	42.8 a	48.6 a	0.139	0.864	0.708
	Lamar	22.4 a	20.5 a	19.6 a	18.6 a	18.6 a	25.2 a	0.967	0.796	0.278
	Orchard	38.9 a	58.8 a	47.2 a	51.3 a	38.9 a	54.6 a	0.646	0.721	0.394
	Roggen	15.0 a	4.4 a	21.4 a	18.0 a	14.5 a	8.3 a	0.276	0.569	0.378
	Sheridan Lake	13.6 a	10.5 a	24.2 a	<b>24.6 a</b>	<b>8.3 b</b>	<b>15.4 ab</b>	0.165	0.053	<b>0.033</b>
	Yuma	<b>40.6 a</b>	<b>43.3 a</b>	<b>32.5 b</b>	<b>43.8 a</b>	<b>41.6 b</b>	<b>31.0 c</b>	<b>&lt;0.001</b>	<b>0.001</b>	<b>&lt;0.001</b>
2019 Combined ANOVA		<b>34.4 b</b>	<b>46.2 a</b>	<b>44.9 ab</b>	46.3 a	37.6 a	41.6 a	<b>0.020</b>	0.810	0.384
2020	Akron	24.6 a	37.8 a	29.8 a	22.3 a	39.8 a	30.2 a	0.208	0.431	0.811
	Arapahoe	36.6 a	55.5 a	63.9 a	40.3 a	54.5 a	63.9 a	0.367	0.492	0.315
	Burlington	58.7 a	86.4 a	75.9 a	<b>102.1 a</b>	<b>62.3 b</b>	<b>54.5 b</b>	0.173	<b>&lt;0.001</b>	<b>0.001</b>
	Julesburg	8.2 a	8.1 a	11.5 a	11.5 a	16.8 a	9.1 a	0.405	0.227	0.613
	Lamar	64.2 a	73.3 a	66.8 a	62.5 a	71.1 a	70.7 a	0.885	0.791	0.275
	Orchard	24.9 a	50.4 a	60.5 a	40.5 a	40.3 a	55.0 a	0.132	0.345	0.366
	Roggen	53.8 a	87.8 a	81.1 a	69.3 a	87.2 a	81.1 a	0.360	0.557	0.368
	Sheridan Lake	27.3 a	72.0 a	41.1 a	50.7 a	47.7 a	42.0 a	0.058	0.793	0.084
Yuma	6.7 a	12.9 a	14.7 a	12.6 a	9.5 a	12.2 a	0.124	0.558	0.219	
2020 Combined ANOVA		<b>33.7 b</b>	<b>54.6 a</b>	<b>49.8 a</b>	45.7 a	46.9 a	45.4 a	<b>0.001</b>	0.940	0.093
2-yr Combined ANOVA		<b>34.0 b</b>	<b>50.4 a</b>	<b>47.3 a</b>	46.0 a	42.2 a	43.5 a	<b>&lt;0.001</b>	0.421	0.077

**Table 19.** Post-harvest residual NO<sub>3</sub>-N ANOVA using fixed effects combined over two years.

Effect	Significance
<b>Year</b>	<b>0.014</b>
<b>Environment</b>	<b>&lt;0.001</b>
Variety	0.739
Environment x Variety	0.600
<b>N application rate</b>	<b>0.001</b>
Environment x N application rate	0.960
Variety x N application rate	0.474
Environment x Variety x N application rate	0.976

Tukey-Kramer mean separation test for differences in post-harvest residual NO<sub>3</sub>-N for the two years ( $\alpha \leq 0.05$ ).

Year	Estimate (lbs NO <sub>3</sub> - N ac <sup>-1</sup> )
<b>2019</b>	<b>37.85 b</b>
<b>2020</b>	<b>46.78 a</b>

residual soil NO<sub>3</sub>-N in the 2019, 2020, or the two-year combined data. The N application rate x variety interaction was most often non-significant at all locations over both years. In the water limited year, residual nitrate was the greatest and yields were the lowest, indicating that soil N was not used by the crop. Comparatively, the high yielding year had significantly less post-harvest residual NO<sub>3</sub>-N indicating that N was used by the crop.

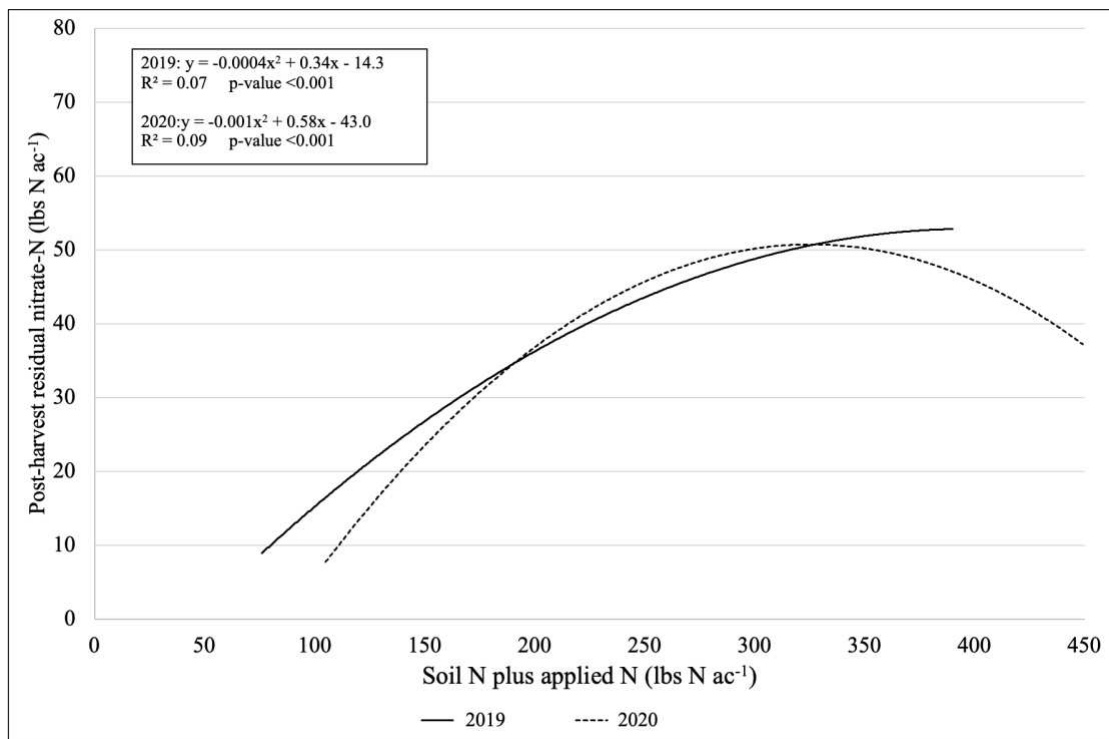
Year, environment, and N application rate had significant effects on post-harvest residual soil NO<sub>3</sub>-N for the two years (Table 19). In 2020, under water-limited conditions, post-harvest residual soil NO<sub>3</sub>-N was greater than under the higher yielding conditions of 2019. These findings suggest that under water-limited conditions, soil N is simply not able to be utilized. The opposite is true in water “rich” years. As soil NO<sub>3</sub>-N is water soluble and thus moves towards roots via mass flow (Havlin et al., 2014), the lack of soil water simply increases residual soil NO<sub>3</sub>-N.

### Relationship between post-harvest residual nitrate-N and soil N plus applied N

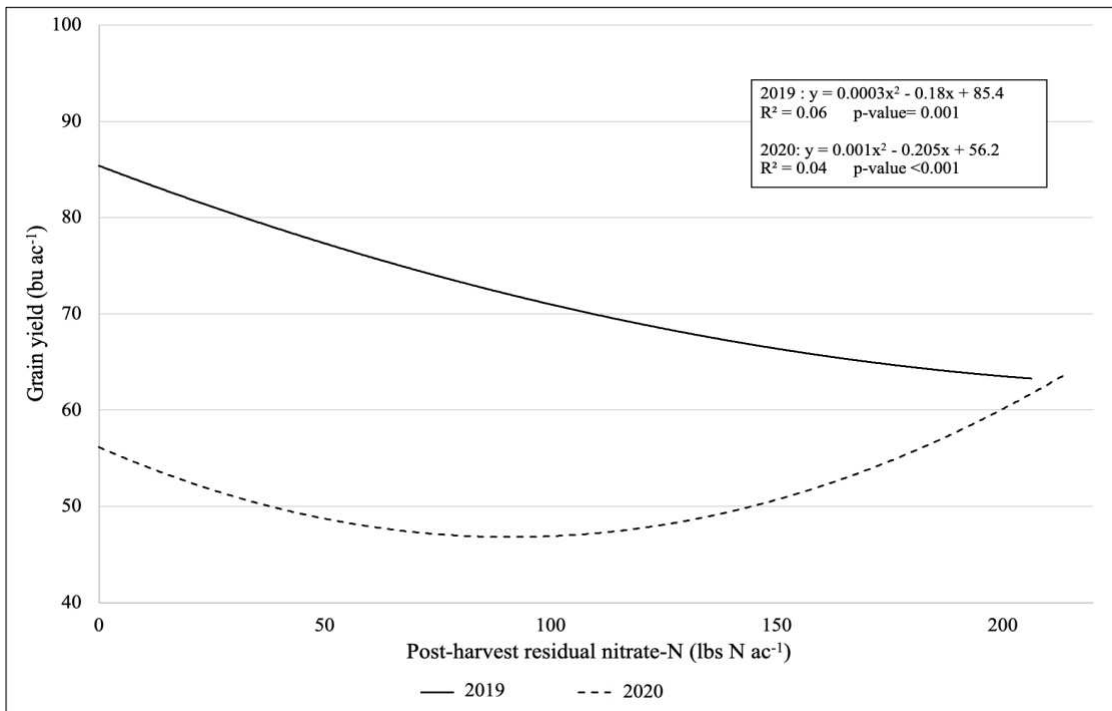
The relationship between post-harvest residual soil NO<sub>3</sub>-N and increasing N fertilizer application rates across all varieties and environments is shown in Figure 10. Quadratic curves fit the data best in both 2019 and 2020. In 2019, the post-harvest residual soil NO<sub>3</sub>-N was nearly maximized at 55 lbs N ac<sup>-1</sup> at a soil + N applied rate of ~ 400 lbs N ac<sup>-1</sup>. In 2020, post-harvest residual soil NO<sub>3</sub>-N reached a maximum of 50 lbs N ac<sup>-1</sup> at a soil + N applied rate of 320 lbs N ac<sup>-1</sup>.

### Relationship between residual nitrate-N and grain yield

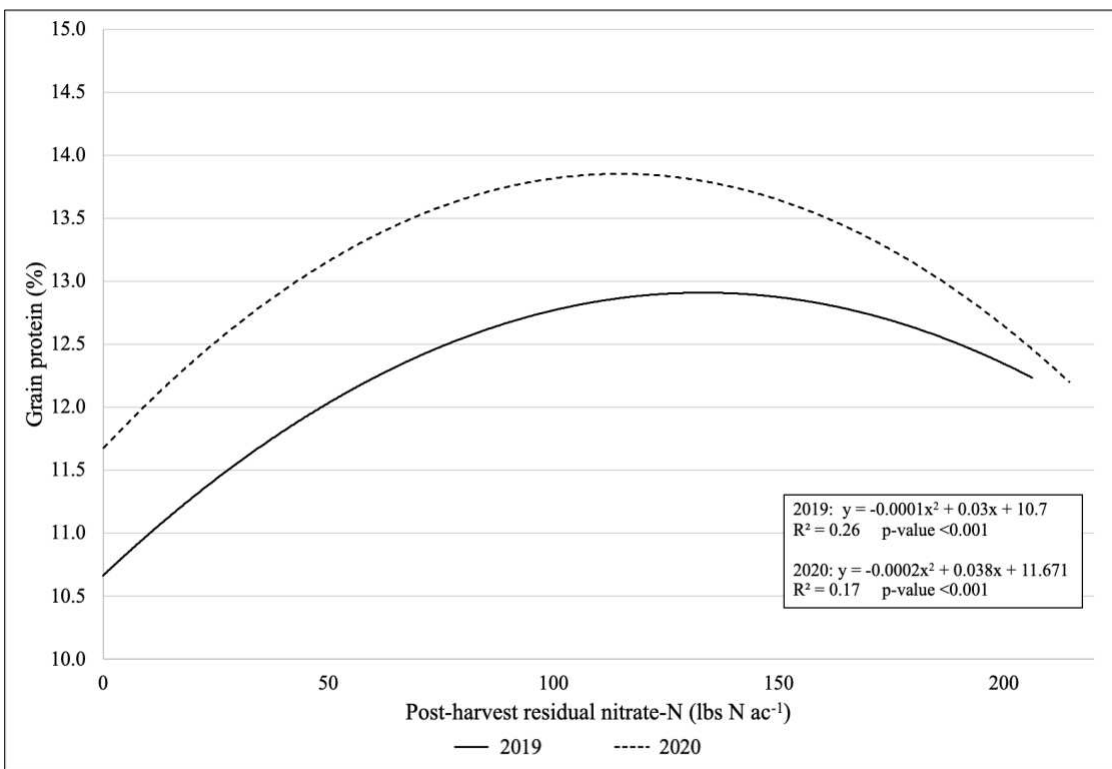
The relationship between post-harvest residual soil NO<sub>3</sub>-N and grain yield across all varieties and environments is shown in Figure 11. Quadratic curves fit the data best in both 2019 and 2020. In 2019, grain yields were lowest when residual soil NO<sub>3</sub>-N was maximized. In 2020, grain yields were lowest when residual soil NO<sub>3</sub>-N was ~ 100 lbs ac<sup>-1</sup>.



**Figure 10.** Relationship between post-harvest residual soil nitrate N and increasing N fertilizer rates by year across all varieties and environments.



**Figure 11.** Relationship between post-harvest residual  $\text{NO}_3\text{-N}$  and grain yield by year across all varieties and environments.



**Figure 12.** Relationship between residual nitrate-N and grain protein content by year across all varieties and environments.

### **Relationship between post-harvest residual nitrate-N and grain protein**

The relationship between post-harvest residual soil  $\text{NO}_3\text{-N}$  and grain protein content across all varieties and environments is shown in Figure 12. Quadratic curves fit the data best in both 2019 and 2020. In both years, as post-harvest residual soil  $\text{NO}_3\text{-N}$  increased, grain protein increased until a maximum of 12.9% protein content at 138 lbs N  $\text{ac}^{-1}$ , and 13.8% protein content at 115 lbs N  $\text{ac}^{-1}$ , was obtained in 2019 and 2020, respectively. In 2019, the high-yielding year, Figure 14 reflects lower grain protein content in response to increasing post-harvest residual soil  $\text{NO}_3\text{-N}$  as compared to the water-limited year (2020). This reflects the results found by Girma et al. (2006) and Walsh and Walsh (2020) that in water limited years grain protein content is concentrated due to the lack of plant growth and lower yields. Meanwhile, in high-yielding years, the opposite is true.

### **Wheat response to sulfur**

The grain yield and protein results for the sulfur application rate of 20 lbs S  $\text{ac}^{-1}$  plus 90 lbs N  $\text{ac}^{-1}$ , as compared to the 90 lbs N  $\text{ac}^{-1}$ , is shown in Table 20. In 2019, there was not a significant grain yield response to S in 17 of the 18 environments, while no response was found for any environment in 2020. The results for grain yield response to S by environment and for the 2019, 2020, and combined two-year ANOVA for S application rate and control showed that none of the environments were significant for yield except Roggen.

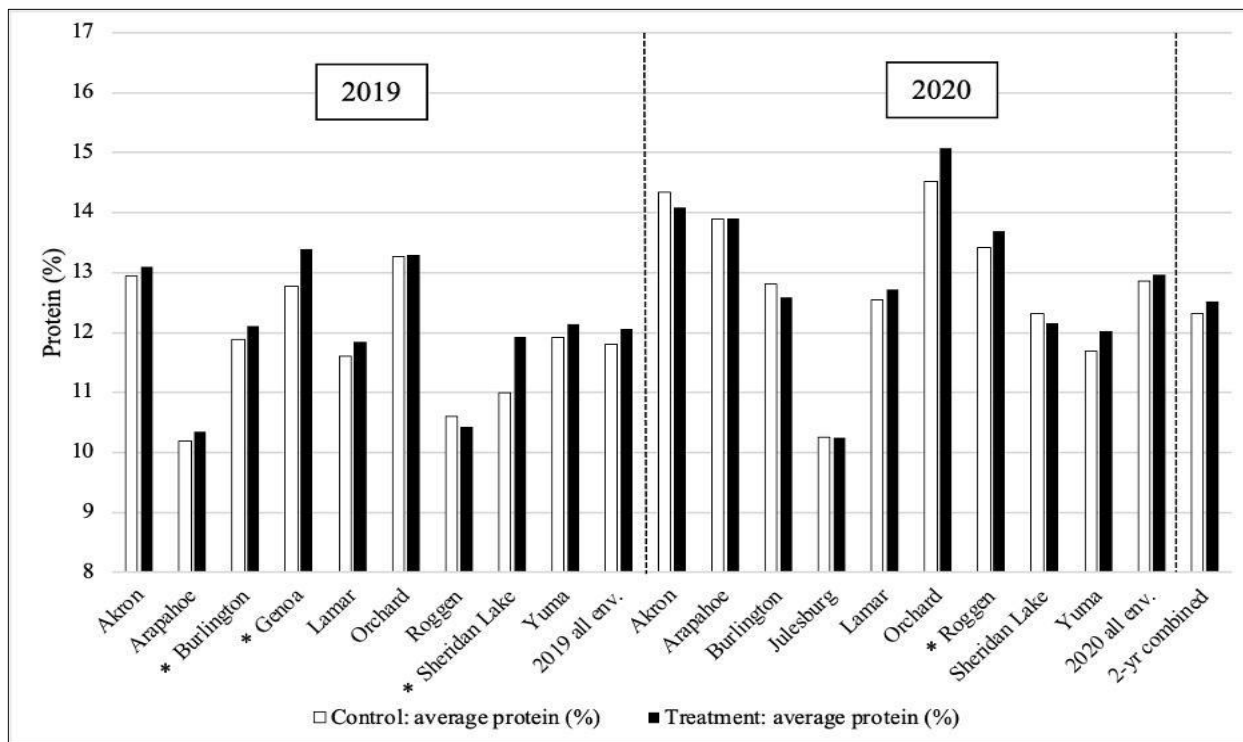
Grain protein increased with an S application in only four of the 18 environments (Table 20 and Figure 13). In 2019, there was a positive response in grain protein due to S application in Burlington, Genoa, and Sheridan Lake. The environment Burlington 2019 had a 0.2% greater protein with the S treatment compared to control. Genoa 2019 had 0.6% higher protein with the

**Table 20.** Effect of sulfur on grain yield and protein from multiple T-test comparisons of 90 lbs N ac<sup>-1</sup> plus 20 lbs S ac<sup>-1</sup> (Trt.) compared to 90 lbs N ac<sup>-1</sup> (Cont.) (significant (Sig.) differences are in bold).

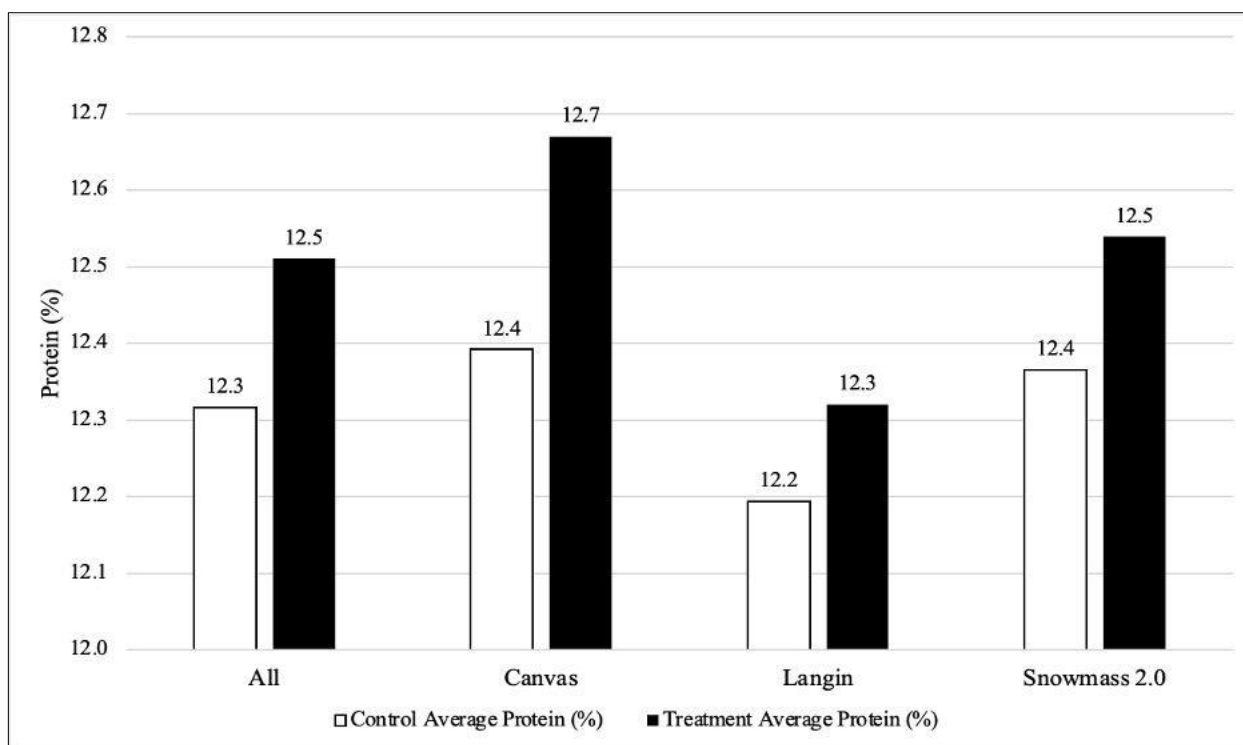
Year	Location	SO <sub>4</sub> -S in fall soil test (0-24") and farmer applied S	Fall N in soil test	SOM (0-24")	pH (0-12")	Soil sand content	Avg. long-term precipitation	Cont.	Trt.	Sig.	Cont.	Trt.	Sig.
		Total lbs S ac <sup>-1</sup>	lbs N ac <sup>-1</sup>	%	%	"	average grain yield bu ac <sup>-1</sup>	average grain protein %					
2019	Akron	7	96	1.5	6.3	29	16.5	66.7	62.6	0.157	12.9	13.1	0.236
	Arapahoe	16	150	1.5	7.9	29	18.2	115.1	111.8	0.230	10.2	10.3	0.181
	Burlington	8	163	1.9	7.5	29	17.7	105.2	106.9	0.359	11.9	12.1	<b>0.014</b>
	Genoa	8	103	1.9	7.2	39	16.6	70.4	69.1	0.372	12.8	13.4	<b>0.040</b>
	Lamar	9	96	1.1	8.1	39	15.8	62.2	63.3	0.344	11.6	11.8	0.181
	Orchard	5	58	1.0	7.6	51	13.5	55.5	53.1	0.239	13.3	13.3	0.456
	Roggen 20	5	68	1.2	7.6	31	14.2	84.9	75.5	<b>0.030</b>	10.6	10.4	0.285
	Sheridan Lake	17	74	1.3	7.4	41	16.4	85.7	83.7	0.153	11.0	11.9	<b>&lt;0.001</b>
	Yuma	10	103	1.5	7.3	49	17.9	90.1	89.4	0.426	11.9	12.1	0.199
2019 all environments		10	101	1.4	7.4	37	16.3	81.7	79.5	0.244	11.8	12.1	0.060
2020	Akron	11	247	1.5	6.9	35	16.5	46.4	48.3	0.301	14.3	14.1	0.176
	Arapahoe	33	158	1.6	8.1	25	18.7	34.9	34.2	0.347	13.9	13.9	0.473
	Burlington	27	142	1.7	7.8	23	17.7	67.7	69.3	0.240	12.8	12.6	0.120
	Julesburg	32	68	1.4	6.3	41	18.2	68.3	68.2	0.479	10.3	10.3	0.488
	Lamar	35	79	1.2	8.3	31	18.5	29.0	30.3	0.253	12.6	12.7	0.150
	Orchard	43	147	1.1	7.1	57	13.5	42.6	43.6	0.388	14.5	15.1	0.150
	Roggen	28	158	1.1	7.3	35	14.2	67.1	66.3	0.383	13.4	13.7	<b>0.044</b>
	Sheridan Lake	42	89	1.2	8.2	41	16.4	40.0	38.9	0.369	12.3	12.2	0.261
	Yuma	28	98	1.8	7.3	37	17.9	69.7	69.0	0.457	11.7	12.0	0.141
2020 all environments		31	132	1.4	7.5	36	16.8	52.0	52.0	0.498	12.8	13.0	0.296
2-yrs combined all environments		20	117	1.4	7.5	36	16.6	67.0	65.8	0.321	12.3	12.5	0.104

S treatment compared to the control. Sheridan Lake 2019 had a 0.9% higher protein with the S treatment compared to control. Roggen 2019 protein was a 0.3% greater protein with the S treatment compared to the control. In 2019, the high yielding year, combined environments for protein showed that S treatment was 0.5% greater than the control. The low yielding year, 2020, combined environments resulted in no increase in grain protein with S. The combined years showed that no differences existed in grain protein content between the S treatment and the control. Most often, wheat grain yields and protein did not respond to the S application at environments. Studies such as Herrera and Dhillon et al. (2012, 2019) did not find meaningful responses to S fertilizer with respect to wheat grain yield or protein content.

The effect of S application on grain yield and protein content as a function of different conditional groupings of environments are shown in Tables 21 and 22. None of the differences among groupings were significant for grain yield or protein. However, these environmental conditions of low soil organic matter, high soil sand content, and low soil S amounts have been suggested as predictors of wheat crops that may respond in grain yield and or grain protein to S applications according to Kansas State University (Lamond, 1997).



**Figure 13.** The grain protein difference between the Control and S Treatment for each environment and for 2019, 2020 and for the two years combined.



**Figure 14.** The grain protein content difference between the Control and S Treatment by variety and overall across all environments.

**Table 21.** Effect of 20 lbs S ac<sup>-1</sup> and 90 lbs ac<sup>-1</sup> compared to 90 lbs N ac<sup>-1</sup> on grain yield for different conditional groupings of environments.

Grouping	Control	Treatment	Significance
	Grain yield (bu ac <sup>-1</sup> )		
North environments	66.2	64.5	0.24
South environments	68.0	67.3	0.45
Sand > 39%	63.5	62.9	0.43
OM ≤ 1.4 %	59.8	58.1	0.28
OM ≤ 1.3	57.2	56.5	0.43
OM < 1.3 and sand > 39%	57.2	56.5	0.43
Long-term precipitation ≤ 16"	62.4	60.4	0.25
Sulfate ≤ 10 lb ac <sup>-1</sup> in fall soil test	74.5	73.0	0.29
Sulfate ≤ 8 lb ac <sup>-1</sup> in fall soil test	68.6	65.7	0.11
pH ≤ 8 (0-24")	47.2	47.2	0.50
pH ≤ 8 (0-12")	41.9	41.7	0.48
Fall S plus trt plus farmer applied S ≤ 10 lbs ac <sup>-1</sup>	73.9	72.5	0.27
Fall S plus trt plus farmer applied S ≤ 8 lbs ac <sup>-1</sup>	72.1	70.6	0.27

**Table 22.** Effect of 20 lbs S ac<sup>-1</sup> and 90 lbs ac<sup>-1</sup> compared to 90 lbs N ac<sup>-1</sup> on grain protein content for different conditional groupings of environments.

Grouping	Control	Treatment	Significance
	Grain protein (%)		
North environments	12.55	12.77	0.17
South environments	12.02	12.20	0.16
Sand > 39%	12.09	12.42	0.10
OM ≤ 1.4 %	12.14	12.39	0.28
OM ≤ 1.3	12.58	12.73	0.12
OM < 1.3 and sand > 39%	12.50	12.86	0.10
Long-term precipitation ≤ 16"	12.64	12.85	0.28
Sulfate ≤ 10 lb ac <sup>-1</sup> in fall soil test	12.02	12.22	0.10
Sulfate ≤ 8 lb ac <sup>-1</sup> in fall soil test	11.97	11.97	0.50
pH ≤ 8 (0-24")	12.64	12.64	0.48
pH ≤ 8 (0-12")	12.59	12.66	0.38
Fall S plus trt plus farmer applied S ≤ 10 lbs ac <sup>-1</sup>	12.29	12.45	0.15
Fall S plus trt plus farmer applied S ≤ 8 lbs ac <sup>-1</sup>	12.33	12.48	0.18

## CONCLUSIONS

The objectives of this study were to: 1) determine if varieties respond differently to N and what the optimum N rate would be for each of the three studied winter wheat varieties currently raised in Colorado, 2) determine if we could make new and better N fertilizer rate recommendations, 3) determine the effect of N and variety on grain yield and quality parameters; test weight, thousand kernel weight, and protein at 18 environments in Eastern Colorado, and 4) determine how S fertilization affects winter wheat grain yield and protein content in multiple locations in Eastern Colorado. Outlined below are the conclusions of each of these objectives.

Objective 1: The two study years were climatically different such that study conclusions can only be applied to each year separately. Grain yield regressed by variety against applied N plus residual soil N showed different response curves by variety in each of the two years. Optimum yields were achieved at different N rates for each variety and each of the years. Our hypothesis that varieties have different N response curves was accepted. Canvas required greater N applications to reach optimum yield in both years. In 2019, under higher yielding conditions, Canvas required about 100 lbs more of N  $\text{ac}^{-1}$  (323 lbs N  $\text{ac}^{-1}$ ) to achieve 100 bu  $\text{ac}^{-1}$  compared to Langin and Snowmass 2.0 (requiring 210 and 229 lbs N  $\text{ac}^{-1}$  to reach 100 bu  $\text{ac}^{-1}$ , respectively). In 2020, there was no difference between Langin and Snowmass 2.0 for yield, although 2020 was a moisture limited year that impacted the wheat response to N.

Grain protein content was highly affected by variety and N fertilization. Again, the two study years were climatically different such that study conclusions can only be applied to each year separately. In 2019, under higher producing climatic conditions, increased soil N plus applied N increased both yield and grain protein up to 12.2% grain protein for Canvas, 11.9% for Langin, and 12.1% for Snowmass 2.0. After these points, the relationship of the greater the yield,

the lower the protein content existed. Canvas had the highest protein content in 2019 and Langin had the lowest. Langin never achieved greater than 12% protein content in 2019. In 2020, N fertilization did not affect grain yield but did significantly increase protein content. In 2020, Snowmass 2.0 was more efficient at making 12% protein at 55.7 bu ac<sup>-1</sup> and 194 lbs of soil N plus applied N ac<sup>-1</sup> compared to Canvas at 46.5 bu ac<sup>-1</sup> at 193 lbs of soil N plus applied N ac<sup>-1</sup> for 12% protein. Remarkably, Snowmass 2.0 had high yields and high protein content in 2020 (same protein content as Canvas at high yields, and higher protein than Langin across all N levels). Langin required more N to reach 12% at 219.5 lbs N ac<sup>-1</sup> but achieved a yield of 51 bu ac<sup>-1</sup> at 12% at 219.5 lbs N ac<sup>-1</sup>. These results show that Snowmass 2.0 maintained protein at higher yield levels.

NDVI readings were affected by variety and soil N plus applied N in both years. Langin was found to have the highest NDVI and Canvas the lowest. The two years showed that in a high yielding year, the average NDVI index was 0.2 higher than the water limited year. In a water limited year, NDVI was shown to respond to N however, the actual yield response to N was much lower than predicted by NDVI index. Based on the results, it appears that NDVI can be useful for making in-season N application suggestions in a high moisture year but less useful in dry years.

Objective 2: Since excessive N fertilizer applications rates were used, we were not able to determine new fertilizer recommendations for yield. Thus, the CSU fertilizing winter wheat fact sheet was not able to be updated from this study. However, this research gave insight into the importance of environment and variety in maximizing yields using N and the usefulness of understanding variety response. Breeding research could move in the direction of testing N

efficiency to increase variety performance. This research also provided valuable insight into the future of making S recommendations for Colorado winter wheat production.

Objective 3: test weight, thousand kernel weight, and protein were affected by N, variety and environments. Snowmass 2.0 was able to maintain desirable test weight, protein, and thousand kernel weights at higher yields when compared to similar yield for Langin. Therefore, there is a variety effect on these parameters for grain quality. In the future, breeding efforts could target these responses to create varieties that are able to maintain high yields while also maintaining milling and baking quality.

This study showed that grain yield and protein content are highly affected by environmental conditions within years, applied N fertilizer, and variety response to N. Our hypothesis that N could increase grain protein above a 12% threshold at high N rates in high moisture years was rejected. Precipitation was found to affect N utilization. Evidence of this was supported by year in grain yield, post-harvest residual  $\text{NO}_3\text{-N}$ , and the NDVI. In years where higher than average precipitation was received, yield increased. The NDVI was shown to increase with increasing N, and moisture limited years responded less to NDVI with increasing N, providing evidence that N was utilized more in a high moisture year. However, grain protein was not able to be improved beyond a certain point by variety. There was more post-harvest residual  $\text{NO}_3\text{-N}$  overall remaining in the soil in a moisture-limiting year, showing that N was not able to be utilized in those years for increasing grain yield.

Objective 4: Our hypothesis that S will increase grain yield and protein at environments with low soil organic matter and low S in the fall soil tests was not accepted. Yield was not improved at any environments with the additions of S. Future research on additional S rates,

application timing, and environmental conditions on S improving grain yield and grain protein would be beneficial for Eastern Colorado wheat farmers.

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## APPENDIX

## APPENDIX

**Table A1.** SAS code for split plot ANOVA for a single environment

```
data Akron19yield;
input variety $ trt $ yield rep;
cards;

;
proc mixed;
class rep variety trt;
model yield=variety trt variety*trt;
random rep rep*trt;
lsmeans variety*trt/pdiff
adjust=tukey;
ods output diffs=diffs;
run;

proc print data=Akron19yield;
var lsd estimate stderr df;
run;
```

**Table A2.** SAS code split plot ANOVA for combined years and 2-year combined ANOVA

```
data multifertwheat;
input loc $ variety $ Nrate $ yield rep;
cards;

proc mixed;
class loc rep variety Nrate;
model yield=loc|variety|Nrate;
random rep(loc) rep*Nrate(loc);
lsmeans loc*variety*Nrate/pdiff cl
slice=variety*loc;
ods output diffs=diffs;
run;

proc print data=multifertwheat;
var lsd estimate stderr df;
run;
```

**Table A3. 2019 dryland wheat field characteristics and management practices**

		Akron	Arapahoe	Burlington	Genoa	Julesburg	Lamar	Orchard	Roggen	Sheridan Lake	Walsh	Yuma
Average Yield (bu/ac)		67	104	100	72	54	53	48	73	60	90	86
Location	GPS Coordinates (Lat/Long)	40.1493 -103.1373	39.0015 -102.2461	39.2852 -102.2795	39.3516 -103.5093	40.8356 -102.3429	38.0026 -102.6135	40.4817 -104.1099	40.0727 -104.3019	38.5345 -102.4712	37.4312 -102.3104	40.1907 -102.6611
	County	Washington	Cheyenne	Kit Carson	Lincoln	Sedgwick	Prowers	Morgan	Weld	Kiowa	Baca	Yuma
	Soil Type	Rago Silt Loam	Keith-Richfield silt loam	Kuma-Keith silt loam	Weld silt loam	Keith-Kuma silt loams	Wild silt loam	Briggsdale clay loam	Weld loam	Wiley loam	Wiley loam	Haxtun sandy loam
Soil	Sand-Silt-Clay %	29-50-21	29-52-19	29-54-17	31-50-19	31-50-19	39-44-17	51-26-23	31-44-25	41-40-19	21-60-19	49-32-19
	Soil Organic Matter	1.5 %	1.6 %	1.9 %	2.0 %	1.6 %	1.1 %	1.2 %	1.4 %	1.3 %	1.7 %	1.4 %
	Soil pH	6.3	7.9	7.5	7.2	6.3	8.1	7.6	7.6	7.4	7.9	7.3
	Soil Nutrients at planting (N-P lb/ac)	111-15	166-7	182-10	123-20	102-27	80-5	70-5	82-10	87-10	137-11	117-18
	Applied Fertilizer in Season (N-P-K lb/ac)	8-28-0	73-46-0-7S-0.5Z	92-49-0	62-38-0-1S	23-28-0-1Zn	45-36-0	59-37-0-2S-0.25Z	8-28-0	54-28-0-8.25S	56-20-0	37-28-0-2S
Management	Tillage	No-Till	Verticle Tillage	Tilled	2x Tilled	No-Till	No-Till	No-Till	No-Till	No-Till	Tilled	Min-Tilled
	Previous Crop 2017/2018	Proso millet/ Chemfallow	Corn/ Chemfallow	Corn/ Chemfallow	Oil Sunflowers/ Fallow	Corn/ Fallow	Wheat/ Chemfallow	Proso Millet/ Chemfallow	Corn/ Fallow	Grain Sorghum/ Chemfallow	Wheat / Fallow	Proso Millet / Fallow
	Planting Date	3-Oct-2018	13-Sep-2018	13-Sep-2018	18-Sep-2018	2-Oct-2018	12-Sep-2018	2-Oct-2018	1-Oct-2018	12-Sep-2018	18-Sep-2018	19-Sep-2018
Development	Harvest Date	26-Jul-2019	16-Jul-2019	17-Jul-2019	18-Jul-2019	30-Jul-2019	8-Jul-2019	29-Jul-2019	25-Jul-2019	12-Jul-2019	8-Jul-2019	13-Jul-2019
	Heading Date (Avg)	4-Jun-2019	26-May-2019	28-May-2019	2-Jun-2019	8-Jun-2019	16-May-2019	9-Jun-2019	5-Jun-2019	20-May-2019	19-May-2019	30-May-2019
	Days from Planting to Heading (Avg)	244	255	257	257	249	246	250	247	250	243	253
	Biotic Stress	Minor sawfly, trace stripe rust, tansy mustard present in May	Some WSMV noted mid-May	Sprayed for stripe rust June 1	-	Tan Spot, Cephalosporium stripe, WSMV, stripe rust present	-	Severe sawfly	Low level stripe rust noted June; sprayed for rust in June	-	Minor leaf and stripe rust, barley yellow dwarf virus, tan spot	Low levels stripe and leaf rust
Environment	Abiotic Stress	Freeze - possible* Some lodging	-	-	Freeze-minor*	Freeze - suspected**	Freeze - severe*	Freeze - moderate* Slight hail damage in June	Freeze - moderate*	Freeze - severe*	-	Leaf rolling mid-May indicated drought stress 2.8" rain late May
	Total Rain: April 1 to Harvest	9.25 in	6.99 in	7.56 in	8.22 in	7.46 in**	5.76 in	8.42 in	7.4 in	9.07 in	6.89 in	5.97 in
	Last Spring Freeze Dates	May 22: 2 hr < 30 F Temp to 29 F	No Freeze Events	No Freeze Events	May 22: 2 hr < 30 F Temp to 29 F	May 22: Suspected**	May 19: 15 min <31 F Temp to 30.6 F May 22: 3 hr < 30 F Temp to 28.2 F	May 22: 1 hr < 30 F Temp to 28.5 F	May 22: 1hr < 30 F Temp to 28.7 F	May 22: 3 hrs < 30 F Temp to 28 F	No Freeze Events	No Freeze Events

\*Freeze severity estimated from Kansas State Wheat Freeze Damage Publication ([https://www.sunflower.k-state.edu/agronomy/docs/c646\\_Whole\\_Wheat\\_Freeze\\_Publication.pdf](https://www.sunflower.k-state.edu/agronomy/docs/c646_Whole_Wheat_Freeze_Publication.pdf))

\*\*Weather station limited data: Missing rain prior to May 17, missing Temp - May 22 midnight to 6:20 am

WSMV: Wheat streak mosaic virus

**Table A4. 2019 dryland wheat field characteristics and management practices**

	Akron	Arapahoe	Burlington	Genoa	Julesburg	Lamar	Orchard	Roggen	Sheridan Lake	Walsh	Yuma	
<b>Average Yield (bu/ac)</b>	45	35	60	26*	56	26	41	58	32	Abandoned	66	
<b>Location</b>	<b>GPS Coordinates (Lat/Long)</b>	40.1494	38.9066	39.3152	39.3577	40.8245	38.0026	40.4804	40.0842	38.5340	37.4312	40.1866
		-103.1385	-102.3141	-102.2578	-103.5103	-102.3063	-102.6135	-104.1095	-104.3013	-102.4357	-102.3104	-102.6543
	<b>County</b>	Washington	Cheyenne	Kit Carson	Lincoln	Sedgwick	Prowers	Morgan	Weld	Kiowa	Baca	Yuma
<b>Soil</b>	<b>Soil Type</b>	Rago Silt Loam	Wiley Complex	Kuma-Keith silt loam	Weld silt loam	Keith-Kuma silt loams	Manvel silt loam	Platner Loam	Weld loam	Fort Collins sandy loam	Wiley loam	Haxtun sandy loam
	<b>Sand-Silt-Clay %</b>	35-46-19	25-62-13	23-62-15	27-52-21	41-42-17	31-56-13	57-22-21	35-44-21	41-44-15	21-60-19	21-60-19
	<b>Soil Organic Matter</b>	1.5 %	1.8 %	1.7 %	1.7 %	1.4 %	1.3 %	1.2 %	1.2 %	1.2 %	1.7 %	1.7 %
	<b>Soil pH</b>	6.9	8.1	7.8	6.8	6.3	8.3	7.1	7.3	8.2	8.2	8.2
	<b>Soil Nutrients at planting (N-P-K lb/ac)</b>	153-11	108-2	86-9	51-8	37-12	46-3	98-16	94-7	52-3	78-4	78-4
<b>Management</b>	<b>Applied Fertilizer in Season (N-P-K lb/ac)</b>	8-28-0	63-28-0-4.6S	88-48-0	38-28-0-5S	48-28-0-5S	50.5-28-0	62.4-28-0	8-28-0	60-28-0-11S	-	8-28-0
	<b>Tillage</b>	No-Till	Vertical Tillage	Tilled	2x Tilled	Tilled	No-Till	No-Till	No-Till	No-Till	No-Till	Tilled
	<b>Previous Crop 2017/2018</b>	Proso millet/ Chemfallow	Corn/ Chemfallow	Corn/ Chemfallow	Oil Sunflowers/ Fallow	Corn/ Fallow	Wheat/ Chemfallow	Wheat/ Chemfallow	Wheat/ Fallow	Grain Sorghum/ Chemfallow	Wheat / Fallow	Wheat / Fallow
<b>Development</b>	<b>Planting Date</b>	19-Sep-2019	13-Sep-2019	12-Sep-2019	25-Sep-2019	17-Sep-2019	13-Sep-2019	17-Sep-2019	25-Sep-2019	13-Sep-2019	18-Sep-2019	18-Sep-2019
	<b>Harvest Date</b>	2-Jul-2019	3-Jul-2020	8-Jul-2020	18-Jul-2020	7-Jul-2020	1-Jul-2020	30-Jun-2020	10-Jul-2020	1-Jul-2020	-	3-Jul-2020
	<b>Heading Date (Avg)</b>	24-May-2020	19-May-2020	18-May-2020	3-Jun-2020	29-May-2020	14-May-2020	25-May-2020	26-May-2020	17-May-2020	-	22-May-2020
	<b>Days from Planting to Heading (Avg)</b>	248	249	249	252	255	244	251	244	247	-	247
<b>Environment</b>	<b>Biotic Stress</b>	Heavy sawfly pressure	-	-	-	barley yellow dwarf virus, tan spot, cephalosporium stripe	-	Severe sawfly	-	-	-	-
	<b>Abiotic Stress</b>	Freeze** Moderate hail in May	Freeze** Heat and Drought stress in early May and worsening into June	Freeze** Heat and Drought stress in early May and worsening into June	Drought	Freeze**	Freeze** Drought stress in early spring and worsening into June	Freeze** Drought - moderate by end of May	Freeze**	Freeze** Drought - April worsening through June	Severe Drought	Freeze**
	<b>Total Rain: April 1 to Harvest</b>	4.1 in	4.5 in	6.4 in	4.8 in	1.6 in	3.6 in	2.1 in	2.1 in	3.2 in	-	3.1 in
	<b>Days temp reached above 90 F (April 1 - Harvest)</b>	17	26	26	17	26	30	26	32	28	-	26

\*Data not used for analysis due to drought, patch stands, and poor emergence.

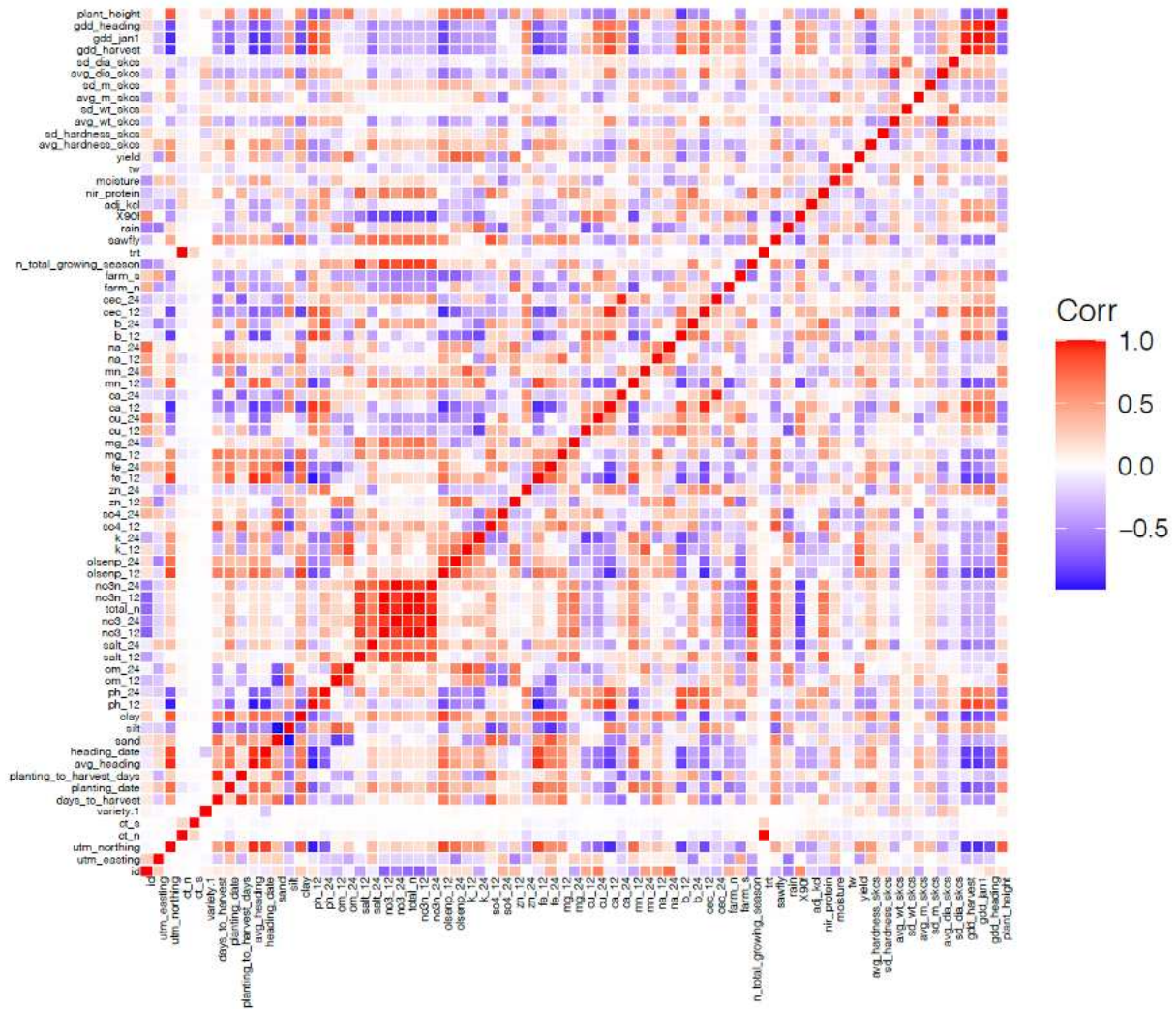
\*\*Freeze April 12-14 caused leaf damage across the growing region. Damage was severe to moderate leaf burn. Due to the timing of the freeze little damage to the developing wheat head was found.

**Table A5.** Correlation Matrix results across all years and environments.

	nir_protein	yield	so4_12	so4_24
id	-0.22	-0.03	-0.04	0.27
id_yr	-0.2	0	-0.05	0.26
utm_easting	-0.18	-0.4	0.12	0.43
utm_northing	-0.08	0.53	0.2	-0.33
ct_n	0.21	-0.07	0.01	0.06
ct_s	0.05	-0.02	0.01	0.03
variety	0	0.21	0	0
variety_2	-0.08	0.16	0	-0.01
days_to_harvest	0.44	-0.02	0.8	0.24
planting_date	0.08	0.24	0.23	-0.09
planting_to_harvest_days	0.42	-0.09	0.73	0.27
avg_heading	-0.14	0.39	0.21	-0.28
heading_date	-0.1	0.29	0.19	-0.25
sand	0.34	-0.45	0.8	0.58
silt	-0.37	0.35	-0.84	-0.55
clay	0.33	0.26	0.57	0.13
ph_12	0.19	-0.42	-0.11	0.37
ph_24	0.54	-0.36	0.19	0.38
om_12	-0.25	0.71	-0.57	-0.63
om_24	-0.31	0.73	-0.61	-0.57
salt_12	0.76	-0.07	0.55	0.41
salt_24	0.4	-0.08	0.33	0.39
X03_12	0.68	-0.04	0.5	0.23
X03_24	0.51	0.14	0.23	0.05
total_n	0.63	0.02	0.41	0.17
olsenp_12	0.16	0.45	0.42	-0.15
olsenp_24	-0.08	0.77	-0.06	-0.52
k_12	-0.23	0.69	-0.45	-0.45
k_24	-0.44	0.62	-0.57	-0.68
so4_12	0.71	-0.43	1	0.71
so4_24	0.56	-0.72	0.71	1
zn_12	-0.01	0.64	-0.18	-0.39
zn_24	0.01	0.12	-0.23	-0.17
fe_12	-0.09	0.24	0.25	-0.23
fe_24	0.13	-0.18	0.58	0.44
mg_12	0.48	0.12	0.69	0.21
mg_24	0.45	-0.3	0.44	0.48

cu_12	0.27	-0.43	0.37	0.46
cu_24	0.07	-0.48	0.06	0.59
ca_12	0.04	-0.35	-0.32	0.15
ca_24	-0.03	0.1	-0.51	-0.32
mn_12	0	0.22	0.09	-0.19
mn_24	-0.33	0.47	-0.56	-0.3
na_12	0.32	0.06	0.48	0.17
na_24	0.16	0.12	0.18	0.27
b_12	0.39	-0.57	0.26	0.45
b_24	0.72	-0.21	0.51	0.33
cec_12	-0.1	-0.28	-0.47	-0.02
cec_24	0.01	0.12	-0.44	-0.27
farm_n	-0.12	0.26	-0.17	-0.1
farmer_app_n	-0.36	0.03	-0.15	-0.05
farm_s	-0.34	-0.47	-0.23	0.33
n_total_growing_season	0.67	0.16	0.39	0.15
trt	0.21	-0.07	0.01	0.06
sawfly	0.7	-0.23	0.84	0.51
drought	0.36	-0.46	0.33	0.45
rain	-0.28	0.6	-0.5	-0.68
X90f	-0.3	-0.15	-0.18	-0.04
ndvi	0.32	0.54	0.32	-0.1
tillage	-0.51	0.8	-0.64	-0.8
adj_kcl	0.24	-0.21	0	0.04
nir_protein	1	-0.42	0.71	0.56
moisture	-0.39	0.33	-0.15	-0.44
tw	-0.31	0.19	-0.19	-0.2
yield	-0.42	1	-0.43	-0.72
avg_hardness_skcs	0.2	0.03	0.34	0.22
avg_wt_skcs	-0.41	0.22	-0.44	-0.34
avg_m_skcs	-0.38	0.47	-0.28	-0.59
avg_dia_skcs	-0.39	0.08	-0.43	-0.25
gdd_harvest	-0.06	-0.27	-0.36	-0.03
gdd_jan1	-0.05	-0.29	-0.39	0.03
gdd_heading	-0.18	-0.21	-0.48	0.01
plant_height	-0.36	0.76	-0.29	-0.56

\*red indicates positive correlation above 0 and blue indicates a negative correlation below 0



**Figure A1.** Correlation matrix across all years and environments

**Table A6.** R sample code for the correlation matrix across years and environments

```
# Load the packages used
library(bReeze)
library(corrplot)
library(skimr)

# Read dataset to R
nrcorr<- read.csv("/Users/danicakluth/Documents/Wheat Data/Corr Matrix/Corr Matrix Data NR
Study.csv", na="n/a")
head(nrcorr)
colnames(nrcorr)
# Data management for visualization analysis
```

```
nr.cor<-as.data.frame(lapply(nrcorr, as.numeric)) # Make variables numeric for cor analysis
# Descriptive statistics of dataset
skim(nr.cor)

# Create correlation matrix of data
res <- cor(nr.cor) # Corr matrix
round(res, 2)

corrplot(cor(nr.cor), method = "circle")
#plots with other shapes
corrplot(cor(nr.cor), method = "ellipse")
corrplot(cor(nr.cor), method = "square")
corrplot(cor(nr.cor), method = "color")

#only show upper square
corrplot(cor(nr.cor), method = "color", type = "upper")
```