Technical Research Report:

Optimum Nitrogen Fertilizer Management Strategies for High-Yielding Spring Wheat in Manitoba

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Executive Summary

The overall purpose of this project was to determine the optimum nitrogen (N) fertilization strategies for high-yielding spring wheat in Manitoba. This information is needed because Manitoba farmers are growing new varieties of spring wheat (eg. AAC Brandon and Prosper) and using other crop management tools (e.g., fungicides) that have resulted in much higher yield potential than for the spring wheat production systems on which our traditional N recommendations were developed.

To address this need, researchers at the University of Manitoba, in collaboration with other partners, completed 8 site-years of field trials during the 2016-2017 growing seasons, using AAC Brandon (Canadian Western Red Spring class, CWRS) and Prosper (Canadian Northern Hard Red class, CNHR) spring wheat. High intensity gold level experiments were conducted at Carman and Brunkild during both season (4 site-years), and silver level experiments were conducted at Melita in both seasons, Carberry in 2016 and Grosse Isle in 2017 (4 site-years). Results of the project are discussed by the following specific research objectives:

Determine appropriate rates for N, based on realistic yield and protein goals for these new varieties (e.g., the overall supply of N required on a per bushel basis)

Prosper produced 4.3 – 16.3 bu/ac higher grain yields, compared to AAC Brandon across sites, while AAC Brandon had 0.7 – 2.0 % higher grain protein content across sites. There were no biophysical interactions between N rate and variety, indicating that Prosper consistently out-yielded Brandon, while Brandon had constantly higher grain protein content across all N rates. Economic optimum rates were determined using a 5-year average price for urea and wheat prices that assumed access to low protein markets. The total N supply (soil test NO_3-N + fertilizer N) required to obtain economic optimum yield and protein varied from 1.5 – 2.3 lbs N/bu at silver level sites and 1.7 – 3.0 lbs N/bu at gold level sites. However, if we exclude the hail damaged site at Carman 2016, the average total N supply at the optimum yield and protein content was 1.99 lb N/bu. This requirement is less than the current recommendation of 2.5 lbs N/bu.

Determine the most effective and efficient combinations of timing, placement and source, especially for midseason top-up applications

Midseason split N application at planting and at stem elongation or flag leaf stages yielded at least as much grain as equivalent rates applied entirely at planting. At gold level sites, there was a small but significant yield increase by splitting N applications between planting and stem elongation, compared to other application timings. At gold level sites, grain protein content increased with stem elongation split applications, compared to when N was applied entirely at planting. Flag leaf split applications consistently increased grain protein content compared to equivalent rates of N applied at planting and stem elongation split applications (0.3 – 0.7%). Late season post-anthesis N applications consistently increased grain protein content (1.1 – 1.8%), regardless of N source. However, post-anthesis applications of urea solution increased grain yield (4.5 bu/ac) and protein content (0.6%) above that for post-anthesis applications of UAN. There was no advantage for using an ESN blend, compared to conventional urea, when applied banded at seeding under the environmental conditions of our study, which were generally dry in spring. If conditions had been wetter and more favorable for early season losses during this study, we might have observed an advantage for ESN.

Evaluate some innovative soil tests for measuring the amount of organic soil N that can be released by mineralization during the growing season

None of the N mineralization indices tested in this experiment had a significant relationship with growing season mineralization in field trials. These results indicate that although these indices have shown promise in laboratory and growth chamber experiments, the reliability of these tests broke down when tested in the field. The variation in soils, management history and environmental conditions of the field trials are thought to be the main reasons for the large variation of predicted N mineralization compares to actual estimated N mineralization. Therefore, although these mineralization tests may hold promise for predicting “potential” mineralization, their ability to predict “actual” mineralization under field conditions appears to be very limited.

Develop decision tools for midseason evaluation of yield and protein sufficiency

Reflectance from vegetation, measured as normalized differential vegetative index (NDVI), was usefully related to spring wheat grain yield for individual sites and varieties as well as when combined across sites and varieties, at all three timings of sensing (stem elongation, flag leaf, and anthesis). The relationship between NDVI and grain protein content was acceptable for individual site-years, but the relationship was lost when site-years were combined, severely limiting the value of this measurement for this purpose.

Similar to NDVI, SPAD meter readings (chlorophyll content) had good relationships with grain yield when combined across site-years and varieties at all timings for sensing. However, SPAD readings had poor relationships with grain protein content for individual site-years and varieties. When combined across site-years and varieties, SPAD readings had a very poor relationship with protein, but this relationship was improved slightly by normalizing data relative to the high N treatment.

Flag leaf N concentration had a significant relationship with grain yield at 7 out of 8 individual site-years and all individual site-years for grain protein content. When data were combined across site-years, flag leaf N concentration had significant relationships with grain yield and protein but relationships were very weak.

Midseason soil sampling for nitrate N resulted in highly variable analyses, which resulted in an unreliable range of estimates for economic optimum rates of N. Post-harvest soil residual NO_3-N levels indicated that residual N typically did not begin to climb until N fertilization rates exceeded the economic optimum. The residual N at economic optimum rates of fertilizer N ranged from 22 – 53 lbs N/ac across site-years.

Project Synthesis

The average total supply of N (spring NO_3-N + fert) required to obtain economic optimum yield across sites-years in this project was 1.99 lbs N/bu, but optimum rates per bushel varied with site-year, especially at silver level sites. Growing season mineralization from soil organic N reserves was highly variable across site-years, which resulted in large deviations of actual N supply from expected N supply from the soil and, therefore, variable economic optimum rates of N. Traditional methods of determining the total supply of N to recommend for a wheat crop do not take into account the soil N that is released through mineralization during the growing season. Our study revealed that predicting growing season mineralization from a soil sample taken before planting is extremely difficult.

Due to this uncertainty in the soil's N supply, it could be beneficial to apply enough N at planting to meet a modest yield goal and re-visit the question of N sufficiency for yield and protein potential once the crop is established. However, in order for this strategy to work, first, we need to be able to predict the potential grain yield and protein content in-season, and secondly, we need to be confident that the in-season intervention with N fertilizer will result in a positive yield or protein response.

Indices used to predict grain yield (GreenSeeker and SPAD Meter) were reasonably reliable when combined across site-years and varieties for all measurement timings, but especially at flag leaf, which coincided well with the crop responses when N fertilizer was applied at these timings. Grain protein content was much more difficult to predict across site-years and varieties, partly due to the uncertainty of late season N supply for soil mineralization.

Post-anthesis N applications target protein increases, alone, rather than both yield and protein, as for the earlier season split applications. Post-anthesis applications consistently increased protein content, but to warrant an application that solely targets protein increases would require the ability to predict absolute protein content before application. For example, the economic benefits of late season applications are often greatest if the crop's protein levels are raised above a minimum market threshold (e.g., 13%). However, the tools tested in this project did not demonstrate the ability to predict protein content.

Post-harvest soil NO_3-N can be used as an auditing tool to determine if the supply of N was excessive for meeting the yield and protein of wheat in a particular field and year. When comparing economic optimum N rates to the amount of post-harvest NO_3-N in the top 60 cm of soil, we determined that if residual levels were greater than 55 lbs N/ac, the N supply was likely more than adequate for reaching the optimum economic yield of spring wheat at that field site in that year.

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Introduction

Manitoba farmers are growing new varieties of spring wheat (e.g. Brandon, Prosper, Faller, etc) that have very high yield potential, some with farm-wide average yields in excess of 80 bu/ac and field averages in excess of 100 bu/ac. These high yielding varieties of spring wheat have brought about challenges for our current nitrogen management strategies. Our provincial guidelines provide N recommendations for spring wheat yield as high as 65 bu/ac (online N recommendations, developed from Western Cooperative Fertilizer trials conducted from 1989-2004) or 50 bu/ac (Manitoba Soil Fertility Guide, based on U of M research conducted in the 1970s). The standard recommendation of 2.5 lbs N/bu for milling quality spring wheat indicates that 200 lbs of soil and fertilizer N per acre are required for 80 bu/ac crops, which represents a large financial risk to wheat growers, as well as a substantial agronomic and environmental risk (eg. lodging, leaching and nitrous oxide emissions). When high yields are achieved, these new varieties will often produce low protein, frequently below thresholds (e.g. 13.5%) that are acceptable for selling this wheat at milling wheat prices. Although midseason fertilization might be regarded as a means of reducing these risks, research in the Prairies has shown that there is risk of midseason N being “stranded” on the soil surface. Also, there is substantial debate about the best method for midseason applications of N. Researchers in North Dakota recommend applying a true foliar application of 30 lbs N/acre approximately one week after anthesis, using a 50:50 mix of urea ammonium nitrate (28-0-0) and water. However, research at AAFC-Brandon and the University of Manitoba has shown very poor uptake of N through wheat leaves. Therefore, many Canadian Prairie agronomists would prefer to recommend applying N earlier in the growing season and in “dribble bands” to minimize contact with foliage and to maximize efficiency of fertilizer N uptake from the soil.

To address these issues, the 4Rs (Right Rate, Source, Placement and Timing) approach for nutrient management must be determined for these new, high-yielding spring wheat varieties, with the following more detailed research objectives:

- Determine appropriate rates for N, based on realistic yield and protein goals for these new varieties (e.g., the overall supply of N required on a per bushel basis)
- Determine the most effective and efficient combinations of timing, placement and source, especially for midseason top-up applications
- Evaluate some innovative soil tests for measuring the amount of organic soil N that can be released by mineralization during the growing season
- Develop decision tools for midseason evaluation of yield and protein sufficiency

Part 1: Nitrogen rate, timing, source and placement for spring wheat production in Manitoba

Manitoba produces approximately 18% of the spring wheat grown across Canada each year with 2.7 million acres grown across the province in 2017 (Statistics Canada 2017). Due to advancements in breeding and agronomic management practices spring wheat yields have more than doubled over the past 50 years and continue to increase. The current provincial nitrogen (N) fertilizer recommendations were developed using varieties with much lower yield potential than varieties currently being grown across the province. New varieties of spring wheat have very high yield potential and when these high yields are achieved, grain protein concentrations often fall short of typical protein targets or thresholds for hard red spring wheat (~13.5%). The existing recommendation of providing a total of 2-3 lbs fertilizer plus soil test residual N/bu spring wheat target yield paired with current varieties with yield potential >90 bu/ac creates large financial, agronomic and environmental risks. There are a number of fertilization strategies that producers in Manitoba may be able to utilize to mitigate the risks associated with these large requirements for N fertilizer, such as alternative sources and split applications, but there has been very little research into using these strategies for growing wheat under Manitoba's growing conditions.

Midseason, split application is a fertilization strategy where a base rate of N is applied at or before planting to meet early season crop requirements and the remainder is applied midseason. The objective of split application is to match crop uptake demands and minimize risk of losses from applying large quantities several months in advance of crop demand. Applying a portion of total N fertilizer within the growing season aims to match N supply to timing of crop demand and in turn increase N use efficiency. Holzapfel et al.(2007) evaluated delaying N fertilizer application in Saskatchewan for canola and spring wheat and saw no effect on spring wheat grain protein content but measured reduced yield in one of three years due to very dry conditions following the midseason application, which resulted in surface stranding of the fertilizer. Karamanos et al. (2004) observed that across 49 trials with in-season split N applications of wheat, a consistent increase in grain protein content was observed only when in-season N applications were correcting a N deficiency and applications were rarely economical.

Late season, foliar applications of N after flowering (post-anthesis) have potential to be used to increase grain protein content in wheat. Woolfolk et al. (2002) found significant grain N content increases when N was applied to winter wheat as a post-anthesis foliar application in Oklahoma. Previous work has indicated little to no influence on yield from N applications this late in the season; therefore, adequate N must be supplied earlier in the growing season to meet yield potential. Urea ammonium nitrate (UAN) diluted 50% with water is commonly used in North Dakota as an N source for foliar post-anthesis N applications to spring wheat. Dissolved urea solution is another potential source for foliar N application; this source is widely used in Europe on winter wheat due to lower risk of leaf burn compared to UAN.

Environmentally Smart Nitrogen (ESN) is a controlled-release urea granular fertilizer (44-0-0) that was developed to match N supply to crop demand throughout the season. Each urea granule is encapsulated in a polymer coating which allows moisture into the granule and N release is controlled during the growing season by soil temperature (Nutrien Ltd. 2018). The use of ESN could potentially replace an additional in-season application of N fertilizer, when labour and equipment might be scarce, while still providing the benefits of matching N supply to the demand of the growing crop.

The objectives of this study were to (1) determine appropriate rates of N, based on yield and protein goals for current, high yielding varieties of spring wheat being grown widely across Manitoba and (2) determine the most effective and efficient combination of rate, timing and source of N fertilization.

MATERIALS & METHODS

Field experiments were divided into two levels of research sites based on treatment and measurement intensity: gold level sites that were managed by the Department of Soil Science at the University of Manitoba and silver level sites that were contracted out to provincial and independent research stations. Gold level sites were located at Carman and Brunkild, MB during both the 2016 and 2017 growing seasons (4 site-years). Silver level sites were located at Melita and Carberry, MB in 2016 and Melita and Grosse Isle, MB in 2017 (4 site-years). Trial location soil types, spring soil residual NO_3-N along with select agronomic information is provided in Table 1.1. A factorial design was used at all experiments with spring wheat variety and N fertilizer treatment as the main effects. Treatments were arranged in the field using a randomized complete block design. Spring soil samples (0 - 60 cm) were taken by replicate across the trial location (15 samples per replicate) either before or at seeding using a Giddings soil probe. Samples were divided into top soil (0 - 15 cm) and sub-soil (15 - 60 cm) before being combined and homogenized across each replicate. The topsoil was divided into 4 separate subsamples per replicate. One subsample was sent to Farmers Edge Laboratories for a complete nutrient analysis (Nitrate-N, Olsen-P; NH_4OAc exchangeable K, Ca, Mg, Na; water-extractable S and Cl; DTPA-extractable Fe, Cu, Cu, Zn, Mn, and B; pH; EC; soil organic matter; base saturation; and CEC) and the other subsamples were used to evaluate tests for estimating potential growing season soil N mineralization (Chapter 2).

Two spring wheat varieties were used, AAC Brandon (Canadian Western Red Spring class, CWRS) and Prosper (Canadian Northern Hard Red class, CNHR). AAC Brandon is a well-known spring wheat variety in Manitoba and is known for high yields and high grain protein content. Prosper is a US-bred variety of wheat that has a very high yield potential but a more modest grain protein content than AAC Brandon.

Nitrogen treatments applied across both varieties included increasing rates of N applied at planting in 30 lbs N/ac increments from 0 – 200 lbs N/ac at gold level sites and 0 – 170 lbs N/ac at silver level sites. Nitrogen at planting was applied midrow banded as conventional urea at gold level sites and at silver sites N was applied by hand broadcast shortly after seeding as Agrotain-treated urea. In-season N application timing was evaluated by applying a base rate of 80 lbs N/ac at planting and an addition 30 or 60 lbs N/ac at stem elongation or flag leaf timing as Agrotain-treated urea. These midseason applications were broadcasted on the soil surface by hand at both gold and silver level sites. Late season N applications of 30 lbs N/ac post-anthesis were also applied in addition to a base rate of 80 lbs N/ac that had been applied at planting. Post-anthesis N applications were foliar-applied using flat fan herbicide nozzles as diluted UAN (diluted 50:50 with water to 14% N solution) at both gold and silver sites, while gold sites had an additional treatment using dissolved urea solution (9% N solution). Gold level trials also had additional treatments to examine ESN blended with conventional urea applied as midrow bands at seeding. Two rates of ESN:Urea blends were tested, a suboptimal rate of 80 lbs N/ac (50:50, ESN:Urea) and a higher rate of 140 lbs N/ac (100:40, ESN:Urea). Gold level sites also included a placement check treatment of 80 lbs N/ac applied surface broadcast by hand as Agrotain-treated urea (Table 1.2).

Seeding dates varied from April 28 to May 10 (Table 1.1); certified seed was used for all sites and treated with Raxil Pro Shield (375 mL/100kg) in a single batch for each variety in the 2016 and 2017 growing seasons to ensure consistency across sites. Gold level sites were seeded using the University of Manitoba Department of Soil Science's small plot airseeder which has 8 rows spaced 20.3 cm apart, with knife openers and midrow banding capability. Melita locations in 2016 and 2017 were seeded using a 6-row small plot seeder with 24 cm row spacing and dual knife openers. A 4-row seeder with 30 cm spacing and disc openers was used in Carberry and a 7-row double disc seeder with 19 cm row spacing was used in Grosse Isle. A blanket application of seed placed MAP (11-52-0) was applied at 40 lbs P_2O_5/ac across all treatments at each site. Seeding rate was determined for a target plant population of 250 plants per square meter, which is the current provincial recommendation. Herbicides were applied as needed throughout the season depending on the weed spectrum present at each site. Twinline fungicide (200 mL/ac) was applied at flag leaf for leaf disease control and Caramba (400 mL/ac) was applied at anthesis for fusarium head blight control at all sites.

At soft dough, all plots were visually rated for lodging using the rating scale developed by Berry et al. (2003). At physiological maturity, biomass samples were taken from the middle four rows, 0.5 m row length (Note: at Carberry, only the middle 2 rows were sampled, to avoid outer rows in those four row plots). Two samples were taken for each plot, one near the front and the other near back, at a minimum of 1 m from plot border. Biomass samples from each plot were air-dried for 1 week, weighed and threshed using a stationary Wintersteiger Classic small plot combine. Entire grain samples were collected from each biomass sample, while a subsample of homogenized straw was taken from each plot sample. Plot harvest Index (HI) was determined for each plot using equation 1. Grain and straw samples were then oven dried and re-weighed. Straw samples were ground in the Wiley mill (2mm), while a grain sample (~50 g) from each plot was ground using a Tecator Cyclotec mill. Ground samples of grain and straw were sent to AGVISE Laboratories for total N analysis, determined by Dumas combustion.

(1) **Harvest Index (HI)** = Grain Biomass (g) / Total Biomass (g)

Gold level locations had outside rows removed prior to grain harvest to avoid any possible edging effects from neighboring plots. Trial grain harvest by plot was conducted with a Wintersteiger Classic small plot combine equipped with a HarvestMaster that was calibrated to measure grain weight and moisture during harvest at all gold level experiment sites. Silver level sites were also harvested with Wintersteiger Classic small plot combines and whole samples were retained. For the silver level sites, grain weight and moisture content were determined manually on an individual plot basis, following completion of harvest. Grain yields and protein concentrations were corrected to 13.5 % moisture content. Grain protein content was determined by multiplying grain N content by 5.7 which corresponds to the standard method used by the Canadian Grain Commission for milling quality wheat. Nitrogen uptake and removal was calculated for each plot using combine grain yields, harvest index and straw/grain total N % determined from biomass sampling (equations 2-7).

(2) **Total Biomass (lbs/ac)** = Grain Yield (lbs/ac) / Harvest Index

(3) **Straw Yield (lbs/ac)** = Total biomass (lbs/ac) – Grain yield (lbs/ac)

(4) **Grain N (lbs/ac)** = Grain yield (lbs/ac) * (Grain N % / 100)

(5) **Straw N (lbs/ac)** = Straw yield (lbs/ac) * (Straw N % / 100)

(6) **N Uptake (lbs/ac)** = Grain N (lbs/ac) + Straw N (lbs/ac)

(7) **N Removal (lbs/ac)** = Grain N (lbs/ac)

Agronomic efficiency (AE) was also determined for each plot using equation 8.

(8) **FNUE** = ((Fertilized Plot Grain Yield – Check Plot Grain Yield) / Applied N)

Economic analysis was completed on N rate treatments using a 5-year average urea price of $0.43/lbs N and wheat prices for #1 CWRS and CNHR in southern Manitoba on Jan 8, 2017. Wheat prices included protein premiums and we assumed that the market accessibility for both classes of wheat was not restricted by a defined threshold for protein content (e.g., 13%). Return to N inputs ($) was determined for each plot using equation 9.

(9) **Return to N ($/ac)** = (Yield (bu/ac) * Selling Price ($/bu)) – (N applied (lbs N/ac) * $0.43/lbs N)

Statistical analysis

A factorial design was used; site-year, variety and N treatment and their interactions were fixed factors. All experiments were arranged as a randomized complete block design; therefore, block was a random factor within each site-year. Proc GLIMMIX was used to complete lsmeans comparisons using Tukey's method to determine means groupings for grain yield, grain protein content, N uptake, N removal, FNUE and Return to N. Predetermined linear contrasts were used for biologically relevant comparisons to determine the most effective timing, source and placement of N fertilizer. Proc nlmixed was used to test numerous segmented models to yield compared to total N supply for each variety within each site-year.

RESULTS & DISCUSSION

Nitrogen Rate

The relationship between grain yield and total N supply (spring residual NO_3-N + fertilizer N applied) was explored using regression analysis for each site-year and variety through non-linear mixed models with the goal of determining a common model for yield response to N across sites and varieties. The suitability of the models was measured using AICC values to compare linear-plateau, linear-linear, quadratic-plateau, quadratic and linear models. The lowest AICC value was used to determine best fit (Table 1.3). Do to the high variability in which yield response model was best across site years and varieties, we determined that regression analysis was not suitable for determining N rate response.

A global analysis of variance (ANOVA) was used to determine the influence of N treatment, variety and site-year on grain yield and protein content. The global ANOVA analysis of yield responses of gold level sites indicated all main effects (N treatment, variety, site-year) were significant ($p<0.05$), as were the 2-way interactions (N treatment*variety, N treatment*site-year, Variety*Site-year). However, the 3-way interaction between site-year*N treatment*variety was not significant (Appendix Table 4.1). Analysis of silver level sites also indicated that all main effects and the two way interactions with site-year played a significant role in determining grain yield. However, for the silver level sites, the two-way interaction of N treatment*variety and the 3-way interaction did not have significant effects on grain yield (Appendix Table 2). For both gold and silver level sites, the ANOVA for grain protein content indicated a significant effect of all three main effects and the 2-way interactions with site-year, while the two-way interaction of N treatment*variety and the 3-way interaction did not have significant effects (Appendix Table 4-5).

AAC Brandon spring wheat variety produced grain yields that were significantly lower and grain protein contents that were significantly higher than for Prosper at all site-years, across both levels of trials (Figure 1.1-1.8). At gold level sites, Prosper yielded 7.8 – 15.4 bu/ac more than AAC Brandon, while AAC Brandon had 1.3 – 2.0 % higher grain protein content (Appendix Tables 1, 4). Prosper yielded 4.3 – 16.3 bu/ac more and had 0.7 – 1.3% lower grain protein content than AAC Brandon across silver level sites (Appendix Tables 1, 5).

The minimum rate of N fertilizer required to match the biological maximum grain yield and protein content across varieties was determined using means separations at each site-year. At Brunkild 2016, the equivalent of biological maximum yield was reached at 110 lbs N/ac and maximum protein content at 170 lbs N/ac (Figure 1.1). This site had satisfactory yields for the 2016 growing season but relatively low grain protein content, with AAC Brandon at 200 lbs N/ac being the only treatment that reached 13%. At Carman 2016, the minimum rate of N required to match maximum yield and protein was 140 lbs N/ac (Figure 1.2). This site had modest yield and high grain protein content across all N rates, most likely due to hail damage at flag leaf timing which probably limited yield potential at this site. Brunkild 2017 was the highest yielding site-year across both growing seasons, with yields reaching 130 bu/ac and the equivalent of maximum yield was reached at 110 lbs N/ac (Figure 1.3). Grain protein content was low regardless of the rate of N applied at this site, with no treatment reaching 13% for either variety; however, the equivalent of maximum protein within this range of N rates was obtained at 140 lbs N/ac. At Carman 2017 there was little yield response to applied N fertilizer with the maximum equivalent yield being reached at 50 lbs N/ac (Figure 1.4A). The minimum rate of N required to maximize grain protein content was 140 lbs N/ac (Figure 1.4B). Across gold level site-years, there were similar residual NO_3-N levels ranging from 40-47 lbs N/ac in the 0-60 cm depth (Table 1.1) and the sites were in close geographic proximity (~40 km) to each other. The variation in N responsiveness across these sites is likely due to differences in soil N mineralization potential (Chapter 2) and yield potential across these sites.

Silver level sites had larger variation in soil residual NO_3-N levels ranging from 11 lbs N/ac at Melita 2017 to 89 lbs N/ac in Carberry 2016. In Melita 2016, there were relatively modest yields with the minimum rate of N required to match maximum yield being only 80 lbs N/ac (Figure 1.5A). Protein contents were high across all N rates, with a protein content of 13% reached without applying any N fertilizer, which may indicate influence of other yield limiting factors at this site other than N supply (e.g., high disease pressure, moisture stress, etc.). The equivalent of maximum protein content was reached at 110 lbs N/ac for this site-year (Figure 1.5B). Carberry 2016 had little response to N fertilizer with maximum yield and protein being obtained at 50 lbs N/ac (Figure 1.6). This small response can be contributed to high residual NO_3-N (89 lbs N/ac) as well as high growing season N mineralization (130 lbs N/ac, Chapter 2) at this site. At Melita 2017 the minimum rate of N required to match maximum yield and protein was 110 lbs N/ac (Figure 1.7). Protein content was low across all N treatments at this site; AAC Brandon with 170 lbs N/ac applied was the only treatment to reach 13% grain protein content. At Grosse Isle 2017, the equivalent of maximum grain yield was obtained at 80 lbs N/ac while grain protein content was only maximized at 140 lbs N/ac (Figure 1.8).

The economic optimum rate of N was determined for each site year and is summarized in Table 1.4. Across all site-years, both varieties had similar economic responses to N rates applied resulting in no significant interaction between N treatment*Variety (Appendix Tables 7-8). The economic optimum N rate was 140 lbs N/ac at all gold level sites. The total N supply (spring NO_3-N + fertilizer N) required per bushel of grain yield varied from 1.7 lbs N/bu at Brunkild 2017 where grain yield were very high to 3.0 lbs N/bu at Carman 2016 where grain yield was limited by midseason hail damage. Total N supply per bushel at silver sites varied from 1.5 lbs N/bu at Carberry where large amounts of N were supplied from the soil to 2.3 lbs N/bu at Grosse Isle 2017. Overall, if the hail-damaged site at Carman 2016 is excluded from the analysis, the average total N supply per bushel at the optimum rate of fertilizer N was 1.99 lbs N/bu.

Nitrogen Timing

To determine significant differences between N timing treatments, predetermined linear contrasts were used to compare specific sets of N treatments across varieties and site-years. When comparing N applied at planting as broadcast Agrotain-treated urea to equivalent cumulative rates of N applied as split applications at planting and at stem elongation or flag leaf there was a 3.3 bu/ac increase with stem elongation timing at gold level sites (Figure 1.9A, 1.10A). All other midseason application timings at gold and silver level sites had similar yields the equivalent rates applied at planting.

Split application of N at planting and flag leaf resulted in higher grain protein content than equivalent rates of N applied entirely at planting or as split applications at stem elongation for gold and silver level sites (Figures 1.9B, 1.10B). Split applications of N at planting and stem elongation resulted in an increased grain protein content compared to N applied entirely at planting at gold level sites (Figures 1.10B). Nitrogen uptake was increased by 14.2 and 10.6 lbs/ac when N applications were split between planting and stem elongation and flag leaf, respectively, compared to N applications entirely at planting at gold level sites (Figure 1.11A). There were no significant differences between N uptake when N was applied entirely at planting or split with stem elongation or flag leaf timing at the silver level sites (Figure 1.12A). At both gold and silver level sites, N removal rates increased when N application was applied using a stem elongation split timing compared to entirely at planting. Flag leaf split applications increased N removal at gold level sites but removal rates were similar to applications entirely at planting for silver level sites (Figure 1.11B, 1.12B). Fertilizer agronomic efficiency (AE) was similar regardless of N application timing at gold and silver level sites (Appendix Table 17).

These results indicate that it is possible in Manitoba growing conditions to delay a portion of total N applied into the growing season without detrimental effects to final grain yield or protein. Additionally, applying N as late as the flag leaf stage may allow for increased grain protein content compared to when N is applied entirely at or near planting. However, to avoid stranding N fertilizer on the surface of the soil, rainfall is required soon after midseason applications of N. In our study, growing season rainfall varied greatly between 2016 and 2017, with ~50% less growing season rainfall in 2017 than in 2016. Nevertheless, regardless of the total growing season rainfall across all site-years there was a rainfall event of at least 5 mm within one week of N application (Appendix Figures 1-4). This rainfall occurrence after application is likely a major contributing factor to the success of the midseason N applications in this study, compared to others in the Canadian Prairies. Additionally, we must reinforce that the midseason applications at stem elongation and flag leaf were applied using Agrotain-treated urea to minimize volatilization losses that may have occurred in the event that rainfall did not occur soon after application.

Post-anthesis, foliar applications of N are typically used as a method to increase grain protein content rather than yield due to the application timing being so late in the season. Therefore, it is important to compare post-anthesis split applications to the base rate as well as the equivalent rate applied at planting. At gold level sites, yield for the base rate of 80 lbs N/ac was similar to that for the post-anthesis split application of 80 + 30 lbs N/ac (Figure 1.13A). However, at silver level sites, yield was decreased by 3.3 bu/ac by post-anthesis application compared to the base rate (Figure 1.14A). Yields of equivalent rates of N applied in the spring were greater than for the base rate plus post-anthesis split application, which indicates 80 lbs N/ac was insufficient for yield potential across site-years. Post-anthesis split applications increased protein content compared to both the base rate and the equivalent rate of N applied entirely at seeding for both gold and silver level sites (Figure 1.13B, 1.14B). At gold level sites post-anthesis applications increased protein content by 1.8% and at silver sites 1.1% compared the base rate of N applied at planting.

Nitrogen uptake was increased by 21 and 16 lbs N/ac at gold and silver sites, respectively, by post-anthesis application compared to the base rate (Figure 1.15A, 1.16A). Post-anthesis application increased N removal compared to the base rate at gold level sites, but not at silver level sites. At silver level sites, N removal was larger for equivalent rates of N applied entirely at planting, compared to post-anthesis split applications, while these treatments resulted in similar rates of N removal at gold level sites (Figure 1.15B, 1.16B). At gold level sites, post-anthesis split applications resulted in AE that was similar to the base rate or the equivalent rate of N applied entirely at planting. At silver level sites, AE was reduced by 6.7% compared to the base rate and 7.5% compared to equivalent rates of N applied entirely at planting. A reduction of AE would be expected with an application targeted towards increasing protein content as the calculation for AE considers only grain yield and not grain protein content.

This research indicates consistent increases in grain protein content by post-anthesis fertilizer application with little to no influence on grain yield compared to the base rate of N applied. Therefore, adequate N must be applied earlier in the season to meet yield potential and post-anthesis applications would be warranted if there is risk of inadequate protein content. However, two of the challenges with this strategy are to predict whether the protein content would be “inadequate” without the post-anthesis application and whether the increase in protein content after the post-anthesis application would be sufficient to improve market access, based on meeting minimum protein content for milling quality wheat (e.g., 13%).

Nitrogen Source

Dissolved urea solution was tested as an additional N source for post-anthesis applications at gold level sites and significant differences between UAN and urea solution were determined using predetermined linear contrasts. Compared to post-anthesis applications of UAN, application of dissolved urea resulted in a 4.1 bu/ac increase in grain yield and a 0.6% protein increase (Table 1.5). Applications of urea solution also resulted in 11.0 and 10.3 lbs/ac more N uptake and removal, respectively, compared to applications of UAN. Both sources resulted in similar AE. Leaf burn was observed for both sources of post-anthesis foliar-applied N, but foliar damage was considerably less for dissolved urea solution than UAN, which may be responsible for a decrease in grain yield with UAN compared to urea solution (Figure 1.17). Both post-anthesis sources were applied at the same rate of N (30 lbs/ac) but because the urea solution was more dilute, it was applied at a water volume that was 17 US GAL/ac more than the UAN solution. This large water volume could have contributed to the decreased leaf burn with the urea solution compared to the UAN.

ESN blends produced grain yield, protein, N uptake, N removal and AE that were similar to those for conventional urea when applied midrow banded at seeding (Table 1.6). This indicates that there was no significant benefit to blending ESN with urea when applied at planting by midrow banding, under the environmental conditions of our study. However, early season moisture conditions during our study were relatively dry; if conditions had been wetter, the ESN blends might have outperformed conventional urea due to smaller leaching and denitrification losses.

Nitrogen Placement

At gold level sites, N was applied at a suboptimal rate of 80 lbs N/ac using two different placement methods, banding and broadcast. Contrary to our expectations, banded conventional urea resulted in 4.9 bu/ac lower grain yield than broadcast Agrotain-treated urea. Nitrogen uptake and removal was also reduced by 14.5 and 10.6 lbs N/ac when N was banded rather than broadcast and AE was 5.9% less (Table 1.7). The reasons for the poorer performance of banded conventional urea, compared to broadcast Agrotain-treated urea are not known.

CONCLUSIONS

The total N supply (soil test NO_3-N + fertilizer N) required to obtain economic optimum yield and protein varied from 1.5 – 2.3 lbs N/bu at silver level sites and 1.7 – 3.0 lbs N/bu at gold level sites. However, if we exclude the hail damaged site at Carman 2016, the average total N supply at the optimum yield and protein content was 1.99 lb N/bu. This requirement is less than the current recommendation of 2.5 lbs N/bu.

Midseason split application at planting and at stem elongation or flag leaf stages yielded at least as much as equivalent rates applied entirely at planting. Flag leaf split applications consistently increased grain protein content compared to equivalent rates of N split applied at planting and stem elongation.

Late season post-anthesis N applications consistently increased grain protein content, regardless of N source. However, post-anthesis applications of urea solution increased grain yield and protein content above that for post-anthesis applications of UAN. There was no advantage to ESN blends over conventional urea when applied banded at seeding under the environmental conditions of our study.

REFERENCES

Holzapfel, C.B., Lafond, G.P., Brandt, S.A., May, W.E., and Johnston, A.M. 2007. In-soil banded versus post-seeding liquid nitrogen applications in no-till spring wheat and canola. Can. J. Plant Sci. **87**: 223–232. doi:10.4141/P05-224.

Nutrien Ltd. 2018. How ESN works. [Online] Available: http://www.smartnitrogen.com/what-esn/how-esn-works [2018 Feb. 26].

R.E. Karamanos, N.A. Flore, J.T.H. 2004. Effect of post-emergence nitrogen application on the yield and protein content of wheat. Can. J. Plant Sci. **22**: 54. doi:10.1071/EA9820054.

Statistics Canada 2017. Principle field crop area. [Online] Available: http://www.statcan.gc.ca/daily-quotidien/170629/dq170629c-eng.htm [2018 Feb. 26].

Woolfolk, C.W., Raun, W.R., Johnson, G. V, Thomason, W.E., Mullen, R.W., Wynn, K.J., and Freeman, K.W. 2002. Influence of Late-Season Folier Nitrogen Applications on Yiled and Grain Nitrogen in in Winter Wheat. Agron. J. **94**: 429–434. doi:10.2134/agronj2002.0429.

Part 1: Tables

Table 1.1. Field research site characteristics and nitrogen fertilizer application dates for all site-years.

	Carman 2016	**Brunkild 2016**	**Carman 2017**	**Brunkild 2017**	**Melita 2016**	**Carberry 2016**	**Melita 2017**	**Grosse Isle 2017**
Level	Gold	Gold	Gold	Gold	Silver	Silver	Silver	Silver
GPS Coordinates	49.498, -98.031	49.592, -97.605	49.494, -98.041	49.610, -97.539	49.251, -101.032	49.9059, -99.3555	49.2247, -101.051	50.085, -97.429
Residual Spring Soil NO_3-N (0 - 60 cm)	47	40	43	43	43	89	11	65
Soil Texture	SCL	HC	LS	HC	SL	SL	L	C
Stubble Type:	Wheat	Canola	Flax	Soybeans	W. Wheat	Canola	Fall Rye	Canola
Seeding Date	28-Apr-16	05-May-16	02-May-17	05-May-17	06-May-16	05-May-16	10-May-17	02-May-17
Stem Elongation N Application	08-Jun-16	14-Jun-16	09-Jun-17	09-Jun-17	17-Jun-16	17-Jun-16	23-Jun-17	09-Jun-17
Flag Leaf N Application	21-Jun-16	29-Jun-16	22-Jun-17	22-Jun-17	23-Jun-16	23-Jun-16	29-Jun-17	26-Jun-17
Post-Anthesis N Application	04-Jul-16	06-Jul-16	07-Jul-17	07-Jul-17	08-Jul-16	08-Jul-16	06-Jul-17	10-Jul-17
Harvest date	01-Sept-16	29-Aug-16	24-Aug-17	25-Aug-17	22-Aug-16	02-Sept-16	28-Aug-17	29-Aug-2017

Table 1.2. Treatment list for gold and silver level experiments.

Variety	N Rate		Source		Timing/Placement	
	Planting	**In-season**	**Planting**	**In-season**	**Planting**	**In-season**
	------------- lbs N/ac ------------					
AAC Brandon (CWRS) Prosper (CNHR)	0		Urea (Gold), Agrotain-treated urea (Silver)		Midrow band at planting (Gold) Broadcast immediately after planting (Silver)	
	50					
	80					
	110					
	140					
	170					
	200*					
	80*		ESN:Urea (40:40)			
	140*		ESN:Urea (100:40)			
	80	30	Urea (Gold), Agrotain-treated urea (Silver)	Agrotain-treated urea		Stem Elongation, Broadcast
	80	60				
	80	30				Flag Leaf, Broadcast
	80	60				
	80	30		UAN		Post Anthesis, Foliar
	80*	30		Urea Solution		
	80*			Agrotain-treated Urea	Broadcast	

Note: * indicates treatments only included in Gold Level Experiments

Table 1.3. AICC values for yield regression models for total N supply (Spring NO_3-N + fertilizer) vs. yield for best-fit determination. Bolded numbers in each row indicate models with the lowest AICC values for determining the model with the best fit.

Site-Year	**Variety**	**Model**				
		Linear - Plateau	Linear - Linear	Quadratic - Plateau	Quadratic	Linear
Brunkild 2016	Brandon	**176.1**	178.4	235.9	201.7	177.2
Brunkild 2016	Prosper	176.3	**172.8**	240.7	180.6	180.8
Carman 2016	Brandon	187.2	191.9	205.4	188.5	**182.5**
Carman 2016	Prosper	182.0	183.1	240.6	181.3	**176.4**
Brunkild 2017	Brandon	197.3	192.8	211.5	**188.6**	209.7
Brunkild 2017	Prosper	214.2	**204.4**	247.3	211.0	211.6
Carman 2017	Brandon	200.3	198.3	205.6	198.2	**196.1**
Carman2017	Prosper	199.8	**198.6**	203.9	203.0	202.8
Melita 2016	Brandon	129.2	129.6	152.0	**127.6**	133.2
Melita 2016	Prosper	**141.2**	144.8	174.7	145.1	143.5
Carberry 2016	Brandon	171.7	177.8	182.9	**171.7**	167.1
Carberry 2016	Prosper	188.8	192.0	192.4	188.2	**180.1**
Melita 2017	Brandon	152.9	153.9	183.1	152.0	**150.8**
Melita 2017	Prosper	163.6	159.0	194.4	157.4	**154.6**
Grosse Isle 2017	Brandon	**162.4**	165.6	223.7	166.3	167.4
Grosse Isle 2017	Prosper	165.1	171.7	233.2	**156.3**	170.7

Table 1.4. Summary of economic optimum N rate and total N supply at each site year. Economic margins for N fertilizer based off grain pricing from Jan 5, 2018 and 5-year average urea price of \$0.43/lbs N, with market access assumed to be unrestricted by minimum protein thresholds.

Level	Site-year	Spring NO_3-N (0-60 cm)	Fertilizer N Rate at Economic Optimum	Total N supply at Economic Optimum	Yield at Economic Optimum	Nitrogen Supply per bushel
		lbs N/ac	lbs N/ac	lbs N/ac	bu/ac	lbs N/bu
Gold	Brunkild 2016	40	140	180	75	2.4
	Carman 2016	47	140	187	62	3.0
	Brunkild 2017	43	140	183	110	1.7
	Carman 2017	43	140	183	96	1.9
Silver	Melita 2016	43	80	123	60	2.1
	Carberry 2016	89	50	139	95	1.5
	Melita 2017	11	140	151	74	2.0
	Grosse Isle 2017	65	110	175	75	2.3

Table 1.5. Predetermined linear contrasts between UAN and urea solution when applied at post anthesis for yield, protein, nitrogen uptake, nitrogen removal, fertilizer nitrogen use efficiency (FNUE) and post-harvest soil residual NO_3-N.

Response Variable	UAN	vs.	Urea Sol'n	Std. Err	P-Value
Yield (bu/ac)	75.62		79.67	1.78	0.0235
Protein (%)	12.91		13.51	0.14	<0.0001
N Uptake (lbs N/ac)	134.6		145.6	4.77	0.0190
N Removal (lbs N/ac)	100.1		110.3	2.93	0.0005
FNUE	24.61		27.52	2.69	0.2788

Table 1.6. Predetermined linear contrasts between ESN blends and urea when applied at planting for yield, protein, nitrogen uptake, nitrogen removal, agronomic efficiency (AE) and post-harvest soil residual NO_3-N.

Response Variable	Urea	vs.	ESN Blend	Std. Err	P-Value
Yield (bu/ac)	80.50		82.50	2.63	0.1294
Protein (%)	11.97		12.09	0.21	0.2345
N Uptake (lbs N/ac)	135.3		138.5	7.14	0.3792
N Removal (lbs N/ac)	100.0		103.5	4.34	0.1047
AE	27.99		27.92	3.96	0.9693

Table 1.7. Predetermined linear contrasts between band and broadcast applications of nitrogen fertilizer for yield, protein, nitrogen uptake, nitrogen removal and agronomic efficiency (AE).

Response Variable	Band	vs.	Broadcast	Std. Err	P-Value
Yield (bu/ac)	77.99		82.9	1.83	<0.0001
Protein (%)	11.45		11.43	0.15	0.9224
N Uptake (lbs N/ac)	118.9		133.4	4.96	0.0190
N Removal (lbs N/ac)	88.2		98.8	3.04	0.0006
AE	30.09		36.02	2.73	0.0311

Part 1: Figures

Figure 1.1. Spring wheat yield (A) and protein (B) response to nitrogen fertilizer rates at Brunkild 2016. The yield advantage for Prosper and protein advantage for Brandon were consistent across N rates. Similar letters at the top of each bar indicate statistically similar values for the average of Brandon and Prosper varieties.

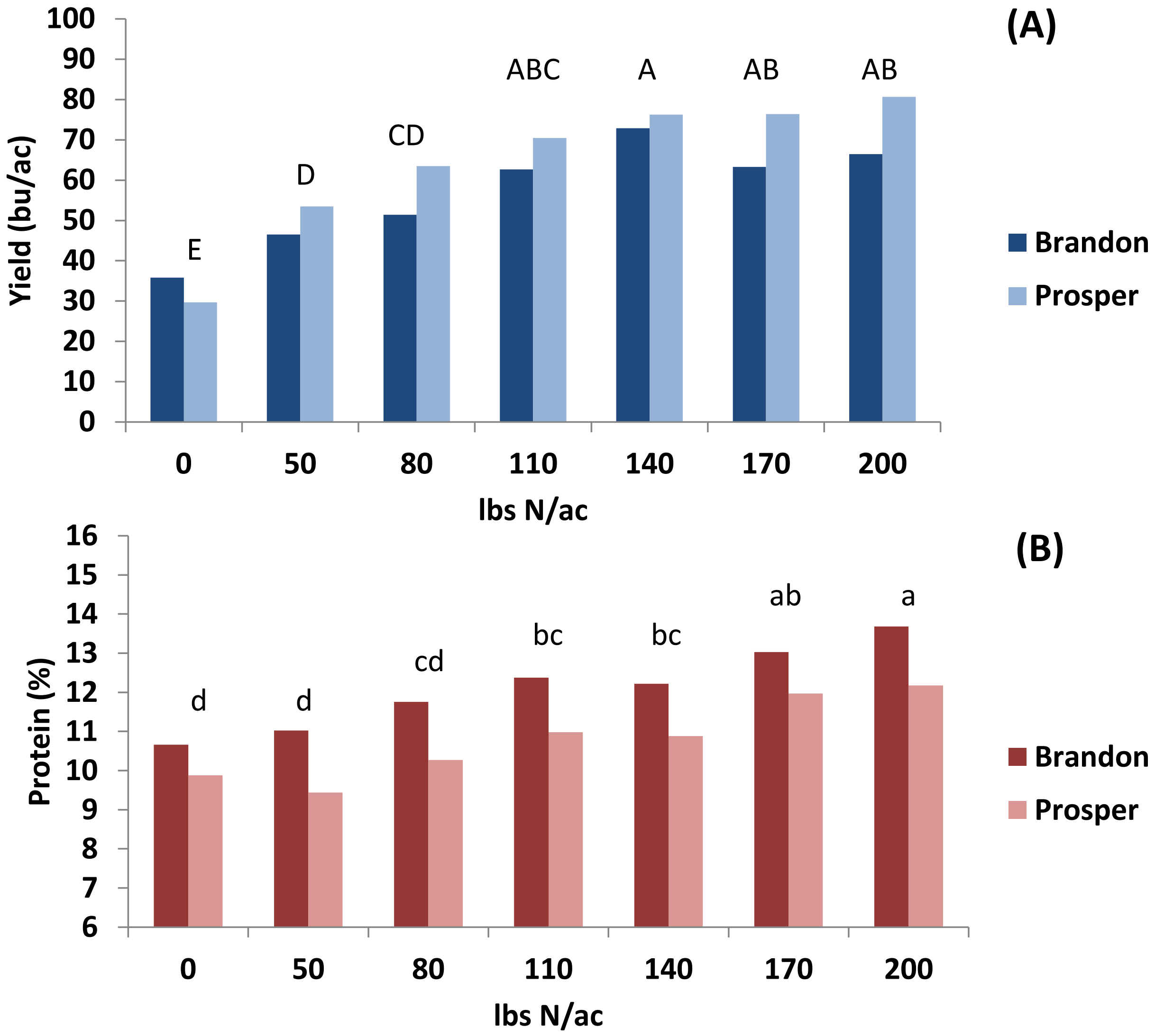


Figure 1.2. Spring wheat yield (A) and protein (B) response to nitrogen fertilizer rates at Carman 2016. The yield advantage for Prosper and protein advantage for Brandon were consistent across N rates. Similar letters at the top of each bar indicate statistically similar values for the average of Brandon and Prosper varieties.

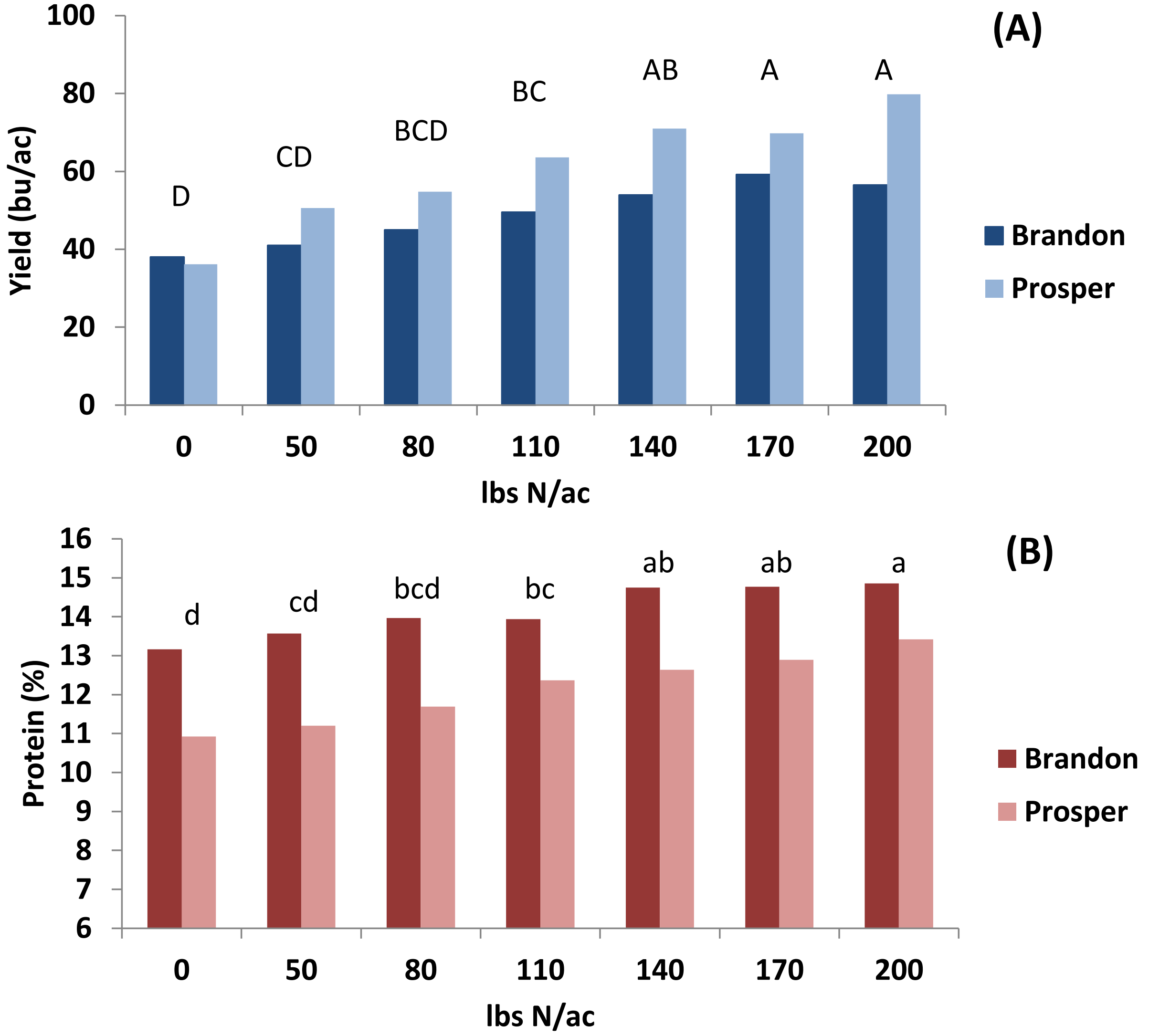


Figure 1.3. Spring wheat yield (A) and protein (B) response to nitrogen fertilizer rates at Brunkild 2017. The yield advantage for Prosper and protein advantage for Brandon were consistent across N rates. Similar letters at the top of each bar indicate statistically similar values for the average of Brandon and Prosper varieties.

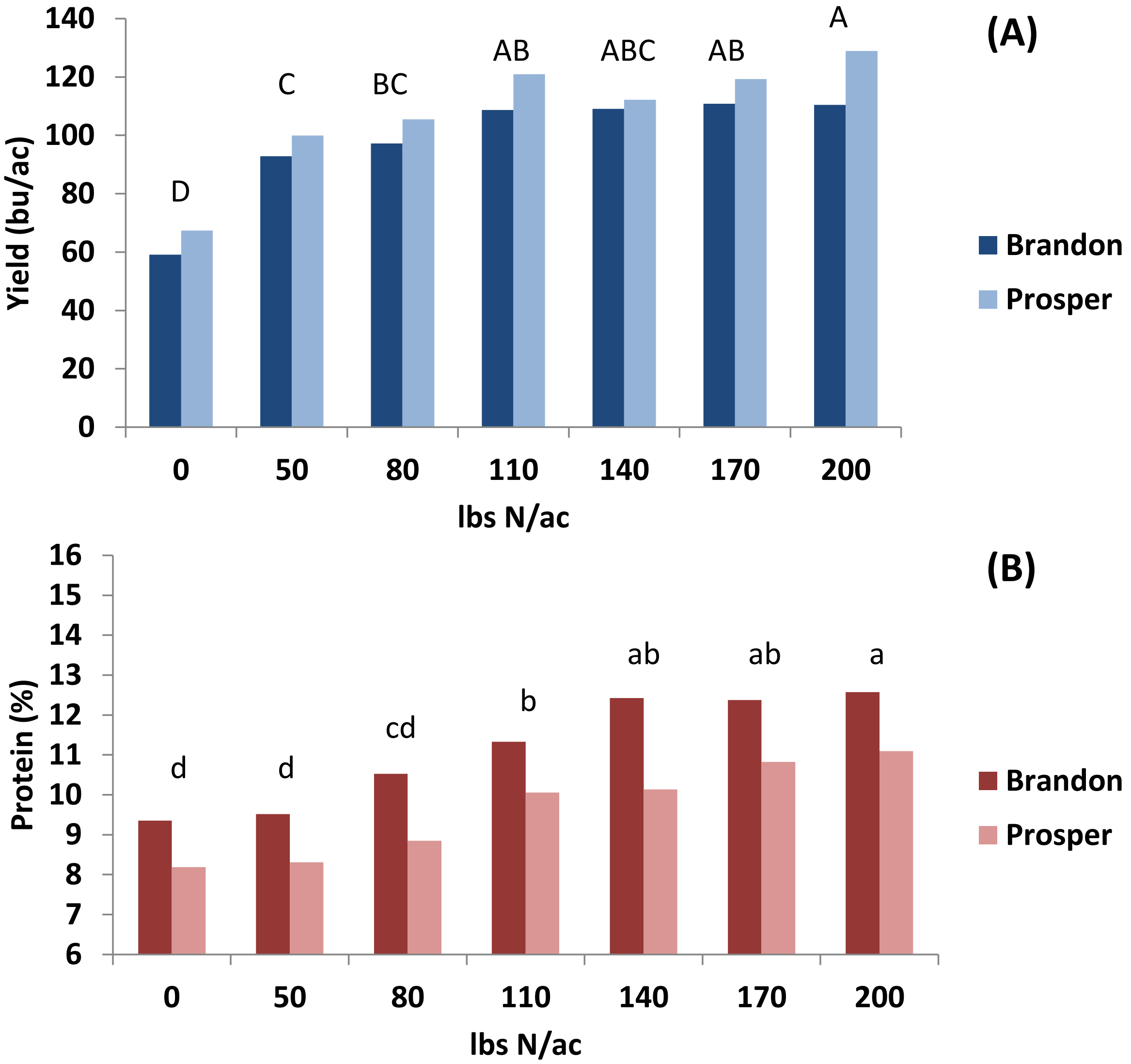


Figure 1.4. Spring wheat yield (A) and protein (B) response to nitrogen fertilizer rates at Carman 2017. The yield advantage for Prosper and protein advantage for Brandon were consistent across N rates. Similar letters at the top of each bar indicate statistically similar values for the average of Brandon and Prosper varieties.

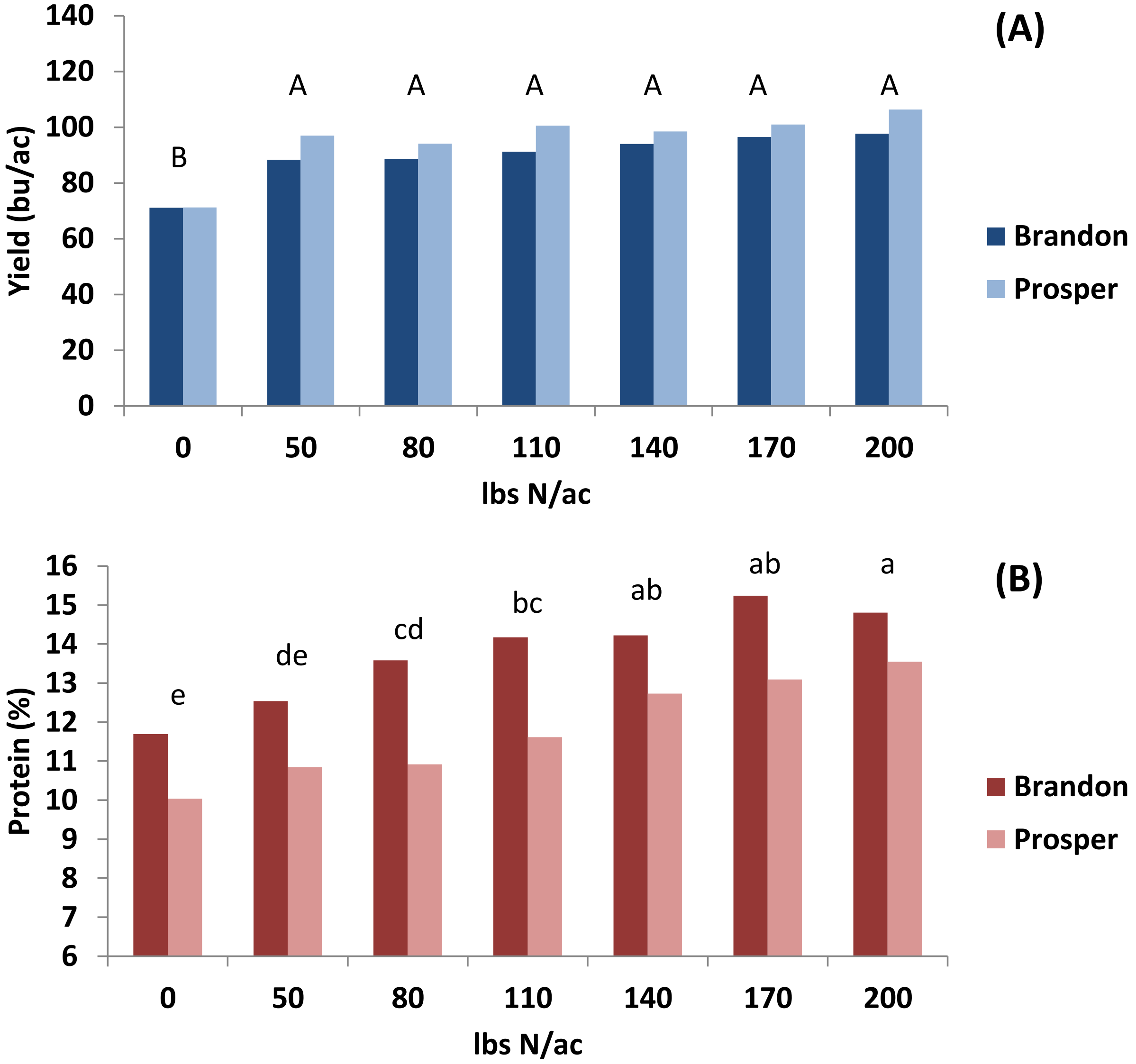


Figure 1.5. Spring wheat yield (A) and protein (B) response to nitrogen fertilizer rates at Melita 2016. The yield advantage for Prosper and protein advantage for Brandon were consistent across N rates. Similar letters at the top of each bar indicate statistically similar values for the average of Brandon and Prosper varieties.

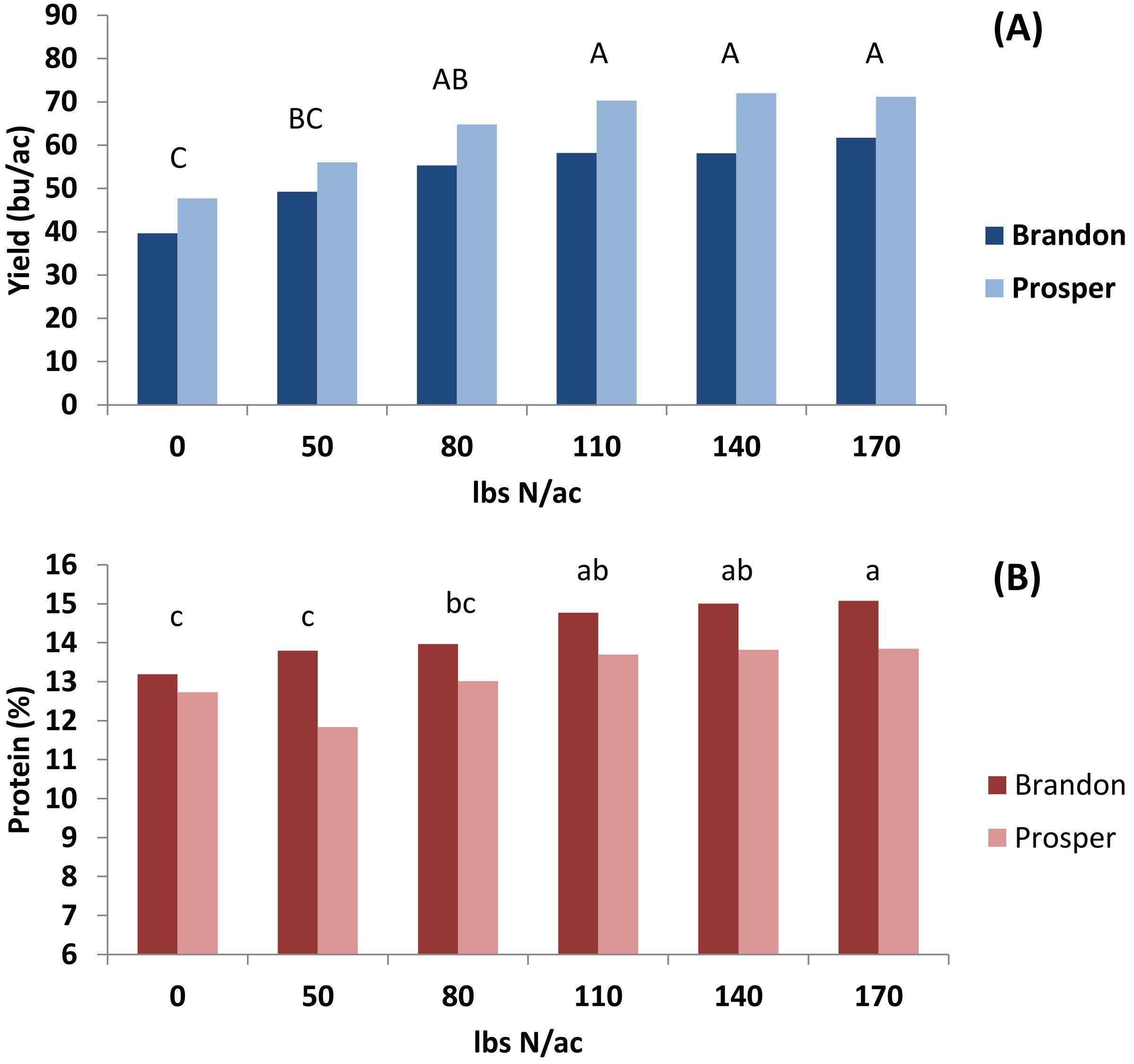


Figure 1.6. Spring wheat yield (A) and protein (B) response to nitrogen fertilizer rates at Carberry 2016. The yield advantage for Prosper and protein advantage for Brandon were consistent across N rates. Similar letters at the top of each bar indicate statistically similar values for the average of Brandon and Prosper varieties.

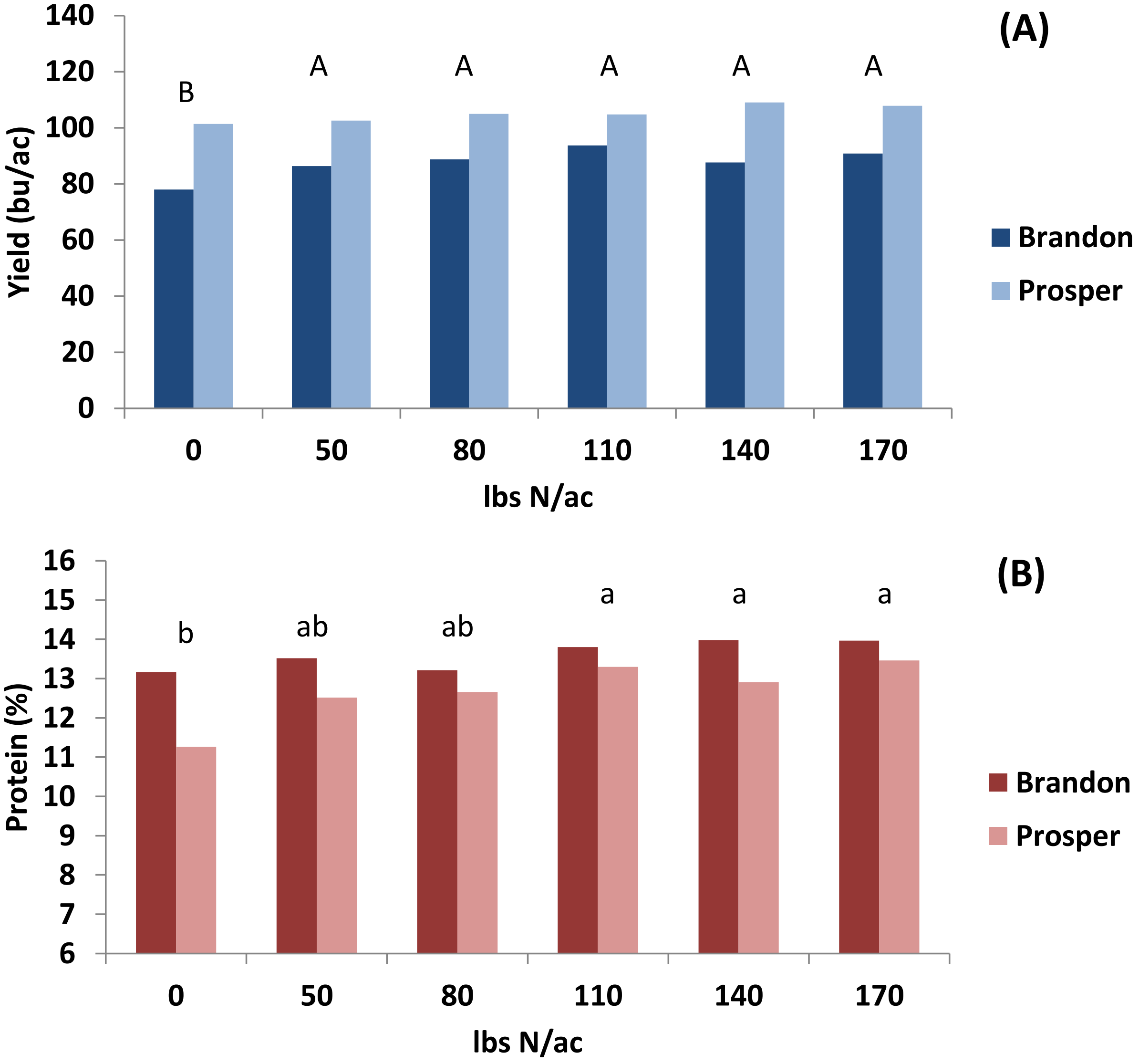


Figure 1.7. Spring wheat yield (A) and protein (B) response to nitrogen fertilizer rates at Melita 2017. The yield advantage for Prosper and protein advantage for Brandon were consistent across N rates. Similar letters at the top of each bar indicate statistically similar values for the average of Brandon and Prosper varieties.

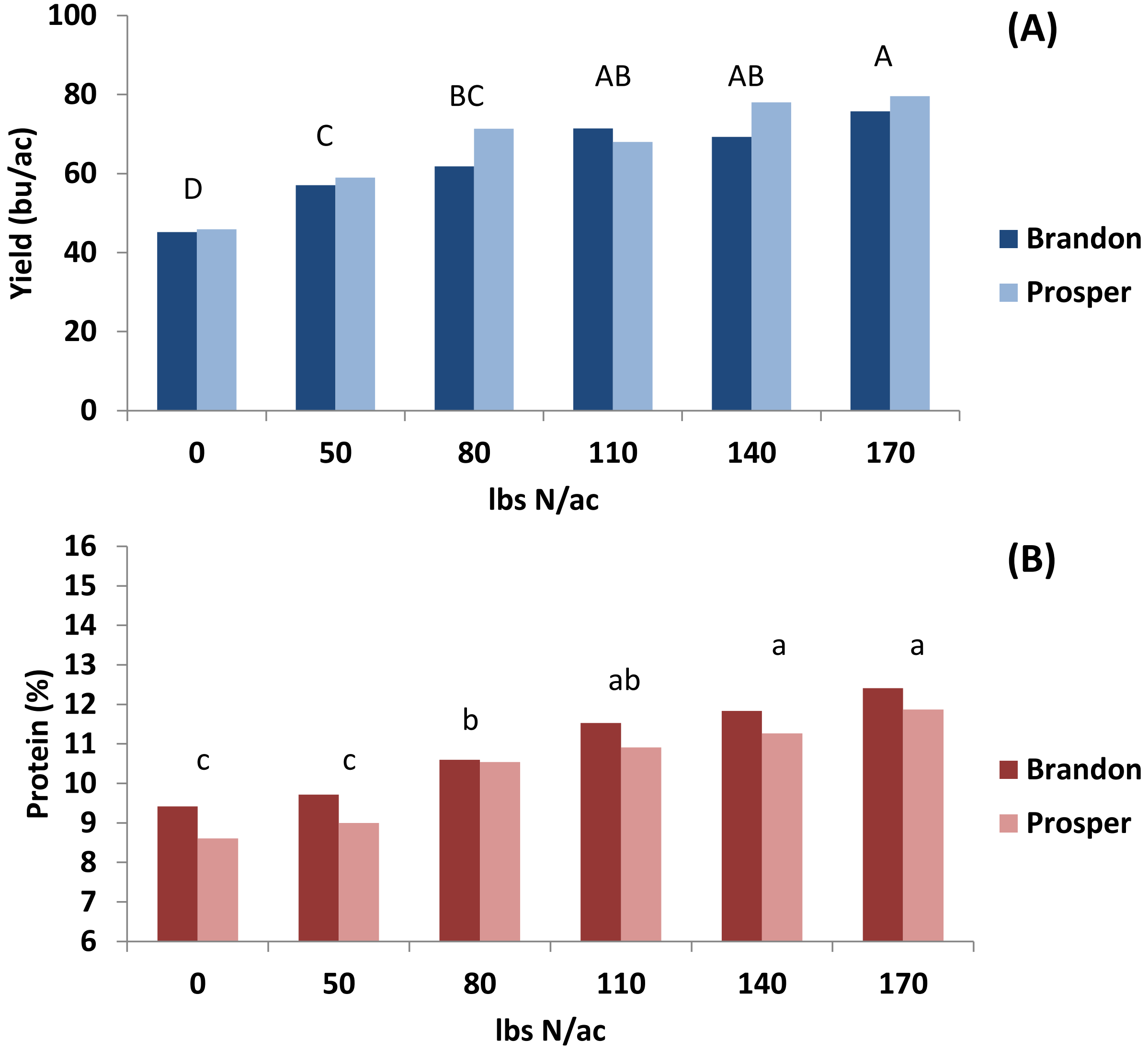


Figure 1.8. Spring wheat yield (A) and protein (B) response to nitrogen fertilizer rates at Grosse Isle 2017. The yield advantage for Prosper and protein advantage for Brandon were consistent across N rates. Similar letters at the top of each bar indicate statistically similar values for the average of Brandon and Prosper varieties.

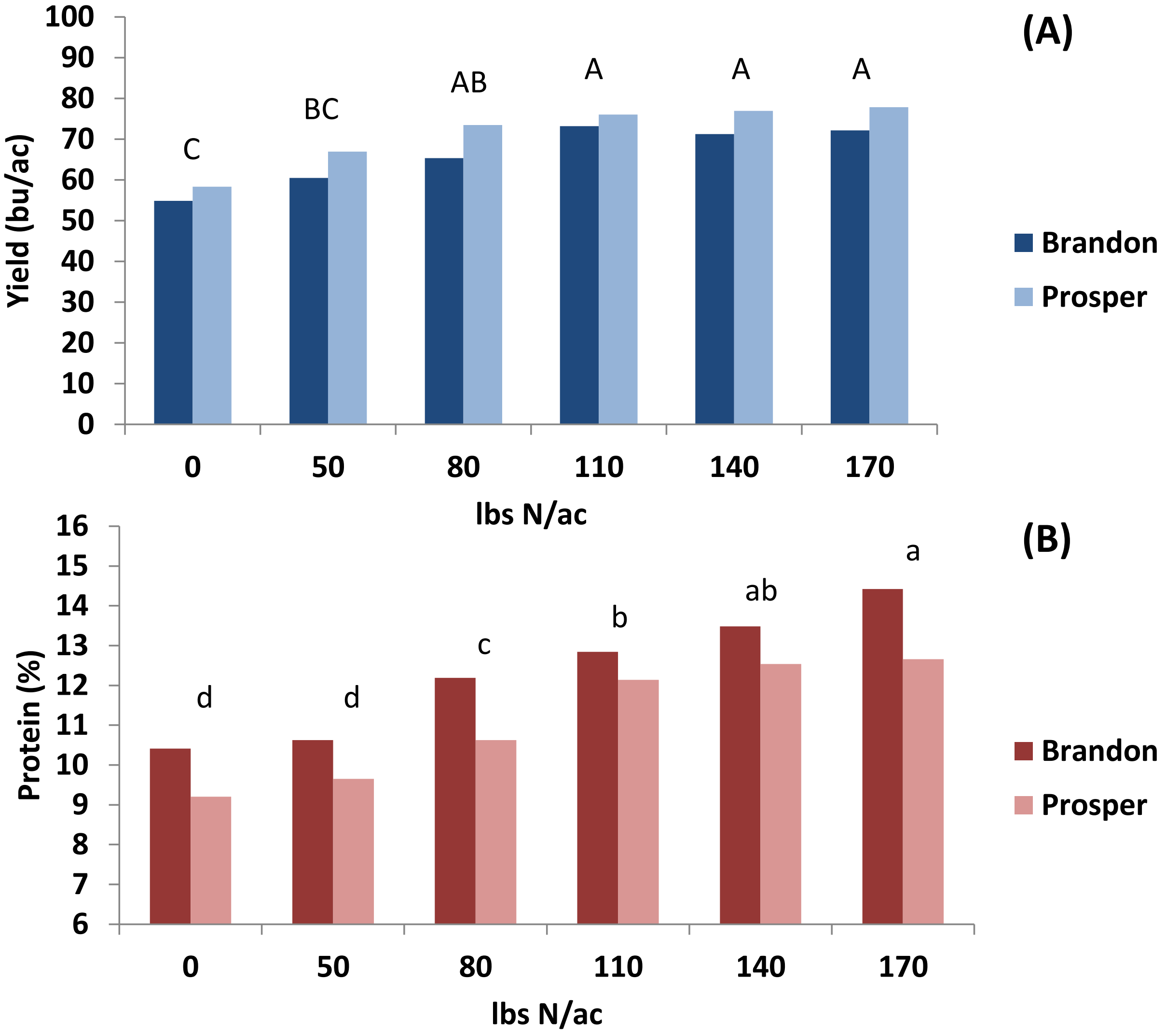


Figure 1.9. Grain yield (A) and protein content (B) response to midseason split nitrogen applications at gold level sites using predetermined linear contrasts across site-years and varieties to determine significant differences across timing treatments. Similar letters at the top of each bar indicate statistically similar values for the average of the two rates of N.

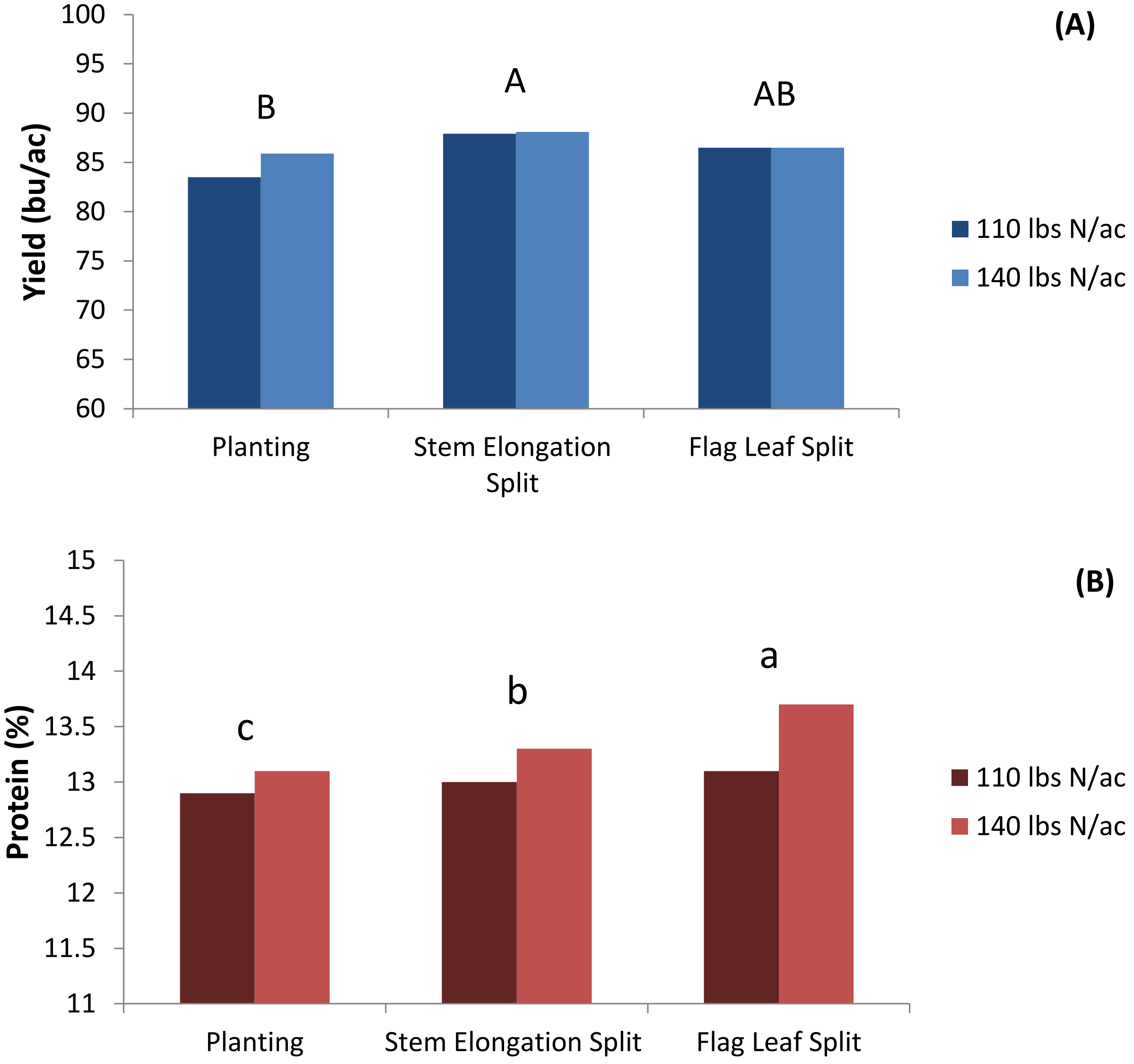


Figure 1.10 . Grain yield (A) and protein content (B) response to midseason split nitrogen applications at silver level sites using predetermined linear contrasts across site-years and varieties to determine significant differences across timing treatments. Similar letters at the top of each bar indicate statistically similar values for the average of the two rates of N.

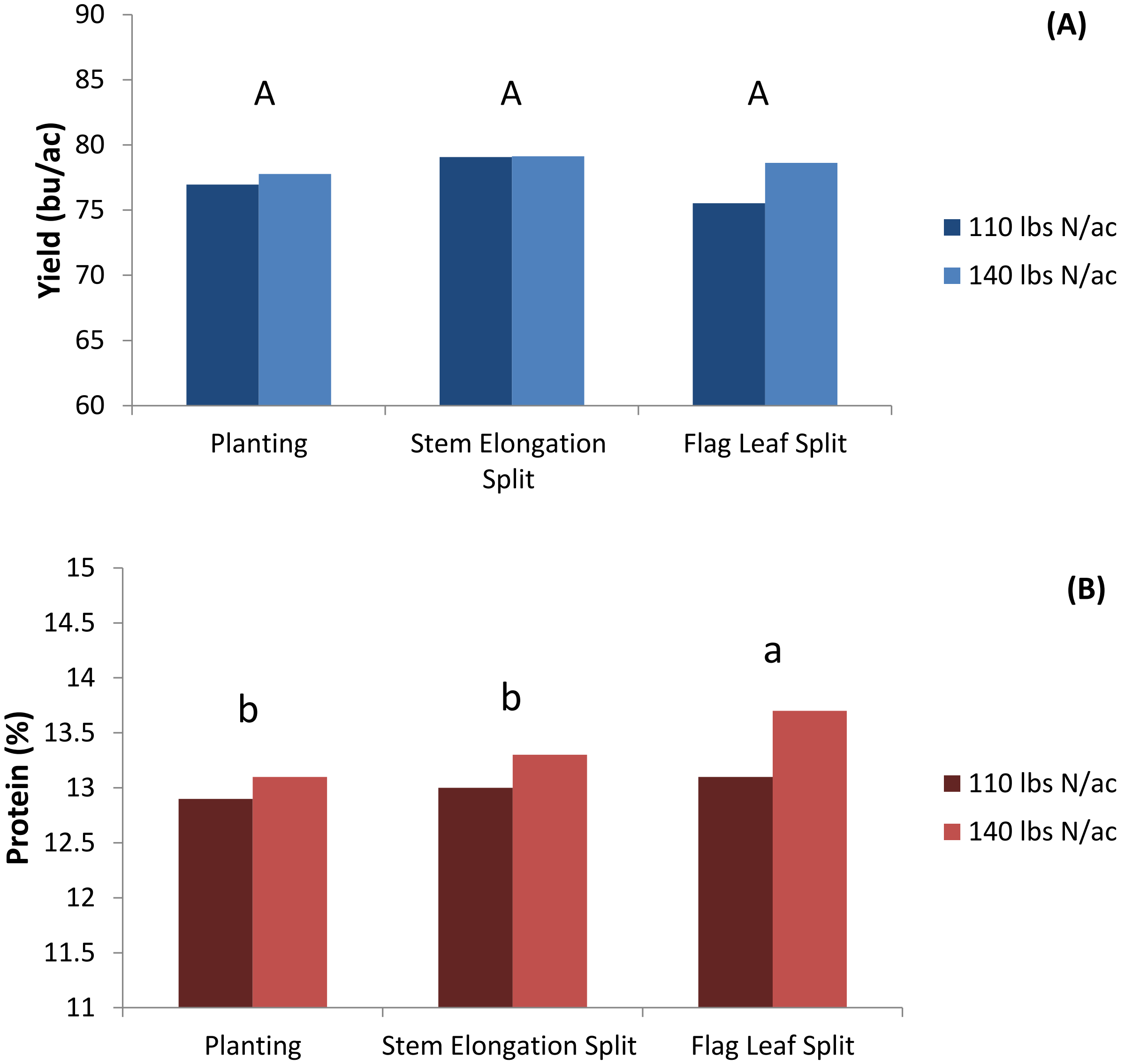


Figure 1.11. Nitrogen uptake (A) and removal (B) response to midseason split nitrogen applications at gold level sites using predetermined linear contrasts across site-years and varieties to determine significant differences across timing treatments. Similar letters at the top of each bar indicate statistically similar values for the average of the two rates of N.

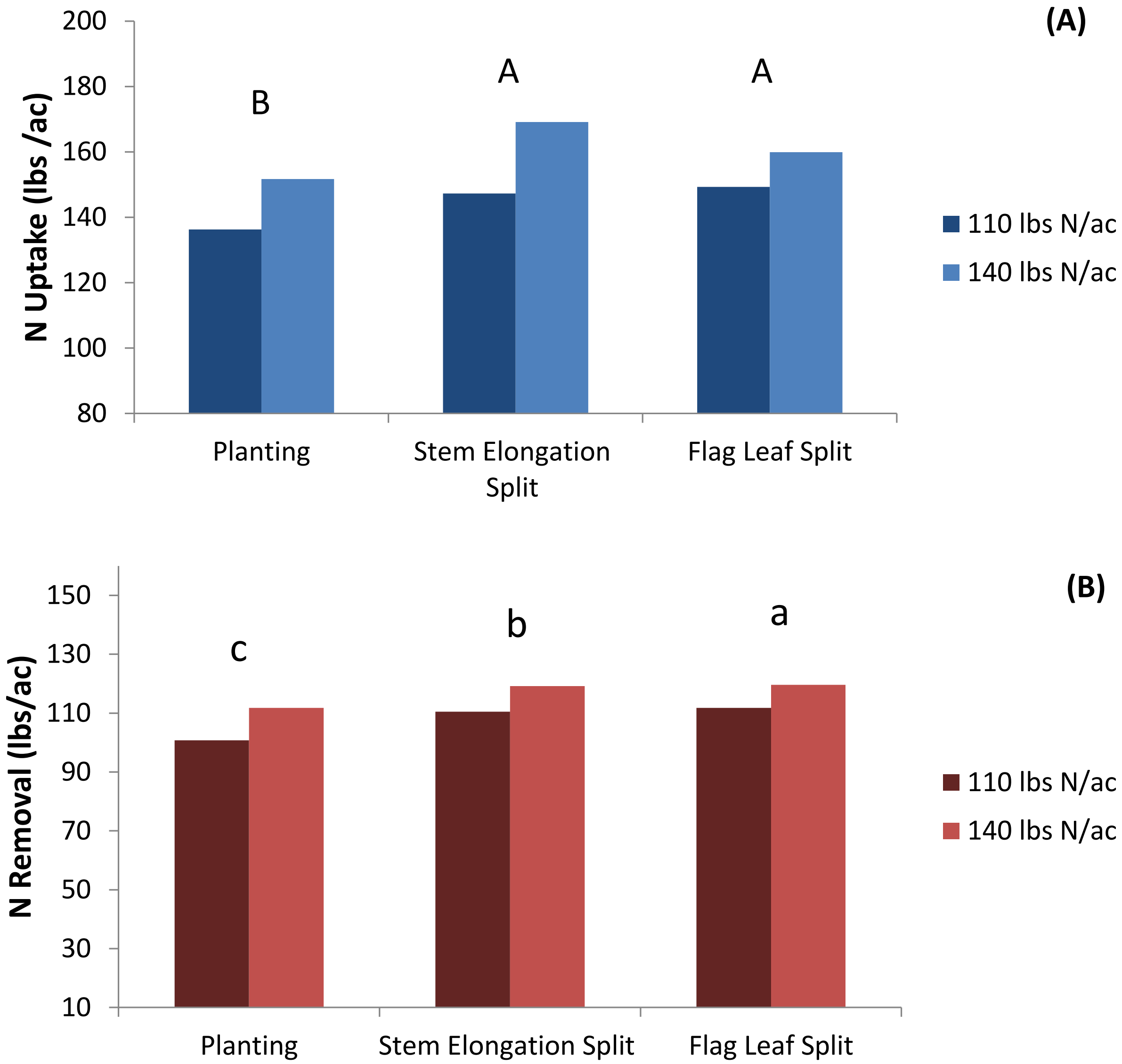


Figure 1.12. Nitrogen uptake (A) and removal (B) response to midseason split nitrogen applications at silver level sites using predetermined linear contrasts across site-years and varieties to determine significant differences across timing treatments. Similar letters at the top of each bar indicate statistically similar values for the average of the two rates of N.

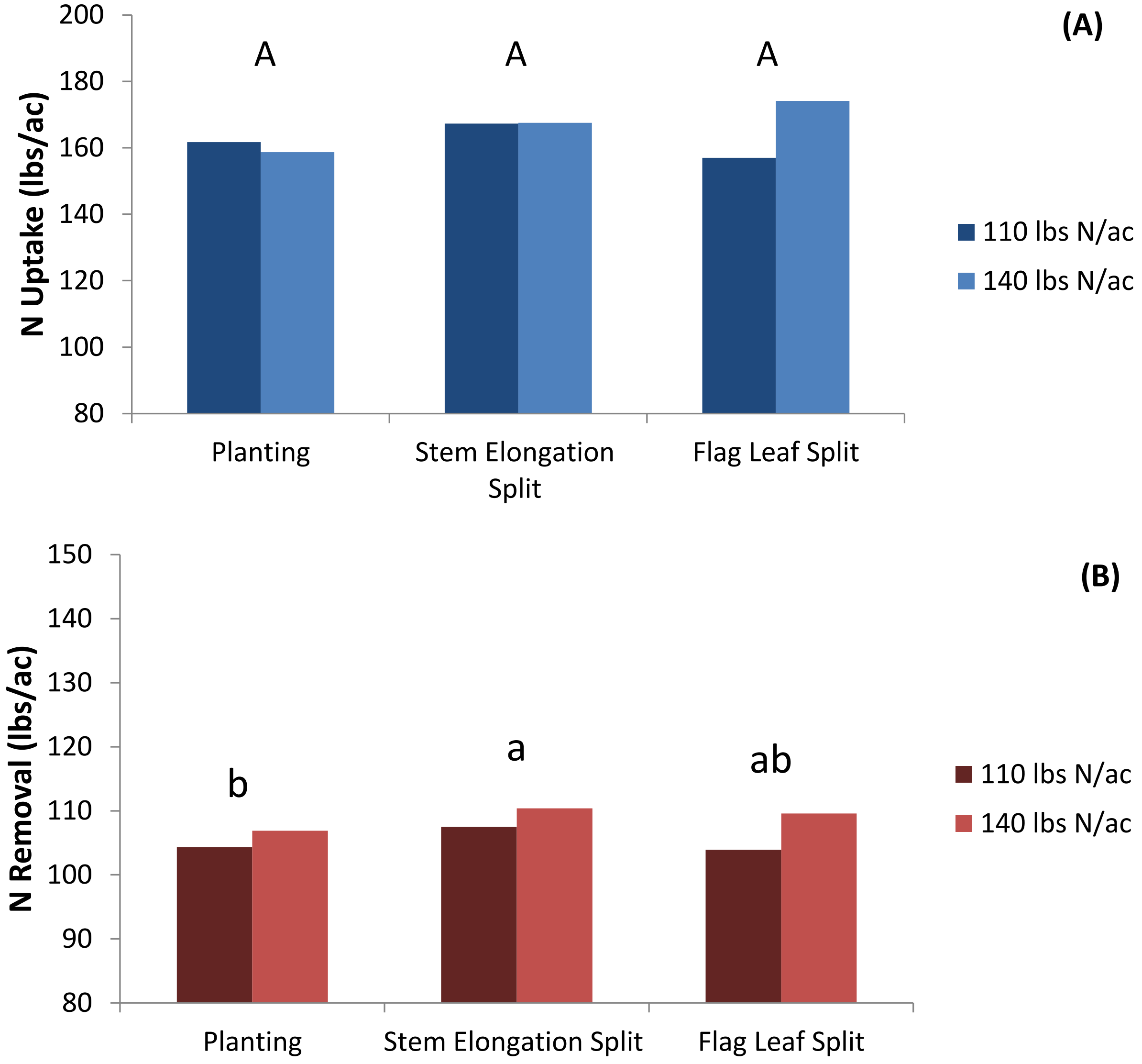


Figure 1.13. Grain yield (A) and protein content (B) response to post-anthesis split nitrogen applications at gold level sites using predetermined linear contrasts across site-years and varieties to determine significant differences across treatments. (*) indicates significant differences for N applied entirely at planting, compared to split application

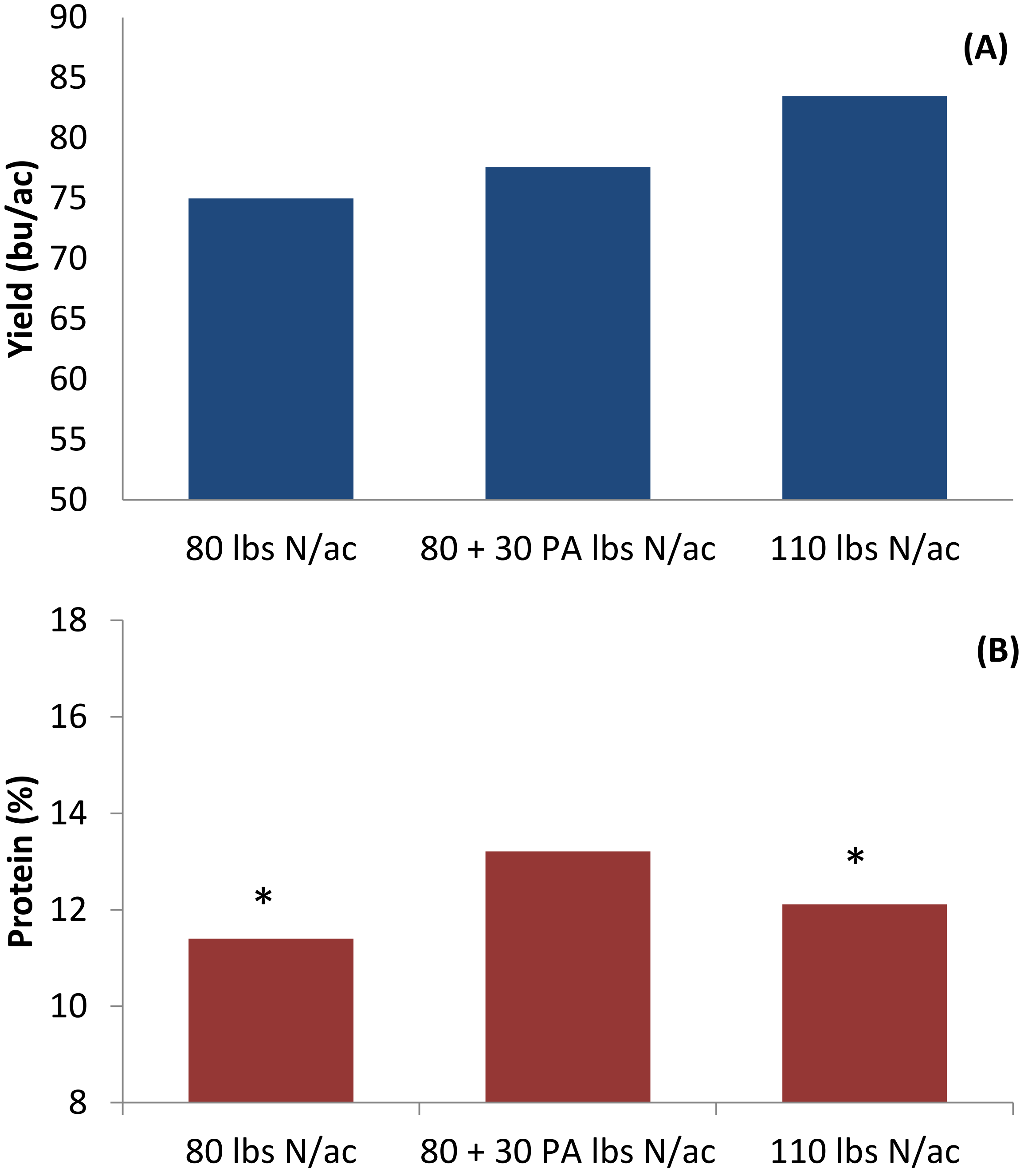


Figure 1.14. Grain yield (A) and protein content (B) response to post-anthesis split nitrogen applications at silver level sites using predetermined linear contrasts across site-years and varieties to determine significant differences across treatments. (*) indicates significant differences for N applied entirely at planting, compared to split application

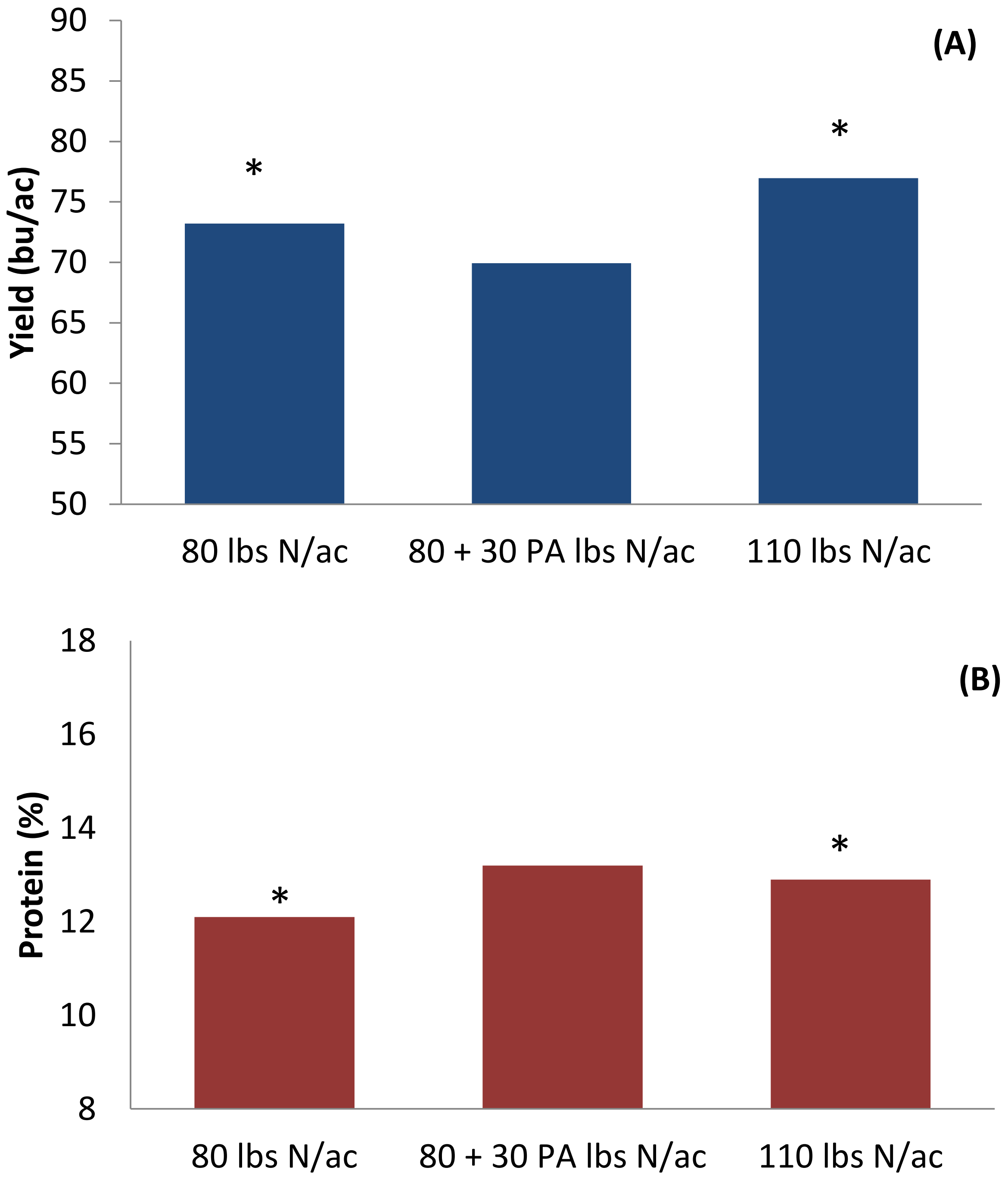


Figure 1.15. Nitrogen uptake (A) and removal (B) response to post-anthesis split nitrogen applications at gold level sites using predetermined linear contrasts across site-years and varieties to determine significant differences across treatments. (*) indicates significant differences for N applied entirely at planting, compared to split application

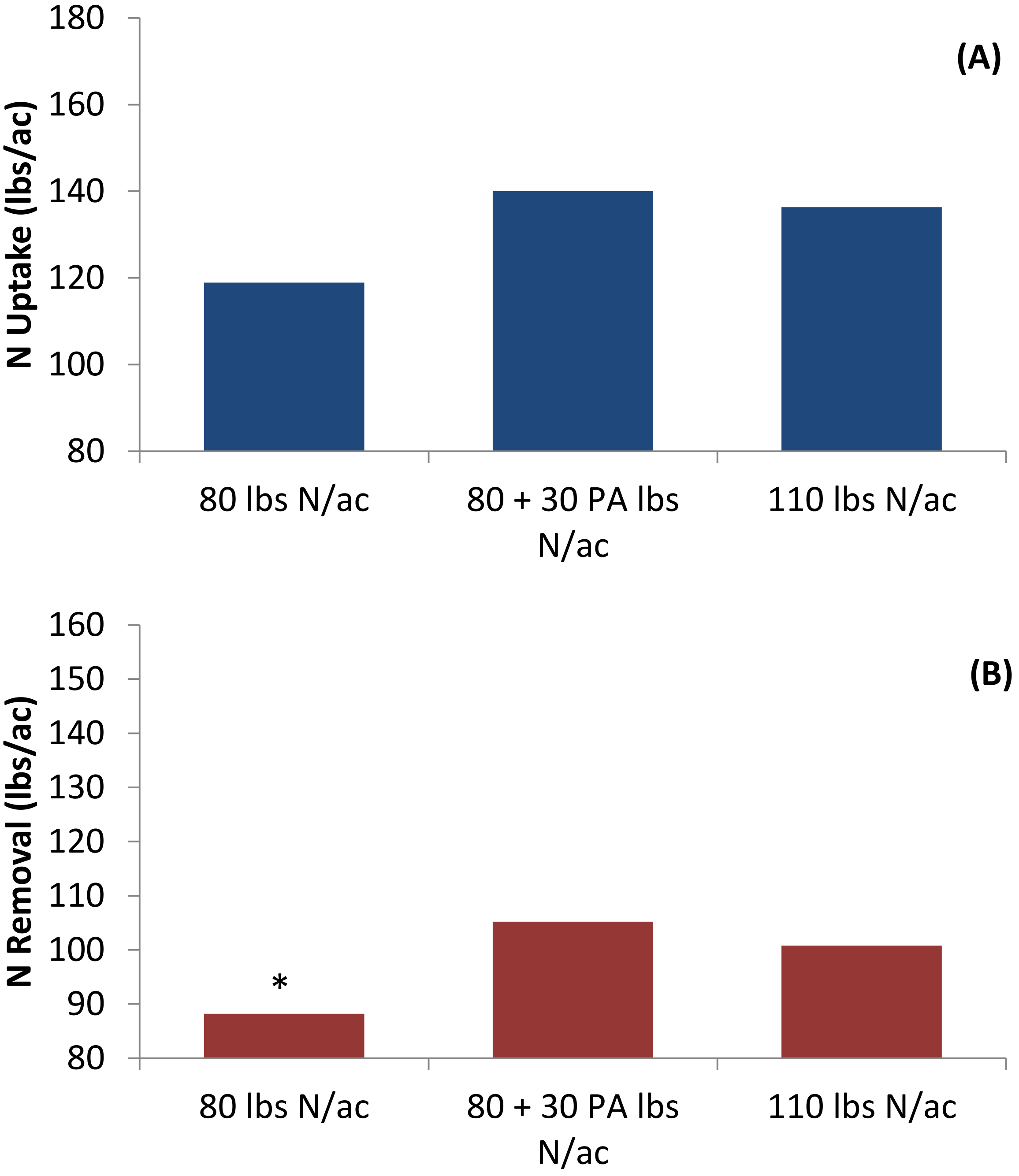


Figure 1.16. Nitrogen uptake (A) and removal (B) response to post-anthesis split nitrogen applications at silver level sites using predetermined linear contrasts across site-years and varieties to determine significant differences across treatments. (*) indicates significant differences for N applied entirely at planting, compared to split application

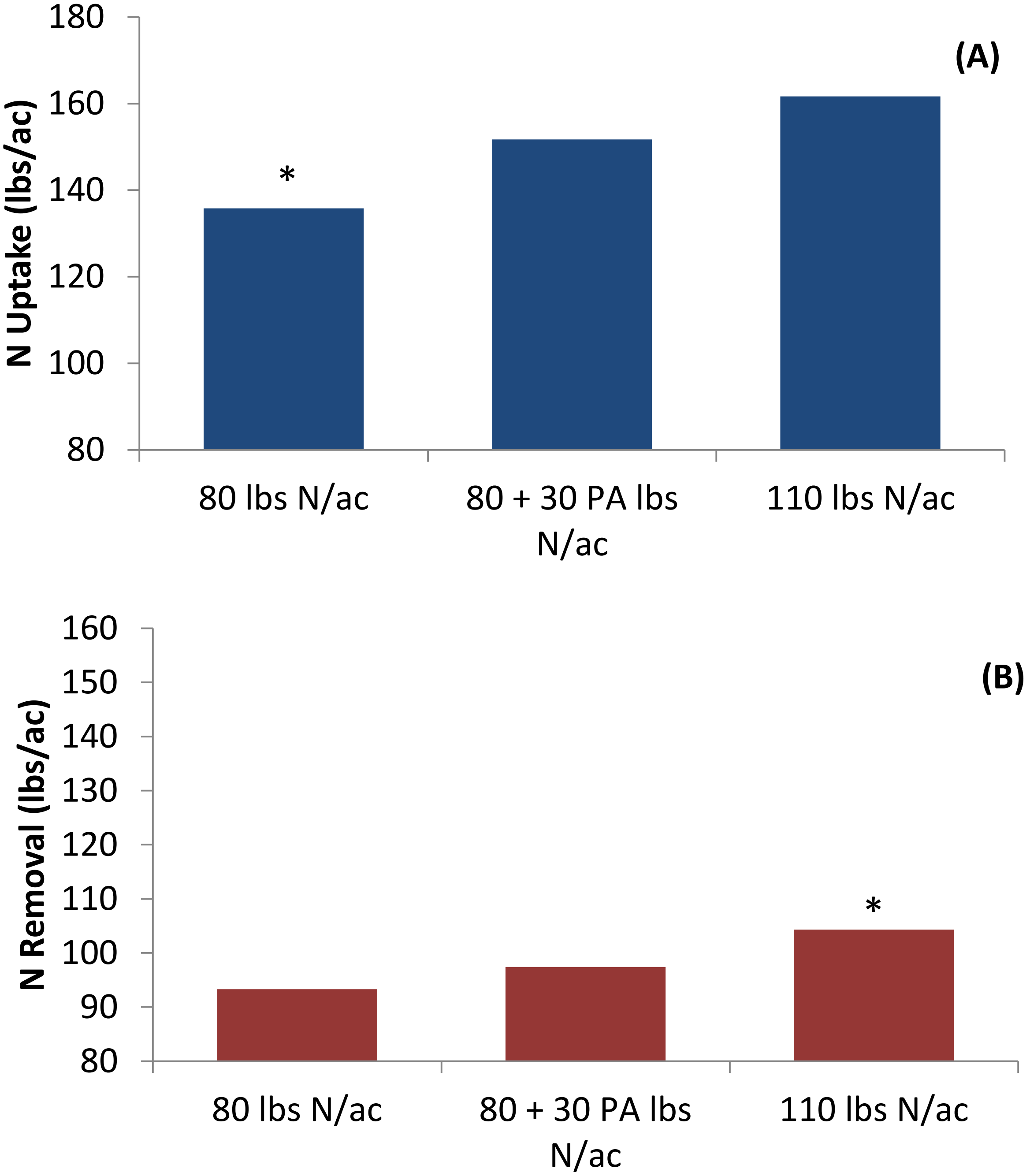
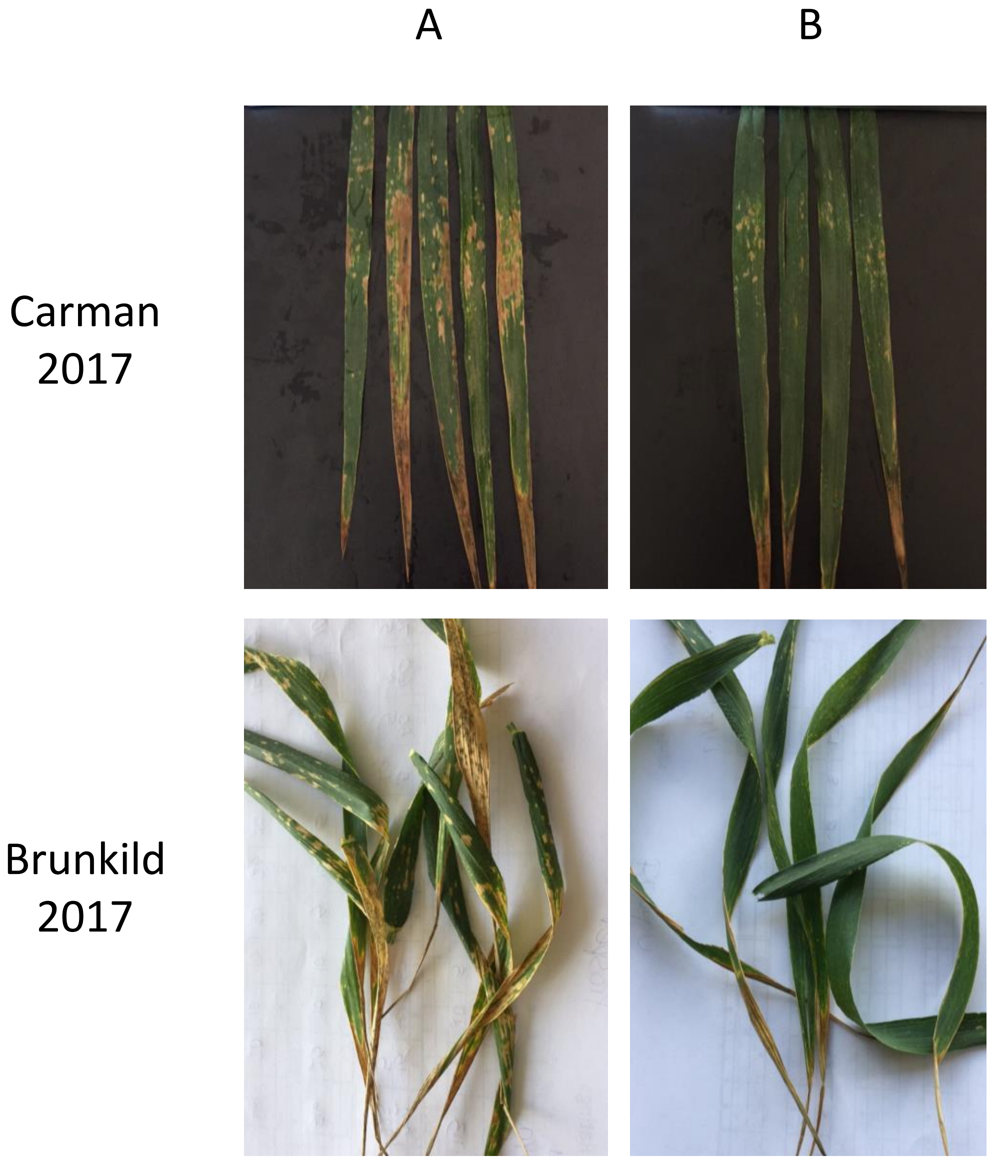


Fig 1.17. Leaf burn from 30 lbs N/ac foliar applied post-anthesis nitrogen with (A) UAN and (B) dissolved urea solution at Carman 2017 (top) and Brunkild 2017 (bottom).



Part 2: Nitrogen supply: Indices for estimating growing season N mineralization

Pre-plant soil NO_3-N tests are traditionally the basis on which fertilizer N rates are recommended in the Canadian Prairies, but soils also provide plant available N through mineralization of N from the soil's organic reserves during the growing season. The amount of N that's mineralized may vary greatly from place to place and time to time, due to soil type, management history and environmental conditions. Currently there is no reliable indicator that can be used prior to or at seeding to predict a soil's ability to mineralize N during the upcoming growing season. However, several 'quick tests' have shown promise as predictors for estimated mineralizable N in growth chamber work (Seward 2016).

In 2015, Les Henry, Professor Emeritus from the University of Saskatchewan, indicated that placing field moist soil in a plastic bag for a month and then testing for NO_3-N released during this time would be a potential indicator for estimating growing season mineralization (Henry 2015). During a subsequent growth chamber study at the University of Manitoba, Jeff Seward (2016), found that the Les Henry Net mineralization test was able to predict recoverable N in a 7-week biomass study in soils collected from annual cropping systems (R^2 = 0.92) and perennial systems (R^2 = 0.86) that had a variety of different histories of nutrient management practices and capacities for N mineralization.

Chemical extraction methods such as $NaHCO_3$ extraction and absorbance at 205nm and 260nm have shown success as an indicator for potential soil N mineralization across a several Canadian and American soils (R^2 = 0.74)(Sharifi et al. 2007). In Seward's study, $NaHCO_3$ indices had good relationships with recoverable N in annual cropping systems (R^2 = 0.85-0.81), but a weak relationship in perennial systems (R^2 = 0.20-0.26). Solvita CO_2 burst test is a method to measure microbial respiration in soils and it is thought to be directly related to potential N mineralization of soils (Solvita & Woods End Laboratories) and mineralizable N is estimated from the CO_2 respiration of soils using Solvita online calculator (Solvita & Woods End Laboratories). Soil organic matter content (SOM) as well as pre-plant NO_3-N levels have been used as indicators by crop advisors to estimate available growing season N but this relationship has not been proven.

The utility of these 'quick tests' across a range of Manitoba field conditions is yet to be tested but if growing season mineralization could be accurately estimated at seeding it would allow for fine tuning of fertilizer N rate recommendations to account for mineralizable N. The objective of this portion of the study was to evaluate these tests as indicators of N mineralization during the growing season.

MATERIAL & METHODS

Field trials were conducted during the 2016 and 2017 growing seasons across southern Manitoba to investigate a number of N fertilizer strategies on two spring wheat varieties (Chapter 1). Spring soil samples (0 - 60 cm) were taken by replicate across each trial location (15 samples per replicate) either before or at seeding using a Giddings soil probe. Samples were divided into surface soil (0 - 15 cm) and sub-soil (15 - 60 cm) before being combined and homogenized across each replicate. Each replicate's sample of surface soil was divided into 4 separate subsamples. One subsample was sent to Farmers Edge Laboratories immediately for a complete nutrient analysis (Nitrate-N, Olsen-P; NH_4OAc exchangeable K, Ca, Mg, Na; water-extractable S and Cl; DTPA-extractable Fe, Cu, Cu, Zn, Mn, and B; pH; EC; soil organic matter; base saturation; and CEC). Within these general soil fertility analyses, measurements of NO_3-N (0 - 60 cm) and soil organic matter were tested as predictor indices of estimated mineralizable N. The remaining soil samples were used to evaluate specialized tests as indicators of growing season soil mineralization.

One subsample of surface soil (0 - 15 cm) from each replicate was placed into sealed plastic bag for the Les Henry incubation test. Bags were filled with ~500 g of field moist soil and then perforated multiple times with the tip of a pen and placed in a box without a lid and stored at room temperature for a 4-week incubation period. After incubation, soil was removed from the plastic bag, homogenized and sent to Farmers Edge Laboratories for NO_3-N analysis (= Les Henry Gross Mineralization). The net amount of N mineralized during the incubation (referred to as Les Henry Net Mineralization or LHN) was determined by subtracting the amount of NO_3-N measured prior to incubation from the amount of NO_3-N measured after incubation (Equation 1).

(1) **Les Henry Net Mineralization** = Les Henry Gross Mineralization – Initial pre-plant NO_3-N

The second method used to estimate the potential N mineralization was UV absorbance of $NaHCO_3$ soil extract at 205 and 260 nm. One gram of oven-dried surface soil plus 20.0 mL of 0.01M $NaHCO_3$ was placed into a 50.0 mL centrifuge tube and shaken for 15 minutes at 150 excursions per minute (EPM) (Fox and Piekielek 1978). The solution was passed through Whatman No. 42 filter paper into scintillation vials. A sample of the filtered solution was added to cuvettes and then analyzed in a spectrophotometer at 205 nm and 260 nm (Ultrospec 2100 pre UV/Visible Spectrophotometer, General Electric, Buckinghamshire, UK) for absorbance, measured as milli-absorbance units (mAU).

Field moist subsamples were sent to AGVISE Laboratories for a Solvita CO_2 burst test. Soils were dried, weighted and moistened to trigger production of CO_2. The amount of CO_2 produced in a 24 hour period after rewetting was measured using a Solvita Digital Color reader (Solvita & Woods End Laboratories).

Following harvest of the field sites, soil samples were taken from 0 - 15 cm and 15 - 60 cm in each plot. A total of six samples from the 0 - 15 cm depth and two from the 15 - 60 cm depth were taken for each plot and homogenized before being sent to Farmer's Edge Laboratories for NO_3-N analysis. N mineralization was estimated at each site using post-harvest soil samples and plant N uptake of check plots that had not received N fertilizer. Nitrogen uptake was calculated for each check plot (Chapter 1) at harvest and mineralization, after accounting for changes in soil NO_3-N, was estimated by equation (2). Although this is regarded as a practical method for estimating N mineralization, it does not account for gains or losses of plant available soil N in deep subsoil (e.g., 60 – 120 cm), nor does it account for losses of plant available soil N due to denitrification or leaching.

(2) **Estimated Mineralization (lbs N/ac)** = (N Uptake + Post-harvest soil NO_3-N) – (Spring NO_3-N)

Statistical Analysis

Simple linear regression between the predictor indices (Spring NO_3-N, SOM, Les Henry Gross, Les Henry Net, $NaHCO_3$-205 nm, $NaHCO_3$-260 nm and Solvita Burst test) and estimated mineralization during the growing season for each trial site-year was conducted using Proc REG in SAS to determine the usefulness of each predictor for estimating growing season mineralization.

RESULTS & DISCUSSION

Estimated mineralization during the growing season varied from 35 lbs N/ac at Brunkild 2016 to 130 lbs N/ac at Carberry 2016 (Table 2.1). Spring NO_3-N (Figure 2.1) and SOM (Figure 2.2) did not have a significant relationship with estimated growing season mineralization with *P*-values of 0.32 and 0.95, respectively (Table 2.2). Therefore, it does not seem appropriate to regard these measurements as useful predictors for N mineralization, even though these measurements are used by some agronomists for this purpose.

The incubation measurements for Les Henry Net (Figure 2.3) and Les Henry Gross did not have significant relationships with growing season mineralization, with model *P*-values of 0.486 and 0.483, respectively, and R^2 values of 0.08 (Table 2.2). These results contrast dramatically with the excellent performance of these incubation tests for predicting N mineralization in a recent growth chamber study by Seward (2016). However, Seward's study focused on different management histories for one soil type. Therefore, in addition to the variability in environmental conditions for the field vs. the growth chamber experiment, another reason for the difference in performance of the Les Henry incubation test might be the variation of soil types in the field experiment.

The chemical extractions and absorbance measurements with $NaHCO_3$-205 nm (Figure 2.4) and $NaHCO_3$-260 nm (Figure 2.5) also failed to show significant relationships with estimated growing season mineralization (Table 2.2).

Solvita CO_2-C Burst test did not have a significant relationship with estimated mineralization during the growing season (*P*-value=0.4490 and R^2 = 0.099). The Solvita online calculator allows for input of Solvita CO_2-C results and an estimated soil N mineralization value is calculated (Table 2.1). However, similar to the results for using the raw values for the Solvita CO_2-C Burst test, there was no significant relationship between the Solvita calculated estimated growing season mineralization and the estimated growing season mineralization for the field trials (Table 2.2).

CONCLUSION

Out of the N mineralization indices tested in this experiment, none had as significant relationship with growing season mineralization in field trials. These results indicate that although these indices have shown promise in laboratory experiments, the reliability of these tests broke down when tested in the field. The variation in soils, management history and environmental conditions of the field trials are thought to be the main reasons for the large variation of predicted N mineralization compares to actual estimated N mineralization. Therefore, although these mineralization tests may hold promise for predicting "potential" mineralization, their ability to predict "actual" mineralization under field conditions appears to be very limited.

REFERENCES

Fox, R.H., and Piekielek, W.P. 1978. A rapid method for estimating the nitrogen-supplying capability of a soil. Soil Sci. Soc. Am. J. **42**: 751–753. doi:10.2136/sssaj1978.03615995004200050019x.

Henry, L. 2015. Make sure you test the right pound of ground: Les Henry. [Online] Available: http://www.agcanada.com/2015/09/make-sure-you-test-the-right-pound-of-ground-les-henry?sidebar=browse&utm_source=AGCanada.com+Newsletter&utm_campaign=c7a852088f-Editors+Choice+-+August+22&utm_medium=email&utm_term=0_6452d60ad7-c7a852088f-87947193 [2018 Feb. 26].

Seward, R.J. 2016. Soil tests for predicting nitrogen mineralization in manured soils. University of Manitoba.

Sharifi, M., Zebarth, B.J., Burton, D.L., Grant, C.A., Porter, G.A., Cooper, J.M., Leclerc, Y., Moreau, G., and Arsenault, W.J. 2007. Evaluation of laboratory-based measures of soil mineral nitrogen and potentially mineralizable nitrogen as predictors of field-based indices of soil nitrogen supply in potato production. Plant Soil **301**: 203–214. doi:10.1007/s11104-007-9438-7.

Solvita & Woods End Laboratories (n.d.). Guide for Interpreting CO2 Burst Results. [Online] Available: https://solvita.com/soil/potential-min-n-calc/ [2018a Feb. 26].

Solvita & Woods End Laboratories (n.d.). CO2-Burst Method - Standard Lab Protocol. [Online] Available: https://solvita.com/co2-burst/ [2018b Feb. 26].

Part 2: Tables

Table 2.1. Soil NO3-N supply, N uptake and predictor indices for estimating growing season soil N mineralization

Site-Year	SOM	0-15 cm Spring NO_3-N	0-60 cm Spring NO_3-N	Les Henry Gross Incubation	Les Henry Net Incubation	NaHCO3-205nm	NaHCO3-260nm	Solvita	Solvita Estimated Mineralization	N Uptake	0-60 cm Fall NO_3-N	0 - 60cm Δ Soil N	Estimated Growing Season Mineralization
	%	lbs/ac	lbs/ac	lbs/ac	lbs/ac	mAU	mAU	ppm CO_2-C	lbs N/ac	lbs/ac	lbs/ac	lbs./ac	lbs N/ac
Melita 2016	3.7	11	43.9	28.6	17.6	84	137.1	127.9	52	62.1	28.3	-15.5	46.6
Carberry 2016	6.2	27.5	88.5	57.5	30	123.3	197	128.8	52.5	178.5	42.6	-45.9	129.6
Carman 2016	5.7	14.3	46.8	32.5	18.3	152.5	237.3	114.2	50	71.3	42.7	-4.1	67.2
Brunkild 2016	4.6	17.1	38.1	28	10.9	68.3	110.9	121.1	51	48.9	29.9	-8.2	35.2
Carman 2017	2.3	21.3	42.5	75	53.8	167.8	237.3	75.8	41	99.6	17.4	-26.7	72.9
Brunkild 2017	6	21	43.3	64.8	43.8	88.8	144	254.5	59	69.1	19.5	-23.7	45.4
Grosse Isle 2017	7.7	33	65.3	57.2	24.2	173.3	264	234	59.5	72.3	38.6	-26.6	45.7
Melita 2017	4.6	7.8	10.8	42.4	34.7	96.8	153.5	163.8	56.5	64.2	31.9	21.1	85.3

Table 2.2 . Results of regression analysis (n=8) for the relationship between predictor indices and estimated mineralized soil N (lbs N/ac) using a simple linear model: y = a + (b*x). Numbers in parentheses indicate that Standard Error of the mean.

Parameter Estimates			Pr>F	R^2
x	a	b		
Spring NO_3-N (0-60 cm)	39.5 (26.6)	0.56 (0.52)	0.320	0.164
Soil Organic Matter	63.5 (40.1)	0.48 (7.5)	0.951	0.001
Les Henry Net	47.9 (26.7)	0.62 (0.83)	0.486	0.084
Les Henry Gross	41.7 (34.3)	0.50 (0.67)	0.483	0.085
$NaHCO_3$-205nm	45.8 (37.6)	0.17 (0.29)	0.593	0.051
$NaHCO_3$-260nm	40.8 (41.5)	0.13 (0.22)	0.552	0.062
Solvita CO_2-C	89.8 (31.5)	-0.15 (0.19)	0.449	0.099
Solvita Estimated Mineralization	113.8 (110.1)	-0.91 (2.1)	0.677	0.031

Part 2: Figures

Figure 2.1. Regression analysis of soil organic matter and estimated N mineralization (lbs N/ac) during the growing season across site-years in the 2016 and 2017 growing seasons.

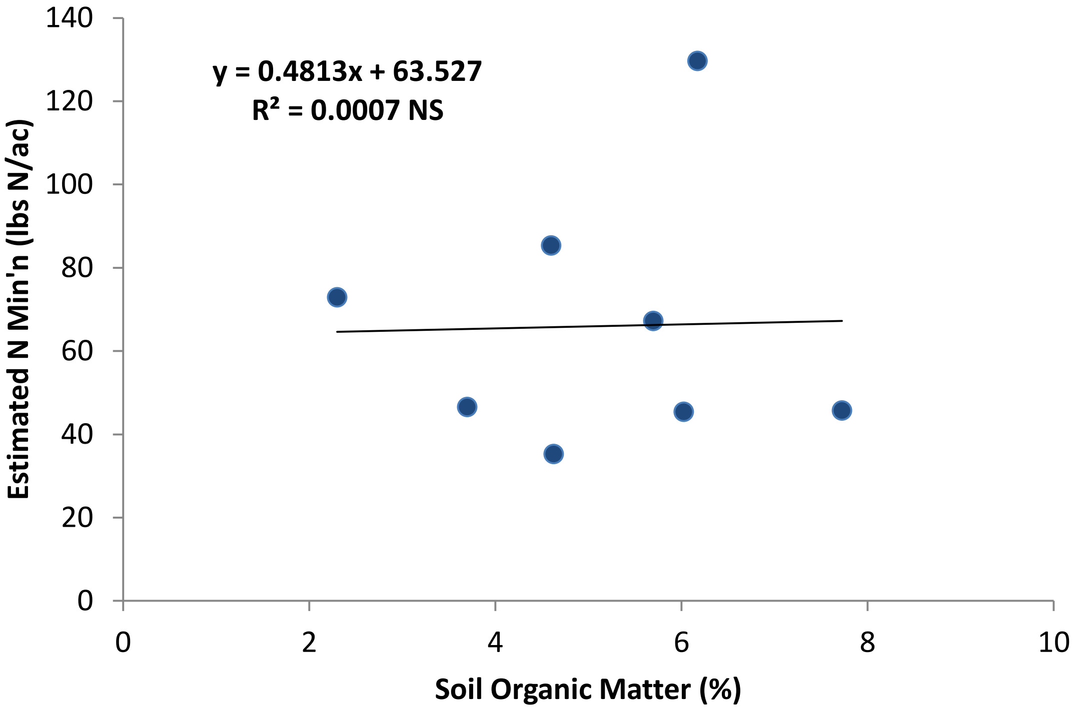


Figure 2.2. Regression analysis of spring soil NO_3-N (0-60cm) and estimated N mineralization (lbs N/ac) during the growing season across site-years in the 2016 and 2017 growing seasons.

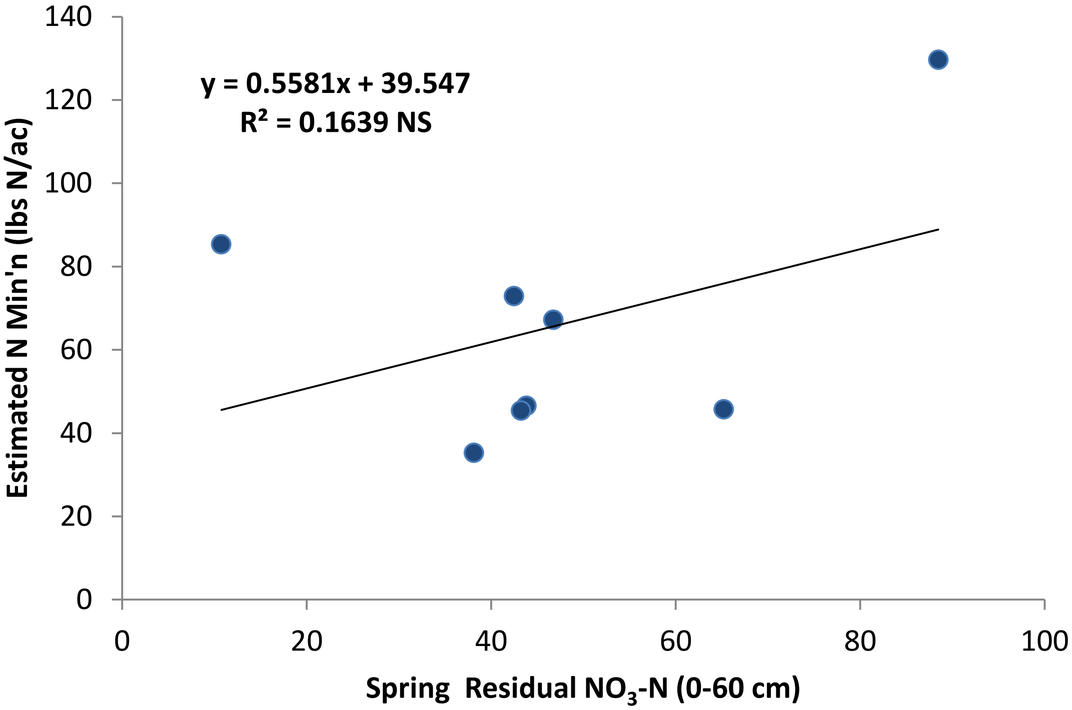


Figure 2.3. Regression analysis of Les Henry incubation test (lbs N/ac) and estimated N mineralization (lbs N/ac) during the growing season across site-years in the 2016 and 2017 growing seasons.

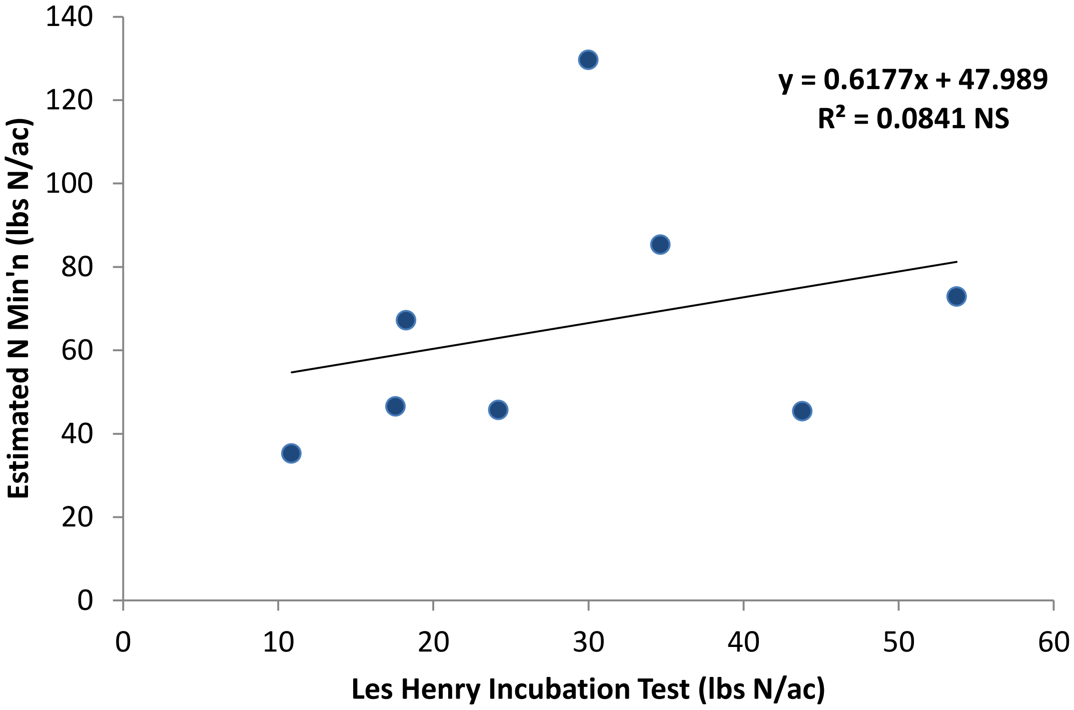


Figure 2.4. Regression analysis of $NaHCO_3$ extraction at 205 nm absorbance and estimated N mineralization (lbs N/ac) during the growing season across site-years in the 2016 and 2017 growing seasons.

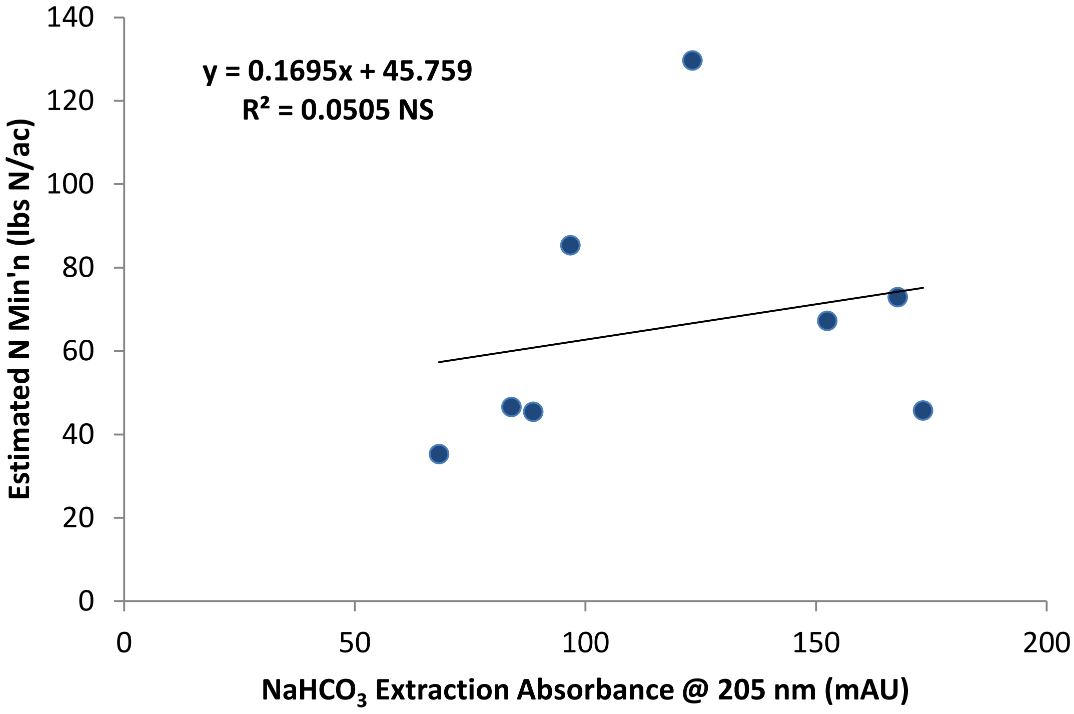


Figure 2.5. Regression analysis of $NaHCO_3$ extraction at 260 nm absorbance and estimated N mineralization (lbs N/ac) during the growing season across site-years in the 2016 and 2017 growing seasons.

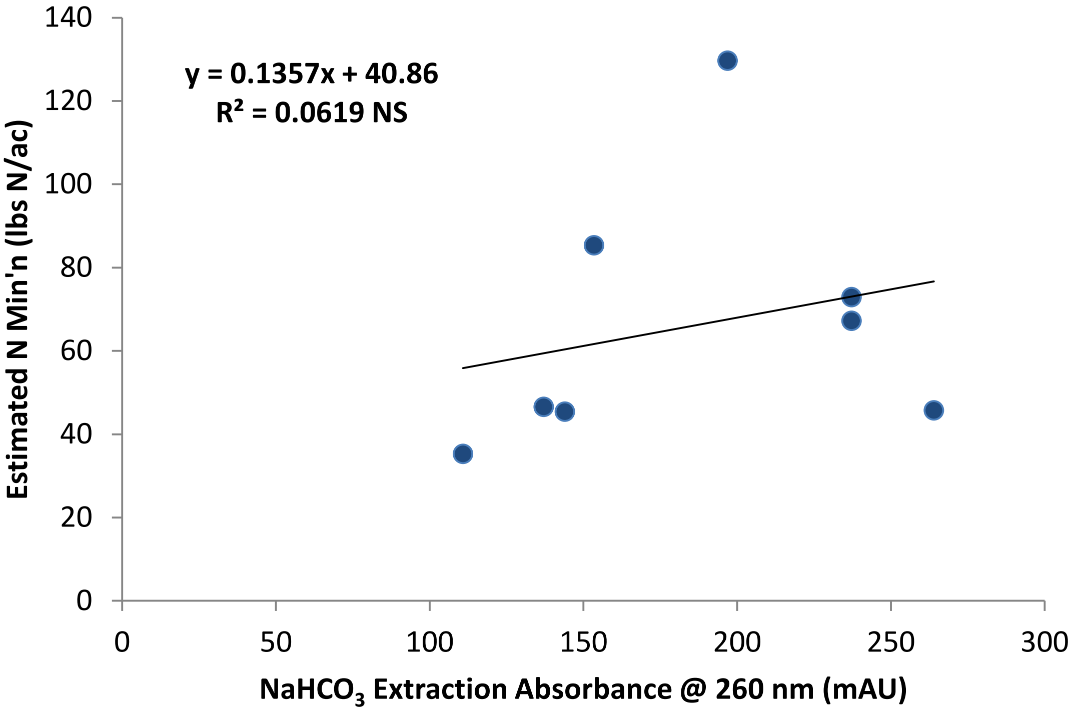
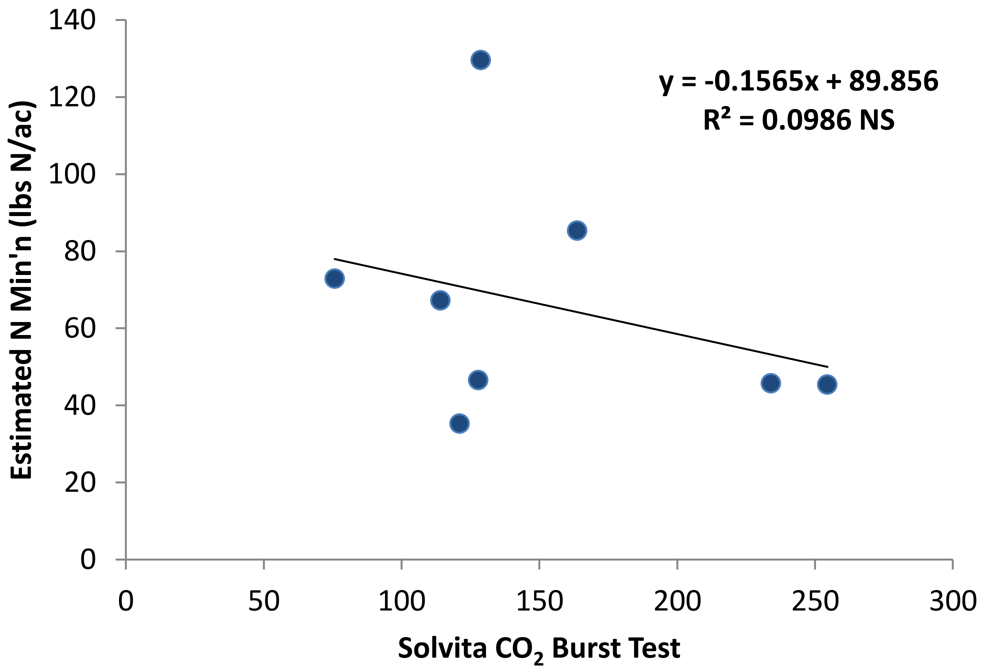


Figure 2.6. Regression analysis of Solvita CO_2 burst test and estimated N mineralization (lbs N/ac) during the growing season across site-years in the 2016 and 2017 growing seasons.



Part 3: Indicators of nitrogen sufficiency for yield and protein

Current spring wheat varieties being grown across the province have very high yield potential and consequently large requirements for nitrogen. Substantial agronomic, economic and environmental risks are involved when applying the entire crop nitrogen requirement at or before planting, because of the uncertainty of upcoming growing conditions. In the Prairies, nitrogen recommendations are traditionally based on fall or spring pre-plant NO_3-N soil samples and do not take into account nitrogen mineralization during the growing season. Lack of accurate tools to predict in-season soil N mineralization can result in large deviations in expected N supply from soil, compared to actual growing season N supply from soil (Chapter 2). Furthermore, the crop's yield potential and demand for nitrogen is also difficult to predict before the crop is established and growing. Therefore, it could be advantageous for a wheat grower to delay a portion of the total nitrogen fertilizer application until later in the season, to allow for the crop N status to be evaluated and to determine an appropriate supplemental rate of N application, if needed. The most appropriate method and time to evaluate N sufficiency during the growing season has not yet been developed for Manitoba spring wheat production systems, but is expected to vary from other regions of the world.

A number of tools have been developed to estimate grain yield and, perhaps, protein content of spring wheat during the growing season. GreenSeeker® (Trimble) is a common in-season sensor that has been used successfully to estimate yield potential of a crops and estimate in-season N recommendations using normalized differential vegetative index (NDVI). Holzapfel et al. (2009b) developed a canola yield response curve using a GreenSeeker in Saskatchewan and then went on to successfully use in-season measurements to determine nitrogen fertilizer application rates(Holzapfel et al. 2009a). The SPAD meter is an optical sensor that measures chlorophyll content of crop leaves, which is then used to estimate crop N status. Flag leaf N content is a direct measurement of N content in flag leaf tissue which might be able to determine N deficiency in crops. Current lab logistics and analyses have shortened the time between tissue sampling and return of results, so it is now possible to have analyses from flag leaf sampling in time for in-season intervention with N applications. Soil sampling for NO_3-N in-season may also be a way of estimating remaining plant available N and aiding in the decision to apply additional N to carry the crop out to harvest. Post-harvest NO_3-N soil sampling may allow us to audit our N application program to determine if sufficient N was present to maximize yield and protein.

To be able to use any of these tools to determine N sufficiency and predict response of yield or protein to in-season applied N, we must first determine if they have a significant relationship with grain yield or protein content. The overall objective of this portion of the study was to determine which tools had the strongest and most consistent relationship with final grain yield and protein content, not only within a site-year, but also across site-years. Additionally, we attempted to determine the most suitable time of sensing for optical sensors (GreenSeeker and SPAD) to optimize their relationship with final yield and/or protein.

MATERIALS & METHODS

Field trials were established in the 2016 and 2017 growing seasons across southern Manitoba to evaluate rates, timing and sources of N fertilizer applications (Chapter 1). Nitrogen rates were applied at planting as conventional urea applied through midrow banders at gold level sites and broadcast as Agrotain®-treated urea at silver level sites. Rates increased in intervals of 30 lbs N/ac, from 0 – 200 lbs N/ac and 0 - 170 lbs N/ac at gold and silver level sites, respectively. Throughout the growing season, measurements were taken on plots with increasing rates of spring applied N to evaluate relationships between predictor tools and final grain yield and protein content.

A handheld GreenSeeker optical sensor (NTech Industries 2009) was used to determine NDVI of each plot by actively emitting radiation in the visible red (660 nm) and near infrared (770 nm) bandwidths and measuring the proportion of emitted radiation that was reflected from the canopy. NDVI is calculated by: NDVI = (NIR-red)/(NIR+red), where red and NIR are the spectral reflectance measurements for the visible red and near-infrared regions. Each plot was measured along its entire length and the GreenSeeker sensor was handheld 0.5 – 0.8 m above the crop canopy. Depending on the plot length, approximately 10-20 individual NDVI values were logged for each plot; the average of these values was used to represent the plot for a given timing. NDVI readings were taken on every plot at each site shortly before each N application (stem elongation, flag leaf, and anthesis) (Table 3.1).

Measurements with a SPAD 502 Plus® chlorophyll meter (Spectrum Technologies, Inc.) were collected for N rate plots at gold level sites at the same crop timings as the GreenSeeker. The SPAD meter gives an indirect assessment of leaf N status by measuring chlorophyll content using a calibrated LED (light-emitting diode) to measure transmission of red light to infrared light through the leaf (Uddling et al. 2007). The device was clamped onto the newest fully unrolled leaf of five plants from the middle rows of each plot and the average value of the readings (Index of relative chlorophyll content) was used. To normalize NDVI and SPAD readings for the stage of crop development, values from each plot were divided by the number of accumulated Growing Degree Days (GDD) (base 4.7) from the date of planting to the date of sensing (Table 3.1).

Flag leaf samples were taken from the inside rows of each plot at all sites when the flag leaves were unrolled with a full visible collar; 30 leaves were collected from each plot. Samples were oven dried, and ground using a Tecator Cyclotec mill. Dried and ground samples were then sent to AGVISE Laboratories for total N analysis by combustion.

Soil samples were taken from 0 - 15 cm and 15 - 60 cm depth at flag leaf timing and post-harvest within each plot area. Six subsamples were taken from the 0 - 15 cm depth and 2 from the 15 - 60 cm depth for each plot near the inner rows. Samples were combined by common depth and homogenized before being sent to Farmers Edge Laboratories for NO_3-N analysis.

Statistical Analysis

Analysis of covariance was completed in Proc GLIMMIX to determine if interactions with site-year and variety had a significant effect on the relationship of the predictor indices (GreenSeeker, SPAD and flag leaf) and the response variable, grain yield or protein content. If the predictor indices or its interactions in the analysis of covariance were significant, linear regression analysis was done using proc REG to describe relationships between predictor indices and the response variables. Soil NO_3-N data was analysed using proc GLIMMIX to complete lsmeans comparisons using Tukey's significant difference method to determine means groupings. All data was checked for normality prior to statistical analyses.

Soil NO_3-N data was not normally distributed; therefore a lognormal transformation was required before analysis and data was back transformed for presentation.

RESULTS & DISCUSSION

GreenSeeker (NDVI)

Analysis of covariance (ANCOVA) indicated that when NDVI readings were taken at stem elongation, the interaction of NDVI/GDD*site-year*variety was significant for grain yield, i.e., site-year and variety significantly influenced the relationship between NDVI/GDD and yield (Table 3.2). Therefore, regression models were fit for each variety within each individual site-year (Table 3.3). The relationship of NDVI/GDD and yield was significant for at least one variety at all site-years, except Grosse Isle 2017. The R^2 values for significant models ranged from 0.20 for AAC Brandon at Brunkild 2017 to 0.58 for Prosper at Melita 2016. Figure 3.1 illustrates that at Brunkild 2016, when NDVI was measured at stem elongation, only the Prosper variety had a significant relationship with yield. The relationship between protein content and NDVI/GDD when measured at stem elongation was not influenced my an interaction with site-year or variety, as determined by the ANCOVA results (Table 3.2). Therefore, a single linear regression models was fit combined across site-years and varieties. A significant (*P*-value 0.0002) but weak (R^2 = 0.03) linear relationship was observed (Table 3.9, Figure 3.7).

When NDVI was measured at flag leaf timing, the relationship to grain yield and protein content was significantly influenced only by the site-year interaction (Table 3.2). Therefore, linear models were fit for each site-year and combined across varieties. Linear regression models between NDVI/GDD and grain yield and protein content were significant for all site-years except Carberry 2016 (Table 3.4, 3.5) where a large amount of N was supplied from the soil through pre-plant residual NO_3-N and growing season mineralization (Chapter 2). The R^2 values for NDVI/GDD and grain yield for site-years with significant relationships varied from 0.30 at Melita 2017 to 0.67 at Brunkild 2017 (Table 3.4). Figure 3.2 illustrates the relationship of NDVI/GDD to grain yield at Brunkild 2016 which had a reasonably useful R^2 value of 0.60. The relationship between NDVI/GDD and final grain protein content ranged in strength from Melita 2016 (R^2=0.19) to Carman 2017 (R^2= 0.52, Table 3.5). Figure 3.3 illustrates this relationship at Brunkild in 2016.

For measurements of NDVI at anthesis, the relationship of NDVI/GDD to grain yield was influenced by site-year*NDVI/GDD, variety*site-year, and NDVI/GDD*site-year*variety (Table 3.2). Due to these interactions, linear models were fit for each variety within a site-year. Strongly significant models were identified for all site-years and varieties except for both varieties at Carberry 2016 (Table 3.6). Relationships of NDVI/GDD to grain yield were strong for the remaining sites and varieties, with R^2 values ranging from 0.54 - 0.96 across responsive site-years (Table 3.6). An example of the effect of the NDVI/GDD*site-year*variety interaction on grain yield is illustrated in Figure 3.4 for AAC Brandon and Prosper at Brunkild 2016.

The relationship between protein content and NDVI/GDD when measured at anthesis was influenced only by the interaction of site-year and NDVI/GDD, as indicated by the ANCOVA (Table 3.2). Therefore, linear regression models were fit to individual site-years and combined across varieties. Linear models were significant at every site-year, with R^2 values ranging from 0.20 at Melita 2016 to 0.55 at Grosse Isle 2017 (Table 3.7); Figure 3.5 illustrates this relationship at Brunkild 2016.

In order for these NDVI measurement tools to be utilized widely across the province, these data must be considered across multiple site-years that represent a range of growing conditions and also across a range of wheat varieties. When combined across all site-years and varieties, the relationship between yield and NDVI/GDD was highly significant (*P*-value <0.0001) regardless of the timing of NDVI measurement (Table 3.8). The greatest R^2 value was observed when reflectance was measured at flag leaf timing (0.60), followed by anthesis (0.55) and stem elongation (0.52) (Figure 3.6). Another important requirement for tools that predict yields is that they should enable an effective management response. In this case, the optimum timing at flag leaf for measuring NDVI as a predictor for grain yield coincided very well with the excellent yield and protein responses to midseason applications of supplemental N fertilizer at the flag leaf stage (Chapter 1).

When combined across site-years and varieties, the relationship between protein content and NDVI/GDD was significant, but very weak, with R^2 values ranging from 0.01 – 0.03 (Table 3.9, Figure 3.7). Normalizing the data on the basis of measuring grain yield and protein content relative to the high N treatments did not improve the relationships when combining site-years and varieties (Appendix Tables 18-19). This leads us to believe that the relationship between NDVI and protein content was largely influenced by variation among site-years; therefore, the relationship dissolves when combined across site-years. This poor relationship between NDVI and protein content across site-years also indicates that it is unlikely NDVI will be used as an indicator of final protein content alone because of difficulty in developing a reliable response model.

In summary, the relationship of grain yield to NDVI/GDD held quite well when combined across sites and varieties which indicates that there is potential to use NDVI for estimating grain yield using NDVI active sensors. These results agree with previous studies looking at GreenSeeker utility in spring wheat. In particular, the NDVI measurements at flag leaf may be especially useful, not only due to their relatively strong relationship with grain yield, but also due to this timing enabling an effective opportunity to apply supplemental N fertilizer.

SPAD (Chlorophyll Meter)

The results of the ANCOVA indicated that SPAD chlorophyll meter readings and their relationship to yield and protein was not influenced by interactions with variety or site-year when measured at stem elongation and flag leaf timings (Table 3.10). Therefore, one linear regression model was fit across site-years and varieties for yield and protein when SPAD chlorophyll was measured at stem elongations and flag leaf timings. The SPAD/GDD and yield regression model was highly significant when measured at stem elongation or flag leaf timings, with R^2 values of 0.67 and 0.60, respectively (Table 3.11, Figure 3.8). The linear model for SPAD/GDD and protein was significant, but weak ($R^2 = 0.08$) when measured at stem elongation and not significant when measured at flag leaf timing (Table 3.12, Figure 3.9).

The relationship between SPAD/GDD and yield when measured at anthesis was dependent on site-year*SPAD/GDD interaction (Table 3.10); therefore, linear models were fit to each site-year and varieties were combined (Table 3.13). The linear relationship between yield and SPAD was highly significant at all site-years (P<0.0001) with R^2 values ranging from 0.29 at Carman 2017 and 0.67 at Brunkild 2017. Figure 3.10 demonstrates this relationship at Brunkild in 2016.

The relationship between SPAD/GDD measured at anthesis and grain protein was dependent on the site-year*SPAD/GDD interaction (Table 3.10). Due to this interaction regression models had to be fit to each site-year for SPAD/GDDs relationship to protein. The relationship was significant for all site years expect Carman 2017 and R2 values ranged from 0.25 – 0.33 across sites with a significant relationship (Table 3.14, Figure 3.11).

Even though the relationships between anthesis measurements of SPAD/GDD and final grain yield or protein were affected by an interaction with site-year, the practical utility of this tool was tested across site years and varieties. When site-years and varieties were combined, SPAD/GDD measured at anthesis had a good relationship with yield similar to other sensing timings with an R^2 value of 0.60 (Table 3.11, Figure 3.8). Combined analysis of SPAD/GDD (measured at anthesis) and grain protein resulted in no significant linear relationship (Table 3.12, Figure 3.9), similar to combined data from SPAD/GDD when measured at flag leaf timing.

Normalizing protein and SPAD/GDD data by the high N treatment and using values expressed relative to those for the highest rate of applied N slightly improved their relationship when data were combined across site-years and varieties for sensing at flag leaf and anthesis. As a result, R^2 values increased to 0.25 and 0.34, respectively (Table 3.15). Even with this slight improvement, the relationships are not strong enough to confidently use as a prediction method. Other attempts to normalize data with relative values to improve relationships were also unsuccessful (Appendix Table 20-21). This indicates that SPAD readings are similar to NDVI and show promise in predicting yield across a range of site-years and varieties, but do not appear to be reliable as a predictor of protein across varieties or locations.

Flag Leaf Nitrogen Content

The results of the ANCOVA for flag leaf nitrogen content's relationship to yield and protein indicated that the site-year*flag leaf N interaction had a significant effect on the model but variety and its interactions did not (Table 3.16). Therefore, linear models were fit to individual site-years and varieties were combined for analysis of both grain yield and protein content. Significant relationships between flag leaf N and grain yield were observed at all site-years except Carberry 2016 where there was limited response to applied nitrogen (Table 3.17, Chapter 1). The strength of this relationship varied from R^2=0.11 at Brunkild 2017 to R^2=0.65 at Brunkild 2016 (Table 3.17). Relationships between flag leaf nitrogen content and protein were highly significant at all site-years with R^2 values ranging from 0.17 – 0.67 (Table 3.18). An example of the relationship between flag leaf N content and grain yield and protein content is illustrated for Brunkild 2016 in Figure 3.12.

Although the relationship between flag leaf N and grain yield or protein was affected by an interaction with site-year, the practical value of this tool was tested across a combination of all site-years and varieties. When flag leaf nitrogen content was combined across site-years and varieties, we observed significant but weak relationships with both yield and protein (Table 3.19, Figure 3.13). Normalizing data by using the relative values improved these relationships slightly by increasing R^2 values from 0.14 to 0.38 for yield and from 0.10 to 0.20 for protein (Table 3.20) but overall, the relationships were still weak. This indicates that flag leaf N concentration relationships to yield and protein were very dependent on site-year and relationships were lost when employed across locations.

Midseason Soil Samples

Global ANOVA analysis of midseason soil NO_3-N samples indicated that there was a significant effect of N rate and site-year as well as the N rate*site-year interaction, but there was no effect of variety or interactions containing variety (Appendix Table 27). Therefore, N rates were compared by combining varieties within each site-year. Midseason soil samples were used to determine the amount of soil NO_3-N required at flag leaf timing to produce the economic optimum yield at each site. The rate of fertilizer N applied at planting for the economic optimum yield at all gold level sites was 140 lbs N/ac fertilizer N. At Carman in 2017, there was an average of 133 lbs soil NO_3-N /ac in the 0 – 60 cm depth across both varieties at the economic optimum rate of fertilizer N (Figure 3.14A). Brunkild 2016 had much less, with only 75 lbs NO_3-N /ac available at flag leaf timing across both varieties (Figure 3.14B). In 2017, midseason soil NO_3-N at the economic optimum rate of fertilizer N ranged from 92 to 219 lbs NO_3-N /ac at Carman and Brunkild, respectively (Figure (3.14C-D). Part of the reason for this variability across site-years is that soil samples taken at flag leaf timing for soil NO_3-N were highly variable due to "hot-spots" of N from midrow banding of fertilizer and high rep-to-rep variability (raw data CV 82%). Although these results don't appear to be useful due to high variability, we collected only 6 samples for top-soil and 2 samples for sub-soil per plot, while the recommendation for field composite sampling is 15-20 samples; therefore, a field composite sample could have reduced variability and provide more meaningful results.

Post-Harvest Soil Sampling

Post-harvest soil NO_3-N concentrations may be useful for determining whether or not an excessive rate of fertilizer was applied to a crop. Although this tool is not predictive, it can be used to evaluate the appropriateness of historic practices. In this study, the amount of NO_3-N measured in soil after harvest was used to determine the rate of spring-applied fertilizer N at which soil residual N began to rise, as well as the amount of post-harvest NO_3-N that matched up with the economic optimum rate of fertilizer N for each site. At silver level sites, there was a significant site-year*N treatment interaction, but no effect of variety or its interactions (Appendix Table 25). Therefore, post-harvest residual N levels were compared for N treatments at each site-year and averaged across varieties. Figure 3.15A shows that there was no significant effect of increasing fertilizer N rates on soil residual NO_3-N at Carberry 2016 and the residual NO_3-N at the economic optimum rate of N was 45 lbs N/ac. At Melita 2016, the only fertilizer N rate which had residual NO_3-N levels higher than the minimum rate was 140 lbs of fertilizer N/ac, which resulted in 38 lbs residual NO_3-N/ac. At this same site-year, the economic optimum rate of N (80 lbs N/ac) had 29 lbs N/ac residual NO_3-N at post-harvest sampling (Figure 3.15B). At Grosse Isle 2017, only the highest rate of N applied (170 lbs N/ac) resulted in an increased level of post-harvest residual NO_3-N. At this site-year, the economic optimum rate (110 lbs N/ac) had a residual NO_3-N level of 43 lbs N/ac, which was similar to the lowest residual NO_3-N levels for that site (Figure 3.15C). Figure 3.15D shows that 170 lbs N/ac fertilizer N application was required to significantly increase residual soil NO_3-N at Melita 2017. The economic optimum rate of 140 lbs N/ac at this site-year resulted in 34 lbs N/ac residual NO_3-N, which was not significantly different from treatments with the lowest or highest levels of residual soil NO_3-N (Appendix Table 25).

At gold level sites there was a significant 3-way interaction between N treatment*variety*site-year; therefore, N treatments were compared within each site-year for each variety (Appendix Table 26). At Carman 2016, there were no significant differences in post-harvest soil residual NO_3-N (Figure 3.16A) and residual N at economic optimum was 30 and 40 lbs N/ac for AAC Brandon and Prosper, respectively. Brunkild 2016, also had no significant increases in soil residual NO_3-N levels across N rates

(Figure 3.16B). For this site-year, soil residual NO_3-N levels at economic optimum N rates were 30 lbs N/ac for AAC Brandon and 52 lbs N/ac for Prosper (Appendix Table 26). At Carman 2017, the lowest N rate that resulted in a significant increase in residual NO_3-N was 170 lbs N/ac for AAC Brandon and 140 lbs N/ac for Prosper (Figure 3.16C). The economic optimum rate for both varieties at this site-year was 140 lbs N/ac (Chapter 1) and residual NO_3-N levels were 23 lbs N/ac for AAC Brandon and 53 lbs N/ac for Prosper at that rate (Appendix Table 26). At Brunkild 2017, there was no significant increase in residual soil NO_3-N for Prosper. The lowest rate of N that significantly increase soil residual NO_3-N for AAC Brandon was 170 lbs N/ac (Figure 3.16D). The economic rate of N for both varieties was 140 lbs N/ac and soil residual NO_3-N levels at this rate were 35 and 22 lbs N/ac for AAC Brandon and Prosper, respectively. This indicates that in 2017, soil residual NO_3-N began to increase sooner for AAC Brandon than Prosper at Carman, but vice versa for Brunkild.

These results indicate that at all combinations of sites and varieties, except for Prosper at Carman 2017, soil residual N levels began to increase at N rates applied that were higher than the economic rate of N. The amount of post-harvest soil test NO_3-N at the economic rate of N ranged from 22 lbs N/ac at Brunkild 2017 (Prosper) to 53 lbs N/ac at Carman 2017 (Prosper). Therefore, if the amount of fall residual NO_3-N exceeded 60 lbs N/ac in the top 60 cm, the supply of soil plus fertilizer N probably exceeded the crop's N requirement for economic optimum yield in that particular growing season.

CONCLUSIONS

NDVI has a useful relationship with spring wheat final grain yield for individual sites and varieties as well as when combined, regardless of timing of sensing at stem elongation, flag leaf or anthesis. The relationship between NDVI and grain protein content was good for individual site-years but the relationship was lost when site-years were combined, severely limiting the value of this measurement for this purpose.

Similar to NDVI, SPAD meter reading (chlorophyll content) had good relationships with grain yield when combined across site-years and varieties at all sensing timings. However, SPAD readings had poor relationships with grain protein content, unless measured at anthesis timing for individual site-years and varieties. When combined across site-years and varieties, SPAD readings had a very poor relationship with protein, but this relationship was improved slightly by normalizing data relative to the high N treatment.

Flag leaf N content had a significant relationship with grain yield at 7 out of 8 individual site-years and all individual site-years for grain protein content. When data were combined across site-years, flag leaf N content had a significant relationship with grain yield and protein but relationships were very weak.

Midseason soil sampling for nitrate N resulted in highly variable analyses, which resulted in an unreliable range of estimates for economic optimum rates of N. Post-harvest soil residual NO_3-N levels indicated that residual N typically does not begin to climb until N fertilization rates exceed the economic optimum. The residual N at economic optimum rates of fertilizer N ranged from 22 – 53 lbs N/ac across site-years.

REFERENCES

Holzapfel, C.B., Lafond, G.P., Brandt, S.A., Bullock, P.R., Irvine, R.B., James, D.C., Morrison, M.J., and May, W.E. 2009a. Optical sensors have potential for determining nitrogen fertilizer topdressing requirements of canola in Saskatchewan. Can. J. Plant Sci. **89**: 411–425. doi:10.4141/CJPS08127.

Holzapfel, C.B., Lafond, G.P., Brandt, S.A., Bullock, P.R., Irvine, R.B., Morrison, M.J., May, W.E., and James, D.C. 2009b. Estimating canola (*Brassica napus* L.) yield potential using an active optical sensor. Can. J. Plant Sci. **89**: 1149–1160. doi:10.4141/CJPS09056.

Uddling, J., Gelang-Alfredsson, J., Piikki, K., and Pleijel, H. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth. Res. **91**: 37–46. doi:10.1007/s11120-006-9077-5.

Part 3: Tables

Table 3.1. Dates and accumulated Growing Degree Days (GDD) (base 4.7°C) for timing of in-season predictors of spring wheat grain yield and protein sufficiency at all sites-years during the 2016-2017 growing seasons.

		Seeding	**Stem Elongation**	**Flag Leaf**	**Anthesis**
Carman 2016	Date	Apr 28, 16	Jun 8, 16	Jun 21, 16	Jul 4, 16
	Accumulated GDD	0	362.5	538.2	702.1
Brunkild 2016	Date	May 5, 16	Jun 14, 16	Jun 29, 16	Jul 6, 16
	Accumulated GDD	0	418.6	616.2	708.4
Carman 2017	Date	May 2, 17	Jun 9, 17	Jun 22, 17	Jul 7, 17
	Accumulated GDD	0	345.8	492.7	673.9
Brunkild 2017	Date	May 5, 17	Jun 9, 17	Jun 22,17	Jul 7, 17
	Accumulated GDD	0	306.7	447.3	620.9
Melita 2016	Date	May 6, 16	Jun 17, 16	Jun 23, 16	Jul 8, 16
	Accumulated GDD	0	442.3	516.3	706.6
Carberry 2016	Date	May 5, 16	Jun 17, 16	Jun 23, 16	Jul 8, 16
	Accumulated GDD	0	436.1	510.5	690.2
Melita 2017	Date	May 10, 16	Jun 23, 17	Jun 29, 17	Jul 6, 17
	Accumulated GDD	0	428.4	491.6	589.8
Grosse Isle 2017	Date	May 2, 17	Jun 9, 17	Jun 26, 17	Jul 10, 17
	Accumulated GDD	0	327.4	493.0	678.1

Table 3.2. Results of ANCOVA for the relationship of spring wheat grain yield and protein content to NDVI/GDD by variety and site-year.

	Stem Elongation		Flag Leaf		Anthesis	
	Yield	Protein	Yield	Protein	Yield	Protein
	Pr > F					
NDVI/GDD	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Variety	0.3140	0.2409	0.4775	0.4420	0.6813	0.4485
NDVI/GDD*Variety	0.9322	0.0863	0.9090	0.1572	0.3195	0.9977
Site-year	0.0826	0.0122*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
NDVI/GDD*Site-year	0.0949	0.0784	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Variety*Site-year	0.0417*	0.1267	0.1313	0.3871	0.0234*	0.0878
NDVI/GDD*Variety*Site-year	0.0272*	0.2366	0.0759	0.5576	0.0387*	0.1122

Table 3.3. Results of regression analysis for the relationship between spring wheat grain yield and NDVI (measured at stem elongation) divided by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x). Numbers in parentheses indicate the Standard Error of the mean.

		Parameter Estimates						**Pr>F**	**R^2**
		a			*b*				
Site-year	*Variety*	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Carman 2016	Brandon	39.73 (13.2)	12.85	66.6	8151.4 (11321)	-15213	31516	0.4784	0.02
	Prosper	26.54 (13.3)	-0.8	53.9	30053 (12003)	5280	54826	0.0195*	**0.21**
Brunkild 2016	Brandon	31.13 (19.7)	-10.6	70.8	24890 (18033)	-12414	62194	0.1808	0.08
	Prosper	19.74 (13.8)	-8.7	48.2	45917 (13454)	18149	73685	0.0023*	**0.33**
Melita 2016	Brandon	23.40 (9.1)	4.4	42.5	21236 (6326)	8079	34393	0.0030*	**0.35**
	Prosper	22.81 (7.8)	6.4	39.2	29176 (5449)	17843	40509	<0.0001*	**0.58**
Carberry 2016	Brandon	93.50 (21.4)	49.0	137.9	-3251 (11762)	-27710	21209	0.7850	0.00
	Prosper	40.20 (30.3)	-22.8	103.2	36164 (16884)	1149	71179	0.0435*	**0.17**
Carman 2017	Brandon	14.93 (25.2)	-36.9	66.7	39712 (13340)	12292	67133	0.0062*	**0.25**
	Prosper	-18.67 (21.8)	-63.6	26.2	61814 (11753)	37608	86021	<0.0001*	**0.53**
Brunkild 2017	Brandon	-10.53 (43.3)	-99.9	78.8	54549 (21770)	9713	99384	0.0191*	**0.20**
	Prosper	148.10 (64.9)	14.5	281.5	-21011 (33725)	-90334	48313	0.5387	0.01
Melita 2017	Brandon	7.90 (20.4)	-34.4	50.2	59587 (21785)	14408	104767	0.0121*	**0.25**
	Prosper	22.50 (18.7)	-16.3	61.3	47548 (19850)	6381	88716	0.0256*	**0.21**
Grosse Isle 2017	Brandon	47.50 (16.9)	12.6	82.3	14457 (14260)	-14856	43769	0.3200	0.04
	Prosper	85.50 (22.3)	39.6	131.4	-16413 (23097)	-63890	31063	0.4836	0.02

Table 3.4. Results of regression analysis for the relationship between spring wheat grain yield and NDVI (measured at flag leaf) divided by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x). Numbers in parentheses indicate the Standard Error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
	a			*b*				
Site-year	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Brunkild 2016	-60.7 (14.2)	-89.3	-32.2	94425 (10927)	72466	116385	<0.0001	**0.60**
Brunkild 2017	-261.9 (35.0)	-332.2	-191.6	192932 (18527)	155772	230092	<0.0001	**0.67**
Carberry 2016	143.9 (59.1)	24.9	262.9	-28815 (35912)	-101145	43516	0.4266	0.01
Carman 2016	5.8 (10.4)	-15.1	26.8	41041 (8757)	23450	58631	<0.0001	**0.31**
Carman2017	-93.5 (25.3)	-144.3	-42.7	108287 (14722)	78759	137815	<0.0001	**0.51**
Grosse Isle 2017	-25.5 (17.1)	-59.8	8.9	60914 (11254)	38351	83477	<0.0001	**0.35**
Melita 2016	1.0 (8.3)	-15.8	17.8	40972 (5846)	29188	52755	<0.0001	**0.53**
Melita 2017	-3.9 (15.4)	-34.7	27.4	64352 (14345)	35477	93228	<0.0001	**0.30**

Table 3.5. Results of regression analysis for the relationship between spring wheat grain protein content and NDVI (measured at flag leaf) divided by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x). Numbers in parentheses indicate the Standard Error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
	a			*b*				
Site-year	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Brunkild 2016	4.8 (1.5)	1.8	7.8	5151 (1163)	2811	7490	<0.0001	**0.29**
Brunkild 2017	-9.7 (3.5)	-16.8	-2.7	10636 (1861)	6902	14369	<0.0001	**0.38**
Carberry 2016	5.5 (4.3)	-3.2	14.2	4638 (2618)	-633	9910	0.0832	0.06
Carman 2016	7.4 (0.97)	5.4	9.4	4886 (831)	3214	6558	<0.0001	**0.42**
Carman2017	-12.8 (3.4)	-19.6	-6.0	14925 (1973)	10966	18883	<0.0001	**0.52**
Grosse Isle 2017	-8.2 (2.9)	-14.1	-2.4	12937 (1920)	9084	16788	<0.0001	**0.46**
Melita 2016	10.3 (1.1)	8.2	12.5	2415 (752)	901	3930	0.0024	**0.19**
Melita 2017	4.07 (1.7)	0.6	7.6	6161 (1610)	2917	9405	0.0004	**0.25**

Table 3.6. Results of regression analysis for the relationship between spring wheat grain yield and NDVI (measured at anthesis) divided by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x). Numbers in parentheses indicate the Standard Error of the mean.

		Parameter Estimates						**Pr>F**	**R^2**
		a			*b*				
Siteyr	*Variety*	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Carman 2016	Brandon	-30.8 (15.1)	-61.4	0.318	78826 (14831)	48217	109435	<0.0001	**0.54**
	Prosper	-79.8 (12.4)	-105.5	-54.3	142015 (12617)	115974	168056	<0.0001	**0.84**
Brunkild 2016	Brandon	-66.6 (36.2)	-141.4	8.2	109326 (31912)	43312	175341	0.0023	**0.34**
	Prosper	-72.0 (13.5)	-99.8	-44.2	123287 (11980)	98562	148012	<0.0001	**0.82**
Melita 2016	Brandon	-1.1 (4.7)	-10.8	8.6	54202 (4575)	44687	63716	<0.0001	**0.87**
	Prosper	-25.0 (8.1)	-41.9	-8.1	84652 (7647)	68748	100555	<0.0001	**0.85**
Carberry 2016	Brandon	-71.6 (101.9)	-283.7	140.5	134610 (86424)	-44739	313959	0.1335	0.10
	Prosper	143.8 (94.2)	-51.6	339.2	-32825 (79988)	-198709	133059	0.6855	0.01
Carman 2017	Brandon	-98.4 (27.8)	-155.7	-41.1	161045 (23839)	112043	210047	<0.0001	**0.64**
	Prosper	-127.9 (22.9)	-175.1	-80.8	192950 (19738)	152300	233600	<0.0001	**0.79**
Brunkild 2017	Brandon	-185.9 (26.1)	-239.8	-132.2	214371 (19703)	173792	254950	<0.0001	**0.83**
	Prosper	-316.0 (33.2)	-384.2	-247.9	314243 (24578)	263723	364763	<0.0001	**0.96**
Melita 2017	Brandon	-32.5 (15.0)	-63.7	-1.3	112922 (17633)	76352	149491	<0.0001	**0.65**
	Prosper	-33.6 (10.6)	-55.5	-11.6	121098 (12687)	94786	147410	<0.0001	**0.81**
Grosse Isle 2017	Brandon	-57.5 (9.9)	-77.8	-37.3	110219 (8891)	91942	128496	<0.0001	**0.86**
	Prosper	-74.1 (9.8)	-94.4	-53.8	128725 (8809)	110616	146834	<0.0001	**0.89**

Table 3.7. Results of regression analysis for the relationship between spring wheat grain protein content and NDVI (measured at anthesis) divided by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x). Numbers in parentheses indicate the Standard Error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
	a			*b*				
Site-year	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Brunkild 2016	3.12 (1.6)	-0.19	6.4	7422 (1464)	4477	10368	<0.0001	**0.35**
Brunkild 2017	-4.56 (3.2)	-10.9	1.8	11167 (2388)	6375	15957	<0.0001	**0.29**
Carberry 2016	-13.9 (5.5)	-24.9	-2.9	22972 (4641)	13629	32314	<0.0001	**0.35**
Carman 2016	3.9 (1.5)	0.9	6.9	9216 (1489)	6222	12211	<0.0001	**0.44**
Carman2017	-9.4 (3.6)	-16.6	-2.3	19113 (3062)	12972	25255	<0.0001	**0.42**
Grosse Isle 2017	-8.5 (2.6)	-13.7	-3.4	17950 (2294)	13349	22551	<0.0001	**0.54**
Melita 2016	9.9 (1.1)	7.6	12.2	3678 (1094)	1475	5882	0.0016	**0.20**
Melita 2017	1.5 (1.2)	-0.9	4.0	10886 (1468)	7928	13845	<0.0001	**0.55**

Table 3.8. Results of regression analysis for the relationship between spring wheat grain yield and NDVI by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x), combined across site-years and varieties for three times of measurement during crop development (T1 = stem elongation; T2 = flag leaf; AN = anthesis). Numbers in parentheses indicate the Standard Error of the mean.

x	**Parameter Estimates**						**Pr>F**	**R^2**
	A			*b*				
	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
NDVI/GDD (T1)	21.6 (2.6)	16.4	26.8	38116 (1802)	34573	41658	<0.0001	**0.52**
NDVI/GDD (T2)	-13.9 (3.6)	-21.1	-6.8	60464 (2428)	55690	65237	<0.0001	**0.60**
NDVI/GDD (AN)	-40.4 (5.3)	-50.7	-30.0	104824 (4729)	95527	114121	<0.0001	**0.55**

Table 3.9. Results of regression analysis for the relationship between spring wheat grain protein content and NDVI by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x), combined across site-years and varieties for three times of measurement during crop development (T1 = stem elongation; T2 = flag leaf; AN = anthesis). Numbers in parentheses indicate the Standard Error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
x	*A*			*b*				
	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
NDVI/GDD (T1)	10.9 (0.3)	10.4	11.6	757 (204)	354	1159	0.0002	**0.03**
NDVI/GDD (T2)	11.0 (0.5)	10.1	11.9	709 (305)	109	1310	0.0206	**0.01**
NDVI/GDD (AN)	10.3 (0.6)	9.1	11.5	1560 (555)	467	2653	0.0053	**0.02**

Table 3.10 Results of ANCOVA for spring wheat grain yield and protein content relationship to SPAD/GDD by variety and site-year for gold level sites only

	Stem Elongation		Flag Leaf		Anthesis	
	Yield	Protein	Yield	Protein	Yield	Protein
	Pr > F					
SPAD/GDD	0.0001*	0.0009*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Variety	0.8352	0.0917	0.2964	0.0516	0.1355	0.0265*
SPAD/GDD*Variety	0.7928	0.4946	0.1272	0.3971	0.0860	0.3105
Site-year	0.1529	0.3768	0.3460	0.2789	0.0388*	<0.0001*
SPAD/GDD*Site-year	0.1162	0.9870	0.3547	0.1417	0.0036*	0.0033*
Variety*Site-year	0.7421	0.4794	0.5002	0.3939	0.9745	0.0907
SPAD/GDD*Variety*Site-year	0.7362	0.5206	0.4422	0.4670	0.9759	0.1306

Table 3.11. Results of regression analysis for the relationship between spring wheat grain yield and SPAD by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x), combined across site-years and varieties for three times of measurement during crop development (T1 = stem elongation; T2 = flag leaf; AN = anthesis). Numbers in parentheses indicate the Standard Error of the mean

	Parameter Estimates						**Pr>F**	**R^2**
x	*A*			*b*				
	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
SPAD/GDD (T1)	-1.64 (3.9)	-9.5	6.2	724.3 (34.7)	655.7	792.8	<0.0001	**0.67**
SPAD/GDD (T2)	-47.4 (7.1)	-61.4	-33.4	1737.1 (97.0)	1545.0	1928.0	<0.0001	**0.60**
SPAD/GDD (AN)	-121.9 (11.3)	-144.3	-99.6	3265.1 (17.8)	2902.0	3627.0	<0.0001	**0.60**

Table 3.12. Results of regression analysis for the relationship between spring wheat grain protein content and SPAD by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x), combined across site-years and varieties for three times of measurement during crop development (T1 = stem elongation; T2 = flag leaf; AN = anthesis). Numbers in parentheses indicate the Standard Error of the mean.

x	**Parameter Estimates**						**Pr>F**	**R^2**
	A			*b*				
	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
SPAD/GDD (T1)	13.9 (0.5)	12.9	14.8	-18.1 (4.1)	-26.2	-9.9	<0.0001	**0.08**
SPAD/GDD (T2)	10.6 (0.8)	9.0	12.1	18.5 (10.7)	-2.6	39.7	0.0860	0.01
SPAD/GDD (AN)	12.2 (1.3)	9.7	14.7	-5.4 (20.5)	-45.8	34.9	0.7920	0.00

Table 3.13. Results of regression analysis for the relationship between spring wheat grain yield and SPAD (measured at anthesis) by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x). Numbers in parentheses indicate the Standard Error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
	A			*b*				
Site-year	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Carman 2016	-43.2 (14)	-72.2	-14.2	1704.0 (251)	1198	2209	<0.0001	**0.48**
Brunkild 2016	-84.2 (16)	-116.3	-52.0	2430.2 (265)	1895	2964	<0.0001	**0.63**
Carman 2017	-19.3 (24)	-67.7	29.1	1830.3 (393)	1040	2620	<0.0001	**0.29**
Brunkild2017	-105.1 (20)	-145	-65	3105.9 (297)	2509	3702	<0.0001	**0.67**

Table 3.14. Results of regression analysis for the relationship between spring wheat grain protein content and SPAD (measured at anthesis) by growing degree days (GDD) accumulated between seeding and sensing using a simple linear model: y = a + (b*x). Numbers in parentheses indicate the Standard Error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
	a			*b*				
Site-year	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Carman 2016	6.20 (1.7)	2.7	9.6	121.2 (30)	61.0	181.3	0.0002	**0.25**
Brunkild 2016	3.04 (1.7)	-0.36	6.4	140.6 (28)	83.9	197.2	<0.0001	**0.34**
Carman2017	9.76 (3.8)	1.9	17.5	50.0 (63)	-76.7	176.7	0.4321	0.01
Brunkild2017	-0.33 (2.1)	-4.5	3.9	159.7 (31.1)	97.3	222.1	<0.0001	**0.33**

Table 3.15. Results of regression analysis for the relationship between spring wheat grain protein content relative to high N treatment and SPAD by growing degree days (GDD) accumulated between seeding and sensing relative to high N treatment using a simple linear model: y = a + (b*x), combined across site-years and varieties for three times of measurement during crop development (T1 = stem elongation; T2 = flag leaf; AN = anthesis). Numbers in parentheses indicate the standard error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
x	*A*			*b*				
	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
SPAD/GDD (T1)	0.74 (0.06)	0.62	0.86	0.16 (0.06)	0.04	0.28	0.0078	0.03
SPAD/GDD (T2)	0.36 (0.06)	0.23	0.49	0.57 (0.07)	0.43	0.70	<0.0001	0.25
SPAD/GDD (AN)	0.17 (0.07)	0.04	0.31	0.77 (0.07)	0.62	0.91	<0.0001	0.34

Table 3.16. Results of ANCOVA for spring wheat grain yield and protein content relationship to flag leaf nitrogen content, variety and site-year

	Yield	Protein
	Pr > F	
Flag N	<0.0001*	<0.0001*
Variety	0.3072	0.7367
Flag N*Variety	0.0619	0.7712
Site-year	<0.0001*	<0.0001*
Flag N*Site-year	0.0005*	<0.0001*
Variety*Site-year	0.6785	0.6609
Flag N*Variety*Site-year	0.5886	0.8300

Table 3.17. Regression analysis of spring wheat grain yield and flag leaf nitrogen content combined across varieties. Results of regression analysis for the relationship between grain yield and flag leaf N content using a simple linear model: y = a + (b*x). Numbers in parentheses indicate the Standard Error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
	a			*b*				
Site-year	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Brunkild 2016	-50.6 (11.8)	-74.3	-26.9	27.5 (2.9)	21.8	33.3	<0.0001	**0.65**
Brunkild 2017	21.3 (31.7)	-42.3	84.9	20.3 (7.9)	4.5	36.1	0.0127	**0.11**
Carberry 2016	194.5 (58.6)	76.4	312.5	-23.8 (14.3)	-52.6	4.9	0.1016	0.06
Carman 2016	-15.0 (12.0)	-39.1	9.1	18.2 (3.2)	11.9	24.5	<0.0001	**0.40**
Carman2017	-3.3 (17.2)	-37.8	31.2	22.1 (3.9)	14.2	29.9	<0.0001	**0.37**
Grosse Isle 2017	-16.8 (13.4)	-43.7	10.2	19.5 (3.1)	13.3	25.7	<0.0001	**0.43**
Melita 2016	-22.4 (12.9)	-48.4	3.8	20.6 (3.3)	14.0	27.2	<0.0001	**0.48**
Melita 2017	-93.7 (18.5)	-131.1	-56.4	36.1 (4.2)	27.7	44.6	<0.0001	**0.62**

Table 3.18. Regression analysis of spring wheat grain protein content and flag leaf nitrogen content combined across varieties. Results of regression analysis for the relationship between protein content and flag leaf N content using a simple linear model: y = a + (b*x). Numbers in parentheses indicate the Standard Error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
x	*a*			*b*				
	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Brunkild 2016	3.0 (1.0)	0.9	5.1	2.1 (0.25)	1.6	2.6	<0.0001	**0.58**
Brunkild 2017	2.9 (2.2)	-1.5	7.4	1.8 (0.55)	0.7	2.9	0.0016	**0.17**
Carberry 2016	-7.7 (3.0)	-13.8	-1.6	5.1 (0.74)	3.6	6.6	<0.0001	**0.51**
Carman 2016	5.6 (1.1)	3.4	7.8	1.9 (0.28)	1.4	2.5	<0.0001	**0.49**
Carman2017	-4.2 (1.8)	-7.8	-0.6	3.9 (0.41)	3.1	4.7	<0.0001	**0.63**
Grosse Isle 2017	-8.2 (1.9)	-12.1	-4.4	4.6 (0.45)	3.7	5.5	<0.0001	**0.67**
Melita 2016	5.7 (1.3)	3.2	8.2	2.0 (0.31)	1.4	2.7	<0.0001	**0.48**
Melita 2017	-6.5 (1.9)	-10.4	-2.6	3.9 (0.43)	3.02	4.8	<0.0001	**0.64**

Table 3.19. Regression analysis of spring wheat grain yield and protein content to flag leaf nitrogen content combined across varieties and site-years. Results of regression analysis for the relationship between grain yield and flag leaf N content using a simple linear model: y = a + (b*x), combined across site-years and varieties. Numbers in parentheses indicate the Standard Error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
	a			*b*				
	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Yield (bu/ac)	-13.8 (10.7)	-34.9	7.4	21.6 (2.6)	16.5	26.7	<0.0001	**0.14**
Protein (%)	6.1 (0.9)	4.4	7.8	1.5 (0.2)	1.0	1.9	<0.0001	**0.10**

Table 3.20. Regression analysis of spring wheat grain yield and protein content relative to high N treatment to flag leaf nitrogen content relative to high N treatment combined across varieties and site-years. Results of regression analysis for the relationship between grain yield and flag leaf N content using a simple linear model: y = a + (b*x), combined across site-years and varieties. Numbers in parentheses indicate the Standard Error of the mean.

	Parameter Estimates						**Pr>F**	**R^2**
	a			*b*				
	Estimate	95% Confidence Interval		Estimate	95% Confidence Interval			
Yield (bu/ac)	-0.15 (0.09)	-0.32	0.03	1.07 (0.09)	0.89	1.26	<0.0001	**0.38**
Protein (%)	0.51 (0.05)	0.40	0.62	0.41 (0.06)	0.30	0.53	<0.0001	**0.20**

Part 3: Figures

Figure 3.1. Linear relationship between yield and NDVI (sensed at stem elongation) divided by growing degree days (GDD) accumulated between seeding and sensing for (A) Brandon and (B) Prosper at Brunkild 2016.

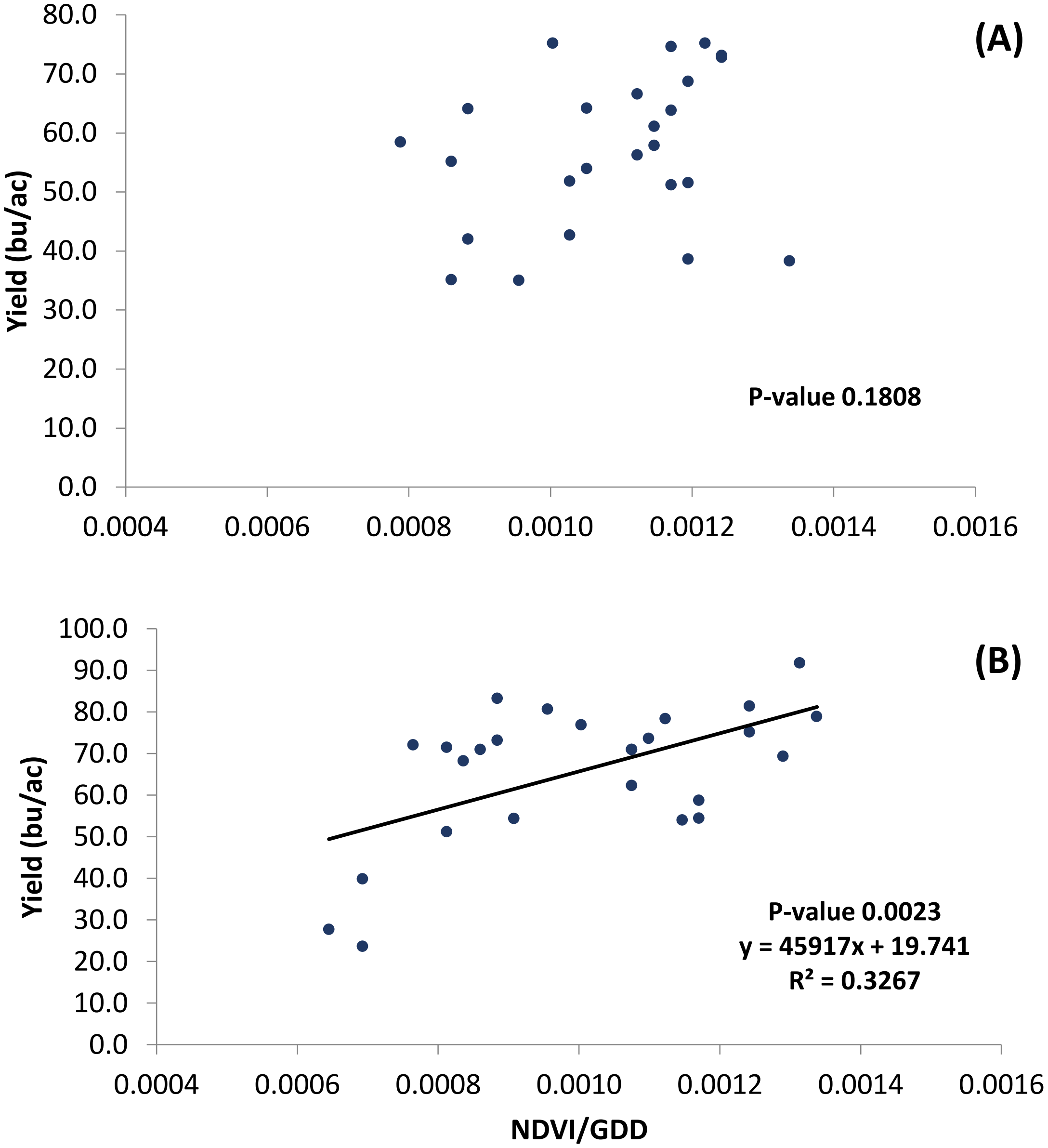


Figure 3.2. Linear relationship between yield and NDVI (sensed at flag leaf) divided by growing degree days (GDD) accumulated between seeding and sensing combined across varieties at Brunkild 2016.

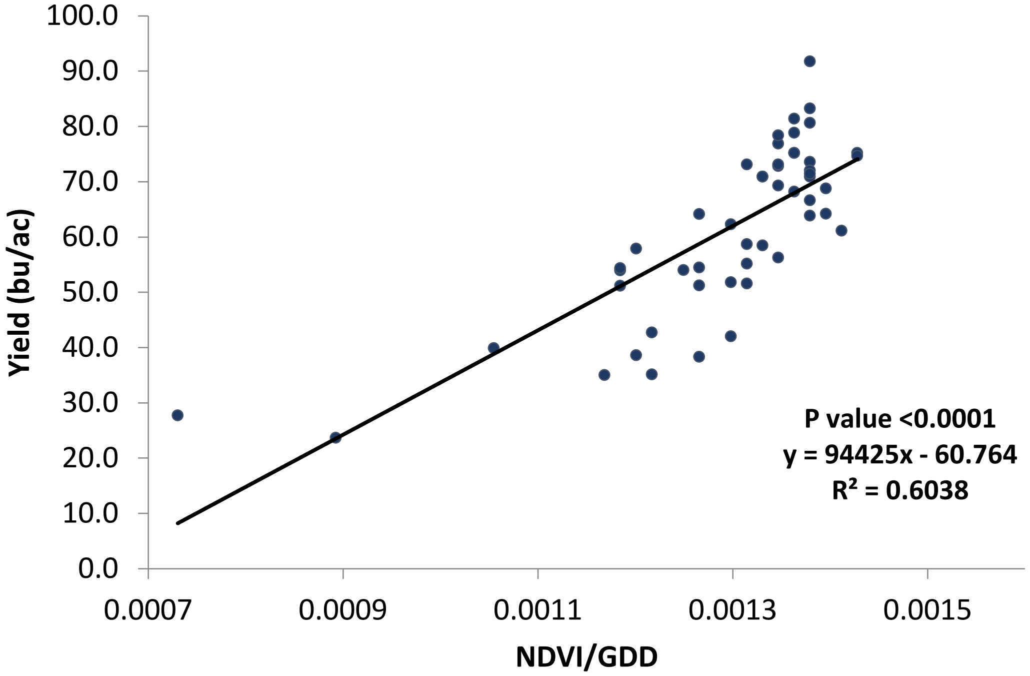


Figure 3.3. Linear relationship between protein and NDVI (sensed at flag leaf) divided by growing degree days (GDD) accumulated between seeding and sensing combined across varieties at Brunkild 2016.

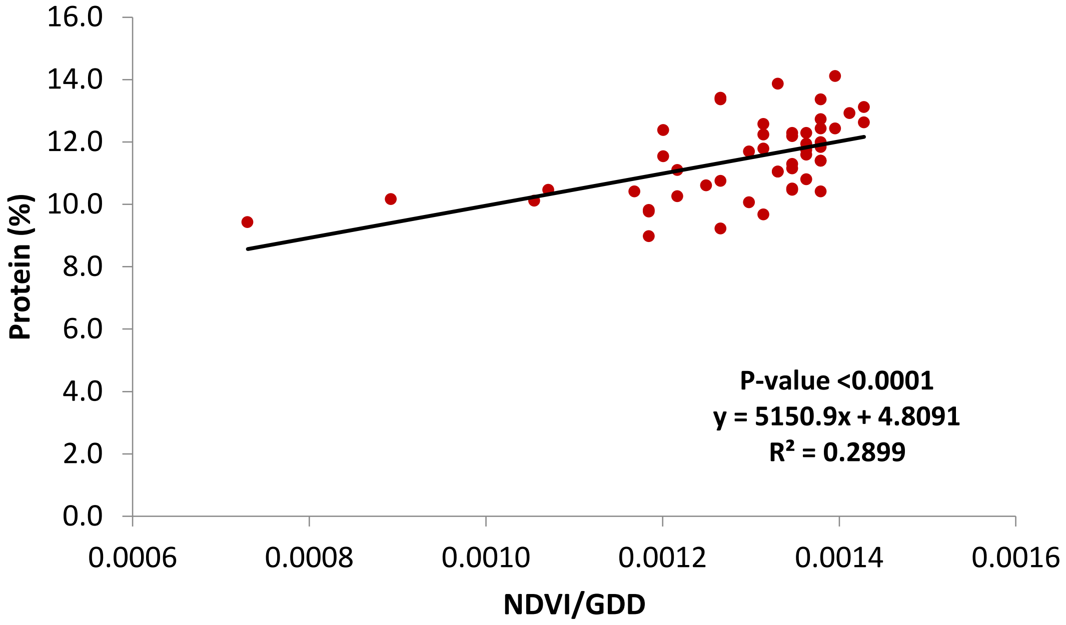


Figure 3.4. Linear relationship between yield and NDVI (sensed at anthesis) divided by growing degree days (GDD) accumulated between seeding and sensing for (A) Brandon and (B) Prosper at Brunkild 2016.

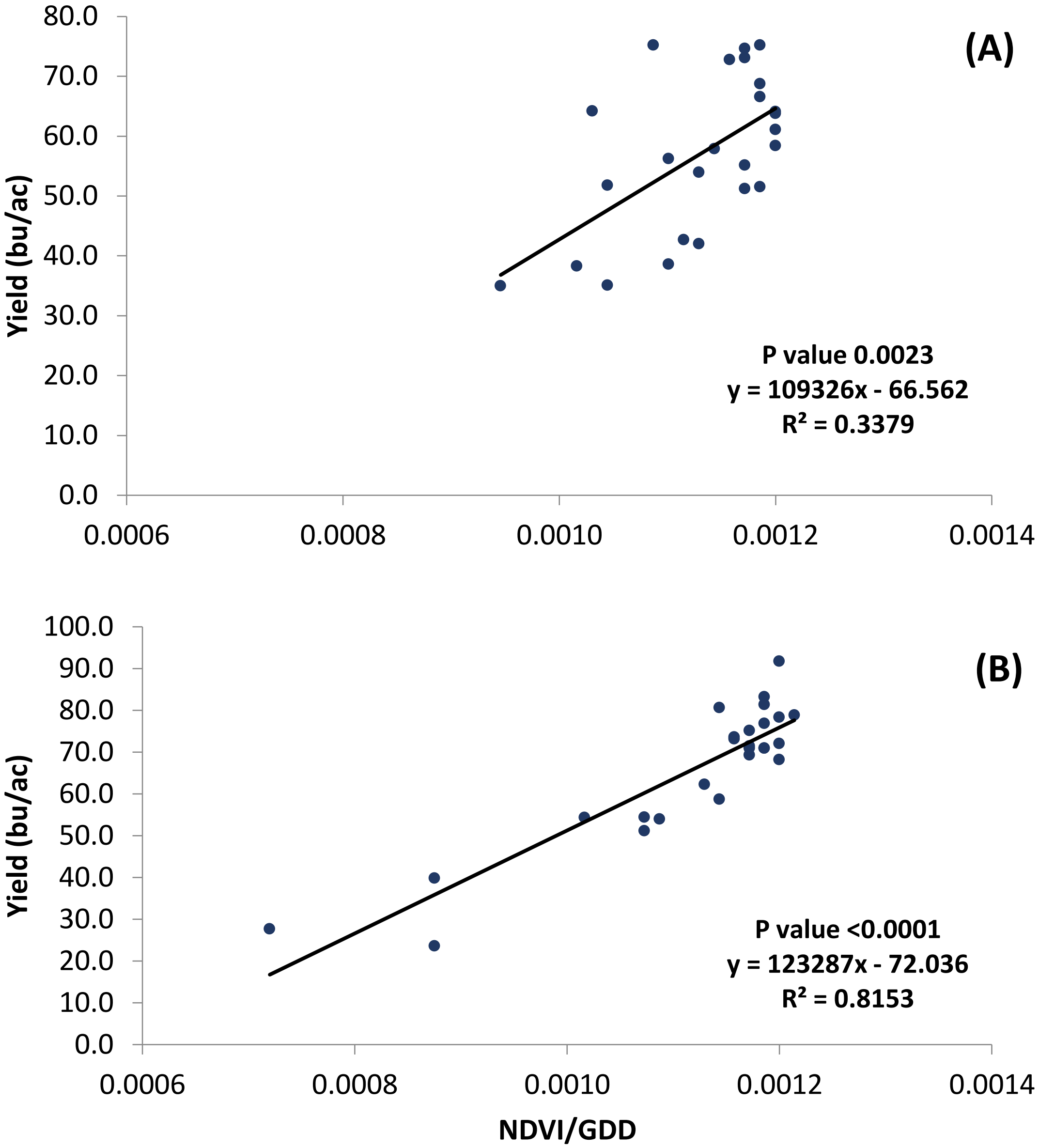


Figure 3.5. Linear relationship between protein and NDVI (sensed at anthesis) divided by growing degree days (GDD) accumulated between seeding and sensing combined across varieties at Brunkild 2016.

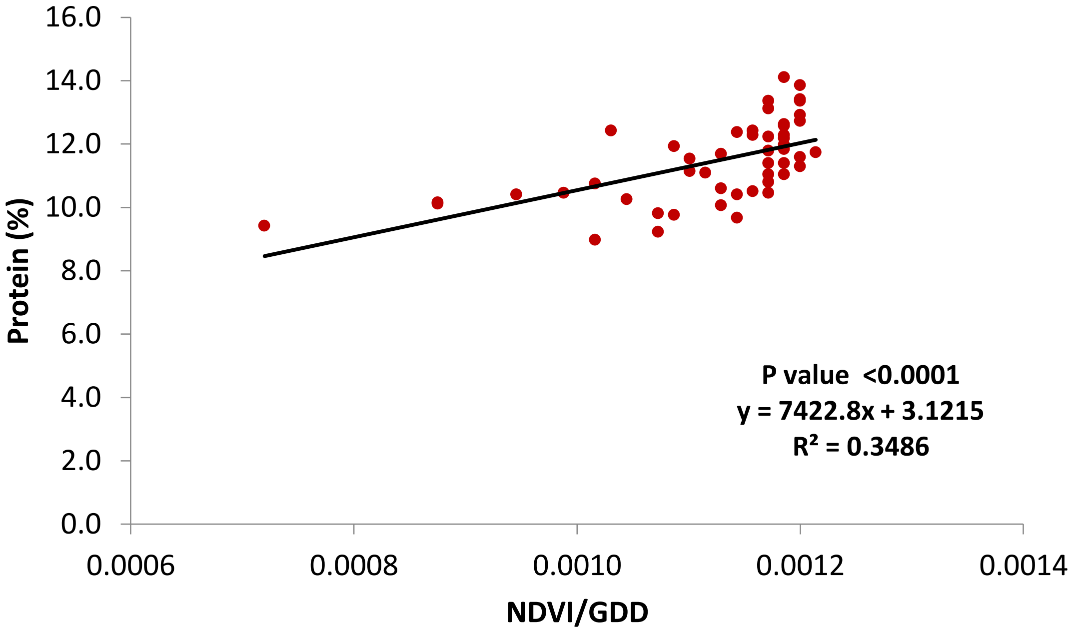


Figure 3.6. Linear relationship between yield and NDVI divided by growing degree days (GDD) accumulated between seeding and sensing combined across site-years and varieties when sensed at (A) stem elongation, (B) flag leaf and (C) anthesis.

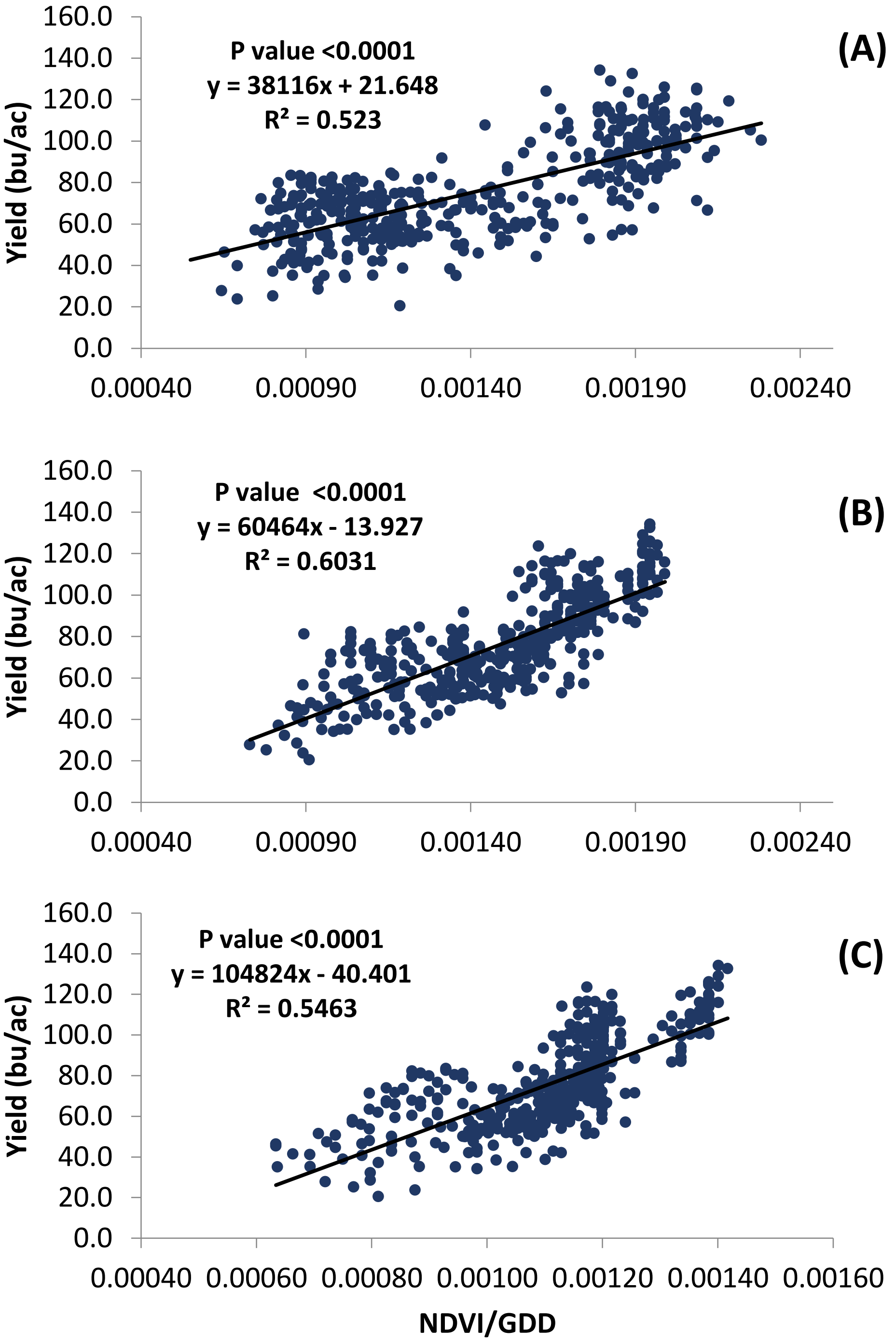


Figure 3.7. Linear relationship between protein content and NDVI divided by growing degree days (GDD) accumulated between seeding and sensing combined across site-years and varieties when sensed at (A) stem elongation, (B) flag leaf and (C) anthesis.

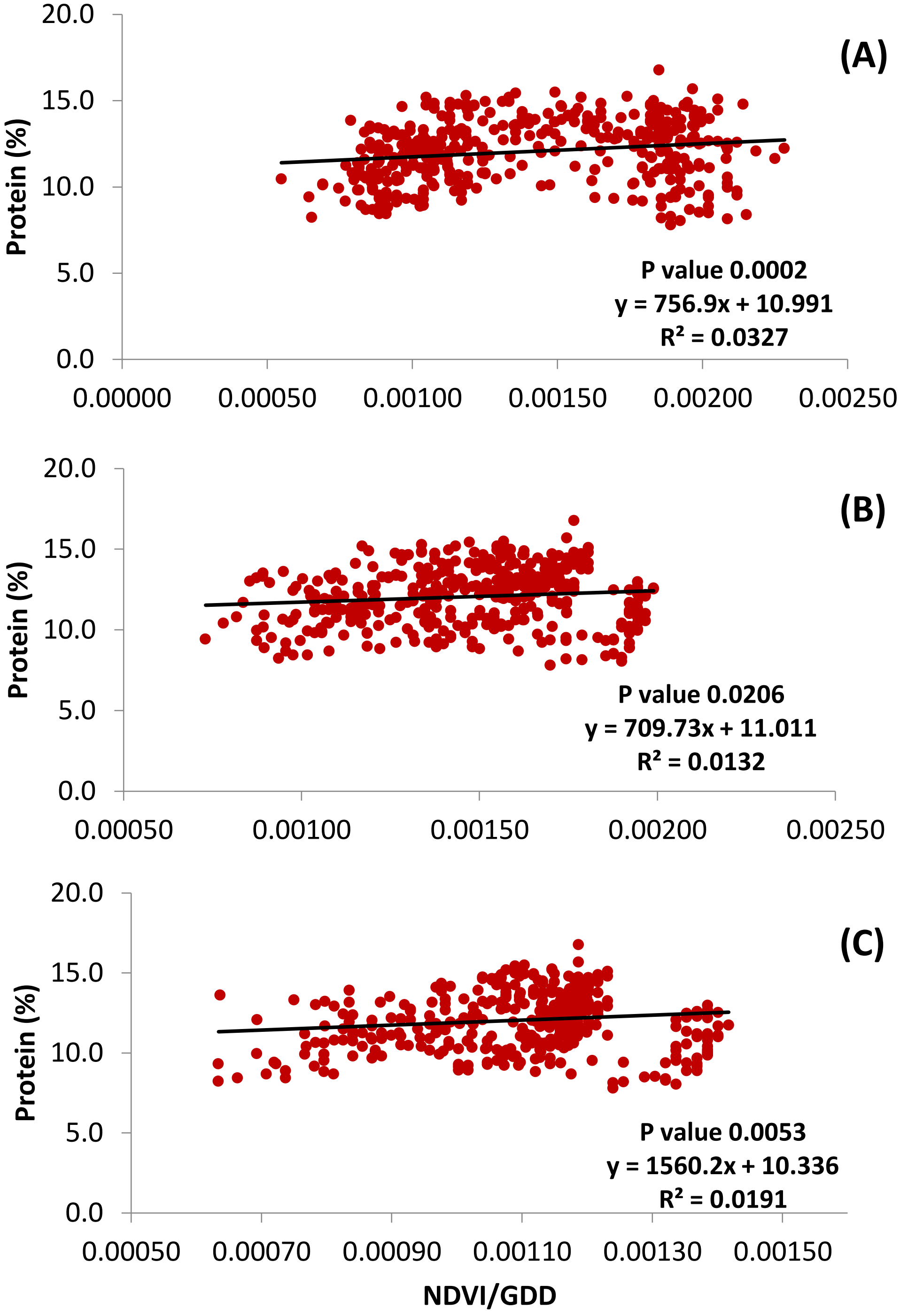


Figure 3.8. Linear relationship between yield and SPAD divided by growing degree days (GDD) accumulated between seeding and sensing combined across site-years and varieties when sensed at (A) stem elongation, (B) flag leaf and (C) anthesis.

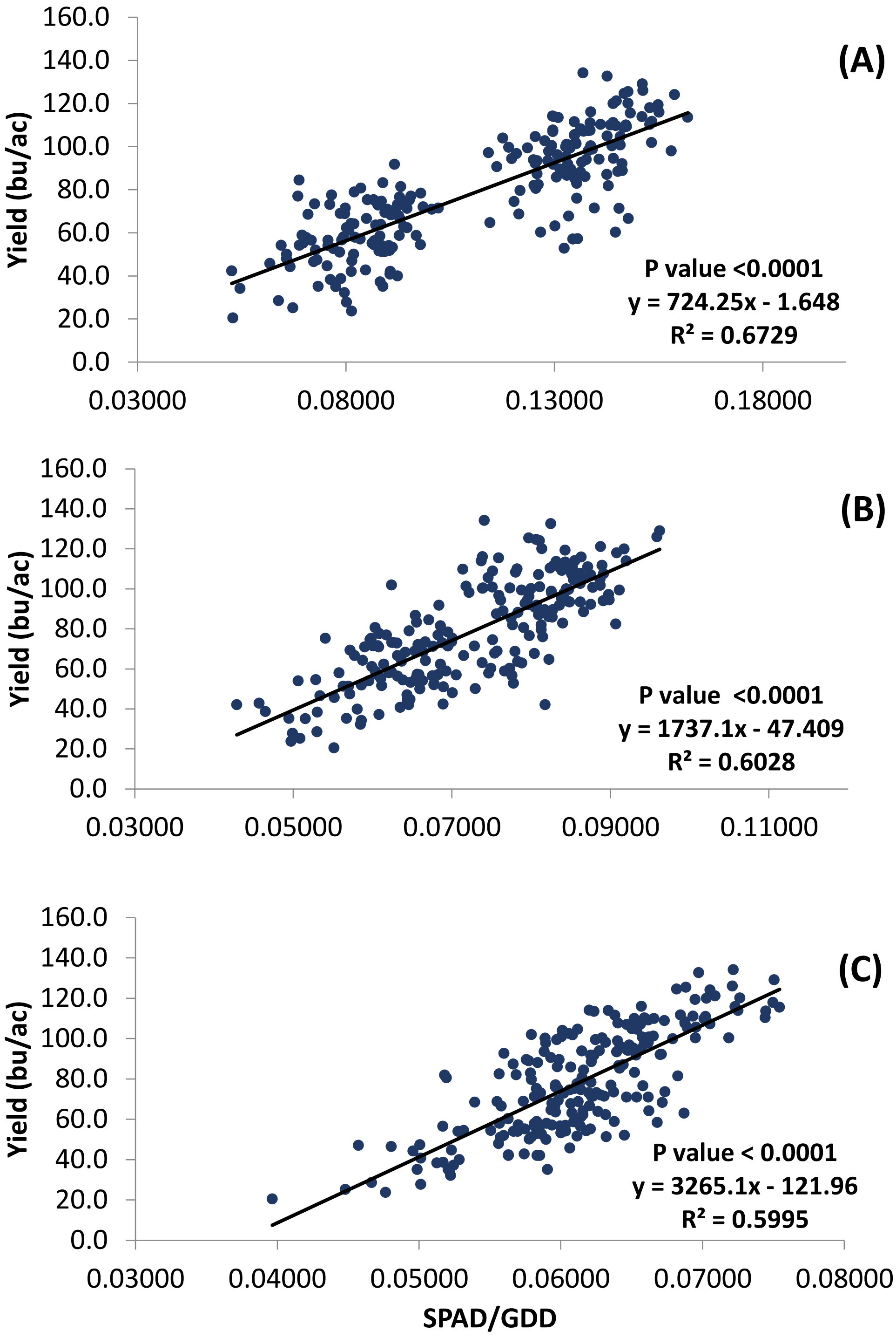


Figure 3.9. Linear relationship between protein and SPAD divided by growing degree days (GDD) accumulated between seeding and sensing combined across site-years and varieties when sensed at (A) stem elongation, (B) flag leaf and (C) anthesis.

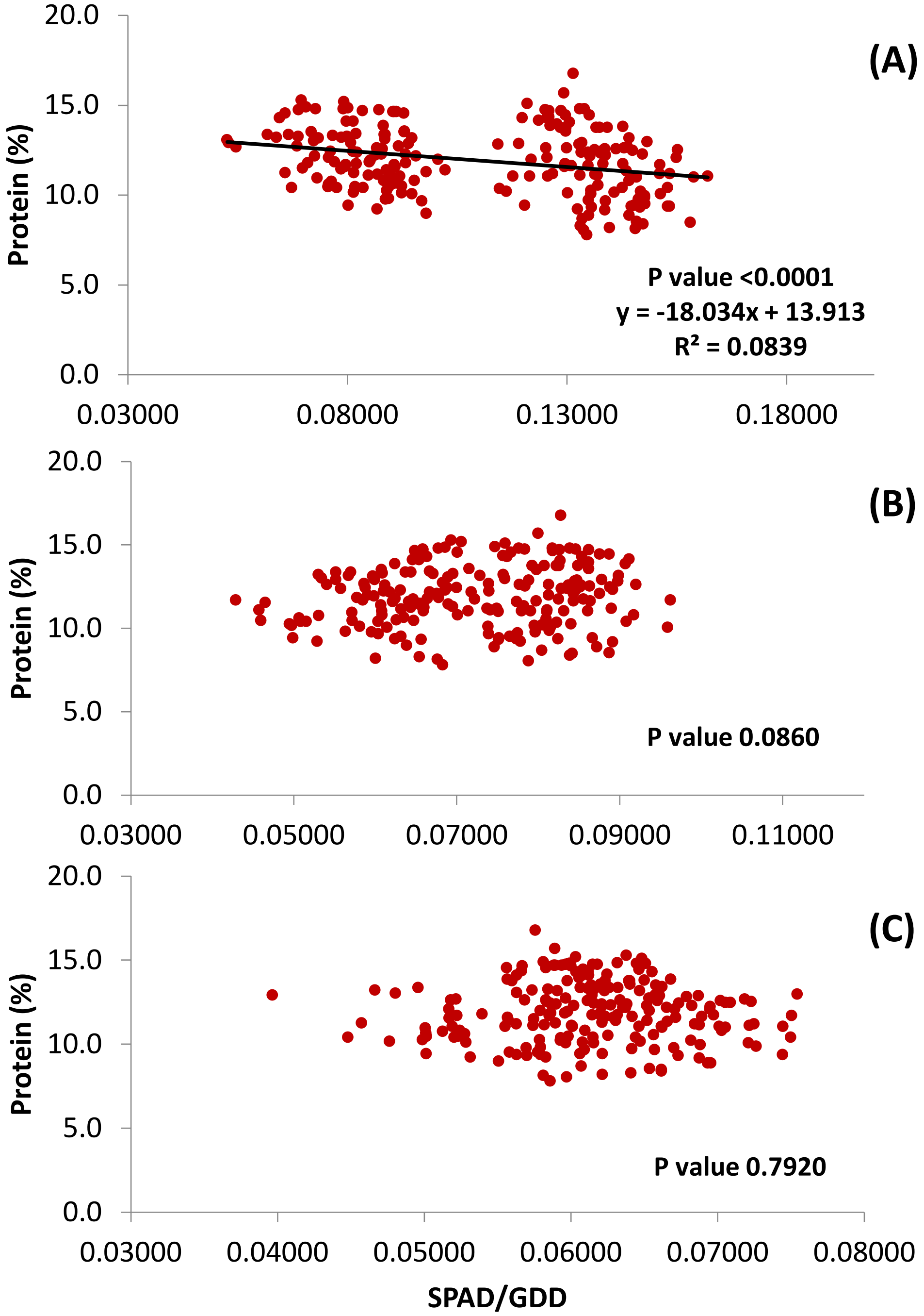


Figure 3.10. Linear relationship between yield and SPAD (sensed at anthesis) divided by growing degree days (GDD) accumulated between seeding and sensing at Brunkild 2016 combined across varieties.

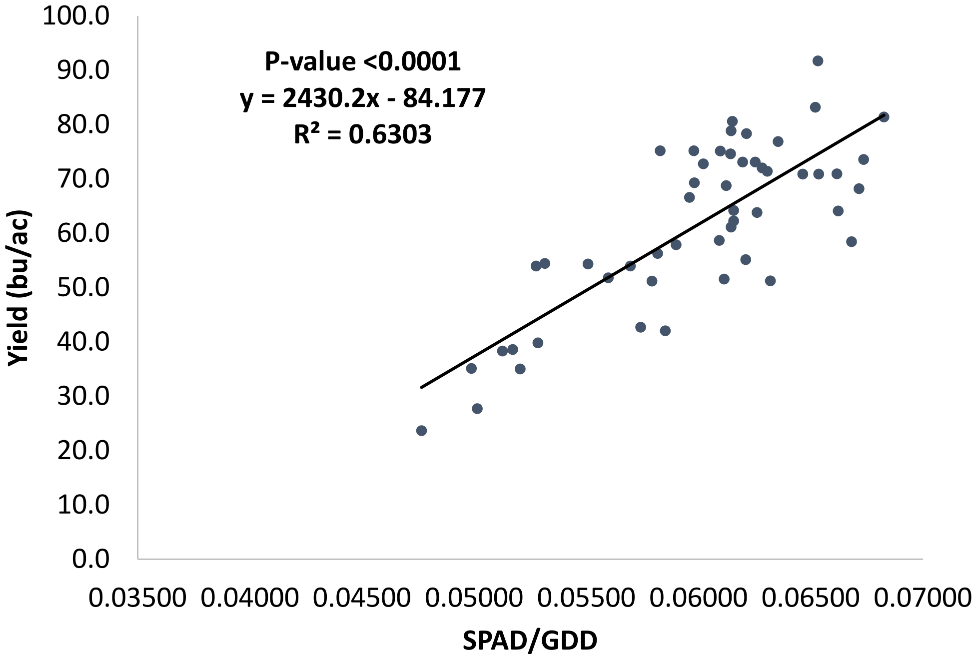


Figure 3.11. Linear relationship between protein and SPAD (sensed at anthesis) divided by growing degree days (GDD) accumulated between seeding and sensing at Brunkild 2016 combined across varieties.

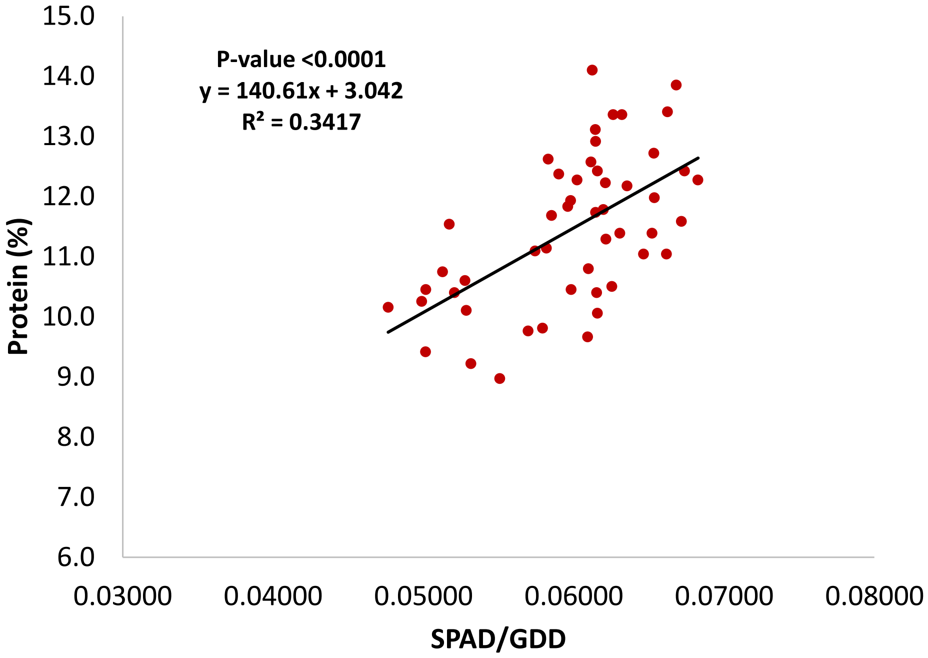


Figure 3.12. Linear relationship between (A) yield and flag leaf nitrogen content and (B) protein and flag leaf content at Brunkild 2016.

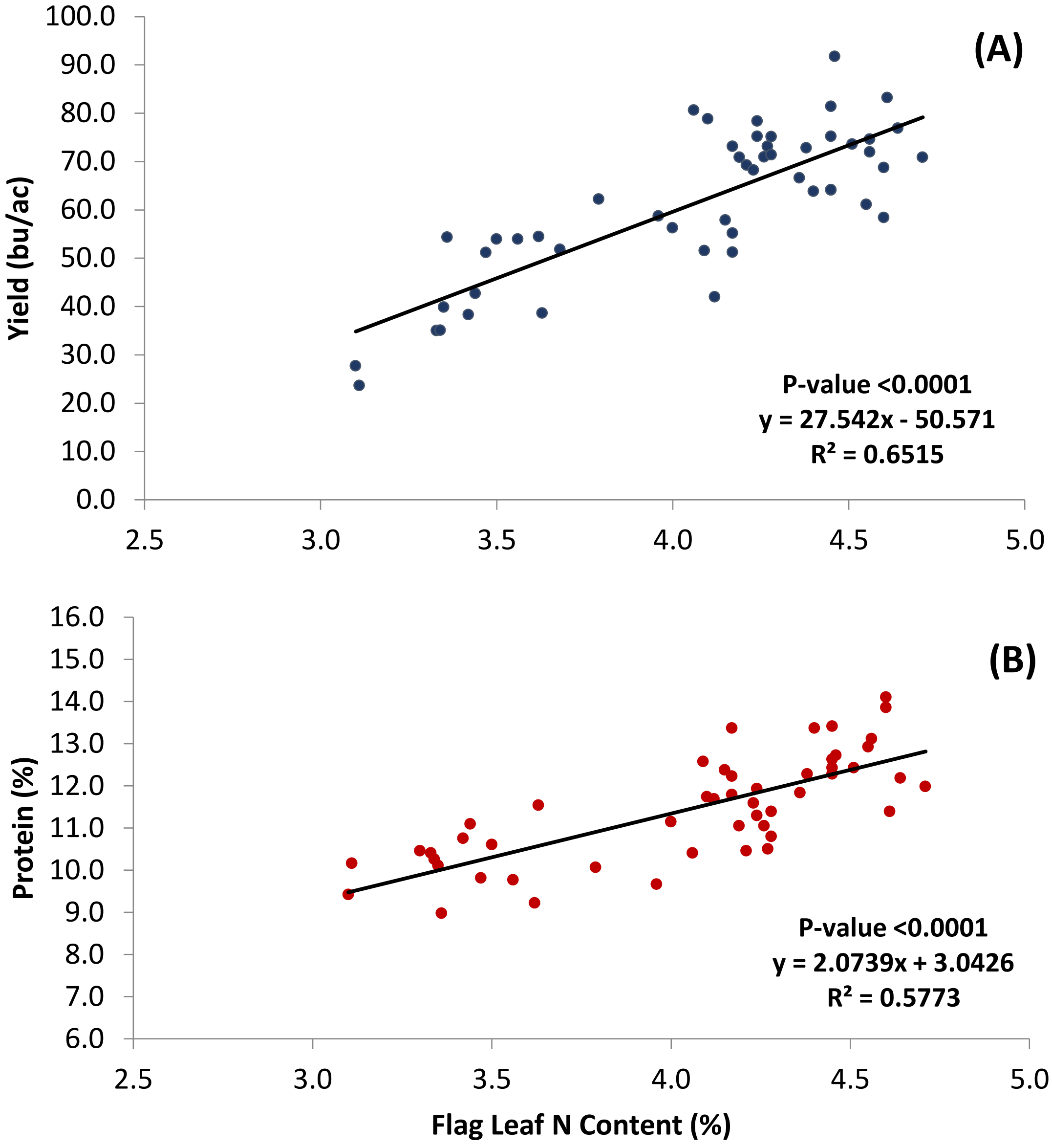


Figure 3.13. Linear relationship between (A) yield and flag leaf nitrogen content and (B) protein and flag leaf content combined across site-years and varieties.

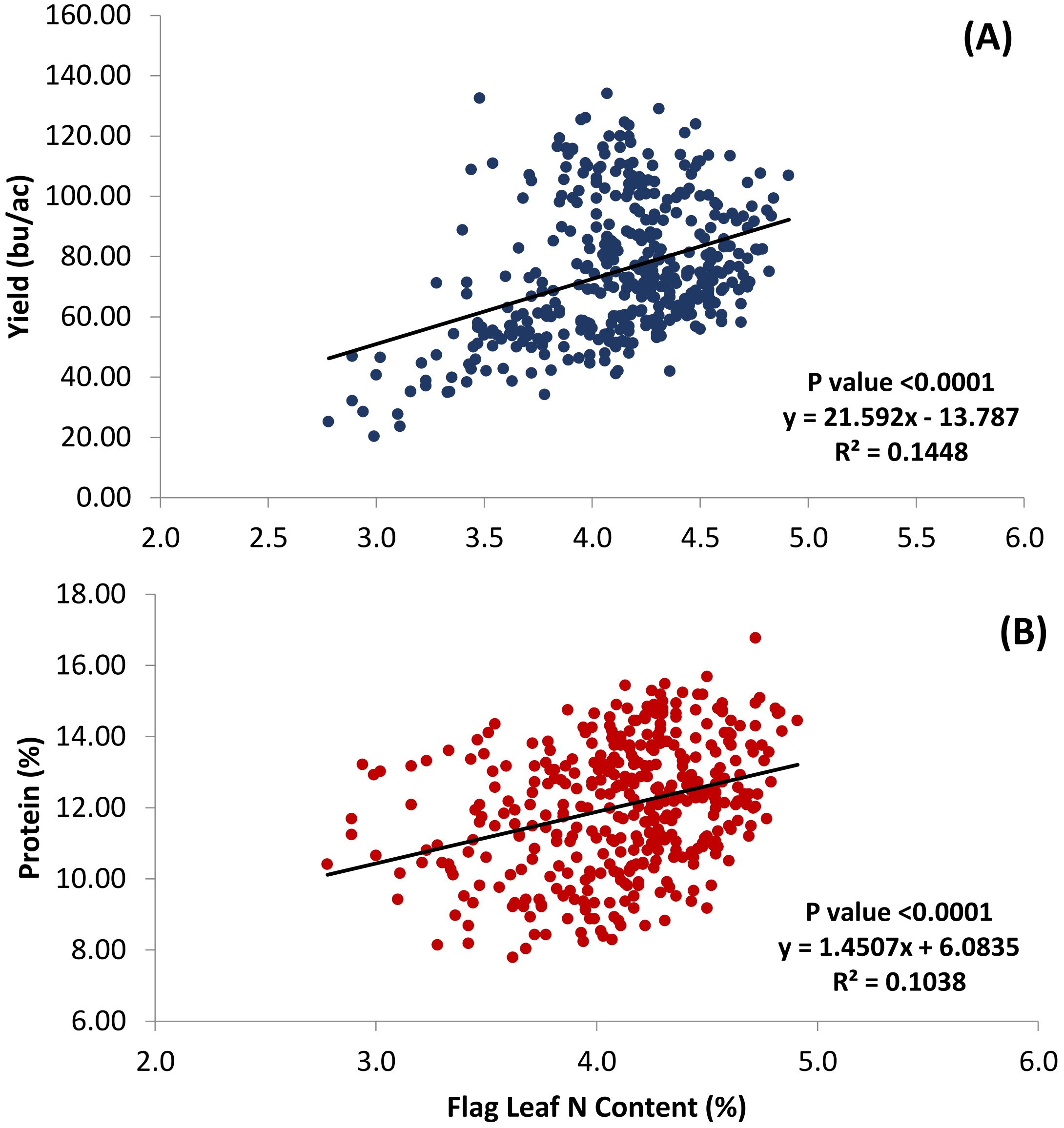


Figure 3.14. Midseason soil nitrate N (sampled at flag leaf, 0-60 cm depth) at gold level sites (A) Carman 2016, (B) Brunkild 2016, (C) Carman 2017, and (D) Brunkild 2017, similar letters over bars indicate values that do not differ significantly from each other, averaged over varieties at each N rate. Economic optimum N rates at each site indicated with a (*).

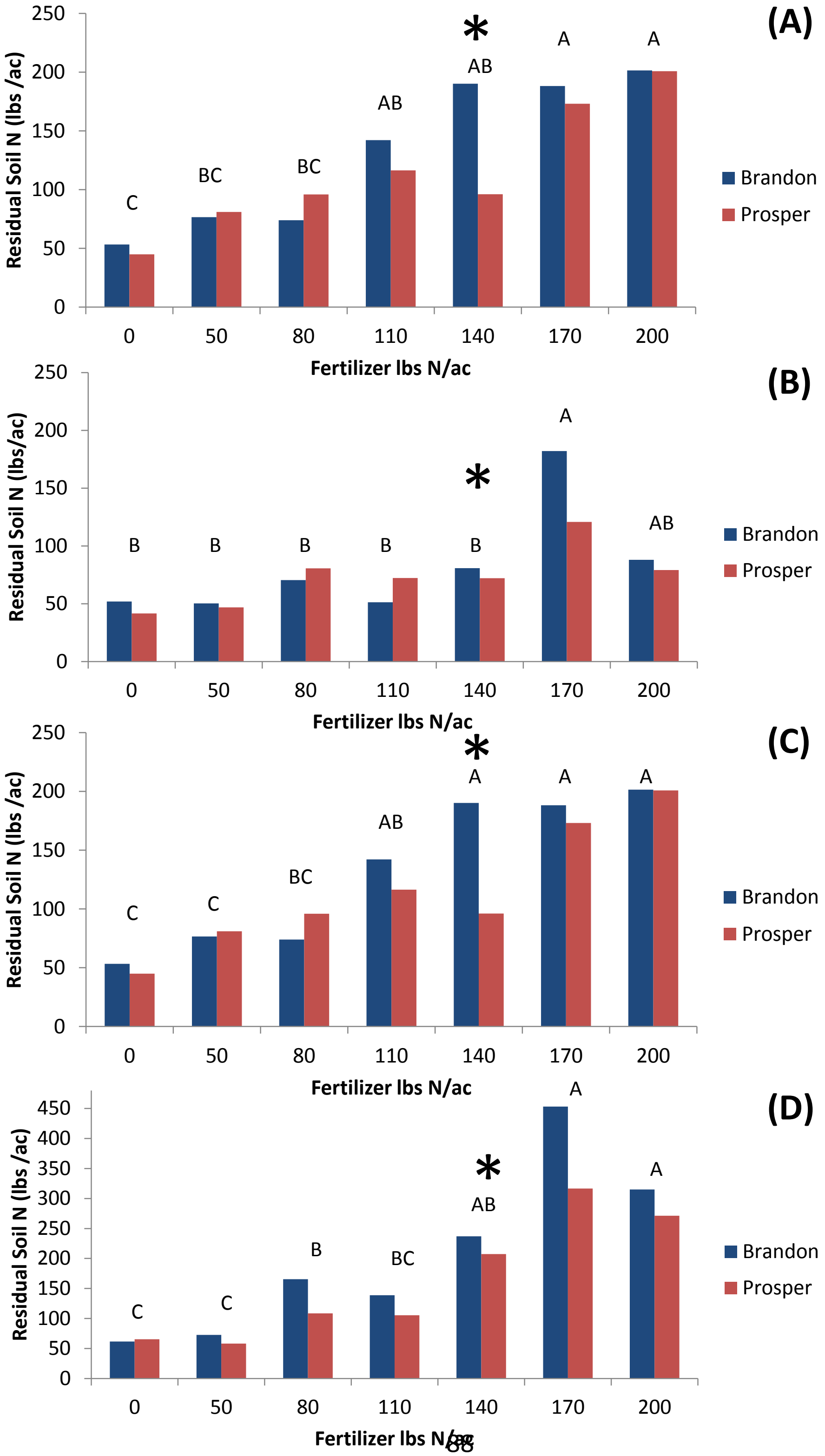


Figure 3.15. Post-harvest soil residual nitrate N (0-60 cm depth) at silver level sites (A) Carberry 2016, (B) Melita 2016, (C) Grosse Isle 2017, and (D) Melita 2017, similar letters over bars indicate values that do not differ significantly from each other, averaged over varieties at each N rate. Economic optimum N rates at each site indicated with a (*).

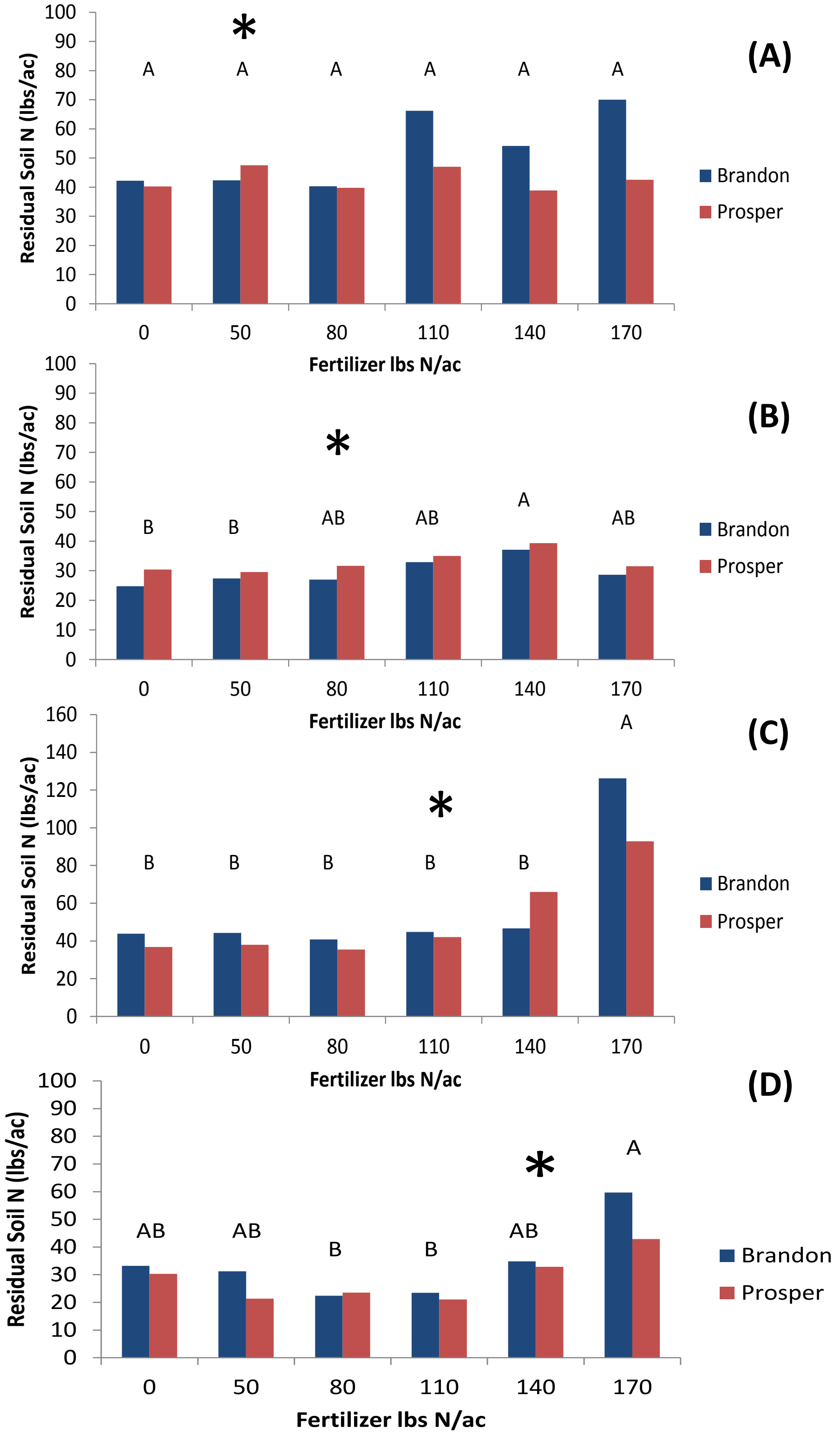
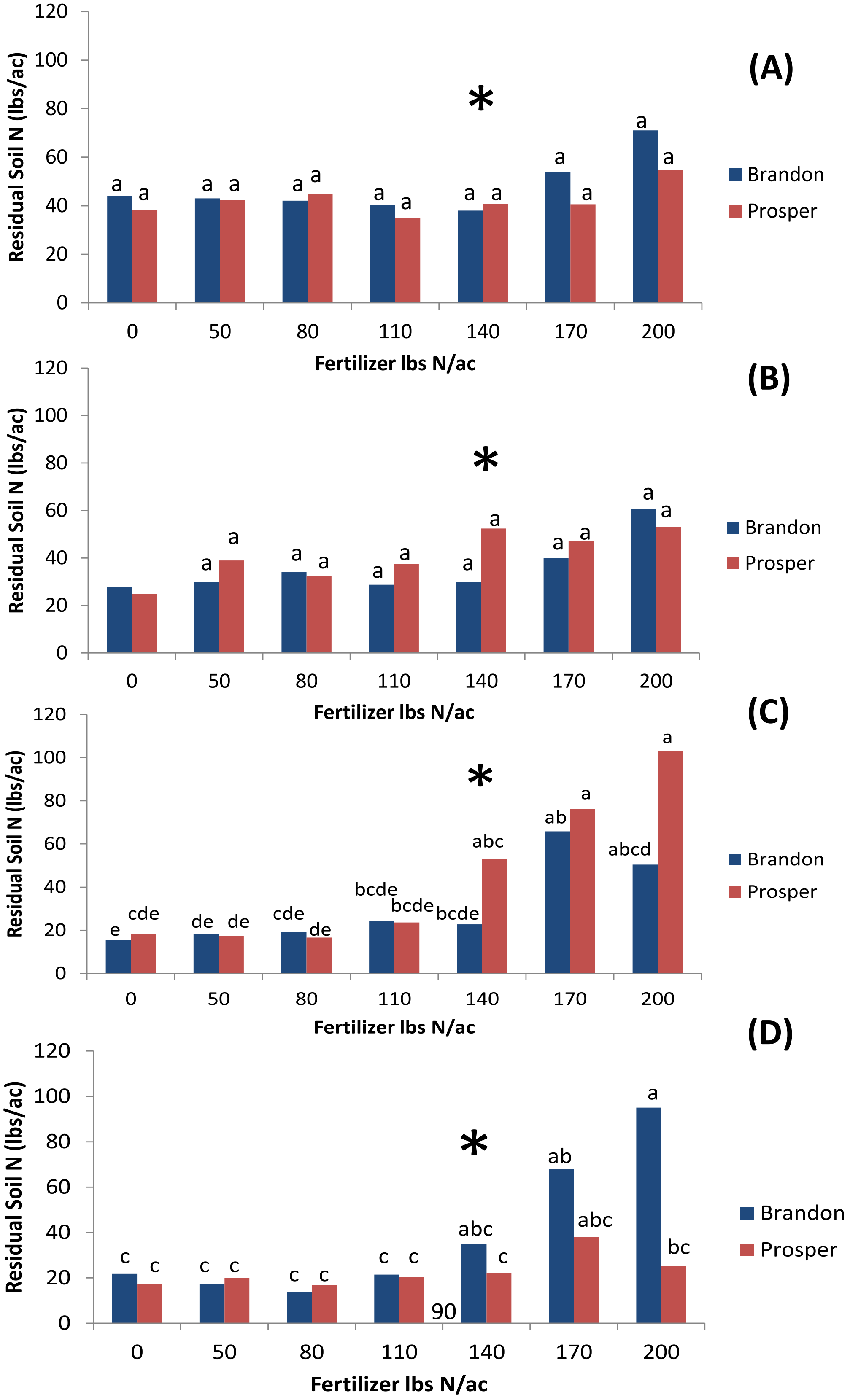


Figure 3.16. Post-harvest soil residual nitrate N (0-60 cm depth) at gold level sites (A) Carman 2016, (B) Brunkild 2016, (C) Carman 2017, and (D) Brunkild 2017, similar letters over bars indicate values that do not differ significantly from each other. Economic optimum rates at each site indicated with a (*).



Overall Summary and Conclusions

The potential yields for current varieties of spring wheat being grown across Manitoba are much higher than what they have been in the past and as a result, large amounts of N are required to achieve these yields. Pre-plant soil NO_3-N tests reveal the amount of early season available N and, paired with the target yield for a particular field and year, are used to determine N recommendations. The average total supply of N (spring NO_3-N + fert) required to obtain economic optimum yields across site-years in this project was 1.99 lbs N/bu, but economic rates per bushel varied substantially, especially at silver level sites.

One of the reasons for this variability in optimum rates of N was the variability in growing season mineralization of soil N, especially across silver level sites, which resulted in large deviations from expected N supply from the soil. Conventional recommendations for the total supply of N do not take into account the organic reserves of soil N that are released through mineralization during the growing season. Our study revealed that it is extremely difficult to use a pre-plant soil test to predict the amount of N that will be mineralized during the growing season across locations, probably due to variability in environmental conditions and management histories.

Due to this uncertainty in soil N supply during the growing season it could be beneficial to apply enough N at planting to meet a modest yield goal and re-visit the question of N sufficiency for yield and protein potential once the crop is established. However, in order for this strategy to work we first need to be able to evaluate yield and protein potential in-season, and secondly we need to be confident that the in-season intervention with N fertilizer will result in a yield or protein response.

Indices used to predict grain yield (GreenSeeker and SPAD Meter) were relatively reliable when combined across site-years and varieties, regardless of when there were measured in our study. In-season N applications at stem elongation and flag leaf timing obtained similar yields as equivalent rates applied entirely at planting. This indicates that there is potential for delaying a portion of N fertilizer in-season without decreasing yield. NDVI measured by the GreenSeeker had the best relationship with final grain yield, in particular when it was measured at flag leaf timing, which coincided well with the responses to midseason applications of N fertilizer at this timing.

Grain protein content was much more difficult to predict across site-years and varieties, probably due to the uncertainty of late season N supply from soil N mineralization. In-season N applications resulted in increased protein content, especially when applied at flag leaf and post-anthesis timings. Post-anthesis N applications solely target protein increases, rather than increases in both yield and protein, as for earlier season split applications. Post-anthesis applications consistently increased protein content but to warrant an application that solely targets protein increases would require the ability to predict absolute protein content before application. This would help a wheat producer to determine if the expected grain protein content was likely to be below a target market threshold (e.g., 13%) and also if the response to late season application would be enough to raise protein levels over that threshold. However, the tools that we tested did not predict absolute concentrations of grain protein across site-years.

Post-harvest soil NO_3-N can be used as an auditing tool to determine if the supply of N was excessive for meeting the yield and protein of wheat in a particular field and year. When comparing economic optimum N rates to post-harvest N supply, we determined that if residual levels were greater than 55 lbs N/ac, the supply of N was likely more than adequate for achieving optimum economic yields of spring wheat at that site and in that year.

Appendices

Appendix Table 1. Gold level sites lsmeans analysis of grain yield (bu/ac**).** Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05).

	Treatment					Site-Year		
Variety	Timing	Source	Rate	Brunkild 2016	Carman 2016	Brunkild 2017	Carman 2017	Combined
			lbs N/ac			bu/ac		
Brandon			0	35.8	38.0	59.1	71.1	51.0 o
	Seeding	Urea	50	46.5	41.0	92.8	88.3	67.1 n
	Seeding	Urea	80	51.4	45.0	97.2	88.5	70.5 mn
	Seeding	Urea	110	62.7	49.5	108.7	91.2	78.0 ijklm
	Seeding	Urea	140	72.9	53.9	109.1	94.0	82.5 efghijkl
	Seeding	Urea	170	63.3	59.2	110.8	96.5	82.4 efghijkl
	Seeding	Urea	200	66.5	56.5	110.4	97.7	82.8 efghijk
	Seeding	ESN/Urea	80	58.3	47.3	97.9	85.8	72.3 lmn
	Seeding	ESN/Urea	140	64.3	53.4	113.1	85.9	79.2 hijklm
	Seeding/T1	Urea	110	67.6	59.8	106.0	94.0	81.9 fghijkl
	Seeding/T1	Urea	140	73.2	59.6	109.0	93.3	83.8 defghijk
	Seeding/T2	Urea	110	62.3	54.3	113.9	86.3	79.2 hijklm
	Seeding/T2	Urea	140	61.6	51.8	108.8	90.4	78.2 ijklm
	Seeding/PA	Urea/UAN	110	56.1	35.9	101.9	87.9	70.4 mn
	Seeding/PA	Urea/Urea Sol	100	56.3	40.8	105.1	93.6	73.9 klmn
	Seeding	Urea (BC)	80	61.5	61.7	84.7	94.7	75.6 ijklmn
Prosper			0	29.7	36.2	67.4	71.2	51.1 o
	Seeding	Urea	50	53.5	50.6	99.9	97.0	75.2 jklmn
	Seeding	Urea	80	63.5	54.8	105.5	94.1	79.5 ghijklm
	Seeding	Urea	110	70.5	63.6	120.9	100.6	88.9 abcdefgh
	Seeding	Urea	140	76.3	71.0	112.2	98.5	89.5 abcdefgh
	Seeding	Urea	170	76.4	69.8	119.3	101.0	91.7 abcdef
	Seeding	Urea	200	80.7	79.8	128.9	106.4	99.0 a
	Seeding	ESN/Urea	80	66.9	63.6	110.9	97.2	84.7 cdefghij
	Seeding	ESN/Urea	140	76.8	76.8	126.6	95.1	93.9 abcd
	Seeding/T1	Urea	110	80.8	72.0	121.0	102.3	94.0 abc
	Seeding/T1	Urea	140	70.6	71.6	122.7	104.4	92.4 abcde
	Seeding/T2	Urea	110	72.1	75.2	127.2	100.9	93.8 abcd
	Seeding/T2	Urea	140	75.8	73.2	125.6	104.7	94.8 ab
	Seeding/PA	Urea/UAN	110	60.2	59.7	109.7	93.7	80.8 ghijkl
	Seeding/PA	Urea/Urea Sol	110	67.2	58.3	114.7	101.4	85.4 bcdefghi
	Seeding	Urea (BC)	80	64.6	78.3	123.4	94.1	90.1 abcdefg
Brandon				60.0 bC	50.5 bD	101.8 bA	89.9 bB	75.6
Prosper				67.8 aC	65.9 aC	114.7 aA	97.7 aB	86.7
			0	32.7 fB	37.1 fB	63.3 eA	71.1 bA	52.4
	Seeding	Urea	50	49.9 eB	45.7 efB	96.3 dA	92.6 aA	71.2
	Seeding	Urea	80	57.4 deB	49.8 cdefB	101.3 dcA	91.4 aA	75.0
	Seeding	Urea	110	66.6 abcdC	56.5 bcdeC	114.8 abcA	95.9 aB	83.5
	Seeding	Urea	140	74.6 aC	62.2 abcdC	110.6 abcdA	96.3 aB	85.9
	Seeding	Urea	170	69.8 abcC	64.5 abC	115.1 abcA	98.7 aB	87.0
	Seeding	Urea	200	73.7 abcC	68.1 abC	119.7 abA	102.0 aB	90.9
	Seeding	ESN/Urea	80	62.6 bcdC	55.5 bcdeC	104.4 bcdA	91.5 aB	78.5
	Seeding	ESN/Urea	140	70.5 abcC	65.1 abC	119.9 abA	90.5 aB	86.5
	Seeding/T1	Urea	110	74.2 aC	65.9 abC	113.5 abcA	98.2 aB	87.9
	Seeding/T1	Urea	140	71.8 abC	65.6 abC	115.9 abcA	98.9 aB	88.1
	Seeding/T2	Urea	110	67.2 abcdC	64.8 abC	120.5 aA	93.6 aB	86.5
	Seeding/T2	Urea	140	68.7 abcdC	62.5 abcC	117.2 abA	97.5 aB	86.5
	Seeding/PA	Urea/UAN	110	58.1 dceC	47.8 defC	105.8 abcdA	90.8 aB	75.6
	Seeding/PA	Urea/Urea Sol	110	61.7 bcdeC	49.6 efD	109.9 abcdA	97.5 aB	79.7
	Seeding	Urea (BC)	80	62.9 abcdB	70.0 aB	104.1 bcdA	94.4 aA	82.9
Site-Year				63.9	58.2	108.6	93.8	
ANOVA		df				Pr> F		
Variety								<0.0001
N Trt								<0.0001
Variety* N Trt								0.0202
SiteYr								<0.0001
SiteYr*Variety								<0.0001
SiteYr*N trt								<0.0001
SiteYr*N trt*Variety								0.1326
Coeff var (C.V.)								30.1

Appendix Table 2. Silver level sites lsmeans analysis of grain yield (bu/ac)**.** Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05).

Treatment				Site-year				
Variety	Timing	Source	Rate	Melita 2016	Carberry 2016	Melita 2017	Grosse Isle 2017	Combined
			lbs N/ac	bu/ac				
Brandon			0	39.7	78.0	45.2	54.9	54.4
	Seeding	Urea	50	49.2	86.4	57.1	60.5	63.3
	Seeding	Urea	80	55.3	88.7	61.8	65.3	67.8
	Seeding	Urea	110	58.7	93.8	71.4	73.2	74.1
	Seeding	Urea	140	58.1	87.7	69.3	71.2	71.6
	Seeding	Urea	170	61.7	90.8	75.8	72.2	75.1
	Seeding/T1	Urea	110	59.7	98.7	71.8	72.9	75.6
	Seeding/T1	Urea	140	61.5	90.1	73.4	74.8	74.9
	Seeding/T2	Urea	110	57.4	87.6	66.0	72.3	70.8
	Seeding/T2	Urea	140	63.4	91.3	71.5	69.5	73.9
	Seeding/PA	Urea/UAN	110	52.1	76.4	66.2	69.3	66.0
Prosper			0	47.7	101.4	45.9	58.4	63.3
	Seeding	Urea	50	56.0	102.6	59.0	66.9	71.1
	Seeding	Urea	80	64.8	104.9	71.3	73.5	78.6
	Seeding	Urea	110	70.3	104.8	68.0	76.0	79.8
	Seeding	Urea	140	72.0	109.0	78.0	76.9	84.0
	Seeding	Urea	170	71.2	107.8	79.6	77.8	84.1
	Seeding/T1	Urea	110	67.2	107.9	77.5	77.7	82.6
	Seeding/T1	Urea	140	71.9	107.4	76.8	77.2	83.4
	Seeding/T2	Urea	110	69.5	102.7	74.3	74.4	80.2
	Seeding/T2	Urea	140	72.7	110.0	76.0	74.6	83.3
	Seeding/PA	Urea/UAN	110	63.1	90.1	69.0	73.4	73.9
Brandon				56.0 bC	88.1 bA	66.3 bB	68.7 bB	69.8
Prosper				66.0 aC	104.4 aA	70.5 aB	73.3 aB	78.6
			0	43.7 dC	89.7 bcA	45.6 dC	56.6 cB	58.9
	Seeding	Urea	50	52.6 dcC	94.5 abA	58.0 cBC	63.7 bcB	67.2
	Seeding	Urea	80	60.0 abcC	96.8 abA	66.6 bcBC	69.4 abB	73.2
	Seeding	Urea	110	64.2 abC	99.3 abA	69.7 abBC	74.6 aB	77.0
	Seeding	Urea	140	65.1 abC	98.3 abA	73.7 abBC	74.0 aB	77.8
	Seeding	Urea	170	66.5 abC	99.3 abA	77.7 aB	75.0 aB	79.6
	Seeding/T1	Urea	110	63.1 abC	103.3 aA	74.7 abB	75.3 aB	79.1
	Seeding/T1	Urea	140	66.7 abC	98.8 abA	75.1 abB	76.0 aB	79.1
	Seeding/T2	Urea	110	63.5 abC	95.1 abA	70.1 abBC	73.3 abB	75.5
	Seeding/T2	Urea	140	68.1 aB	100.7 aA	73.7 abB	72.1 abB	78.6
	Seeding/PA	Urea/UAN	110	57.6 bcC	83.3 cA	67.6 bcB	71.3 abB	69.9
Site-Year				61.0	96.3	68.4	71.0	

ANOVA	**df**	**Pr > F**
Variety	1	<0.0001
N Trt	10	<0.0001
Variety*N Trt	10	0.7301
SiteYr	3	<0.0001
SiteYr*Variety	30	<0.0001
SiteYr*N Trt	3	<0.0001
SiteYr*Variety*N Trt	30	0.9958
Coeff var (C.V.)		22.6

Appendix Table 3. Pre-determined linear contrasts for in-season timing of N applications on grain yield (bu/ac)

Gold Level Sites						
	Estimate			Estimate	Std. Err	P-Value
	bu/ac			bu/ac		
Planting	84.7	vs.	Stem Elongation Split	88.0	2.54	0.0156
Planting	84.7	vs.	Flag Leaf Split	86.5	2.52	0.1843
Planting (80 lbs N/ac)	75.0	vs.	Post-Anthesis Split	77.6	3.08	0.0947
Planting (110 lbs N/ac)	83.5	vs.	Post-Anthesis Split	77.6	3.16	0.0004
Stem Elongation Split	75.0	vs.	Flag Leaf Split	86.5	2.50	0.2610
Stem Elongation Split	88.0	vs.	Post-Anthesis Split	77.6	3.05	<0.0001
Flag Leaf Split	86.5	vs.	Post Anthesis Split	77.6	3.06	<0.0001
Silver Level Sites						
Planting	77.4	vs.	Stem Elongation Split	79.1	0.19	0.1135
Planting	77.4	vs.	Flag Leaf Split	77.1	2.20	0.789
Planting (80 lbs N/ac)	73.2	vs.	Post-Anthesis Split	69.9	1.56	0.0376
Planting (110 lbs N/ac)	77.0	vs.	Post-Anthesis Split	69.9	1.56	<0.0001
Stem Elongation Split	79.1	vs.	Flag Leaf Split	77.1	2.21	0.0664
Stem Elongation Split	79.1	vs.	Post-Anthesis Split	69.9	1.56	<0.0001
Flag Leaf Split	78.6	vs.	Post Anthesis Split	69.9	1.56	0.0004

Appendix Table 4. Gold level sites lsmeans analysis of grain protein content (%)**.** Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05).

Treatment				Site-Year				
Variety	Timing	Source	Rate	Brunkild 2016	Carman 2016	Brunkild 2017	Carman 2017	Combined
			lbs N/ac	----------	----------	%	----------	----------
Brandon			0	10.7	13.2	9.4	11.7	11.2
	Seeding	Urea	50	11.0	13.6	9.5	12.5	11.7
	Seeding	Urea	80	11.8	14.0	10.5	13.6	12.5
	Seeding	Urea	110	12.4	13.9	11.3	14.2	13.0
	Seeding	Urea	140	12.2	14.7	12.4	14.2	13.4
	Seeding	Urea	170	13.0	14.8	12.4	15.2	13.9
	Seeding	Urea	200	13.7	14.9	12.6	14.8	14.0
	Seeding	ESN/Urea	80	12.0	14.0	10.6	13.1	12.4
	Seeding	ESN/Urea	140	13.0	14.6	12.3	13.6	13.4
	Seeding/T1	Urea	110	12.6	14.3	11.0	13.4	13.8
	Seeding/T1	Urea	140	13.4	15.0	12.2	15.0	13.9
	Seeding/T2	Urea	110	13.3	14.8	11.5	14.3	13.5
	Seeding/T2	Urea	140	14.0	15.0	12.3	15.3	14.2
	Seeding/PA	Urea/UAN	110	13.6	15.0	11.9	14.2	13.7
	Seeding/PA	Urea/Urea Sol	110	14.7	16.0	12.2	15.0	14.4
	Seeding	Urea (BC)	80	11.5	13.8	9.8	13.9	12.3
Prosper			0	9.9	10.9	8.2	10.0	9.8
	Seeding	Urea	50	9.4	11.2	8.3	10.8	10.0
	Seeding	Urea	80	10.3	11.7	8.9	10.9	10.4
	Seeding	Urea	110	11.0	12.4	10.1	11.6	11.3
	Seeding	Urea	140	10.9	12.6	10.1	12.7	11.6
	Seeding	Urea	170	12.0	12.9	10.8	13.1	12.2
	Seeding	Urea	200	12.2	13.4	11.1	13.5	12.6
	Seeding	ESN/Urea	80	9.9	11.6	9.4	11.6	10.6
	Seeding	ESN/Urea	140	11.5	13.4	11.1	12.0	12.0
	Seeding/T1	Urea	110	10.9	12.5	10.3	12.1	11.4
	Seeding/T1	Urea	140	11.9	13.5	10.9	12.5	12.2
	Seeding/T2	Urea	110	12.1	12.6	10.0	12.1	11.7
	Seeding/T2	Urea	140	13.5	13.1	11.1	12.9	12.6
	Seeding/PA	Urea/UAN	110	12.2	13.7	10.4	12.2	12.1
	Seeding/PA	Urea/Urea Sol	110	13.0	13.4	10.9	13.0	12.6
	Seeding	Urea (BC)	80	9.9	11.8	9.5	11.2	10.6
Brandon				12.7 aC	14.5 aA	11.4 aD	14.0 aB	13.1
Prosper				11.3 bC	12.5 bA	10.1 bD	12.0 bB	11.5
			0	10.3 gB	12.0 gA	8.8 fC	10.9 hB	10.5
	Seeding	Urea	50	10.2 gB	12.4 fgA	8.9 fC	11.7 ghA	10.8
	Seeding	Urea	80	11.0 efgB	12.8 defgA	9.7 efC	12.3 fgA	11.4
	Seeding	Urea	110	11.7 defB	13.2 defA	10.7 cdC	12.9 defA	12.1
	Seeding	Urea	140	11.6 defB	13.7 abcdeA	11.3 abcB	13.5 abcdeA	12.5
	Seeding	Urea	170	12.5 bcdB	13.8 abcdA	11.6 abcC	14.2 abA	13.0
	Seeding	Urea	200	12.9 abB	14.1 abcA	11.8 aC	14.2 aA	13.3
	Seeding	ESN/Urea	80	10.9 efgB	12.8 efgA	10.0 deC	12.3 fgA	11.5
	Seeding	ESN/Urea	140	12.3 bcdBC	14.0 abcA	11.7 abC	12.8 defB	12.7
	Seeding/T1	Urea	110	11.7 cdeB	13.4 bcdeA	10.6 cdeC	12.8 efA	12.1
	Seeding/T1	Urea	140	12.7 bcB	14.3 abA	11.6 abcC	13.8 abcdA	13.1
	Seeding/T2	Urea	110	12.7 bB	13.7 bcdeA	10.8 bcdC	13.2 cdefAB	12.6
	Seeding/T2	Urea	140	13.8 aA	14.1 abcA	11.7 abB	14.1 abcA	13.4
	Seeding/PA	Urea/UAN	110	12.9 abB	14.3 abA	11.2 abcC	13.2 bcdefB	12.9
	Seeding/PA	Urea/Urea Sol	110	13.8 aB	14.7 aA	11.5 abcC	14.0 abcAB	13.5
	Seeding	Urea (BC)	80	10.7 fgB	12.8 efgA	9.6 efC	12.6 efgA	11.4
Site-Year				12.0	13.5	10.7	13.0	

ANOVA	df	Pr> F
Variety	1	<0.0001
N Trt	15	<0.0001
Variety* N Trt	15	0.6775
SiteYr	3	<0.001
SiteYr*Variety	3	<0.001
SiteYr*N trt	45	<0.001
SiteYr*N trt*Variety	45	0.2531
Coeff var (C.V.)		14.29

Appendix Table 5. Silver level sites lsmeans analysis of grain protein content (%)**.** Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05).

Variety	Timing	Source	Rate	Melita 2016	Carberry 2016	Melita 2017	Grosse Isle 2017	Combined
			lbs N/ac	------------------------- % -------------------------				
Brandon			0	13.2	13.2	9.4	10.4	11.5
	Seeding	Urea	50	13.8	13.5	9.7	10.6	11.9
	Seeding	Urea	80	14.0	13.2	10.6	12.2	12.5
	Seeding	Urea	110	14.8	13.8	11.5	12.8	13.2
	Seeding	Urea	140	15.0	14.0	11.8	13.5	13.6
	Seeding	Urea	170	15.1	14.0	12.4	14.4	14.0
	Seeding/T1	Urea	110	14.4	13.3	12.1	14.0	13.4
	Seeding/T1	Urea	140	15.1	12.9	12.7	13.6	13.8
	Seeding/T2	Urea	110	14.9	14.4	11.8	13.7	13.7
	Seeding/T2	Urea	140	15.0	14.1	12.8	14.6	14.1
	Seeding/PA	Urea/UAN	110	15.6	14.5	11.7	13.2	13.7
Prosper			0	12.7	11.3	8.6	9.2	10.5
	Seeding	Urea	50	11.8	12.5	9.0	9.7	10.8
	Seeding	Urea	80	13.0	12.7	10.5	10.6	11.7
	Seeding	Urea	110	13.7	13.3	10.9	12.1	12.5
	Seeding	Urea	140	13.8	12.9	11.3	12.5	12.6
	Seeding	Urea	170	13.8	13.5	11.9	12.7	13.0
	Seeding/T1	Urea	110	13.8	13.1	11.3	11.8	12.5
	Seeding/T1	Urea	140	13.8	13.2	11.7	12.6	12.8
	Seeding/T2	Urea	110	13.5	13.3	10.8	12.2	12.5
	Seeding/T2	Urea	140	14.1	13.4	11.9	13.4	13.2
	Seeding/PA	Urea/UAN	110	14.1	13.7	10.9	12.2	12.7
Brandon				14.6 aA	13.8 aAB	11.5 aC	13.0 aB	13.2
Prosper				13.5 bA	13.0 bA	10.8 bB	11.7 bB	12.2
			0	13.0 cA	12.2 cA	9.0 eB	9.8 eB	11.0
	Seeding	Urea	50	12.8 cA	13.0 bcA	9.4 eB	10.1 eB	11.3
	Seeding	Urea	80	13.5 bcA	12.9 bcA	10.6 dB	11.4 dB	12.1
	Seeding	Urea	110	14.2 abA	13.6 abAB	11.2 cdC	12.5 cB	12.9
	Seeding	Urea	140	14.4 abA	13.4 abAB	11.5 abcC	13.0 abcB	13.1
	Seeding	Urea	170	14.5 aA	13.7 abAB	12.1 abcB	13.5 abA	13.5
	Seeding/T1	Urea	110	14.1 abA	13.2 abAB	11.7 abcB	12.9 bcAB	13.0
	Seeding/T1	Urea	140	14.4 abA	13.5 abAB	12.2 abC	13.1 abcBC	13.3
	Seeding/T2	Urea	110	14.2 abA	13.8 abAB	11.3 bcdB	13.0 bcAB	13.1
	Seeding/T2	Urea	140	14.6 aA	13.7 abAB	12.4 aB	14.0 aA	13.7
	Seeding/PA	Urea/UAN	110	14.9 aA	14.1 aA	11.3 bcdC	12.7 bcB	13.2
Site-Year				14.0	13.4	11.2	12.4	

ANOVA	**df**	**Pr > F**
Variety	1	<0.0001
N Trt	10	<0.0001
Variety*N Trt	10	0.8558
SiteYr	3	<0.0001
SiteYr*Variety	30	<0.0001
SiteYr*N Trt	3	0.0028
SiteYr*Variety*N Trt	30	0.349
Coeff var (C.V.)		13.11

Appendix Table 6. Pre-determined linear contrasts for in-season timing of N applications on grain protein content (%)

Gold Level Sites						
	Estimate			Estimate	Std. Err	P-Value
	%			%		
Planting	12.3	vs.	Stem Elongation Split	12.6	0.2053	0.0035
Planting	12.3	vs.	Flag Leaf Split	13.0	0.2039	<0.0001
Planting (80 lbs N/ac)	11.4	vs.	Post-Anthesis Split	13.2	0.2524	<0.0001
Planting (110 lbs N/ac)	12.1	vs.	Post-Anthesis Split	13.2	0.2556	<0.0001
Stem Elongation Split	12.6	vs.	Flag Leaf Split	13.0	0.2001	0.0001
Stem Elongation Split	12.1	vs.	Post-Anthesis Split	13.2	0.2439	<0.0001
Flag Leaf Split	12.6	vs.	Post Anthesis Split	13.2	0.2424	<0.0001
Silver Level Sites						
Planting	13.0	vs.	Stem Elongation Split	13.1	0.2046	0.1463
Planting	13.0	vs.	Flag Leaf Split	13.4	0.2057	0.0003
Planting (80 lbs N/ac)	12.1	vs.	Post-Anthesis Split	13.2	0.1436	<0.0001
Planting (110 lbs N/ac)	12.9	vs.	Post-Anthesis Split	13.2	0.1436	0.0119
Stem Elongation Split	13.1	vs.	Flag Leaf Split	13.4	0.2050	0.0281
Stem Elongation Split	13.0	vs.	Post-Anthesis Split	13.2	0.1436	0.0631
Flag Leaf Split	13.1	vs.	Post Anthesis Split	13.2	0.1436	0.2610

Appendix Table 7. Economic analysis (Return to N, $) for gold level sites. Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05). Analysis is based on grain pricing from Jan 5, 2018 and 5-year average urea price of $0.43/lbs. N and assumed access to lower protein wheat markets.

Treatment				Site-Year				
Variety	Timing	Source	Rate	Brunkild 2016	Carman 2016	Brunkild 2017	Carman 2017	Combined
			lbs N/ac	---------- Return to N ($) ----------				
Brandon			0	182.5	251.3	280.9	412.5	281.8
	Seeding	Urea	50	211.6	254.7	418.9	535.2	355.3
	Seeding	Urea	80	260.1	277.9	450.1	558.7	386.7
	Seeding	Urea	110	322.4	304.0	541.9	609.7	444.5
	Seeding	Urea	140	377.3	343.9	612.2	617.1	487.6
	Seeding	Urea	170	326.6	372.5	602.6	659.5	490.3
	Seeding	Urea	200	373.9	344.3	604.6	640.6	490.9
Prosper			0	144.0	183.7	316.4	339.1	245.8
	Seeding	Urea	50	232.8	242.9	453.8	474.4	351.0
	Seeding	Urea	80	280.3	261.1	467.3	452.4	365.3
	Seeding	Urea	110	316.7	309.2	527.9	496.4	412.6
	Seeding	Urea	140	325.5	348.8	494.3	505.8	418.6
	Seeding	Urea	170	350.6	336.6	538.2	532.6	439.5
	Seeding	Urea	200	364.7	406.2	586.0	575.3	483.1
Brandon				293.5 aC	306.9 aC	501.6 aB	576.3 aA	419.6
Prosper				287.8 aB	289.4 aB	483.4 aA	482.3 bA	388.0
			0	163.3	217.5	298.6	375.8	263.8 d
	Seeding	Urea	50	222.2	248.8	436.4	505.3	353.2 c
	Seeding	Urea	80	270.2	269.5	458.7	505.6	376.0 c
	Seeding	Urea	110	319.6	306.6	534.9	553.0	428.5 b
	Seeding	Urea	140	351.4	346.4	553.3	561.4	453.1 ab
	Seeding	Urea	170	338.6	354.5	570.4	596.1	464.9 ab
	Seeding	Urea	200	369.3	375.3	595.3	607.9	487.0 a
Site-Year				290.7	302.6	492.5	529.3	

ANOVA	df	Pr> F
Variety	1	<0.0001
N Trt	6	<0.0001
Variety* N Trt	6	0.1879
SiteYr	3	<0.0001
SiteYr*Variety	3	<0.0001
SiteYr*N trt	18	0.0536
SiteYr*N trt*Variety	18	0.4737
Coeff var (C.V.)		34.9

Appendix Table 8. Economic analysis (Return to N, $) for silver level sites. Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05). Analysis is based on grain pricing from Jan 5, 2018 and 5-year average urea price of $0.43/lbs. N and assumed access to lower protein wheat markets.

Treatment				Site-Year				
Variety	Timing	Source	Rate	Melita 2016	Carberry 2016	Melita 2017	Grosse Isle 2017	Combined
			lbs N/ac	Return to N ($)				
Brandon			0	260.7	510.7	214.8	269.3	313.9
	Seeding	Urea	50	314.7	568.8	241.7	279.6	351.2
	Seeding	Urea	80	354.4	543.7	282.4	354.0	383.6
	Seeding	Urea	110	390.5	595.3	351.2	415.8	438.2
	Seeding	Urea	140	386.0	561.9	336.6	421.3	426.4
	Seeding	Urea	170	401.5	562.6	383.2	452.0	449.8
Prosper			0	277.9	523.6	218.4	277.8	324.4
	Seeding	Urea	50	283.4	562.7	258.9	296.9	350.5
	Seeding	Urea	80	355.3	579.2	318.5	328.2	395.3
	Seeding	Urea	110	399.0	592.6	296.5	378.4	416.6
	Seeding	Urea	140	403.5	579.8	345.7	381.1	427.5
	Seeding	Urea	170	389.7	592.8	364.1	372.5	430.0
Brandon				351.3 aB	571.8 aA	301.7 aC	365.3 aB	393.9
Prosper				351.5 aB	557.2 aA	300.4 aC	339.3 bB	390.7
			0	269.3 bBC	517.2 bA	216.6 cC	273.5 cB	319.2
	Seeding	Urea	50	299.0 bB	565.8 aA	250.3 cB	288.2 cB	350.8
	Seeding	Urea	80	354.9 aB	561.4 abA	300.4 bC	341.1 bBC	389.5
	Seeding	Urea	110	394.8 aB	594.0 aA	323.9 bC	397.1 aB	427.4
	Seeding	Urea	140	394.7 aB	570.8 aA	341.2 abC	401.2 aB	427.0
	Seeding	Urea	170	395.6 aB	577.7 aA	373.7 aB	412.8 aB	439.9
Site-Year				351.4	564.5	301.0	352.3	

ANOVA	df	Pr> F
Variety	1	0.5169
N Trt	5	<0.0001
Variety* N Trt	5	0.1793
SiteYr	3	<0.0001
SiteYr*Variety	3	0.0262
SiteYr*N trt	15	0.0001
SiteYr*N trt*Variety	15	0.2492
Coeff var (C.V.)		30.3

Appendix Table 9. Gold level sites lsmeans analysis of nitrogen uptake (lbs N/ac)**.** Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05).

Treatment				Site-Year				
Variety	Timing	Source	Rate	Brunkild 2016	Carman 2016	Brunkild 2017	Carman 2017	Combined
			lbs N/ac	--- lbs. N/ac	---			
Brandon			0	53.1	81.1	68.7	108.2	77.8
	Seeding	Urea	50	67.2	91.9	114.7	148.0	105.5
	Seeding	Urea	80	89.9	104.7	132.5	164.6	122.9
	Seeding	Urea	110	106.7	120.9	157.6	176.0	140.3
	Seeding	Urea	140	137.0	134.2	170.4	184.6	156.5
	Seeding	Urea	170	122.1	153.7	179.6	202.0	164.4
	Seeding	Urea	200	141.8	161.1	188.9	198.3	172.5
	Seeding	ESN/Urea	80	102.6	105.5	131.2	149.5	122.2
	Seeding	ESN/Urea	140	126.7	137.1	178.1	150.9	148.2
	Seeding/T1	Urea	110	110.7	151.1	153.2	170.4	145.8
	Seeding/T1	Urea	140	120.6	139.2	174.2	191.0	172.4
	Seeding/T2	Urea	110	143.2	181.2	166.9	157.5	147.3
	Seeding/T2	Urea	140	118.8	145.9	167.9	186.9	157.3
	Seeding/PA	Urea/UAN	110	128.4	146.1	153.6	171.2	133.1
	Seeding/PA	Urea/Urea Sol	110	109.3	98.3	159.3	188.8	139.9
	Seeding	Urea (BC)	80	97.0	114.3	107.0	174.7	135.9
Prosper			0	42.8	60.0	70.1	85.5	64.6
	Seeding	Urea	50	78.4	97.9	107.0	142.7	106.5
	Seeding	Urea	80	106.9	99.0	119.0	135.1	115.0
	Seeding	Urea	110	116.9	97.4	155.5	159.8	132.4
	Seeding	Urea	140	122.9	144.8	146.0	173.6	146.8
	Seeding	Urea	170	151.5	149.4	169.2	183.0	163.3
	Seeding	Urea	200	157.8	189.5	189.8	203.5	185.1
	Seeding	ESN/Urea	80	100.1	116.0	129.4	153.6	124.8
	Seeding	ESN/Urea	140	136.3	160.4	182.7	155.1	158.6
	Seeding/T1	Urea	110	125.6	141.3	159.6	168.6	148.8
	Seeding/T1	Urea	140	132.3	172.2	178.3	180.4	165.8
	Seeding/T2	Urea	110	132.2	149.2	162.6	161.7	151.4
	Seeding/T2	Urea	140	138.6	157.3	173.5	180.5	162.5
	Seeding/PA	Urea/UAN	110	111.4	133.0	145.1	152.9	135.6
	Seeding/PA	Urea/Urea Sol	110	125.5	139.5	162.9	177.4	151.3
	Seeding	Urea (BC)	80	93.6	138.9	151.9	139.6	131.0
Brandon				110.9 aD	129.2 aC	150.2 aB	170.2 aA	140.1
Prosper				117.1 aC	134.1 aB	150.2 aAB	159.6 bA	140.2
			0	48.0 gB	70.6 fAB	69.4 fAB	96.9 gA	71.2
	Seeding	Urea	50	72.8 fgC	94.9 efBC	110.9 eB	145.4 fA	106.0
	Seeding	Urea	80	98.4 efB	101.8 efB	125.7 deAB	149.8 efA	118.9
	Seeding	Urea	110	111.8 bcdeB	109.2 deB	156.5 bcdA	167.9 abcdefA	136.3
	Seeding	Urea	140	130.0 abcdeB	139.5 bcdB	158.2 abcdAB	179.1 abcdeA	151.7
	Seeding	Urea	170	136.8 abcC	151.6 abBC	174.4 abAB	192.5 abA	163.8
	Seeding	Urea	200	149.8 aB	175.3 aAB	189.3 aA	200.9 aA	178.8
	Seeding	ESN/Urea	80	101.3 defC	110.8 deBC	130.3 cdeAB	151.6 defA	123.5
	Seeding	ESN/Urea	140	131.5 abcdeB	148.8 abB	180.4 abA	153.0 cdefAB	153.4
	Seeding/T1	Urea	110	123.1 abcdeC	140.3 bcdBC	156.4 bcdAB	169.5 abcdefA	147.3
	Seeding/T1	Urea	140	137.8 abB	176.7 aA	176.2 abA	185.7 abcA	169.1
	Seeding/T2	Urea	110	125.5 abcdeB	147.6 abcAB	164.7 abA	159.6 cdefA	149.3
	Seeding/T2	Urea	140	133.5 abcdC	151.7 abBC	170.7 abAB	183.7 abcdA	159.9
	Seeding/PA	Urea/UAN	110	110.3 bcdeB	115.7 cdeB	149.3 bcdA	162.0 bcdefA	134.3
	Seeding/PA	Urea/Urea Sol	110	111.3 bcdeB	126.9 bcdeB	161.1 abcA	183.1 abcdeA	145.6
	Seeding	Urea (BC)	80	102.2 cdefB	145.0 abcA	129.4 cdeAB	157.1 cdefA	133.4
Site-Year				114.0	131.6	150.2	164.9	

ANOVA	df	Pr> F
Variety	1	0.9548
N Trt	15	<0.0001
Variety* N Trt	15	0.2152
SiteYr	3	<0.0001
SiteYr*Variety	3	0.0025
SiteYr*N trt	45	0.0012
SiteYr*N trt*Variety	45	0.3687
Coeff var (C.V.)		26.8

Appendix Table 10. Silver level sites lsmeans analysis of nitrogen uptake (lbs N/ac)**.** Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05).

Treatment				Site-year				
Variety	Timing	Source	Rate	Melita 2016	Carberry 2016	Melita 2017	Grosse Isle 2017	Combined
			lbs N/ac	-- lbs N/ac --				
Brandon			0	65.2	175.0	70.2	75.1	96.4
	Seeding	Urea	50	100.5	190.0	75.3	82.6	112.1
	Seeding	Urea	80	126.0	209.4	94.1	105.8	133.8
	Seeding	Urea	110	165.5	247.9	122.1	124.9	165.1
	Seeding	Urea	140	149.4	216.5	118.4	131.2	153.9
	Seeding	Urea	170	199.3	247.4	137.3	142.2	181.5
	Seeding/T1	Urea	110	194.8	229.8	121.0	134.9	170.1
	Seeding/T1	Urea	140	159.3	227.1	144.8	139.3	167.6
	Seeding/T2	Urea	110	147.8	225.4	110.7	129.9	153.5
	Seeding/T2	Urea	140	191.3	236.1	144.9	131.7	176.0
	Seeding/PA	Urea/UAN	110	166.3	238.2	105.4	118.3	157.1
Prosper			0	58.7	183.0	58.2	69.5	92.4
	Seeding	Urea	50	143.2	203.4	76.5	83.9	126.7
	Seeding	Urea	80	115.7	222.8	109.6	102.8	137.7
	Seeding	Urea	110	166.3	232.8	111.9	122.0	158.3
	Seeding	Urea	140	168.5	228.0	129.8	128.0	163.6
	Seeding	Urea	170	220.4	242.4	138.6	129.9	182.8
	Seeding/T1	Urea	110	179.0	221.0	137.2	120.6	164.4
	Seeding/T1	Urea	140	177.1	228.0	137.8	126.9	167.5
	Seeding/T2	Urea	110	172.8	232.9	115.3	121.0	160.5
	Seeding/T2	Urea	140	167.4	244.2	144.1	133.1	172.2
	Seeding/PA	Urea/UAN	110	148.8	212.2	108.4	115.8	146.3
Brandon				151.4	222.1	113.1	119.6	151.6
Prosper				156.2	222.8	115.2	113.9	152.0
			0	62.0 eB	179.0 cA	64.2 dB	72.3 cB	94.4
	Seeding	Urea	50	121.9 cdB	196.7 bcA	75.9 cdC	83.2 bcC	119.4
	Seeding	Urea	80	120.8 dB	216.1 abcA	101.8 bcdB	104.3 abcB	135.8
	Seeding	Urea	110	165.9 bB	240.4 aA	117.0 abC	123.4 aC	161.7
	Seeding	Urea	140	159.0 bcdB	222.2 abA	124.1 abC	129.6 aBC	158.7
	Seeding	Urea	170	209.8 aB	244.9 aA	138.0 abC	136.0 aC	182.2
	Seeding/T1	Urea	110	186.9 abB	225.4 abA	129.1 abC	127.7 aC	167.3
	Seeding/T1	Urea	140	168.2 bB	227.5 abA	141.3 aBC	133.1 aC	167.5
	Seeding/T2	Urea	110	160.3 bcB	229.2 abA	113.0 abcC	125.4 aC	157.0
	Seeding/T2	Urea	140	179.4 abB	240.2 aA	144.5 aC	132.4 aC	174.1
	Seeding/PA	Urea/UAN	110	157.6 bcdB	225.2 abA	106.9 abcC	117.1 abC	151.7
Site-Year				153.8	222.4	114.2	116.8	

ANOVA	**df**	**Pr > F**
Variety	1	0.8538
N Trt	10	<0.0001
Variety*N Trt	10	0.6416
SiteYr	3	<0.0001
SiteYr*Variety	30	0.5134
SiteYr*N Trt	3	0.0043
SiteYr*Variety*N Trt	30	0.5134
Coeff var (C.V.)		37.06

Appendix Table 11. Pre-determined linear contrasts for in-season timing of N applications on N Uptake (lbs N/ac)

Gold Level Sites						
	Estimate			Estimate	Std. Err	P-Value
	lbs N/ac			lbs N/ac		
Planting	144.0	vs.	Stem Elongation Split	158.2	6.93	<0.0001
Planting	144.0	vs.	Flag Leaf Split	154.6	6.88	0.0022
Planting (80 lbs N/ac)	118.9	vs.	Post-Anthesis Split	140.0	8.59	<0.0001
Planting (110 lbs N/ac)	136.3	vs.	Post-Anthesis Split	140.0	8.37	0.3882
Stem Elongation Split	158.2	vs.	Flag Leaf Split	154.6	6.73	0.2888
Stem Elongation Split	158.2	vs.	Post-Anthesis Split	140.0	8.19	0.0738
Flag Leaf Split	149.3	vs.	Post Anthesis Split	140.0	8.14	0.218
Silver Level Sites						
Planting	160.2	vs.	Stem Elongation Split	167.4	8.56	0.0929
Planting	160.2	vs.	Flag Leaf Split	165.5	8.56	0.2122
Planting (80 lbs N/ac)	135.8	vs.	Post-Anthesis Split	151.7	6.10	0.0095
Planting (110 lbs N/ac)	161.7	vs.	Post-Anthesis Split	151.7	6.10	0.1030
Stem Elongation Split	167.4	vs.	Flag Leaf Split	165.5	8.57	0.6636
Stem Elongation Split	167.3	vs.	Post-Anthesis Split	151.7	6.16	0.0120
Flag Leaf Split	174.1	vs.	Post Anthesis Split	151.7	6.10	0.3879

Appendix Table 12. Gold level sites lsmeans analysis of nitrogen removal (lbs N/ac)**.** Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05).

Treatment				Site-Year				
Variety	Timing	Source	Rate	Brunkild 2016	Carman 2016	Brunkild 2017	Carman 2017	Combined
			lbs N/ac	------------------------------	------------------------------	lbs N/ac	------------------------------	------------------------------
Brandon			0	40.5	52.8	58.3	87.8	59.9 o
	Seeding	Urea	50	51.3	58.8	92.9	117.3	80.0 mn
	Seeding	Urea	80	63.7	64.1	107.8	126.4	90.5 klmn
	Seeding	Urea	110	81.2	73.1	129.6	136.2	105.0 defghik
	Seeding	Urea	140	93.9	83.7	142.7	140.9	115.3 abcdef
	Seeding	Urea	170	86.8	92.0	144.4	154.3	119.4 abcd
	Seeding	Urea	200	95.7	88.4	146.3	152.2	120.7 abcd
	Seeding	ESN/Urea	80	73.9	69.6	108.7	118.5	92.7 jklmn
	Seeding	ESN/Urea	140	87.9	82.6	145.9	123.1	109.9 bcdefghi
	Seeding/T1	Urea	110	89.4	90.6	123.5	132.7	109.0 bcdefghi
	Seeding/T1	Urea	140	103.0	94.3	139.9	147.0	121.0 abc
	Seeding/T2	Urea	110	87.2	84.4	138.3	130.6	110.1 bcdefghi
	Seeding/T2	Urea	140	91.4	82.3	140.7	145.3	114.9 abcdef
	Seeding/PA	Urea/UAN	110	79.9	56.8	127.9	131.8	99.1 fghijkl
	Seeding/PA	Urea/Urea Sol	110	86.7	68.8	134.9	147.3	109.4 bcdefghi
	Seeding	Urea (BC)	80	73.9	90.3	88.3	138.4	97.7 ghijk
Prosper			0	30.7	41.8	58.2	75.2	51.5 o
	Seeding	Urea	50	53.1	59.9	87.4	111.6	78.0 n
	Seeding	Urea	80	68.9	67.6	98.3	109.1	86.0 lmn
	Seeding	Urea	110	81.5	54.0	128.1	122.9	96.6 hijkl
	Seeding	Urea	140	87.3	93.9	119.8	132.1	108.3 cdefghij
	Seeding	Urea	170	98.1	94.7	135.8	139.0	116.9 abcde
	Seeding	Urea	200	103.7	112.1	150.9	151.5	129.5 a
	Seeding	ESN/Urea	80	69.7	78.0	109.3	118.4	93.8 jklm
	Seeding	ESN/Urea	140	93.2	108.3	148.6	121.2	117.8 abcd
	Seeding/T1	Urea	110	92.2	95.1	130.5	130.3	112.0 bcdefg
	Seeding/T1	Urea	140	88.7	101.8	141.1	137.8	117.4 abcd
	Seeding/T2	Urea	110	91.8	100.0	133.8	128.2	113.4 bcdefg
	Seeding/T2	Urea	140	107.9	101.0	146.3	141.9	124.3 ab
	Seeding/PA	Urea/UAN	110	77.4	86.0	120.3	120.3	101.0 bcdefgh
	Seeding/PA	Urea/Urea Sol	110	91.8	82.2	132.0	138.9	111.2 efghijkl
	Seeding	Urea (BC)	80	67.4	98.0	123.3	111.2	100.0 fghijkl
Brandon				80.4 aB	77.0 bB	123.1 aA	133.1 aA	103.4
Prosper				81.5 aB	85.9 aB	122.7 aA	124.4 bA	103.6
			0	35.6 eC	47.3 eBC	58.3 fB	81.5 fA	55.7
	Seeding	Urea	50	52.2 edC	59.3 edC	90.2 eB	114.5 eA	79.0
	Seeding	Urea	80	66.3 cdB	65.9 edB	103.1 eA	117.8 deA	88.2
	Seeding	Urea	110	81.3 abcB	63.5 edC	128.9 abA	129.6 bcdeA	100.8
	Seeding	Urea	140	90.6 abB	88.8 abcB	131.3 abA	136.5 abcdA	111.8
	Seeding	Urea	170	92.5 aB	93.3 abB	140.1 abA	146.6 abA	118.1
	Seeding	Urea	200	99.7 aB	100.3 aB	148.6 aA	151.8 abA	125.1
	Seeding	ESN/Urea	80	71.8 bcdB	73.8 bcdB	109.0 cdeA	118.5 deA	93.3
	Seeding	ESN/Urea	140	90.6 abC	95.4 aC	147.3 aA	122.1 cdeB	113.8
	Seeding/T1	Urea	110	90.8 abB	92.9 abB	127.0 bcA	131.5 bcdeA	110.5
	Seeding/T1	Urea	140	95.9 aB	98.1 aB	140.5 abA	142.4 abcA	119.2
	Seeding/T2	Urea	110	89.5 abB	92.2 abB	136.1 abA	129.4 bcdeA	111.8
	Seeding/T2	Urea	140	99.7 aB	91.7 abB	143.5 abA	143.6 abcA	119.6
	Seeding/PA	Urea/UAN	110	78.7 abcB	71.4 cdB	124.1 bcdA	126.0 cdeA	100.1
	Seeding/PA	Urea/Urea Sol	110	89.2 abB	75.5 bcdB	133.5 abA	143.1 abcA	110.3
	Seeding	Urea (BC)	80	70.6 bcdC	94.1 abB	105.8 deB	124.8 cdeA	98.8
Site-Year				80.9	81.5	122.9	128.7	
ANOVA		df				Pr> F		
Variety		1						0.8546
N Trt		15						<0.0001
Variety* N Trt		15						0.0257
SiteYr		3						<0.0001
SiteYr*Variety		3						<0.0001
SiteYr*N trt		45						<0.0001
SiteYr*N trt*Variety		45						0.0762
Coeff var (C.V.)								30.17

Appendix Table 13. Silver level sites lsmeans analysis of nitrogen removal (lbs N/ac)**.** Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05).

Treatment				Site-year				
Variety	Timing	Source	Rate	Melita 2016	Carberry 2016	Melita 2017	Grosse Isle 2017	Combined
			lbs N/ac	-- lbs N/ac	--			
Brandon			0	55.3	108.0	44.8	60.2	67.1
	Seeding	Urea	50	71.1	123.1	42.3	67.7	76.1
	Seeding	Urea	80	81.4	122.6	69.0	83.8	89.2
	Seeding	Urea	110	90.4	135.9	86.8	99.1	103.0
	Seeding	Urea	140	91.8	128.9	86.4	101.2	102.1
	Seeding	Urea	170	97.9	132.9	99.1	109.5	109.9
	Seeding/T1	Urea	110	89.2	137.5	91.5	107.2	106.3
	Seeding/T1	Urea	140	97.6	130.7	97.8	107.1	108.3
	Seeding/T2	Urea	110	90.1	132.4	81.9	104.5	102.2
	Seeding/T2	Urea	140	100.2	134.2	96.0	106.5	109.2
	Seeding/PA	Urea/UAN	110	85.5	120.0	81.2	96.2	95.7
Prosper			0	63.8	120.2	41.5	56.6	70.5
	Seeding	Urea	50	69.8	134.7	55.9	68.0	82.1
	Seeding	Urea	80	88.8	139.1	79.3	82.0	97.3
	Seeding	Urea	110	101.3	145.8	78.0	97.1	105.5
	Seeding	Urea	140	104.9	148.1	92.5	101.5	111.7
	Seeding	Urea	170	103.7	152.0	99.3	103.7	114.7
	Seeding/T1	Urea	110	97.7	148.3	92.5	96.4	108.7
	Seeding/T1	Urea	140	104.1	149.3	94.6	102.2	112.6
	Seeding/T2	Urea	110	98.6	143.1	84.8	96.0	105.6
	Seeding/T2	Urea	140	106.5	154.7	73.7	104.9	110.0
	Seeding/PA	Urea/UAN	110	93.6	128.9	79.3	94.5	99.1
Brandon				86.4 bC	127.8 bA	79.7 aC	94.8 aB	97.2
Prosper				93.9 aB	142.2 aA	79.2 aC	91.2 aB	101.6
			0	59.5 dB	114.1 cA	43.2 eC	58.4 dB	68.8
	Seeding	Urea	50	70.4 cdB	128.9 abA	49.1 eC	67.8 cdB	79.1
	Seeding	Urea	80	85.1 bcB	130.9 abA	74.2 dB	82.9 bcB	93.3
	Seeding	Urea	110	95.8 abB	140.8 aA	82.4 bcdC	98.1 abB	104.3
	Seeding	Urea	140	98.3 abB	138.5 abA	89.5 abcdB	101.3 aB	106.9
	Seeding	Urea	170	100.8 aB	142.5 aA	99.2 aB	106.6 aB	112.3
	Seeding/T1	Urea	110	93.5 abB	142.9 aA	92.0 abcB	101.8 aB	107.5
	Seeding/T1	Urea	140	100.9 aB	140.0 abA	96.2 abB	104.6 aB	110.4
	Seeding/T2	Urea	110	94.4 abBC	137.8 abA	83.4 bcdC	100.2 aB	103.9
	Seeding/T2	Urea	140	103.3 aB	144.5 aA	84.8 abcdC	105.7 aB	109.6
	Seeding/PA	Urea/UAN	110	89.6 abA	124.4 bcA	80.2 cdC	95.3 abB	97.4
Site-Year				90.2	135.0	79.5	93.0	

ANOVA	**df**	**Pr > F**
Variety	1	<0.0001
N Trt	10	<0.0001
Variety*N Trt	10	0.8173
SiteYr	3	<0.0001
SiteYr*Variety	30	<0.0001
SiteYr*N Trt	3	<0.0001
SiteYr*Variety*N Trt	30	0.7717
Coeff var (C.V.)		28.07

Appendix Table 14. Predetermined linear contrasts for in-season timing of N applications on N removal (lbs N/ac)

Gold Level Sites						
	Estimate lbs N/ac			Estimate lbs N/ac	Std. Err	P-Value
Planting	106.3	vs.	Stem Elongation Split	114.9	4.19	<0.0001
Planting	106.3	vs.	Flag Leaf Split	115.7	4.16	<0.0001
Planting (80 lbs N/ac)	88.2	vs.	Post-Anthesis Split	105.2	5.27	<0.0001
Planting (110 lbs N/ac)	100.8	vs.	Post-Anthesis Split	105.2	5.07	0.0850
Stem Elongation Split	114.9	vs.	Flag Leaf Split	115.7	4.13	0.6904
Stem Elongation Split	110.5	vs.	Post-Anthesis Split	105.2	5.03	0.0342
Flag Leaf Split	111.8	vs.	Post Anthesis Split	105.2	4.99	0.0086
Silver Level Sites						
Planting	150.6	vs.	Stem Elongation Split	109.0	3.38	0.0459
Planting	150.6	vs.	Flag Leaf Split	106.8	3.40	0.4921
Planting (80 lbs N/ac)	93.3	vs.	Post-Anthesis Split	97.4	2.40	0.8610
Planting (110 lbs N/ac)	104.3	vs.	Post-Anthesis Split	97.4	2.40	0.0044
Stem Elongation Split	109.0	vs.	Flag Leaf Split	106.8	3.39	0.1909
Stem Elongation Split	107.5	vs.	Post-Anthesis Split	97.4	2.40	<0.0001
Flag Leaf Split	103.9	vs.	Post Anthesis Split	97.4	2.40	0.0069

Appendix Table 15. Gold level sites lsmeans analysis of Harvest Index (HI)**.** Means with similar lowercase letters are not significantly different within a column (P<0.05).

Treatment				Site-year				
				Brunkild 2016	Carman 2016	Brunkild 2017	Carman 2017	Combined
Brandon			0	0.39	0.34	0.47	0.44	0.41
	Seeding	Urea	50	0.34	0.31	0.45	0.45	0.39
	Seeding	Urea	80	0.39	0.35	0.46	0.44	0.41
	Seeding	Urea	110	0.40	0.33	0.48	0.45	0.41
	Seeding	Urea	140	0.41	0.33	0.49	0.44	0.42
	Seeding	Urea	170	0.38	0.35	0.47	0.45	0.41
	Seeding	Urea	200	0.36	0.32	0.48	0.47	0.41
	Seeding	ESN/Urea	80	0.39	0.36	0.47	0.45	0.42
	Seeding	ESN/Urea	140	0.38	0.34	0.49	0.47	0.42
	Seeding/T1	Urea	110	0.40	0.35	0.47	0.44	0.41
	Seeding/T1	Urea	140	0.37	0.29	0.48	0.45	0.40
	Seeding/T2	Urea	110	0.41	0.31	0.49	0.47	0.42
	Seeding/T2	Urea	140	0.39	0.31	0.50	0.45	0.41
	Seeding/PA	Urea/UAN	110	0.38	0.30	0.48	0.45	0.40
	Seeding/PA	Urea/Urea Sol	110	0.38	0.32	0.49	0.45	0.41
	Seeding	Urea (BC)	80	0.41	0.34	0.47	0.45	0.42
Prosper			0	0.42	0.35	0.46	0.47	0.43
	Seeding	Urea	50	0.42	0.33	0.46	0.47	0.42
	Seeding	Urea	80	0.42	0.36	0.47	0.48	0.43
	Seeding	Urea	110	0.40	0.37	0.49	0.48	0.43
	Seeding	Urea	140	0.40	0.36	0.48	0.47	0.43
	Seeding	Urea	170	0.35	0.37	0.49	0.48	0.42
	Seeding	Urea	200	0.39	0.33	0.47	0.48	0.42
	Seeding	ESN/Urea	80	0.44	0.37	0.48	0.47	0.44
	Seeding	ESN/Urea	140	0.39	0.39	0.49	0.49	0.44
	Seeding/T1	Urea	110	0.41	0.37	0.48	0.48	0.44
	Seeding/T1	Urea	140	0.37	0.35	0.48	0.47	0.42
	Seeding/T2	Urea	110	0.42	0.38	0.48	0.49	0.44
	Seeding/T2	Urea	140	0.40	0.39	0.52	0.48	0.45
	Seeding/PA	Urea/UAN	110	0.40	0.37	0.47	0.48	0.43
	Seeding/PA	Urea/Urea Sol	110	0.43	0.32	0.46	0.47	0.42
	Seeding	Urea (BC)	80	0.36	0.39	0.48	0.48	0.43
Brandon				0.39 b	0.33 b	0.48 a	0.45 b	0.41
Prosper				0.40 a	0.36 a	0.48 a	0.48 a	0.43
			0	0.41 ab	0.35 a	0.41 a	0.46 a	0.42
	Seeding	Urea	50	0.38 ab	0.32 a	0.37 a	0.46 a	0.40
	Seeding	Urea	80	0.40 ab	0.35 a	0.41 a	0.46 a	0.42
	Seeding	Urea	110	0.40 ab	0.35 a	0.39 a	0.46 a	0.42
	Seeding	Urea	140	0.41 ab	0.35 a	0.39 a	0.46 a	0.42
	Seeding	Urea	170	0.36 b	0.36 a	0.41 a	0.47 a	0.42
	Seeding	Urea	200	0.38 ab	0.33 a	0.41 a	0.47 a	0.41
	Seeding	ESN/Urea	80	0.42 a	0.36 a	0.37 a	0.46 a	0.43
	Seeding	ESN/Urea	140	0.39 ab	0.37 a	0.41 a	0.48 a	0.43
	Seeding/T1	Urea	110	0.41 ab	0.36 a	0.48 a	0.46 a	0.42
	Seeding/T1	Urea	140	0.37 ab	0.32 a	0.48 a	0.46 a	0.43
	Seeding/T2	Urea	110	0.41 a	0.35 a	0.48 a	0.48 a	0.41
	Seeding/T2	Urea	140	0.39 ab	0.35 a	0.51 a	0.47 a	0.43
	Seeding/PA	Urea/UAN	110	0.39 ab	0.34 a	0.48 a	0.46 a	0.43
	Seeding/PA	Urea/Urea Sol	110	0.41 ab	0.32 a	0.47 a	0.46 a	0.42
	Seeding	Urea (BC)	80	0.38 ab	0.37 a	0.48 a	0.47 a	0.41
Site-Year				0.39	0.35	0.48	0.46	

ANOVA	**df**	**Pr > F**
Variety	1	<0.0001
N Trt	15	0.0039
Variety*N Trt	15	0.8336
SiteYr	3	<0.0001
SiteYr*Variety	3	<0.0001
SiteYr*N Trt	45	0.0303
SiteYr*Variety*N Trt	45	0.107
Coeff var (C.V.)		14.9

Appendix Table 16. Silver level sites lsmeans analysis of Harvest Index (HI)**.** Means with similar lowercase letters are not significantly different within a column (P<0.05).

Treatment				Site-year				
				Melita 2016	Carberry 2016	Melita 2017	Grosse Isle 2017	Combined
Brandon			0	0.42	0.34	0.38	0.45	0.40
	Seeding	Urea	50	0.35	0.37	0.43	0.46	0.40
	Seeding	Urea	80	0.25	0.32	0.45	0.46	0.37
	Seeding	Urea	110	0.27	0.32	0.43	0.46	0.37
	Seeding	Urea	140	0.24	0.34	0.45	0.45	0.37
	Seeding	Urea	170	0.24	0.32	0.45	0.46	0.37
	Seeding/T1	Urea	110	0.25	0.35	0.52	0.46	0.40
	Seeding/T1	Urea	140	0.24	0.33	0.43	0.46	0.36
	Seeding/T2	Urea	110	0.26	0.34	0.45	0.47	0.38
	Seeding/T2	Urea	140	0.23	0.34	0.41	0.47	0.36
	Seeding/PA	Urea/UAN	110	0.27	0.31	0.51	0.46	0.39
Prosper			0	0.46	0.34	0.40	0.46	0.41
	Seeding	Urea	50	0.26	0.37	0.44	0.46	0.38
	Seeding	Urea	80	0.23	0.36	0.44	0.47	0.37
	Seeding	Urea	110	0.22	0.37	0.44	0.47	0.37
	Seeding	Urea	140	0.20	0.38	0.45	0.47	0.38
	Seeding	Urea	170	0.20	0.36	0.46	0.48	0.37
	Seeding/T1	Urea	110	0.19	0.39	0.44	0.47	0.37
	Seeding/T1	Urea	140	0.21	0.38	0.43	0.47	0.37
	Seeding/T2	Urea	110	0.22	0.36	0.47	0.47	0.38
	Seeding/T2	Urea	140	0.20	0.38	0.45	0.48	0.38
	Seeding/PA	Urea/UAN	110	0.24	0.36	0.47	0.48	0.39
Brandon				0.28 a	0.33 b	0.45 a	0.46 a	0.38
Prosper				0.24 b	0.37 a	0.44 a	0.47 a	0.38
			0	0.39 a	0.34 a	0.44 b	0.46 a	0.41
	Seeding	Urea	50	0.44 b	0.37 a	0.30 ab	0.46 a	0.39
	Seeding	Urea	80	0.44 bc	0.34 a	0.24 ab	0.47 a	0.37
	Seeding	Urea	110	0.43 bc	0.34 a	0.24 ab	0.47 a	0.37
	Seeding	Urea	140	0.45 c	0.36 a	0.22 ab	0.46 a	0.37
	Seeding	Urea	170	0.46 c	0.34 a	0.22 a	0.47 a	0.37
	Seeding/T1	Urea	110	0.48 c	0.37 a	0.22 a	0.47 a	0.38
	Seeding/T1	Urea	140	0.43 c	0.36 a	0.22 ab	0.46 a	0.37
	Seeding/T2	Urea	110	0.46 bc	0.35 a	0.24 a	0.47 a	0.38
	Seeding/T2	Urea	140	0.43 c	0.36 a	0.21 ab	0.47 a	0.37
	Seeding/PA	Urea/UAN	110	0.49 bc	0.33 a	0.26 a	0.47 a	0.39
Site-Year				0.26	0.35	0.44	0.47	

ANOVA	**df**	**Pr > F**
Variety	1	0.7992
N Trt	10	0.0009
Variety*N Trt	10	0.483
SiteYr	3	<0.0001
SiteYr*Variety	30	<0.0001
SiteYr*N Trt	3	<0.0001
SiteYr*Variety*N Trt	30	0.8865
Coeff var (C.V.)		25.77

Appendix Table 17. Gold level sites lsmeans analysis of agronomic efficiency (AE) (%). Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05).

Treatment				Site-Year				
Variety	Timing	Source	Rate	Brunkild 2016	Carman 2016	Brunkild 2017	Carman 2017	Combined
			lbs N/ac	%				
Brandon			0	-	-	-	-	-
	Seeding	Urea	50	21.7	14.6	67.3	31.5	33.8
	Seeding	Urea	80	31.3	8.8	47.6	20.0	26.9
	Seeding	Urea	110	28.5	10.5	45.1	17.0	25.3
	Seeding	Urea	140	30.7	12.7	35.8	15.4	23.6
	Seeding	Urea	170	26.1	12.5	30.4	14.1	20.8
	Seeding	Urea	200	30.0	9.3	25.7	12.6	19.4
	Seeding	ESN/Urea	80	26.2	7.6	48.5	16.6	24.7
	Seeding	ESN/Urea	140	26.6	12.6	38.5	9.2	21.7
	Seeding/T1	Urea	110	25.7	19.9	43.2	19.5	27.1
	Seeding/T1	Urea	140	30.1	15.5	35.6	13.2	23.6
	Seeding/T2	Urea	110	21.8	14.9	49.7	12.6	24.7
	Seeding/T2	Urea	140	24.0	9.9	35.5	12.8	20.5
	Seeding/PA	Urea/UAN	110	28.6	-1.9	38.9	14.0	19.9
	Seeding/PA	Urea/Urea Sol	110	37.0	2.6	41.8	19.1	25.1
	Seeding	Urea (BC)	80	24.1	29.7	32.0	27.7	28.4
Prosper			0	-	-	-	-	-
	Seeding	Urea	50	30.7	27.6	66.2	51.5	43.4
	Seeding	Urea	80	33.9	20.7	48.3	30.1	33.3
	Seeding	Urea	110	31.4	22.4	49.2	26.7	32.4
	Seeding	Urea	140	39.0	21.8	32.4	19.5	28.2
	Seeding	Urea	170	26.2	19.4	30.9	17.5	23.5
	Seeding	Urea	200	28.6	20.3	31.0	17.6	24.4
	Seeding	ESN/Urea	80	32.0	33.4	55.1	32.4	38.2
	Seeding	ESN/Urea	140	19.0	28.3	42.7	17.8	27.0
	Seeding/T1	Urea	110	30.5	32.0	48.5	28.3	34.8
	Seeding/T1	Urea	140	33.9	24.8	41.2	23.3	30.9
	Seeding/T2	Urea	110	34.2	34.9	54.9	27.0	37.7
	Seeding/T2	Urea	140	37.0	26.0	42.0	23.9	32.2
	Seeding/PA	Urea/UAN	110	37.2	20.8	39.0	20.4	29.3
	Seeding/PA	Urea/Urea Sol	110	29.2	19.5	43.5	27.4	29.9
	Seeding	Urea (BC)	80	23.3	51.9	70.9	28.7	43.7
Brandon				27.5 aB	11.9 bC	41.0 bA	17.0 bBC	24.4
Prosper				31.1 aB	26.9 aB	46.4 aA	26.2 aB	32.6
			0	-	-	-	-	-
	Seeding	Urea	50	26.2 aBC	21.1 bC	66.7 aA	41.5 aB	38.9
	Seeding	Urea	80	32.6 aAB	14.7 bB	47.9 bcA	25.1 abB	30.1
	Seeding	Urea	110	30.0 aAB	16.4 bB	47.2 bcA	20.9 bB	28.9
	Seeding	Urea	140	34.8 aA	17.2 bA	34.1 bcdA	17.5 bA	25.9
	Seeding	Urea	170	26.2 aA	15.9 bA	30.6 cdA	15.8 bA	22.1
	Seeding	Urea	200	29.3 aA	14.8 bA	28.3 dA	15.1 bA	21.9
	Seeding	ESN/Urea	80	29.1 aB	20.5 bB	51.8 abA	24.5 abB	31.5
	Seeding	ESN/Urea	140	22.8 aAB	20.6 bB	40.6 bcdA	13.5 bB	24.4
	Seeding/T1	Urea	110	28.1 aB	25.9 abB	45.8 bcdA	23.9 abB	30.9
	Seeding/T1	Urea	140	32.0 aAB	20.2 bB	38.4 bcdA	18.4 bB	27.2
	Seeding/T2	Urea	110	28.0 aB	24.9 abB	52.3 baA	19.8 bB	31.2
	Seeding/T2	Urea	140	30.5 aAB	17.9 bB	38.8 bcdA	18.3 bB	26.4
	Seeding/PA	Urea/UAN	110	32.9 aA	9.5 bC	38.9 bcdA	17.2 bBC	24.6
	Seeding/PA	Urea/Urea Sol	110	33.1 aAB	11.1 bC	42.7 bcdA	23.3 bBC	27.5
	Seeding	Urea (BC)	80	23.7 aB	40.8 aAB	51.4 abA	28.2 abB	36.0
Site-Year				29.3	19.4	43.7	24.6	0.0

ANOVA	df	Pr> F
Variety	1	<0.0001
N Trt	15	<0.0001
Variety* N Trt	15	0.4893
SiteYr	3	0.0007
SiteYr*Variety	3	0.0003
SiteYr*N trt	45	<0.0001
SiteYr*N trt*Variety	45	0.8782
Coeff var (C.V.)		49.82

Appendix Table 18. Silver level sites lsmeans analysis of agronomic efficiency (AE)(%). Means with similar lowercase letters are not significantly different within a column; means with similar uppercase letters are not significantly different within a row (P<0.05).

Treatment				Site-year				
Variety	Timing	Source	Rate	Melita 2016	Carberry 2016	Melita 2017	Grosse Isle 2017	Combined
			lbs N/ac	%				
Brandon			0	-	-	-	-	-
	Seeding	Urea	50	20.5	17.1	14.4	11.2	15.8
	Seeding	Urea	80	19.5	13.4	-9.0	13.0	9.3
	Seeding	Urea	110	16.8	14.3	4.8	16.6	13.1
	Seeding	Urea	140	13.2	6.9	8.7	11.7	10.1
	Seeding	Urea	170	13.0	7.6	5.0	10.2	8.9
	Seeding/T1	Urea	110	17.6	18.8	11.5	16.3	16.1
	Seeding/T1	Urea	140	15.6	9.0	5.0	14.2	11.0
	Seeding/T2	Urea	110	16.1	8.8	11.0	15.8	12.9
	Seeding/T2	Urea	140	17.0	9.9	5.9	10.4	10.8
	Seeding/PA	Urea/UAN	110	11.3	-18.9	1.5	13.1	1.8
Prosper			0	-	-	-	-	-
	Seeding	Urea	50	15.4	5.0	-10.5	17.2	6.8
	Seeding	Urea	80	20.6	5.2	3.8	18.9	12.1
	Seeding	Urea	110	20.0	3.6	-0.5	16.1	9.8
	Seeding	Urea	140	16.9	5.9	1.5	13.3	9.4
	Seeding	Urea	170	13.4	4.1	2.5	11.5	7.9
	Seeding/T1	Urea	110	17.2	6.4	1.3	17.5	10.6
	Seeding/T1	Urea	140	16.9	3.2	2.9	13.5	9.1
	Seeding/T2	Urea	110	19.2	1.7	2.7	14.6	9.5
	Seeding/T2	Urea	140	15.9	5.0	2.6	11.6	8.8
	Seeding/PA	Urea/UAN	110	13.4	-9.7	7.7	13.7	6.3
Brandon				16.1 aA	8.7 aA	5.9 aA	13.3 aA	11.0
Prosper				16.9 aA	3.0 bAB	1.4 bB	14.8 aAB	9.0
			0	-	-	-	-	-
	Seeding	Urea	50	17.9	11.1	2.0	14.2	11.3 ab
	Seeding	Urea	80	20.1	9.3	-2.6	16.0	10.7 ab
	Seeding	Urea	110	18.4	9.0	2.1	16.3	11.5 ab
	Seeding	Urea	140	15.1	6.4	5.1	12.5	9.8 ab
	Seeding	Urea	170	13.2	5.8	3.4	10.8	8.4 ab
	Seeding/T1	Urea	110	17.4	12.6	6.4	16.9	13.3 a
	Seeding/T1	Urea	140	16.2	6.1	4.0	13.8	10.0 ab
	Seeding/T2	Urea	110	17.7	5.2	6.8	15.2	11.2 ab
	Seeding/T2	Urea	140	16.5	7.4	4.2	11.0	9.8 ab
	Seeding/PA	Urea/UAN	110	12.3	-14.3	4.6	13.4	4.0 b
Site-Year				16.5	5.9	3.6	14.0	

ANOVA	**df**	**Pr > F**
Variety	1	0.0669
N Trt	10	0.0214
Variety*N Trt	10	0.2336
SiteYr	3	0.1030
SiteYr*Variety	30	0.0327
SiteYr*N Trt	3	0.0718
SiteYr*Variety*N Trt	30	0.8598
Coeff var (C.V.)		58.6

Appendix Table 19. Predetermined linear contrasts for timing of N applications on agronomic efficiency

Gold Level Sites						
	Estimate			Estimate	Std. Err	P-Value
	%			%		
Planting	27.4	vs.	Stem Elongation Split	29.1	3.84	0.3709
Planting	27.4	vs.	Flag Leaf Split	28.8	3.83	0.4551
Planting (80 lbs N/ac)	30.1	vs.	Post-Anthesis Split	26.1	4.61	0.2276
Planting (110 lbs N/ac)	28.9	vs.	Post-Anthesis Split	26.1	4.74	0.0902
Stem Elongation Split	29.1	vs.	Flag Leaf Split	28.8	3.76	0.8788
Stem Elongation Split	39.0	vs.	Post-Anthesis Split	26.1	4.58	0.0339
Flag Leaf Split	31.2	vs.	Post Anthesis Split	26.1	4.62	0.0258
Silver Level Sites						
Planting	10.6	vs.	Stem Elongation Split	11.7	3.33	0.5216
Planting	10.6	vs.	Flag Leaf Split	10.5	3.35	0.9525
Planting (80 lbs N/ac)	10.7	vs.	Post-Anthesis Split	4.0	2.35	0.0050
Planting (110 lbs N/ac)	11.5	vs.	Post-Anthesis Split	4.0	2.35	0.0017
Stem Elongation Split	11.7	vs.	Flag Leaf Split	10.5	3.36	0.4867
Stem Elongation Split	13.3	vs.	Post-Anthesis Split	4.0	2.32	<0.0001
Flag Leaf Split	11.2	vs.	Post Anthesis Split	4.0	2.35	0.0024

Appendix Table 20. Results of regression analysis (n=8) for the relationship between predictor indices and grain yield (bu/ac) using simple linear model: y = a + (b*x). Numbers in parentheses indicate that Standard Error of the mean

Parameter Estimates			Pr>F	R^2
x	a	b		
Spring NO_3-N (0-60 cm)				
Soil Organic Matter	48.6 (24.8)	1.24 (4.65)	0.7974	0.0119
Les Henry Net	28.6 (28.6)	0.55 (0.26)	0.0815	0.4215
Les Henry Gross	30.6 (13.5)	0.84 (0.42)	0.0940	0.3971
$NaHCO_3$-205nm	13.6 (13.4)	0.85 (0.26)	0.0174	0.6385
$NaHCO_3$-260nm	35.5 (22.5)	0.16 (0.18)	0.3985	0.1201
Solvita CO_2-C	33.4 (25.0)	0.12 (0.13)	0.4050	0.1179

Appendix Table 21. Results of regression analysis for the relationship between grain yield and NDVI/GDD sensed at three timings in combination with values normalized using values relative to the high N treatment using a simple linear model: y=a+(b*x). Analysis is combined based on significant effects from ANCOVA analysis.

Time of Sensing	**Site-year**	**Variety**	**NDVI/GDD vs. Yield**		**NDVI/GDD vs. Relative Yield**		**Relative NDVI vs. Yield**		**Relative NDVI vs. Relative Yield**	
			P-Value	R2	P-Value	R2	P-Value	R2	P-Value	R2
Stem Elongation	Brunkild 2016	Brandon	0.1808	0.08			0.1749	0.08	0.0001	**0.27**
		Prosper	0.0023	**0.33**			0.0086	**0.25**		
	Brunkild 2017	Brandon	0.1091	0.20			0.0266	**0.18**	0.8356	0.00
		Prosper	0.5387	0.01			0.3443	0.04		
	Carberry 2016	Brandon	0.785	0.00			0.5751	0.02	0.6078	0.01
		Prosper	0.0435	**0.17**			0.4868	0.02		
	Carman 2016	Brandon	0.4784	0.02			0.7808	0	0.6708	0.00
		Prosper	0.0195	**0.21**			0.2201	0.06		
	Carman 2017	Brandon	0.0062	**0.25**			0.0928	0.1	<0.0001	**0.28**
		Prosper	<0.0001	**0.53**			0.0015	**0.34**		
	Grosse Isle 2017	Brandon	0.32	0.04			0.2682	0.05	0.1645	0.04
		Prosper	0.4836	0.02			0.6693	0.01		
	Melita 2016	Brandon	0.003	**0.35**			0.0162	**0.25**	0.4314	0.01
		Prosper	<0.0001	**0.58**			<0.0001	**0.68**		
	Melita 2017	Brandon	0.0121	**0.25**			0.0279	**0.2**	0.0012	**0.21**
		Prosper	0.0256	**0.21**			0.0326	**0.19**		
	COMBINED		**<0.0001**	**0.52**	**<0.0001**	**0.05**	**0.0113**	**0.02**	**0.0061**	**0.02**
Flag Leaf	Brunkild 2016	Brandon	<0.0001	**0.60**	<0.0001	**0.59**	0.4264	0.01	0.964	0.00
		Prosper								
	Brunkild 2017	Brandon	<0.0001	**0.67**	<0.0001	**0.72**	<0.0001	**0.71**	<0.0001	**0.68**
		Prosper								
	Carberry 2016	Brandon	0.4266	0.01	0.6369	0.00	0.0109	**0.14**	0.0091	**0.14**
		Prosper								
	Carman 2016	Brandon	<0.0001	**0.31**	<0.0001	**0.47**	<0.0001	**0.34**	<0.0001	**0.30**
		Prosper								
	Carman 2017	Brandon	<0.0001	**0.51**	<0.0001	**0.66**	<0.0001	**0.58**	<0.0001	**0.69**
		Prosper								
	Grosse Isle 2017	Brandon	<0.0001	**0.35**	0.5786	0.00	<0.0001	**0.44**	0.2498	0.03
		Prosper								
	Melita 2016	Brandon	<0.0001	**0.53**	0.4314	0.01	<0.0001	**0.54**	0.4792	0.01
		Prosper								
	Melita 2017	Brandon	<0.0001	**0.30**	<0.0001	**0.29**	0.0158	**0.12**	<0.0001	**0.29**
		Prosper								
	COMBINED		**<0.0001**	**0.60**	**<0.0001**	**0.11**	**<0.0001**	**0.10**	**<0.0001**	**0.11**
Anthesis	Brunkild 2016	Brandon	0.0023	**0.34**	<0.0001	**0.54**	0.9827	0.00	0.2637	0.03
		Prosper	<0.0001	**0.82**						
	Brunkild 2017	Brandon	<0.0001	**0.83**	<0.0001	**0.71**	<0.0001	**0.70**	<0.0001	**0.80**
		Prosper	<0.0001	**0.96**						
	Carberry 2016	Brandon	0.1335	0.10	0.854	0.00	0.1877	0.04	0.2996	0.02
		Prosper	0.6855	0.01						
	Carman 2016	Brandon	<0.0001	**0.54**	<0.0001	**0.64**	<0.0001	**0.46**	<0.0001	**0.62**
		Prosper	<0.0001	**0.84**						
	Carman 2017	Brandon	<0.0001	**0.64**	<0.0001	**0.67**	<0.0001	**0.58**	<0.0001	**0.74**
		Prosper	<0.0001	**0.79**						
	Grosse Isle 2017	Brandon	<0.0001	**0.86**	0.4436	0.01	<0.0001	**0.77**	0.3921	0.01
		Prosper	<0.0001	**0.89**						
	Melita 2016	Brandon	<0.0001	**0.87**	0.3369	0.02	<0.0001	**0.72**	0.4278	0.01
		Prosper	<0.0001	**0.85**						
	Melita 2017	Brandon	<0.0001	**0.65**	<0.0001	**0.66**	<0.0001	**0.61**	<0.0001	**0.76**
		Prosper	<0.0001	**0.81**						
	COMBINED		**<0.0001**	**0.55**	**<0.0001**	**0.13**	**<0.0001**	**0.19**	**<0.0001**	**0.22**

Appendix Table 22. Results of regression analysis for the relationship between grain protein content and NDVI/GDD sensed at three timings in combination with values normalized using values relative to the high N treatment using a simple linear model: y=a+(b*x). Analysis is combined based on significant effects from ANCOVA analysis

Time of Sensing	**Site-year**	**Variety**	**NDVI/GDD vs. Protein**		**NDVI/GDD vs. Relative Protein**		**Relative NDVI vs. Protein**		**Relative NDVI vs. Relative Protein**	
			P-Value	R2	P-Value	R2	P-Value	R2	P-Value	R2
Stem Elongation	Brunkild 2016	Brandon	0.0263	**0.10**			0.2606	0.05	0.2928	0.02
		Prosper					0.2036	0.07		
	Brunkild 2017	Brandon	0.1234	0.04			0.1049	0.1	0.6655	0.00
		Prosper					0.1944	0.06		
	Carberry 2016	Brandon	0.5743	0.01			0.7633	0	0.7788	0.00
		Prosper					0.1027	0.11		
	Carman 2016	Brandon	0.0045	**0.16**			0.3686	0.04	0.9238	0.00
		Prosper					0.3108	0.04		
	Carman 2017	Brandon	0.0112	**0.12**			0.2515	0.05	0.0025	**0.16**
		Prosper					0.0373	**0.16**		
	Grosse Isle 2017	Brandon	0.0068	**0.13**			0.1583	0.78	0.7115	0.00
		Prosper					0.5904	0.01		
	Melita 2016	Brandon	0.0023	**0.19**			0.0021	**0.35**	0.001	**0.22**
		Prosper					0.0069	**0.3**		
	Melita 2017	Brandon	0.0002	**0.27**			0.0014	**0.39**	<0.0001	**0.35**
		Prosper					0.0748	0.14		
	COMBINED		**0.0002**	**0.03**	**0.1744**	**0.01**	**0.0008**	**0.03**	**0.0058**	**0.02**
Flag Leaf	Brunkild 2016	Brandon		**0.29**	<0.0001	**0.32**	0.2556	0.06	0.7041	0.00
		Prosper	<0.0001				0.7062	0.00		
	Brunkild 2017	Brandon		**0.38**	<0.0001	**0.42**	<0.0001	**0.51**	<0.0001	**0.40**
		Prosper	<0.0001				0.0007	**0.36**		
	Carberry 2016	Brandon		0.06	0.3119	0.02	0.1129	0.12	0.6671	0.00
		Prosper	0.0832				0.3448	0.04		
	Carman 2016	Brandon		**0.42**	<0.0001	**0.52**	<0.0001	**0.64**	0.0004	**0.24**
		Prosper	<0.0001				0.0011	**0.38**		
	Carman 2017	Brandon		**0.52**	<0.0001	**0.58**	<0.0001	**0.61**	<0.0001	**0.51**
		Prosper	<0.0001				0.0001	**0.45**		
	Grosse Isle 2017	Brandon		**0.46**	0.1223	0.04	0.0013	**0.35**	0.1263	0.04
		Prosper	<0.0001				0.003	**0.29**		
	Melita 2016	Brandon		**0.19**	0.006	**0.16**	<0.0001	**0.58**	0.022	**0.11**
		Prosper	0.0024				0.899	0.13		
	Melita 2017	Brandon		**0.25**	0.0012	**0.21**	0.0034	**0.34**	0.0004	**0.25**
		Prosper	0.0004				0.2138	0.07		
	COMBINED		**0.0206**	**0.01**	**0.0017**	**0.02**	**<0.0001**	**0.05**	**<0.0001**	**0.07**
Anthesis	Brunkild 2016	Brandon	<0.0001	**0.35**	<0.0001	**0.40**	0.267	0.03	0.7832	0.00
		Prosper								
	Brunkild 2017	Brandon	<0.0001	**0.29**	<0.0001	**0.48**	<0.0001	**0.38**	<0.0001	**0.55**
		Prosper								
	Carberry 2016	Brandon	<0.0001	**0.35**	<0.0001	**0.31**	0.0047	**0.16**	0.0002	**0.26**
		Prosper								
	Carman 2016	Brandon	<0.0001	**0.44**	<0.0001	**0.67**	0.0001	**0.27**	<0.0001	**0.54**
		Prosper								
	Carman 2017	Brandon	<0.0001	**0.42**	<0.0001	**0.66**	<0.0001	**0.50**	<0.0001	**0.66**
		Prosper								
	Grosse Isle 2017	Brandon	<0.0001	**0.54**	0.0012	**0.18**	<0.0001	**0.55**	0.0002	**0.23**
		Prosper								
	Melita 2016	Brandon	0.0016	**0.20**	0.0057	**0.16**	0.001	**0.22**	0.0079	**0.15**
		Prosper								
	Melita 2017	Brandon	<0.0001	**0.55**	<0.0001	**0.53**	<0.001	**0.62**	<0.0001	**0.66**
		Prosper								
	COMBINED		**0.0053**	**0.02**	**<0.0001**	**0.05**	**<0.0001**	**0.15**	**<0.0001**	**0.19**

Appendix Table 23. Results of regression analysis for the relationship between grain yield and SPAD/GDD sensed at three timings in combination with values normalized using values relative to the high N treatment using a simple linear model: y=a+(b*x). Analysis is combined based on significant effects from ANCOVA analysis

Time of Sensing	**Site-Year**	**Variety**	**SPAD/GDD vs. Yield**		**SPAD/GDD vs. Relative Yield**		**Relative SPAD vs. Yield**		**Relative SPAD vs. Relative Yield**	
			P-Value	R2	P-Value	R2	P-Value	R2	P-Value	R2
Stem Elongation	Brunkild 2016	Brandon					0.2935	0.05		
		Prosper					0.1497	0.08		
	Brunkild 2017	Brandon					0.0266	**0.18**		
		Prosper					0.3443	0.03		
	Carman 2016	Brandon					0.0026	**0.32**		
		Prosper					0.1298	0.09		
	Carman 2017	Brandon					0.0928	0.1		
		Prosper					0.0015	**0.34**		
	COMBINED		**<0.0001**	**0.67**	**0.0048**	**0.03**	**0.0015**	**0.34**	**<0.0001**	**0.08**
Flag Leaf	Brunkild 2016	Brandon			<0.0001	**0.32**	<0.0001	**0.54**	<0.0001	**0.50**
		Prosper								
	Brunkild 2017	Brandon			0.0003	**0.22**	<0.0001	**0.71**	<0.0001	**0.68**
		Prosper								
	Carman 2016	Brandon			<0.0001	**0.37**	<0.0001	**0.49**	<0.0001	**0.35**
		Prosper								
	Carman 2017	Brandon			0.0001	**0.24**	<0.0001	**0.58**	<0.0001	**0.69**
		Prosper								
	COMBINED		**<0.0001**	**0.60**	**<0.0001**	**0.17**	**<0.0001**	**0.31**	**<0.0001**	**0.44**
Anthesis	Brunkild 2016	Brandon	<0.0001	**0.63**			0.0011	**0.38**	<0.0001	**0.37**
		Prosper					<0.0001	**0.7**		
	Brunkild 2017	Brandon	<0.0001	**0.67**			<0.0001	**0.72**	<0.0001	**0.80**
		Prosper					<0.0001	**0.83**		
	Carman 2016	Brandon	<0.0001	**0.48**			0.0005	**0.41**	<0.0001	**0.44**
		Prosper					<0.0001	**0.7**		
	Carman 2017	Brandon	<0.0001	**0.29**			<0.0001	**0.58**	<0.0001	**0.74**
		Prosper					<0.0001	**0.76**		
	COMBINED		**<0.0001**	**0.60**	**<0.0001**	**0.32**	**<0.0001**	**0.37**	**<0.0001**	**0.5**

Appendix Table 24. Results of regression analysis for the relationship between grain protein content and SPAD/GDD sensed at three timings in combination with values normalized using values relative to the high N treatment using a simple linear model: y=a+(b*x). Analysis is combined based on significant effects from ANCOVA analysis.

Time of Sensing	Site-Year	Variety	SPAD/GDD vs. Protein		SPAD/GDD vs. Relative Protein		Relative SPAD vs. Protein		Relative SPAD vs. Relative Protein	
			P-Value	R2	P-Value	R2	P-Value	R2	P-Value	R2
Stem Elongation	Brunkild 2016	Brandon							0.5627	0.01
		Prosper							0.2169	0.07
	Brunkild 2017	Brandon							0.2588	0.05
		Prosper							0.1247	0.09
	Carman 2016	Brandon							0.0099	**0.26**
		Prosper							0.0791	0.128
	Carman 2017	Brandon							0.0477	**0.14**
		Prosper							0.0781	0.12
	COMBINED		**<0.0001**	**0.08**	**0.2237**	**0.01**	**0.2087**	**0.01**	**0.0078**	**0.03**
Flag Leaf	Brunkild 2016	Brandon					0.005	**0.15**	<0.0001	**0.43**
		Prosper								
	Brunkild 2017	Brandon					<0.0001	**0.26**	<0.0001	**0.40**
		Prosper								
	Carman 2016	Brandon					0.0547	0.07	<0.0001	**0.46**
		Prosper								
	Carman 2017	Brandon					<0.0001	**0.45**	<0.0001	**0.52**
		Prosper								
	COMBINED		**0.086**	**0.01**	**0.0021**	**0.05**	**0.0001**	**0.07**	**<0.0001**	**0.26**
Anthesis	Brunkild 2016	Brandon	0.0002	**0.47**	<0.0001	**0.63**	<0.0001	**0.69**	<0.0001	**0.76**
		Prosper	<0.0001	**0.60**			<0.0001	**0.51**	<0.0001	**0.60**
	Brunkild 2017	Brandon	<0.0001	**0.75**	<0.0001	**0.56**	<0.0001	**0.5**	<0.0001	**0.51**
		Prosper	<0.0001	**0.60**			<0.0001	**0.55**	<0.0001	**0.66**
	Carman 2016	Brandon	0.0095	**0.23**	<0.0001	**0.37**	0.0047	**0.3**	0.002	**0.35**
		Prosper	0.0008	**0.37**			<0.0001	**0.6**	<0.0001	**0.52**
	Carman 2017	Brandon	<0.0001	**0.76**	0.0092	**0.12**	<0.0001	**0.67**	<0.0001	**0.74**
		Prosper	<0.0001	**0.61**			<0.0001	**0.55**	<0.0001	**0.59**
	COMBINED		**0.792**	**0.00**	**<0.0001**	**0.14**	**<0.0001**	**0.14**	**<0.0001**	**0.34**

Appendix Table 25. Results of regression analysis for the relationship between grain yield and flag leaf N content in combination with values normalized using values relative to the high N treatment using a simple linear model: y=a+(b*x). Analysis is combined based on significant effects from ANCOVA analysis.

Site-year	Variety	Flag Leaf N vs. Yield		Flag Leaf N vs. Relative Yield		Relative Flag Leaf N vs. Yield		Relative Flag Leaf N vs. Relative Yield	
		P-Value	R2	P-Value	R2	P-Value	R2	P-Value	R2
Brunkild 2016	Brandon Prosper	<0.0001	**0.65**	<0.0001	**0.62**	<0.0001	**0.70**	<0.0001	**0.53**
Brunkild 2017	Brandon Prosper	0.0127	**0.11**	0.0006	**0.20**	0.0053	**0.14**	0.0922	0.05
Carberry 2016	Brandon Prosper	0.1016	0.06	0.2115	0.04	0.5461	0.01	0.9438	0.00
Carman 2016	Brandon Prosper	<0.0001	**0.40**	<0.0001	**0.63**	<0.0001	**0.54**	<0.0001	**0.63**
Carman 2017	Brandon Prosper	<0.0001	**0.37**	<0.0001	**0.54**	<0.0001	**0.37**	<0.0001	**0.57**
Grosse Isle 2017	Brandon Prosper	<0.0001	**0.43**	0.5697	0.00	0.6886	0.00	<0.0001	**0.39**
Melita 2016	Brandon Prosper	<0.0001	**0.48**	0.0466	**0.09**	0.1152	0.06	<0.0001	**0.45**
Melita 2017	Brandon Prosper	<0.0001	**0.62**	<0.0001	**0.64**	<0.0001	**0.50**	<0.0001	**0.70**
COMBINED		**<0.0001**	**0.14**	**<0.0001**	**0.28**	**<0.0001**	**0.38**	**<0.0001**	**0.39**

Appendix Table 26. Results of regression analysis for the relationship between grain protein content and flag leaf N content in combination with values normalized using values relative to the high N treatment using a simple linear model: y=a+(b*x). Analysis is combined based on significant effects from ANCOVA analysis.

Site-year	**Variety**	**Flag Leaf N vs. Protein**		**Flag Leaf N vs. Relative Protein**		**Relative Flag Leaf N vs. Protein**		**Relative Flag Leaf N vs. Relative Protein**	
		P-Value	**R2**	**P-Value**	**R2**	**P-Value**	**R2**	**P-Value**	**R2**
Brunkild 2016	Brandon	<0.0001	**0.58**	<0.0001	**0.70**	<0.0001	**0.42**	<0.0001	**0.71**
	Prosper								
Brunkild 2017	Brandon	0.0016	**0.17**	0.0026	**0.16**	0.1371	0.04	0.2071	0.03
	Prosper								
Carberry 2016	Brandon	<0.0001	**0.51**	<0.0001	**0.45**	0.0006	**0.23**	<0.0001	**0.61**
	Prosper								
Carman 2016	Brandon	<0.0001	**0.49**	<0.0001	**0.58**	<0.0001	**0.37**	<0.0001	**0.71**
	Prosper								
Carman 2017	Brandon	<0.0001	**0.63**	<0.0001	**0.61**	<0.0001	**0.38**	<0.0001	**0.35**
	Prosper								
Grosse Isle 2017	Brandon	<0.0001	**0.67**	<0.0001	**0.26**	0.7037	0.00	0.26	0.01
	Prosper								
Melita 2016	Brandon	<0.0001	**0.48**	<0.0001	**0.29**	0.0089	**0.15**	0.0194	**0.12**
	Prosper								
Melita 2017	Brandon	<0.0001	**0.64**	<0.0001	**0.62**	<0.0001	**0.46**	<0.0001	**0.53**
	Prosper								
COMBINED		**<0.0001**	**0.10**	**<0.0001**	**0.19**	**0.0003**	**0.06**	**<0.0001**	**0.20**

APPENDIX Table 27. Gold level sites lsmeans analysis of midseason soil NO$_3$-N (lbs N/ac). Means with similar lowercase letters are not significantly different within a column (P<0.05).

Treatment				Site-Year				
Variety	Timing	Source	Rate	Brunkild 2016	Carman 2016	Brunkild 2017	Carman 2017	Combined
			lbs N/ac	-- lbs N/ac	---			
Brandon	Seeding	Urea	0	51.9	53.3	61.6	35.0	48.3
	Seeding	Urea	50	50.3	76.5	72.5	39.3	56.2
	Seeding	Urea	80	70.5	74.0	165.5	44.6	77.0
	Seeding	Urea	110	51.4	142.2	138.9	61.9	87.0
	Seeding	Urea	140	80.8	190.2	236.8	83.3	128.4
	Seeding	Urea	170	182.0	188.2	453.3	117.1	201.9
	Seeding	Urea	200	88.0	201.5	315.1	79.0	141.5
Prosper	Seeding	Urea	0	41.6	44.9	65.3	28.9	42.3
	Seeding	Urea	50	46.9	81.0	58.3	32.3	50.6
	Seeding	Urea	80	80.7	95.9	108.7	53.3	79.7
	Seeding	Urea	110	72.3	116.3	105.6	90.6	92.4
	Seeding	Urea	140	72.1	96.0	207.5	103.2	107.9
	Seeding	Urea	170	120.8	173.1	316.7	116.3	162.9
	Seeding	Urea	200	79.2	200.9	271.4	123.8	148.5
Brandon				72.5	115.1	162.7	59.2	93.9
Prosper				68.1	102.2	131.5	66.6	87.6
	Seeding	Urea	0	45.9 b	48.4 c	62.7 c	31.5 c	45.1
	Seeding	Urea	50	48.0 b	77.7 bc	64.3 c	35.2 c	53.1
	Seeding	Urea	80	74.5 b	83.1 bc	132.6 b	48.1 bc	78.1
	Seeding	Urea	110	60.2 b	126.9 ab	119.7 bc	74.0 ab	89.4
	Seeding	Urea	140	75.3 b	133.3 ab	218.8 ab	91.7 a	117.3
	Seeding	Urea	170	146.7 a	178.5 a	374.7 a	115.4 a	180.9
	Seeding	Urea	200	82.4 ab	198.6 a	289.2 a	97.8 a	144.5
Site-Year				70.2	108.3	146.1	62.7	

ANOVA	df	Pr> F
Variety	1	0.2349
N Trt	6	<0.0001
Variety* N Trt	6	0.7681
SiteYr	3	0.0033
SiteYr*Variety	3	0.2234
SiteYr*N trt	18	0.0273
SiteYr*N trt*Variety	18	0.8617
Coeff var (C.V.)		33.6

Appendix Table 28. Silver level sites lsmeans analysis of post-harvest residual soil NO_3-N (lbs N/ac). Means with similar lowercase letters are not significantly different within a column (P<0.05).

Treatment				Site-Year				
Variety	Timing	Source	Rate	Melita 2016	Carberry 2016	Melita 2017	Grosse Isle 2017	Combined
			lbs N/ac	---- lbs N/ac ----				
Brandon	Seeding	Urea	0	24.8	42.2	33.2	43.8	34.7
	Seeding	Urea	50	27.4	42.4	31.2	44.2	35.0
	Seeding	Urea	80	27.0	40.3	22.4	40.8	31.1
	Seeding	Urea	110	32.9	66.2	23.5	44.8	38.4
	Seeding	Urea	140	37.1	54.2	34.8	46.6	41.9
	Seeding	Urea	170	28.7	70.0	59.7	126.2	61.5
Prosper	Seeding	Urea	0	30.4	40.2	30.3	36.8	33.7
	Seeding	Urea	50	29.6	47.5	21.4	38.0	32.2
	Seeding	Urea	80	31.7	39.8	23.5	35.4	31.6
	Seeding	Urea	110	35.0	47.0	21.1	42.1	34.3
	Seeding	Urea	140	39.3	38.8	32.9	66.0	42.1
	Seeding	Urea	170	31.5	42.5	42.9	92.9	47.4
Brandon				29.0	50.6	31.9	51.9	39.3
Prosper				32.3	42.0	27.4	47.8	36.3
	Seeding	Urea	0	27.2 b	40.9 a	31.5 ab	40.0 b	34.1
	Seeding	Urea	50	28.3 b	44.5 a	25.6 ab	40.7 b	33.5
	Seeding	Urea	80	29.0 ab	39.8 a	22.8 b	37.7 b	31.3
	Seeding	Urea	110	33.7 ab	55.4 a	22.1 b	43.1 b	36.2
	Seeding	Urea	140	37.9 a	45.5 a	33.6 ab	55.1 b	41.9
	Seeding	Urea	170	29.8 ab	54.2 a	50.2 a	107.4 a	53.9
Site-Year				30.6	46.0	29.5	49.7	

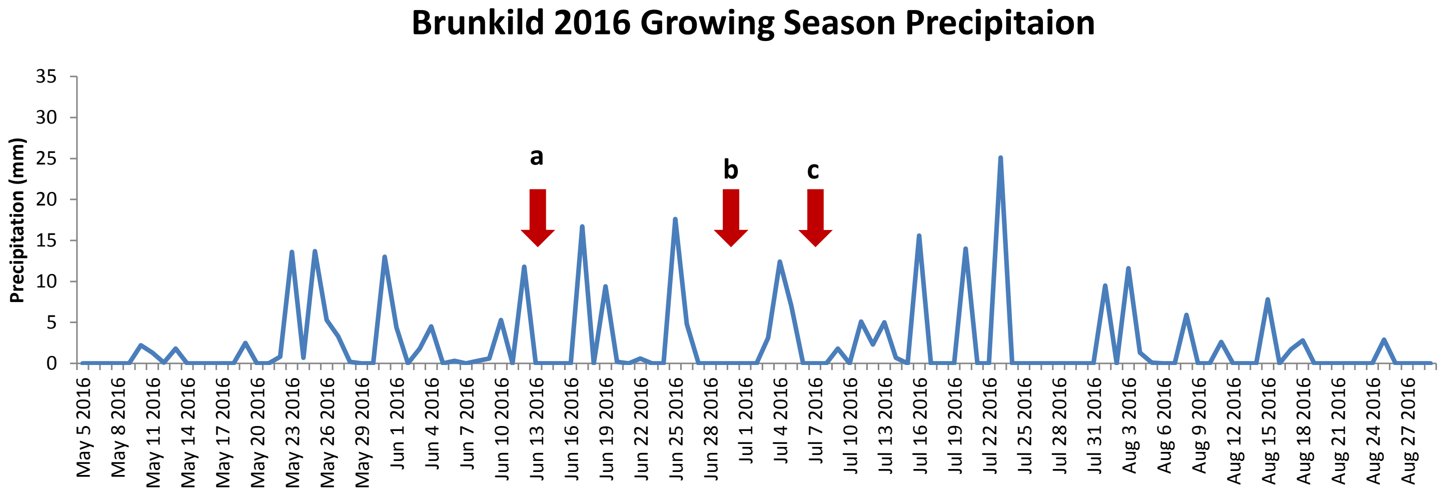
ANOVA	df	Pr> F
Variety	1	<0.0001
N Trt	5	0.1210
Variety* N Trt	5	0.6323
SiteYr	3	0.0056
SiteYr*Variety	3	0.1739
SiteYr*N trt	15	0.0006
SiteYr*N trt*Variety	15	0.9102
Coeff var (C.V.)		21.3

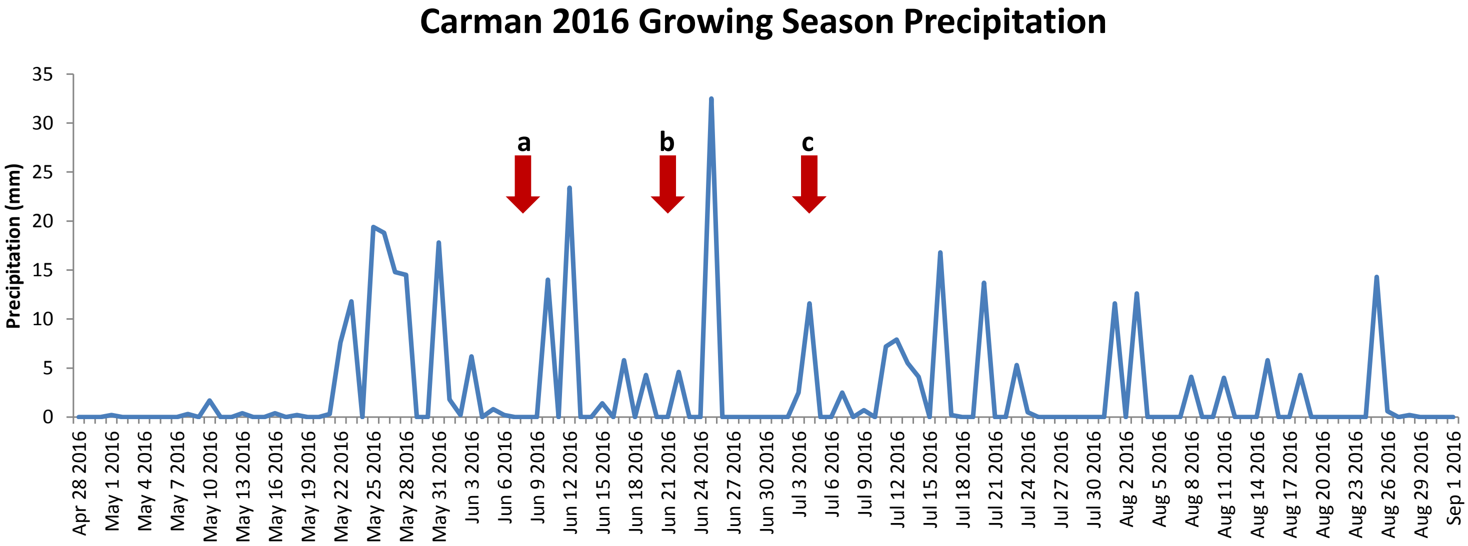
Appendix Table 29. Gold level sites lsmeans analysis of post-harvest residual soil NO_3-N (lbs N/ac). Means with similar lowercase letters are not significantly different within a column (P<0.05).

Treatment				Site-Year				
Variety	Timing	Source	Rate	Brunkild 2016	Carman 2016	Brunkild 2017	Carman 2017	Combined
			lbs N/ac	---------- lbs N/ac ----------				
Brandon	Seeding	Urea	0	27.7 a	44.1 a	21.8 c	15.5 e	25.0
	Seeding	Urea	50	30.0 a	43.0 a	17.3 c	18.2 de	24.9
	Seeding	Urea	80	34.0 a	42.1 a	13.8 c	19.4 cde	24.6
	Seeding	Urea	110	28.8 a	40.2 a	21.5 c	24.4 bcde	27.5
	Seeding	Urea	140	29.9 a	38.0 a	34.9 abc	22.8 bcde	30.3
	Seeding	Urea	170	39.9 a	54.1 a	67.9 ab	65.9 ab	55.0
	Seeding	Urea	200	60.5 a	71.0 a	95.0 a	50.4 abcd	66.4
Prosper	Seeding	Urea	0	24.8 a	38.2 a	17.3 c	18.3 cde	23.1
	Seeding	Urea	50	39.0 a	42.3 a	19.9 c	17.4 de	27.1
	Seeding	Urea	80	32.3 a	44.7 a	16.8 c	16.6 de	24.8
	Seeding	Urea	110	37.5 a	35.0 a	20.3 c	23.6 bcde	27.8
	Seeding	Urea	140	52.4 a	40.8 a	22.3 c	53.1 abc	39.4
	Seeding	Urea	170	47.0 a	40.6 a	37.9 abc	76.2 a	47.8
	Seeding	Urea	200	53.0 a	54.6 a	25.1 bc	102.9 a	51.6
Brandon				34.0	45.7	30.1	26.4	33.3
Prosper				39.0	41.3	21.7	33.4	32.8
	Seeding	Urea	0	25.9	40.7	19.2	16.7	24.0
	Seeding	Urea	50	33.9	42.2	18.3	17.7	25.9
	Seeding	Urea	80	32.8	42.9	15.1	17.7	24.6
	Seeding	Urea	110	32.6	37.1	20.7	23.8	27.6
	Seeding	Urea	140	39.2	39.0	27.6	34.5	34.5
	Seeding	Urea	170	42.9	46.4	50.3	70.3	51.2
	Seeding	Urea	200	56.1	61.7	48.4	71.4	58.4
Site-Year				36.3	43.4	25.5	29.7	

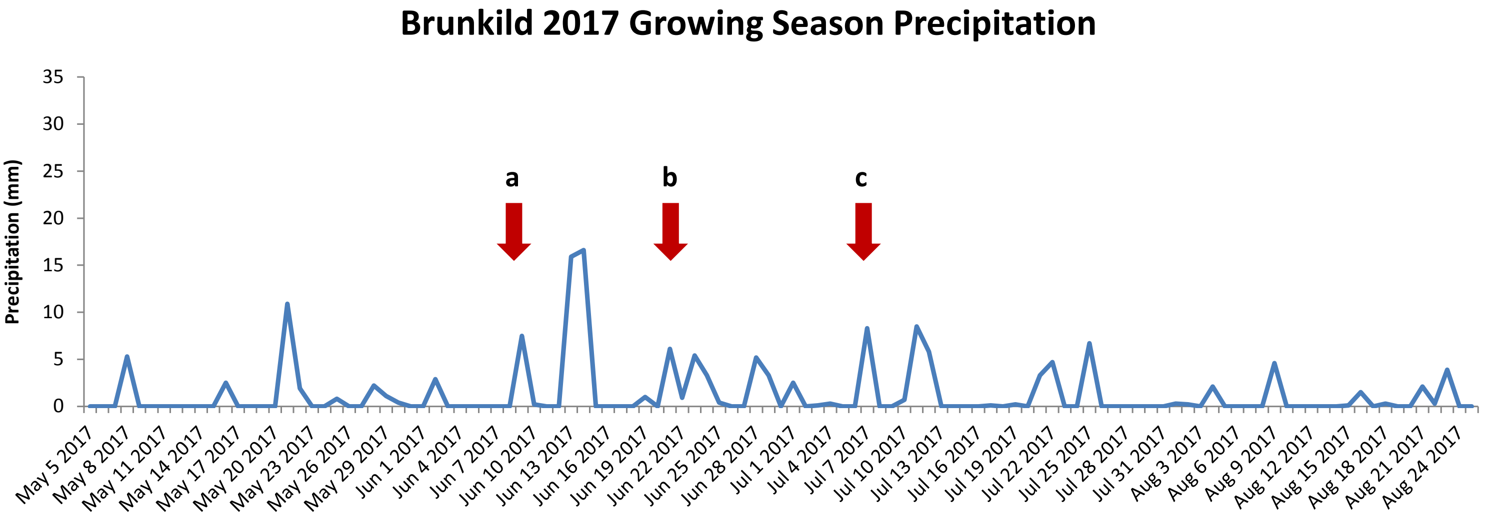
ANOVA	df	Pr> F
Variety	1	0.7651
N Trt	6	<0.0001
Variety* N Trt	6	0.1921
SiteYr	3	<0.0001
SiteYr*Variety	3	0.0004
SiteYr*N trt	18	<0.0001
SiteYr*N trt*Variety	18	0.0176
Coeff var (C.V.)		25.9

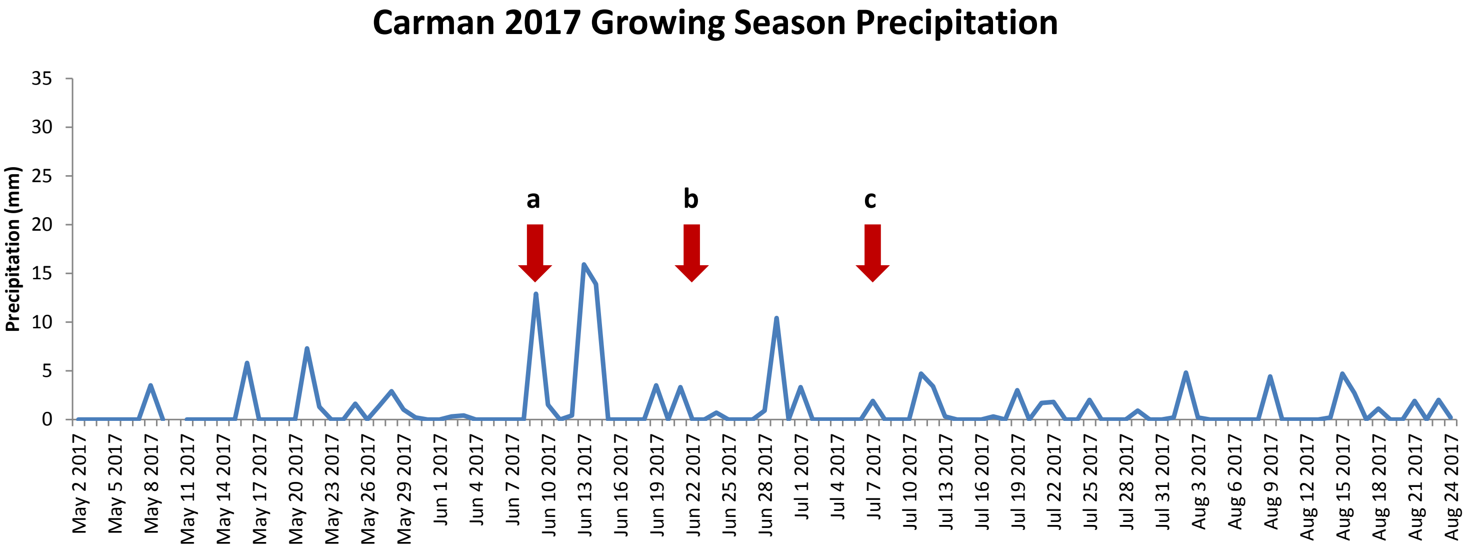
Appendix Figure 1. Growing season precipitation at 2016 gold level sites with nitrogen applications marked with red arrows: (a) stem elongation application, (b) flag leaf application, (c) post-anthesis application



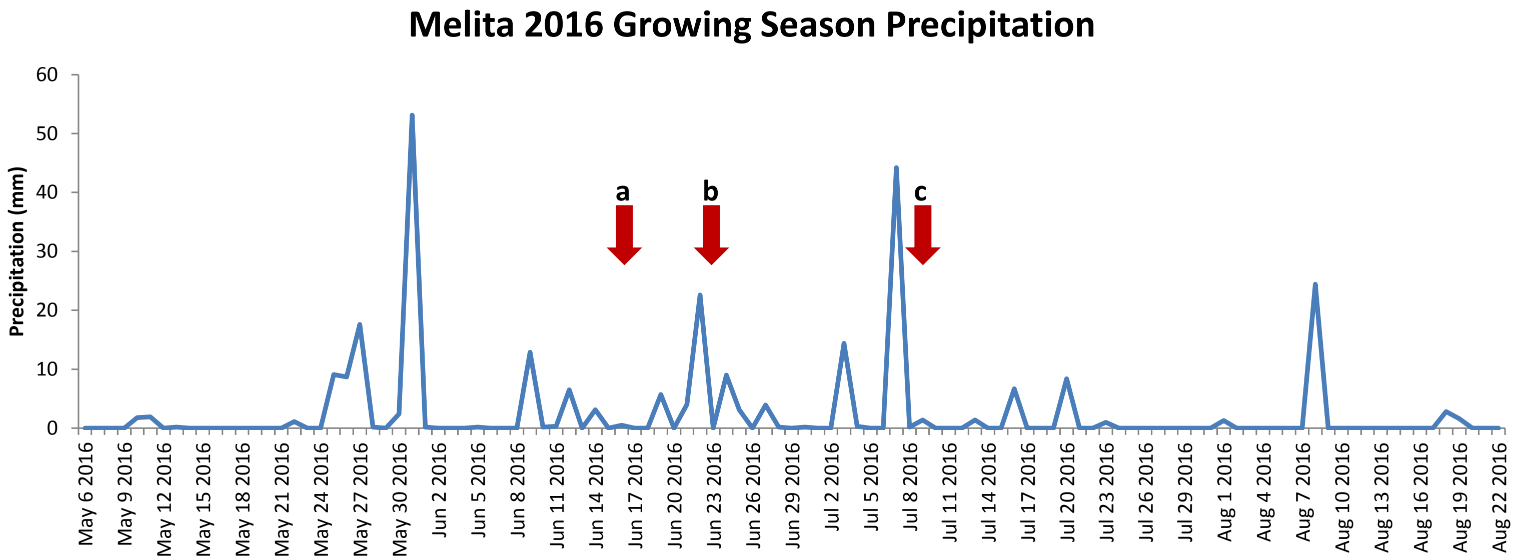


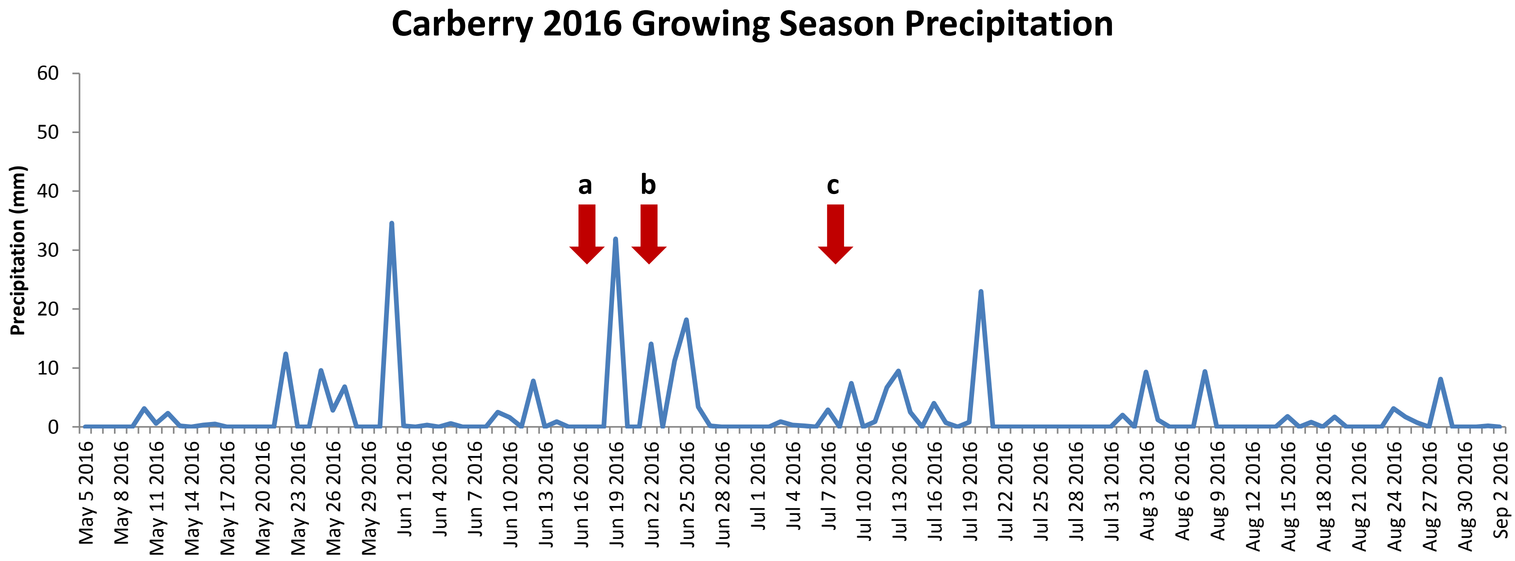
Appendix Figure 2. Growing season precipitation at 2017 gold level sites with nitrogen applications marked with red arrows: (a) stem elongation application, (b) flag leaf application, (c) post-anthesis application





Appendix Figure 3. Growing season precipitation at 2016 silver level sites with nitrogen applications marked with red arrows: (a) stem elongation application, (b) flag leaf application, (c) post-anthesis application


Melita 2016 Growing Season Precipitation


Carberry 2016 Growing Season Precipitation

Appendix Figure 4. Growing season precipitation at 2017 silver level sites with nitrogen applications marked with red arrows: (a) stem elongation application, (b) flag leaf application, (c) post-anthesis application