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In-Season Crop Management Effect On Soybean Yield and Grain Quality in Eastern South Dakota

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IN- SEASON CROP MANAGEMENT EFFECT ON SOYBEAN YIELD AND GRAIN

QUALITY IN EASTERN SOUTH DAKOTA

BY

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THESIS ACCEPTANCE PAGE

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This thesis is dedicated to my family and friends for supporting me.

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CONTENTS

ABBREVIATIONS

MG   maturity group

MRML   Most recent matured leaves

PDate   planting date

R1   beginning flower

R2   full flower

R3   beginning pod

R5   beginning seed fill

R6   Seed fill

R7   Beginning maturity

R8   Physical maturity

SERF   South East Research Farm- Beresford, SD

V4   four trifoliate

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ABSTRACT

IN-SEASON CROP MANAGEMENT EFFECT ON SOYBEAN YIELD AND GRAIN

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Soybean [Glycine max (L.) Merr.] yield has increased over time by introduction of newer varieties and improved agronomic practices. However, grain protein concentration has been decreasing at the same time. New field studies in 2018 and 2019 investigated in-season crop management practices effect such as use of foliar protection application, fertilizer application, different maturity length or different planting dates on grain yield and grain quality. The studies were established near Brookings, SD and Beresford, SD. In-season treatments were targeting nutrient availability or protecting soybean canopy during the grain filling period. Such treatments included the use of fungicide, insecticide, or supplying additional nutrients through foliar applications at the beginning of grain fill of the soybeans. Nitrogen and S applications were also made at different timings either at pre-season, V4, and R3 growth stages. Biomass samples were taken at R5, R6, and R7 and partitioned into parts of the plant. Grain yield, grain protein concentration, and grain oil concentration were taken at harvest and analyzed. While foliar protection application or fertilizer treatment effects did not impact grain yield or seed composition, year, location, and maturity group often influenced these parameters. Applying what is needed for each field is important when trying to maintain grain yield and quality.

CHAPTER 1

INTRODUCTION

Soybeans [*Glycine max* (L.) Merr.] have been produced and harvested on nearly

36 million hectares in 2018 in the United States (USDA-NASS, 2019). Over the last

century, soybean yields increased by 0.0234 Mg ha^{-1} yr^{-1}, and with this yield gain, a slow

dilution of soybean grain protein concentration occurred (Rowntree et al., 2013a).

Soybeans seeds are generally composed of approximately 34 percent protein and

approximately 19 percent oil at the 13% moisture content (Stowe, 2017, Kaur et al.,

2017). Grain protein and oil concentrations usually are negatively correlated. Soybean

pricing is only based on the yield along with grain grading quality which currently does

not include seed composition such as levels of oil and protein, like for other crops.

Changing grain composition has not been an issue on the soybean grain market yet, as it

has in small grains, such as wheat (*Triticum aestivum* L.; Kaur et al., 2017,

Weidenbenner et al., 2014). Many wheat producers receive grain price reduction because

they did not meet the protein requirement. Soybean meal is one of the many uses for

soybeans produced in the United States. Lower concentration levels of protein often

affect meal processors trying to meet quality standards, and also impacts farmers who

feed soybean meal to their animals. If protein concentration levels are lower, then the

farmer will have to either supplement protein or use, and buy, more soybean meal in

order to supply the same protein amount as it would be with higher grain protein

concentration soybeans. Research over the years has shown that there are many factors

that influence seed protein concentration levels. There are three main categories that

these factors fall under; genetics, management, and the environment influenced factors

also known as the G x M x E. The realized grain protein level is the result of the interactions of the three factors mentioned above.

Looking at the genetics portion, in recent years soybean protein levels have declined partially due to breeding effort; that selecting varieties with higher or high protein levels generally were lower priority due to the fact that it is easier to improve grain yield (Anthony et al., 2012). While protein and oil concentration are not specifically bred or improved due to a larger demand for higher yield, this has led to an increase in oil concentration levels over time. Yield increase also contributed to changes in soybean growth; research comparing historical varieties documented that modern soybean varieties spend more time in reproductive growth allowing longer time to develop pods and seeds than older varieties (Rowntree et al., 2013b). Knowing when to select varieties that are known to be less susceptible to pathogens, or certain pests can help maintain foliar canopies through the growing season. While selecting for genetics is important for increasing yield and grain quality, the environment is vital as well.

The environment plays a key role in seed quality and development through weather related events. High air temperatures, and moderate to low amounts of rainfall during the seed filling period and during the reproductive growth generally result in higher protein concentration in soybean seeds (Rotundo and Westgate, 2009). Increased temperatures and high amounts of precipitation are said to increase yield (Rowntree et al., 2013a). The air temperature effect on grain oil concentration showed mixed results; Rowntree et al, (2013a) documented oil concentration increase with lower air temperatures, while Anthony et al. (2012) with higher air temperature, especially if these temperatures occur after the R5 (beginning seed) growth stage (Naeve and Huerd, 2008,

Kaur et al., 2017,). Drought conditions during late reproductive stages have been found to decrease grain protein concentration in the seeds (Rotundo and Westgate, 2009). The magnitude of the increase in protein synthesis level depends on the timing and extent of the environmental stress. Seed mass also can be heavily influenced by environmental factors such as water availability and pest pressure. Stress during seed fill will have the most abundant increase in protein (Naeve and Huerd, 2008). This increase in protein could be because the seed size decreases and could be why protein is not normally correlated with higher yield (Rotundo and Westgate, 2009). Seeds at lower parts of the plant tend to have higher amounts of oil, while at the upper parts of the plant have more protein because the accumulation of oil in seeds often starts earlier in seed development than protein (Rotundo and Westgate, 2009; Saldivar et al., 2011, Huber et al. 2016). With greater amounts of N, there is likely to be more protein formed in the seeds (Rotundo and Westgate, 2009). If rain is abundant early in the growing season, there would be an increase in N leaching in the soil making it unavailable to the soybean later in the growing season. Along with temperature and water stress, other weather events that can remove or damage leaves can have an impact on photosynthesis and protein synthesis, such as hail. Hail damage can also lead to foliar disease development later in the growing season. Foliar diseases can reduce seed mass when not treated (Weidenbenner et al., 2014). Disease management through fungicide applications can help reduce soybean pathogens during the season.

Though a producer has minimal control over the weather conditions during the grain fill period, there is some ability to influence the timing and length of the grain fill period through the choice of planting date and the maturity length of the planted variety.

Previous research has shown that planting date can have a major impact on soybean protein levels. When it comes to planting soybeans, later planted soybeans tend to have a higher protein concentration then earlier planted soybeans (Jaureguy et al., 2013, Rowntree et al., 2013a). When planted earlier in the growing season, the soybeans will have longer time before flowering. When planted later, the soybeans have to mature faster. Often times, there is much shorter time difference in soybean flowering time than the time difference between planting in early and delayed planted soybeans, as soybean flowering is partially triggered by the day length time following the summer solstice. Length of the growing season forces certain parts of the country, like South Dakota, to plant shorter maturity groups of soybeans to enable them to reach physiological maturity due to generally later planting dates and an earlier typical first frost dates compared to growing environments further south. Finding the right maturity group for the area is key to reaching full yield potential and maintain grain quality. Just like the maturity group, nutrient applications are another management practice that can influence grain protein levels. Soybean crops normally derive between 60 and 89 percent of their total N uptake from N fixation (Abendroth et al., 2006; Tien et al., 2002). However, more conservative estimates suggest that the contribution of N fixation can meet from 25 to 75 percent of total plant N demand (Deibert et al., 1979; Salvagiotti et al., 2008; Collino et al., 2015). Protein synthesis is the process of forming amino acids into proteins. Nitrogen is one of the major components of these amino acids that are the building blocks of proteins (Galili and Galini, 2008). Nitrogen and S tend to interact at the metabolic level in such a way that an imbalance in their supply reduces the crop yield (Jamal et al., 2005). According to

Jamal et al. (2005), the inclusion of S fertilizer recommendation (up to 20 kg S ha^{-1}) for optimum soybean yield in S-deficient soils is necessary.

Protecting the canopy through management practices (e.g. supplying nutrient, or protection against pests) can prolong leaf senescence. By delaying leaf senescence and maintaining photosynthesis, soybean plants will have a longer time to produce protein in the seeds (Garcia and Hanway, 1976). Protein development starts much later then oil synthesis and by extending physiological maturity will help increase protein concentrations in the seeds (Saldivar et al., 2011, Huber et al. 2016). Adding inoculum during planting is another way to potentially increase protein. Abel and Erdman (1964) and Caldwell and Vest (1970) state that inoculation with *Bradyrhizobium japonicum* successfully increase soybean nodulation with increases in plant fresh weight, seed yield, and protein concentration in soils with a low or absent native population. "*B. japonicum* improved plant development and growth and the grain and protein yields of soybean crops." Co-inoculation with *Azospirillum spp*. has also been successful in increasing root density and length, root biomass, root hair development, shoot biomass, nodule number, and the fresh weight of soybeans" (Molla et al. 2001). Management practices are potentially the easiest ways to increase yield and maintain the percent of protein in the seed.

Yield is the main determining factor to determine income, along with market price. Since there is no economic incentive for seed oil or protein level at the grain elevator, it is harder to convince farmers to produce a crop that has higher seed protein (or better seed composition). The main question is, what can be done to maintain protein levels while still increasing yield?

Investigation of these management practices that could influence grain quality led to a multi-state collaborative research project. The goal of this project is to identify management factors affecting soybean seed protein and its composition, and to define the best management practices for increasing soybean seed quality. To accomplish this goal, field studies were established spanning across the Midwest to provide new information on how to increase soybean seed protein and essential amino acids by implementation of specific cultural practices. Field experiments were targeting one of the following components of management decisions: 1) altering growing environment conditions during seed filling (SF) using different maturity groups, and different planting dates, 2) altering nutritional status of the plant with applications of N and S at different timings of application (pre-plant, early or late growing season) or different fertilizer rates, or 3) maintaining the plant health through protection of leaf, extending leaf retention, and delaying nutrient remobilization. In South Dakota, objective of the field experiments were to 1) determine the effects of foliar protection applications at beginning of seed filling on protein levels in soybean seeds in different maturity group varieties, and 2a) determine the effects of N and S application rate and timing on protein levels in soybean seeds in different maturity group varieties, and 2b) different planting dates.

CHAPTER 2.

FOLIAR PROTECTION EFFECT ON SEED COMPOSITION AND CANOPY
RETENTION

2.1 INTRODUCTION

Foliar protection applications done in-season can help maintain canopies. Canopy

research determined that increase in light interception with narrow rows spacing for

soybeans during grain filling strongly correlated with the grain yield increase (Board et

al., 1990; Bullock et al., 1998; and Egli, 1994). An increase in aboveground biomass,

light interception, and assimilate utilization are all contributed to yield increases in

narrow rows compared to wide-rows production system (Andrade et al., 2002, Board et

al., 1990; Bullock et al., 1998; and Egli, 1994).

Foliar diseases or insects can reduce leaf area and reduced leaf area will lower

photosynthesis, and reduce grain yield (Bassanezi et al., 2001). Foliar protection

applications can aid in maintaining healthy crop canopies during grain fill period, which

relieves crop stress and extends photosynthetic production. Fungicides are often marketed

as the "cure all" for soybean diseases, but have been shown to mainly benefit fields that

have a problem/disease present (Jordan, 2010).

If foliar diseases or insects become a problem within a field early in the growing

season, then the soybeans express their stress by reduction in canopy height, and the

failure of closing the canopy between rows (Hansen et al., 2003). Typical preventive

pesticide application timing recommended around the early reproductive stages (either

due to insects presence or probability of pathogen infection and prevention) which also

coincide when canopies start to close, to prevent major losses and keep stress levels to a

minimum. In South Dakota, white mold (*Sclerotinia sclerotiorum)* is a disease that can

cause severe yield losses. One of the management practices to help reduce the amount of

white mold in a field is by increasing row spacing (typically .75 meter) or reducing plant

population to lower relative humidity in the crop canopy. Row spacing also affects light

interception in the lower portion of the soybean canopy. With the wider row spacing

there is more light that can reach the lower parts of the soybean plant and the ground

which will increase the air temperatures within the canopy during seed-fill due to

decreased albedo effect. This effect has shown to result in higher soybean protein

concentrations (Naeve and Huerd, 2008). There is little evidence of yield increases in

soybean production with foliar fungicide application, however, it does help maintain

yield when disease is present in the field. According to Jordan (2010), a yield increase is

not seen when diseases or insects are not present. Foliar protection applications directly

help maintain yield potential when there are problems in the growing season. The yield

benefit from fungicide applications has been attributed to maintaining healthier plants

that can function better compared to the untreated control and infected plants (Badenoch-

Jones et al., 1996; Bryson et al., 2000; Grossmann et al., 1999; Grossmann

and Retzlaf, 1997; Kuroda et al., 1996). Increasing seed mass while maintaining seed

number could explain how fungicides have contributed to grain yield in some

environments but not others (Weidenbenner et al., 2014). Maintaining healthy canopy

through the early reproductive stages can help to achieve the crop's yield potential.

    Those who use fungicides are those who most commonly found a problem in their

fields or have a recurring problem. Wrather and Koenning (2006) found that foliar

diseases accounted for only about 7.2 percent of total yield reduction over the three-year

period of their study in 2005. Other publication however, has shown that fungicide applications are profitable even when disease problems are not present (Orlowski et al. 2016). However, following the integrated pest management principles, fungicide application should only be used when there are known or anticipated problems in the field to manage fungicide resistance development.

Another way to help maintain healthy canopies is by preventing nutrient deficiencies. Foliar fertilizers sometimes contribute to observed yield differences (Jordan, 2010). These nutrient deficiencies could be from lack of fertilization before planting, not supplying adequate amount of the nutrient that the crop needs or plants not being able to acquire it from the soil. Garcia and Hanway (1976) speculated that minimizing the nutrient depletion of soybean leaves caused by remobilization was the cause of yield increases from foliar fertilization. They hypothesized that this reduction in nutrient depletion delayed senescence of the soybean and extended the leaf photosynthetic activity and improved seed fill. A study by Rotundo and Westgate (2009) showed fewer seeds per plant resulted in greater availability of nitrogen per seed that yielded high protein concentration seeds. When there are fewer seeds per plant, yield is often reduced (Rees et al., 2019). The objective of this project is to determine the effects of foliar protection applications at the beginning of seed filling on protein levels in soybean seeds in different maturity group varieties.

2.2 MATERIAL AND METHODS

2.2.1 Site description and experimental design

This study was conducted in the eastern part of South Dakota at two of the South Dakota State University Research Farms; near Brookings, SD (44.3114° N, 96.7984° W), and at the Southeast Research Farm (SERF; 43.0805° N, 96.7737° W) near Beresford, SD. The soil types were Divide (fine-loamy over sandy, mixed, superactive, frigid Aeric Calciaquolls), and Egan-Wentworth complex, which is a fine-silty, mixed, superactive, mesic Udic Haplustolls at Brookings and Egan-Clarno-Tetonka complex, which is a fine-loamy/fine-silty, mixed, superactive, mesic Typic Haplustolls; Egan-Clarno-Trent complex, which is a fine-silty, mixed, superactive, mesic Udic Haplustolls at SERF in 2018 and 2019, repsectively.

Two soybean varieties, GH1024X, GH2041X (maturity group 1.0 and 2.0 respectively, Golden Harvest Seed, Minnetonka, MN) were planted in this research. Six different foliar protection treatments were applied on each variety at R3 (beginning pod) growth stage: un-treated control, fungicide only, insecticide only, foliar fertilizer only, fungicide plus insecticide, and a combination of fungicide, insecticide, and foliar fertilizer. Plots received the following products according to treatment assignment: Trivapro [Benzovindiflupyr 2.9%, Azoxystrobin 10.5%, Propiconazole 11.9%, Group 11, 3(DeMethylation Inhibitors), 7(Succinate dehydrogenase inhibitors)] fungicide at 0.172L ha^{-1}; and Miravis [Pydiflumetofen 18.3%, Group 7] fungicide at 0.172L ha^{-1}; Endigo [Lambda-Cyhalothrin 9.48%, Thiamethoxam 12.6%, Group 3A, 4A; demethylation inhibitors and phenlamides)] insecticide at 0.052 L ha^{-1}, and Generate (0.28% Fe, 0.11%

Mn, 0.14% Cu, 0.11% Zn, 0.001% Mo, 0.52% Co, 0.11% Na) foliar fertilizer at 0.366L

ha^{-1} rates when sprayed at 140 L ha^{-1}. The combination of variety and foliar product

treatments were arranged in a randomized complete block design.

Corn (*Zea mays L.*) was the preceding crop at both locations in 2019 and the

Brookings site in 2018. These sites utilized conventional tillage practices and prior to

planting fields were field cultivated, while at Beresford in 2018 soybeans were planted in

no-till ground following oats (*Avena sativa* L.). In 2019, soybeans at the Beresford site

required replanting due to very poor plant stand caused by wet weather conditions during

planting and emergence window. Soybean varieties were planted at 346,000 seeds ha^{-1} in

76-cm rows (Table 2-1). Plots were maintain weed free. Plots sizes were 3m x 18.3m in

Brookings and 4.5m x 13.7m at SERF in 2018 and at both locations in 2019.

2.2.2 Field data collection

Before planting, soil samples were taken from each replication (15 cores of 2 cm

diameter in each replication) and separated to 0-15cm, 15-60cm; soil samples were air

dried with forced air then ground to pass through a 2 mm sieve, and sent off to a certified

commercial laboratory (AgSource Laboratories, Lincoln, NE) to determine nutrient

concentrations, soil pH and organic matter (OM). The laboratory used the 1:1 soil-water

slurry method for soil pH, the loss of ignition method for OM, the cadmium reduction

method for NO_3^--N and the monocalcium phosphate method for SO_4^{2-}-S concentration

determination (AgSource Laboratories, 2020).

At both locations, the foliar treatments were applied at the R3 growth stage in end

of July 2018 and 2019 (Table 2-1). In 2018, at the SERF site the foliar fertilizer and the

combination of all product treatments were discarded, due to an initial planting error that caused these foliar treatments to apply on the same maturity group treatment resulting 8 plots, all the same maturity group variety, with the same foliar treatment (the switch of maturity groups between treatments was noticed only at the beginning of leaf senescence). Visual plant damage ratings were taken before foliar application, 14, and 28 days after application for extent of leaf damage and presence and extent of disease based on percentage of leaf damage and percentage of the plant they made up.

After emergence (around V3 growth stage) and at physiological maturity, plant population was estimated through stand counts at four locations in the center rows within each plot. The number of plants were counted on one meter length of a row and plant population were calculated from.

At R5 (beginning seed), R6 (full seed), and R7 (beginning maturity) growth stages biomass samples were taken from 0.5 meter section of a non-border row. Sampling times presented in Table 2-1. The R5 and R6 samples were partitioned into leaves, stems, branches (including petioles), pods, and fallen leaves. The R7 samples were partitioned into leaves, stems, branches (including petioles), pod shells, seeds, and fallen leaves. Fallen leaves were any leaves or petioles that fell in the area biomass was collected from (0.38 m from each side of the row). All samples were dried at 60 $^\circ$C until constant weight and the dry biomass accumulation was calculated.

Grain harvest was done in late October (Table 2-1). The middle two rows were harvested with a Massey Ferguson 8XP plot combine. Grain weight, grain moisture and test weight readings were recorded. Plot lengths were measured to determine grain yield.

Grain protein and oil concentrations were determined with InfraTec Nova (FOSS

Analytics, Hillerød, Denmark) instrument. Seed mass (200-seed weight) were estimated

by weighing grain samples collected from the harvested plots. Grain yield, grain protein

and oil, and the 200 seed weight data was adjusted to 130 g kg^{-1} moisture content.

2.2.3 Statistical analysis

Results are analyzed in R Studio in ANOVA and Fishers-LSD at 0.05

significance level. The foliar application treatments, maturity group, year, and location,

were all considered fixed factors in the statistical model. The SERF site in 2018 was

analyzed separately due to the planting and foliar application error. The other three site

years were combined for statistical analysis.

2.3 RESULTS

2.3.1 Weather

Daily minimum and maximum temperatures, and precipitation in 2018 and 2019

for each site are shown in Figure 2-1 and monthly mean minimum and maximum

temperatures and monthly precipitation are presented in Table 2-2. Average highs

temperatures ranged from 11.0 to 28.89 °C while mean low temperatures ranged from 0.6

to 17.87 °C in the May - October period.

Monthly total precipitation data were also calculated. From May until October

rainfall was ranging from 19 to 221 millimeters a month. Precipitation was higher in

2018 at the SERF site. SERF also had higher precipitation early in the 2019 season. The

largest monthly precipitation was recorded in 2019 September at the Brookings site with

220.98mm among the four site-years. Brookings also had the largest amount of precipitation in one day (159.3mm) which occurred in July 2018 in Brookings. Each site received above normal precipitation from planting until harvest (e.g. the SERF site in 2018 was nearly doubled the 30 years normal).

2.3.2 Field Characteristics

Soil samples taken before planting and analyzed for pH, OM, N, S are shown in Table 2-3. Soil pH ranged from 5.7 to 7.6 in the 0-15cm soil layer and from 6.5 to 7.6 in the 15-60 cm depth. Organic matter content in the top 15 cm depth ranged from 3.2% to nearly 4.5% while in the 15-60 cm soil depth it ranged from 2.6 to 3.0% among the sites. Soil NO_3^--N ranged between 2.5 and 22.5ppm, and between 1.0 and 8.0 ppm, while SO_4^{2-}-S ranged between 3.0 and 11.3 ppm and between 2.0 and 8.8 ppm in the 0-15 cm and 15-60 cm soil depths, respectively (Table 2-3).

2.3.3 Stand counts

There were no statistical differences in early season or final plant stand among the foliar treatments (Table 2-4 and Table 2-5). However, the ANOVA analysis detected statistical differences between the MGs as main factors and the year x location interaction both for early-season and final stand counts (Table 2-4). While plant stands were statistically different between the MGs, the differences was 12-13,000 plants ha^{-1} across the site years, and treatments at either time (Table 2-5). The 2019 SERF site had approximately 21,000 plants ha^{-1} smaller plant stand at the early part of the growing season averaged across the other factors (Table 2-5).

At the SERF site in 2018 early season plant stand did not differ, while the final plant stand was approximately 6,000 plants ha^{-1} larger in MG2 variety plots averaged across the foliar treatments (Table 2-7).

2.3.4 Grain yield, protein, and oil concentrations

Both grain yield, grain protein and oil concentration were influenced by the interaction of growing season and the location (Table 2-4). However, only the grain yield and the grain oil concentration differed due to the MG, and to the year x location x MG interaction (Table 2-4). The foliar application treatments did not have yield differences in grain yield, grain protein concentration or grain oil concentration (Table 2-4). Only a few significant interactions were detected among the other interactions and their impact on grain yield and grain components were inconsistent (Table 2-4). For example, the location x MG x foliar application treatment influenced the grain yield, while the year x MG and year x location x foliar application treatment influenced the grain oil concentration at the p=0.05 significance level (Table 2-4). Data on grain yield, grain protein, and oil concentrations are shown in Table 2-5. Maturity group 2 variety out yielded MG 1 variety by about 0.50 Mg ha^{-1} across the foliar treatments and site years. Oil concentration was also numerically higher with fungicide and insecticide treatment compared to the other foliar treatments averaged across the maturity groups and site years. Brookings MG1 had the significantly highest protein (0.8 percent higher) while all other MG in Brookings and SERF were significantly higher in oil (0.4 percent higher) this could be why we saw the year by location by treatment interaction (Table 2-6). 2019 at SERF numerically was higher than Brookings in both years and fungicide and treatments with fungicide tend to be the highest yielding (Table 2-6).

Yield in 2018 at Brookings MG2 was the highest than all other treatments in the year by location by MG interaction. Protein concentration was the highest in MG1 variety in Brookings across the foliar treatment and oil concentrations was the opposite with Brookings MG1 being the lowest (Table 2-6). At the Brookings sites the MGs differed in grain protein and oil concentrations, while at the SERF site the MGs had similar grain composition.

The SERF site in 2018 that was analyzed separately (Table 2-7) fungicide and treatments that had fungicide tended be significantly the highest yielding. MG2 was significantly higher in grain yield, grain protein concentration, and grain oil concentration (Table 2-7).

2.3.5 Biomass accumulation and partitioning

Biomass partitions did not differ among foliar application treatments at either growth stages across the three site-years (Table 2-8). There were no significant differences among the treatments at R5 growth stage in any part of the plant, but the year by location interaction was significant (Table 2-8). Soybean plants tended to have the largest amount of biomass when the plant was at R6; this is before they start dropping their leaves. Biomass in 2019 was lower approximately by ~1000kg ha^{-1} at both locations and at all three growth stages than in 2018 (Figure 2-2). Treatments did not change the amount of biomass that the plant maintains through the late reproductive (R5-R7) stages except for the leaves with the foliar fertilizer treatment at R6 growth stage which was lower than the foliar fertilizer, fungicide, insecticide combination, but did not differ from the untreated control (Table 2-9). Only the leaves biomass differed at the R6 growth stage, while the leaves, and pods biomass accumulation differed at the R7 growth stage

(Table 2-8). Leaves with either fungicide only or combination but containing fungicide treatments had the three largest leaves biomass across MGs and site years (Table 2-9). Only the fungicide and insecticide treatment did not differ from the other three non-fungicide containing treatments. At the R5 and R6 growth stages, MG1 variety's stems, were higher in biomass, while the branches were higher in MG2 (Table 2-10). R7 stems were significantly higher in MG1 while the rest of the partitions, branches, pod shells, seeds were higher in MG2 (Table 2-10). R5 and R7 leaves, stems, and branches were the highest in 2018 at Brookings, while the R5 pods were significantly higher in 2019 Brookings, and the R7 seeds and pod shells were higher in 2019 at the SERF in the year by location interactions (Table 2-10). All R6 biomass partitions and total biomass accumulation were significantly higher in 2018 Brookings compared to the other site years. Table 2-11 shows the proportional distribution of the plant partitions relative to the total biomass.

Over time, pod/seed biomass increased while other parts of the plant biomass decreased due to resources being moved to into the pods and away from the leaves and stems.

2.3.6 Disease and pest damage assessment

There were some seasonal differences in the pre application pest ratings comparing between 2018 and 2019. In 2018, initial pest damage ratings were (more leaf damage) higher prior to the R3 spraying then in 2019. There was no significant differences between the years in diseases pressure pre application. After application, there was higher insect damage in the untreated control and the foliar fertilizer only treatments at both locations. Fungicide treatment also had more insect damage at

Brookings in both years. However, no noticeable disease pressure differences were

observed 14 to 28 days after application. (Table 2-12, 2-13)

2.4 DISCUSSION

2.4.1 Weather

Negative monthly mean temperatures only occurred in April 2018 at both sites,

and only reached subzero temperatures on May 10, 2019 at the SERF. These early spring

low temperatures prevent growers from early planting (generally planting does not occur

in April in South Dakota) and achieving a higher soybean grain yield. In addition, April

25th is the first date that soybeans can be planted in South Dakota and be protected by

crop insurance. Large amounts of rainfall could affect plant stand during the season

which would lead to less yield. Rotundo and Westgate (2009) found protein increases

when there is water stress (lack of water) during grain fill. While none of these site years

had these type of water stress, at the Brookings site in 2018 had some of the lowest

amounts of precipitation (still higher than the 30 year average) during the late

reproductive stages, during the two growing seasons and had higher levels of protein then

in 2019. At SERF in 2018, the trial had large amount of rainfall during grain fill and

warmer weather throughout the growing season and ended up having the highest yield

and protein concentration in this studying agreeing with the findings of Rotundo and

Westgate (2009).

2.4.2 Grain yield, and quality

The location effect on yield is related to environmental factors such as the higher early season precipitation and warmer temperatures (Naeve and Huerd, 2008) during the growing season at the SERF site.

Picking a MG that is right for each location is important when trying to maximize the light interception which will influence yield. Brookings does not commonly plant MG2 due to how far north it is (Hall et al. 2012). Though unlike the nitrogen and sulfur study (Chapter 3), the MG2 variety did better than the MG1. The MG2 variety takes a longer time to mature allowing the leaves to stay on longer than the MG1 varieties. Because of this, the yield and protein levels benefited.

South Dakota has a problem with white mold in soybeans. One of the fungicides used in this study was targeted to control white mold. However, the R3 application timing is late to prevent white mold infection, but the grain filling period was the main focus of this study. Future studies could address the effect of different fungicide (or plant protection product) application timings effects on grain quality and disease development and their interaction. There were no statistical differences in disease pressure in the 14 and 28 days after application ratings. There could have been more differences later in the season that were not evaluated.

Previous research has found that applications of fungicide are profitable even when disease problems are not shown (Orlowski et al., 20016). However, this study did not show yield benefit in relatively low disease pressure conditions. In addition, spraying fungicide and insecticide below the economical threshold of the pest (e.g. not following the integrated pest management approach) can accelerate the resistance development problems.

2.4.3 Biomass

Biomass was collected at the R5, R6, and R7 growth stages. Some of the biomass weights differences occurred may be because the timing of sample collection may not have happened at the exact plant developmental stages due to the large amount of plots that needed to collected and partitioned during the grain filling period. The two MG samples were collected at the same time at the R5 growth stage while in later stages they were collected separately when the different MGs reach the target growth stage. The same sampling time can explain biomass accumulation differences between MGs at the R5 growth stage.

Branches and petioles partitions were collected together and could be why there was a dramatic decrease from 1210 to 714kg ha^{-1} when going from R6 to R7 growth stage as the petioles fall from the plant. In 2018, there was lodging in the fields which also caused some difficulties to precisely define the area and collect the fallen leaves and petioles which can explain the larger decline in total biomass from R6 to R7 growth stages in that year. Fungicide application delayed leaf senescence which resulted leaf biomass differences at R7 growth stage, similarly to Parker and Boswell (1980) observations.

Both growing seasons had above normal precipitation, which affected the stand uniformity, even though the plant stand did not substantially differ among the treatments. However, the not completely uniform plant stand could vary the number of plants collected in a sample (plants were cut from a 0.5 m section of the row) impacting total biomass accumulation and even the partitioning of the biomass. When a lower number of plant was collected, the soybean plants had more branches which meant that there were

more pods per plant. These plants also tended to have more leaves due to the number of branches. Planting time can also affect how many pods a plant has. Planting occurred at close to optimal time in the season in 2018 and had a longer time before the summer solstice, which leads to a longer amount of time that the plant can grow and produce nodes. Higher numbers of nodes leads to a larger number of pods per plant.

2.5 CONCLUSION

Foliar protection application at the beginning of the grain fill period generally did not impact grain yield or grain protein in our four site years. While we did not see a common trend in our findings, we had very low insect and disease pressure at each site year. This was also coupled with fairly challenging growing seasons during the two years of the study, including a wet growing season with delayed planting in 2019. Even though leaf senescence was somewhat delayed with fungicide applications, overall biomass accumulation or partitioning was not affected by the in-season crop management treatments, nor was grain yield. With currently no incentive on the grain market for the higher protein content, the likelihood of foliar applications used without pest pressure is unlikely to become adapted as a common practice to increase protein levels. Along with this is the thought of resistance development becoming more prevalent. If a field was found to reach economical threshold of insect pressure or prone to disease problems year after year, applying fungicide and/or insecticide could lead to maintaining higher grain yield levels or increase yield compared to the untreated control, and potentially can help to increase grain protein levels as well.

In the future, finding areas that have been known to have pest/disease presence would be important to find out if these foliar applications would work more consistently.

Also, applying fungicides at different timings, or using different fungicides and insecticides could change some of the findings. Delaying disease and insect damage ratings following the fungicide application would be important to see if there would be a significant change in protein concentration and yield. Analyzing nutrient composition of the biomass collected would be the short term goal to see if by applying these foliar treatments affected the nutrient composition in each or either part of the plant.

Table 2-1 Experimental design, varieties used and other crop production parameters, along with planting, treatment application, biomass and machine harvest dates.

Field Activities	2018 Brookings	SERF	2019 Brookings	SERF
Varieties		GH1024X, GH2041X		
Experimental Design		RCBD		
Row spacing		0.76m		
Replications		4		
Seeding Rate		346,000 viable seeds ha^{-1}		
Plot dimensions	3m x 18.3m		4.5m x 13.7m	
Tillage	Conventional	No-till	Conventional	
Rotation	Corn-Soybean	Oat-Soybean	Corn-Soybean	
Planting Date	May 15 [†]	May 17	June 2	June 8
R3 application	July 21	July 20	July 25	July 25
R5 Biomass	Aug. 9 Aug. 13	Aug. 6	Aug. 13 Aug. 20	Aug. 15 Aug. 19
R6 Biomass	Aug. 30 Sep. 7	Aug. 20 Aug. 23	Sep. 4 Sep. 10	Sep. 3 Sep. 5
R7 Biomass	Sep. 14 Sep. 19	Sep.5 Sep.12	Sep. 23 Oct. 4	Sep. 23 Sep. 30
Machine harvest	Oct. 19	Oct. 18	Oct. 19-20	Oct. 18

[†] multiple dates for a field activity indicate that the MG1 and MG2 varieties have been sampled on separately on the days presented

Table 2-2. Monthly mean minimum and maximum air temperature and monthly precipitation accumulation near Brookings and Beresford, SD in 2018 and 2019.

	----2018 Brookings----			-----2018 SERF-----			----2019 Brookings----			----2019 SERF----		
	Mean Max	Mean Min	Precip.	Mean Max	Mean Min	Precip.	Mean Max	Mean Min	Precip.	Mean Max	Mean Min	Precip.
	----°C----		mm	---- °C ----		mm	---- °C ----		mm	----°C----		mm
April	6.8	-3.2	19.3	9.8	-2.7	45.5	N/A	N/A	N/A	14.1	2.1	88.7
May	23.8	11.1	18.8	24.6	12.1	94.5	N/A	N/A	N/A	18.5	7.0	156.7
June	26.1	16.4	102.1	28.0	17.4	209.3	26.5	13.9	70.4	27.0	14.4	98.3
July	26.9	15.8	215.7	28.6	16.4	77.7	28.0	16.5	192.0	28.9	17.9	109.0
August	26.1	15.3	86.9	26.8	15.4	96.5	25.1	14.0	109.5	26.6	15.1	82.3
September	21.6	11.8	108.0	23.2	12.5	172.5	23.2	12.6	221.0	25.2	13.7	74.2
October	11.2	0.6	46.0	13.1	1.1	51.6	11.0	1.9	45.2	13.4	2.0	82.0

Table 2-3 Pre plant field characteristics of soil pH, organic matter (OM), and soil nitrate-N (NO_3^--N), and sulfate-S (SO_4^{2-}-S) concentration in the 0-15cm and 15-60cm soil zones in 2018 and 2019 near Brookings and Beresford, SD.

	Soil depth (cm)	pH	OM (%)	NO_3^--N (ppm)	SO_4^{2-}-S (ppm)
2018 Brookings	0-15	6.58 ± 0.35	4.45 ± 0.39	22.50 ± 7.54	11.25 ± 0.96
	15-60	7.60 ± 0.43	2.73 ± 0.08	8.00 ± 3.16	8.75 ± 0.50
2018 SERF	0-15	6.28 ± 0.88	3.38 ± 0.48	4.00 ± 2.58	7.00 ± 4.00
	15-60	6.88 ± 0.71	3.00 ± 0.47	5.25 ± 1.26	7.50 ± 2.52
2019 Brookings	0-15	5.73 ± 0.16	3.33 ± 0.32	2.50 ± 0.58	3.00 ± 0.00
	15-60	6.53 ± 0.09	2.75 ± 0.24	2.75 ± 0.50	2.00 ± 0.00
2019 SERF	0-15	6.20 ± 0.16	3.20 ± 0.00	2.63 ± 0.22	5.00 ± 1.41
	15-60	7.63 ± 0.45	2.63 ± 0.22	1.00 ± 0.50	5.25 ± 1.50

Table 2-4. Analysis of Variance (ANOVA) significance levels of foliar protection application, year, location, and maturity group (MG) main effects and their interactions on early-season and final plant stand, grain yield, and grain protein and oil concentrations in eastern SD in 2018 and 2019.

	Plant Stand		Grain Yield	Grain Protein concentration	Grain Oil concentration
	Early	Late			
Year	0.007	<.0001	<.0001	<.0001	0.98
Location	<.0001	<.0001	<.0001	<.0001	<.0001
MG	0.04	0.002	<.0001	0.40	<.0001
Treatment	0.67	0.74	0.46	0.24	0.16
Year*Location	0.01	<.0001	<.0001	<.0001	<.0001
Year*MG	0.22	0.59	0.73	0.91	0.001
Location*MG	0.54	0.95	0.12	<.0001	0.0005
Year* Treatment	0.58	0.33	0.97	0.57	0.53
Location* Treatment	0.84	0.53	0.74	*0.05*	0.55
MG* Treatment	0.92	*0.05*	0.46	0.96	0.76
Year*Location*MG	0.29	*0.09*	0.02	0.19	<.0001
Year*Location* Treatment	0.97	0.17	0.82	0.15	0.02
Year*MG* Treatment	0.97	0.98	0.64	0.81	0.62
Location*MG* Treatment	0.34	0.52	0.67	0.46	0.55
Year*Location*MG*Treatment	0.87	0.65	0.87	0.565	0.32

Table 2-5. Main treatment effect on early season and final plant stands in 2018 and 2019 near Brookings and Beresford, SD

	Early season	Final stand	Grain yield	Grain Protein concentration	Grain Oil concentration
	(plants ha^{-1})		Mg ha^{-1}		%
Foliar application treatment					
Control	270,600	245,900	4.56	35.2	17.9
Fungicide	266,400	240,900	3.95	35.2	17.9
Insecticide	268,100	243,100	4.57	35.0	18.0
Fungicide and Insecticide	266,600	240,700	4.46	35.0	18.1
Foliar Fertilizer	266,900	251,100	5.17	35.1	18.0
FFI[††]	278,700	242,700	4.35	35.1	18.0
Maturity Group					
MG1	273,800a	250,100a	4.44b	35.3	17.8b
MG2	265,100b	237,700b	4.58a	35.2	18.1a
*Year*Location*					
2018 Brookings	275,300a	196, 207c	4.51a	36.5a	17.9b
2019 Brookings	274,200a	272,500a	3.59b	34.6b	17.8b
2019 SERF	253,300b	254,500b	3.94ab	34.7b	18.1a

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

[††] Combination of foliar fertilizer, fungicides and insecticide application

Table 2-6. Interactions for grain yield, protein and oil concentrations.

		Grain yield Mg ha^{-1}	Grain protein concentrations %	Grain oil concentrations
Year*Location*MG				
2018 Brookings MG1		4.4b[†]		
2018 Brookings MG2		4.8a		
2019 SERF MG1		3.7c		
2019 SERF MG2		4.2b		
2019 Brookings MG1		3.4d		
2019 Brookings MG2		3.8c		
Location*MG				
Brookings MG1			35.7a	17.6b
Brookings MG2			35.3b	18.1a
SERF MG1			34.5c	18.0a
SERF MG2			34.9bc	18.1a
Year*Location*Treatment				
2018 Brookings	Control			17.8abc
	Fungicide			17.8abc
	Insecticide			17.9abc
	Fungicide and Insecticide			18.2a
	Foliar Fertilizer			17.9abc
	FFI[††]			18.0abc

Table 2-6 cont.

		Grain yield Mg ha⁻¹	Grain protein concentrations %	Grain oil concentrations
2019 Brookings	Control			17.8bc
	Fungicide			17.9abc
	Insecticide			17.8bc
	Fungicide and Insecticide			17.8bc
	Foliar Fertilizer			17.9abc
	FFI			17.8abc
2019 SERF	Control			18.2a
	Fungicide			18.0abc
	Insecticide			18.1abc
	Fungicide and Insecticide			18.2ab
	Foliar Fertilizer			18.1abc
	FFI			18.0abc

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level
[††] Combination of foliar fertilizer, fungicides and insecticide application

Table 2-7. Foliar application effect on stand counts, grain yield, protein and oil concentrations in Beresford, SD in 2018.

Maturity Group	Foliar application treatment	Early Stand	Final Stand	Yield	Protein	Oil
		(plants ha^{-1})		(Mg ha^{-1})	(%)	
MG1	Control	247200	220200	5.53b	35.1 bc[†]	18.5
	Fungicide	252600	231800	5.62a	34.7 c	18.7
	Insecticide	260000	241700	5.51b	34.9 c	18.4
	Fungicide and Insecticide	251000	238400	5.64a	34.9 c	18.5
MG2	Control	244400	233800	5.52c	35.0 bc	18.7
	Fungicide	236200	241700	5.91a	35.2 ab	18.9
	Insecticide	251800	246100	5.71b	35.0 bc	18.8
	Fungicide and Insecticide	254300	235100	5.93a	35.5 a	18.7
	p<F					
	MG	0.98	**0.01**	**0.05**	**<.0001**	**<.0001**
	Treatment	0.92	0.33	**0.003**	0.10	**0.01**
	MG* Treatment	0.85	0.88	0.55	**0.04**	0.14

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

[††] Combination of foliar fertilizer, fungicides and insecticide application

Table 2-8 Analysis of Variance (ANOVA) significance levels of foliar protection application, year, location, and maturity group (MG) main effects and their interactions on leaves, main stem, branches and petioles, pods biomass, and total biomass accumulation at R5, R6, and R7 growth stages in eastern SD in 2018 and 2019.

	Leaves	Main Stem	Branches + Petioles	Pods	Total biomass
			(p<F)		
R5					
Year	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Location	**<.0001**	**<.0001**	**0.001**	**<.0001**	**0.02**
MG	**0.04**	0.23	**<.0001**	**0.008**	**0.01**
Treatment	0.98	0.11	0.56	0.17	0.53
Year*Location	**0.003**	**<.0001**	0.26	**<.0001**	**<.0001**
Year*MG	0.77	**0.006**	*0.10*	*0.08*	0.61
Location*MG	**0.02**	**0.02**	0.27	*0.05*	0.39
Year* Treatment	0.70	0.30	0.25	0.20	0.78
Location* Treatment	0.21	0.64	0.20	0.44	0.36
MG* Treatment	0.6	0.42	0.33	0.61	0.71
Year*Location*MG	0.86	0.12	0.26	0.03	0.41
Year*Location* Treatment	0.12	0.86	0.18	0.76	0.69
Year*MG* Treatment	0.03	**0.0001**	0.29	0.34	0.01
Location*MG* Treatment	0.81	0.20	0.98	0.71	0.56
Year*Location*MG*Treatment	0.19	*0.08*	0.40	0.43	0.17

Table 2-8 cont.

	Leaves	Main Stem	Branches + Petioles	Pods	Total biomass
	(*p*<F)				
	R6				
Year	**<.0001**	**<.0001**	**0.0001**	**<.0001**	**<.0001**
Location	**<.0001**	**<.0001**	**<.0001**	**0.02**	**0.03**
MG	*0.06*	**0.002**	0.51	0.75	0.29
Treatment	0.20	**0.008**	0.45	0.26	0.11
Year*Location	**0.006**	**<.0001**	**<.0001**	0.36	0.34
Year*MG	**<.0001**	**0.004**	*0.05*	0.17	0.34
Location*MG	**<.0001**	**0.002**	**0.003**	*0.08*	0.11
Year* Treatment	0.52	0.13	0.10	0.20	0.43
Location* Treatment	**0.04**	*0.05*	0.28	0.14	*0.06*
MG* Treatment	0.59	0.25	0.95	0.70	0.83
Year*Location*MG	**<.0001**	**<.0001**	**0.04**	0.91	**0.03**
Year*Location* Treatment	0.58	0.60	0.88	0.69	0.70
Year*MG* Treatment	0.62	0.33	0.70	0.57	0.81
Location*MG* Treatment	0.75	0.70	0.49	0.69	0.76
Year*Location*MG*Treatment	0.24	0.23	0.12	0.66	0.30

Table 2-8 cont.

	Leaves	Main Stem	Branches + Petioles	Seeds / Pod Shells	Total biomass
	(kg ha⁻¹)				
	R7				
Year	**<.0001**	**<.0001**	**<.0001**	**<.0001/<.0001**	0.94
Location	**<.0001**	**<.0001**	0.12	**0.02/<.0001**	**<.0001**
MG	**0.007**	*0.06*	0.99	**<.0001**/0.18	0.93
Treatment	**<.0001**	0.35	0.36	**0.02**/0.11	0.15
Year*Location	0.78	**<.0001**	**<.0001**	0.62/**<.0001**	**<.0001**
Year*MG	**0.001**	**0.02**	*0.08*	0.45/0.66	**<.0001**
Location*MG	0.17	0.26	**0.03**	0.97/0.26	0.97
Year* Treatment	0.47	0.55	0.98	0.12/*0.08*	*0.09*
Location* Treatment	*0.05*	0.16	0.35	0.89/0.72	0.37
MG* Treatment	0.69	0.68	0.92	0.15/0.66	0.66
Year*Location*MG	0.78	0.79	0.46	0.69/0.24	0.63
Year*Location* Treatment	0.30	0.40	0.85	0.72/0.64	0.23
Year*MG* Treatment	0.52	0.38	0.98	0.53/0.12	0.50
Location*MG* Treatment	0.58	0.85	0.42	0.99/0.99	0.77
Year*Location*MG*Treatment	0.60	0.68	0.65	0.94/0.88	0.38

Table 2-9. Foliar application, effect on R5, R6, and R7 biomass weights in eastern SD in 2018.

	Leaves	Main Stem	Branches + Petioles	Pods; Seeds / Pod Shells	Total biomass
			(kg ha^{-1})		
			R5		
UTC	2051	1606	1343	2047	7047
Fungicide	2020	1444	1396	1852	6712
Insecticide	2031	1477	1435	2095	7038
Fungicide and Insecticide	2045	1528	1387	2156	7116
Foliar Fertilizer	1963	1331	1352	1678	6324
FFI[††]	1969	1519	1237	1770	6495
			R6		
UTC	1657ab[†]	1697	1254	4287	8895
Fungicide	1629ab	1519	1180	3895	8223
Insecticide	1606ab	1642	1213	4123	8584
Fungicide and Insecticide	1654ab	1642	1241	4192	8729
Foliar Fertilizer	1482b	1506	1123	3876	7987
FFI[††]	1720a	1797	1248	4258	9023
			R7		
UTC	206b	1192	694[*]	4431b	6523
Fungicide	348a	1236	800	4737ab	7121
Insecticide	203b	1333	717	4737ab	6990
Fungicide and Insecticide	284ab	1237	715	5026a	7262
Foliar Fertilizer	215b	1297	645	4916ab	7073
FFI[††]	325a	1258	714	4500b	6797

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

[††] Combination of foliar fertilizer, fungicides and insecticide application

[*] Branches and petiole fractions at R7 growth stage contained mainly branches, as petioles have fallen

Table 2-10. Foliar protection maturity group differences, and year by location interaction on leaves, stems, branches, pods, seeds, pod shells, and total biomass.

			Leaves	Stems	Branches	Pods; Seeds/Pod Shells	Total
R5	*MG*						
	MG1		1935b	1534a	1306b	1606a	6382b
	MG2		2030a	1529b	1510a	1599b	6669a
	Year x Location						
	2018	Brookings	2603a	2484a	1727a	1784b	8598a
	2019	Brookings	1597b	1196b	1192b	5057a	4756b
		SERF	1562b	847b	1040b	789b	4239b
R6	*MG*						
	MG1		1842a	1820a	1435b	4065a	9471a
	MG2		1797b	1690b	1470a	4012b	9348b
	Year x Location						
	2018	Brookings	2244a	3020a	1785a	5057a	12601a
	2019	Brookings	1530b	1282b	1259b	3287b	7656b
		SERF	1549b	940b	1138b	3157b	7072b
R7	*MG*						
	MG1		297b	1536a	750b	3521b/1653b	8540b
	MG2		328a	1453b	827a	3635a/1725a	8696a
	Year x Location						
	2018	Brookings	458a	2331a	848a	2169b/792b	7813b
	2019	Brookings	126c	1021b	632b	2945b/2311a	7587b
		SERF	317b	962b	652b	4354a/1760a	8024a

Table 2-11. Foliar application treatment effect on the relative biomass partitioning at the different growth stages across maturity groups and site-years in 2018 and 2019.

	Leaves	Main Stem	Branches and Petioles	Pods; Seeds/Pod Shells	Fallen Leaves
			%		
R5					
UTC	0.36	0.24	0.19	0.14	N/A
Fungicide	0.34	0.21	0.24	0.14	N/A
Insecticide	0.33	0.24	0.22	0.14	N/A
Fungicide and Insecticide	0.33	0.23	0.23	0.14	N/A
Foliar Fertilizer	0.32	0.21	0.22	0.17	N/A
FFI††	0.31	0.23	0.19	0.18	N/A
R6					
UTC	0.19	0.18	0.14	0.47	0.03ab
Fungicide	0.21	0.17	0.14	0.46	0.02ab
Insecticide	0.18	0.19	0.13	0.47	0.03ab
Fungicide and Insecticide	0.18	0.18	0.15	0.47	0.02b
Foliar Fertilizer	0.18	0.18	0.14	0.46	0.04a
FFI††	0.20	0.19	0.14	0.45	0.03ab
R7					
UTC	0.02	0.13	0.07	0.44/0.20	0.13
Fungicide	0.04	0.14	0.08	0.44/0.21	0.10
Insecticide	0.02	0.15	0.08	0.46/0.21	0.09
Fungicide and Insecticide	0.02	0.15	0.07	0.45/0.19	0.11
Foliar Fertilizer	0.04	0.12	0.06	0.45/0.23	0.12
FFI††	0.03	0.15	0.06	0.45/0.22	0.11

†† Combination of Fungicide, Insecticide, and Foliar Fertilizer

Table 2-12 Analysis of Variance (ANOVA) significance levels of pest and pathogen on foliar protection application, year, location, and maturity group (MG), and timing of rating (SBS, Septoria Brown Spot; SDS, Sudden Death Syndrome) in eastern SD in 2018 and 2019.

	Pest	SBS	SDS
Year	<.0001	<.0001	0.01
Location	<.0001	0.35	0.01
Treatment	<.0001	0.68	0.74
MG	<.0001	0.93	0.37
Timing	<.0001	<.0001	0.002
Year*Location	<.0001	*0.06*	0.02
Year*Treatment	<.0001	0.92	0.48
Location*Treatment	0.01	0.65	0.38
Year*MG	<.0001	0.63	0.35
Location*MG	0.30	0.48	0.36
Treatment*MG	<.0001	0.74	0.99
Year*Timing	<.0001	<.0001	0.002
Location*Timing	<.0001	0.02	0.002
Treatment*Timing	<.0001	0.94	0.85
MG*Timing	0.03	0.99	0.44
Year*Location*Treatment	<.0001	0.64	0.49
Year*Location*MG	0.33	0.69	0.38
Year*Treatment*MG	0.002	0.52	0.99
Location*Treatment*MG	*0.07*	0.27	0.99
Year*Location*Timing	<.0001	0.15	0.004
Year*Treatment*Timing	<.0001	0.94	0.53
Location*Treatment*Timing	0.74	0.80	0.38
Year*MG*Timing	0.02	0.80	0.42
Location*MG*Timing	0.77	0.62	0.43
Treatment*MG*Timing	0.23	0.89	1.00
Year*Location*Treatment*MG	0.15	0.82	0.99
Year*Location*Treatment*Timing	0.008	0.77	0.54
Year*Location*MG*Timing	0.83	0.90	0.46
Year*Treatment*MG*Timing	0.20	0.46	0.99
Location*Treatment*MG*Timing	0.68	0.52	0.99
Year*Location*Treatment*Timing	0.89	0.89	0.99

Table 2-13. Pest and pathogen disease (SBS Septoria Brown Spot, SDS Sudden Death Syndrome) ratings between Treatments, Timing of ratings, MG in Brookings and Beresford, SD in 2018 and 2019.

	Pest	SBS	SDS
Location			
Brookings	2.98a	4.51	0.89
SERF	2.35b	4.13	N/A
Timing			
Pre	1.05c	0.70b	N/A
14 DAA	2.98b	1.83b	N/A
28 DAA	3.97a	10.43a	1.33
Timing Treatments*			
0 DAA Control	1.13de	0.81b	0.41c
0 DAA Fungicide	1.03e	0.69b	0.34c
0 DAA Insecticide	1.03e	0.63b	0.31c
0 DAA Fungicide and Insecticide	1.06e	0.69b	0.34c
0 DAA Foliar Fertilizer	1.13de	0.69b	0.34c
0 DAA FFI††	0.91e	0.69b	0.34c
14 DAA Control	4.46ab	2.22b	2.06ab
14 DAA Fungicide	3.41b	1.84b	1.93ab
14 DAA Insecticide	1.81cde	2.06b	2.13ab
14 DAA Fungicide and Insecticide	1.91cde	1.63b	1.75b
14 DAA Foliar Fertilizer	4.34ab	1.84b	1.94ab
14 DAA FFI††	1.97cde	1.41b	1.81ab
28 DAA Control	5.69a	11.13a	2.4ab
28 DAA Fungicide	4.19ab	9.09a	2.22ab
28 DAA Insecticide	2.63cd	11.38a	2.66a
28 DAA Fungicide and Insecticide	2.94bc	9.13a	1.91ab
28 DAA Foliar Fertilizer	5.41a	12.44a	2.53a
28 DAA FFI††	3.00bc	9.43a	2.03ab
*Year*location*			
2018 Brookings	0.84c	5.90a	1.77a
2019 Brookings	5.12a	3.12b	0b
2019 SERF	3.20b	1.99b	0b

†† Combination of Fungicide, Insecticide, and Foliar Fertilizer

Figure 2-1. Daily minimum and maximum air temperature and precipitation amount near Brookings and Beresford, SD in 2018 and 2019.
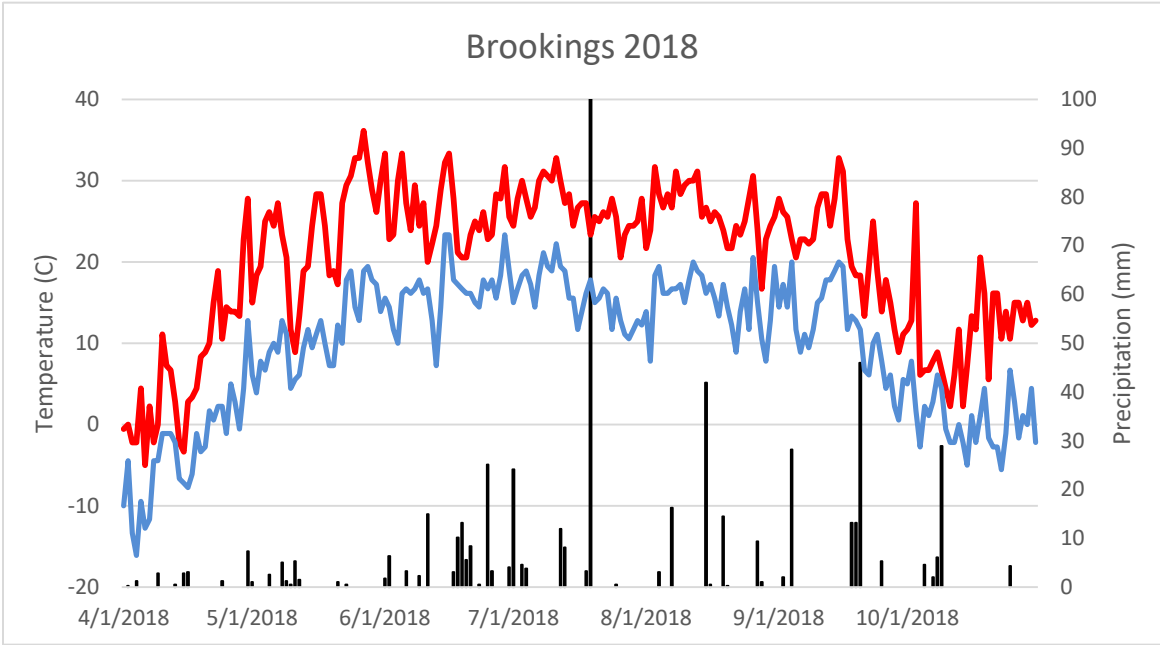
* 159.3mm precipitation on July 19th
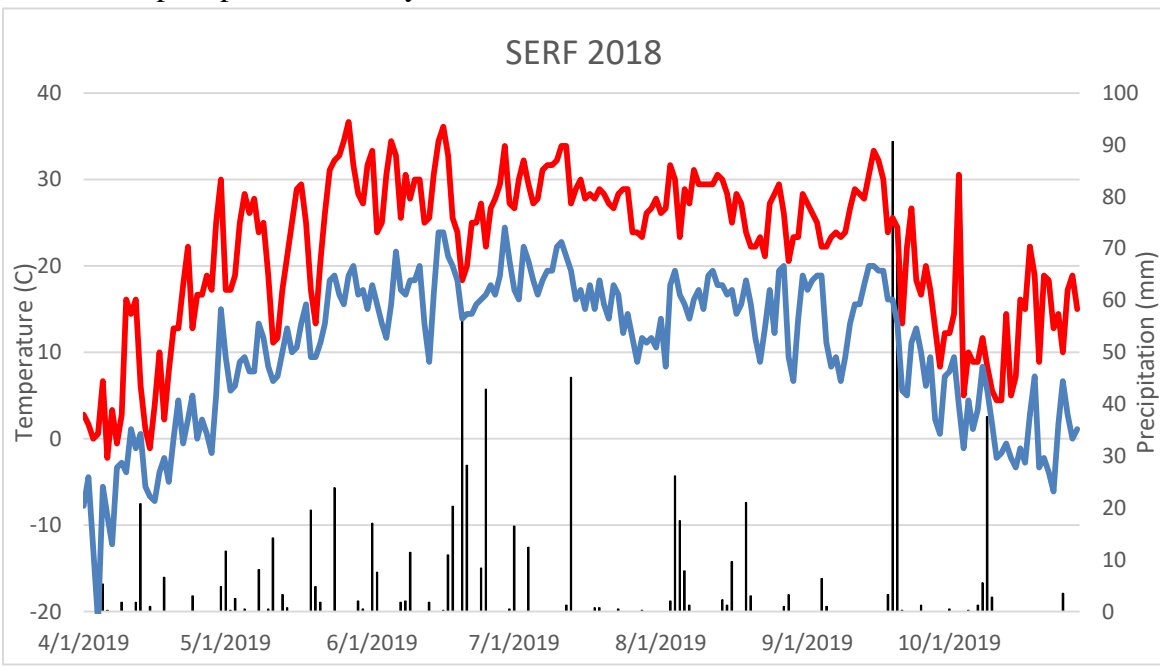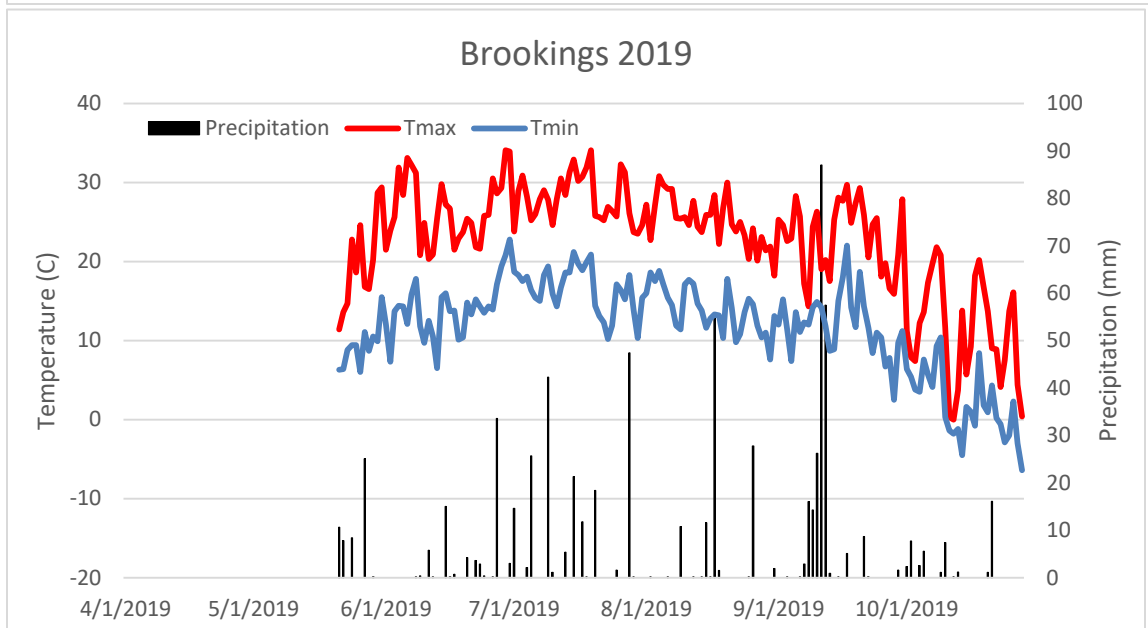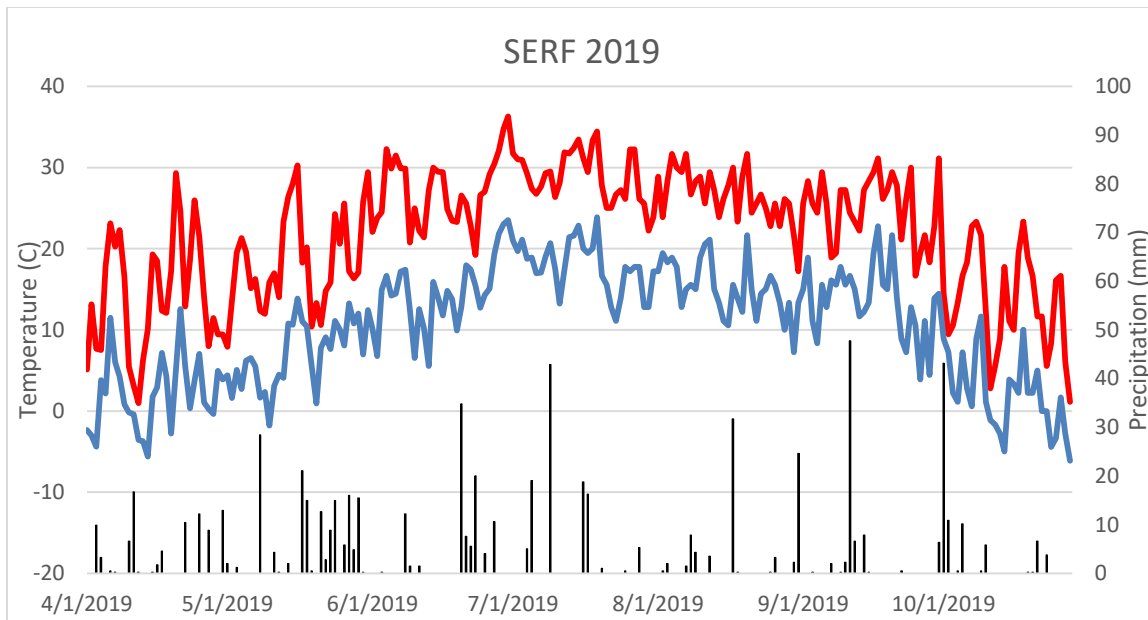
Figure 2-2. Foliar application, R5, R6, R7 biomass weights in eastern SD in 2018 and 2019.
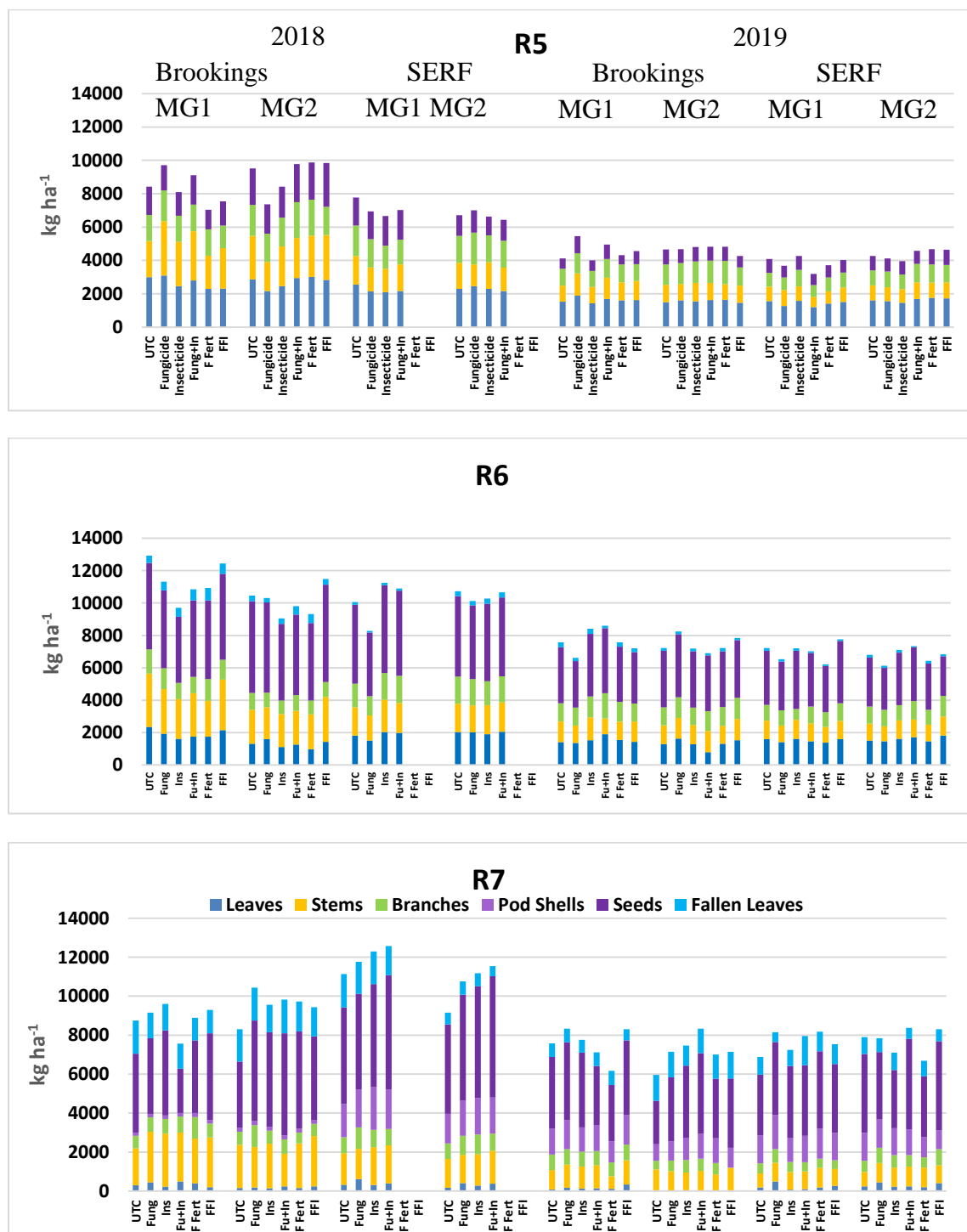
†† Combination of foliar fertilizer, fungicides and insecticide application

CHAPTER 3

NITROGEN AND SULFUR EFFECT ON SOYBEAN SEED COMPOSITION

3.1 INTRODUCTION

The amount of fixed N used by a plant is often largely dependent on N availability in the soil, with the plants utilizing available soil N prior to N gained from N fixation (Salvagiotti et al., 2009a). Soybeans fix about from 25 to 75 percent of their N according Salvagiotti et al. (2008), and from approximately two to three weeks after planting to pod fill (Flynn et al., 2015). Adding N fertilizer to the soybeans later during the growing season can aid with the needs of the crop when it is unable to fix enough for itself. Protein synthesis is the process of forming amino acids into proteins. Nitrogen is one of the major components of these amino acids that are the building blocks of proteins (Galili and Galili., 2008). Nitrogen and S applications during the growing season could help increase grain quality, therefore, applying N can cause the soybeans to be less stressed in the reproductive stages which may aid in the achievement of a higher seed protein percentage. Late application of N around R3 (beginning seed) tended to increase the percentage of protein in the seed, but yield was not affected (Wesley et al. 1997). In a study conducted using several cultivars with different maturity lengths, remobilization of N from the plant to the seed ranged from 30 to almost 100 percent, with more N being remobilized in southern regions due to a longer growing season (Zeiher et al., 1982). While there has not been a considerable amount of research done that focuses solely on the composition of soybeans; a study by Schmitt et al. (2001) showed a positive response in grain protein concentration when late season (R2-R5) N is applied. Available N in the soil is crucial during periods of peak N demand, such as grain fill period. "In general,

soybean yield correlates closely with total N in the plant because of the large amount of N accumulation in the seed" (Kuwahara 1986). Modern cultivars have a larger amount of biomass and N accumulation than older cultivars (Schmitt et al., 2001). Modern cultivars' nutrient uptake occurs longer during the grain fill period than older varieties (Kovács and Casteel, unpublished). While N composition of the soybean is important, there are additional S requirements needed for the soybean plant to reach its full potential. Sulfur is a constituent of key enzymes in N metabolism (Campbell, 1999; Friedrich and Schrader, 1978) and in legumes is associated with the enzymes that are responsible for biological N fixation (Benton and Peters, 2004). Sulfur is key in plant metabolism and part of essential amino acids in seed storage proteins (Losak et al., 2010; Sexton et al., 1998; Takahashi et al., 2011). Pulse applied ^{35}S research documented that more S translocated to the seed when taken up later, during seed development (Naeve et al., 2005). Salvagiotti et al. (2009b) showed a rise in N uptake in response to S addition that increased biomass production, via a larger radiation capture, which then increased soybean yield (Salvagiotti et al., 2009b).

Historically, S fertilization has not been a concern for most of the farmers due to enough sulfate (SO_4^{2-}) deposition from the environment for the crop to flourish. Typically, low organic matter and coarse textured soils were considered to be at risk for S deficiency (Brady and Weil, 1999). There has been a reduction in sulfur deposition because of the increase in control of S released from industry and the use of low S grade fertilizers (Scherer, 2001). With recent pollution reductions, research has found that applications of sulfur are needed in other soil conditions as well (Brady and Weil, 1999).

In addition, with the increased grain yield, S uptake also increased in modern soybean varieties (Kovács and Casteel, unpublished).

Nitrogen and S should be given in balanced doses to obtain optimum yield (Jamal et al., 2005). "In seeds of Ivy (*Hedera helix* L.), Obeso et al. (2012) showed an allometric relationship between S concentration and seed mass. Isometric variations between seed mass and N concentration in Ivy seeds suggest allometric relationships between N and S. In soybeans Salvagiotti et al., (2012) found that for a given amount of N accumulated in seeds, S deficient soybeans tended to accumulate 12% less S in seeds than soybeans with no S limitations.

Protein quality can change: "If increased demand for S-containing amino acids within the seed did create a source limitation in S uptake or reduction, then either protein yield would drop to maintain quality, or the seed would synthesize greater amounts of poorer quality protein than expected" (Sexton et al., 1998). Not only is increasing protein levels important, but maintaining the quality of the protein is important when considering fertilizing soybeans.

The objectives of this study were to 1) determine the effects of N and S application rate and timing on grain yield and protein levels in soybean seeds in different maturity group varieties, and 2) determine the effect of planting dates.

3.2 MATERIAL AND METHODS

3.2.1 Site description and experimental design

This study was conducted at two of the South Dakota State University Research Farms; near Brookings (44.3114° N, 96.7984° W), and at the Southeast Research Farm (SERF; 43.0805° N, 96.7737° W) near Beresford, SD. The soil types were Divide (fine-loamy over sandy, mixed, superactive, frigid Aeric Calciaquolls), and Egan-Wentworth complex, which is a fine-silty, mixed, superactive, mesic Udic Haplustolls at Brookings and Egan-Clarno-Tetonka complex, which is a fine-loamy/fine-silty, mixed, superactive, mesic Typic Haplustolls; Egan-Clarno-Trent complex, which is a fine-silty, mixed, superactive, mesic Udic Haplustolls at SERF in 2018 and 2019, repsectively.

Two soybean varieties, AG11X8, AG24X7 (maturity group 1.1 and 2.4, respectively, AsGrow Seed Co LLC, St. Kalamazoo, MI) were included in this research. Different N S fertilizer application timings were compared in each variety: untreated control, application at planting, application at V4 (four trifoliate), at R3 (beginning pod), or at V4 and R3 growth stages. Assigned plots received 11 kg S ha^{-1} and 44.8 kg N ha^{-1} at each application timing. Sulfur was applied as ammonium-sulfate (AMS; 21-0-0-24S) and urea (46-0-0) was used to supplement the remainder N at pre-plant and V4 growth stage. These applications were broadcast applied by hand. The R3 applications were done with a one nozzle wand next to the base of the plant with AMS and Urea-ammonium nitrate (UAN 28-0-0) at a total of 140 L ha^{-1} application rate. At the Brookings sites an additional, delayed, second planting date was also included. Treatments were arranged in a randomized complete block design at SERF and with split-plot arrangement in Brookings, with 4 replications at each location. At the Brookings site the main plot was the planting date and sub plots were the fertilizer application timing and soybean maturity group combinations.

Corn (*Zea mays L.*) was the preceding crop at both locations in 2019 and the Brookings site in 2018. These sites utilized conventional tillage practices and prior to planting fields were field cultivated, while at Beresford in 2018 soybean were planted in no-till ground following oats (*Avena sativa* L.). In 2019, soybeans at the Beresford site required replanting due to very poor plant stand caused by wet weather conditions during planting and emergence window. All plots were maintained weed free. Soybean varieties were planted at 346,000 seeds ha^{-1} in 76-cm rows; the planting dates for each sites are presented in Table 3-1. Plot sizes were 3m x 18.3m in Brookings in 2018 and 4.5m x 13.7m at SERF both years and Brookings in 2019.

3.2.2 Data collection

Before planting, soil samples were taken from each replication (15 cores of 2 cm diameter in each replication) and separated to 0-15cm, 15-60cm; soil samples were air dried with forced air then ground to pass through a 2 mm sieve, and sent off to a certified commercial laboratory (AgSource Laboratories, Lincoln, NE) to determine nutrient concentrations, soil pH and organic matter (OM). The laboratory used the 1:1 soil-water slurry method for soil pH, the loss of ignition method for OM, the cadmium reduction method for NO_3^--N and the monocalcium phosphate method for SO_4^{2-}-S concentration determination (AgSource Laboratories, 2020).

Pre-plant fertilizer application was done on the same days as planting. The in-season fertilizer application times presented in Table 3-1.

After emergence (around V3 growth stage) and at physiological maturity, plant population was estimated through stand counts at four locations within each plot. The

number of plants were counted on one meter length of a non-border row and plant population were calculated from it.

Most recent matured trifoliate leaf (MRML) samples were taken from 25 plants in each plot in Brookings and at the SERF in July and August in 2018 (Table 3-1). Samples were dried then ground to pass through a 1 mm sieve, and analyzed by a certified commercial laboratory (AgSource Laboratories, Lincoln, NE) to determine nutrient concentrations. Nitrogen concentration was determined by the Kjeldahl method, P, K, Mg, Ca, S, Zn, Mn, Cu, Fe, B, Al concentrations were determined by the inductively coupled argon plasma spectrometry method (AOAC International, 2000; AgSource Laboratories, 2020). At R5 (beginning seed), R6 (full seed), and R7 (beginning maturity) growth stages biomass samples were collected from a 0.5m section of a non-border row. Sampling times are presented in Table 3-1. The R5 and R6 samples were partitioned into leaves, stems, branches (including petioles), pods, and fallen leaves. The R7 samples were partitioned into leaves, stems, branches (including petioles), pod shells, seeds, and fallen leaves. Fallen leaves were any leaves or petioles that fell in the area biomass was collected from 0.38 m each side of the sampled row. All samples were dried at 60 degrees Celsius until constant weight then weighed.

Grain harvest was done in late October (Table 3-1). The middle two rows being harvested with a Massey Ferguson 8XP plot combine. Grain weight, grain moisture and test weight readings were recorded. Plot lengths were measured to determine grain yield. Grain protein and oil concentrations were determined with InfraTec Nova (FOSS Analytics, Hillerød, Denmark) instrument. Seed mass (200-seed weight) were measured

by weighing grain samples collected from the harvested plots. Grain yield, grain protein and oil, and the 200 seed weight data was adjusted to 130 g kg^{-1} moisture content.

3.2.3 Statistical analysis

Results were then analyzed in R Studio and interpreted using ANOVA and Fishers-LSD at 0.05 significance level. The two site years from SERF, and the first planting dates at the Brookings site were analyzed together for treatment differences. Further ANOVA analysis were carried out for the Brookings sties, where both planting dates from both year have been included in the analysis to compare the effect of planting dates. All treatment factors (fertilizer application timing, MG, year, location, and planting date) were considered fixed factors.

3.3 RESULTS

3.3.1 Weather

These experiments located side by side with the experiments presented in Chapter 2. Detailed information about weather conditions (daily and monthly minimum and maximum air temperature, and daily and monthly total precipitation) were described in Chapter 2.3.1.

3.3.2 Field Characterization

Preplant soil sample tests indicated soil pH levels between 6.1 and 6.9, and between 6.2 and 7.5, while OM levels ranged between 3.0% and 4.8%, and between 2.6% and 3.6%; in the 0-15cm and 15-60cm soil depths, respectively, among the experimental sites (Table 3-2). Soil nutrient levels ranged between 3.3ppm and 19.3ppm and between 2.8ppm and 8.0 ppm for NO_3^--N, and nutrient levels were between 3.0ppm and 12.3ppm

and between 2.5ppm and 8.5ppm for SO_4^{2-}–S in the 0-15cm and 15-60cm soil layers, respectively, among the sites (Table 3-2).

3.3.3 Stand Counts

When comparing SERF and Brookings, neither early season nor final plant stands differed among the fertilizer application treatments (Table 3-3). Early season plant stand differed only between the locations and the MGs (Table 3-3). Plant stands were approximately 12,400 plants ha^{-1} larger in MG2 compared to MG1 when plots were averaged across locations, growing seasons and fertilizer treatments. Early season plant stands were approximately 24,700 plants ha^{-1} higher at the Brookings site relative to the SERF site averaged across the fertilizer treatments, MGs, and growing season (Table 3-4). Final plant stands varied between the MGs and by the year by location interaction (Table 3-3). Maturity group 1 variety had nearly 10,000 plants ha^{-1} larger final plant stand than MG2 varieties across fertilizer application timings, growing seasons and locations, while final plant stand was 30,000 plants ha^{-1} larger in 2019 than in 2018 averaged across fertilizer application timings, MGs, and locations (Table 3-4).

When comparing the two planting dates at Brookings, larger plant population were established with the second planting date compared to the first planting date (Tables 3-6 and 3-7); plant population differed by 30,000 plant ha^{-1} at the early stand counts and by 10,000 plants ha^{-1} for the harvest stand counts averaged across the fertilizer applications, MGs and growing seasons. Plant stands in 2019 were higher (by about 10,000 plants ha^{-1} for the early season estimation and by 60,000 plants ha^{-1} in the harvest stand counts) when averaged across fertilizer treatments, MGs, and planting dates (Table 3-6 and 3-7).

3.3.4 Grain Yield, Protein and Oil concentrations

When comparing across locations, as shown in Table 3-3, statistical analysis showed that there were no significant differences in grain yield among nutrient application timings across locations. However, the year by location interaction (Table 3-3) revealed that grain yield was nearly 0.9 Mg ha^{-1} higher at SERF in 2018 compared to the Brookings site averaged across fertilizer treatments and MGs (Table 3-5). While numerically 0.35 Mg ha^{-1} higher but statistically not different yield was achieved in the same comparison in 2019 (Table 3-5). When we look at the location by MG interaction (Table 3-3), MGs did not differ within a location averaged across the fertilizer application timings and growing seasons, but numerically MG2 varieties had higher yield at Brookings, while MG1 varieties achieved numerically higher yield at the SERF site (Table 3-5). The SERF site in 2018 had the highest grain yield and grain oil concentration (Table 3-4). For grain protein, the Brookings site had nearly 1% higher grain protein concentration compared to the SERF site in 2018, while in 2019 grain protein was numerically higher at SERF (by about 0.45%) but not statistically different between the sites (Table 3-5). Grain protein concentration ranged from 33.6-36.2 percent, in both locations (Tables 3-5. The year by location by MG interaction for grain oil concentration (Table 3-3) indicated that grain oil concentration were higher at the SERF site in 2018 compared to the Brookings location and MGs did not differ, while in 2019 MG2 varieties had higher grain oil concentration by about 0.5% in both locations (Table 3-5). As shown on Table 3-4, grain oil concentration were higher by about 0.15% in V4+R3 fertilizer treatment than the pre-plant application, but neither of these treatments were different from the untreated check and other fertilizer application timings.

When comparing the planting dates at the Brookings site, fertilizer application timing treatments were also not different for grain yield, protein, or oil concentrations (Table 3-6). Only when looked at on the 0.1 significance level is fertilizer application timing impacted grain protein concentration (Table 3-6). The year by planting date interaction indicated a 0.55 Mg ha^{-1} yield advantage for early planting in 2018 while there were no differences among the planting dates in 2019 averaged across the fertilizer timing and MGs (Tables 3-6 and 3-8). The year by fertilizer application interaction showed no yield differences between the growing seasons when N and S fertilizer was applied, but the non-treated control had 0.45 Mg ha^{-1} lower yield in 2019 averaged across the MGs and planting dates (Table 3-8). In 2019, the first planting date yielded higher grain oil concentration than any other planting dates in the two years. Grain yield and protein concentrations were the highest in 2018 with the first planting date (Table 3-8). Grain protein concentration ranged from 33.8-36.0 percent, over both planting dates (Tables 3-8). In 2018, grain protein concentration was higher in the first planting date; while in 2019, it was higher in the second planting date (Table 3-8).

3.3.5 Biomass

When comparing the two locations, biomass partitions did not differ due to fertilizer application timing treatments at either growth stages across the four site-years (Table 3-9 and 3-10). Branches and petioles were collected together and could be why we see a dramatic decrease (1722 to 817kg ha^{-1}) from R6 to R7 biomass weights (Table 3-10). While Brookings was similar to SERF in leaves and pods biomass at R5 and R6; there was significantly higher (~500 kg ha^{-1} in R5 ~1000 kg ha^{-1} R6) stems and branches biomass in the Brookings site than at the SERF site (Figure 3-1). Total biomass in 2019

was lower (more than 1000 kg ha^{-1}) than in 2018. The biomass partitioning was also

calculated as the relative proportion of the biomass accumulation for each treatment

(Table 3-11) to see if there were difference between the biomass allocation relative to the

total biomass accumulation. Results indicated that even though total biomass

accumulation may had differed, the proportion of biomass allocation within the plant was

not significant (Table 3-11).

When comparing the planting dates, biomass accumulation was influenced by the

year by planting date interaction (Table 3-12). At the R6 growth stage, in 2018 the

second planting date accumulated less (~1000 kg ha^{-1}) total biomass than the first planting

date (Table 3-13, Figure 3-2). Overall, in 2019 there were lower biomass accumulation

then in 2018. The biomass partitioning was also calculated as the relative proportion of

the biomass accumulation for each treatment (Table 3-14).There were no noticeable

differences between the planting dates on biomass partitioning in this study (data not

shown).

3.3.6 Most Recent Matured Leaf

Results from the MRML samples in 2018 when comparing the fertilizer

application timings showed differences between N and S concentrations (at the 0.10

significance level (Table 3-15). Sulfur tissue concentration was also higher at the SERF

site by 0.03% (Table 3-15). Nitrogen concentration in the control treatments were lower

than the fertilized plots averaged across MGs, locations, and growing seasons (Table 3-

15).

When comparing the planting dates at Brookings, planting date influenced the leaf K nutrient concentration (p=0.06; Table 3-16). Only the S nutrient concentrations varied due to fertilizer application timings, which was also significant at the 0.10 significance levels averaged across planting dates, and MGs (Table 3-17). Nitrogen concentration in the unfertilized treatments (control and R3) were numerically lower than the fertilized plots (Table 3-16).

3.4 DISCUSSION

3.4.1 Field Characteristics

The pre-season soil tests taken are shown in Table 3-2 to help show there were adequate pre-season nutrients in the field which can be the reason why we did not see a large increase in yield from applying nitrogen and sulfur throughout the growing season. If fertilizing with N and S would improve grain quality to the field that were not lacking proper amounts in the soil, then we could assume N and S increase protein levels.

3.4.2 Grain yield, and quality

Location effect on yield is related to environmental factors such as the higher early season precipitation and warmer temperatures (Rowntree et al., 2013a) during the 2018 growing season at the SERF location. Brookings received a one-time large amount of rain (nearly 160 mm on July 19), which caused substantial lodging in those plots. Lower grain yield with delayed planting is well documented (De Bruin and Pedersen, 2008), which is caused by shorter time for the plant to grow before it starts the reproductive growth and reaches maturity. Later planted soybeans also produce a lower number of nodes per plant (Specht, 2007). The nodes are where the soybeans produce its

pods so with fewer pods will cause lower yield. Delayed planting of long or longer maturity group varieties increase the chances that the soybeans will not fully mature before the first frost event. In 2018, the first frost occurred before the MG2 variety fully matured (October 4, Figure 1-2) causing the lower grain yields and protein levels for those treatments compared to the late planted shorter variety or the treatments planted at the optimal planting time (first planting date). The early frost disrupts photosynthesis, and grain filling which led these MG2 variety treatments with lower yields and lower protein concentrations even though later planting is said to have lower yield and higher protein concentration (Jaureguy et al., 2013, Rowntree et al, 2013a).

Contrary to some research (Kane et al., 1997; Robison et al., 2009), seed protein concentration did not increase within the maturity group when planted in June rather than in May (Pedersen and Lauer, 2003). Grain oil concentration on the other hand, had a larger concentration in MG2 variety than in the MG1 variety regardless of the fertilizer application timing. The pre-plant fertilizer application lowered grain oil concentration compared to the control treatments.

In Brookings 2019, lower levels of residual NO_3^--N and SO_4^{2-}-S were found in the soil at the pre-plant sampling time (Table 3-2) which would also considered to be close to low levels according to the current recommendation guidance (Clark, 2020). This could attribute to the lower amounts of protein in these treatments, even though they were planted later which is supposed to increase grain protein concentration (Jaureguy et al., 2013, Rowntree et al., 2013a).

While plant stand was not a problem in this study, making sure that your planter is properly calibrated will help ensure that the field is planted uniformly and that it will not affect yield. Yield and grain quality can also be impacted by maturity groups and planting dates.

Selecting the right maturity group or variety can help increase the productivity of the soybeans in the field. Selecting the right maturity group also depends on when you are planting. Planting dates can affect soybean germination. Soybeans need a soil temperature of at least 10 °C (Hall et al., 2012) in order for them to germinate meaning that if they are planted too early they will have problems with germination which will lead to an uneven stand. This was also observed through higher early season plant stand when planting was delayed and temperatures were also warmer (Table 3-7). The longer the planting is delayed, the shorter the maturity group for the area should be planted. Planting dates that crop insurance covers in South Dakota range from April 25th to June 10th, and planting before or after this time frame it is not recommended. In 2019, the second planting date at Brookings was planted three days after what would been covered by crop insurance. While all of the others were within the dates of crop insurance. As in the Assefa et al. (2019) article, the two locations were located between the 40 and 45 degree latitude, which showed a decline in oil concentration and protein concentrations as planting date was delayed. Maturity group selection is also easy to control to help insure the best results from the field.

3.4.3 Nutrients and Plant Biomass

Some of the biomass weights differences occurred because timing of sample collection may not have happened at the exact plant developmental stages desired due to

the large amount of plots that needed to be collected and partitioned during the grain filling period. Both maturity groups' samples at the R5 growth stage were collected at the same time while in later stages they were collected separately when the different MGs reached the target growth stage. The same sampling time can explain biomass accumulation differences between MGs at the R5 growth stage. There were more distinct time separation between locations and maturity groups as the grain filling period progressed. Node number could have also affected the pods per plant from the two years. Planting occurred earlier in the season in 2018 and planting date one had a longer time before the summer solstice, this leads to a longer amount of time that the plant can grow and produce nodes. Higher numbers of nodes likely lead to a larger number of pods per plant. This could be why there was higher grain yield in 2018.

Some of the treatments that are higher in Figure 2-1 and 2-2 are due to a lower number of plants collected (1 or 2 plants). When there were less plants collected, the soybean plants had more branches. These plants also tended to have more leaves due to the number of branches.

Due to all of the rain received during the growing season, the nitrogen that was plant available may have leached rather than being utilized by the plant. Nitrogen is not normally applied to soybeans due to the fact that soybeans are legumes. This means that they produce their own N through nodules. Abendroth et al. (2006) and Tein et al. (2002) found that soybeans normally acquire between 60 and 89 percent of their total N uptake from N-fixation. Early season N application can interfere with nodule formation on the plant when large amounts of N are applied due to adequate amounts of available N in the soil. Soybeans use the available soil N before initiating nodule formation and N fixation.

This could lead to lower amounts of N fixation, especially later in the growing season, when early season N (from fertilizer application or from mineralization) has been utilized by the plant or leached away.

Sulfur in the past was not a problem due to the amount of S deposition from the atmosphere. Now with emission restrictions, S deficiencies and response to S application have become more prevalent (Scherer, 2001). Sulfur levels are also not typically a problem in South Dakota unless weather is cool and wet, or when production is on coarse textured soils or on low organic matter soil (Gelderman et al., 2000). Soil pre-plant S levels were sufficient according to current South Dakota recommendation guidance (Clark, 2020) which helps to explain why we did not see grain yield response when applying sulfur during the season similarly to the observations of Flavio et al. (2007).

Future research will have to be conducted too further investigate if different rates or different types of N, and S, and their ratios will increase the amount of grain protein found in the soybean seed.

3.5 CONCLUSION

When looking at both locations and both planting dates in Brookings, there might have been more significant differences in protein and yield levels had the fields that were in the study were deficient in N and S. While N and S application timings did not seem to affect fields with adequate amounts of nutrients in the soil it could potentially increase if the field was known to have these deficiencies. In this study application of supplemental N and S fertilizers did not improve grain yield or protein concentrations on fields that were already sufficient in these nutrients. While Brookings in 2019 had the lowest

amount of N and S in the pre-season soil samples, applications of N and S did not affect grain yield or quality. In the future, looking for nodules on the roots and taking soil samples after harvest would be a way to find out how these applications influenced the soybean.

Table 3-1.Experimental design, used varieties and other crop production parameters, along with planting, treatment application, biomass and machine harvest dates.

Field activities	2018 Brookings	SERF	2019 Brookings	SERF
Varieties	AG11X8 and AG24X7			
Row spacing	0.76m			
Replications	4			
Seeding Rate	346,000 viable seeds ha^{-1}			
Plot dimensions	3m x 18.3m		4.5m x 13.7m	
Tillage	Conventional	No-till	Conventional	
Rotation	Corn-Soybeans	Oat-Soybean	Corn-Soybeans	
Experimental Design	Split Plot Design	RCBD	Split Plot Design	RCBD
Planting Date	May 15†, June 4	May 17	June 3, June 13	June 8
Pre plant application	May 15, June 4	May 17	June 3, June 13	June 8
V4 application	June 18 July 9	June 19	July 8 July 15	July 15
R3 application	July13 Aug. 8	July 10	July 25 Aug 7	July 25
MRML	July 9 Aug. 1	July 10	July 18 July 26	July 18
R5 Biomass	Aug. 10, Aug. 13, Aug. 27	Aug. 6	Aug. 13, Aug. 20, 22	Aug. 15, 19
R6 Biomass	Aug.31, Sep.7, Sep. 14	Aug. 20, 23	Aug. 29, Sep.10, Aug. 29, Sep.10	Sep. 3, 5
R7 Biomass	Sep. 11, 17, Sep. 24, Oct. 1	Sep.5,12	Sep. 23,Oct. 4, Sep.23, Oct. 4	Sep. 23, 30
R8 Biomass	Oct. 15	Oct. 5	Oct. 9, Oct. 15, 16	Oct. 11
Harvest Date	Oct. 19-20	Oct. 18	Oct. 20	Oct. 18

† multiple dates in the line for a field activity indicate that the MG1 and MG2 varieties have been sampled separately on the days indicated for all experimental site, while different date in different lines for a field activity indicate the sampling time differences for the different planting dates at the Brookings site.

Table 3-2. Pre plant field characteristics of soil pH, organic matter (OM), and soil nitrate-N (NO$_3^-$-N), and sulfate-S (SO$_4^{2-}$-S) concentration in the 0-15cm and 15-60cm soil zones in 2018 and 2019 near Brookings and Beresford, SD.

	Soil depth (cm)	pH	OM (%)	NO$_3^-$-N (ppm)	SO$_4^{2-}$-S (ppm)
2018 Brookings	0-15	6.13 ± 0.53	4.73 ± 0.57	19.25 ± 4.3	12.25 ± 1.69
	15-60	6.25 ± 0.12	3.63 ± 0.25	7.75 ± 0.41	8.50 ± 0.57
2018 SERF	0-15	6.63 ± 0.79	3.40 ± 0.57	7.50 ± 2.52	8.00 ± 2 .16
	15-60	7.18 ± 0.64	2.98 ± 0.52	5.50 ± 1.91	12.00 ± 3.74
2019 Brookings	0-15	6.53 ± 0.24	3.95 ± 0.17	3.25 ± 1.26	3.00 ± 0.00
	15-60	7.25 ± 0.31	2.80 ± 0.47	2.75 ± 0.50	2.50 ± 0.58
2019 SERF	0-15	6.88 ± 0.70	2.95 ± 0.31	6.00 ± 2.45	5.25 ± 1.50
	15-60	7.48 ± 0.56	2.60 ± 0.36	8.00 ± 4.32	6.50 ± 4.36

Table 3-3. Analysis of Variance (ANOVA) significance levels of nitrogen and sulfur application, year, location, and maturity group (MG) main effects and their interactions on early-season and final plant stand, grain yield, and grain protein and oil concentrations in eastern SD in 2018 and 2019.

	Plant Stand		Grain Yield	Grain Protein concentration	Grain Oil concentration
	Early	Late			
Year	*0.08*	<.0001	<.0001	<.0001	**0.03**
Location	**<.0001**	0.18	**<.0001**	**<.0001**	**<.0001**
MG	**<.0001**	**<.0001**	*0.07*	**<.0001**	**<.0001**
Treatment	0.67	0.26	0.70	0.15	**<.0001**
Year*Location	0.23	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Year*MG	0.27	0.15	0.24	**0.04**	**<.0001**
Location*MG	0.16	*0.05*	**0.0005**	**<.0001**	**<.0001**
Year* Treatment	0.74	0.68	0.14	*0.06*	0.64
Location* Treatment	0.37	0.69	0.79	0.52	0.31
MG* Treatment	0.20	0.64	0.81	0.73	0.95
Year*Location*MG	0.14	**0.03**	0.84	0.65	**0.01**
Year*Location* Treatment	0.68	0.89	0.79	0.42	0.97
Year*MG* Treatment	0.17	0.29	0.79	0.75	0.50
Location*MG* Treatment	0.61	0.50	0.70	0.96	0.85
Year*Location*MG*Treatment	0.37	0.40	0.83	0.39	0.40

Table 3-4. Main treatment effect on early season and final plant stands in 2018 and 2019 near Brookings and Beresford, SD

	Early Stand	Final Stand	Yield	Protein	Oil
	(plants ha^{-1})		Mg ha^{-1}	%	
UTC[†]	245,600	244,900	4.00	34.74	18.22ab[††]
Pre	257,700	240,900	4.01	34.82	18.04b
V4	250,800	233,800	3.98	34.71	18.13ab
R3	248,300	230,800	4.09	34.87	18.13ab
V4+R3	252,300	241,900	4.05	34.67	18.22a
MG1	263,200b	246,900a	4.09b	34.6b	18.0
MG2	275,800a	237,200b	4.33a	35.0a	18.3
2018	255,500b[†††]	221,200b	4.54a	35.4a	18.2
2019	283,400a	255,700a	3.53b	34.1b	18.1
Brookings	263,400a	235,500	3.73b	34.9a	17.9b
SERF	238,500b	241,400	4.40a	34.7b	18.3a

[†]Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at pre-plant;V4, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at V4 growth stage; R3,11 kg S ha^{-1} and 44.8 kg N ha^{-1}fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at both V4 and R3 growth stage
[††] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level
[†††] different lower-case letters indicate statistically different results within a column at p=0.10 confidence level

Table 3-5. Nitrogen and Sulfur study; Year by location, and location by maturity group interaction effects on grain yield grain protein concentrations, and the year by location by maturity group interaction effects on grain oil concentration near Brookings, SD and Beresford, SD in 2018 and 2019.

Year	Location	Maturity group	Grain Yield (Mg ha^{-1})	Protein	Oil (%)
2018	Brookings		4.10b	35.96a	17.83b
	SERF		4.97a	34.91b	18.51a
2019	Brookings		3.35c	33.83c	18.13b
	SERF		3.71bc	34.42bc	18.12b
	Brookings	MG1	3.68b	34.88a	17.83c
		MG2	3.77b	34.91a	18.13b
	SERF	MG1	4.49a	34.31b	18.25ab
		MG2	4.19a	35.02a	18.38a
2018	Brookings	MG1			17.78b
		MG2			17.88b
	SERF	MG1			18.66a
		MG2			18.36a
2019	Brookings	MG1			17.88b
		MG2			18.38a
	SERF	MG1			17.84b
		MG2			18.40a

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

Table 3-6. Analysis of Variance (ANOVA) significance levels of foliar protection application, year, location, planting date (PDate), and maturity group (MG) main effects and their interactions on early-season and final plant stand, grain yield, and grain protein and oil concentrations in the planting date comparison near Brookings, SD in 2018 and 2019.

	Plant Stand		Grain Yield	Grain Protein concentration	Grain Oil concentration
	Early	Late			
Year	0.02	<.0001	0.0008	<.0001	0.0002
PDate	<.0001	0.003	<.0001	0.13	0.02
MG	0.13	0.19	0.14	0.003	<.0001
Treatment	0.49	0.48	0.48	*0.07*	0.13
Year*PDate	<.0001	0.83	<.0001	<.0001	0.005
Year*MG	0.59	0.002	0.007	0.48	<.0001
Year*Treatment	0.01	0.65	0.005	0.0003	<.0001
PDate*MG	0.55	*0.10*	0.14	0.31	0.99
PDate*Treatment	0.38	*0.05*	0.43	0.28	0.19
MG*Treatment	0.50	0.78	0.97	0.86	0.91
Year*Pdate*MG	0.31	0.59	*0.05*	0.04	0.04
Year*Pdate*Treatment	0.95	0.28	0.34	0.97	0.95
Year*MG*Treatment	0.30	0.007	0.51	0.66	0.50
PDate*MG*Treatment	0.11	0.46	0.46	0.90	0.98
Year*Pdate*MG*Treatment	0.48	0.75	0.54	0.53	0.55

Table 3-7. Effect on early season and final plant stands in 2018 and 2019 near Brookings, SD

| | | Stand counts | |
| | | Early | Final |
		(plants ha^{-1})	
Year			
2018		273,900b[†]	210,800b[†]
2019		283,300a	269,800a
Planting date			
Pdate 1		263,500b	235,400b
Pdate 2		293,800a	245,200a
*Year*Planting Date*			
2018	Pdate 1	271,300bc[††]	
	Pdate 2	276,500b	
2019	Pdate 1	255,700c	
	Pdate 2	311,000a	
*Year*Treatment*			
2018	UTC[†††]	272,900bc	
	Pre	275,000bc	
	V4	276,600bc	
	R3	275,200bc	
	V4+R3	269,800c	
2019	UTC[†††]	271,700bc	
	Pre	289,300a	
	V4	289,700a	
	R3	277,800b	
	V4+R3	288,100a	

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

[††] different lower-case letters indicate statistically different results within a column at p=0.10 confidence level

[†††]Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at pre-plant;V4, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at V4 growth stage; R3,11 kg S ha^{-1} and 44.8 kg N ha^{-1}fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at both V4 and R3 growth stage

Table 3-8. Nitrogen and Sulfur application timing and planting date effect on grain yield, protein and oil concentrations in Brookings SD in 2018 and 2019.

		Yield	Protein	Oil
		Mg/ha^{-1}	%	
Year x Treatment				
2018	**UTC**[††]	3.70a[†]	35.8a	18.0ab
	Pre-plant	3.42ab	36.0a	17.9ab
	V4	3.39ab	35.8a	18.0ab
	R3	3.68a	35.9a	18.0ab
	V4+R3	3.71a	35.7a	18.1a
2019	**UTC**	3.25b	34.2b	17.8ab
	Pre-plant	3.37ab	34.0bc	17.7b
	V4	3.39ab	33.8c	17.9ab
	R3	3.33ab	34.2b	17.9ab
	V4+R3	3.40ab	33.9bc	17.9ab
*Year*Pdate*				
2018	**PDate1**	4.10a	36.0a	17.8b
	PDate2	3.05c	35.7b	17.9b
2019	**PDate1**	3.34b	33.8d	18.1a
	PDate2	3.36b	34.2c	17.9b

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

[††]Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at pre-plant;V4, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at V4 growth stage; R3,11 kg S ha^{-1} and 44.8 kg N ha^{-1}fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at both V4 and R3 growth stage

Table 3-9 Analysis of Variance (ANOVA) significance levels of N and S application, year, location, and maturity group (MG) main effects and their interactions on biomass in eastern SD in 2018 and 2019.

	Leaves	Main Stem	Branches + Petioles	Pods; Seeds/ Pod Shells	Total Biomass
			R5		
Year	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Location	**0.003**	**<.0001**	0.75	0.6	**<.0007**
MG	0.37	0.63	**<.0001**	**<.0001**	0.70
Treatment	0.39	0.8	0.19	0.66	0.69
Year*Location	**0.002**	0.41	0.21	0.18	0.65
Year*MG	0.25	0.22	**0.03**	**<.0001**	0.15
Location*MG	0.89	0.57	*0.05*	**<.0001**	*0.10*
Year* Treatment	0.87	0.61	0.94	0.42	0.77
Location* Treatment	0.6	0.41	0.62	0.99	0.28
MG* Treatment	0.28	0.34	0.5	0.53	0.41
Year*Location*MG	0.17	*0.07*	0.16	**<.0001**	0.72
Year*Location* Treatment	0.87	0.74	0.93	0.68	0.87
Year*MG* Treatment	0.71	0.74	0.88	0.79	0.65
Location*MG*Treatment	0.51	0.29	0.81	0.82	0.82
Year*Location*MG*Treatment	0.81	0.5	0.57	0.49	0.88
			R6		
Year	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Location	**<.0001**	**<.0001**	**<.0001**	0.35	**<.0001**
MG	0.87	*0.1*	**0.03**	0.27	0.54
Treatment	0.96	0.89	0.96	0.65	0.80
Year*Location	**<.0001**	**<.0001**	**<.0001**	**0.03**	**<.0001**
Year*MG	*0.1*	0.14	0.93	**<.0001**	**0.003**
Location*MG	**0.003**	**0.02**	0.85	**0.008**	0.57
Year* Treatment	0.63	0.35	0.75	0.79	0.31
Location* Treatment	0.16	0.25	0.34	0.67	0.30
MG* Treatment	0.65	*0.05*	0.82	0.4	*0.06*
Year*Location*MG	0.66	**0.004**	0.61	*0.08*	0.44
Year*Location* Treatment	0.13	0.14	0.13	**0.04**	*0.06*
Year*MG* Treatment	0.54	*0.05*	0.52	0.17	0.29
Location*MG*Treatment	0.78	0.54	0.7	0.99	0.95
Year*Location*MG*Treatment	0.55	0.87	0.57	0.58	0.60

Table 3-9 cont.

	Leaves	Main Stem	Branches + Petioles	Pods; Seeds/ Pod Shells	Total Biomass
			R7		
Year	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Location	**0.01**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
MG	**<.0001**	0.15	**<.0001**	0.37	0.36
Treatment	0.77	0.45	0.11	0.26	0.85
Year*Location	**<.0001**	**<.0001**	0.55	**<.0001**	0.30
Year*MG	**0.001**	**0.02**	**<.0001**	0.79	0.29
Location*MG	0.69	**<.0001**	**<.0001**	*0.06*	0.12
Year* Treatment	0.51	0.64	0.42	0.45	0.86
Location* Treatment	**<.0001**	0.88	*0.08*	0.35	0.45
MG* Treatment	0.61	0.91	0.19	0.48	0.70
Year*Location*MG	0.6	**<.0001**	**<.0001**	*0.05*	0.46
Year*Location* Treatment	*0.1*	0.74	*0.07*	0.46	0.86
Year*MG* Treatment	0.98	0.91	0.37	0.34	0.85
Location*MG*Treatment	0.19	0.39	*0.08*	0.66	0.20
Year*Location*MG*Treatment	*0.06*	0.55	*0.1*	0.45	0.24

Table 3-10. Effect of Nitrogen and Sulfur applications, on R5, R6, and R7 biomass in eastern SD in 2018 and 2019.

		Leaves	Main Stem	Branches and Petioles	Pods; Seeds/Pod Shells	Total Biomass
			(kg ha^{-1})			
R5	UTC[†]	1902	1558	1438	1370	6268
	Preplant	1913	1551	1474	1284	6222
	V4	1861	1514	1400	1236	6011
	R3	1856	1516	1364	1300	6036
	V4+R3	1860	1731	1421	1317	6329
R6	UTC	1799	1981	1718	4018	9516
	Preplant	1782	1926	1717	3693	9118
	V4	1813	2002	1712	3859	9386
	R3	1813	1967	1682	3974	9436
	V4+R3	1818	1999	1780	3886	9483
R7	UTC	284	1341	819[*]	3406/2186	8036
	Preplant	297	1290	842	3106/2207	7742
	V4	303	1267	861	3251/2276	7958
	R3	297	1259	767	3106/2221	7650
	V4+R3	283	1203	795	3289/2218	7788

Table 3-10 cont.

		Leaves	Main Stem	Branches and Petioles	Pods; Seeds/Pod Shells	Total Biomass
				(kg ha^{-1})		
Year Location*						
R5						
2018	Brookings	2498a††	2394a	1641a	1735a	10102a
	SERF	2108b	1725b	1769a	1615a	7219b
2019	Brookings	1595c	1344bc	1224b	727b	4892c
	SERF	1600c	875c	1140b	780b	4397c
R6						
2018	Brookings	3014a	3682a	2637a	5045a	14889a
	SERF	1996b	1786b	1736b	4561a	7219b
2019	Brookings	1599b	1333b	1231b	3022b	7262b
	SERF	1554b	780b	1231b	3223b	4397b
R7						
2018	Brookings	684a	2227a	990a	4040b/1580c	10438a
	SERF	313b	1637b	1171a	4617a/1956a	11345a
2019	Brookings	95c	1008c	596b	3349c/1592c	6632b
	SERF	401b	975c	731b	4329ab/1772b	8086b

† Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at pre-plant;V4, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at V4 growth stage; R3,11 kg S ha^{-1} and 44.8 kg N ha^{-1}fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at both V4 and R3 growth stage

†† different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

*Branches and petiole fractions at R7growth stage contained mainly branches, as petioles have fallen

Table 3-11. Percentages of biomass plant weights in eastern SD in 2018 and 2019.

		Leaves	Main Stem	Branches and Petioles	Pods; Seed/ Pod Shells	Fallen Leaves
R5	UTC	0.33	0.25	0.24	0.19	N/A
	Pre	0.33	0.25	0.25	0.18	N/A
	V4	0.34	0.23	0.25	0.18	N/A
	R3	0.32	0.25	0.24	0.20	N/A
	V4+R3	0.32	0.24	0.24	0.20	N/A
R6	UTC	0.21	0.18	0.18	0.39	0.04
	Pre	0.23	0.20	0.16	0.38	0.07
	V4	0.21	0.19	0.17	0.40	0.04
	R3	0.20	0.18	0.17	0.39	0.04
	V4+R3	0.20	0.19	0.17	0.39	0.04
R7	UTC	0.04	0.16	0.09	0.36/0.17	0.13
	Pre	0.06	0.17	0.11	0.37/0.17	0.15
	V4	0.04	0.15	0.10	0.35/0.17	0.13
	R3	0.04	0.15	0.07	0.39/0.17	0.14
	V4+R3	0.04	0.15	0.10	0.32/0.17	0.14

[†]Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at pre-plant;V4, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at V4 growth stage; R3,11 kg S ha^{-1} and 44.8 kg N ha^{-1}fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at both V4 and R3 growth stage

Table 3-12. Analysis of Variance (ANOVA) significance levels of N and S application, year, location, planting date (PDate), and maturity group (MG) main effects and their interactions on biomass concentrations in Brookings, SD in 2018 and 2019.

	Leaves	Main Stem	Branches + Petioles	Pods; Seeds/ Pod Shells	Total Biomass
			R5		
Year	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Pdate	**<.0001**	**<.0001**	**0.003**	**<.0001**	0.32
MG	0.35	0.75	**<.0001**	**<.0001**	0.70
Treatment	0.94	0.81	0.96	0.60	0.96
Year*Pdate	**<.0001**	**<.0001**	**<.0001**	**0.03**	**0.01**
Year*MG	0.87	**0.007**	0.20	**0.004**	0.21
Year*Treatment	0.85	0.33	0.66	**<.0001**	0.15
Pdate*MG	0.84	0.36	0.90	0.70	0.76
Pdate*Treatment	0.44	0.44	0.65	0.45	0.61
MG*Treatment	0.19	0.89	0.28	0.30	0.39
Year*Pdate*MG	0.94	0.79	0.74	0.02	0.64
Year*Pdate*Treatment	0.85	0.63	0.32	0.93	0.70
Year*MG*Treatment	0.80	0.75	0.80	0.48	0.69
Pdate*MG*Treatment	0.63	0.28	0.81	0.64	0.49
Year*Pdate*MG*Treatment	0.75	0.85	0.42	0.82	0.78
			R6		
Year	**<.0001**	**<.0001**	**<.0001**	**0.007**	**<.0001**
Pdate	**<.0001**	**<.0001**	**<.0001**	0.98	**<.0001**
MG	**0.04**	0.22	**<.0001**	0.96	0.35
Treatment	0.64	0.45	0.65	0.96	0.84
Year*Pdate	**<.0001**	**<.0001**	0.48	**<.0001**	**<.0001**
Year*MG	0.05	**<.0001**	0.50	0.33	0.15
Year*Treatment	**<.0001**	**0.003**	0.06	0.10	0.73
Pdate*MG	0.09	0.03	0.51	0.33	*0.08*
Pdate*Treatment	0.57	0.75	0.90	0.97	0.97
MG*Treatment	0.94	0.11	0.85	0.53	0.45
Year*Pdate*MG	0.81	0.57	0.78	0.62	0.70
Year*Pdate*Treatment	0.21	0.71	0.26	0.93	0.65
Year*MG*Treatment	0.77	**0.02**	0.99	0.90	0.67
Pdate*MG*Treatment	0.49	0.58	0.14	0.62	0.65
Year*Pdate*MG*Treatment	0.99	0.90	0.79	0.83	0.99

Table 3-12 cont.

	Leaves	Main Stem	Branches + Petioles	Pods; Seeds/ Pod Shells	Total Biomass
			R7		
Year	**<.0001**	**<.0001**	**<.0001**	*0.05*/**0.01**	**<.0001**
Pdate	**0.003**	**<.0001**	**0.02**	**0.01/0.09**	**0.002**
MG	**<.0001**	**0.02**	**<.0001**	0.37/0.21	0.65
Treatment	0.07	0.60	0.20	0.12/0.28	0.38
Year*Pdate	**<.0001**	**<.0001**	**<.0001**	0.11/**<.0001**	**<.0001**
Year*MG	**<.0001**	**<.0001**	**0.02**	0.06/0.62	0.17
Year*Treatment	**<.0001**	**0.03**	**<.0001**	0.68/0.16	0.70
Pdate*MG	0.11	0.41	*0.07*	0.84/0.63	0.81
Pdate*Treatment	*0.05*	0.96	0.17	*0.05*/0.49	0.15
MG*Treatment	0.27	0.71	0.28	0.53/0.42	0.53
Year*Pdate*MG	**0.004**	0.11	**<.0001**	*0.06*/0.32	0.48
Year*Pdate*Treatment	*0.06*	0.93	0.46	0.58/0.19	0.59
Year*MG*Treatment	0.64	0.74	0.86	0.98/0.84	0.99
Pdate*MG*Treatment	0.28	0.74	0.35	0.45/0.45	0.46
Year*Pdate*MG*Treatment	0.50	0.92	0.42	0.25/0.62	0.47

Table 3-13. Effect of Nitrogen and Sulfur applications, on R5, R6, R7 biomass in Brookings, SD in 2018 and 2019.

		Leaves	Main Stem	Branches and Petioles	Pods; Seeds/ Pod Shells	Total Biomass
					(kg ha^{-1})	
R5	UTC	1902	1558	1438	1370	6268
	Preplant	1913	1551	1474	1284	6222
	V4	1861	1514	1400	1236	6011
	R3	1856	1516	1364	1300	6036
	V4+R3	1860	1731	1421	1317	6329
R6	UTC	1799	1981	1718	4018	9516
	Preplant	1782	1926	1717	3693	9118
	V4	1813	2002	1712	3859	9386
	R3	1813	1967	1682	3974	9436
	V4+R3	1818	1999	1780	3886	9483
R7	UTC	284	1341	819[*]	3406/2186	8036
	Preplant	297	1290	842	3106/2207	7742
	V4	303	1267	861	3251/2276	7958
	R3	297	1259	767	3106/2221	7650
	V4+R3	283	1203	795	3289/2218	7788

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at pre-plant;V4, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at V4 growth stage; R3,11 kg S ha-1 and 44.8 kg N ha-1fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at both V4 and R3 growth stage.

Table 3-14. Percentages of biomass plant weights in Brookings, SD in 2018 and 2019 on two planting dates.

		Leaves	Main Stem	Branches and Petioles	Pods; Seed/ Pod Shells	Fallen Leaves
R5	UTC	0.30	0.26	0.22	0.20	N/A
	Pre	0.28	0.28	0.23	0.20	N/A
	V4	0.31	0.24	0.23	0.20	N/A
	R3	0.30	0.26	0.22	0.21	N/A
	V4+R3	0.30	0.26	0.22	0.20	N/A
R6	UTC	0.18	0.22	0.18	0.39	0.05
	Pre	0.21	0.22	0.18	0.35	0.05
	V4	0.18	0.21	0.17	0.39	0.05
	R3	0.18	0.22	0.17	0.39	0.04
	V4+R3	0.17	0.22	0.18	0.38	0.04
R7	UTC	0.03	0.18	0.08	0.34/0.18	0.11
	Pre	0.08	0.17	0.10	0.32/0.17	0.14
	V4	0.05	0.17	0.10	0.33/0.17	0.11
	R3	0.05	0.18	0.09	0.35/0.16	0.12
	V4+R3	0.04	0.16	0.09	0.33/0.17	0.13

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at pre-plant;V4, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at V4 growth stage; R3,11 kg S ha-1 and 44.8 kg N ha-1fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at both V4 and R3 growth stage.

Table 3-15. Nitrogen and Sulfur application effect on N, P, K, S, concentration in most recently matured leaves (MRML) tissue samples taken at R2 growth stage in eastern SD in 2018.

Year/Location	Treatment	N-	P	K	S
			(ppm)		
Brookings		5.37	0.37	2.26a	0.27b
SERF		5.42	0.36	2.06b	0.30a
	UTC	5.26c	0.36	2.17	0.27b
	Preplant	5.41ab	0.37	2.12	0.29a
	V4	5.45a	0.36	2.11	0.29a
	R3	5.35b	0.37	2.22	0.28ab
	V4+R3	5.49a	0.37	2.16	0.29a
	p<F				
	Location	0.25	0.24	**<.0001**	**<.0002**
	MG	0.27	0.89	0.26	0.39
	Treatment	*0.08*	0.33	0.80	*0.09*
	Location*MG	0.43	0.96	0.79	0.96
	Location* Treatment	0.96	0.49	0.62	0.46
	MG* Treatment	0.62	0.91	0.96	0.54
	Location*MG*Treatment	0.47	0.62	0.53	0.98

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at pre-plant;V4, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at V4 growth stage; R3,11 kg S ha-1 and 44.8 kg N ha-1fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at both V4 and R3 growth stage

Table 3-16. Nitrogen and Sulfur application effect on N, P, K, S, concentration in most recently matured leaves (MRML) tissue samples taken at the R2 growth stage in Brookings SD in 2018.

Year/Location	Treatment	N	P	K	S
				ppm	
Pdate 1		5.36	0.37	2.26a	0.27
Pdate 2		5.36	0.38	2.19b	0.27
UTC		5.27	0.36	2.20	0.26b
Pre-plant		5.36	0.38	2.19	0.28a
V4		5.43	0.38	2.25	0.28a
R3		5.28	0.37	2.22	0.26b
V4+R3		5.47	0.37	2.25	0.28a
	p<F				
	PDate	0.87	0.38	*0.06*	0.30
	MG	0.35	0.52	0.40	0.68
	Treatment	0.17	0.12	0.93	*0.09*
	PDate*MG	0.20	0.46	*0.06*	0.15
	PDate*Treatment	0.50	0.20	0.41	0.53
	MG*Treatment	0.48	0.64	0.64	0.44
	PDate*MG*Treatment	0.65	0.39	0.20	0.91

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at pre-plant;V4, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at V4 growth stage; R3,11 kg S ha-1 and 44.8 kg N ha-1fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at both V4 and R3 growth stage.

Table 3-17. Nitrogen and Sulfur application effect on N, K, S, in most recently matured leaves (MRML) tissue samples taken at the R2 growth stage in eastern SD in 2018.

		Brookings/ SERF			PDates	
		N	S	K	S	K
			(ppm)			
Location						
Brookings			0.27b	2.22a		
SERF			0.30a	2.05b		
Planting Date						
Pdate1						2.26a
Pdate2						2.19b
Planting Date MG*						
Pdate 1	MG1					2.24a
	MG2					2.27a
Pdate2	MG1					2.24a
	MG2					2.13b

[†] different lower-case letters indicate statistically different results within a column at p=0.10 confidence level

Figure 3-1. Nitrogen and Sulfur applications, R5, R6, R7 biomass weights in eastern SD in 2018 and 2019.
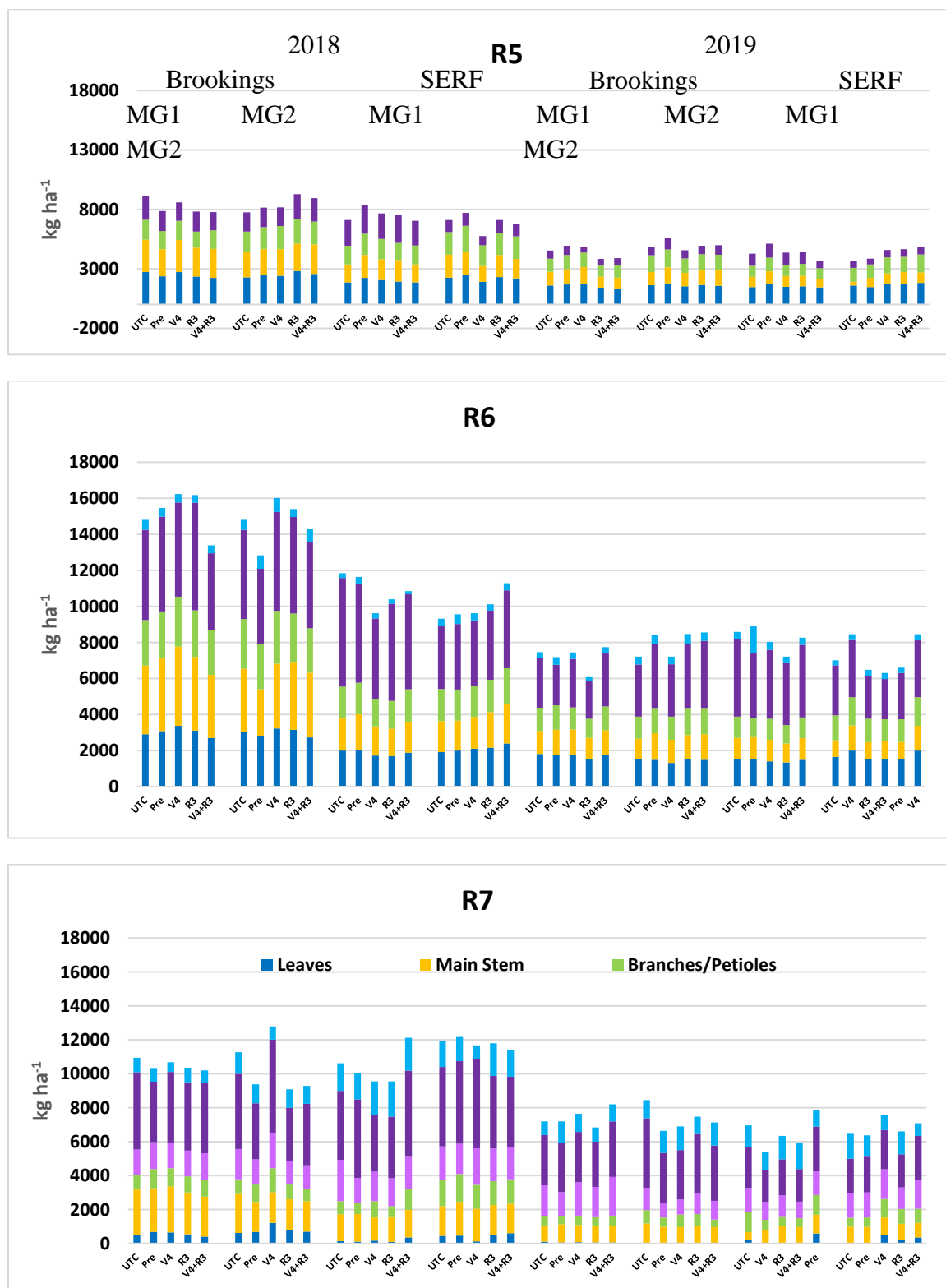
Figure 3-2. Nitrogen and Sulfur applications, R5, R6, R7 biomass weights in Brookings SD in 2018 and 2019.
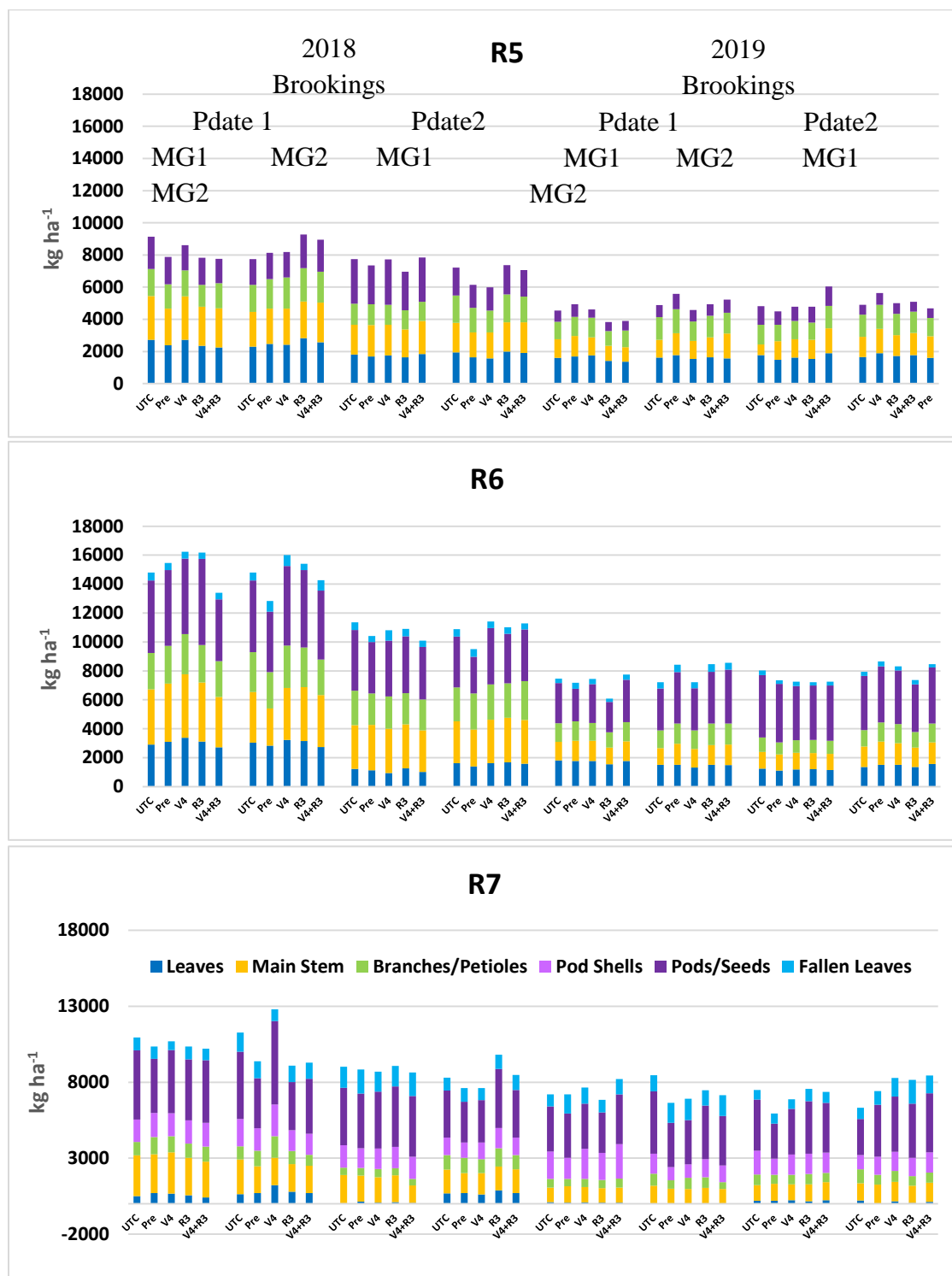
CHAPTER 4

4.1 OVERALL CONCLUSIONS

Overall, 2018 was not easily comparable to 2019 due to the delayed planting at Brookings and replanting at SERF in 2019 as a result of inclement weather conditions and the late spring. While comparing these two studies is difficult due to varieties used from different seed companies in the two studies and the varieties had somewhat different maturity lengths (1.0 vs 1.1 and 2.0 vs 2.4), there is a way to do so logically. When approaching a field, knowing some of its history is the key to finding what could increase the amount of protein in the plant. If a field is known to have low S, then applications of S may increase the amount of protein you receive while still maintaining or increasing yield. If a field is known to have a problem with a disease, then appropriate fungicide application could potentially help increase the amount of protein and grain yield at harvest.

The more products applied on a field, the more the production cost is. If there is not a problem in the field, there is not a reason to add unnecessary costs that will not help increase the grain quality or help benefit the amount of grain yield that is harvested. Only when there is a problem will benefits be seen when applying fungicide, insecticides, or fertilizer applications on both grain quality and grain yield.

The current study showed that the crop management practices evaluated had small influence on grain yield or seed composition during the two years of the project. We have experienced above normal precipitation amount during the early part of the growing season, and plenty of rainfall through the season which may influenced the results we have seen. With current soybean grain pricing structures at the elevators, there is no

economic incentive for producing higher seed protein concentration while maximizing

yield. It is not practical to convince farmers to produce a crop that has higher seed protein

(or better seed composition) without providing some premium for their effort.

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APPENDIX

Table A-1. Foliar application effect on grain yield, protein and oil concentrations in eastern SD in 2018 and 2019.

Year/Location	MG	Treatment	Yield (Mg ha⁻¹)	Protein (%)	Oil (%)
2018 Brookings	MG1	Control	4.40	36.9a	17.8b[†]
		Fungicide	3.93	36.8a	17.7b
		Insecticide	4.21	36.6a	17.8b
		Fungicide and Insecticide	4.07	36.4ab	18.0ab
		FFI††	4.58	36.3ab	18.1ab
		Foliar Fertilizer	4.79	36.8a	17.8b
	MG2	Control	4.74	36.6a	17.8b
		Fungicide	5.29	36.4ab	18.0ab
		Insecticide	4.92	36.5ab	17.9ab
		Fungicide and Insecticide	4.86	35.8b	18.4a
		FFI	4.14	36.5ab	18.0ab
		Foliar Fertilizer	4.90	36.4ab	18.1ab
2019 Brookings	MG1	Control	3.36	34.9ab	17.5b
		Fungicide	3.45	34.8ab	17.3b
		Insecticide	3.32	34.8abc	17.4b
		Fungicide and Insecticide	3.46	34.8abc	17.4b
		FFI	3.51	34.8abc	17.5b
		Foliar Fertilizer	3.27	34.9a	17.5b
	MG2	Control	3.65	34.4abcd	18.1a
		Fungicide	3.93	34.2cd	18.4a
		Insecticide	3.80	34.3bcd	18.2a
		Fungicide and Insecticide	3.82	34.3abcd	18.2a
		FFI	4.02	34.6abcd	18.1a
		Foliar Fertilizer	3.47	34.1d	18.3a

Table A-1 cont.

Year/Location	MG	Treatment	Yield (Mg ha⁻¹)	Protein (%)	Oil (%)
2019 SERF	MG1	Control	3.64	34.9ab	18.1b
		Fungicide	3.85	34.8ab	17.9b
		Insecticide	3.66	34.8abc	18.1b
		Fungicide and Insecticide	3.64	34.8abc	18.0b
		FFI	3.77	34.8abc	18.0b
		Foliar Fertilizer	3.69	34.9ab	18.0b
	MG2	Control	3.92	34.4abcd	18.1a
		Fungicide	4.24	35.1a	18.1a
		Insecticide	4.30	34.3bcd	18.2a
		Fungicide and Insecticide	4.28	34.3abcd	18.3a
		FFI	4.15	34.6abcd	18.2a
		Foliar Fertilizer	4.18	34.1d	18.2a

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

[††] Combination of foliar fertilizer, fungicides and insecticide application

Table A-2. Foliar application effect on grain yield, protein and oil concentrations in Beresford, SD in 2018.

		Yield Mg ha^{-1}	Protein %	Oil %
Maturity Group	*Foliar application treatment*			
MG1	Control	5.53	35.1 bc[†]	18.5c[†]
	Fungicide	5.62	34.7 c	18.7a
	Insecticide	5.51	34.9 c	18.4c
	Fungicide and Insecticide	5.64	34.9 c	18.5bc
MG2	Control	5.52	35.0 bc	18.7a
	Fungicide	5.91	35.2 ab	18.9a
	Insecticide	5.71	35.0 bc	18.8a
	Fungicide and Insecticide	5.93	35.5 a	18.7ab
	p<F			
	Treatment	0.80	*0.06*	0.31
	MG	*0.01*	0.21	0.11
	Treatment x MG	0.11	0.75	0.81

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

Table A-3. Foliar application, percentage of weights compared to total on R5, R6, and R7.

Stage	Year	Location	MG	Treatment	2018					2019				
					Leaves	Main Stem	Branches and Petioles	Pods	Fallen Leaves	Leaves	Main Stem	Branches and Petioles	Pods	Fallen
R5	2018	Brookings	MG 1	UTC	0.36	0.26	0.19	0.2	N/A	0.37	0.24	0.24	0.15	N/A
				Fungicide	0.32	0.3	0.19	0.16	N/A	0.35	0.25	0.22	0.19	N/A
				Insecticide	0.3	0.33	0.19	0.18	N/A	0.36	0.24	0.24	0.16	N/A
				Fungicide and Insecticide	0.31	0.32	0.18	0.19	N/A	0.34	0.26	0.23	0.17	N/A
				Foliar Fertilizer	0.33	0.28	0.22	0.17	N/A	0.35	0.24	0.24	0.18	N/A
				FFI††	0.31	0.32	0.18	0.19	N/A	0.36	0.25	0.22	0.17	N/A
			MG 2	UTC	0.3	0.27	0.2	0.23	N/A	0.32	0.22	0.26	0.19	N/A
				Fungicide	0.3	0.24	0.23	0.24	N/A	0.34	0.21	0.27	0.18	N/A
				Insecticide	0.29	0.28	0.21	0.22	N/A	0.32	0.23	0.27	0.18	N/A
				Fungicide and Insecticide	0.3	0.21	0.22	0.23	N/A	0.34	0.21	0.28	0.17	N/A
				Foliar Fertilizer	0.31	0.25	0.22	0.23	N/A	0.34	0.19	0.29	0.17	N/A
				FFI††	0.29	0.27	0.17	0.27	N/A	0.35	0.24	0.25	0.16	N/A

Table A-3 cont.

Stage	Year	Location	MG	Treatment	2018						2019				
					Leaves	Main Stem	Branches and Petioles	Pods	Fallen Leaves	Leaves	Main Stem	Branches and	Pods	Fallen Leaves
	SERF		MG 1	UTC	0.41	0.28	0.03	0.27	N/A	0.27	0.15	0.14	0.15	N/A
				Fungicide	0.30	0.20	0.24	0.23	N/A	0.3	0.03	0.18	0.16	N/A
				Insecticide	0.31	0.21	0.21	0.27	N/A	0.26	0.15	0.16	0.14	N/A
				Fungicide and Insecticide	0.31	0.23	0.20	0.25	N/A	0.26	0.13	0.15	0.15	N/A
				Foliar Fertilizer	N/A	N/A	N/A	N/A	N/A	0.28	0.14	0.16	0.14	N/A
				FFI††	N/A	N/A	N/A	N/A	N/A	0.27	0.15	0.16	0.13	N/A
			MG 2	UTC	0.34	0.23	0.24	0.19	N/A	0.28	0.15	0.16	0.15	N/A
				Fungicide	0.35	0.19	0.27	0.19	N/A	0.28	0.15	0.17	0.14	N/A
				Insecticide	0.35	0.24	0.24	0.17	N/A	0.27	0.15	0.17	0.14	N/A
				Fungicide and Insecticide	0.34	0.22	0.25	0.20	N/A	0.27	0.16	0.18	0.12	N/A
				Foliar Fertilizer	N/A	N/A	N/A	N/A	N/A	0.29	0.15	0.18	0.15	N/A
				FFI††	N/A	N/A	N/A	N/A	N/A	0.27	0.15	0.16	0.14	N/A

Table A-3 cont.

Stage	Year	Location	MG	Treatment	2018						2019				
					Leaves	Main Stem	Branches and Petioles	Pods	Fallen Leaves	Leaves	Main Stem	Branches and	Pods	Fallen Leaves
R6		Brookings	MG 1	UTC	0.18	0.26	0.12	0.41	0.03	0.19	0.17	0.15	0.46	0.04
				Fungicide	0.18	0.22	0.12	0.44	0.05	0.21	0.16	0.17	0.44	0.03
				Insecticide	0.17	0.25	0.10	0.42	0.06	0.18	0.17	0.15	0.46	0.04
				Fungicide and Insecticide	0.16	0.25	0.90	0.44	0.06	0.22	0.11	0.18	0.47	0.02
				Foliar Fertilizer	0.16	0.20	0.12	0.44	0.07	0.20	0.15	0.16	0.45	0.04
				FFI††	0.17	0.25	0.10	0.43	0.05	0.20	0.18	0.15	0.44	0.03
			MG 2	UTC	0.12	0.20	0.10	0.54	0.03	0.18	0.16	0.15	0.48	0.02
				Fungicide	0.16	0.19	0.09	0.54	0.03	0.20	0.15	0.15	0.47	0.02
				Insecticide	0.12	0.23	0.09	0.52	0.04	0.18	0.17	0.15	0.48	0.03
				Fungicide and Insecticide	0.13	0.21	0.10	0.51	0.05	0.11	0.19	0.18	0.5	0.02
				Foliar Fertilizer	0.10	0.23	0.09	0.51	0.06	0.18	0.15	0.16	0.48	0.03
				FFI††	0.12	0.24	0.08	0.52	0.03	0.2	0.17	0.17	0.45	0.02

Table A-3 cont.

Stage	Year	Location	MG	Treatment	2018					2019				
					Leaves	Main Stem	Branches and Petioles	Pods	Fallen Leaves	Leaves	Main Stem	Branches and	Pods	Fallen Leaves
		SERF	MG 1	UTC	0.18	0.17	0.14	0.48	0.02	0.22	0.16	0.13	0.46	0.02
				Fungicide	0.18	0.18	0.15	0.48	0.01	0.27	0.16	0.14	0.46	0.02
				Insecticide	0.18	0.18	0.15	0.48	0.01	0.22	0.17	0.1	0.5	0.02
				Fungicide and Insecticide	0.18	0.17	0.15	0.48	0.01	0.21	0.16	0.15	0.47	0.01
				Foliar Fertilizer	N/A	N/A	N/A	N/A	N/A	0.22	0.16	0.15	0.46	0.02
				FFI††	N/A	N/A	N/A	N/A	N/A	0.21	0.15	0.14	0.5	0.01
			MG 2	UTC	0.19	0.16	0.16	0.46	0.03	0.22	0.15	0.16	0.44	0.03
				Fungicide	0.20	0.16	0.16	0.45	0.03	0.24	0.15	0.17	0.42	0.02
				Insecticide	0.18	0.18	0.14	0.47	0.03	0.23	0.16	0.14	0.45	0.02
				Fungicide and Insecticide	0.19	0.17	0.15	0.46	0.03	0.23	0.15	0.16	0.45	0.01
				Foliar Fertilizer	N/A	N/A	N/A	N/A	N/A	0.23	0.16	0.14	0.44	0.03
				FFI††	N/A	N/A	N/A	N/A	N/A	0.27	0.17	0.19	0.36	0.02

Table A-3 cont.

Stage	Year	Location	MG	Treatment	2018					2019				
					Leaves	Main Stem	Branches and Petioles	Seeds/Pod Shells	Fallen Leaves	Leaves	Main Stem	Branches and	Seeds/Pod Shells	Fallen Leaves
R7		Brookings	MG 1	UTC	0.02	0.15	0.05	0.34/0.31	0.13	0.01	0.13	0.10	0.17/0.49	0.09
				Fungicide	0.03	0.20	0.06	0.32/0.30	0.10	0.02	0.14	0.10	0.18/0.48	0.08
				Insecticide	0.02	0.19	0.05	0.33/0.31	0.10	0.02	0.15	0.10	0.16/0.49	0.09
				Fungicide and Insecticide	0.05	0.26	0.08	0.25/0.23	0.13	0.02	0.15	0.09	0.17/0.49	0.09
				Foliar Fertilizer	0.03	0.16	0.08	0.31/0.35	0.08	0.11	0.08	0.08	0.18/0.46	0.09
				FFI††	0.01	0.18	0.05	0.34/0.32	0.09	0.04	0.15	0.08	0.18/0.46	0.07
			MG 2	UTC	0.01	0.19	0.06	0.31/0.29	0.14	N/A	0.19	0.08	0.15/0.39	0.24
				Fungicide	0.01	0.13	0.07	0.35/0.33	0.11	N/A	0.14	0.08	0.14/0.46	0.18
				Insecticide	0.01	0.17	0.05	0.39/0.37	0.01	N/A	0.13	0.09	0.16/0.52	0.14
				Fungicide and Insecticide	0.02	0.11	0.05	0.37/0.35	0.11	0.003	0.12	0.08	0.15/0.50	0.15

Table A-3 cont.

Stage	Year	Location	MG	Treatment	2018					2019				
					Leaves	Main Stem	Branches and Petioles	Seeds/Pod Shells	Fallen Leaves	Leaves	Main Stem	Branches and	Seeds/Pod Shell	Fallen Leaves
				Foliar Fertilizer	0.01	0.15	0.04	0.36/0.34	0.10	N/A	0.11	0.08	0.17/0.50	0.16
				FFI††	0.02	0.19	0.05	0.33/0.31	0.11	N/A	0.15	0.002	0.16/0.55	0.17
		SERF	MG 1	UTC	0.03	0.15	0.07	0.15/0.44	0.15	0.02	0.09	0.07	0.19/0.51	0.12
				Fungicide	0.05	0.14	0.09	0.16/0.42	0.14	0.06	0.12	0.09	0.21/0.46	0.06
				Insecticide	0.03	0.16	0.07	0.18/0.43	0.14	0.01	0.13	0.07	0.17/0.51	0.11
				Fungicide and Insecticide	0.03	0.16	0.07	0.16/0.47	0.12	0.01	0.11	0.06	0.17/0.46	0.19
				Foliar Fertilizer	N/A	N/A	N/A	N/A	N/A	0.02	0.12	0.06	0.19/0.48	0.12
				FFI††	N/A	N/A	N/A	N/A	N/A	0.04	0.11	0.06	0.19/0.47	0.13
			MG 2	UTC	0.02	0.16	0.09	0.17/0.50	0.07	0.03	0.09	0.07	0.18/0.51	0.11
				Fungicide	N/A	0.14	0.09	0.17/0.52	0.07	0.05	0.11	0.09	0.16/0.52	0.08
				Insecticide	0.03	0.14	0.09	0.17/0.51	0.06	0.03	0.12	0.08	0.17/0.51	0.11

Table A-3 cont.

Stage	Year	Location	MG	Treatment	2018					2019				
					Leaves	Main Stem	Branches and Petioles	Seeds/ Pod Shells	Fallen Leaves and	Leaves	Main Stem	Branches and	Seeds/Pod Shell	Fallen Leaves and
				Fungicide and Insecticide	0.03	0.15	0.08	0.16/0.54	0.04	0.03	0.12	0.07	0.16/0.55	0.07
				Foliar Fertilizer	N/A	N/A	N/A	N/A	N/A	0.01	0.08	0.02	0.19/0.56	0.14
				FFI††	N/A	N/A	N/A	N/A	N/A	0.05	0.11	0.09	0.16/0.52	0.07

†† Combination of foliar fertilizer, fungicides and insecticide application

Table A-4. Nitrogen and Sulfur applications, effect on grain yield, protein and oil concentrations in eastern SD in 2018 and 2019.

| | | Brookings | | | | SERF | |
| | | PDate 1 | | PDate 2 | | | |
		MG1	MG2	MG1	MG2	MG1	MG2
		Grain Yield Mg ha^{-1}					
2018	UTC	4.08	4.10	3.69	2.91	5.23	4.81
	Pre-plant	3.98	4.33	3.22	2.16	5.11	4.72
	V4	3.81	3.87	3.25	2.79	5.11	4.74
	R3	4.16	4.37	3.25	2.93	5.14	4.77
	V4+R3	4.38	3.97	3.52	2.98	5.22	4.88
2019	UTC	3.13	3.28	3.20	3.41	3.86	3.51
	Pre-plant	3.22	3.39	3.43	3.44	3.81	3.57
	V4	3.43	3.49	3.30	3.38	3.92	3.65
	R3	3.31	3.50	3.41	3.08	3.81	3.71
	V4+R3	3.27	3.42	3.38	3.53	3.71	3.57

Table A-4 cont.

		Brookings				SERF	
		PDate1		PDate2			
		MG1	MG2	MG1	MG2	MG1	MG2
		Protein (%)					
2018	UTC	35.80	35.9	36.1	35.3	34.5	35.2
	Pre-plant	36.1	36.2	36.2	35.5	34.6	35.5
	V4	36.0	35.9	35.8	35.3	34.5	35.5
	R3	36.0	36.2	36.1	35.2	34.5	35.1
	V4+R3	35.6	35.9	36.0	35.5	34.4	35.3
2019	UTC	34.1	33.8	34.6	34.4	34.1	34.6
	Pre-plant	33.6	33.9	34.3	34.2	34.1	34.7
	V4	33.6	33.8	34.2	34.2	33.9	34.6
	R3	34.2	33.9	34.5	34.2	34.3	34.9
	V4+R3	33.8	33.6	34.3	34.1	34.2	34.8
		Oil (%)					
2018	UTC	17.8	17.8	17.4	18.2	18.6	18.4
	Pre-plant	17.5	17.8	17.5	18.2	18.6	18.3
	V4	17.8	18.0	17.5	18.3	18.7	18.3
	R3	17.8	17.9	17.5	18.3	18.7	18.4
	V4+R3	18.0	17.9	17.6	18.3	18.7	18.4
2019	UTC	17.7	18.5	17.4	18.3	17.8	18.4
	Pre-plant	17.7	18.2	17.4	18.4	17.8	18.4
	V4	17.7	18.4	17.5	18.4	17.9	18.3
	R3	17.7	18.4	17.4	18.4	17.8	18.4
	V4+R3	17.8	18.6	17.4	18.4	17.9	18.5

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

Table A-5. R5, R6, R7 biomass by percentages of total weight in eastern SD in 2018 and 2019.

						2018					2019				
Stage	Year	Location	Pdate	MG	Treatment	Leaves	Main Stem	Branches and Petioles	Pods	Fallen Leaves	Leaves	Main Stem	Branches and Petioles	Pods	Fallen Leaves
R5	2018	Brookings	Pdate 1	MG 1	UTC†	0.30	0.30	0.18	0.22	N/A	0.37	0.24	0.24	0.15	N/A
					Pre	0.30	0.29	0.19	0.21	N/A	0.34	0.26	0.34	0.16	N/A
					V4	0.32	0.32	0.19	0.18	N/A	0.35	0.23	0.24	0.15	N/A
					R3	0.30	0.31	0.17	0.22	N/A	0.34	0.26	0.24	0.16	N/A
					V4+R3	0.29	0.31	0.20	0.20	N/A	0.34	0.23	0.26	0.15	N/A
				MG 2	UTC	0.30	0.28	0.22	0.21	N/A	0.33	0.25	0.27	0.14	N/A
					Pre	0.30	0.27	0.23	0.20	N/A	0.35	0.23	0.27	0.15	N/A
					V4	0.30	0.27	0.24	0.19	N/A	0.33	0.23	0.29	0.15	N/A
					R3	0.30	0.25	0.22	0.23	N/A	0.32	0.24	0.27	0.17	N/A
					V4+R3	0.29	0.28	0.21	0.22	N/A	0.33	0.25	0.26	0.15	N/A
			Pdate 2	MG 1	UTC	0.22	0.23	0.16	0.34	0.04	0.32	0.25	0.23	0.21	N/A
					Pre	0.22	0.25	0.17	0.31	0.05	0.23	0.46	0.19	0.12	N/A
					V4	0.22	0.24	0.15	0.35	0.04	0.37	0.14	0.25	0.24	N/A
					R3	0.23	0.24	0.16	0.33	0.04	0.33	0.26	0.23	0.18	N/A
					V4+R3	0.22	0.25	0.14	0.33	0.05	0.34	0.24	0.24	0.19	N/A

Table A-5 cont.

Stage	Year	Location	Pdate	MG	Treat-ment	2018					2019				
						Leaves	Main Stem	Branches and Petioles	Pods; Seeds/Pod	Fallen Leaves	Leaves	Main Stem	Branches and Petioles	Pods; Seeds/Pod	Fallen Leaves
				MG 2	UTC	0.24	0.23	0.21	0.22	0.09	0.34	0.29	0.24	0.13	N/A
					Pre	0.24	0.23	0.23	0.21	0.09	0.31	0.26	0.23	0.20	N/A
					V4	0.24	0.25	0.20	0.22	0.09	0.34	0.26	0.28	0.13	N/A
					R3	0.26	0.23	0.22	0.23	0.05	0.34	0.29	0.24	0.13	N/A
					V4+R3	0.25	0.25	0.21	0.22	0.06	0.33	0.27	0.27	0.13	N/A
R6			Pdate 1	MG 1	UTC	0.20	0.26	0.17	0.34	0.04	0.26	0.19	0.17	0.34	0.04
					Pre	0.24	0.31	0.02	0.40	0.04	0.37	0.20	0.29	0.14	0.06
					V4	0.21	0.27	0.17	0.32	0.03	0.24	0.17	0.17	0.37	0.05
					R3	0.19	0.25	0.16	0.37	0.03	0.25	0.19	0.19	0.31	0.04
					V4+R3	0.20	0.26	0.18	0.32	0.03	0.23	0.19	0.16	0.36	0.05
				MG 2	UTC	0.20	0.24	0.19	0.33	0.04	0.18	0.16	0.18	0.42	0.06
					Pre	0.22	0.20	0.20	0.33	0.06	0.23	0.18	0.17	0.38	0.06
					V4	0.20	0.22	0.18	0.34	0.05	0.21	0.16	0.17	0.40	0.06
					R3	0.20	0.24	0.18	0.35	0.03	0.18	0.17	0.17	0.42	0.06
					V4+R3	0.19	0.25	0.17	0.33	0.05	0.18	0.18	0.18	0.4	0.06

Table A-5 cont.

Stage	Year	Location	Pdate	MG	Treatment	2018						2019					
						Leaves	Main Stem	Branches and Petioles	Branches and Shells; Pods; Seeds/Pod		Fallen Leaves	Leaves	Main Stem	Branches and Petioles	Pods; Seeds/Pod		Fallen Leaves
			Pdate 2	MG 1	UTC	0.11	0.27	0.21	0.37		0.05	0.17	0.15	0.13	0.52		0.04
					Pre	0.11	0.30	0.21	0.34		0.04	0.17	0.17	0.17	0.44		0.03
					V4	0.09	0.28	0.21	0.36		0.07	0.15	0.14	0.12	0.54		0.04
					R3	0.12	0.28	0.20	0.36		0.05	0.15	0.15	0.11	0.55		0.03
					V4+R3	0.10	0.28	0.21	0.36		0.04	0.16	0.16	0.12	0.52		0.04
				MG 2	UTC	0.15	0.27	0.22	0.32		0.05	0.18	0.18	0.15	0.45		0.04
					Pre	0.15	0.27	0.26	0.27		0.06	0.16	0.15	0.12	0.53		0.04
					V4	0.14	0.26	0.21	0.34		0.04	0.17	0.18	0.14	0.47		0.03
					R3	0.15	0.28	0.22	0.31		0.04	0.17	0.19	0.15	0.45		0.04
					V4+R3	0.14	0.27	0.24	0.32		0.04	0.18	0.18	0.16	0.44		0.03
R7			Pdate 1	MG 1	UTC	0.05	0.25	0.08	0.13/ 0.42		0.08	0.01	0.14	0.08	0.26/ 0.39		0.11
					Pre	0.07	0.25	0.11	0.15/ 0.34		0.08	0.01	0.15	0.07	0.19/ 0.40		0.17
					V4	0.06	0.25	0.10	0.14/ 0.39		0.05	0.02	0.13	0.08	0.25/ 0.41		0.14
					R3	0.05	0.24	0.09	0.15/ 0.39		0.08	0.01	0.15	0.07	0.19/ 0.40		0.12
					V4+R3	0.04	0.23	0.10	0.15/ 0.40		0.08	0.01	0.13	0.07	0.26/ 0.39		0.12

Table A-5 cont.

Stage	Year	Location	Pdate	MG	Treat-ment	2018					2019				
						Leaves	Main Stem	Branches and Petioles	Seeds/Pod Shells	Fallen Leaves	Leaves	Main Stem	Branches and Petioles	Seeds/Pod Shells	Fallen Leaves
				MG 2	UTC	0.06	0.20	0.08	0.16/0.39	0.11	N/A	0.14	0.09	0.16/0.47	0.13
					Pre	0.07	0.19	0.10	0.16/0.35	0.12	0.01	0.12	0.07	0.28/0.40	0.2
					V4	0.10	0.14	0.11	0.16/0.43	0.06	N/A	0.13	0.09	0.16/0.49	0.2
					R3	0.09	0.20	0.09	0.15/0.35	0.12	N/A	0.15	0.08	0.13/0.44	0.14
					V4+R3	0.08	0.19	0.08	0.15/0.39	0.11	N/A	0.14	0.11	0.13/0.42	0.19
			Pdate 2	MG 1	UTC	0.01	0.21	0.05	0.16/0.42	0.15	0.02	0.15	0.09	0.18/0.46	0.09
					Pre	0.02	0.19	0.06	0.15/0.41	0.18	N/A	0.13	0.06	0.15/0.45	0.11
					V4	0.01	0.19	0.06	0.16/0.43	0.15	0.03	0.14	0.09	0.21/0.45	0.09
					R3	0.01	0.19	0.05	0.15/0.44	0.15	0.03	0.19	0.10	0.18/0.39	0.11
					V4+R3	0.01	0.13	0.05	0.17/0.46	0.18	0.03	0.15	0.09	0.19/0.44	0.1

Table A-5 cont.

Stage	Year	Location	Pdate	MG	Treatment	2018					2019				
						Leaves	Main Stem	Branches and Petioles	Seeds/ Pod Shells	Fallen Leaves	Leaves	Main Stem	Branches and Petioles	Seeds/ Pod Shells	Fallen Leaves
				MG 2	UTC	0.08	0.19	0.12	0.14/ 0.37	0.10	0.01	0.14	0.08	0.025/ 0.41	0.12
					Pre	0.09	0.17	0.13	0.13/ 0.35	0.12	0.03	0.16	0.08	0.18/ 0.45	0.12
					V4	0.08	0.18	0.12	0.14/ 0.37	0.10	0.03	0.18	0.14	0.15/ 0.38	0.1
					R3	0.09	0.16	0.12	0.14/ 0.40	0.10	N/A	0.17	0.09	0.16/ 0.46	0.11
					V4+R 3	0.08	0.18	0.11	0.14/ 0.37	0.12	0.02	0.15	0.09	0.15/ 0.45	0.14

[†]Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at pre-plant;V4, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at V4 growth stage; R3,11 kg S ha^{-1} and 44.8 kg N ha^{-1}fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at both V4 and R3 growth stage.

Table A-6. R5, R6, R7 biomass by percentages of total weight in Bookings SD in 2018 and 2019.

Stage	Year	Location	MG	Treatment	2018						2019					
					Leaves	Main Stem	Branches and Petioles	Pod Shells	Pods; Seeds/	Fallen Leaves	Leaves	Main Stem	Branches and Petioles	Pod Shells	Pods; Seeds/	Fallen Leaves
R5	2018	Brookings	MG1	UTC†	0.30	0.30	0.18		0.22	N/A	0.37	0.24	0.24		0.15	N/A
				Pre	0.30	0.29	0.19		0.21	N/A	0.34	0.26	0.34		0.16	N/A
				V4	0.32	0.32	0.19		0.18	N/A	0.35	0.23	0.24		0.15	N/A
				R3	0.30	0.31	0.17		0.22	N/A	0.34	0.26	0.24		0.16	N/A
				V4+R3	0.29	0.31	0.20		0.20	N/A	0.34	0.23	0.26		0.15	N/A
			MG2	UTC	0.30	0.28	0.22		0.21	N/A	0.33	0.25	0.27		0.14	N/A
				Pre	0.30	0.27	0.23		0.20	N/A	0.35	0.23	0.27		0.15	N/A
				V4	0.30	0.27	0.24		0.19	N/A	0.33	0.23	0.29		0.15	N/A
				R3	0.30	0.25	0.22		0.23	N/A	0.32	0.24	0.27		0.17	N/A
				V4+R3	0.29	0.28	0.21		0.22	N/A	0.33	0.25	0.26		0.15	N/A
		SERF	MG1	UTC	0.26	0.21	0.22		0.30	N/A	0.35	0.20	0.22		0.23	N/A
				Pre	0.27	0.23	0.21		0.29	N/A	0.35	0.27	0.26		0.12	N/A
				V4	0.27	0.23	0.22		0.28	N/A	0.34	0.21	0.22		0.24	N/A
				R3	0.26	0.24	0.19		0.31	N/A	0.34	0.21	0.23		0.23	N/A
				V4+R3	0.27	0.21	0.23		0.29	N/A	0.34	0.21	0.22		0.24	N/A
			MG2	UTC	0.32	0.27	0.27		0.14	N/A	0.38	0.21	0.28		0.14	N/A
				Pre	0.32	0.26	0.28		0.14	N/A	0.39	0.19	0.25		0.16	N/A
				V4	0.33	0.23	0.30		0.13	N/A	0.44	0.08	0.33		0.15	N/A
				R3	0.32	0.26	0.26		0.15	N/A	0.38	0.20	0.30		0.12	N/A
				V4+R3	0.32	0.24	0.28		0.15	N/A	0.37	0.20	0.29		0.14	N/A

Table A-6 cont.

Stage	Year	Location	MG	Treatment	2018						2019					
					Leaves	Main Stem	Branches and Petioles	Pod Shells	Pods; Seeds/	Fallen Leaves	Leaves	Main Stem	Branches and Petioles	Pod Shells	Pods; Seeds/	Fallen Leaves
R6		Brookings	MG1	UTC	0.20	0.26	0.17		0.34	0.04	0.26	0.19	0.17		0.34	0.04
				Pre	0.24	0.31	0.02		0.40	0.04	0.37	0.2	0.29		0.14	0.06
				V4	0.21	0.27	0.17		0.32	0.03	0.24	0.17	0.17		0.37	0.05
				R3	0.19	0.25	0.16		0.37	0.03	0.25	0.19	0.19		0.31	0.04
				V4+R3	0.20	0.26	0.18		0.32	0.03	0.23	0.19	0.16		0.36	0.05
			MG2	UTC	0.20	0.24	0.19		0.33	0.04	0.18	0.16	0.18		0.42	0.06
				Pre	0.22	0.20	0.20		0.33	0.06	0.23	0.18	0.17		0.38	0.06
				V4	0.20	0.22	0.18		0.34	0.05	0.21	0.16	0.17		0.40	0.06
				R3	0.20	0.24	0.18		0.35	0.03	0.18	0.17	0.17		0.42	0.06
				V4+R3	0.19	0.25	0.17		0.33	0.05	0.18	0.18	0.18		0.40	0.06
		SERF	MG1	UTC	0.17	0.15	0.17		0.51	0.02	0.19	0.14	0.14		0.47	0.05
				Pre	0.18	0.17	0.15		0.47	0.03	0.19	0.17	0.16		0.46	0.17
				V4	0.18	0.17	0.16		0.47	0.03	0.18	0.14	0.14		0.50	0.05
				R3	0.17	0.14	0.16		0.52	0.02	0.17	0.14	0.12		0.40	0.05
				V4+R3	0.18	0.15	0.17		0.49	0.02	0.18	0.15	0.15		0.48	0.05
			MG2	UTC	0.20	0.18	0.19		0.38	0.05	0.24	0.14	0.20		0.36	0.04
				Pre	0.21	0.19	0.18		0.38	0.06	0.18	0.15	0.14		0.49	0.04
				V4	0.22	0.18	0.18		0.38	0.04	0.24	0.13	0.20		0.40	0.04
				R3	0.21	0.19	0.18		0.38	0.05	0.23	0.14	0.19		0.39	0.06
				V4+R3	0.22	0.19	0.17		0.39	0.03	0.24	0.16	0.19		0.37	0.06

Table A-6 cont.

Stage	Year	Location	MG	Treatment	2018 Leaves	2018 Main Stem	2018 Branches and Petioles	2018 Pod Shells / Pods; Seeds	2018 Fallen Leaves	2019 Leaves	2019 Main Stem	2019 Branches and Petioles	2019 Pod Shells / Pods; Seeds	2019 Fallen Leaves
R7		Brookings	MG1	UTC	0.05	0.25	0.08	0.13/0.42	0.08	0.01	0.14	0.08	0.26/0.39	0.11
				Pre	0.07	0.25	0.11	0.15/0.34	0.08	0.24	0.16	0.19	0.35	0.17
				V4	0.06	0.25	0.10	0.14/0.39	0.05	0.02	0.13	0.08	0.25/0.41	0.14
				R3	0.05	0.24	0.09	0.15/0.39	0.08	0.01	0.15	0.07	0.19/0.40	0.12
				V4+R3	0.04	0.23	0.10	0.15/0.40	0.08	0.01	0.13	0.07	0.26/0.39	0.12
			MG2	UTC	0.06	0.20	0.08	0.16/0.39	0.11	N/A	0.14	0.09	0.16/0.47	0.13
				Pre	0.07	0.19	0.10	0.16/0.35	0.12	0.01	0.12	0.07	0.28/0.40	0.20
				V4	0.10	0.14	0.11	0.16/0.43	0.06	N/A	0.13	0.09	0.16/0.49	0.20
				R3	0.09	0.20	0.09	0.15/0.35	0.12	N/A	0.15	0.08	0.13/0.44	0.14
				V4+R3	0.08	0.19	0.08	0.15/0.39	0.11	N/A	0.14	0.11	0.13/0.42	0.19
		SERF	MG1	UTC	0.01	0.14	0.08	0.23/0.40	0.14	N/A	0.11	0.08	0.17/0.50	0.15
				Pre	0.01	0.16	0.07	0.15/0.46	0.16	0.01	0.15	0.08	0.16/0.46	0.15
				V4	0.02	0.15	0.11	0.18/0.33	0.21	N/A	0.12	0.07	0.17/0.50	0.20
				R3	0.01	0.15	0.06	0.18/0.39	0.21	N/A	0.12	0.06	0.16/0.51	0.17
				V4+R3	0.03	0.15	0.11	0.15/0.39	0.17	N/A	0.14	0.07	0.14/0.45	0.21
			MG2	UTC	0.04	0.15	0.13	0.16/0.37	0.14	0.05	0.11	0.11	0.17/0.50	0.16
				Pre	0.04	0.17	0.15	0.16/0.35	0.13	N/A	0.13	0.07	0.13/0.45	0.17
				V4	0.01	0.15	0.12	0.19/0.46	0.07	0.03	0.11	0.11	0.17/0.42	0.10
				R3	0.04	0.14	0.12	0.17/0.37	0.16	0.03	0.06	0.04	0.20/0.50	0.15
				V4+R3	0.05	0.15	0.13	0.16/0.36	0.14	0.06	0.11	0.12	0.19/0.43	0.10

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at pre-plant;V4, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at V4 growth stage; R3,11 kg S ha^{-1} and 44.8 kg N ha^{-1}fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at both V4 and R3 growth stage.

Table A-7. MRML samples 2018

Year/Location	MG	Treatment	N[-]	P	K	S
					(ppm)	
Brookings	MG1	UTC†	5.17	0.35	2.20	0.25
		Pre-plant	5.36	0.38	2.28	0.28
		V4	5.45	0.40	2.38	0.30
		R3	5.24	0.36	2.17	0.25
		V4+R3	5.39	0.36	2.17	0.27
	MG2	UTC	5.28	0.35	2.26	0.26
		Pre-plant	5.43	0.36	2.13	0.27
		V4	5.43	0.37	2.24	0.28
		R3	5.42	0.39	2.41	0.28
		V4+R3	5.48	0.36	2.31	0.28
SERF	MG1	UTC	5.21	0.37	2.14	0.29
		Pre-plant	5.42	0.37	2.06	0.31
		V4	5.43	0.34	1.87	0.29
		R3	5.43	0.36	2.08	0.28
		V4+R3	5.56	0.36	1.97	0.30
	MG2	UTC	5.37	0.35	2.09	0.28
		Pre-plant	5.42	0.36	2.02	0.29
		V4	5.50	0.33	1.93	0.30
		R3	5.31	0.38	2.22	0.29
		V4+R3	5.52	0.38	2.20	0.32

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at pre-plant;V4, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at V4 growth stage; R3,11 kg S ha^{-1} and 44.8 kg N ha^{-1}fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha^{-1} and 44.8 kg N ha^{-1} fertilizer application rate at both V4 and R3 growth stage.

Table A-8. MRML samples between planting dates.

	Treatment	N⁻	P	K	S
			(ppm)		
Pdate1	UTC	5.17	0.35	2.2	0.25
	Pre-plant	5.36	0.38	2.28	0.28
	V4	5.45	0.4	2.38	0.3
	R3	5.22	0.36	2.17	0.25
	V4+R3	5.39	0.36	2.17	0.28
	UTC	5.28	0.35	2.26	0.26
	Pre-plant	5.43	0.36	2.13	0.27
	V4	5.43	0.37	2.24	0.29
	R3	5.42	0.39	2.41	0.28
	V4+R3	5.48	0.36	2.31	0.28
Pdate2	UTC	5.39	0.38	2.29	0.27
	Pre-plant	5.31	0.39	2.2	0.29
	V4	5.42	0.37	2.22	0.27
	R3	5.23	0.37	2.22	0.26
	V4+R3	5.51	0.39	2.27	0.28
	UTC	5.25	0.35	2.05	0.26
	Pre-plant	5.35	0.39	2.14	0.27
	V4	5.4	0.38	2.14	0.27
	R3	5.26	0.36	2.09	0.26
	V4+R3	5.5	0.38	2.24	0.27

[†]Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha⁻¹ and 44.8 kg N ha⁻¹ fertilizer application rate at pre-plant;V4, 11 kg S ha⁻¹ and 44.8 kg N ha⁻¹ fertilizer application rate at V4 growth stage; R3,11 kg S ha⁻¹ and 44.8 kg N ha⁻¹fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha⁻¹ and 44.8 kg N ha⁻¹ fertilizer application rate at both V4 and R3 growth stage.